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CONTRIBUTED ABSTRACTS AND INVITED SESSION INFORMATION FOR

31st Annual Fall Meeting

THE AMERICAN PHYSIOLOGICAL SOCIETY

A Joint Meeting with the:
CANADIAN
PHYSIOLOGICAL
SOCIETY

AMERICAN
SOCIETY OF
ZOOLOGISTS,
Division of Comparative
Physiology and Biochemistry

CANADIAN
SOCIETY OF
ZOOLOGY,
Section of Comparative
Physiology and Biochemistry

October 12-17, 1980
Sheraton-Centre
Toronto, Canada

SCHEDULE FOR REFRESHER, SYMPOSIA, TUTORIALS AND SPECIAL SESSION

Oct. 13, Monday, AM & PM

Refresher Course: Physiology of calcium and Phosphate Regulation, F. G. Knox. (no other activities will be scheduled for this day)

Oct. 14, Tuesday, AM

- Poster Symposium: Mechanics of Breathing I
- Symposium: Quantitative dynamic regulation of glucose metabolism in physiology and diabetes, M. Vranic.
- Symposium: New perspectives on calcium antagonists, Session I Basic Interactions, G. B. Weiss.
- Tutorials:
  - Follicle stimulating hormone, J. H. Dorrington.
  - Computer assisted tomography, E. H. Wood.

Oct. 14, Tuesday, PM

- Symposium: Control of breathing during fetal and early post-natal life, V. Chernick.
- Symposium: Morphological aspects of renal function, A. P. Evan.
- Bowditch Lecture, (4:30): Seasonal Breeding: A Saga of Reversible Fertility and the Golden Fleece. F. J. Karsh
- Tutorials:
  - Radioactive iodine metabolism nuclear accidents, L. Van Middlesworth.
  - The mechanism and biological value of fever and the significance of endogenous antipyresis, K. E. Cooper.
  - Elements of locomotor control, S. Rossignol.
- APS Annual Banquet (6:30 PM), Ontario Place.

Oct. 15, Wednesday, AM

- Tutorial: Review of the history of respiratory gasses. R. H. Kellogg.
- Symposium: (follows above lecture) Teaching of respiratory physiology, J. B. West.
- Symposium: New perspectives on calcium antagonists, Session II Excitation - Contraction Coupling, R. P. Rubin.

Oct. 15, Wednesday, PM

- Symposium: Man in the cold, R. F. Goldman.
- Symposium: Hormonal regulation of foregut development, B. T. Smith.
- Symposium: Neurophysiology of autonomic preganglionic neurons, F. R. Calaresu.
- Special Opening of the Ontario Science Center (5 PM)

Oct. 16, Thursday, AM

- Symposium: Comparative physiology of diffusion in respiratory gas exchange, J. Piiper and P. Scheid
- Symposium: Physiology of splanchnic circulation, E. D. Jacobson.
- Symposium: New perspectives on calcium antagonists, Session III Stimulus - Secretion Coupling, F. R. Goodman.
- Tutorials:
  - Specialization in movement systems: Lessons learned from examination of the physiology of head movement, V. C. Abrahams.
  - Vestibular physiology: The peripheral response and its central significance, G. Melville Jones
  - The evaluation of the Sympathetic System in physiological & pathological conditions in animals & humans. J. DeChamplain.

Oct. 16, Thursday, PM

- Poster Symposium: Control of Breathing.
- Tutorial: Special adaptations in marine mammals, F. R. Engelhardt.

  (the following lectures are included)
  - Hormonal strategies of adaptation, F. R. Engelhardt.
  - Integrative metabolic functions during diving and recovery, P. W. Hochachka.
  - Growth, starvation and thermoregulation, N. A. Ortisland.
- APS Business Meeting (4:30 PM)

Oct. 17, Friday, AM

- Symposium: Endocrine regulation of vertebrate seasonal reproductive cycles, R. E. Peter.

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1

RESPIRATORY MECHANICS DURING VENTILATION BY HIGH FREQUENCY OSCILLATION (HFO). D.J. Gillespie,* J.R. Rodarte and R.E. Hyatt. Mayo Clinic and Mayo Foundation, Rochester, MN 55901

HFO has been shown to be effective in maintaining ventilation but the effects of HFO on the mechanical properties of the lung and chest wall are unknown. We studied pentobarbital anesthetized dogs in a volume displacement plethysmograph and compared lung volume and static inflation-deflation pressure volume (PV) curves during spontaneous breathing and HFO. Ventilation was maintained, up to 9 hours, by a piston pump with a stroke volume of 2.5 ml/Kg and a frequency between 25 and 30 Hz. With these settings ABG's were acceptable and the mean airway pressure ranged between 4 and 12 cm H_2O. During HFO there was no change in the shape of the PV curves, however, small decreases were noted in TLC (inflation to 30 cm H_2O airway pressure) with a slight parallel shift of the total respiratory PV curve to the right. These changes could be reversed with muscle paralysis (succinylcholine) and can be explained by alterations in tone of the expiratory muscles. In some animals an unexplained small shift to the right of the lung PV curve occurred which reversed with return to spontaneous breathing. Comparison of FRC before and after HFO showed no consistent change. "FRC" during HFO was stable and corresponded to the increased airway pressure. We conclude that HFO, for periods up to 9 hours, causes no detrimental changes in respiratory mechanics. (Supported by NIH grant #HL 21584; DJG recipient of NHLBI Clinical Investigator Award #1-K08-HL00708).

2

THE BEHAVIOUR OF THE RESPIRATORY MUSCLES DURING SLEEP. E. Tabachnik,* N. Muller, H. Levison, and A.C. Bryan. Res Inst Hospital for Sick Children, Toronto, Canada.

We studied the behaviour of the respiratory musclesduring sleep in 8 normal adolescents (mean age 14 yrs). Sleep state was determined by electroencephalographic (EEG) and electro-occulographic criteria. Tidal volume (V_t), as well as the relative contribution of rib cage (RC) and abdomen (Abd) to V_t was monitored by a respiratory inductive plethysmograph. Intercostal and diaphragmatic EMG's were recorded using surface electrodes. In the awake supine posture the mean RC and Abd contribution to V_t was 40% and 60% respectively. During quiet sleep the intercostal activity increased substantially, leading to a reversal in the relative contribution of the RC (65%) and Abd (35%) to V_t. Rapid eye movement sleep (REM) was associated with a marked reduction in the EMG activity of the intercostal muscles and a concomitant increase in the diaphragmatic activity (mean increase of 33%). The RC contribution to V_t decreased to a mean of 34% in REM sleep. Tidal volume was maintained due to the increase in diaphragmatic activity. We conclude that respiratory muscle activity changes with sleep and that REM sleep is associated with a substantial increase in the diaphragmatic work load.

(supported in part by Canadian Cystic Fibrosis Foundation)

3

EFFECTS OF METABOLIC INHIBITORS ON RESPIRATORY MUSCLE BLOOD FLOW. Michael Reid* and Robert L. Johnson. University of Texas Health Science Center, Dallas, TX 75235

Liang (J Clin Invest 60:61-69, '77) has shown Krebs-cycle inhibition by monofluoroacetate (MFA) mimics hypoxia by causing maximal coronary vasodilation; inhibition of glycolysis by iodoacetate (IA) has no effect. We have examined the effect of MFA and IA on respiratory muscle blood flow. Dogs were instrumented to measure blood flow (to diaphragm, intercostals, transverse abdominis and triceps), using radioactive microspheres, and diaphragmatic pressure volume work, with lower-body plethysmography. Control measurements were made at rest and during loading breathing through an inspiratory resistor, after which either MFA (2 mg/kg) or IA (25 mg/kg) was administered. After equilibration, 'rest' and 'work' measurements were repeated, the dogs sacrificed, and muscles removed for scintillation counting. MFA produced a marked increase in resting diaphragmatic blood flow that was not further affected by inspiratory loading. Blood flow increases to the other respiratory muscles (130-360% above 'rest') were also insensitive to work. IA produced no changes in respiratory muscle blood flow. Triceps blood flow was unaffected throughout. We conclude that oxidative inhibition is a potent vasodilator in the respiratory muscles.

	Diaphragmatic Blood Flow in ml/gm/min			
	Rest	Work	Drug Rest	Drug Work
MFA dogs	.159±.069	.553±.174	1.787±.408	1.543±.361
IA dogs	.159±.042	.456±.105	.265±.035	.576±.070

4

RIB CAGE AND ABDOMEN-DIAPHRAGM RESPONSE TO HYPOXIA, HYPERCAPNIA AND EXERCISE. A.Perl*, N.Zamel, and A.S. Rebuck.Trihosp. Respir. Serv.,Toronto,Canada M5G 1X5

It is known that increased ventilation during exercise or CO_2 rebreathing is due largely to augmentation of the rib cage (RC) and to a lesser degree of the abdomen-diaphragm (AD) compartment. No information is available regarding the response to hypoxia or whether any correlation exists between the responses within individuals. We used inductive plethysmography to monitor the total ventilatory,as well as compartmental response to CO_2, hypoxia and treadmill exercise in 11 healthy subjects. In all but 2 subjects during the CO_2 and hypoxia response and in all during exercise the RC compartment predominated the ventilatory response (mean RC slope/AD slope for ventilation: CO_2=3.5, hypoxia=5.2, exercise=3.5).Comparing the RC response at a given level of ventilation in each individual we found the following: CO_2 vs hypoxia $R=0.98; p<0.01$, CO_2 vs exercise $R=0.73$; $p<0.05$, and hypoxia vs exercise $R=0.68$; $p<0.05$. We conclude that the RC dominates the ventilatory response to hypercapnia, hypoxia and exercise, and that for CO_2 and hypoxia the compartmental response is determined by the ventilatory demand and not by the type of the stimulus; however, during exercise the RC movement for the same level of ventilation was higher than during CO_2 and hypoxic stimuli.

5

EFFECT OF MECHANICAL VENTILATION ON RESPIRATORY MUSCLE BLOOD FLOW DURING SHOCK. N. Viires,*G.Sillye§ A.Rassidakis§M.Aubier§ E.Donovan*& Ch. Roussos. Meakins Christie Labs., McGill Univ. Clinic, Royal Victoria Hospital, Montreal, Canada H3A 2B4

We studied blood flow to the respiratory muscles during shock in 2 groups of dogs; one was paralyzed and mechanically ventilated (Mv), the other was breathing spontaneously (Sb). Shock was induced by cardiac tamponade. Cardiac output ($\dot{Q}c$) and muscle blood flow were measured by the radioactive microsphere technique. $\dot{Q}c$ during shock fell to 30% of control values in both groups and was maintained constant. None of the dogs were hypoxic. Ventilation in the Mv group remained unchanged throughout the experiment at 4.75±0.25 L/min, while in Sb it increased during shock by 140% (6.35±1.30 L/min to 15.23±2.01 L/min). Absolute respiratory muscle blood flow decreased with shock in Mv by 79% (36.93± 2.22 ml/min control to 7.77± 0.98 ml/min shock).By contrast, in Sb it increased by 90% (55.82± 3.24 ml/min to 106.07± 9.06 ml/min). The fraction of $\dot{Q}c$ distributed to respiratory muscles during control amounted to 1.85% in Mv and 2.79% in Sb. With shock this decreased to 1.55% in Mv while in Sb it increased by 21.21%. We conclude that mechanical ventilation during shock preserves a large portion of $\dot{Q}c$ that was used by the respiratory muscles during spontaneous breathing, thus making it available to other organs. Supported by the MRC.

6

WHAT MECHANICAL VARIABLES GOVERN THE PERCEIVED MAGNITUDE OF RESPIRATORY LOADS? K.J. Killian* and E.J.M. Campbell. Cardiorespiratory Unit, McMaster University, Hamilton, Ontario L8N 3Z5

Although discrimination experiments have shown that the detection of small resistive loads is a function of resistance ($R=P/\dot{V}$) there is little information on the relative importance of pressure (P), flow ($\dot{V}$) or resistance (R) in assessing the perceived magnitude of larger loads. We have studied the perceived magnitude of added resistive loads in normal subjects inspiring through a range of externally added resistances (3.4-24 cms H_2O/L/s) at various flow rates (0.4-1.2 L/s). Using open magnitude scaling we found that the perceived magnitude of added loads was a function of both flow rate and resistance. The exponents calculated for added resistance alone increased from .84±.10 (S.E.) at a flow rate of 0.4 L/s to 1.36±.19 (S.E.) at a flow rate of 1.2 L/s. However, when the exponents were calculated against the product of $\dot{V}xR$ we found a unique exponent [1.02±.17 (S.E.)] for all five flow rates. As the product of $\dot{V}xR=P$ the results suggest that pressure or inspiratory muscle force is dominant in the genesis of the perceived magnitude of resistive loads. (Supported by Medical Research Council #MA6333).

7

EFFECTS OF INSPIRATORY LOADING ON RESPIRATORY MUSCLE ACTIVITY DURING EXPIRATION. J.Martin*, M.Aubier*, and L.A. Engel. (SPON: Ch. Roussos). Meakins Christie Labs., McGill Univ. Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada.

We studied respiratory muscle activity during expiration in 4 normal subjects during inspiratory resistive(Rin) and elastic(El) loading, with and without positive end-expiratory pressure(PEEP). Pleural(Ppl), abdominal(Pg) and transdiaphragmatic (Pdi) pressures were measured with balloon catheters. Net respiratory muscle activity(Pmus) was inferred by relating Ppl to the chest wall relaxation pressure-volume curve. Abdominal muscle activity (Pabd) was quantitated by relating Pg to the relaxation pressure-volume curve of the abdomen. Subtracting Pabd from Pmus when Pdi =0 allows calculation of intercostal/accessory muscle activity (Pic). Inspiratory muscle relaxation (Pmus and Pdi) was faster during loading than quiet breathing. Expiratory muscles were recruited so that end-expiratory volume (EEV) fell during Rin and El by 0.29(0.1-0.5)l and 0.48(0.25-1.1)l, resp. PEEP increased EEV to 1.2(1.0-1.6)l while the increases on PEEP + Rin and PEEP + El were only 0.95(0.5-1.4)l and 0.78(0.5-1.3)l, resp. In 14 of 20 maneuvers, muscle activity (>2 cmH_2O) was present at end-expiration and in 7, inspiratory Pic and Pabd were present together. We conclude that inspiratory resistive and elastic loading results in (a) enhanced inspiratory muscle relaxation, (b) expiratory muscle recruitment and (c) frequently antagonistic muscle activity, during expiration. (Supported by the MRC of Canada.)

8

EFFECTS OF DIFFERENT RESTRICTIVE PROCEDURES ON LUNG MECHANICS. Monika Scheidt* and R.E. Hyatt. Mayo Clinic, Rochester, MN 55901.

Chest strapping increases lung recoil (Pst) and maximal expiratory flow (Vmax). It is uncertain whether the increase in Pst is caused by breathing at low lung volumes and/or by distortion of the lung. We measured lung volumes, Pst and $\dot{V}max$ in 10 healthy male subjects during 1)Control and 2)Partial maneuvers, 3)Rib cage restriction (RCR) and 4)predominate Abdominal restriction (AR). Esophageal pressure was measured simultaneously at three sites, 6 cm apart. Three estimates of Pst yielded three pressure-volume curves and two estimates of pleural pressure gradient (PPG) in each state. Lung volumes were decreased and flows increased with RCR and AR by approximately the same amount. Compared to the partial maneuvers the overall PPG was increased with RCR. Compared to RCR the PPG with AR was increased high in the esophagus but decreased low in the esophagus. Thus the increase in Pst high in the esophagus was the same with RCR and AR but in the mid-esophagus the change in Pst was less with AR. Nevertheless, based on the mean of the three Pst estimates, the increases in recoil were not different for RCR and AR and correlated with the degree of reduction of lung volumes for both types of restriction. We conclude that low lung volume breathing determines the increase in Pst while shape changes appear to alter the PPG. (Supported in part by HL 21584 from NHLBI).

9

DIAPHRAGMATIC MUSCLE TONE IN DOGS. J. Lopes*, N. Muller & A.C.Bryan, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada.

Based on several experiments in anesthetized animals it is commonly accepted that there is no tonic activity in the diaphragm. However, using surface electrodes after intercostal nerve block we have recently demonstrated that anesthesia depresses tonic diaphragmatic EMG activity in humans. To assess the presence of tonic diaphragmatic activity and to avoid contamination by other respiratory muscles we recorded the diaphragmatic EMG using chronically implanted electrodes located near the central tendon in three dogs. A total of 9 experiments were done. The dogs were studied in the awake state, with and without sedation with acepromazine and after a dose of phenobarbital. We defined tonic diaphragmatic activity as electrical activity present in the EMG throughout expiration. In all instances there was a significant decrease in tonic diaphragmatic activity with anesthesia ($p < .001$). In the experiments with no sedation prior to phenobarbital, the changes were much greater than after sedation, indicating that sedation also decreases tonic diaphragmatic activity. We conclude that in the awake dog there is small, but significant tonic activity in the diaphragm. This activity is greatly diminished or abolished after sedation or barbiturate anesthesia.

(Supported in part by Ontario Thoracic Society)

10

RELATIONSHIP OF DIRECT DIAPHRAGMATIC EMG TO MUSCLE LENGTH IN A CANINE DIAPHRAGM STRIP PREPARATION. M. J. Kim*, W. S. Druz*, W. Machnach* and J.T.Sharp, Hines V. A. Hospital and Univ. of Illinois, Colleges of Medicine and Nursing.

The relationship between direct diaphragm EMG, force and length was studied in the canine diaphragm strip with intact blood supply and innervation under 2 conditions: supramaximal tetanic phrenic stimulation (STPS) and CO_2 stimulation with phrenic nerve intact. In 5 dogs, under both conditions EMG's were recorded from two bipolar silver wire electrodes sutured into the strip, one near its central end and the other near its costal end. A third EMG (central to costal) summed potentials from the whole strip by recording potentials between the central site and the costal site. When muscle length was changed from 50 to 140% of resting in-situ length (Lo), the three EMG's decreased curvilinearly by 40 to 50% in the STPS series and by 30% in the CO_2 stimulation series at constant phrenic neurogram amplitude. Minimal and maximal force outputs were observed at 40 and 120% of Lo for STPS and 70% and 150% of Lo for CO_2 stimulation. The force output at comparable muscle length by the STPS was three times greater than that with CO_2 stimulation. The different force-length relationships found in the CO_2 breathing series may reflect differences in recruitment of various sized muscle fibers. The study indicates that the direct diaphragm EMG may not be an accurate reflection of the phrenic neurogram. The length-tension state of the muscle is another variable to consider in its interpretation. Supported by USPHS Grant No. HL-08789-12.

11

CAPSAICIN STIMULATES BOTH PULMONARY AND BRONCHIAL C-FIBERS TO EVOKE TRACHEAL CONTRACTION. H.M. Coleridge, J.C.G. Coleridge, A.M. Roberts*, M.P. Kaufman* and D.G. Baker. Cardiovascular Research Institute, UCSF, San Francisco, CA. 94143

Capsaicin injected into the right heart of dogs causes reflex bronchoconstriction by stimulating pulmonary C-fibers, but injected into the left heart it has little effect on airway diameter although it is known to stimulate bronchial C-fibers (Russell & Lai-Fook, J. Appl. Physiol. 47: 961, 1979). In anesthetized dogs, we injected capsaicin into the circulation at various sites and examined the reflex changes in tension in an upper tracheal segment innervated by the superior laryngeal nerves. Capsaicin (100-200 µg) injected into the right atrium caused tracheal contraction; left atrial injection had variable effects. Capsaicin (1-5 µg) injected into the bronchial artery evoked dose-dependent increases in tension that were abolished by cutting or cooling (0-1°C) the vagus nerves. Injection into the descending aorta or femoral artery reflexly decreased tension. Atropine abolished the contraction evoked by right atrial or bronchial arterial injection of capsaicin. After atropine, left atrial injection of capsaicin either decreased tracheal tension or had no effect. We conclude that both pulmonary and bronchial C-fibers evoke reflex tracheal contraction, and that the variable effects of left atrial injection result from the interplay of two opposing reflex mechanisms activated by the chemical as it passes through the systemic circulation. (Supported by NIH grants HL-06285, HL-24136 and HL-07192.)

12

STIMULATION OF BRONCHIAL C-FIBERS BY BRADYKININ REFLEXLY CONTRACTS TRACHEAL MUSCLE. A.M. Roberts*, H.M. Coleridge, J.C.G. Coleridge, D.G. Baker and M.P. Kaufman*. Cardiovascular Research Institute, UCSF, San Francisco, CA. 94143

Bradykinin injected into the bronchial artery stimulates afferent vagal endings of bronchial C-fibers: it has little direct effect on other vagal afferents in the lung. We have investigated reflex effects of stimulating bronchial C-fibers in anesthetized dogs. We recorded transverse tension in the posterior wall of an upper cervical tracheal segment innervated by the superior laryngeal nerves (the recurrent laryngeal nerves were cut). Injection of bradykinin (0.02-1.5 µg) into the bronchial artery caused a dose-dependent increase in tracheal muscle tension which still occurred when myelinated fibers from the lung were blocked by cooling the cervical vagus nerves to 7°C but was abolished when myelinated fibers were blocked by cooling to 0-1°C. The efferent supply to the tracheal segment was unaffected by cooling because it was rostral to the vagal block. When the bradykinin-induced contraction was abolished by vagal cooling, reflex contraction of the tracheal segment could still be evoked by stroking the larynx or by ventilating the lungs with 5% O_2 in N_2. The bradykinin-induced reflex was also abolished by cutting the vagal or superior laryngeal nerves, or by administering atropine. Tracheal contraction was usually accompanied by cardiac slowing. Our results provide support for the hypothesis that stimulation of bronchial C-fibers causes bronchoconstriction. (Supported by NIH grants HL-06285, HL-24136 and HL-07192).

13

EXERCISE INCREASES SULFUR DIOXIDE-INDUCED BRONCHOCONSTRICTION IN SUBJECTS WITH ASTHMA. D. Sheppard,* J.A. Nadel, and H.A. Boushey* (SPON: M.B. McIlroy). Cardiovascular Research Institute, University of California, San Francisco, CA 94143

We undertook a study to determine whether moderate exercise modifies the rise in specific airway resistance (SRaw) produced by sulfur dioxide (SO_2) in subjects with mild asthma. In seven subjects, we compared the bronchomotor effects of exercise alone (400 Kpm/min on a cycle ergometer), inhalation of SO_2 alone, and the combination of exercise and SO_2. The temperature (21-23°C) and relative humidity (69-73%) of the inspired gas were the same for each experiment. Neither inhalation of 0.50 ppm of SO_2 at rest nor exercise alone had any effect on SRaw ($p > 0.5$). Inhalation of SO_2 during exercise, however, produced a significant increase in SRaw [from 8.46 ± 1.35 L x cm H_2O/L/s (mean ± SE) to 18.16 ± 3.80 with 0.50 ppm and from 8.07 ± 1.02 to 10.48 ± 1.70 with 0.25 ppm ($p < 0.05$)]. In the two most responsive subjects, inhalation of 0.10 ppm of SO_2 also caused a significant increase in SRaw ($p < 0.05$). We conclude that moderate exercise increases the bronchoconstriction produced by a given concentration of SO_2 in subjects with asthma and that concentrations as low as 0.10 ppm can cause bronchoconstriction in these subjects. (Supported in part by USPHS Program Project Grant HL-24136)

14

EFFECT OF FITNESS ON EXERCISE INDUCED BRONCHOSPASM (EIB). F. Haas, A. Haas*, H. Pineda* and D. Gaudino*. Depts. of Physiol. and Rehab. Med. N.Y.U. Med. Ctr., N.Y., N.Y. 10016

Maximal expiratory flow-volume (MEFV) curves were performed at 3 min. intervals before, during and after graded treadmill exercise by 28 subjects with a history of EIB. 15 had an average maximum O_2 consumption ($\dot{V}O_{2max}$) of 49 ml O_2/kg/min (FIT); 13 had $\dot{V}O_{2max}$ 37 ml O_2/kg/min (UNFIT). Both groups had similarly impaired resting MEFV (mid-vital capacity flow reduced to 60% of predicted). In exercise both reached heart rates of 180/min. FIT ran for 15 and UNFIT for 12 min. In exercise: MEFV increased 10 and 9% in peak expiratory flow (PEF), 28 and 24% and 40 and 18% in maximum expiratory flow at 50 and 75% of vital capacity ($\dot{V}_{max50}$ and $\dot{V}_{max25}$) in FIT and UNFIT, respectively. Post-exercise: MEFV decreased 7 and 17% in PEF, 15 and 22% in $\dot{V}_{max50}$, and 11 and 22% in $\dot{V}_{max25}$, in FIT and UNFIT, respectively. Analysis of variance showed: 1) greater flow increases in MEFV in FIT than in UNFIT ($P<.01$) and 2) post-exercise flows in FIT did not decrease as much as in UNFIT ($P<.01$). EIB onset (indicated by reduced $\dot{V}_{max50}$) occurred 4 min. post-exercise in FIT and significantly earlier (1 min. prior to the end of exercise) in UNFIT ($P<.01$). Similarly, maximum EIB occurred significantly later in FIT than UNFIT (9 and 6 min., respectively, $P<.01$). The data suggests that physical fitness promotes bronchodilation during exercise as evidenced by the greater flows, and diminishes the magnitude of EIB as well as retarding both the onset of EIB and the time of maximum bronchoconstriction. (Supported by RSA 16-P-56801/2/18.)

15

BREATHING PATTERN AND TRACHEAL SMOOTH MUSCLE TONE. Enrico M. Camporesi and John V. Salzano, Dept. of Physiology and Anesthesiology. Duke University, Durham, N.C. 27710.

Changes in tracheal smooth muscle tone were measured in anesthetized paralyzed dogs from the changes in pressure of a compliant cuff of an endotracheal tube inserted in the upper cervical trachea Dogs (16-22 kg) were ventilated through a tightly fitting cannula inserted in the lower third of the cervical trachea. Ventilatory frequency (Vf) was adjusted to maintain normocapnia with tidal volumes (VT) of 15 ml/kg. Breathing patterns were altered for 3-5 minute durations by stepwise changes in one of the following parameters: VT, inspiratory duration (Ti) and expiratory duration (Te). Two sets of experiments were performed at a constant Vf. In the first series VT was maintained constant and Ti was shortened from 50% of Ttot to 20% of Ttot (Ttot = Ti + Te), resulting in a progressively increasing tracheal constriction. Insertion of end-inspiratory pauses (EIP) lasting from 0 to 30% of Ttot resulted instead in a stepwise increase in tracheal dilation. Maximal dilation was obtained with the pattern: 20% Ti 30% EIP, 50% Te. In the second set of experiments Ti/Te was maintained constant while VT was stepwise either decreased or increased from control. This resulted respectively in constriction or dilation of the trachea. All tone changes were abolished by vagotomy or atropine infusion. These data indicate that reflexly sustained tracheal tone is promptly altered by the breathing pattern. The results suggest that tone-inhibitory vagal sensory afferents are recruited at high lung volume and in late inspiration.
Supported by NIH Grant HL 23812.

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PATHWAYS OF SYMPATHETIC INNERVATION OF CERVICAL TRACHEALIS MUSCLE IN DOGS. James K. Brown*, Robert Shields*, Donald Munster*, and Warren M. Gold. CVRI and Dept. of Med., Univ. of Calif., San Francisco, CA 94143

In each of 11 anesthetized dogs, we monitored isometric tension in discrete segments of the cervical trachealis muscle in situ, as described previously (Physiologist 21:70, 1978). To induce tone in each segment, histamine (5-25 µg/kg)was injected into the tracheal circulation. Electrical stimulation (20v, 60Hz, 3msec) of specific nerves partially relaxed (47 ± 8%, mean ± SE) histamine-induced contractions after pre-treatment with atropine (to block contractions caused by simultaneous stimulation of parasympathetic fibers carried in these nerves). Relaxations occurred during stimulation of the superior laryngeal nerves (SLNs) in 7/11 dogs, during stimulation of the central ends of the cervical vagosympathetic trunks (with SLNs cut) in 11/11, and during stimulation of recurrent laryngeal nerves in 11/11. In 3 dogs pre-treated with both atropine and phentolamine (to block alpha-adrenergic contractions), administration of propranolol prevented relaxant responses, which therefore were the result of stimulation of beta-adrenergic receptors presumably by neurotransmitter released from adrenergic nerves. We conclude that in dogs sympathetic fibers are carried to the trachea by 3 pathways: SLNs, a cranial pathway different from SLNs, and recurrent laryngeal nerves. (Supported by NIH Grant HL-24136, the Parker B. Francis Foundation, and the Francis S. North, Sr., Foundation.)

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EFFECTS OF STRETCH ON ACTIVELY CONTRACTED CANINE TRACHEAL SMOOTH MUSCLE. S. Gunst* and J. Russell. (SPON: R.E. HYATT) Mayo Clinic and Foundation, Rochester, MN 55901.

Trachealis strips were mounted horizontally in a tissue bath, between a force transducer and a steel rod which could be moved to alter muscle length using a variable speed motor driven screw. Muscle length and tension could be continuously measured during stretch and simultaneously plotted on an X-Y recorder. L_o was first determined as the length at which a standard electrical stimulus produced maximal active tension. Active tension during stretch was then investigated as follows: An initial length was set with the muscle in a relaxed state. The muscle was then contracted isometrically using 10^{-5}M ACh. When active tension reached a steady state, muscle length was reduced to below 10% L_o. The strip was then slowly stretched (3.5 mm/sec) to obtain a continuous length-active tension curve. This procedure was repeated for isometric contractions performed at 4 or more different lengths on each muscle. Results show that active tension during stretch is higher in muscles stretched after isometric contraction at short lengths (<.75 L_o) as compared with muscles initially contracted at lengths close to or greater than L_o. The decreased contractility found in muscles initially contracted at long lengths where significant passive tension is present may be due to inability of the contractile element to shorten maximally against an afterload. (Supported by HL 21584 and HL 07222).

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CONTRACTIONS OF CANINE INTRAPULMONARY AIRWAYS AND THEIR DEPENDENCE ON CHOLINE UPTAKE. Mark C. Hyatt* and James A. Russell. Mayo Clinic and Foundation, Rochester, MN 55901.

The importance of the high-affinity choline uptake process in sustaining acetylcholine synthesis in isolated canine intrapulmonary airways was examined by using tissue bath techniques. Prolonged electrical stimulation (ES) at 5 or 15 Hz of control airways caused contractions which decayed during the first few minutes of stimulation but eventually reached plateaus. In contrast, contractions decayed rapidly and continuously in airways exposed to 10^{-4}M hemicholinium-3 (HC-3). However, maximal responses to brief periods of ES were not significantly different between control airways and airways exposed to HC-3. Exogenous choline (10^{-4}M), neostigmine ($2x10^{-6}$M), and physostigmine ($2x10^{-6}$M) all caused contractions of resting airways, which we conclude were mediated by the spontaneous release of acetylcholine since these responses either were prevented or abolished by atropine and HC-3 depending on when the airways were exposed to these antagonists. We conclude that two distinct "pools" of acetylcholine are present in the parasympathetic nerves innervating canine airways and that both depend on choline uptake for sustaining acetylcholine synthesis. One pool represents the primary source of acetylcholine for nerve mediated release and the other is available for spontaneous release. (Supported in part by NHLBI grant # HL 21584.)

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CHARACTERIZATION OF MEMBRANE FRACTION FROM BOVINE TRACHEAL SMOOTH MUSCLE. Kannan, M.,* M. Kornegay* and J. F. Souhrada. Natl. Jewish Hospital Res. Ctr., Denver CO 80206

A method has been developed to obtain plasma membrane (PM)-enriched fraction from bovine tracheal smooth muscle. It involves homogenizing the muscle with a polytron; differential centrifugation of the homogenate to obtain microsomes and fractionation of the microsomes into three membrane fractions on discontinuous sucrose density gradient. The three fractions were: F1, F2, and F3 at 20-36%(W/W), 36-45% and 45-60% sucrose interfaces respectively. The isolated membranes were characterized to assess their purity by measuring marker enzymes associated with PM and mitochondria (MI). The PM markers 5'-nucleotidase, Mg^{++}-ATPase, $(Na^{+}+K^{+})$-ATPase and p-Nitrophenyl-phosphatase had the highest specific activities in F1, followed by F2 and F3. MI markers cytochrome C oxidase and succinate cytochrome C reductase had the highest specific activities in F3, followed by F2 and F1. Outer MI marker enzyme , NADH-cytochrome C reductase activity was highest in F1, followed by F2 and F3. Based on the distribution and specific activities of the PM and MI marker enzymes, F1 was identified as PM with outer MI membrane; F2 as PM with inner MI membrane; and F3 as mostly inner MI membrane. Ca^{++} uptake studies, using ^{45}Ca, revealed that F3 showed the highest (>90%) inhibition by azide; F2 lower (50%) inhibition; and F1 the least (<10%). The interpretations based on marker enzyme activity were consistent with the results of Ca^{++} uptake studies.

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EFFECT OF TEMPERATURE ON THE PHYSIOLOGICAL PROPERTIES OF AIRWAY SMOOTH MUSCLE. M. Souhrada*, J. F. Souhrada* and R. M. Cherniack. Natl. Jewish Hospital and Res. Ctr. Denver, CO 80206

We tested the hypothesis that temperature may directly influence resting membrane potential (Em) and ATP-ase activity of bovine and guinea pig airway smooth muscle (ASM). To determine the resting membrane potential (Em) of ASM, glass microelectrodes filled with 3M KCl were used. The Em and ATP-ase activity of ASM was assessed 60 minutes after incubation in normal oxygenated physiological salt solution (PSS) (pH = 7.38 $\pm$ 0.02) at each of the following temperatures: 21°, 23°, 29°, 37° and 40°C. The effect of Ouabain (10^{-3}M) and histamine (10^{-3}M) on the Em of ASM was also determined at each temperature. As a measure of ATP-ase activity, K^{+}-induced relaxation was determined using the method of Webb and Bohr (Blood Vessels 15: 198, 1978). It was found that: 1) Em of ASM was directly proportional to the temperature of the incubating medium; the Em decreased with the decreasing temperature; 2) the administration of Ouabain reduced the dependency of Em on the temperature of the experimental medium; 3) at lower temperatures, histamine-induced depolarization of ASM was significantly potentiated; 4) decrease in the temperature significantly decreased ATP-ase activity of ASM. It is concluded that a change in temperature has a direct influence on the airway smooth muscle cells, affecting both resting membrane potential and ATPase activity of ASM cells.

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CORONARY SINUS ADENOSINE CONCENTRATION AND OUTPUT BY THE CANINE MYOCARDIUM DURING EXERCISE. J.E. McKenzie*, R.P. Steffen*, R.B. Price*, and F.J. Haddy. Dept. Physiology, Uniformed Services University, Bethesda, MD 20014

In the unanesthetized closed chest dog preparation, arterial and coronary sinus adenosine (Ado) concentrations were measured during rest and during treadmill exercise (for 7 min at 5 miles /hour at a 20% slope). Using a new technique developed by Schrader et al, blood was mixed 1:1 with Dipyridamole (Persantine) during collection to decrease uptake of adenosine by red blood cells. The blood was then deproteinized with 8% perchloric acid and adenosine separated by thin layer chromatography. Adenosine concentrations were determined spectrophotometrically. Exercise increased heart rate from 115±9 to 196±5 beats/min, cardiac output (microsphere withdrawal technique) from 4.5±0.4 to 10.3±0.9 l/min, myocardial oxygen consumption from 12.9±2.6 to 35.9±6.2 ml $O_2 \cdot min^{-1} \cdot 100g^{-1}$ and coronary flow (15μ microspheres) from 105±13 to 221±40 ml/min 100g. Coronary vascular resistance (CVR) decreased from 1.10±0.20 to 0.79±0.11 mmHg.ml^{-1}.min^{-1}.100g and this was associated with a two fold increase incoronary sinus Ado from 0.10±0.01 to 0.19±0.02 nM/ml plasma ($P<0.05$). There was no significant difference between arterial Ado during control and exercise. There was a significant correlation between coronary sinus Ado concentration and CVR ($r=-0.58$; $P<0.05$) and myocardial Ado output and CVR ($r=-0.75$; $P<0.01$). These data support a role for Ado in the local control of coronary blood flow during exercise. (Supported by USUHS Grant #CO 7635)

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PARALLEL CHANGES IN MYOCARDIAL OXYGEN CONSUMPTION AND ADENOSINE PRODUCTION. Robert M. Knabb*, Alban N. Bacchus*, Rafael Rubio, and Robert M. Berne. University of Virginia School of Medicine, Charlottesville, VA. 22908

Myocardial oxygen consumption ($M\dot{V}O_2$) and coronary blood flow (CBF) are uniquely related. It has been proposed that altered production of adenosine (ADO) by the myocardium may be the means whereby CBF and $M\dot{V}O_2$ are matched. To test this hypothesis we measured ADO production and $M\dot{V}O_2$ in open chest dogs. ADO concentration was measured in Krebs-Henseleit solution (37°C, pH 7.4, equilibrated with 95% O_2 and 5% CO_2) injected into the intact pericardial sac where it remained for 4.5 min. ADO concentration in the infusate has been shown to be a good index of tissue ADO levels. $M\dot{V}O_2$ was altered by aortic constriction, I.V. $CaCl_2$ infusion, or I.V. norepinephrine infusion. Increases of $M\dot{V}O_2$ resulted in increases of infusate ADO. Significant positive correlations ($p<0.05$) were obtained between infusate ADO and $M\dot{V}O_2$ ($r=0.79$), infusate ADO and CBF ($r=0.67$), and $M\dot{V}O_2$ and CBF ($r=0.80$). These results suggest that ADO production is coupled to $M\dot{V}O_2$ in a manner that is independent of the condition which alters $M\dot{V}O_2$, and support the adenosine hypothesis for the regulation of CBF. (Supported by USPHS Grant HL10384)

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LACK OF EVIDENCE FOR INVOLVEMENT OF ADENOSINE (ADO) IN THE HYPEREMIA INDUCED BY PACING. J.P. Manfredi* and H.V. Sparks, Jr. Michigan State Univ., E. Lansing, MI 48824

We looked for production of adenosine ($\dot{P}ado$), calculated as plasma flow times venous-arterial plasma [ado] differences, from blood perfused dog hearts before, during, and after increases in heart rate via atrial pacing. Both myocardial $\dot{V}O_2$ and vascular conductance increased up to twofold with pacing ($p<0.02$). Although baseline $\dot{P}ado$ was > 0 in five of six dogs, $\dot{P}ado$ did not increase during pacing (1.0 ± 0.8 nmol/min/100g) when compared to either the initial (0.8 ± 0.7 nmol/min/100g) or final (1.4 ± 0.5 nmol/min/100g) controls. Linear regression analysis showed no dependence of either $\dot{P}ado$ on $\dot{V}O_2$ ($R^2=.05$) or conductance on $\dot{P}ado$ ($R^2=.001$). Using our blood collection techniques, we have found that 20% of ^3H-ado added to blood is removed from plasma each minute. Since the mean transit time from capillary to collecting site is approximately 1 minute, metabolism of ado in blood cannot account for the lack of dependence of $\dot{P}ado$ on pacing. It is unlikely that the constancy of $\dot{P}ado$ can be explained by degradation of ado as it passes through pores in the capillary wall: calculated estimations of ado uptake within capillary pores indicate that a measurable increase in $\dot{P}ado$ would result from an increase in interstitial [ado] of >0.06μM. Our results therefore suggest that ado does not mediate the hyperemia induced by pacing. (Supported by NIH grant HL24232-02)

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ENHANCEMENT OF HYPOTHERMIC CARDIAC PERFORMANCE WITH ADENOSINE Roy F. Burlington and Mark Zook*. Dept. Biol., Central Michigan Univ., Mt. Pleasant, MI 48859

Adenosine, a potent vasodilator, increases during myocardial hypoxia or ischemia at 37°C. Exogenous adenosine returns tissue ATP to normal in postischemic hearts. In nonhibernating mammals, severe hypothermia causes ischemia, decreased heart rate (HR) and heart failure between 15 and 12°C. Adenosine may alleviate this dysfunction. To test this hypothesis, we perfused the aorta (60 mmHg) of rat hearts with oxygenated ($95\%O_2$-$5\%CO_2$) Krebs Ringer Bicarbonate solution containing 5 mM glucose (G) or G + 50 μM adenosine (Ad). A latex balloon was inserted to monitor left ventricular systolic pressure (LVSP) and to maintain end diastolic pressure at 10 mmHG. Hearts were initially perfused at 27°C with G or G + Ad and thereafter temperature (temp) was decreased until cardiac arrest occurred. Control hearts became arrhythmic below 17°C and they failed at a mean ($\bar{X}$) temp of 12°C. After arrest, increasing coronary flow (CF) by increasing perfusion pressure (120 mmHg) did not restore function in G hearts. The $\bar{X}$ arrest temp for Ad hearts was 7°C. Some Ad hearts exhibited rhythmic contractions at 3°C. Compared to G hearts at all temperatures preceding arrest, Ad treated hearts had significantly higher CF ($P = <.01$), O_2 consumption ($P = <.01$) and LVSP ($P = <.05$). Continuous perfusion of exogenous Ad during progressive hypothermia maintains a relatively high CF which supports enhanced cardiac function. (Partially supported by The Faculty Research and Creative Endeavors Committee, Central Mich. Univ.)

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VASOACTIVITIES OF INOSINE AND ADENOSINE ON ISOLATED CANINE CORONARY ARTERIAL RINGS. Loren R. Mayer*, Terry W. Hurst* and Carl E. Jones, Dept. of Med. Physiology, Texas A&M College of Medicine, College Station, Texas 77843.

The effects of inosine (IN) and adenosine (AD) on relaxation of 15 isolated canine coronary arterial rings were investigated in an attempt to describe possible interactions of IN with the AD receptor. The effective dose for half maximal relaxation (ED_{50}) for AD was 0.81 ± 0.15 (S.E.) μM and for IN was 239.3 ± 25.2 μM. The molar potency ratio, ($ED_{50}AD/ED_{50}IN$) was 0.0043 ± .0009. These values are similar to those reported in in vivo dog hearts. However, in one-third of the vessels there was no response to AD, while IN caused substantial relaxation in all vessels. Furthermore, in the vessels that responded to both nucleosides, the maximal relaxation caused by IN was 160% that caused by AD($P<0.05$); the maximal relaxation response to IN, when expressed as a percentage of the increase in vessel tension caused by 1 μM prostaglandin F2α, was 72.9 ± 5.6%. Further studies showed that the AD response was blocked by aminophylline, but the response to IN was unaffected. Thus in coronary vessels of the size used (1.0-1.9 mm o.d.), In seems to act on a different receptor than does AD. This vasodilation of larger coronary vessels may explain the increased collateral blood flow and reduced infarct size observed when IN is infused after coronary ligation. (Supported by NIH grants HL-22243 and HL-20286 and by Texas A&M Organized Research funds.)

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INFLUENCE OF ADENOSINE ON CALCIUM UPTAKE BY CULTURED VASCULAR SMOOTH MUSCLE CELLS. R.A.Fenton*, S.P.Bruttig, R.Rubio, and R.M.Berne. Univ. VA. Sch. Med., Charlottesville, VA 22908.

Adenosine (ADO) is thought to play a central role in the metabolic regulation of blood flow. A diminution of Ca influx into vascular smooth muscle cells (VSM) may be the mechanism by which the ADO-induced vasodilation is manifest (A.J.P. 230: 1239,1976). However, Dutta, et al. (Fed.Proc. 39:530,1980) were unable to support this conclusion. Consequently, we studied the effect of ADO on transmembrane Ca fluxes in cultured VSM, where neural influences are absent and maximal cellular-medium interaction can be achieved. VSM monolayers from rat aorta were incubated in 5ml PSS (37°C, pH=7.4) consisting of (mM) $MgSO_4$,1.0; $CaCl_2$,0.9; K_2SO_4,4.6; NaCl,136; MOPS,9.0; dextrose, 10; and ^{45}Ca, 0.3 uCi/ml. Following a 30 minute incubation and 5 washes (1 min each) with 20mM Ca-EGTA (5°C) to remove extracellular ^{45}Ca, cells were dissolved in 1 N NaOH. Aliquots of cellular digest and incubation medium were counted using standard liquid scintillation techniques. Cellular Ca with 4.6mM K was 61.6 ± 2.7 pmoles per 10^5 cells. Verapamil (10^{-6}M) significantly decreased (22%) and 100mM K (NaCl replacement) increased (19%) this value. Norepinephrine (10^{-6}M) was without effect. Elevation of the extracellular K (25mM) increased cellular Ca to 75.1 ± 4.7 pmoles per 10^5 cells. ADO (10^{-7}M) completely reversed the effect of this K depolarization on Ca influx. These data suggest that adenosine mediated vasodilation may result from reduced Ca influx.
(NIH Grant HL 10384 and F32 HL 06024)

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INTERACTIONS OF POTASSIUM-INDUCED CORONARY VASODILATION WITH ADENOSINE, HYPOXIA, AND HYDROGEN ION. Mark A. Young* and Gary F. Merrill, Rutgers University, New Brunswick, NJ 08903.

We studied the effects of adenosine, hydrogen ion, and hypoxia on K^+ induced vascular dilation in 27 isolated, perfused, spontaneously beating guinea pig hearts. The hearts were perfused with Krebs-Ringers-bicarbonate solution equilibrated with 95% O_2 and 5% CO_2. One minute K^+ infusions (final concentration 12,14,16 mM) were performed in the presence of adenosine (0.05μM-0.5 μM), hypoxia (70% and 45% O_2), combined adenosine and hypoxia, and combined adenosine and hydrogen ion (pH 7.2). In the absence of adenosine, K^+ resulted in small, but insignificant flow increases. In contrast, 0.05 μM and 0.1 μM adenosine resulted in K^+ responses (16 mM) 58% and 43% above control flow ($P < .05$). The same adenosine concentrations at pH 7.2 induced significant K^+ responses (35% above control), although less than those observed at pH 7.4. Hypoxia (70% and 45% O_2) resulted in normokalemic flow values comparable to those seen with adenosine. However, K^+ elevation during hypoxia did not increase flow and, in some instances, constricted the vessels. Hearts perfused with 0.05 μM and 0.1 μM adenosine in the presence of 70% O_2 demonstrated significant K^+ responses only at the higher adenosine concentration. These results indicate that (1) hypoxia does not influence the coronary response to K^+, (2) adenosine significantly enhances the dilating action of K^+, and (3) neither hypoxia nor hydrogen ion further enhances this action of adenosine. Supported in part by N. J. Heart Assn.

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INDOMETHACIN CAUSES INCREASED VASCULAR RESISTANCE IN ISCHEMIC MYOCARDIUM. Mark W. Gorman and Harvey V. Sparks. Michigan State University, E. Lansing, MI 48824

Under certain conditions, myocardial ischemia results in a progressive increase in vascular resistance within the ischemic area over the first 3 hrs. These experiments test the hypothesis that the release of a vasoconstrictor prostaglandin (PG) or thromboxane A_2 (TXA_2) causes this rise in resistance. Regional ischemia was produced in 7 open-chest dogs by partial occlusion of the left anterior descending coronary artery (LAD). A hydraulic occluder on the LAD was continuously adjusted so as to maintain distal LAD pressure at 50 mm Hg. Changes in flow therefore reflect changes in vascular resistance. The hearts were paced at 180 beats/min. Myocardial blood flows were measured with 15 μ microspheres after 30 min and 180 min of ischemia. During this time flow (ml/min/100g) in the ischemic subepicardium (epi) fell from 80±8 to 60±7 while subendocardial (endo) flow fell from 41±7 to 21±5. Flow in non-ischemic areas was unchanged. We then gave indomethacin (5mg/kg,i.v.) and after 30 min measured flows again. In the ischemic area epi flow fell to 48±7 and endo flow fell to 16±4. Indomethacin increased flow in non-ischemic areas from 121±7 to 148±6. These results argue that the time-dependent flow decrease in the ischemic area is not due to release of TXA_2 or a vasoconstrictor PG. Instead, arachidonate metabolites appear to cause vasodilation in the ischemic area of this preparation.

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EFFECT OF AN ENDOPEROXIDE ANALOG ON RIGHT CORONARY BLOOD FLOW IN THE PIG. Jerry B. Scott, Stephen W. Ely*, Donald L. Anderson*, Donald C. Sawyer*. Depts. of Physiology & Small Animal Surgery & Medicine. Mich. State Univ., E. Lansing, 48824

The purpose of this study was to examine the in vivo effects of a synthetic PGH_2 analog, (15S) hydroxy-9α,11α-(epoxymethano) prosta-5Z,13E dienoic acid or 9,11-EMP (Upjohn Co.) on the porcine coronary circulation. Eight Poland-China pigs (45-55 kg) were anesthetized with thiamylal Na and N_2O, and mechanically respirated. The chest was opened and the right coronary artery (RCA) was perfused via an extracorporeal circuit at constant pressure (120 mmHg). 9,11-EMP (1 μg/min intracoronary) had no effect on heart rate (151±6 to 149±8), mean aortic pressure (87±6 to 85±7), right ventricular pressure (32/2±2.4 to 33/3±4/1.1) and dP/dt max (1187±156 to 1106 ±222). RCA blood flow was significantly decreased (113±21 to 93±20 ml/min/100g) while coronary resistance was increased (1.31±0.2 to 1.53±0.2 PRU 100). Higher dosages of the drug produced further decreases in coronary blood flow and lethal arrhythmias. In vitro studies have shown this H_2 analog capable of producing smooth muscle contraction and platelet aggregation. The elevation in coronary resistance seen could have resulted from either or both actions of the drug. These studies suggest that endoperoxides can cause immediate and marked reductions in coronary flow and interfere with cardiac rhythm. (Supported by NIH HL10879-11A1 and Mich. Heart Assoc.)

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EFFECTS OF VERAPAMIL AND NIFEDIPINE ON PLATELET ACTIVATION. V. Paul Addonizio, Carol A. Fisher* and L. Henry Edmunds, Jr. U. of PA, Dept. of Surgery, Phila., PA 19104.

The extent to which thromboxanes, potent vasoconstrictors released from activated platelets, contribute to prinzmetal's and unstable angina is currently unknown. We, therefore, evaluated the platelet inhibiting properties of two drugs efficacious in the treatment of variant angina. In concentrations tested (20 to 500 μg/ml), in 12 random, aspirin abstaining donors, verapamil completely prevented release of Thromboxane B_2, (<0.1 pmol/ml) measured by radioimmunoassay, from platelets challenged by epinephrine; inhibited epinephrine induced aggregation and release of ^{14}C serotonin; and demonstrated a dose dependent inhibition of ^{14}C serotonin uptake. Verapamil was equally effective against platelets devoid of their plasma environment and did not alter plasma or buffer pH or inonized Ca++ concentrations. Interestingly, verapamil's effects could be reversed by gel filtration of treated platelets, demonstrating that its antiplatelet activity does not outlast its presence in plasma. Verapamil was nearly ineffective against ADP and ineffective against thrombin induced platelet activation. Nifedipine produced a similar pattern of platelet inhibition. As antiplatelet agents, verapamil and nifedipine appear to be functioning as potent relatively specific epinephrine antagonists. This observation suggests an in vivo mechanism of action for these drugs and provides insight into the genesis of variant and unstable angina pectoris. (Support - HL-22346)

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MYOCARDIAL TRANSPORT OF DIATRIZOATE MEGLUMINE. J.W. Whitaker*, R.G. Tancredi*, T. Yipintsoi, and D. Donald. Department of Physiology and Biophysics, Mayo Foundation, Rochester, MN 55901

We studied the myocardial transport of ^{125}I-diatrizoate meglumine (D) in order to assess its suitability as a radiographic contrast agent for measuring myocardial blood flow. 48 injections containing D, ^{131}I-antipyrine (A), and a vascular reference tracer ^{99m}Tc-albumin (R) were made into the coronary artery inflow of three isolated Langendorf heart preparations perfused with blood at various flows. Transport functions (h) derived from venous tracer-dilution curves were used to calculate area-weighted fractional extractions (E_D) for D. At low plasma flows ($F_p < 0.5$ ml/min·g), mean E_D was 0.44; at high $F_p > 1.0$ ml/min·g, mean E_D decreased to 0.23 ($p < 0.001$). At all $F_p < 2.21$ ml/min·g for each heart, $h_A(t)$ were transformed to a nearly coincident function $\phi_A(\mu) = \bar{t} \cdot h_A(t/\bar{t})$, where $\bar{t}$ was the mean transit time for $h_A(t)$. In contrast, $\phi(\mu)$ for D were heterogenous. Residue function obtained from probes positioned directly over the left ventricle confirmed the heterogenous transport of D through the myocardium. We concluded that diatrizoate meglumine was diffusion-limited in its exchange across myocardial capillaries. Therefore, it is not a suitable agent for measuring myocardial blood flow. (Supported by grant HL 04664).

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ENERGETICS OF DOG HEART. J. Grayson, C. Bayliss*, M. Leveson,* Department of Physiology, University of Toronto.

We used a right heart by-pass technique to measure energy utilization in anaesthetized dog heart. We measured cardiac output, aortic pressure, coronary flow, arterial and coronary venous oxygen. External work and oxygen consumption were calculated. Afterload increase - no effect on oxygen extraction or consumption, external work increased. Preload increase decreased oxygen extraction but increased consumption (due to flow increase). External work increase was even greater. Thus both increase in afterload and preload increased myocardial efficiency. Over range of cardiac output from 1 to 3 litres, efficiency changed linearly from 2 to 25%. Relation to afterload was also linear when cardiac output was kept constant. We propose that mechanical efficiency may be an important aspect of cardiac reserve.
Work supported by Ontario Heart Foundation Grant No. T1-35.

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CARDIOPULMONARY DYSFUNCTION IN NORMAL AND NEUTROPENIC PIGS IN RESPONSE TO PSEUDOMONAS AERUGINOSA BACTEREMIA. D.O.EDDY*, S.H.CROCKER*, R.N.OBENAUF*, B.L.WISMAR*, B.D.LOWERY*, (Sponsor: T.A.LESH). The Ohio State University, Columbus, Ohio 43210.

We have previously reported a number of dose-dependent changes in cardiopulmonary function and lung morphology of pigs subjected to varying infusion concentrations of Pseudomonas aeruginosa. One notable change evidenced by electron micrographs was the sequestration of neutrophils in the pulmonary vasculature. We hypothesized that the neutrophils were associated with the severe hypoxemia, increased pulmonary shunt fraction and vascular resistance. Each group of pigs (n=5) received a continuous 6-hr infusion of either sterile saline (Group C) or $1x10^9$ bacteria/min/20 kg body wt (Groups A&B). Group B had been pretreated with cyclophosphamide which resulted in a marked neutropenia. Groups A&B, in contrast to C, were noted to have significant ($p<.01$) changes in: cardiac index (-50%), PaO_2 (from 83 to 48 mmHg), pulmonary shunt fractions (19 to 42%), pulmonary artery pressure (13 to 47 mmHg), and vascular resistance (+400%) and a femoral artery pressure decline to 40 mmHg. Electron microscopy revealed areas of interrupted and fragmented capillary endothelium in both A&B and virtually no neutrophils in B. These data indicate that the pulmonary failure and lung damage are not due to neutrophil sequestration in the lungs. (Supported by NIGMS Grant 1R23GM26865-01 and the Central Ohio Heart Association).

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PREVENTION OF PERIPHERAL EDEMA WITH BLOOD COLLOID REPLACEMENT FOLLOWING HYPOVOLEMIC SHOCK IN DOGS. John B. Cologne*, Alan R. Hargens, and Richard M. Peters*. Division of Cardio-Thoracic Surgery, University of California Medical Center, San Diego, CA 92103

Edema is often cited as a complication which requires colloid replacement following hypovolemic shock in humans. To test the effects of blood colloid replacement on peripheral edema formation, interstitial fluid pressure was measured in skeletal muscle (IMP) and subcutaneous tissue (SQP) of ten dogs anesthetized with pentobarbital sodium. Following 30 minutes of shock, shed blood was rapidly replaced in five dogs, while an equal volume of crystalloid (lactated Ringer's) was rapidly infused into the other five. Lactated Ringer's was then infused into all dogs for 24 hours to maintain pulmonary capillary wedge pressure (PCWP) and cardiac output at baseline levels. IMP and SQP rose significantly within 2 hours in the crystalloid group (-1.7 ± 0.5 to 0.6 ± 1.1 mmHg and -3.3 ± 0.3 to -1.0 ± 0.9 mmHg, respectively) and remained elevated. Following whole blood replacement, IMP and SQP rose at a slower rate, and then declined to near baseline levels by 24 hours. PCWP was elevated to 4-5 mmHg above baseline value in the whole blood group despite cardiac output levels below baseline. Thus the extent of peripheral edema secondary to crystalloid infusion is diminished by replacement of blood colloids. Such replacement, however, elevates PCWP which can lead to pulmonary edema. (Supported by USPHS/NIH grants GM 24901, HL 13172, and GM 17284).

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TOTAL HYPOTHERMIC BLOOD EXCHANGE IN ACUTE ENDOTOXIN SHOCK. Frank Gollan and Joanne McDermott.* Miami VA Medical Center and University Miami School of Medicine, Miami, Fl. 33125.

It has been suggested that the high mortality of endotoxin shock may be prevented by an early and total blood exchange. This rapid procedure at 30°C does not carry any mortality in our laboratory. After the i.v. injection of endotoxin (10 mg/kg) and blood transfusions into 33 dogs, severe hemoconcentration, tachypnea, tachycardia, low cardiac output, metabolic acidosis and hyperpyrexia led uniformly to death within five hours. All of these symptoms were prevented in five dogs by total hypothermic blood exchange, instituted five minutes after the endotoxin injection. Nevertheless, all of these treated animals died a delayed death, although their symptoms were ameliorated. Since the endotoxin particles were rapidly phagocytized by the reticulo-endothelial system, even very early total blood exchange could not dislodge them anymore from their intracellular site. Total hypothermic blood exchange has been used successfully in poisoning with short-acting barbiturates and carbon monoxide, incompatible blood transfusions, Reyes Syndrome and some cases of postnecrotic hepatic coma, but as a single treatment it does not affect the mortality of acute endotoxin shock in the dog. (Supported by the Veterans Administration Research Service.)

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CIRCULATING RETICULOENDOTHELIAL DEPRESSING SUBSTANCE ACTIVITY FOLLOWING THERMAL INJURY. D.J. Loegering, Department of Physiology, Albany Medical College, Albany, New York 12208.

This laboratory has previously shown that a reticuloendothelial (RE) depressing substance is present in portal vein blood following thermal injury or intestinal ischemia. (Circ. Shock 7:212,1980). This study extends the work on RE depressing substance following thermal injury. Thermal injury of 30% body surface area (30 sec in 90°C water) was produced in anesthetized dogs or rats. Arterial or portal vein blood was collected and plasma extracts were prepared by protein precipitation with TCA and ether extraction. RE depressing substance activity was assayed by measuring the colloidal carbon clearance rate in rats or mice following i.v. injection of plasma extracts. At 3 hr after thermal injury, RE depressing activity was present in portal vein plasma extracts from each of 6 dogs. Pre-burn arterial blood samples had no activity and 3 hr post-burn arterial samples had RE depressing activity. Plasma extracts from rats contained activity at 3 and 24 hr after thermal injury. Non-injured dogs or rats never had detectable RE depressing activity. Acid hydrolysis of plasma extracts destroyed RE depressing activity, suggesting a peptide structure which is similar to that described for the RE depressing substance formed during intestinal ischemia. These data suggest that the formation of an RE depressing substance following thermal injury may contribute to the impairment of RE function and host defense following thermal injury (GM-26297).

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DEPRESSION OF THE RETICULOENDOTHELIAL SYSTEM FOLLOWING THE PHAGOCYTOSIS OF ERYTHROCYTE GHOSTS. M.J. Schneidkraut and D.J. Loegering, Dept. Physiol., Alb. Med. Col., Albany, NY 12208.

Previous studies from this laboratory have shown that the phagocytosis of crude erythrocyte stroma preparation by the reticuloendothelial system (RES) can cause an RE depression and increased shock susceptibility. The present study determined if homologous, hemoglobin-free, erythrocyte ghosts are removed from the circulation by the RES and if their phagocytosis depressed RE function. Erythrocyte ghosts labelled with ^{125}I and injected i.v. (1.5mg/100g ghost protein) into anesthetized, inbred rats were cleared with a half-time of 13.9±1.5 min (mean ± SEM). Ghost localization in the liver, spleen and lungs was 20.6±2.5% injected dose per total organ (%ID/TO), 17.4±6.5% %ID/TO, and 0.9±0.2 %ID/TO, respectively, 30 min after injection. Similarly, labelled erythrocytes showed no detectable clearance over 30 min. Phagocytic index, determined from the clearance rate of sheep erythrocytes, was depressed 37% ($p<.01$) 30 min after the injection of erythrocyte ghosts and was due to a 42.4% ($p<.01$) decrease in hepatic and a 26.7% ($p<.05$) decrease in splenic localization of sheep erythrocytes. It is concluded that the phagocytosis of pure erythrocyte membrane material can cause a RE depression. It is postulated that burn injury releases into the circulation erythrocyte membranous debris, the phagocytosis of which may result in an RES depression and increased susceptibility to shock and infection. (Mid-Hudson Chapter of Amer. Heart Assoc., GM-26102, HL-07194)

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ASPIRIN (ASA) IMPROVES SURVIVAL IN ENDOTOXIN (LPS) INDUCED SHOCK IN RATS: A ROLE FOR THROMBOXANE (Tx). W.C. Wise, P.V. Halushka* and J.A. Cook. Depts. Physiology, Pharmacology and Medicine. Medical University of South Carolina, Charleston, South Carolina 29403.

Endotoxic shock is associated with elevations in plasma Tx and thrombocytopenia. We evaluated the potential beneficial effects of ASA in S. enteritidis LPS (20 mg/kg iv) induced shock in Long-Evans rats. LPS produced a 90% (N=36) mortality in control rats at 24 hrs. Pretreatment with ASA (0.5 to 30 mg/kg iv) 30 min. prior to LPS significantly ($P<0.001$) improved survival. ASA, 15 mg/kg, produced an optimal survival rate of 75% ($P<0.001$, N=15). LPS elevated plasma TxB_2 at 30 min. from <200 to 2159±252 pg/ml (N=18). ASA (15 mg/kg, 30 min. pre LPS) lowered TxB_2 to 827±102 pg/ml (N=19 $P<0.01$) and reduced LPS induced increases in serum fibrin/fibrinogen split products and lysosomal enzymes. This dose of ASA also lowered thrombin (5 U/ml) induced in vitro platelet synthesis of TxB_2 from 48±12 (N=13) to 19±4 ng/ml PRP/$5x10^5$ (N=4, $P<0.05$). Since ASA produces a prolonged inhibition of platelet Tx synthesis, rats were pretreated with 15 mg/kg ASA 24 hrs. pre LPS. ASA improved survival from 11% to 41% (N=12, $P<0.005$) and lowered TxB_2 levels to 636±66 pg/ml (N=6, $P<0.001$). These results are consistent with the hypothesis that TxA_2 plays a role in the pathogenesis of endotoxic shock and that the platelet may represent an important source of TxA_2. (Supported by NIH GM27673, GM20387, RR1079, S.C. and American Heart Association, and BGRS RR05420.)

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DECREASED ARACHIDONATE (AA) METABOLISM: A ROLE IN THE RESISTANCE OF METHYL PALMITATE (MP) PRETREATED RATS TO ENDOTOXIC (LPS) SHOCK. J.A. Cook, W.C. Wise, and P.V. Halushka*. Departments of Physiology, Pharmacology, and Medicine, Medical University of South Carolina, Charleston, S.C. 29403.

Rats pretreated with MP exhibit depressed RE phagocytic function and are paradoxically refractory to shock induced by LPS. Since metabolism of AA to thromboxane A_2 (TxA_2) and PGI_2 is markedly increased in LPS shock, we sought to determine if depressed AA metabolism may be involved in MP induced resistance to LPS. Rats were pretreated with MP 1 g/kg iv for 2 days and then given S. enteritidis LPS (20 mg/kg iv). Plasma levels of thromboxane A_2 (TxA_2) and PGI_2 were measured via RIA of their stable metabolites TxB_2 and 6-keto-$PGF_{1\alpha}$. Thirty minutes after LPS, both TxB_2 and 6-keto-$PGF_{1\alpha}$ increased in control rats from <200 pg/ml to 2,207 ± 282 pg/ml (N=16) and 840 ± 50 pg/ml (N=8), respectively. In MP pretreated rats, LPS increased plasma TxB_2 to only 484 ± 50 pg/ml and 6-keto-$PGF_{1\alpha}$ was <200 pg/ml (N=10) (P<0.01). LPS induced increases in serum fibrinogen/fibrin degradation products from <0.05 to 32.9 ± 2.2 µg/ml (N=7) in control rats but was only 16.2 ± 4.4 µg/ml (N=10) (P<0.05) in MP treated rats. These observations raise the possibility that decreased AA metabolism may contribute to the protective effect of MP in endotoxic shock. (Supported by NIH GM 27673, GM 20387, RR 1070, American Heart Association and BGRS RR05420)

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CAN STEROID/ANTIBIOTIC TREATMENT PREVENT DEATH FROM LD_{100} E. COLI WHEN ADMINISTERED AFTER PROLONGED SYSTEMIC HYPOTENSION? L.B. Hinshaw, L.T. Archer*, B.K. Beller-Todd*, B.A. Benjamin*, D.J. Flournoy*, and R.B. Passey*. VA Medical Center and Univ. of Oklahoma Health Sci. Ctr., Oklahoma City, OK 73104.

We previously reported (J. Surg. Res. 28:151, 1980) that infusion of methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS) increased survival of baboons given a 2-hr infusion of LD_{100} E. coli. The purpose of this study was to determine if baboons would survive if MPSS/GS was postponed until 4 hr after the initiation of E. coli infusion. Baboons (P.c. cynocephalus) were given a 2-hr infusion of LD_{100} E. coli and monitored for 12 hr: Group I (N=8) was given E. coli only and Group II (N=4) was given E. coli plus MPSS (75 mg/kg) and GS (18 mg/kg). Death was prevented in 3 of the 4 fully-treated baboons whereas all untreated animals died within 42 hr. Mean systemic arterial pressures (MSAP) in Group II baboons were significantly decreased compared with control values for a minimum of 3 hr before the initial MPSS/GS infusions were begun. Blood glucose concentrations were decreased ($p<0.02$) by 4 hr in both groups but treatment resulted in higher values from 8-12 hr ($p<0.05$). Lactic acid and pCO_2 values were inversely related, pO_2, pH and hematocrit remained relatively constant, and BUN and creatinine concentrations increased in all animals. Data demonstrate that survival is increased in lethal E. coli shock even when MPSS/GS is postponed until 4 hr after the initiation of E. coli. (Supported by VAMC and NIH HL24590.)

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A THERAPEUTIC STUDY OF PREDNISOLONE SODIUM SUCCINATE COMPARED WITH DEXAMETHASONE FOR THE TREATMENT OF E. COLI SEPSIS IN THE CANINE. G.L. White*, G.S. Koehn*, S.D. Kosanke* and L.B. Hinshaw (SPON: R.D. Stith). Univ. of Oklahoma Health Sciences Center and V.A. Medical Center, Oklahoma City, Oklahoma 73190.

Although corticosteroids have been intensively studied for the treatment of septic shock, much controversy over their use still remains. This study was designed to compare the therapeutic value of post-treatment with prednisolone sodium succinate (PSS) in sterile water or dexamethasone (DEX) in a polyethylene glycol carrier both used with gentamicin sulfate (GS) for E. coli septic shock. Four groups of dogs were studied: Group A-Control group received E. coli with no treatment; Group B-Treated with PSS and GS after E. coli administration; Group C-Administered DEX and GS following E. coli infusion; and Group D-Treated with GS only after E. coli. Survival results revealed 75% mortality in Group A (6/8), Group C (6/8) and Group D (6/8) while 100% survived in Group B (8/8). The Group B dogs had significantly less (<.05) hemoconcentration than the other groups. Results suggest that PSS with GS significantly increases survival of dogs subjected to E. coli septic shock when compared to DEX. (Supported by NIH Grant P40-RR-01098-03AR, the Upjohn Company and V.A. Medical Center.)

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IN-VITRO EFFECTS OF PREDNISOLONE SODIUM SUCCINATE ON E. COLI DESTRUCTION, NEUTROPHIL LOSS, AND GLUCOSE UTILIZATION IN DOG BLOOD. G.S. Koehn*, S.D. Kosanke*, and G.L. White* (SPON: K.J. Dormer). Animal Resources and Facilities, Univ. of Oklahoma Health Sciences Center and V.A. Medical Center, Oklahoma City, Oklahoma 73190.

Increased survival in the dog subjected to experimental septic shock has been reported with treatment with prednisolone sodium succinate (PSS); however, certain corticosteroids have been reported to potentiate infections. This study was designed to determine if PSS alters E. coli mortality, glucose utilization or neutrophil destruction in whole dog blood studied in-vitro (N=6) which was subjected to live E. coli (9×10^6 organisms/ml of blood). Group 1 (control) received E. coli only, Group 2 had E. coli and 15 µg/ml of PSS and Group 3 had E. coli plus 150 µg/ml of PSS. Results revealed that neither low nor high concentrations of PSS had any influence on E. coli destruction, glucose utilization or neutrophil loss compared to control $P>0.05$. Findings in this study suggest that PSS does not enhance E. coli infections in dog blood studied in-vitro. (Supported by NIH Grant P-40-RR01098-03 AR and Oklahoma City V.A. Medical Center.)

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PRIMARY CULTURE OF THE CHICK DUODENUM. A HORMONE RESPONSIVE MODEL OF DEVELOPMENT. Howard M. Zinman* and Barry T. Smith. Queen's Univ., Kingston, ON K7L 3N6 and Harvard Med. Sch., Boston, MA 02115

A simple and reproducible technique for growing chick duodenal epithelial cells and fibroblasts in bilayer culture has been established. The methodology is based on the observation that minimal essential medium supplemented with 10% fetal calf serum promotes the active proliferation of fibroblasts, whereas a 6% chicken serum and 4% fetal calf serum supplement triggers the active proliferation of epithelial cells. Trypsin dissociated epithelial cells and fibroblasts reaggregate and proliferate as an epithelial-fibroblast bilayer, which responds to 10^{-6}M cortisol by increasing the amount of alkaline phosphatase and by elongation of epithelial microvilli. The maintenance of a close association between the two cell types suggests that epithelial-mesenchymal interactions may play a role in duodenal development. Epithelial cells (grown on a collagen substrate) and fibroblasts (isolated by differential adherence) have been cultured separately allowing this hypothesis to be tested.

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THE EFFECT OF AMILORIDE ON ACID DISPOSAL IN THE DUODENUM. G. M. Johnson* and R.S.K. Chung. Dept. of Surgery, VAMC and Univ. of Iowa, Iowa City, IA 52240.

Previous studies of H+ movement in many levels of the GI tract indicated a proportional, but oppositely directed relationship with net Na+ movement. If H+ movement occurs through the same pathway as Na+, it may be affected by amiloride, which blocks the sodium channel in the apical cell membrane. Under anesthesia the rat duodenum was cannulated and perfused at 38°C. Perfusates contained 138mM Na+, 106mM Cl^-, 17mM [C-14] erythritol, 0.2% [H-3] PEG 4,000, 10^{-4}M amiloride, with osmolality adjusted to 308 mOsm/Kg H_2O with mannitol. Luminal [H+] was maintained by pH stat and H+ disposal determined from titrant added. Net ion fluxes in μmoles/30 min/mg dry weight were:

Luminal [H+]	Control		10^{-4} M Amiloride	
(mM)	H+	Na+	H+	Na+
0	-0.23±0.03	-0.23±0.06*	-0.18±0.05	+0.07±0.09*
1	-0.49±0.03	+0.24±0.07*	-0.41±0.02	+0.58±0.04*
5	-1.32±0.09	+0.61±0.11	-1.18±0.08	+0.92±0.13

(- represents absorption; *p<0.01)

Amiloride caused a significant decrease in Na+ and H_2O absorption, indicating the presence of the sodium channel in the duodenal mucosa. Since there is no concurrent decrease in H+ disposal, we conclude that H+ back diffusion is mainly via the paracellular shunt pathway, rather than through the sodium channel in the apical cell membrane.

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INTESTINAL ABSORPTION IN THE OBSTRUCTED ILEUM OF GERMFREE DOGS. James B. Heneghan and John W.L. Robinson* La. State Univ. Sch. Med., New Orleans, LA 70112 and Central Hospital Univ. Vaud, Lausanne, Switzerland

Our previous experiments in conventional mongrel dogs showed that mechanical obstruction of the ileum induced a *SECRETORY STATE* with net loss of water and electrolytes into the intestinal lumen. Now, four germfree beagles (9.6 - 11.8 kg) were anesthetized and a 20 cm loop of ileum (control loop), 30 cm above the ileo-cecal valve, was perfused with Krebs bicarbonate buffer containing 0.2% glucose for 1 hr. The loop was then excised, passed out of the isolator, and dissected for study. The transport capacity for L-phenylalanine and β-methyl-D-glucoside was assessed by incubating samples of mucosa for 1 hr. at 37°C in 1mM solutions of the radioactive substrate in Krebs bicarbonate buffer. Mechanical obstruction was produced by closing the ends of the bowel with surgical staples. The dogs were allowed to recover for 7 days when similar experiments were performed on the loop above and the loop below the obstruction. The ileal mucosa above the obstruction in germfree beagles *ABSORBED* water and electrolytes. Functionally, the control and below loops of germfree dogs were very similar to their respective conventional loops. However, the percentage of mucosa in all germfree dogs was lower than that observed in conventionals, and indicated smooth muscle hypertrophy and/or reduced mucosal mass. These data emphasize the importance of the "normal" microbial flora in the pathophysiology of the secretory state produced by intestinal obstruction.(Supported in part by NIH Grant AM-18886)

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SEPARATION OF MUCUS SECRETION FROM WATER AND ELECTROLYTE SECRETIONS IN CHOLERA ENTEROTOXIN TREATED RABBIT ILEUM IN VIVO. Gordon J. Leitch, Oliver O. Njoku* and Michael H. George*. Dept. of Physiology, Sch. Med. Morehouse College, and Dept. of Biology, Atlanta University, Atlanta, GA 30314.

In vivo rabbit ileal loops were prepared and filled with an isotonic NaCl-$NaHCO_3$ solution. At hourly intervals the solutions were replaced, and the net fluxes of water and electrolytes were measured. Loop fluid mucus concentrations were assessed by measuring fluid viscosities at several shear rates after normalizing the fluid volumes to loop lengths. When the in vivo rabbit ileum was exposed to cholera enterotoxin (CT) there was a gradual increase in the secretions of water and electrolytes to dose related sustained maximal levels, and after a lag period there was a dose related transient secretion of mucus into the loop lumen. Mucosal exposure to a crude E. coli heat stable enterotoxin preparation produced similar patterns of water, electrolytes and mucus secretions. CT-induced secretions of water, electrolytes and mucus were inhibited by cycloheximide, colchicine, cytochalasin B, indomethacin, verapamil and tetracaine. In every case the inhibition of mucus secretion occurred at lower drug doses than did the inhibition of water and electrolyte secretions. On the other hand acetazolamide could be shown to inhibit water and electrolyte secretions while not affecting mucus secretion.

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CHARACTERISTICS OF INTESTINAL GLUCOSE SECRETION. Gary M. Levine and Yih-Fu Shiau. VA Medical Center and University of Pennsylvania, Philadelphia, PA 19104

While intestinal glucose absorption has been extensively investigated, little is known about glucose secretion. The discrepancy in glucose absorption rates when radioactive tracers are used compared to simultaneous chemical measurements suggest that such a phenomenon exists. We developed a method to investigate glucose secretion in rats. 10-25% dextrose with ^{14}C (U) glucose was infused intravenously and the appearance of ^{14}C glucose measured in jejunum perfused with buffer, 21 mM glucose or 2 mM phlorizin. TLC of blood and perfusate samples was employed to determine the amount of ^{14}C present as glucose. Blood glucose was measured by glucose oxidase. Over a range of blood glucose from ∿ 4-18 mM, glucose secretion was linear with blood glucose (r=0.86, p<0.01). During jejunal perfusion with glucose, secretion accounted for ∿ 20% of the absorptive flux (318 ± 22 vs 1538 ± 136 nM/cm/hour). Perfusion with phlorizin significantly increased recovery of secreted glucose compared to buffer (p<0.01). Volume expansion with dextran at a constant blood glucose resulted in an increase in glucose secretion by 25 nM/cm/hour/cm H_2O (p<0.01). We believe these data indicate that glucose secretion is a quantitatively important phenomenon that appears to be a passive process. A significant portion of secreted glucose is reabsorbed by active transport. Supported by VA Research Grants MRIS 1449 and 7864.

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Inhibition of Intestinal Fatty Acid Permeation by Ethanol. Verney L. Sallee. Texas Coll. Osteo. Med, Ft. Worth, Tx. 76107

Uptake of the homologous series of fatty acids by everted sacs of rat intestine shows a variable dependence on addition of methylene groups. Short chain fatty acid (SCFA) uptake is slightly increased by addition of a methylene group but long chain fatty acid (LCFA) uptake is increased about 3.3 fold for each added methylene group. SCFA are taken up more rapidly than would be predicted from LCFA uptake. Two general mechanisms could explain this pattern of SCFA uptake. (1) SCFA uptake may occur by an alternate pathway in addition to a second pathway shared with LCFA. (2) The rate of FA uptake may be determined by molecular size as well as lipid solubility. The influence of ethanol on FA uptake was studied to distinguish between these two mechanisms. Uptake rates determined for FA4:0, FA10:0 and FA12:0 are presented in the table as means ± SEM(n=6-12). Units are nmoles·min^{-1} per 100 mg for 1 mM conc.

	FA4:0	FA10:0	FA12:0
Control	30.9 ± 4.5	145.6 ± 4.0	302.6 ± 27.2
1% EtOH	31.2 ± 5.5	140.4 ± 8.1	325.6 ± 39.3
5% EtOH	22.4 ± 5.5	108.5 ± 5.5	283.7 ± 29.0
10% EtOH	5.6 ± 3.0	61.4 ± 4.8	248.7 ± 26.7

The marked inhibition by ethanol of FA4:0 uptake but only slight inhibition of FA12:0 uptake indicated that an alternate pathway for SCFA uptake, inhibited by ethanol, may be present in rat intestine. Supported by TCOM Faculty Research Funds

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ROLES OF NON-MAST CELL HISTAMINE AND HEPARIN IN FAT ABSORPTION. L.B. Jaques, A. Wollin and S. Wice. Department of Physiology, College of Medicine, Department of Oral Biology, College of Dentistry, University of Saskatchewan, Saskatoon, Sask., S7N OWO.

Feeding of olive oil to rats results in a marked increase in vascular permeability in the small intestine (shown by high concentrations of Evans blue and plasma protein in the lymph) and a marked increase in intestinal lymph flow for several hours. These changes are due to histamine release as they are blocked by the combined action of H_1 (pyrilamine) and H_2 (Burimamide®) receptor antagonists and by the i.v. injection of heparin, which releases DAO (diamine oxidase, histaminase) from adjacent sites. Fat feeding also causes the release of DAO which evidently terminates the histamine effect. Repeated feeding of fat increases all these effects. On feeding rats a carbohydrate diet, three types of mucopolysaccharide are found in the intestine - heparitins (sulfate), multisulfated chondroitins and heparins. Feeding of fat causes the disappearance of the heparin in the small intestine, but not in the large intestine, with no change in appearance or number of mast cells. These results suggests that release of non-mast cell histamine is an important factor for promoting transport of dietary fat and its effects are regulated by the concomitant release of non-mast cell heparin which releases DAO. (Supported by the Saskatchewan Heart Foundation).

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THE MAXIMAL CHYLOMICRON TRANSPORT RATE OF THE RAT SMALL INTESTINE. Patrick Tso*, Kenneth Buch*, John A. Balint, and John B. Rodgers*. Division of Gastroenterology, Department of Medicine and Department of Physiology, Albany Medical College, Albany, NY 12208

Studies from this laboratory showed inhibition of lymphatic chylomicron transport in the small intestine by the hydrophobic surfactant, Pluronic L-81. This blockade was quickly reversed (~30 min) after the termination of intraduodenal L-81 infusion. By infusing a triolein emulsion labelled with glycerol tri [9-10 ^3H (n)] oleate, together with 0.5 mg/h of L-81, the first quarter of small intestinal mucosa was saturated with absorbed lipid, ~125 µmol triglyceride (TG). After the termination of L-81 infusion, lymphatic chylomicron output was monitored and maximal output measured. Radioactive lipid output into thoracic duct lymph was measured hourly during the 8 h of lipid infusion and every 15 min during the subsequent 5 h of saline infusion. Lymphatic chylomicron output rose quickly after termination of L-81 infusion and reached a steady TG output of 32.3 µmol/h by 2 h. Phospholipid (PL) and cholesterol outputs followed the TG output closely and reached a steady output of 4.8 µmol/h and 2.8 µmol/h respectively. The increase of TG output was associated with the transport of large chylomicron particles 2000-6000 Å and a decrease in PL/TG ratio. This model provides for the first time an approach for quantitation of maximal export of lipoproteins from a segment of the upper small intestine. (This work was supported by Grant HL-17332).

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BILIARY RECOVERY OF ABSORBED BILE ACID IN THE PRESENCE OF DIETARY NUTRIENTS. Joseph D. Fondacaro. Dept. Physiology, Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio 45267.

Earlier studies from this laboratory indicated that, of the three major food types, only triglyceride (TGR) inhibited intestinal villus uptake of cholic and taurocholic acid in vitro while starch (S) and albumin (A) had no effect on this process. To substantiate these finds, in vivo experiments were conducted using biliary recovery of taurocholic acid (TC) in rats as an index of intestinal absorption. Rats were anesthetized with pentobarbital and ilea were perfused with Krebs buffer containing TC with and without A, S, or TGR. TC levels in bile reached a plateau at approx. 30 min after start of perfusion. Approx. 2/3 of the dose of TC in the perfusate was recovered by this method. Following a 60 min perfusion, neither S nor A significantly affected biliary TC content and neither of these substrates delayed the time sequence of recovery of TC when compared to controls. TGR significantly reduced biliary TC levels on an average of 34% from controls. TGR however did not adversely influence the time sequence of recovery. These data indicate that of the 3 major nutrient classes, only triglyceride inhibits bile acid absorption in vivo and support our earlier findings with in vitro techniques. In cystic fibrosis (CF), accompanying pancreatic insufficiency is thought to cause bile acid malabsorption. From our studies we conclude that bile acid malabsorption in CF cannot be accounted for solely on the basis of an intraluminal event involving undigested dietary nutrients. (Supported by NIH grant AM 26650.)

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MAXIMAL ACID SECRETION FROM HEIDENHAIN POUCH. R.J. Joehl*, R.C. Rose, and D.L. Nahrwold. The Pennsylvania State University, Hershey, PA 17033

The intravenous(IV) dose of pentagastrin(PG) and histamine (HIST) which stimulates maximal acid secretion in dogs has been reported to be 8 µg/kg-hr and 80 µg/kg-hr, respectively. We studied the maximal acid secretory response to PG and HIST in 3 dogs with antrectomy, Heidenhain pouch(HP) and gastric fistula(GF). In experiments, PG 4, 8 or 16 µg/kg-hr, or HIST 80, 160 or 320 µg/kg-hr, was infused IV for 3 hr. Only one dose of secretagogue was administered per day. HP acid secretion was collected every 15 min and analyzed. Gastric juice was drained through an open GF. Highest acid secretion was attained within 1 hr and 2 hr after PG and HIST, respectively. To minimize differences among dogs due to HP size, highest acid output(HAO) is expressed as % of peak maximal HIST response per 15 min (%MAX). HAO is also expressed in µEq/15 min (µEq). Results are mean ± SE, n=6. † denotes significant difference from HIST 80 and HIST 320, $P < 0.05$.

	PG 4	PG 8	PG 16	HIST 80	HIST 160	HIST 320
%MAX	35.7±4.6	40.8±6.6	26.1±8.9	83.6±4.5	95.5±4.5†	80.2±2.6
µEq	379±90	411±97	206±110	757±139	858±161	729±205

We conclude that in these dogs: 1) the dose of PG and HIST that stimulates maximal acid secretion from HP is 8 µg/kg-hr and 160 µg/kg-hr, respectively; 2) it is necessary to infuse PG and HIST for 1 hr and 2 hr, respectively, to attain maximal acid secretion from HP. (Supported by NIH Grant #AM 23085).

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Mg^{++} ENHANCEMENT OF Ca^{++}-DEPENDENT BINDING OF VITAMIN B·12 TO ILEAL BRUSH BORDER MEMBRANE VESICLES. Robert C. Beesley and Cheryl D. Bacheller*. Wayne State Univ., Detroit, MI 48201

Brush border membrane vesicles isolated from hamster small intestine were used to investigate binding of vitamin B·12 (B·12). Ileal vesicles bound 25 times more B·12 than did jejunal vesicles. Binding of B·12 to ileal vesicles was dependent on intrinsic factor (IF) and required Ca^{++}. Complete removal of Ca^{++} reduced B·12 binding to <5% of that observed with 10mM Ca^{++}. Increasing the Ca^{++} concentration resulted in a progressive increase in B·12 binding which reached an apparent maximum at 6-8mM Ca^{++}. Mg^{++} alone (0 Ca^{++}) had no effect on binding. However, at low (0.1-2mM) Ca^{++} concentrations, Mg^{++} stimulated B·12 binding. At 0.1, 0.5, 2 and 8mM Ca^{++}, Mg^{++} (2mM) enhanced B·12 binding 4.3, 2.1, 1.4 and 1.1 fold respectively. Mn^{++} and, to a lesser extent, Sr^{++} also stimulated B·12 binding but Zn^{++}, Ba^{++}, La^{+++}, Na^+ and K^+ did not. B·12 was not metabolized and was bound as the IF-B·12 complex which could be removed with EDTA. EGTA was less effective in removing IF-B·12. These results suggest that at least two types of divalent cation reactive sites are involved in binding of IF-B·12 to its ileal receptor. One type is Ca^{++} specific and must react with Ca^{++} if IF-B·12 is to be bound. The other type is less specific, reacting with Mg^{++}, Mn^{++}, Sr^{++} and perhaps Ca^{++} itself thereby enhancing binding of IF-B·12. This enhancement of Ca^{++}-dependent binding of IF-B·12 could result in increased absorption of B·12 when the availability of Ca^{++} is limited. (Supported by USPHS NIH grant #AM 25343)

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EFFECT OF DIET COMPOSITION UPON INTESTINAL SUGAR AND AMINO ACID TRANSPORT IN SEMI-STARVED RATS. R.J. Bridges* and T.Z. Csaky. Univ. of Missouri, Columbia, MO 65212.

Male Sprague-Dawley rats (initial body weight 200-250 g) were kept on a standard laboratory diet consisting of 67% starch, 20% protein, 5% lipid and 8% fiber, fortified with minerals and vitamins. The animals were semi-starved to produce an 8% reduction in body weight in 5 days. 3-O-Methylglucose (3MG) and α-aminoisobutyric acid (AIBA) transport was measured in the isolated everted jejunum. Semi-starvation produced a significant increase in the uphill, active transport of both 3MG (80%) and AIBA (60%). Results were the same when the starch in the diet was replaced by glucose. Removal of the carbohydrate and changing the protein content of the diet to 87% produced only a 20% increase in 3MG transport, but further enhanced (from 60 to 210%) the active transport of AIBA. The results suggest the semi-starvation not only enhances intestinal transport of sugars and amino acids, but the increase also reflects an adaptation to the composition of the diet. (Supported in part by USPH Grant).

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INTRACEREBROVENTRICULARLY (ICV) VS. INTRAVENOUSLY (IV) ADMINISTERED ENDOGENOUS PYROGEN (EP): EFFECTS ON BODY TEMPERATURE AND PLASMA IRON IN RABBITS. D.H. Olster* and M.J. Kluger. Department of Physiology, The University of Michigan Medical School, Ann Arbor, MI 48109

In mammals and reptiles infection is characterized by fever and a fall in plasma iron (Fe), responses thought to be mediated by EP or leukocyte endogenous mediator (LEM), a protein released by activated leukocytes. Although EP (LEM) is known to induce a fever by elevating the thermal "set-point" in the central nervous system, the mechanism by which plasma Fe concentration is reduced is unknown. Bailey et al. (Proc. Soc. Exp. Biol. Med. 153, 419, 1976) reported that LEM isolated from rabbits would, when injected into rats ICV, result in both fever and a fall in plasma Fe concentration. However, since rabbit LEM might be antigenic and therefore inflammatory to rats, we opted to inject EP (LEM) isolated from rabbit leukocytes into rabbits IV and ICV. The results were as follows:

	AVERAGE 4-HOUR ΔT (°C)	PLASMA Fe (μg/100 ml)
EP (IV)	0.49 ± 0.05 SEM n = 6	128.2 ± 13.4 SEM n = 6
Saline (IV)	-0.36 ± 0.13 SEM P<0.01	163.2 ± 14.9 SEM P<0.01
EP (ICV)	1.24 ± 0.21 SEM n = 7	233.7 ± 15.4 SEM n = 7
CSF (ICV)	0.18 ± 0.06 SEM P<0.005	220.0 ± 8.7 SEM P>0.5

Although EP ICV produced a larger fever than EP IV (P<0.005), EP ICV did not result in a reduction in plasma Fe concentration. These data support the hypothesis that in rabbits EP (LEM) acts centrally to cause a fever and systemically to reduce plasma Fe concentration. (Supported by NIH 015298).

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URINARY EXCRETION OF TRACE METALS DURING ACUTE INFECTION IN DOG. Allan P. Klaiman*, Winona Victery*, Matthew J. Kluger and Arthur J. Vander. Univ. of Mich., Ann Arbor, MI. 48109.

A decrease in the plasma concentrations of Zn and Fe occurs during bacterial infection in many species. To evaluate the possible contribution of renal excretion to such a fall, we investigated whether acute infection alters urinary excretion of Zn and Fe. Pentobarbital-anesthetized dogs were prepared for standard renal clearance experiments; following a 1-hour control period, the animals received either heat-killed Pasteurella multocida or vehicle. Rectal temperature in bacteria-treated animals increased significantly by 2 hrs following injection. Excretion rates of Zn and Fe were also significantly elevated by this time and by the end of 4 hrs they had approximately doubled. The changes in Zn and Fe excretion correlated significantly with changes in rectal temperature. K excretion and urine flow over the 4 hrs tended to increase, but the rises were not statistically significant. Plasma concentrations of Zn, Fe, and K did not change, nor did GFR. We conclude that, in the anesthetized dog, acute bacterial infection can increase urinary trace-element excretion. However, the dog, unlike most species, does not manifest a decrease in plasma Zn or Fe. Supported by grants from Mich. Kidney Found. and NIH.

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DIFFERENTIAL EFFECTS OF ENDOTOXIN ON BODY TEMPERATURE AND PLASMA TRACE METAL LEVELS IN NEONATAL GUINEA PIGS. C. M. Blatteis, R. A. Ahokas*, and T. A. Mashburn, Jr.*. Univ. of Tenn. Center for the Health Sciences, Memphis, TN 38163

Plasma levels of Fe and Zn generally fall, and of Cu rise during infection. It has been suggested that these changes, coupled with fever, have survival value. Infected neonates generally do not develop fever during the first week of life; survival could be affected. We studied ontogenic changes in the plasma levels of these trace metals induced by S. enteritidis endotoxin (SE, 2 μg/kg, ip) in conscious 0- to 16-day-old and adult guinea pigs; controls received apyrogenic saline. While fever could not be evoked by this dose of SE until the recipients were 8 days-old, their plasma levels of Fe and Zn (measured 8 h after SE, when these are maximally reduced by analogy with adult rats and rabbits) could be lowered significantly from birth. Plasma Cu was increased following SE from 2 days postnatally. This delay, as compared to the appearance of the responses of Fe and Zn at 0-1 day, may be due to insufficient liver stores of Cu available for mobilization at birth. These results indicate that, contrary to the refractoriness to endotoxin stimulation of the febrigenic system of neonatal guinea pigs, their ability to alter trace metal levels exists from birth. Thus, fever and trace metal changes may not necessarily be coupled for survival during infection. (Supported in part by USPHS NIH grants NS #14929 and HL #21641.)

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MONOPHASIC ENDOTOXIN FEVER IN THE AGED NEW ZEALAND WHITE RABBIT. A.V. Ferguson*, W.L. Veale & K.E. Cooper, Div. Med. Physiol., Faculty of Med., University of Calgary.

Recently, it has been suggested that the febrile response of older rabbits may be markedly reduced when compared to that of younger animals (1). The present work was carried out to further clarify such differences between old and young rabbits. All animals were restrained by use of conventional neck restrainers and colonic temperature was continuously monitored by use of a thermistor probe, and recorded by a Beckman Dynograph. A 1 hr baseline recording was made prior to all injections of pyrogen, and a dose of 1.5 μg/kg endotoxin was administered. Rabbits <12 months old develop a typical biphasic fever following injection of endotoxin with a thermal response index (TRI) of 6.7±0.4°C/hr. In contrast, rabbits over 2 yrs of age develop a significantly reduced fever following injection of pyrogen (TRI=2.72±0.7 $p<0.001$). When the febrile responses of these two groups are more closely examined we find no significant differences between the temperature responses of the two groups during the first 2 hrs after endotoxin (TRI 1.36±0.17 vs 1.6±0.17°C.hr). However, the TRI of the older group is significantly reduced when measured 120-270 minutes after endotoxin injection (1.35±0.6 vs 5.03±0.2°C. hr. $p<0.001$). These data strongly suggest that the changes in the febrile response system which occur with age are specifically related to the second peak of fever. (1) LIPTON, J.M. & TICKNOR, C.B. (1979) J. Physiol. 295: 263-272. Supported by MRC of Canada.

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THE LACK OF A FEBRILE RESPONSE TO BACTERIAL ENDOTOXIN IN THE BRATTLEFORO RAT. P.C. Eagan, N.W. Kasting, W.L. Veale and K.E. Cooper. Div. Med. Physiol., Faculty of Med., Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.

The rat develops fever following peripheral or central administration of bacterial endotoxin. The neuropeptide, arginine vasopressin, is believed to be an endogenous antipyretic in both the sheep and in the rat. The work reported here is designed to investigate the temperature responses of the Brattleboro rat to bacterial endotoxin and prostaglandin E_2 (PGE_2). The Brattleboro rat, which is genetically deficient in arginine vasopressin, is a mutant strain of the Long Evans strain. Microinjection of 1 μg of bacterial endotoxin into a lateral cerebral ventricle produced a fever in the Long Evans rat but not in the Brattleboro rat. Daily injections of 1 μg of endotoxin into a lateral ventricle of the Brattleboro rat did not result in a fever. Intraperitoneal injections of 50 μg of bacterial endotoxin resulted in a fever in the Long Evans rat but the Brattleboro rat showed no such response. Both groups of animals did produce a fever in response to intracerebral ventricular administration of 20 ng of PGE_2. The lack of arginine vasopressin in the Brattleboro rat is postulated to play a role in this observed lack of a febrile response to endotoxin.

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REFLEX AND LOCAL THERMAL CONTROL OF RAT TAIL BLOOD FLOW. M.F. Roberts. John B. Pierce Fndn Lab, Yale Univ. Sch. Med. New Haven, CT. 06519

Skin blood flow is influenced by reflex and local thermal effects, but the interaction between these effects is not known. This study was designed to determine how the reflex control of rat tail blood flow is affected by the level of tail temperature. A loop of PE 60 tubing was inserted into the jugular, advanced to the vena cava and exteriorized at the back of the neck. This loop served as a heat exchanger for altering core temperature. Experiments were performed on unanesthetized animals at least one day following surgery. Tail blood flow (TBF) was measured by venous occlusion plethysmography with a mercury-in-silastic strain gauge. Rectal (T_{re}), back skin (T_s), and tail (T_t) temperatures were measured with thermocouples. T_t was controlled by a heated or cooled air stream, independently of changes in T_{re} or TBF. In each experiment, T_t was held at 23°, 30°, and 37°, respectively, in three core heating transients at an air temperature of 25°C. In different experiments, the order of T_t was randomized. During the core heating transients, T_{re} was raised to 39°C; T_s remained nearly constant. At T_t = 37°, TBF increased rapidly with core heating. At T_t = 23°, the increase in TBF was delayed until high T_{re}. At T_t = 30°, the pattern was intermediate. Since reflex thermal stimulation was presumably the same in all three transients, the results suggest that local temperature acts by altering the response of tail vessels to the reflex signal. (Supported by NIH Grants ES-00354 and HL-17732).

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TAILLESS RATS: THERMAL RESPONSES TO ISOPROTERENOL. Donald E. Spiers*, Christopher C. Barney and Melvin J. Fregly. Dept. of Physiology, University of Florida, Gainesville FL 32610.

Tails were removed from male Sprague-Dawley rats (400-600g) and the animals allowed to recover for 4 weeks before testing. Restrained rats were maintained at an ambient temperature (T_a) of 26°C and administered isoproterenol (ISO), a β-adrenergic agonist, in doses of 0, 12.5, 25, 50 and 100 µg/kg body weight. Colonic temperature (T_{co}) was recorded before injection and for 120 minutes after administration of drug. There was no difference in T_{co} of tailed or tailless rats prior to administration of isoproterenol. Isoproterenol produced an increase in T_{co} in both groups at every dose used. At doses of 25, 50 and 100 µg ISO/kg, the tailless rats had significantly greater increases in T_{co} than the tailed rats. At 100 µg ISO/kg, the T_{co} of the tailless rats remained significantly above that of the tailed rats throughout the postinjection period. Oxygen consumption ($\dot{V}_{O2}$) was measured after administration of 50 µg ISO/kg. An increase in $\dot{V}_{O2}$ occurred in both groups after 10 minutes, with no significant difference between groups over the 120 minute test period. Exposure of rats to a T_a of 36°C produced a significantly higher T_{co} in tailless than in tailed rats during the first 60 minutes. These results suggest that tails are required by adult rats for short-term dissipation of excess heat and maintenance of normal thermal balance when metabolism is increased acutely. (Supported by Contract N00014-75-C-0199 with the Office of Naval Research).

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HYPOTHALAMIC MEDIATION OF THERMOREGULATION IN THE CAT BY A SEROTONERGIC RECEPTOR MECHANISM. W. D. Ruwe and R. D. Myers. University of North Carolina, Chapel Hill, NC 27514

To further elucidate the role of serotonin (5-HT) in central thermoregulatory mechanisms, guide tubes were implanted above the rostral diencephalon in eleven cats. 1.5 to 10.5 µg of 5-HT were microinjected in a volume of 0.75 to 1.5 µl into sites of the hypothalamus of the unrestrained cat. The region of maximum sensitivity is the anterior hypothalamic/preoptic area (AH/POA), within homologous sites of which norepinephrine and dopamine in similar doses produce a fall in body temperature. Dependent upon the neuroanatomical locus of injection, 5-HT evokes either an increase or a decrease in the cat's core temperature. Anatomically reactive sites (temperature change of ≥ 0.5°C within 30 min) were characterized by antagonists of catecholamine and indoleamine receptors. 1.0 to 10.0 µg of phentolamine, d-butaclamol and methysergide selectively delay the onset and reduce the magnitude of the hypothermic response elicited by 5-HT in the most rostral sites. Within more caudal AH/POA loci, the 5-HT-induced rise in body temperature is modified by prior treatment of the site with the receptor antagonists. Since catecholamine-induced hypothermia is also attenuated by these blocking agents, the 5-HT hypothermia may depend not only on the anatomical locus of injection, but also on the functional integrity of the catecholamine receptors of the POA. (Supported in part by U.S. Office of Naval Research Contract N00014-79-C-0078 and National Science Foundation Grant BNS-78-24491)

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REMOVAL OF SYNAPTIC INPUT ALTERS THE THERMOSENSITIVITY OF PREOPTIC NEURONS IN TISSUE SLICES. S.R. Kelso, M.N. Perlmutter, and J.A. Boulant. College of Medicine, Department of Physiology, Ohio State University.

Recent studies suggest that afferent synaptic input determines the level of firing rate in hypothalamic temperature-sensitive neurons. Furthermore, this firing rate determines whether neurons are sensitive to hypothermic or hyperthermic temperatures. Accordingly, removal of afferent input should result in a low firing hypothalamic neuronal population, which is thermosensitive only to hyperthermic temperatures. To test this hypothesis, preoptic single unit thermosensitivity was studied using the in vitro hypothalamic slice preparation. Hypothalamic slices (300-400 µm thick) were maintained in a nutrient medium (7.4 pH; 300 mOsm/kg). In some slice preparations, even local synaptic input was blocked by replacing the normal medium, with a high Mg^{++}-low Ca^{++} medium. Approximately 50% of the units were classified temperature-insensitive, 40% warm-sensitive, and 10% cold-sensitive. Compared with in vivo studies, these units had lower spontaneous firing rates. Also the in vitro warm- and cold-sensitive neurons were primarily sensitive only to hyperthermic temperatures. These results support the hypothesis that afferent synaptic input strongly affects both the firing rate and range of thermosensitivity of hypothalamic neurons. (Supported in part by grants from NIH (5-R01-N514644) and the American Heart Association.)

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CEREBROVASCULAR PERMEABILITY TO SUCROSE NOT INCREASED BY HYPERTHERMIA IN THE RAT. Edward Preston and Gabrielle Préfontaine*. Div. Biol. Sci., NRC, Ottawa, Canada, K1A 0R6.

The radiotracer technique of Rapoport et al.(Brain Res.150: 653, 1978) was used to verify reports that hyperthermia opens the blood-brain barrier. Anesthetized rats were given an intravenous injection of ^{14}C-sucrose. Arterial blood was then sampled periodically for 25 min, whereupon each rat was decapitated and brain regions removed to determine sucrose content. This procedure was carried out during the final 25 min of a 40 min period during which the rat's rectal temperature was maintained between 41-42°C by whole body exposure to 2450 MHz CW microwaves in an anechoic chamber, or to an infrared lamp. The ratio of brain-sucrose concentration (C_{brain}, dpm/g) relative to the average plasma concentration (over 25 min) was slightly but consistently lower in hyperthermic rats compared to controls. Permeability-area products (PA), calculated as the ratio of parenchymal sucrose concentration relative to average plasma concentration, were lower by 50% or more in hyperthermic rats compared to controls. That the foregoing ratios were not increased by hyperthermia indicates that the barrier maintained its relative impermeability to sucrose. The reduced PA values obtained for hyperthermic rats suggests that a reduced cerebrovascular permeation of sucrose may have occurred. However, hyperthermic rats exhibited elevated blood-sucrose levels compared to controls receiving the same dose; the correction of C_{brain} for intravascular sucrose had a disproportionate effect in lowering calculated PA.

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TEMPERATURE PREFERENCES IN MATURING NORMAL MICE AND IN MICE WITH ALTERED SEROTONIN. Cecilie Goodrich and Carole Wilk*. Cleveland State Univ, Cleveland, OH 44115.

Serotonin (5-hydroxytryptamine;5-HT) is known to be involved in thermoregulatory mechanisms in a number of species, and has been shown in this laboratory to affect body temperature (T_b) in mice early in postnatal maturation. A behavioral component of thermoregulation can be assessed by determining temperature preferences (T_{pref}) in a thermal gradient. Our gradient for young mice is constructed on an aluminum plate with a 20 cm separation between the cool (18° C) and warm (37° C) ends; the width of the gradient is about 5 cm. Mice have been tested to determine T_{pref} at ages of 3, 7, 10, 14 days and compared with 6 week old adults. Mean T_{pref} in untreated young mice (3 - 14 days) appears to be close to the temperatures maintained by the parent mice in the nest (29 - 30° C). Alterations in 5-HT were produced by administration of the precursor 5-hydroxytryptophan (5-HTP; 30 mg/kg) and the inhibitor p-chlorophenylalanine (p-CPA; 400 mg/kg). T_{pref} was determined in a double-blind fashion at appropriate times after injection, compared with vehicle-injected littermate controls. Increased brain 5-HT following 5-HTP was associated with increased T_{pref}, except at 3 days. Decreased brain 5-HT after p-CPA was associated with decreased T_{pref}. Comparison with effects on T_b suggests the mice may compensate for altered T_b by opposite changes in T_{pref}.

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MICROWAVE HYPERTHERMIA AND PHYSIOLOGIC REGULATION. Sol M. Michaelson.* University of Rochester, Rochester, N.Y. 14642

Physiologic regulation represented by thermoregulation, neuroendocrine function, neurochemical activity and immune responses are exquisitely tuned interrelated systems that constitute sensitive indicators of body responses to microwave/radiofrequency energy absorption. Electromagnetic fields may affect neuroendocrine, neurochemical activity and behavior. It is important to understand the underlying mechanisms which bring about these effects. Assessment of the integration and correlation of many body functions relative to the hyperthermia or altered homeokinetic status of the individual subjected to microwave/radiofrequency energy is critical in any evaluation of the potential use of these energies. The analysis of the hyperthermia response to microwave/radiofrequency energy absorption will be developed from the level of cellular and molecular effects, immunological and hematological responses, CNS, behavioral, neuroendocrine, neurochemical and thermal distributions as a result of local and whole-body exposure. (Supported by DOE Contract No. DE-AC02-76EV03490.)

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URINARY METABOLITES OF VASOPRESSIN: CONSEQUENCES IN RADIOIMMUNOASSAY. J. R. Claybaugh, D. P. Brooks,* A. K. Sato,* and K. M. Cornette.* Tripler Army Medical Center, HI 96859 and Univ. of Hawaii School of Medicine, Honolulu, HI 96822.

Vasopressin (VP) immunological activity of human urine and urine extracts has been determined using antisera (AS) with different portions of the VP molecule as the antigenic determinant. AS 09 is directed toward the "tail" where AS 11 is directed toward the "ring" of the molecule. Radioimmunoassay (RIA) of unextracted urine, or urine extracted by CG-50 ion exchange procedures, or Sephadex G-25 chromatography all showed 2-4 times greater activity when AS 11 was used than when AS 09 was used. When urine was passed through Sephadex, the activity detected by AS 09 was eluted slightly earlier than that detected by AS 11. Concentrated urine extracts were subjected to ascending paper chromatography utilizing n-Butanol, acetic acid, water (5:1:4) as the solvent. AS 11 detected two areas of immunological activity; only one corresponded to arginine VP and was also detected by AS 09. Determination of plasma VP concentration yielded similar results with either AS; however, only renal clearance of VP determined by RIA employing AS 11 approximated renal creatinine clearance ($C_{creat}:C_{VP}$ = 1.16:1, n = 4). It is concluded that a significant amount of the VP cleared by the kidney appears in the urine as a metabolite immunologically distinct from arginine VP, and that a "ring" directed AS yields more accurate assessment of total VP filtered by the kidney. (Supported by U.S Army Health Services Command and USPHS grant #HL23434-01 CVB.)

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EFFECTS OF CAROTID AND SYSTEMIC OSMOTIC STIMULI ON PLASMA VASOPRESSIN CONCENTRATIONS. C.E. Wade, P. Bie*, L.C. Keil and D.J. Ramsay. Dept. Physiol., Univ. of Calif., San Francisco, CA 94143 and NASA-Ames Research Ctr., Moffett Field, CA 94035.

Plasma vasopressin concentration (P_{AVP}) is primarily regulated by plasma osmolality (P_{OSM}) via hypothetical central osmoreceptors. The effects of intracarotid (IC) and intravenous (IV) infusions of hypertonic saline on P_{AVP} were studied in conscious dogs prepared with bilateral carotid loops. Infusions of solutions were made via both carotids (IC) each at 1.0/ml/min, or via a saphenous vein (IV) at 2 ml/min. The solutions infused were hypertonic NaCl at 0.18 mM/kg/min, 0.09 mM/kg/min, or isotonic saline, 0.15 mM. Blood samples, obtained simultaneously from the external jugular and saphenous veins, were taken prior to the start of the infusions, and after 3 and 6 min. of infusion. Infusions IC raised resting jugular vein P_{OSM} (304 ± 1 mosm/kg H_2O) by 0, 7, and 15 mosm/kg for saline, 0.09, and 0.18 mM NaCl/kg/min, while systemic P_{OSM} was not altered significantly. P_{AVP} was changed 0, 1.7, and 3.0 pg/ml from resting values of 1.8 ± 0.2 pg/ml. Similar IV infusions did not significantly alter jugular vein P_{OSM} or systemic P_{AVP}. P_{AVP} during IC infusions was significantly correlated with jugular vein P_{OSM}(r=0.65, P< 0.001). This finding confirms the existence of central osmoreceptors, and demonstrates their sensitivity to changes in osmolality within the physiological range, uncomplicated by alterations of the volume and composition of the extracellular fluid.
NIH Grant AM06704 & Training Grant AM07265.

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REDUCED MEAN ARTERIAL BLOOD PRESSURE (MABP) AND PLASMA ADH AFTER ALDOSTERONE INFUSION INTO DEHYDRATED CONSCIOUS DOGS. David P. Brooks* and John R. Claybaugh. Univ. of Hawaii School of Medicine, Honolulu, HI 96822 and Clinical Investigation Service, Tripler Army Medical Center, HI 96859.

Aldosterone was infused into 7 conscious dogs while normally hydrated or 48-h dehydrated producing a maximum plasma aldosterone concentration of 1.2 ng/ml. Dehydration increased the mean osmolality from 300 to 312 mOsm/kg, the plasma renin activity (PRA) from 1.05 to 1.78 ng ml^{-1} min^{-1}, and the plasma ADH concentration (PADH) from 2.12 to 7.28 μU/ml. When the dogs were dehydrated, aldosterone infusion (16 ng kg^{-1} min^{-1}) was associated with statistically significant falls in MABP (Δ = -7 mm Hg; P<0.01) and PADH (Δ = -32%; P<0.05). No change in either MABP or ADH was observed when either the same dose of aldosterone was infused into hydrated dogs or a lower dose of steroid (1.6 ng kg^{-1} min^{-1}) was infused into dehydrated dogs, or when vehicle was infused into both hydrated and dehydrated animals. PRA fell to a similar degree throughout all experiments conducted. A significant correlation existed between PRA and ADH (R = 0.81; P<0.005) in the dehydrated state, but not in the hydrated state (R = 0.11, N.S.). It is concluded that in the dehydrated animal aldosterone can cause a fall in MABP, and the data support the concept that the endogenous renin-angiotensin system may play a role in the control of ADH release in situations of increased plasma osmolality. (Supported by U.S. Army Health Services Command and USPHS grant #HL23434-01 CVB.)

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ENHANCED OXYTOCIN SECRETION BY BRATTLEBORO HOMOZYGOTES DURING DEHYDRATION. F.T. LaRochelle, Jr., M. Gellai*, and B.R. Edwards, Department of Physiology, Dartmouth Medical School, Hanover, NH 03755.

Brattleboro rats, homozygous for diabetes insipidus (DI), do concentrate their urine in response to dehydration. In these animals, which lack vasopressin, 24 h dehydration increased urine osmolality from 142 ± 17 ($\bar{x}$ ± SE) to 1130 ± 59 msomol/kg H_2O. Several factors may be involved in this concentrating ability, including oxytocin. Plasma oxytocin levels were 9.9 ± 1.5 pg/ml (range 4.3 to 18.8) in DI rats before dehydration and rose to 25.5 ± 2.8 pg/ml (range 19.3 to 47.0) after 24 h of water deprivation. To determine whether this level of oxytocin can affect the concentration of urine, conscious DI rats were infused with synthetic oxytocin to a plasma concentration of 46.3 ± 4.7 pg/ml to mimic post-dehydration levels of this hormone. In these rats the concentration of urine increased from 114 ± 10 mosmol/Kg H_2O to only 205 ± 22 mosmol/kg H_2O. These results suggest that while oxytocin secretion increases significantly after dehydration in DI rats, and to a level which can be as high as 4 times that of dehydrated LE rats (13.6 ± 0.9 pg/ml), this increase does not appear to play a major role in the concentration of urine in the water-deprived DI rat. (Supported in part by USPHS, NIH Grants HD 12123, AM 08469 and a grant from the Hood Foundation.)

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RAPID AND POTENT NATRIURETIC RESPONSE TO INTRAVENOUS INJECTION OF ATRIAL MYOCARDIAL EXTRACT IN RATS. Harald Sonnenberg, Anthony T. Veress*, Harold B. Borenstein*, and Adolfo J. de Bold*. University of Toronto and Queen's University, Ontario, Canada.

The muscle cells of the atrial myocardium in mammals contain numerous membrane-bound storage granules. The number of these granules appears to vary with changes in fluid and electrolyte balance. We therefore assessed the acute renal effects of an extract of atrial myocardium. Because ventricular muscle cells do not contain granules, extracts of this tissue served as controls. Supernatants of atrial or ventricular myocardial homogenates, obtained from the same freshly excised rat hearts, were injected intravenously into anaesthetized non-diuretic rats. Extracts derived from atrial muscle caused more than 30-fold increases of sodium and chloride excretions within 5 - 10 min from the injection. Urine volume rose 10-fold and potassium excretion doubled. The response was largely complete in 20 min. No such changes in renal function were observed after injection of ventricular extract, or of the homogenization medium alone. Since there was no significant difference in glomerular filtration rate following injection of atrial or ventricular extracts, we conclude that the atrial tissue contained an extremely powerful inhibitor of renal tubular NaCl reabsorption.

Supported in part by grants from the Ontario Heart Foundation.

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UREA HANDLING BY THE DISTAL TUBULE AND COLLECTING DUCT OF THE RAT DURING UREA-SALINE (USD), ISOTONIC SALINE (ISD), OR UREA DIURESIS (UD). D.R. Wilson and H. Sonnenberg. Depts. of Medicine and Physiology, University of Toronto, Toronto.

The aims of the present study were to examine the effects of urea and isotonic saline loads separately and together on urea handling in the medullary collecting duct (MCD) as well as in surface distal tubules. Microcatheterization of the MCD during ISD (5% body weight saline, n=17 paired samples in 6 rats) showed an increase in the remaining fraction of filtered urea (FR urea) from 56.2% at the beginning to 68.8% at the end of the MCD (p<0.05). There was no change in the FR urea along the MCD during UD (P urea 87 mM/l, n=15/6) or during USD (P urea 103 mM/l, n=32/9). Micropuncture of surface distal tubules in the same animals showed an increase in FR urea between end-distal samples and the beginning of the MCD during ISD from 26.2 to 56.2% (p<0.001), and during USD from 51.5 to 75.3% (p<0.001), but no change during UD (70.8 to 60.0%, p=NS). Conclusions: 1) Urea entry into MCD was shown during steady-state diuresis with low intratubular urea concentration (only ISD). 2) Urea entry between the surface distal tubule and the MCD was shown during saline diuresis (ISD or USD) but not UD. The latter finding suggests that isotonic saline loads affect urea transport differently in juxtamedullary nephrons compared to superficial nephrons.

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OBSERVATIONS ON THE ISOLATED PERFUSED HUMAN COLLECTING TUBULE. Norimoto Yanagawa*, Walter Trizna*, Yaacov Bar-Khayim*, and Leon G. Fine. UCLA School of Medicine, Los Angeles, CA 90024.

Cortical collecting tubules (CCT) were dissected from the surviving normal parenchyma of kidneys removed at operation for carcinoma or calculus. The CCTs were perfused in vitro within 60 minutes of removal from the patient. Transtubular potential difference (PD) varied from +3.2 to -2.0 mV. Lowering bath temperature or addition of ouabain to the bath reduced PD towards zero. In the absence of vasopressin, tubules were essentially impermeable to water with extremely low net water fluxes despite a transtubular osmotic gradient of 100 mOsm/kg H_2O. Addition of vasopressin to the bath caused the transtubular osmotic water permeability coefficient to increase to values of 125, 175, and 155 x 10^{-4} cm/sec in three tubules so studied. These results demonstrate close similarities between the human CCT and the more extensively studied rabbit CCT. (Supported by USPHS. NIH Grant # RO1 AM 25301.)

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QUANTITATIVE EFFECT OF SODIUM INTAKE ON RENAL POTASSIUM EXCRETION. David B. Young. University of Mississippi Medical Center, Jackson, MS. 39216

In order to determine quantitatively the importance of Na intake on regulation of renal K excretion the following study was carried out in a group of six adrenalectomzied dogs weighing 22 Kg maintained on a constant replacement of aldosterone (50 µg/day given continuously i.v.). K intake was varied from 10 to 100 mEq/day in three steps of 7 days duration while Na intake was held constant at one of three levels: 10, 30 or 100 mEq/day. At the conclusion of each level of K intake, renal K excretion and plasma potassium (P(K)) were measured and plotted against each other, plasma K being the independent variable. The results yielded a family of three curves relating plasma K to renal K excretion at the three levels of Na intake. Increasing Na intake shifted the curve to the left so that a lower level of P(K) was associated with a given level of K excretion. For example; 10 mEq/day of K as excreted at P(K)'s of 3.5±.3,3.1±.2 and 2.3±.2 mEq/l when Na intake was 10, 30 and 100 mEq/day, respectively. 25 mEq/day was excreted at P(K)'s of 4.5±.1, 4.2±.2 and 3.5±.2 mEq/l when Na intake was 10, 30 and 100 mEq/day, while at the same three levels of Na intake 70 mEq/day of K was excreted at P(K)'s of 4.7±.3,4.3±.1 and 4.1±.1 mEq/l. The data represent a quantitative analysis of the effect of Na intake on steady-state renal K excretion. (Supported by NIH HL 21435 and HL 11678).

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THE CONCURRENT KALIURESIS AND THE DELAYED KALIURESIS PRODUCED BY KCl INFUSION IN SHEEP. L. Rabinowitz and R.L. Sarason*. Dept. Human Physiology, Univ. Calif., Davis, CA, 95616.

The concurrent increases in plasma K and K excretion (U_KV) produced by infusion of K salts have been known for many years; the delayed, secondary kaliuresis now described does not appear to have been previously recognized. 3 mature conscious ewes were each infused i.v. with 50 mEq KCl as an 0.43M solution over 15, 30, and 60 minutes. During all infusions P_K, U_KV & $U_{Na}V$ rose in parallel. After all infusion P_K and $U_{Na}V$ declined rapidly and in parallel. After the 15 min infusion U_KV initially declined and then rose to a second peak 80 min after initiation of the infusion (PI). After the 30 min infusion U_KV initially declined and then either rose to a second peak or plateaued at 55 min PI. After the 60 min inf U_KV tended to plateau for about 40 min. Mean values from 15 min infusion experiments:

	pre-inf.	end-inf.	post-inf.trough	post-inf.peak
P_K mEq/l	3.75	7.26	5.49	4.86
U_KV uEq/min	231	663	400	574
$U_{Na}V$ "	112	1156	585	246

These data support the conclusion that a secondary kaliuresis follows about 55-80 min after initiation of K infusion. Commulative K excretion was the same at 5 hrs after infusion as in control expts without infusion; thus the delayed kaliuresis accelerated return to normal K balance. We have not as yet identified the mechanisms responsible for the second, delayed kaliuresis.

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Na^+-K^+-ATPase DEFICIENCY: A POSSIBLE MECHANISM OF RENAL SALT-WASTING IN A NEWBORN BABY WITH "PSEUDOHYPOALDOSTERONISM". M.M. Popovtzer, Hadassah-Hebrew University School of Medicine, Jerusalem, Israel.

A newborn boy, presented with hyperkalemia (S_K+ 9 mEq/L), hyponatremia (S_{Na}+ 120 mEq/L), urinary loss of Na ($U_{Na}V$>80 mEq/24h), low U_KV (<4 mEq/24h), and high plasma aldosterone (1200 ng/dl) and renin activity (613 ng AngI/ml/h). Neither mineralocorticoids nor diuretics corrected the abnormal renal handling of Na^+ and K^+. Na^+-K^+-ATPase activity in the red blood cell (RBC) ghosts of the baby was extremely low, $10x10^{-3}$ and $13x10^{-3}$ U/mg protein, 3 and 5 weeks after birth, respectively; as compared with $60x10^{-3}$ U/mg protein in a matched control baby. Likewise, Na^+-K^+-ATPase activity in the RBC ghosts of the baby's father, who had no overt electrolyte abnormalities, was extremely low, $6x10^{-3}$ U/mg protein (<10% of matched normal controls). The enzyme activity in the RBC ghosts of the baby's mother was normal, $75x10^{-3}$ U/mg protein. These observations show an association of a defect in renal tubular transport with a deficiency of Na^+-K^+-ATPase. The latter seems to be an inherited abnormality, with a dominant autosomal transmission. These findings suggest that, 1. an inherited Na^+-K^+-ATPase deficiency may be one of the possible mechanisms of congenital "pseudohypoaldosteronism", and 2. the enzyme deficiency may persist until adulthood, however, unknown adaptive mechanisms help compensate for the disorder to make it compatible with life.

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EFFECT OF INCREASING CONCENTRATIONS (CONCS.) OF SUBSTRATE-FREE ALBUMIN (SFA) ON ISOLATED PERFUSED RAT KIDNEY FUNCTION. M.J. Zamlauski-Tucker* and J.J. Cohen. Dept. of Physiol., Univ. of Rochester, Rochester, NY 14642.

Do changes in colloid conc., in the absence of exogenous substrate, affect glomerular filtration rate (GFR) and %Na reabsorption (%T-Na)? In previous studies with the perfused rat kidney, increases in perfusate [albumin], in the presence of substrate, had no consistent effect on GFR while %T-Na increased. In vivo, increases in rat plasma [albumin] increased GFR and had a variable effect on %T-Na in the proximal tubule. We perfused rat kidneys for 92 min with KRB buffer at increasing SFA concs. (Table) in the absence of substrate. We measured GFR, T-Na, oxygen consumption (Q-O_2) and % distal Na delivery [(C-H_2O/GFR + C-Na/GFR) x 100, C=clearance]. There was: a negative correlation (r=-0.9) between perfusate SFA conc. and GFR; a positive correlation (r=0.9) between perfusate SFA conc. and %T-Na. We conclude that increased perfusate [albumin], in the absence of substrate, decreases GFR and increases %T-Na. The passive increase in %T-Na occurs only in the proximal tubule. (USPHS-NIH AM-03602).

SFA conc. g/100 ml	GFR µl/min·g	%T-Na	T-Na µEq/min·g	Q-O_2 µMol/min·g	%Distal Na Delivery
3	778±36	31±1	35.6±1.6	-3.7±0.1	72±1
5	609±68	38±2	33.5±1.5	-3.8±0.5	69±1
6	440±141	61±1	37.1±12	-4.2±1.0	45±2
8	219±26	75±2	24.4±2.7	-3.6±0.7	29±2
10	41±17	83±2	4.9±2.3	-4.9±0.9	

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BONE POTASSIUM. John M. Bowman*, John T. McCall and Patrick J. Kelly. Mayo Clinic and Mayo Foundation, Rochester, MN 55901

The concept of a "membrane" dividing bone interstitial fluid into two discrete compartments has been accepted because of the reports of high total bone potassium in rat calvaria. Studies from this laboratory in dog cortical bone indicate that the V_{DK} (volume of distribution of K^+) is 1.27. Exchangeable bone K^+ equals V_{DK} x plasma K^+ ion or 4 x 1.27 = 5.1 meq/liter bone. This can be accounted for by a cell volume of 5% if bone cells contain 150 meq/liter of K^+ ion. The present study was performed to see whether total bone K^+ determination by spectrophotometric analysis agrees with exchangeable bone K^+ determined by measurement of ^{42}K in bone in the steady state. If they are similar, it would argue for compartmentalization of K^+ by cells rather than by a special membrane that divides fluid compartments beyond the capillary into two separate physiologic fluid spaces. Results: Exchangeable bone K^+ (V_{DK} x plasma K^+ ion) = 6.7 ± 3.0, and total bone K^+ = 7.21 ± 0.99 meq/liter in cortical bone of five dogs; total bone K^+ in human cortical bone was 6.95 ± 1.6 meq/liter, N = 10. The data suggest that cells act to compartmentalize bone K^+ ion rather than that there is a separate potassium-rich interstitial space. (This project was supported in part by USPHS NIH grant AM 15980.)

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RECORDING MOTOR ACTIVITY OF THE TREMATODE, HAEMATOLOECHUS MEDIOPLEXUS, IN RESPONSE TO DRUG TREATMENT. William Craelius, Jeffrey Pomerantz*, David Greene*, and Bernard Fried.* Dept. of Biology, Lafayette College, Easton, Pa. 18042.

Little is known about nerve and muscle physiology of trematodes. Here we report simple method for recording motor and electrical activity of the frog lung fluke, Haematoloechus medioplexus, in response to putative neurotransmitters. The posterior end of the worm was immobilized in one suction electrode, while the anterior end was free to move in a second electrode. This arrangement permits worm behavior that resembles what is seen in the frog lung. Worms in situ and in vitro display charateristic anterior probing movements, and show arhythmic peristaltic contractions. Exposure of worms to serotonin (10^{-4}M) caused peristaltic contractions to occur at a regular frequency of 15 per minute. The amplitude of the contractions was enhanced by serotonin, and the effects were reversible. Exposure of worms to GABA (10^{-5}M) abolished or markedly diminished motor activity, and this was reversible. Cholinergic agents and dopamine had no observable effects on motor activity.

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NERVOUS CONTROL OF FOOT MOVEMENTS IN THE EDIBLE MUSSEL. Edward Aiello, George Stefano* and Edward Catapane*. Fordham Univ., Bronx, N. Y. 10458.

Foot movements in Mytilus edulis are controlled by nerves originating in the pedal ganglia (PG). Cutting the pedal nerves causes the foot to relax. Serotonin (S) applied to the PG, visceral ganglia (VG), cerebral ganglia (CG) or foot causes the foot to elongate. Hemolymph flows in, as shown by radiolabelling, as longitudinal muscle relaxes and circular muscle contracts. Fluorescence histochemistry reveals a serotonergic innervation of hemolymph channels in the base of the foot, which dilate during elongation. There are peripheral S deposits which may relate to circular muscle contraction, for as the foot elongates it gets narrower. S is deficient and elongation fails to occur after treatment for 6 days with 5, 6-dihydroxytryptamine. Conversely, dopamine (DA) applied to the PG, VG, CG or foot causes foot withdrawal and the expression of hemolymph. DA fibers innervate the foot musculature. DA is deficient and contraction fails to occur after treatment for 6 days with 6-hydroxyDA. Applied to the PG, epinine mimics and haloperidol blocks the DA system for foot withdrawal. Clonidine acts like DA but is blocked specifically by phentolamine, suggesting an additional, adrenergic receptor. HT fibers from the CG and DA fibers from the VG exert a tonic influence on the foot. (Supported in part by grants from Fordham Univ. Res. Council and from NIMH (RR-08171) to Medgar Evars College).

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NEUROHUMORS AFFECT [illegible] RESPONSE TO SHUTDOWN OF SODIUM PUMP. Robert B. Hill and Peter Licis*. Univ. of Rhode Island, Kingston, R.I. 02881.

Acetylcholine, 5-hydroxytryptamine, tryptamine, and FRMFamide all have a similar effect in transforming the membrane response observed when buccal muscle of Busycon canaliculatum is exposed to saline lacking potassium ions. In such an external medium the radular protractor muscle first hyperpolarizes, which may be attributed to the change in potassium gradient, and then a depolarization of variable amplitude ensues, which may be attributed to sodium influx during pump shutdown, since both the depolarization and afterhyperpolarization on return to normal external potassium medium are sodium-dependent and ouabain sensitive. Hyperpolarization is maximal at 3 mM bath potassium while depolarization and afterhyperpolarization are maximal at 0 mM potassium. Both ouabain and sodium deprivation produce a steady depolarization which may correspond to the electrogenic pump contribution to resting potential. ACh, 5HT, and tryptamine all increase the depolarizing component in the response to zero potassium. This effect increases with concentration, from 10^{-8} M to 10^{-3} M, to the point where the response appears as frank depolarization with accompanying force. FRMFamide has a similar effect. The alteration in response to zero potassium is sodium dependent. Although the agents have rather different direct effects in normal medium, they all affect the response during pump shutdown similarly.

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DENERVATION AND OUABAIN ALTER TRANSMITTER RELEASE AT LOBSTER NEUROMUSCULAR SYNAPSES. R. Gary Chiang* and C. K. Govind. Scarborough Coll., Univ. of Toronto, West Hill, Ont., Canada M1C 1A4.

The single motoneuron to the limb accessory flexor muscle of lobster (Homarus americanus) forms neuromuscular synapses which release relatively large (high-output) and small (low-output) amounts of transmitter on separate fibers which are regionally located. Transmitter release from these identifiable synapses may be reduced by denervation and increased by exposure to ouabain. Denervation of the muscle by sectioning the main leg nerve caused, within 7 days, an increase in number of transmission failures from high-output synapses which rarely failed in intact preparations and altered the size distribution of their extracellularly recorded junction potentials (ERJPs) to that resembling low-output types. Both observations suggest a decrease in quantal content of synaptic transmission at high-output synapses with denervation. In contrast, application of 10^{-4} M ouabain to the bath saline, coupled with stimulation of the motoneuron, caused, within 2 hr, a reduction in transmission failures at low-output synapses and a shift in the size distribution of their ERJPs to that resembling high-output synapses. Thus nerve stimulation in the presence of ouabain increases quantal content at low-output synapses. Such alterations in transmitter output aid in exploring how synapses differentiate. Supported by NSERCC and Muscular Dystrophy Assoc. of Canada.

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PHARMACOLOGY OF OCTOPAMINE-SENSITIVE ADENYLATE CYCLASE IN INSECT HAEMOCYTES. John W.D. Gole* and Roger G.H. Downer. Department of Biology, University of Waterloo, Ontario, Canada, N2L 3G1.

The adenosine-3', 5'-monophosphate (cyclic AMP) content of whole haemolymph of Periplaneta americana increases by 50-fold within 5 min of injecting 10 µl 10^{-4}M octopamine into the abdominal haemocoel (haemolymph volume approx. 175 µl). The site of aminergic response resides within the plasma membrane of haemocytes. A variety of pharmacological agents (chlorpromazine, α- and β-flupenthixol, dibenamine cyproheptadine, promethazine, phentolamine, yohimbine, phenoxybenzamine, prozosin, propranolol, dichloroisoproterenol clonidine, phenylephrine, methoxamine, isoproterenol) have been used to characterise the system and these studies suggest that the octopamine-sensitive adenylate cyclase exhibits pharmacological properties similar to those of an α-adrenergic receptor.

A 3-5 fold increase in the haemolymph content of guanosine -3', 5'-monophosphate occurs concomitantly with the elevation of cyclic AMP following treatment with octopamine. (supported by Grant A-6084 from NSERC).

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THE EFFECT OF MET-ENKEPHALIN ON THE GILL WITHDRAWAL REFLEX OF APLYSIA AND ASSOCIATED EVOKED NEURAL ACTIVITY BY REPEATED SIPHON STIMULATION. J.A. Thornhill, Ken Lukowiak, K.E. Cooper and W.L. Veale. Div. Med. Physiol., Faculty of Med., Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.

The gill withdrawal reflex (GWR) of Aplysia evoked by tactile stimulation of the siphon is mediated by the integrated activity of the central (CNS) and peripheral (PNS) nervous systems. The CNS exerts suppressive and facilitatory control over the basic reflex mediated by the PNS. This CNS control bestows greater adaptability to the reflex behaviours. We have begun to investigate how the level of CNS control is adjusted by environmental factors and have focused our attention on various neural-active peptides. We report here that methionine enkephalin (m-enk - 10^{-6}M) when infused over the abdominal ganglion of Aplysia caused a significant reduction in the amplitude of the GWR and a decrease in the number of action potentials evoked by the tactile stimuli in central gill motor neurons. The effects brought about by the m-enk could be transiently overcome by a dishabituatory stimulus and were reversible following washout. However, the suppressive effect persisted for up to 5 h. The addition of 10^{-6}M nalaxone reversed m-enk's effects. The results indicate that the vertebrate neuropeptide enkephalin can directly influence behavioral and neural activity of invertebrates.

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NEURONS OF A CIRCADIAN OSCILLATOR: INTRACELLULAR RECORDING AND LUCIFER YELLOW INJECTION. Jon W. Jacklet. Dept. Biology, SUNYA, Albany, N.Y. 12222.

The isolated eye of Aplysia in organ culture and darkness expresses a circadian rhythm in the frequency of compound action potentials (CAP) recorded from the optic nerve. Neurons in the eye responsible for this endogenous dark activity and responses to light stimili were studied by intracellular recording and Lucifer yellow dye injection to trace the morphology of the neurons. Three basic types of neurons were observed, all of which had axons in the optic nerve. The spontaneous dark activity was correlated with pacemaker potentials and attenuated spikes in the D type. These cells receive extensive electrotonic input from other D's, which is the apparent basis for the sychrony of the CAP. The D's are non-receptors corresponding to the neurosecretory cells of the eye. A second type is the R or receptor cell. These cells give graded long lasting depolarization to light but rarely spike. Some receive small electrotonic inputs during spontaneous dark activity. A third type is the H cell and variations of that type. These cells produce large overshooting action potentials when injured by penetration but normally they are silent in the dark and do not contribute to the spontaneous dark activity. They respond to light briskly, giving graded hyperpolarization with depolarization accompanied by spiking. The light response of these cells are quite complex and represent a separate system from the circadian rhythm generators. (Support NSF BNS 11154).

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PHARMACOLOGICAL INVESTIGATION OF CRAYFISH HINDGUT: THE ACTIONS OF BIOGENIC AMINES, ACETYLCHOLINE AND PEPTIDE HORMONE. Robert E. Sullivan. Békésy Laboratory of Neurobiology, Honolulu, HI 96822.

We have investigated the effects of DL-octopamine (OCT), tyramine (TYR), dopamine (DA), acetylcholine (ACh), serotonin (5-HT), and proctolin on the isolated perfused hindgut of Procambarus clarkii. OCT, TYR, and ACh increase the spontaneous gut contractions and effect dose dependent contractures. The effects are TTX (10^{-6}M) resistant suggesting direct action on the hindgut muscle. DL-octopamine was the most potent agonist exhibiting a $K_d \simeq 5x10^{-6}$M. Tyramine ($K_d \simeq 1x10^{-4}$M) and ACh ($K_d \simeq 5x10^{-4}$M) were less effective. The octopamine response was blocked by phentolamine ($3x10^{-5}$M) and the acetylcholine response was blocked by atropine ($3x10^{-4}$M). We are currently investigating a phentolamine resistant DA (10^{-6}M) response which is also not affected by TTX. Proctolin (10^{-7}M) and 5HT (10^{-6}M) had little if any effect.

Radioisotopic procedures designed to detect biogenic amine synthesis indicated minimal levels of octopamine production in the hindgut and rectum. It seems that a more likely source of octopamine is from pericardial organs which store large quantities of octopamine for release as a neurohormone. The results suggest that in Procambarus, the hindgut may be a prime target for circulating levels of octopamine.

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RELEASE OF NORADRENALINE FROM PERIPHERAL ADRENERGIC NERVES INDUCED BY INHIBITION OF THE SODIUM PUMP IN THE ABSENCE OF EXTERNAL CALCIUM. V. Palatý. Dept. of Anatomy, Univ. of Brit. Columbia, Vancouver, B.C., Canada V6T 1W5.

A marked increase in the rate of noradrenaline release from the isolated rat tail artery (assayed by the radioenzymatic method of Henry et al. (1975)) can be detected after inhibition of the Na pump (removal of external K and 1 mM ouabain) even in the absence of external Ca^{2+}. It differs, however, from the increase observed in the presence of Ca not only in the magnitude and the time course (the maximum rate of 2.3 ± 0.3 nmole hr^{-1} g^{-1} is attained after 100 min), but also in that it can be inhibited almost completely by cocaine and phenoxybenzamine, and that it is approximately doubled if monoamine oxidase has been inhibited by pargyline. The release of noradrenaline is accompanied by a significant decrease in the proportion of storage vesicles containing an electron dense core. It can be concluded tentatively that the release observed under the title conditions is primarily non-exocytotic and that the transfer of noradrenaline across the neuronal membrane involves reversal of uptake$_1$. (Supported by a grant from the British Columbia Heart Foundation.)

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ACTION OF LITHIUM ON ADRENERGIC NERVE ENDINGS. Orren Beaty III, Michael G. Collis, John T. Shepherd. Kirksville College of Osteopathic Medicine, Kirksville, Missouri 63501; Mayo Clinic and Foundation, Rochester, Minnesota 55901.

The effect of lithium (0.5-14.4 mEq/l) on adrenergic nerve endings was studied using the lateral saphenous vein of the dog as a model of the adrenergic neuroeffector process. Lithium-induced changes in adrenergic neurotransmission depended upon the concentration of lithium and the stimuli employed to release the transmitter. Lithium (0.5-1.5 mEq/l) attenuated contractions evoked by electrical stimulation (ES) (0.5-10 Hz), while those to tyramine (TYR) (3-60μM) were unaffected. Lithium (3.6-14.4 mEq/l) augmented contractions evoked by ES (0.5-1.0 Hz) or TYR (3-6μM). However, with higher frequencies (5-10 Hz), the contractile responses were attenuated, while those to TYR (30-60μM) were not affected. Respective changes in the overflow of ^{3}H norepinephrine evoked by ES were consistent with the tension changes. The basal tension and basal overflow of ^{3}H norepinephrine were not affected by lithium (0.5-14.4 mEq/l). Inhibition of monoamine oxidase (MAO) abolished the lithium-induced augmentation of the contractile response to ES and TYR. However, the overflow of ^{3}H norepinephrine continued to be augmented though not sufficiently to change the contractile response. Thus, at these concentrations, lithium may reduce or augment adrenergic transmitter release through an effect on the exocytotic process and on MAO. (NIH Support: HL-5883, HL-0168, RR09130).

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DRUG AND IONIC INFLUENCE ON NOREPINEPHRINE METABOLISM IN THE CANINE SAPHENOUS VEIN. William Freas,* and Sheila M. Muldoon Depts. Anesth. and Physiol., USUHS, Bethesda, MD 20014

Neuronal amine uptake and metabolism is of primary importance in the termination of the action of norepinephrine (NE). A model of a sympathetically innervated vascular system frequently used for these studies is the canine saphenous vein. Vein strips were incubated in Krebs-Ringer's containing L-^{3}H-NE ($2x10^{-8}$ to $2x10^{-3}$ M), 15 to 150 minutes. The tissue and medium were analyzed for total tritium, ^{3}H-NE and ^{3}H-metabolite content. At $2x10^{-8}$ M, the following distribution was observed, deaminated metabolites (DOMA + DOPEG; 8%), O-methylated metabolite (NMN; 9%), O-methylated deaminated metabolites (MHPG + VMA; 13%) and ^{3}H-NE, 69%. By contrast at $2x10^{-3}$ M NE, ^{3}H-NMN comprised 67.5%, but ^{3}H-NE accumulation was only 6.1% of the amount taken up. At $2x10^{-7}$ M NE, accumulation of ^{3}H-NE in the vein increased linearly at a rate of 0.023 pmoles/mg/min and dihydroxphenylglycol formation at a rate of 0.005 pmoles/mg/min. 50mM K^+ reduced ^{3}H-NE accumulation by 42% but did not alter the metabolite pattern. Cocaine 10^{-5}M, ouabain 10^{-4}M, and Krebs with low Na^+ (6mM) reduced NE accumulation 90%. Ouabain and low Na^+ produced a significant increase in NMN formation possibly due to inhibition of intraneuronal NE accumulation. Krebs with low K^+ (1.2mM) also caused a significant increase in NMN formation, but accumulation of NE was not inhibited. These data indicate the ionic requirements of amine uptake and metabolism in the dog saphenous vein. Supported by Grants NIH GM25926, USUHS R07627

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CHARACTERISTICS OF NORADRENERGIC VESICLES OF RAT INTERSCAPULAR BROWN ADIPOSE TISSUE (IBAT). Gloria Zaror-Behrens*, Florent Depocas and Suzanne Lacelle*. Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

An average 63% of the dopamine β-hydroxylase (DBH) activity and 29% of the noradrenaline (NA) in sucrose-TRIS (250 mM, 5 mM, pH 7.4) homogenates of IBAT from 28°C acclimated rats sedimented with the "microsomal" fraction (166500 x g, 60 min). Using DBH as marker, application of the method of Slinde and Flatmark (Anal. Biochem. 56: 324, 1973) revealed two populations of noradrenergic vesicles with average sedimentation coefficients of 80 ± 11 and 255 ± 42 S (4°C) and containing respectively ∿65 and ∿35% of the sedimentable DBH. Upon isopycnic sucrose gradient centrifugation of vesicles, DBH activity peaked at a density of 1.091 with a broad shoulder extending to d = 1.19. During rate zonal centrifugation of IBAT vesicles on sucrose density gradients DBH activity and NA separated into slow and fast moving components. The modal density of the slow moving vesicles upon isopycnic recentrifugation was 1.092 while the fast moving vesicles were almost equally distributed over a range of d = 1.12 to 1.19. In the slow moving vesicles, NA and DBH activity relative to protein were respectively 6.5 and 23 times more concentrated than in the homogenate. It is concluded that both large and small noradrenergic vesicles are present in sympathetic nerves of rat IBAT and that both contain DBH and NA.

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EFFECTS OF ADRENALECTOMY ON SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN THE RAT. John W. Dailey*, Harold D. Battarbee and Laurel McNatt*. Departments of Pharmacology and Physiology, LSU Medical Center, Shreveport, LA. 71130

Adrenalectomy (adx) in the rat produces a decrease in blood pressure and increases in heart monoamine oxidase activity and norepinephrine turnover (Dailey and Westfall, Eur. J. Pharm. 48, 383, 1978). These changes were prevented by daily deoxycorticosterone acetate (DOCA) along with 0.9% saline to drink. In order to determine if the effects of DOCA were related to its effects on sodium metabolism, rats were adx under pentobarbital anesthesia. Controls were sham operated. Following adx all animals had free access to food. In one study, animals received 0.9% saline to drink while in other studies they had water to drink. All animals received daily injections, half of the adx animals received 0.5 mg/kg/day of DOCA. Others received 1 ml/kg/day of vehicle. Nine to ten days after surgery systolic blood pressure was taken and fifteen days after surgery 24 hour urinary norepinephrine was measured. Adx caused a fall in blood pressure in animals drinking water. Saline or saline and DOCA prevented the fall in blood pressure. Urinary norepinephrine was increased 315% in adx animals drinking water and 210% in adx animals on saline. DOCA and saline, but not DOCA alone suppressed the increase. These data suggest that the effects of DOCA on sympathetic function may be related to its effects on sodium metabolism. (Supported in part by a grant from American Heart Assn., Louisiana, Inc.)

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RENAL HUMORAL MECHANISMS OF CARDIOVASCULAR SUPPORT. Elliott M. Badder, M.D., Univ. of Md., Balto., Md. 21201. Sponsored by Timothy S. Harrison, M.D., Penn. State Univ., Hershey, Pa. 17033

The influences of reflex renal and adrenal medullary secretion on cardiac output (CO) and total peripheral vascular resistance (TPR) to rapid hemorrhage were compared. In anesthetized ventilated dogs, a left adrenal femoral venous shunt, right atrial catheter, aortic catheter and root of aorta electromagnetic flow probe (Carolina Medical Electronics) were positioned to monitor pressure (Statham transducers), adrenal flow and CO and sample blood before and 15, 30, 60 min. after acute hemorrhage (50 TORR m.a.p.). Adrenal medullary catecholamine secretion (single isotope radioenzymatic assay) and TPR were calculated. Three groups were studied: (N=5, CON) control; (Nx, N=4) acute bilateral nephrectomy; (AMx, N=5) adrenal medullary deprived (right adrenalectomy, left splanchnic nerve section) and results compared (Student's 't' test). Bleeding volumes were comparable. CO, comparable prior to hemorrhage (CON 136 ml/min/kg±57.3 (stand. dev.), Nx 102±40, AMx 99±43), fell in AMx and CON only (15 min. Nx 100.5% ±5%, CON 48%±26 $p<.01$, AMx 44%±22, $p<.01$). In Nx, TPR declined by about one half while TPR increased in CON ($p<.05$ at 30, 60 min.) and AMx ($p<.02$, 30, 60 min.). Adrenal medullary epinephrine secretion was low in Nx (60 min. .140 ug/min±101, $p<.05$ vs CON .878±.724, $p<.02$ vs AMx 1.592±.921). Failure to block adrenal medullary E secretion in AMx may indicate incomplete splanchnic nerve lysis. The results suggest important renal support of cardiovascular reflexes independent of adrenal medullary secretion.

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LOW RETINAL DOPAMINE AND SERUM PROLACTIN: INHERITED DEFECTS IN BW RATS. J. P. H. Wyse* and F. L. Lorscheider, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4

The Bmn-wys (BW) strain of rat is affected by a complex of inherited neurological abnormalities which include retinal degeneration, reduced lactation and altered motor activity. Results from the present study suggest that these abnormalities reflect a defect in neurotransmission. The Falck-Hillarp catecholamine (CA) histofluorescence technique, together with mean densitometric scanning measurements (cm^2±1 SEM), demonstrated a significant ($p<0.001$) reduction in CA histofluorescence in the inner plexiform layer of 6 BW retinae (0.74±0.14) compared to that present in 6 control retinae (2.47±0.29). It was subsequently found that mean retinal dopamine (DA) concentration (pg/µg total protein±1 SEM), measured by a sensitive CA radioenzymatic assay, was selectively and significantly ($p<0.05$) lower in 6 BW retinae (2.80±0.41) compared to 4 control retinae (6.30±1.60). Mean serum prolactin (PRL) levels (ng/ml±1 SEM), measured by radioimmunoassay, were found to be significantly ($p<0.05$) lower in 8 BW rats (24.7±6.1) than in 8 control rats (80.3±19.4). Since PRL synthesis and release in normal rats are under inhibitory regulation by DA, it is possible that low serum PRL concentrations observed in BW rats may be the result of increased DA turnover. This possibility, in conjunction with the low retinal DA levels, lead us to conclude that an inherited dopaminergic abnormality may be the pathogenic basis for the various neurological defects displayed by BW rats.(Supported by Natl. Retinitis Pigmentosa Found. Can.)

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CARDIAC PRE- AND POSTSYNAPTIC α-ADRENOCEPTOR BLOCKING EFFECTS OF THE TETRAMINE DISULFIDE BENEXTRAMINE. B.G. Benfey, T.J. Benfey*, B. Belleau* and C. Melchiorre*. Dept. of Pharmacology and Therapeutics and Dept. of Chemistry, McGill University, Montreal H3G 1Y6, Canada.

Raising the driving rate of the rat isolated left atrium at threshold voltage had a rate-dependent negative inotropic effect. High voltage stimulation increased contractility, presumably by releasing noradrenaline from adrenergic fibers, and raising the driving rate at high voltage had a rate-dependent positive inotropic effect. Phentolamine (1 µM) reversibly, and benextramine (10 µM; Melchiorre et al., J. Med. Chem. 21: 1126, 1978) irreversibly, enhanced the inotropic effects of high voltage stimulation and of raising the driving rate, presumably by facilitating noradrenaline release through blockade of presynaptic α-adrenoceptors on adrenergic nerve terminals. Phentolamine (K_B = 28 nM) competitively and reversibly, and benextramine (IC_{50} = 260 nM) noncompetitively and irreversibly, inhibited the positive inotropic effect of phenylephrine in rabbit isolated left atrium by blocking postsynaptic α-adrenoceptors. Thus like phentolamine, benextramine had similar affinities for pre- and postsynaptic α-adrenoceptors. (Supported by MRC).

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CHRONIC β-ADRENERGIC RECEPTOR BLOCKADE WITH PROPRANOLOL IN MICE USING OSMOTIC MINIPUMPS. Hurley Myers, Ronald Browning* and Jonas Sode. Southern Illinois Univ. Med. Sch., Carbondale, IL 62901 and VA Med. Ctr., Marion, IL 62959.

Osmotic minipumps (OM) delivering 1-2 mg/kg/day have been used to maintain a steady-state blood level of propranolol (P) in rats. This dose, however, failed to block β-receptors in mice challenged with isoproterenol (ISO). Accordingly, we conducted a series of experiments to determine the dose of P which must be placed in the OM to maintain chronic myocardial β-receptor blockade in mice. Changes in left ventricular (LV) cAMP in response to ISO were used to monitor β-adrenoceptor blockade. Mice were given saline (SAL) or P in OM implanted s.c. for 5 days. Four P regimens were tested. On the 5th day the mice were challenged with an i.v. injection of SAL or ISO (0.75 µg/kg) and sacrificed 10 sec later with focused microwave radiation. LV cAMP was measured by radioimmunoassay. The following data (expressed mean ± SEM pmoles of cAMP/g) suggest that complete β-receptor blockade can be achieved in mice with 16 mg/kg/day.

	SAL-SAL	SAL-ISO	PROPRANOLOL-ISO*			
			I	II	III	IV
$\bar{x}$	1335	3071	2678	2839	1216	1026
SEM	±73	±237	±142	±76	±148	±54
Significance		.001	.001	.001	NS	NS

*Group I = 6 mg/kg/day for 3 days (loading), then 1 OM delivering 2 mg/kg/day; Group II = 2 OM delivering 4 mg/kg/day; Group III = 2 OM delivering 16 mg/kg/day; Group IV = 2 OM (16 mg/kg/day) + daily injection of 6 mg/kg.

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FACTORS AFFECTING ENDOGENOUS CATECHOLAMINE RELEASE FROM HYPOTHALAMIC SLICES. Martha A. Hamlet*, Duane K. Rorie* and Gertrude M. Tyce* (SPON: Charles A. Owen, Jr.). Mayo Fdn., Rochester, MN 55901

Factors affecting release of endogenous catecholamines (CA) from slices of hypothalamus were studied. Slices were placed in cell chambers and superfused continuously at 0.5 ml/min with aerated Krebs-Ringer bicarbonate glucose buffer. Superfusate was collected during 10-min intervals under basal conditions and during release evoked by 40 mM K^+. CA were extracted from superfusate and from extracts of the tissue by column chromatography and were quantitated using reverse-phase liquid chromatography with electrochemical detection. While spontaneous release of dopamine (DA) and norepinephrine (NE) from untreated slices was not consistently demonstrable, high K^+ caused release of from 0.9-1.5 (DA) and 3.5-4.9 (NE) ng/g/min. Inhibition of in vitro prostaglandin synthesis by meclofenamate ($6.3x10^{-6}$M) had no effect on spontaneous or K^+-evoked release. Addition of cocaine (10^{-5}M) caused (a) an increase in the spontaneous efflux of NE but not of DA, (b) enhanced the K^+-evoked release of both NE (67%) and DA (90%), and (c) reduced significantly the hypothalamic content of both amines. The experiments suggest that neuronal reuptake of released transmitter is very efficient in the hypothalamus and is important in the maintenance of CA stores. (Supported by Grants AM 07147, HL 23217 and NS 9143).

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Effects of Donor Age on LHRH Response of Rat Anterior Pituitary Cell Cultures. James L. O'Connery* (Spon. Virendra Mahesh), Med. Coll. of Georgia, Augusta, GA 30912. Pituitary glands from 27 and 60 day Holtzman rats were trypsinized and grown 3 days in Modified Eagle's medium plus horse and fetal calf serum. Following a 3h LHRH challenge, gonadotropin release was determined by RIA. 27 day males gave increases in LH release ($p<.05$ from 3 ng/ml) and total LH ($p<.05$ from 6 ng/ml) through 50 ng/ml LHRH while 60 day males gave no further significant increase in LH release ($p<.05$) and total LH ($p<.05$) beyond 5 ng/ml LHRH. 27 day males gave increased ($p<.05$) FSH release at 3 ng/ml LHRH with no significant increase in total FSH. 60 day males gave no significant increase in FSH release or total FSH. 27 day females showed increased LH release ($p<.01$ from 1.5 ng/ml) and total LH ($p<.05$ from 10 ng/ml) through 50 ng/ml LHRH. 60 day females gave a biphasic response to LHRH at 5 ng/ml ($p<.01$) and at 200 ng/ml ($p<.01$). Total LH increased significantly only in response to 200 ng/ml LHRH ($p<.05$). The FSH release response of 27 day females was significant only at 50 ng/ml LHRH ($p<.05$) whereas 60 day animals plateaued beyond 5 ng/ml ($p<.01$). Total FSH levels did not increase significantly in 27 day females whereas significance was attained in 60 day cultures at 5 ng/ml LHRH ($p<.05$). Whereas younger animals gave step-wise response to increased LHRH, older animals responded in "threshold" fashion in which maximum response was attained at relatively low LHRH levels. These observations may be of value in better understanding the mammalian reproductive cycle. (Supported by NICHD grants HD-13100 and HD-10795.

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THE ROLE OF OPIOD PEPTIDES IN THYROTROPIN AND PROLACTIN SECRETION. A.M. Judd* and C.A. Hedge. Dept. of Physiology, West Virginia University Med. Ctr., Morgantown, WV 26506.

The endogenous opioid peptides (OP) are postulated to be involved in the secretion of anterior pituitary hormones. We have studied the role of OP in the secretion of thyrotropin (TSH) and prolactin (PRL) in female Sprague-Dawley rats. The opioid receptor antagonist naloxone (NAL) (.2 mg/kg, IP) had no significant effect on basal or thyrotropin releasing hormone (TRH)-stimulated TSH (30' and 45' post NAL) in unanesthetized rats. Similarly, infusions of NAL (.44 mg/kg for 90 min., IP) had no effect on TSH in anesthetized rats. In unstressed rats sampled via jugular cannulae a higher dose of NAL (12.5 mg/kg, IV) resulted in significantly less PRL (50%) and slightly greater TSH (20%) than in saline controls (20' post NAP). To test for a pituitary site of action of OP, superfused collagenase-dispersed anterior pituitary cells were treated with β-Endorphin (β-En). β-En increased TSH and PRL within 2' over the dose range of 10^{-9} to 10^{-5}M. β-En (10^{-6}M) and TRH (10^{-8}M, a midrange dose) induced equivalent responses in either TSH or PRL. NAL (10^{-4}M) was ineffective in blocking the β-En (10^{-7}M)-induced rise in TSH and PRL. Conclusions: 1)The NAL sensitive OP receptors of the female rat stimulate basal PRL secretion but play little or no role in basal TSH secretion, 2)In addition to well documented hypothalamic effects, β-En directly stimulates pituitary release of TSH and PRL by NAL insensitive receptors. (Supported by NIH Grant AM 21348)

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INTERACTIONS BETWEEN DEXAMETHASONE, 17β-ESTRADIOL, TRH AND DOPAMINE IN THE CONTROL OF PROLACTIN SECRETION IN RAT ADENOHYPOPHYSEAL CELLS IN CULTURE. Fernand Labrie and Vincent Giguère*, MRC Group in Molecular Endocrinology, CHUL, Quebec G1V 4G2, Canada.

Recent data obtained in our laboratory show that 17β-estradiol (E_2) can almost completely reverse the inhibitory effect of dopamine (DA) on PRL release in rat anterior pituitary cells in culture. Moreover, androgens and progestins exert antiestrogenic effects at the pituitary level on PRL secretion. Using the same system, we next investigated the possible effects of a synthetic glucocorticoid, dexamethasone (DEX), alone, on PRL secretion as well as its interactions with E_2, TRH and DA on the same parameter. Using cells obtained from female rats incubated for 4 days in the presence of steroids, increasing concentrations of DEX lead to a 2-fold increase of basal PRL secretion, the stimulatory effect of the steroid being exerted at an ED_{50} value of 2 nM. Preincubation with 100 nM DEX increases the PRL response to TRH by 3-fold. The more important finding is however that DEX shows an antidopaminergic effect similar to that of E_2 by increasing the ED_{50} value of DA action on PRL release by 10-fold. When used together, E_2 and DEX show no additive stimulatory activity on PRL secretion. The present data clearly demonstrate that DEX exerts direct stimulatory effects at the pituitary level on both basal and TRH-induced PRL secretion. Moreover, DEX has a potent antidopaminergic action in prolactin-secreting cells.

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EFFECTS OF 17β-ESTRADIOL AND 5α-DIHYDROTESTOSTERONE ON LHRH RECEPTOR LEVELS IN RAT ANTERIOR PITUITARY CELLS IN CULTURE. Vincent Giguère* and Fernand Labrie, MRC Group in Molecular Endocrinology, CHUL, Québec G1V 4G2, Canada.

Using rat anterior pituitary cells in primary culture, we have recently shown that estrogens and androgens can exert specific effects on the LH and FSH responses to LHRH. In order to determine whether sex steroids exert their effects at the level of the LHRH receptor, we have studied in detail the binding of the highly potent and stable LHRH agonist [^{125}I]-[D-Ser(TBU)^6] LHRH ethylamide in rat anterior pituitary cells treated with 17β-estradiol (E_2) or 5α-dihydrotestosterone (DHT) under conditions previously found optimal for the effects of these steroids on LH and FSH secretion. The radioactive ligand binds rapidly and reversibly to a single class of high affinity sites (K_d = 0.25 nM) with approximately 4000 binding sites/cell. Specificity studies using a large number of LHRH agonists and antagonists show a good correlation between the ability of the peptides to displace the iodinated tracer and their LH-releasing activity. Cells preincubated for 4 days with increasing doses of DHT show a 50% ($p < 0.01$) decrease in LHRH receptor levels at 1 nM DHT while E_2, at concentrations up to 100 nM, has no effect. The present data demonstrate the presence of a specific LHRH receptor in rat anterior pituitary cells in culture and suggest that the inhibitory effect of androgens on the LH response to LHRH could be explained by a decrease of LHRH receptors while the stimulatory effect of estrogens on the same parameter is probably exerted at a later step.

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EFFECTS OF OVINE PROLACTIN (oPRL) AND LEGOTRILE MESYLATE (LM) ON BLOOD PRESSURE AND BODY FLUID COMPARTMENTS IN THE RAT. D.E. Mills*, M.T. Buckman*, and C.T. Peake*(SPON: D. Robertshaw). Depts. of Res. and Med., VAMC and Univ. of NM Sch. of Med., Albuquerque, New Mexico 87131.

The present study examined the effects of oPRL and LM, a dopamine agonist and PRL suppressor, on systolic blood pressure (BP), plasma volume (PV) by ^{51}Cr dilution, extracellular fluid volume (ECF) by $^{35}SO_4$ dilution, and total body water (TBW) by ^3H_2O dilution in adult male rats. Animals received either 0.9% saline, 1.5 mg oPRL, 1.7 mg LM, or 1.7 mg LM+1.5mg oPRL over 7 days via osmotic pump (Alzet) implanted ip. On day 7 the following results were obtained (X±SEM):

	BP(mmHg)	PV(%BW)	ECF(%BW)	TBW(%BW)
controls (n=8)	122±1	5.3±0.4	24.1±1.7	68.8±1.1
oPRL (n=8)	113±1***	7.3±0.6*	31.5±2.7*	67.8±1.7
LM (n=8)	118±1**	5.7±0.4	29.8±3.0*	71.2±1.4
LM+oPRL (n=8)	113±2***	6.1±0.3*	32.6±4.0*	69.5±1.1

* p< .05 ** p< .01 *** p< .001

The data suggest that 1) chronic oPRL administration decreases BP, while increasing PV and ECF, but has no effect on TBW, 2) LM, while decreasing serum rPRL (x̄ 24 hr. PRL 13 ng/ml vs 20 ng/ml control), mimics oPRL action on BP and ECF, and 3) LM + oPRL shows similar results to oPRL alone. We conclude that oPRL decreases BP and may shift fluid from the intracellular to the extracellular compartment without affecting TBW, and that LM may not be a suitable agent for studying the metabolic effects of hypoprolactinemia. (Supported by NM Heart Assoc.)

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MODIFICATIONS OF EXTRACTION PROCEDURES ALTER FEMALE RAT ANTERIOR PITUITARY GLAND GONADOTROPIN CONCENTRATIONS AS MEASURED BY RIA. Kathleen A. Elias,* and Charles A. Blake. University of Nebraska Medical Center, Omaha, NE 68105.

Rat hemi-anterior pituitary glands (APs) were either 1) sonicated in PBS, 2) homogenized (H) in 0.5% Triton-X. 3) H in a 0.5% deoxycholic acid, Triton-X mixture (deoxy-T) or 4) H in PBS. The pH of all solutions was 7.0. The H APs were centrifuged (1500x G) and the supernatants frozen until RIA. Detergents did not interfere with the assays. LH and FSH values from sonicated APs were similar to those of their pairs H in PBS. The Triton-X homogenates had similar amounts of FSH but 2.6 times more LH than the PBS homogenates. The APs H in deoxy-T contained similar amounts of FSH but 3.9 times more LH than APs H in PBS. In another study, APs were treated the same except that aliquots were ultracentrifuged (UC; 165,000x G). UC-H PBS samples had 41% less LH and 38% less FSH while UC-sonicated samples had 75% less LH and 60% less FSH. UC-H detergent samples showed no changes. The results suggest that after homogenization in PBS, anti-FSH sera may recognize most if not all the FSH originally present in APs but anti-LH sera cannot recognize at least 74% of the LH in such preparations likely because this LH may be located within or attached to membranes which impede its detection. Further, after APs are H in PBS, about 1/3 of the total FSH and 10% of the total LH appears to be contained within or attached to membranes which do not impede their detection by the antisera. Supported by the NIH (HD11011 and HD 07097.)

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CHARACTERIZATION OF A DOPAMINERGIC RECEPTOR IN BOVINE POSTERIOR PITUITARY MEMBRANES. P. Poyet*, T. Di Paolo* and F. Labrie, MRC Group in Molecular Endocrinology, CHUL, Quebec G1V 4G2, Canada.

The binding characteristics of the dopaminergic antagonist [^{3}H]spiroperidol were studied in membranes obtained from bovine posterior pituitary gland. Binding of [^{3}H]spiroperidol is time-dependent and reversible. Since phentolamine gives a biphasic displacement curve with a first plateau at 100 nM, all further studies were performed in the presence of 100 nM unlabeled phentolamine in order to mask the α-adrenergic binding sites. The equilibrium dissociation constant (K_D) of specific binding is 0.3 nM. The rank order of potency of various agonists to compete with [^{3}H]spiroperidol binding is consistant with a typical interaction at dopaminergic sites (apomorphine > dopamine > (-) epinephrine ≥ (-) norepinephrine > (-) isoproterenol - clonidine). [^{3}H]spiroperidol binding shows stereoselectivity, (+) butaclamol being 1,000 times more potent than (-) butaclamol. Dihydroergocryptine and the dopaminergic antagonists (+) butaclamol and haloperidol compete for [^{3}H]spiroperidol binding at nanomolar concentrations. Adrenergic and serotonergic drugs are inactive or only weak competitors for [^{3}H]spiroperidol binding at micromolar concentrations. No effect of GTP is observed either in the presence or absence of phentolamine. The present data show the presence of specific dopaminergic receptors in bovine neurohypophysis.

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DURATION OF MORPHINE-INDUCED ANALGESIA IS INCREASED BY PREVIOUS CHRONIC ADMINISTRATION OF NALORPHINE. D.Mark Wright* (SPON: J.L. Henry). Dept. Anaesthesia Research, McGill Univ. 3655 Drummond St., Montreal, Quebec. H3G 1Y6

The effectiveness of morphine as an analgesic is reduced by its repeated administration (i,e. tolerance develops). This study was designed to determine the effectiveness of morphine as an analgesic in male Wistar rats treated chronically for 14 days with twice-daily injection of the mixed opiate agonist/-antagonist nalorphine (10 mg/kg-1, s.c.). Following this treatment the analgesic effectiveness of 2.5 mg.kg-1 (s.c.) morphine was tested at 7,14,21,28 & 35 days by measuring the duration of the analgesia (taken as the period that time to tail-flick from a noxious heat stimulus remained 100% above baseline reaction time). The difference between nalorphine-pretreated and naive rats was greatest on day 21, when the duration was significantly longer ($p < 0.001$) in the nalorphine-pretreated rats (153.33±18.66 min, $n = 3$) than in naive rats (77.0±8.15 min, $n = 5$); saline pretreated rats in fact showed a decrease (29.0±13.55 min, $n = 5$). This temporary increase in the effectiveness of morphine as an analgesic suggests that chronic blockade of the opiate receptor by nalorphine may have induced a supersensitivity to morphine, a suggestion with possible important implications regarding our understanding of tolerance to opiates.

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STRUCTURE OF PRESUMPTIVE STRETCH RECEPTORS IN DOG TRACHEA. J.M. Krauhs* and N.L. Salinas* (SPON: G. Sant'Ambrogio). Dept. Physiology & Biophysics, Univ. of Texas Medical Branch, Galveston, Tx. 77550.

Slowly adapting mechanoreceptors sensitive to transverse but not longitudinal stretch are located in smooth muscle of the dog trachea (Bartlett et al., J. Physiol. 258: 409, 1976). They are believed to contribute to the Hering-Breuer inflation reflex. Nerve endings typical of mechanoreceptors have now been found in plastic-embedded sections of dog trachealis muscle and their ultrastructure has been studied. The muscle was fixed in glutaraldehyde and processed by standard methods. Thick Epon-embedded sections (3 μm) were cut, stained with p-phenylenediamine and toluidine blue and examined for brown nerve endings. Favorable sections were thin-sectioned and examined in the electron microscope. Nerve endings (from myelinated fibers) contained numerous mitochondria, glycogen, osmiophilic granules and a few small clear vesicles. They did not make direct contact with muscle but were surrounded by connective tissue which was attached to muscle. Nerve ending profiles and Schwann cell nuclei were often aligned in rows parallel to the muscle. Processes containing no mitochondria protruded from some of the nerve ending profiles and were aligned parallel to the muscle. They were usually not completely covered by Schwann cells. Further study of these nerve endings may indicate a structural basis for the directionality of the stretch receptor response. (Supported by USPHS. NIH Grant HL#20122.)

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LIGHT REDUCES RETINAL OXYGEN CONSUMPTION IN VIVO. E. Stefánsson,* M. L. Wolbarsht, M. B. Landers, III,* G. Metz,* and D. Cook.* (Spon: L. M. Mendell). Duke Univ. Med. Ctr., Durham, NC 27710

Occlusion of the retinal circulation in cats by a probe pressing on the optic disc allows the retina to receive O_2 from the choroid only.[1] The ERG "b" wave disappears during occlusion, but reappears when the animal breathes 60% O_2 concentrations at 1 atm. Concurrent measurements by polarographic methods of the pO_2 in the vitreous 0.1 mm from the retina show levels near 0 after occlusion. 60% or more O_2 in the breathing mixture raises the "preretinal" pO_2 far above zero. During occlusion the oxygen tension drop across the retina is the difference between the choroidal (arterial) blood pO_2 and the preretinal pO_2 allowing the O_2 consumption of the retinal tissue to be calculated by Fick's Law of diffusion. The O_2 consumption throughout the retina is not homogenous, with the photoreceptors responsible for most of it. We have found the pO_2 fall across the retina to be 103 ± 33 mm Hg in strong light, and 267 ± 73 mm Hg in dark, corresponding to an O_2 consumption of 1.5 ± 0.5 ml O_2/min 100 g tissue in light, and 3.7 ± 1.0 ml O_2/min 100 g tissue in dark. This shows that light (darkness) markedly reduces (increases) the respiration of retina in vivo, as has been shown in isolated retina.[2,3]

Ref: (1) Landers, M. B., III, Trans. Am. Ophthal. Soc. 76: 528, 1978. (2) Zuckerman, R. and Weiter, J. J., Exp. Eye Res. (in press), 1980. (3) Jaffe, M. J., et al., Exp. Eye Res. 20: 531, 1975.

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AUDITORY EVOKED RESPONSES DISTINGUISH BETWEEN RIGHT AND LEFT CEREBRAL HEMISPHERE DAMAGE IN ADULT MEN. Dennis L. Molfese*, Carl Parsons*, Janice L. Bright*, Dileep Kumar* and Jonas Sode, Southern Illinois University, Carbondale, IL, 62901 and VA Medical Center, Marion, IL, 62959.

Auditory evoked responses (AERs) to a series of word pairs were recorded from temporal and parietal regions of left and right hemispheres (LH and RH) of 8 LH damaged (LHD), 8 RH damaged (RHD) and 8 nondamaged hospitalized adult men. Word pairs were selected in which the second word of the pair was (1) identical to the first, (2) semantically related but phonetically dissimilar, or (3) semantically unrelated (32 word pairs for each of these 3 meaning conditions). Principle components analysis and analysis of variance of averaged AERs (Molfese, Brain and Language 5:25, 1978) revealed that 11 factors accounted for 90% of the total variance. One factor (peak latency 550 ms) discriminated between the identical, related and unrelated meaning conditions across all 3 subject groups. Another factor (peak latency 200 msec) reflected the differences in hemispheric responding between the 3 groups ($p < .01$). The hemispheric differences for the control, LHD and RHD groups were small, absent and very large respectively. In addition, the intact LH of the RHD group responded identically to the LH of the controls. The intact RH of the LHD group, however, did not respond like the intact RH of the control group. (Supported in part by SIU Grant #2-10825.)

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SLEEP-WAKEFULNESS STATE IDENTIFICATION SYSTEM FOR LONG-TERM RECORDING IN RATS. J. Ramazankhani*, A. Hirtenfeld*, A. Sen, H. Galin*, T. Pivik* and M. Mamelak*. (SPON: F. A. Sunahara). University of Toronto, Toronto, M5S 1A1.

A system for identifying the states of quiet and active wakefulness, NREM sleep and REM sleep in the rat during long term recordings was developed utilizing the compressed spectral array technique. EEG data were digitized at a sampling rate of 128 samples/sec and a frequency analysis using an algorithm for the fast Fourier Transform was performed each second to obtain power spectral densities of frequencies from 1-63Hz. The spectral data for consecutive one second samples were averaged to provide a spectral array for each 10 second epoch which served as the basis for state identification. Total power distinguished sleep from wakefulness, and the ratio of power at 5, 6, and 7 cycles/sec to the power at 10, 11 and 12 cycles/second distinguished REM sleep from NREM sleep, and active wakefulness from quiet wakefulness. Sleep recordings were also scored visually in 20 second epochs. Computer and visual scoring of 48 hour recordings did not differ by more than 7%. In addition to the time saved, the computer method had the advantage that EMG data were not necessary to distinguish the different states of sleep and wakefulness.

(Supported by Sunnybrook Medical Centre Research Trust Fund)

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VESTIBULO-OCULAR REFLEX DYNAMICS IN MAN DURING VOLUNTARY HEAD OSCILLATION, AND RELATED VISUAL-VESTIBULAR INTERACTION. R.M. Jell, F.E. Guedry*, and W.C. Hixson*. Dept. of Physiology, Univ. of Manitoba, Winnipeg, Canada and NAMRL, NAS Pensacola, Fl.

A method has been developed for producing voluntary head movements about the yaw axis at various frequencies while measuring head angle and eye-in-head angle. The technique has been used to quantify relationships between head and eye movements under three different conditions of visual stimulation: 1) eyes open while attempting to fixate an Earth-fixed target during oscillation; 2) eyes open while attempting to fixate an imagined Earth-fixed target in the dark; 3) eyes open while attempting to fixate a target fixed relative to the head during oscillation. Data obtained from 13 normal subjects show that in condition 1 eye/head velocity gain was unity or slightly greater over a range of oscillation frequencies from 0.1 to 3.4 Hz with a tendency to rise at higher frequencies. In condition 2, gains were generally lower by about 0.15 than those in condition 1, but remained close to unity between 0.1 and 0.2 Hz. Visual suppression overcame the VOR at frequencies below 2Hz in condition 3 and gains fell toward zero. These findings will be discussed in terms of the significance of factors controlling motor performance under different conditions of reference input.

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PHARMACOLOGIC CONTROL OF RECEPTIVE FIELD SIZE IN MOTOR-SENSORY CORTEX IN CATS. C.F. Tyner, M.Y. Spiegelstein*, and M.L. Howell*. Walter Reed Army Inst. of Research, Wash.D.C. 20012.

We showed that iv. naloxone (0.2-2.0 mg/kg) changes many cells' receptive fields from small, contralateral (S) to whole-body (M) in "motor" area 4γ of chloralose-anesthetized cats, and lowers thresholds to skin stimuli (Neurosci. Abst., 1979; 5:713). In 30 additional cells studied before, during, and after drug injection, and 200 studied only after injection, we now find: A) naloxone does not cause S → M changes in nearby post-dimple "sensory" cortex under chloralose. B) Subconvulsive doses of picrotoxin (150 µg/kg) cause many S → M changes, and lowered thresholds, in 4γ; but few changes in post-dimple tissue -- all under chloralose. C) Neither drug causes overt field change in 4γ, or post-dimple, in awake cats tested with innocuous stimuli. D) Diazepam (.03-.07 mg/kg) causes M → S changes and threshold elevation in 4γ under chloralose; the effect has been seen with M cells found under chloralose alone and with those resulting from naloxone or picrotoxin-induced S → M. With sequential drug doses we have also induced several S → M → S and M → S → M shifts. We think these dramatic receptive field changes reflect modulation of a GABA, not an endorphin, system and that chloralose has played a major role, perhaps as a weak GABA antagonist. The sites of drug action are unknown. Also unknown is whether such field changes, or subthreshold analogs, occur during natural behavior; and if they do, under what circumstances. (Supported by DOD).

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EMG RESPONSE TO DISPLACEMENT OF SEVERAL UPPER LIMB JOINTS IN THE SQUIRREL MONKEY. F.A. Lenz*, R.R.Tasker*, I.C. Bruce* and W.G. Tatton. (SPON J. Grayson). Playfair Neurosciences Unit, Univ of Toronto, Toronto, Ontario M5T 2S8.

Averaged, rectified, electromyographic (EMG) responses to displacements produced by randomly ordered,graded, torque loads about upper limb joints were studied for muscles acting across the elbow, wrist and metacarpal-phalangeal joints of four Squirrel monkeys. Short and long latency components ("M1" and "M2,3" respectively) were observed in the EMG responses of all muscles at onset latencies, ranging from 8 to 12 msec for the "M1" and 17 to 25 msec for the "M2,3", which are similar to those reported in man and in old-world primates. allowing for size differences between the species. "M1/"M2,3" ratios of the integrated EMG above baseline were calculated for short head of biceps (0.65 ± 0.18), flexor carpi ulnaris (0.36 ± 0.16) and flexor digitorum profundus (0.14 ± 0.14). A similar progression was observed in the corresponding extensors. "M1" and "M2,3" were graded with respect to velocity in all muscles studied, but the threshold occurred occurred at higher velocities distally. These results demonstrate progressive predominance of long latency EMG components in distal upper limb muscles as compared to proximal muscles. (Supported by the Multiple Sclerosis Society, Ontario Heart Foundation Grant STR-T-2, Medical Research Council of Canada Grant #5218).

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AFFERENT "ENCODING" BY CAT MOTOR CORTICAL NEURONS. A.G.E. North*, and W.G. Tatton. Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, M5T 2S8.

Pericruciate microelectrode recordings were carried out in ten chronically prepared, awake cats and the "afferent receiving" zone that contained motor cortical neurons (MCN's) responding to imposed elbow movements was mapped for each animal. MCN afferent latencies ranged from 10 to 28 msec and were constant for a given MCN within 2 msec despite systematic variations of initial movement velocity and initial included joint angle. Movement responsive neurons were classified into four "subpopulations" as determined by the correlation between firing, quantified by average response histograms, and the acceleration and velocity traces for the imposed movements. The magnitude of the firing probability and the response durations were uniquely monotonically related to the movement parameters for each "subpopulation". The "subpopulations" were classified as velocity (V-MCN's), acceleration (A-MCN's), initial acceleration (IA-MCN's) and combined (C-MCN's). IA-MCN responses were independent of initial joint angle while the response relationships of the other "subpopulations" could be initial angle dependent. These results establish that the afferent responses of motor cortical neurons can be considered to "encode" specific movement parameters. The possible functional role of the "encoding" will be considered within the framework of a predictive error compensating system. (Supported by MRC Grant #5218).

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INTERACTION BETWEEN VISUALLY-CUED VOLUNTARY MOVEMENT AND REFLEX RESPONSES TO IMPOSED PERTURBATIONS. M.J. EASTMAN* and W. G. TATTON. (SPON: J. HUNTER) Playfair Neuroscience unit,Univ. of Toronto, Canada M5T 2S8.

The interaction between unexpected imposed perturbations of the wrist joint and a two choice,visually-cued wrist movement was studied in trained monkeys. The monkeys maintained an initial wrist position signalled by green light emitting diodes (LEDS) for a random interval(4.5 to 6.0 seconds). Arrays of red or yellow LEDS cued a fast(average velocity 400 degrees /second) extension or flexion movement to the extreme wrist positions. On random trials and at random delays following the visual cue,load perturbations were imposed on the wrist by a coaxial motor. Reaction times ranged between 134 and 261 msec for the paradigm with a well defined mode between 160 and 185 msec. Minimum reaction times increased proportionally to the delay between the visual cue and the imposed perturbation. The input/output relations for the reflex responses(i.e. EMG output for a given velocity of the imposed displacement) showed alterations of gain(maximum 300%) begining between 40 and 60 msec after the visual cue. The alterations were dependent on whether the perturbed muscle was cued to function as an agonist or an antagonist. These findings will be considered in the context of the CNS mechanisms underlying the reaction time preparation for a cued motor program. (Supported by MRC grant #5218)

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OROFACIAL MOTOR REPRESENTATION IN PRIMATE SENSORIMOTOR CORTEX. Michael A. Sirisko*, Darlene Postello* and Barry J. Sessle. Faculty of Dentistry, Univ. of Toronto, Canada M5G 1G6

Low-intensity (<30 µA) microstimulation techniques were used in a systematic three-dimensional mapping of the cortex in five unanesthetized monkeys. Depth stimulation within the cortex was performed in 250 µ vertical steps in penetrations that were 1 mm apart. Evoked muscle twitch movements were monitored by visual observations and EMGs of individual muscles. Supplementary data was obtained from cinetographic and electromagnetic monitoring of jaw movement. Contralateral facial movements were evoked from a laterally facing horseshoe-shaped region of the pre-central cortex which surrounded a core of jaw and tongue movements; ipsilateral movements were represented further laterally. Within this general representation, a particular movement could be represented from a number of loci separated by several mm. In some instances, functionally related muscle groups of different cranial motor nerves could be activated from the same locus, e.g. lateral deviation of the body of the tongue in conjunction with buccinator contraction. At other loci, complex movements such as mastication could be produced by continuous stimuli of low intensity (20 µA, 50 Hz). Sensory input to the cortex had an influence on the evoked movements since jaw-opening movements, for example, were maximal and at lowest threshold when the mandible was open only 2-3 mm; conversely jaw-closing movements were most effectively evoked when the jaw was opened widely. (Supported by the Canadian Medical Research Council).

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ELECTROPHYSIOLOGIC AND ANATOMIC CORRELATES OF CORTICOSPINAL SYSTEM MATURATION. I.C. Bruce* and W.G. Tatton. Playfair Neuroscience Unit, University of Toronto, Ontario, M5T 2S8.

Systematic intracortical and subcortical white-matter microstimulation was carried out in a three-dimensional grid in the motor cortex of five kittens aged 13 to 51 days postnatally. Current intensities up to 100 μA at 300 Hz failed to evoke electromyographic (EMG) activity in proximal and distal forelimb and hindlimb muscles, nor were stimulus-locked twitches observed in facial muscles in animals younger than 41 days. In contrast, at 41 days and older, EMG activity was reliably evoked at currents as low as 8 μA in proximal and distal forelimb and proximal hindlimb muscles. Stimulus-locked facial twitches were also first observed at this age. This extends the previously described late appearance of effective output from motor cortex to triceps brachii motoneurons, and demonstrates that this late maturation is synchronous throughout the motor cortical homunculus. As one test of whether this failure in the cortical linkages to motoneurons is due to the absence of corticospinal terminals, hemicord injections of horseradish peroxidase were made at C3-C4 in seven kittens aged 20 to 156 days. Retrograde filling was found in layer V motor cortical neurons in all animals. These findings suggest that the functional output immaturity lies distal to the motor cortex, i.e. as axonal conduction failure, at the corticospinal-propriospinal synapse, or at the propriospinal motoneuron synapse. (Supported by MRC Grant #5218 to WGT).

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POSTNATAL DEVELOPMENT OF THE BICEPS BRACHII MOTONEURON POOL IN THE RAT. D.S. Rootman* and W.G. Tatton. (SPON: J.Hunter) Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, Canada M5T 2S8.

Previous work indicated a five fold decrease in motoneurons innervating rat biceps brachii (BB) from birth to adulthood together with transient bilateral innervation of BB in neonates. Postnatal changes in motoneuron number and distribution within the ventral horn were studied in the rat using retrograde horseradish peroxidase (HRP) transport following infusion into BB. The spatial organization of BB motoneurons was compared in animals 9-14 days old (N=22),in adult animals (N=11) using computer 3-D reconstruction. This allowed observations of the BB motoneurons from a variety of perspectives in relationship to a number of spinal cord landmarks. A maximum 50-75% reduction in motoneuron number was observed from birth to adulthood (adults 98-150, neonates 172-243). The spatial arrangement of the BB motoneurons with regard to the root entry zones and the dorsolateral convexity of the ventral horn remains constant from birth to adulthood. No evidence was found to support the existence of transient connections from motoneurons in both ventral horns in neonate rats. It is suggested that the comparatively small decrease in motoneurons postnatally is a continuation of the histogenetic processes of cell death begun in utero and is related to the postnatal development of the central and peripheral connections of motoneurons. (Supported by MRC grant #5218)

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THE DEVELOPMENT OF MUSCLE SPINDLES IN THE KITTEN. R. Butler and I.M. Payk*, Department of Anatomy, McMaster University, Hamilton, Ontario, L8N 3Z5.

Intrafusal muscle fibres in the tenuissimus muscle of the cat develop as two anatomically and histochemically defined groups. One group is a single nuclear bag fibre whose ATPase profile is characteristic of slow twitch muscle while the other group consists of a second nuclear bag in close association with all the nuclear chain fibres (usually four) and these all exhibit ATPase activity characteristic of fast twitch muscle (Butler (1980) Develop. Biol. 77, 191). Complete dehiscence of the groups of maturing intrafusal muscle fibres takes 4-5 weeks. We are studying the development of physiological function in the muscle spindles of young kittens (0-45 days) and correlating this with the morphological organization. Spindle afferent responses are recorded in isolated dorsal root filaments in response to linear stretch of the muscle during selective fusimotor stimulation. We find that up to 10 days of age, usually phasic components of the response are seen. At ages around 22 days, some spindles display tonic activity, others remain phasic and some display both tonic and phasic discharges. By 35 days, responses comparable to adults are seen with well developed tonic and phasic components. These results confirm the earlier findings of Skoglund (Acta physiol. scand. (1960) 50, 203).
(Supported by the Hospital for Sick Children Foundation, Toronto, and partly by the M.R.C.)

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DISTRIBUTION OF MOTONEURONES SUPPLYING DIFFERENT HEADS OF THE CAT TRAPEZIUS MUSCLE. J. Keane* and F.J.R. Richmond. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The trapezius muscle is composed of 3 separate heads which subserve different movements, but all 3 heads are supplied by branches of a common nerve trunk from the spinal accessory nerve. The relative distribution of motoneurones supplying different muscle heads has been examined using the method of retrograde transport of horseradish peroxidase (HRP) from single cut nerve ends. Most labelled cells were located in the spinal accessory nucleus between C1 and C7. Cells supplying the rostral head, clavotrapezius, occupied only the rostral part of the motor nucleus from C1 to C4, while the more caudal heads acromiotrapezius and spinotrapezius were located in progressively more caudal regions, from C3 to C5 and C4 to C7 respectively. At the level of C2 to C5, the labelled motor pool was located on the lateral border of the ventral horn, but in the C1 and C5 to C7 segments, the motor pool shifted to a more medial position in central lamina VIII. Present results suggest that the anatomical and functional subdivision of the trapezius muscle is matched by anatomical differences in the organization of motoneurone pools.
(Supported by Medical Research Council of Canada.)

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THE EFFECT OF LOCALIZED COOLING OF MUSCLE AND NERVE ON AUTOGENETIC REFLEXES IN ANEMIC DECEREBRATE CATS. F.M. Liebman and D. Lapadula*. NYU College of Dentistry, NY, NY 10010.

In the anemic decerebrate cat muscle spindles are considered to act passively, but the activity level of autogenetic stretch, tendon organ and crossed extension reflexes are subject to conjecture. In our studies, in conjunction with programmed controlled length and velocity of stretch of soleus muscle, with and without background crossed extension activity, the tendon area, muscle belly and sciatic nerve are respectively cooled. Muscle and nerve temperatures are monitored. EMG's, the compound sciatic nerve action potential and muscle force and displacement are also recorded. The compound action potential is subjected to power spectrum analysis in order to evaluate changes in frequency conduction characteristics of the sciatic. Analysis of data to date indicates that in anemic decerebrate cats autogenetic stretch, tendon organ and crossed extension reflexes are highly active. Tendon cooling results in an enhanced stretch reflex and muscle cooling in a diminished cross extension reflex with the magnitude of the superimposed stretch reflex being related to muscle stiffness. Although the basis for the rigidity in anemic and midcollicular preparations are different, stretch, crossed extension and Golgi tendon reflexes appear to operate similarly.
(Supported by NIH Grant RR05332)

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VARIETIES OF SECONDARY SACCADE. P.E. Hallett, B.D. Adams* and R.P. Kalesnykas*. Univ. of Toronto, Ontario. M5S 1A8.

A stimulus steps horizontally and the subject either tracks it (normal task) or attempts an equal and opposite eye movement ('anti' or A-task). Subjects typically respond with a larger 'primary' and a smaller 'secondary' saccadic eye movement. (1) In successful attempts at the tasks we now recognise two varieties of secondary saccade. The frequencies of the 'fast' F and 'late' L varieties can be altered by selecting the subjects, changing the tasks, blanking the stimulus, by the 'suppress secondaries' instruction, etc. F typically occur less than one reaction time from the start of the primary saccade, and correct the larger errors without using optical feedback as to the effect of the primary. L are typically small responses to small errors which are largely deleted, and partly replaced by very late and often erroneous responses, if optical feedback is denied. The latencies for the F secondaries are described by successive planning (the secondary being triggered internally by the start of the primary) or by the secondary being prepared in parallel to the primary in 7 of 8 subjects. L secondaries are triggered by optical feedback and are heterogeneous. (2) In the rare mistakes at the A-task we now recognize 3 varieties ('early', 'middle' and 'late') which are variously attributed to the above mechanisms in different subjects.
(Supported by M.R.C.)

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THE PHYSIOLOGIC EFFECTS OF HYPERVOLEMIC POLYCYTHEMIA IN NEWBORN DOGS. Michael H. LeBlanc*, Uma R. Kotagal* and Leonard I. Kleinman U. of Cincinnati School of Medicine, Dept. of Pediatrics 45267

The effect of hypervolemic polycythemia (Hct 65-75) on oxygen transport and uptake was studied in 12 unanesthetized newborn dogs 3-10 d. old. A control group of 7 newborn dogs (3-10 d. old) were made hypervolemic but not polycythemic in an otherwise identical experiment. Hypervolemia was attained by infusing 33 ml/kg of either packed red blood cells (polycythemia) or whole blood (controls). In the polycythemic group vascular resistance (VR) increased by 180%($p<.01$) cardiac output (CO) decreased by 50% ($p<.001$), oxygen transport (OT) decreased by 20 % ($p<.05$) oxygen consumption (VO_2) decreased by 14% ($p<.02$), and Base excess (BE) decreased by 5 meq/L ($p<.05$). There were no significant changes in venous saturation (VS), or blood lactate (BL). In the 7 control animals there were no significant changes in CO, VR, VO_2 or OT, VS, BE and BL. The ability of the animals to increase their oxygen transport and uptake was tested by measuring the changes induced by lowering the environmental temperature to 12°C for 20 min. Cold stress produced a 30% increase in VO_2 ($p<.01$) in both the polycythemic and the control animals. Cold stress produced a 31% increase in CO and 33% increase in OT in the polycythemic animals. Thus though oxygen consumption was lowered by polycythemia, these animals were still able to increase oxygen uptake in response to cold stress.

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OXYGEN TENSIONS IN THE EMBRYONIC SPINAL CORD. Bradford T. Stokes. Dept. of Physiology, Ohio State University, Columbus, OH 43210.

The partial pressure of oxygen (PO_2) has been studied in the lumbosacral spinal cord of white leghorn chick embryos. Using a newly modified microelectrode system, we have been able to monitor spontaneous fluctuations in tissue PO_2 during the last week of embryonic development at known, marked spinal loci. Oxygen tensions were generally lower than those found in the adult spinal cord. The decline of PO_2 values as one passes from white to grey matter in the adult cord did not occur in fetal spinal tissue. This uniformity in oxygen fields was age dependant; it varied from a high of 22.1 $\pm$ .67 mmHg at day 15 to low values of 6.0 $\pm$ 1.0 mmHg at day 19. The initiation of true respiration at day 20 led to an immediate increase in PO_2 tensions (10.6 $\pm$ 1.0 mmHg). These spontaneous changes in oxygen tension of the embryonic spinal cord are related to periods of changing sensitivity to embryonic hypoxia and modes of tissue respiration. (Supported in part by USPHS NIH Grant NS-10165 and NSF Grant BNS-7905756).

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BLOOD SUBSTITUTION: I. EFFECTS ON EXTRACELLULAR POTASSIUM AND OXYGEN TENSION IN RAT CEREBRAL CORTEX IN SITU. T.J. Sick* M. Rosenthal, J.C. LaManna, Dept. of Neurology, Univ. of Miami, Miami, FL. 33101.

The efficacy of a perfluorinated hydrocarbon (Fluosol) blood substitute was tested by transfusing pentobarbital anesthetized rats until hematocrit was less than 1%. Cortical microelectrodes were implanted for measuring tissue oxygen tension (PtO_2) and extracellular potassium ion activity (K^+o). Just prior to Fluosol infusion, the animals were respired with 100% O_2 since the oxygen carrying capacity of Fluosol is considerably less than whole blood equilibrated with room air. During blood replacement with Fluosol, PtO_2 increased while K^+o remained at preinfusion levels (2-4 mM). To determine whether potassium transport capability is altered by Fluosol administration, the rates of K^+ reaccumulation following direct cortical stimulation were compared. The rate of K^+ reaccumulation after direct cortical stimulation was unchanged after Fluosol transfusion. Respiration of the rats with 30% O_2-70% N_2 following Fluosol substitution resulted in a prompt fall in PtO_2 and rapid rise in K^+o to greater than 50 mM which was accompanied by cessation of all ECoG activity. These results indicate that Fluosol, equilibrated with 100% oxygen, provides the brain with adequate oxygen for the maintenance of ionic balance both in the resting state and during periods of enhanced neuronal activity. (Fluosol courtesy of Alpha Therapeutics. Supported by NIH grants NS14319, NS14325, NS00399 and NS 06300).

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BLOOD SUBSTITUTION: II. EFFECTS ON CYTOCHROME OXIDASE SPECTROPHOTOMETRY IN VIVO. J.C. LaManna, S.M. Pikarsky, T.J. Sick and M. Rosenthal, Dept. of Neurology, Univ. of Miami, Miami, FL. 33101.

Comparisons of optical signals representing reduction/oxidation changes of cytochrome c oxidase (a,a_3) were made in rat cortex in the presence of hemaglobin and when perfluorinated hydrocarbon (Fluosol) had been substituted for whole blood. This was done to directly assess whether differential measurement based upon "reference" wavelengths adequately compensates for light absorption by hemaglobin. FiO_2 was increased to 100% in order to maintain brain oxygen tension and extracellular potassium concentration at normal levels. Difference spectra between "resting" and hypoxic states were recorded by computer assisted, rapid-scanning of the light reflected between 400-700 nm. A characteristic peak at 605 nm, the absorption maximum of cytochrome oxidase, was exhibited in the presence and absence of hemaglobin. By dual wavelength monitoring, transient reduction of cyt a,a_3 of comparable amplitude and kinetics was recorded when the animals were switched to 100% N_2 respiration. These data confirm directly the adequacy of reference wavelength compensation for monitoring cytochrome a,a_3 in the presence of Hb, and strengthen earlier conclusions that this cytochrome, on a tissue level is partially reduced in contrast to its fully oxidized state under "resting" conditions in isolated mitochondria. (Fluosol courtesy of Alpha Therapeutics Co. Supported by NIH grants NS14319, NS14325, NS00399 and NS06300).

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BRAIN TISSUE OXYGEN CONTENT FOLLOWING ACETAZOLAMIDE P.Grieb*, R.E.Forster and P.C.Pape*; Department of Physiology University of Pennsylvania, PA 19104.

Oxygen content of brain tissue has been estimated with the double indicator dilution technique (Forster et al., Adv. Exp.Biol.Med.75:41,1975) in nembutal-anesthetized mechanically ventilated dogs, before and after iv. administration of acetazolamide, $30mg \cdot kg^{-1}$. After rapid injection of autologous blood containing indocyanine green and oxygen-18 into the carotid artery, sagittal sinus blood was sampled continuously and analysed with on-line densitometry and off-line mass spectrometry, respectively. Dilution space between injection site and sagittal sinus was assumed to represent properties of a well mixed system, for both tracers. Time-frequency distribution of a tracer passing through the sampling system was determined experimentally, and dilution curves corrected for mixing in catheters. Five control measurements and seven measurements after acetazolamide were completed. Results are shown as means$\pm$SE. Following the drug, cerebral blood flow (Kety-Schmidt N_2O technique) rose from 18.4$\pm$2.4 to 37.1$\pm$4.6 $ml \cdot (100g \cdot min)^{-1}$, and brain venous O_2 content rose from 11.3$\pm$1.6 to 14.9$\pm$1.8 $ml \cdot (100ml)^{-1}$. Tissue O_2 content rose from 0.109$\pm$0.024 to 0.215$\pm$0.038 $ml \cdot (100g)^{-1}$, $p<0.05$, t-test for independent means. Taking the solubility coefficient for O_2 as $2.2 \cdot 10^{-2}$ $ml \cdot (ml \cdot atm)^{-1}$, mean tissue PO_2, 36.0 mmHg in control and 71.6 mmHg after the drug, is not significantly different from the respective venous PO_2 values.

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DEPRESSION OF CORTICAL Na-K ATPase ACTIVITY IN RATS EXPOSED TO OXYGEN AT 4 ATM. G.B. Kovachich*, O.P. Mishra* and J.M. Clark. Inst. for Environmental Med., Univ. of Penna., Philadelphia, PA 19104

Cortical brain slices were obtained from rats exposed to oxygen at 4 atm, with average convulsion latency of 2.5 hours. The samples were homogenized in TRIS-HCl pH 7.4. 0.1 ml 2.5% w/v homogenate was incubated in reaction mixture containing 100 mM-NaCl, 20 mM-KCl 12 mM $MgCl_2$, 12-mL TRIS-ATP. Ouabain-sensitive ATPase activity was determined estimating Pi formation from ATP, using the method of Fiske and Subbarow. Exposures of 30-180 min to 4 atm oxygen all produced approximately 35% depression of activity ($p<0.05$), 10 min exposure had no effect. Rats sacrificed 24 hours after decompression showed no enzyme depression. Exposure to normoxic helium-oxygen mixture at 4 atm had no effect. The time course of Na-K ATPase inactivation in vivo was similar to that found in cortical slices from unexposed rats incubated at 1 atm oxygen. Oxygen at 4 atm with 2% CO_2 caused marked reduction in convulsion latency and 50% depression of Na-K ATPase activity in 10 min. Results indicated that depression of Na-K ATPase may be involved in development of hyperoxic seizures, in support of the cellular mechanism of CNS O_2 toxicity proposed by Kovachich et al. (Biochim. Biophys. ACTA, 1977 462: 493). Supported by NIH Grant HL 22259 and ONR Contract N00014-76-C-0248.

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ERRORS CAUSED BY DIFFUSION LAG IN CONTINUOUS HEMOGLOBIN-OXYGEN BINDING CURVES. G. N. Lapennas, J. M. Colacino*, and J. Bonaventura*. Duke University Marine Lab, Beaufort, N. C. 28516

In several modern methods (e.g. Aminco Hemoscan) for deriving hemoglobin-oxygen binding curves (OBC), the sample is spread as a thin layer, with or without being covered by a gas permeable membrane, and exposed to linearly increasing oxygen partial pressure (PO2) in a sealed chamber. An O2 electrode monitors PO2 in the chamber while a spectrophotometric system monitors the average % saturation of the sample. The resulting OBC is plotted continuously on an X-Y recorder. Because of diffusion lag, the O2 electrode will indicate PO2 less than the true chamber PO2. This error,Ee, will by itself cause the plotted OBC to be shifted to the left from its true position. Diffusion lag in the sample (and its bounding membrane, if present) cause the average sample PO2 to be less than the chamber PO2. This error, Es, will by itself cause the plotted OBC to be shifted to the right. A mathematical model of the sample and electrode was constructed to predict Ee and Es from sample and electrode properties. Ee depends primarily on electrode membrane properties and the rate of rise (R) of PO2 in the chamber. Es depends on the concentration, thickness, and O2 affinity of the sample and on sample membrane properties and R. The errors predicted by the model were in excellent agreement with observed errors in a modified Aminco Hemoscan. The model can thus be used to select those membrane and sample parameters which minimize Ee and Es. (Supported by NIH Grant HL-07057 and NSF Grant PCM-7906462)

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THE OXYGENATION OF THE CAT RETINA BY DISSOLVED CHOROIDAL O_2 BELOW 1 ATM PRESSURE. M. B. Landers, III,* M. L. Wolbarsht and E. Stefánsson.* (Spon: J. J. Blum) Duke Univ. Med. Ctr., Durham, NC 27710

The inner part of the cat retina is oxygenated by the retinal circulation, the outer part by the choroidal. With the retinal vessels blocked by pressure at the optic disc, measurements with polarographic oxygen electrodes show that the choroidal circulation equilibrated against 60% O_2/40% N_2 at 1 atm will give normal pO_2 in the inner retina[1]. The blood flow through the choroidal circulation is so great (unlike most other tissues in the body) that O_2 dissolved in blood is significant in amount at arterial pO_2 of less than 1 atm. The solubility of O_2 in whole blood[2] is 0.023 ml O_2 ml^{-1} atm^{-1} and the blood flow through cat's choroid is 0.73 ml/min.[3] The choroidal circulation can carry 16.9 µl O_2 min^{-1} atm^{-1} dissolved in blood. We (and others)[4] have found the retinal oxygen consumption to be of the order of 3 - 4 µl O_2/min. The choroidal circulation can supply this much O_2 from the dissolved state alone with an A-V O_2 fall from 300 mm Hg to 140 mm Hg which keeps the hemoglobin fully saturated with oxygen. Thus, the retina can be fully oxygenated at atmospheric pressures without hemoglobin playing a part, all the O_2 requirements being met by choroidal blood dissolved O_2. Ref: (1) Landers, M. B., III, Trans. Amer. Ophthal. Soc. 76:528, 1978. (2) Sendroy, J., et al., J. Biol. Chem. 105:597, 1934. (3) Alm, A., et al., Acta. Physiol. Scand. 84:306, 1972. (4) Glocklin, V. C., et al., Invest. Ophthal. 4:226, 1965.

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LINEAR ANALYSIS OF FACTORS INFLUENCING $P\bar{v}O_2$. Arthur L. Rosen, Steven A. Gould*, Lakshman R. Sehgal*, Hansa L. Sehgal*, and Gerald S. Moss*. Department of Surgery, Michael Reese Hospital and Medical Center and The University of Chicago, Pritzker School of Medicine, Chicago, Illinois 60616

The quantitative significance of simultaneous changes in O_2 delivery and consumption on $P\bar{v}O_2$ has been difficult to assess. We report on a method of evaluating the effect of changes in O_2 availability (C.O., [Hb], s_a), $\dot{V}O_2$, and Hb-O_2 affinity (P_{50}, n) on $P\bar{v}O_2$. The Fick equation, the O_2 content equation, and the Hill equation can be combined to yield a non-linear expression for $P\bar{v}O_2$ as a function of the 6 independent variables: P_{50},n, C.O., [Hb], $\dot{V}O_2$, and s_a. This equation can be approximated by a linear function in these 6 variables. The technique was applied to a set of 77 observations obtained from 15 baboons undergoing total exchange transfusion with a stroma-free Hb solution ([Hb] = 7 gm/dl; P_{50} = 12 to 24 torr). The coefficients of the linearized equation were determined by least squares. The resultant estimating equation for the change in $P\bar{v}O_2$ was: $\Delta P\bar{v}O_2 = 6.4\Delta C.O. + 4.0\Delta[Hb] + 0.33\Delta P_{50} - 0.67\Delta n - 0.20\Delta\dot{V}O_2 - 89\Delta s_a$. The RMS error was 4.0 torr. The exchange transfusion caused the $P\bar{v}O_2$ to fall 29 torr and a fall in the C.O., [Hb], P_{50}, n, $\dot{V}O_2$, and s_a of 0.7L/m, 6.4 gm/dl, 11.9 torr, 0.75, 25.9 cc/min, and 0 respectively. The calculated contributions to the change in $P\bar{v}O_2$ were -4.5, -25.6, -3.9, +0.5, +5.2, and 0 torr. We conclude that the linearized $P\bar{v}O_2$ equation can calculate the effect of changes in O_2 delivery and consumption on $P\bar{v}O_2$

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EFFECT OF HYPOXIA ON REGIONAL OXYGEN SUPPLY AND CONSUMPTION IN CATS. Ellen Buchweitz* and Harvey R. Weiss. Dept. Physiol. & Biophys., CMDNJ-Rutgers Med. Sch., Piscataway, NJ 08854

This study quantitatively determined O_2 consumption and supply regionally in brain during mild hypoxia (8% O_2). Eighteen adult mongrel cats were anesthetized with α-chloralose. Artificial respiration was begun; left atrial and femoral artery catheters were inserted. Radioactive microspheres (15 ± 3µ in diameter) were injected and cerebral blood flow determined. Nine cats breathed 8% O_2 for 10 min. The cats' heads were quickly guillotined in two places and frozen in liquid N_2. Nine regions were examined. Regional arterial and venous O_2 saturation were measured microspectrophotometrically. O_2 consumption was determined as the product of flow and O_2 extraction. Hypoxia significantly increased blood pressure and heart rate. Pa_{O_2} was significantly decreased while P_aCO_2 was not altered by hypoxia. Cerebral blood flow rose insignificantly after hypoxia. Arterial and venous O_2 saturation decreased uniformly and to the same degree, maintaining the arterial-venous O_2 saturation difference. O_2 extraction and consumption were unaffected. The brain oxygen supply/consumption ratio was well maintained during mild hypoxia indicating adequate protection in this condition. (Supported in part by N.I.H. grant HL 21172).

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BLOOD O_2 DISSOCIATION CURVE AND O_2 TRANSPORT AT HYPOXIC HYPOXIA. Z. Turek*, F. Kreuzer*, P. Scotto* and K. Rakusan. Dept. of Physiology, Univ. of Ottawa, Ottawa, Ont., Canada.

The conductance of O_2 transport in blood is a product of blood flow (cardiac output) and the mean slope of the physiological blood O_2 dissociation curve (ODC). The latter is identical to the capacitance coefficient β and in the whole body equals $(CaO_2-C\bar{v}O_2)/(PaO_2-P\bar{v}O_2)$. The slope of the ODC β is also an index of the efficiency of the O_2 transport in the blood as it gives the amount of O_2 transported per unit of a pressure difference in a volume unit of blood. A theoretical analysis of β as a function of the position of the ODC (P50) at several values of arterial PO_2, blood O_2 capacity and arterio-venous O_2 difference $((a-v)O_2)$ was performed. At hypoxia β increases reaching its maximum at a rather low P50. Similarly, an increase in O_2 capacity and/or $(a-v)O_2$ increases β especially at deep hypoxia. Subsequently, β as a function of arterial PO_2 was calculated for 3 different P50 (corresponding to that of llama, man and cow). An ODC shift to the right (cow) results in a moderate increase of β at mild hypoxia. Conversely, a left shift of the ODC (llama) results in a sizeable increase of β at deep hypoxia. Thus, at severe hypoxia, with the same O_2 capacity, $(a-v)O_2$ and cardiac output, an animal with a left shifted ODC (as observed in the typical high altitude animals) can transport more O_2 in the blood at the same pressure difference or the same amount of O_2 at a lower pressure difference than animals with higher P50.
(Supported in part by the Canadian Heart Foundation)

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THE INFLUENCE OF CARBON MONOXIDE ON SICKLE CELL FORMATION. Mildred J. Wiester. US EPA, Research Triangle Park, N.C. 27711

Sickling of red cells in homozygous sickle cell (SS) disease, depends on the concentration of reduced sickle hemoglobin (HbR) whereas the maintenance of the normal discoidal shape is a function of sickle hemoglobin as oxyhemoglobin (HbO_2) methemoglobin or carboxyhemoglobin (HbCO). The present study examines the protection afforded the sickling red blood cell by conversion of 18 percent of the sickle cell hemoglobin to HbCO. In vitro O_2 dissociation curves were determined on whole blood from 3 individuals with SS disease, with and without HbCO present. The degree of sickling was ascertained at points along the curves and correlated with pO_2, HbR and HbO_2. Curves were repeated on each person 4 times over a period of 9 months. Since sickling occurs in the venous circulation, the blood in the tonometer was equilibrated with gases to maintain pH between 7.1-7.2 and pCO_2 between 46-48 torr, while pO_2 was varied between 110 and 20 torr. HbCO did not alter the percent of irreversible sickle cells (ISC). Sickling was decreased in reversible sickle cells by HbCO. Sickling was reduced 4-11% at pO_2=60, 18-25% at pO_2=50, 30-45% at pO_2=40 and 36-48% at pO_2=20. The proportion of sickled cells in the venous circulation, the percent ISC, the hemologobin concentration and $p_{50}O_2$ values were characteristic for each individual throughout the 9 month study.

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THE NEURAL NATURE OF NON-ADRENERGIC INHIBITORY RESPONSES AT THE GUINEA-PIG FUNDUS? Michael A. Cook and M. Lorraine Paul* Dept. of Pharmacology, University of Western Ontario, London, Ontario, N6A 5C1, Canada.

Fundic strip preparations from guinea-pig stomach exhibit an inhibitory response to electrical field stimulation which is neither adrenergic nor cholinergic. Insensitivity of this response to atropine, to adrenergic neurone-blocking agents and its sensitivity to tetrodotoxin (TTX) have led to the presumption of a 'purinergic' neural origin. We have tested the effects of various other interventions capable of altering autonomic nerve function, among them, the effect of local anaesthetics and of cold storage. The local anaesthetics procaine and procainamide ($2x10^{-4}$M) were effective in abolishing the excitatory responses obtainable from the preparations but were without effect on the inhibitory response. Cold, anoxic storage for 12h to 24h similarly decremented or abolished the excitatory responses without affecting the inhibitory response or the responses to exogenous acetylcholine, noradrenaline or ATP. Electron microscopic examination of these stored preparations suggested that damage to enteric neurons had occurred. Prolonged storage for 48h resulted in abolition of all responses to field stimulation as well as severe decrement in the responses to exogenous agonists. Microscopy on these preparations demonstrated muscle damage. These results do not appear compatible with the suggested purinergic neural origin of this response. (Supported by Medical Research Council of Canada)

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β-ADRENOCEPTORS IN THE MYOMETRIUM OF THE PREGNANT EWE. D.J. Crankshaw (SPON: J.E.T. Fox). McMaster University, Hamilton, Ontario. L8N 3Z5.

Several lines of evidence suggest that adrenoceptor regulation is important in determining changes in myometrial responsiveness to sympathomimetic agents. As a first step in the investigation of adrenoceptor regulation during gestation β-adrenoceptors have been characterized in the myometrium of the pregnant (120-130 days) ewe by biochemical and physiological methods. Binding of [^{3}H]dihydroalprenolol (DHA) to microsomal membranes from myometria of these animals was rapid, reversible and saturable (K_B=3.4nM, capacity=850 fmol mg-1). Competition studies with agonists and antagonists showed the site to be stereoselective and of the β_2 subtype. K_Bs for agonists and antagonists in binding assays were compared to EC_{50}s and K_Bs calculated from pA_2 values determined on the spontaneous activity of isolated myometrial strips incubated in the presence of α blockade. K_Ds for binding and for antagonism of the β response were in close agreement for all antagonists tested. K_Bs and EC_{50}s for agonists showed the same rank order but were numerically different. K_B/EC_{50} for full agonists (eg isproterenol ≈ 1000, suggesting a large receptor reserve. Specific binding of DHA to microsomal membranes from the myometrium of the pregnant ewe represents binding to the β-adrenoceptor and can be used to quantitate changes in the number and affinity of receptors present in different physiological states. Supported by MRC of Canada.

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CONTRACTION AND FATIGUE IN ISOLATED SKELETAL MUSCLES FROM CREATINE DEPLETED RATS. G.W. Mainwood, M.W. Alward* & B. Eiselt*. Dept. of Physiology, Univ. of Ottawa, Ottawa, Ontario, Canada K1N 9A9.

Rats were depleted of creatine by feeding a diet containing 1% β-Guanidinopropionate (GP), Fitch et al. (1974). The question of whether or not these muscles have a decreased effective energy reserve for muscle contraction was examined by the use of inhibitors. Following treatment with NaCN (2mM) and monioadacetate (2mM), diaphragm strips from normal rats gave a series of 16±2.5 contractions in response to brief tetanic stimulation. The response of strips from GP treated rats declined rapidly after treatment with the inhibitors giving only 4±0.6 contractions after which a sustained contracture developed. The functional energy reserve under these conditions thus appears to decline in parallel with the fall in total creatine (-80%). In the absence of inhibitors, muscle contraction and the time course of fatigue, and recovery during repetitive stimulation are not significantly affected.

Fitch, C.D., Jellinek, M. & Mueller, E.J. (1974) J. Biol. Chem. 249, 1060-1063.

(This work was supported by the Canadian Medical Research Council.)

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The Contractile Response of Papillary Muscles from Creatine Depleted Rats. G.V. Forester, G.W. Mainwood & W.J. Keon*. Depts. of Surgery and Physiology, Univ. of Ottawa, Ottawa, Canada.

The decline of contraction tension in the hypoxic myocardium is paralleled by a decrease in creatine phosphate levels. This has led to the hypothesis that creatine (C) acts as a phosphate 'shuttle' between the ATP pools of mitochondria and sarcomeres. The argument is not compelling since a number of other parameters change during hypoxia. The hypothesis may be tested by eliminating C from the myocardium. We fed rats on a diet containing 1% β-guanidino propionate for 4 weeks which reduced myocardial C by 75-80%. At this time isometric mechanical characteristics of isolated papillary muscles at 30-37°C were measured as well as the dynamic response to stimulation trains of 100 pulses at 0.1-5Hz. The C depleted muscles did not behave significantly differently from normal muscles. Energy transfer in this preparation does not appear to be impeded by low C levels and therefore these experiments do not provide clear evidence for the shuttle hypothesis.

(Supported by the Medical Research Council of Canada and the Ontario Heart Foundation).

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INDIRECT EVIDENCE FOR REACTIONS CONTROLLING CONTRACTILE STATE. R. L. Coulson, G. Natarajan*, and D. H. Hanck. Dept. of Medicine, University of Kentucky, Lexington and School of Medicine Southern Illinois University, Carbondale, IL 62901.

The rate (V) of a reaction may be expressed as a function of absolute temperature (T) as follows: $V=(R\cdot T/N\cdot h)(e^{\Delta S/R})(e^{-E/RT})$ where R, N, and h are the constants of gas, Avagadro and Planck respectively. In cat right ventricular papillary muscles the rate of the actin-myosin interaction was estimated by the rate of force development at two contractile states (control and paired stimulation) and at two muscle lengths (lmax and the fraction of lmax at which potentiation produced force equal to control at lmax) designated (CL), (CS), (PL), and (PS) respectively. Five muscles were examined in a myograph at five temperatures (range 22.5°C-32.5°C). Using a nonlinear equation solution the parameters of entropy (ΔS) and activation energy (E) were calculated. ΔS±SEM was -22.76±8.19, -81.65±11.66, -20.87±43.41, and 41.75±10.75 and E±SEMx10^3 was -59.94±2.46, 44.10±3.50, -59.04±13.05, -78.74±3.23 for CL, CS, PL, PS. At short muscle length potentiation altered both ΔS and E. While potentiated, length change altered both ΔS and E. However at long lengths potentiation had no effect upon either ΔS or E. E represents the activated complex fraction of the system. Length change alters this fraction independent of contractile state as does potentiation of contractile state at short lengths. Extra reactions appear involved in augmenting contraction both by length change and contractile potentiation. The additional reactions differ in each case.

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COMPARATIVE STUDIES BETWEEN SUPERPRECIPITATION AND SUPERCONTRACTION. George Kaldor and William J. DiBattista*. VA Medical Center, Allen Park, MI 48101

We have compared certain kinetic parameters of the superprecipitating actomyosin suspensions with those of the supercontracting glycerated fibers. We found that the Km of ATPase activity with both models was close to $1.0x10^{-5}$ moles/l. Similarly the Km of superprecipitation and the Km of the supercontraction were all in the 10^{-5} moles/liter ATP range. If ITP rather than ATP was used as the substrate the velocity of both the superprecipitation and supercontraction were greatly diminished. Both the superprecipitated actomyosin particles and the supercontracted glycerinated fibers show considerable condensation, shrinking and shortening. All these results seem to support the hypothesis that the superprecipitation of the actomyosin suspensions is similar to the supercontraction of the glycerinated fibers. Although supercontraction does not occur under physiological conditions it is an inherent property of the muscle fibers. (Supported in part by Michigan Heart Association).

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CHANGES IN SHORTENING VELOCITY OF FAST-TWITCH RAT MUSCLES IMMOBILIZED IN EXTENDED, NEUTRAL AND FLEXED POSITIONS. S.A.Spector, C.P.Simard, V.P.Stokes, J. Vallieres and V.R.Edgerton. Neuromuscular Research Lab., UCLA, Ca. 90024.

Architectural characteristics, e.g. fiber length (FL), and biochemical properties dictate the maximal rate at which muscle shortens (Vmax). Immobilization of a muscle under varying degrees of stretch, known to alter a muscle's architectural profile, might lead to changes in functional speed, as well. Thus, architectural and in situ contractile analyses of fast-twitch medial gastrocnemius (MG) and tibialis anterior (TA) muscles of female rats were performed after a four week immobilization period under four conditions: extension (E), neutral (N), flexion (F) and sham control (C), each group containing seven animals.

With the MG, there was a 20% increase and 17% decrease in muscle length (ML) of E and F, respectively, compared to C. No significant differences were seen in ML between E, N, F and C in the TA. In contrast, FL:ML ratios for E, N and F were similar to C for both muscles (MG=0.51±0.02; TA=0.59±0.06). Vmax of MG of E (203±8 mm/s) was significantly greater than that of C (139±34), N (162±19) or F (159±31). The only significantly altered Vmax of TA was for N (177±33), reduced 27% of C (243±52).

These findings indicate that FL increases account, in part, for changes in immobilized MG's functional speed, independent of biochemical changes. In the TA, architecture was not significantly altered. Therefore, the decrease in muscle shortening speed presumably results from muscle biochemical changes. NIH # 10423, DGES-FCAC.

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EFFECT OF PHOSPHATE AND BICARBONATE ON ^{45}CA FLUXES AND TENSION DEVELOPMENT IN RAT CAUDAL ARTERY. M.A. Stout* and F.P.J. Diecke, CMDNJ-New Jersey Medical School, Newark, NJ 07103

Unidirectional ^{45}Ca fluxes and tension development in helical strips of caudal arteries of Wistar-Kyoto rats were investigated in phosphate (PO_4) or bicarbonate (HCO_3) free solutions. The rate of ^{45}Ca efflux from intracellular Ca compartments of strips desaturated in PO_4-HCO_3-free solutions was significantly faster than Ca efflux in Krebs solution. The effect of PO_4 and HCO_3 removal were additive and highly temperature sensitive. At 4°C, simultaneous removal of PO_4 and HCO_3 increased ^{45}Ca efflux by approximately 100%. At 38°C removal of both anions increased efflux nearly 10-fold. Tension development in response to potassium depolarization or epinephrine was reduced approximately 10% in the absence of PO_4. However, if the passive Ca influx was inhibited by the Ca antagonist, verapamil, removal of PO_4 and HCO_3 decreased tension development in response to epinephrine by 60-70%. The observations on Ca flux and tension development are consistent with the hypothesis that Ca sequestration by intracellular organelles is significantly affected by the extracellular PO_4 and HCO_3 concentrations and further suggest that these anions influence a rapidly releasable intracellular Ca component. (Supported by NIH Grant #HL-21213 and the American Heart Association, New Jersey Affiliate.)

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COMPOUND ACTION POTENTIALS IN CANINE CARDIAC NERVES ELICITED BY LOCALIZED STIMULATION IN SYMPATHETIC GANGLIA. M. McGill*, D.A. Hopkins*, J.A. Armour. Depts. of Physiology & Biophysics and Anatomy, Dalhousie University, Halifax, Canada B3H 4H7

It has been demonstrated that the majority of canine sympathetic postganglionic neurons projecting in physiologically identified cardiac nerves are located in the middle cervical ganglion; considerably fewer cardiac neurons are located in the stellate ganglion. Therefore, it was considered important to determine if stimulation of various regions of these ganglia would result in specific activation patterns in cardiac sympathetic nerves. Following decentralization of the canine left stellate ganglion and the left cervical vagus, the left middle cervical and stellate ganglia were stimulated in localized regions utilizing fine bipolar or unipolar tungsten electrodes. Multifibre recordings were obtained from the innominate, ventromedial, ventrolateral, and left stellate cardiac nerves. Results were obtained before and after atropinization and an infusion of hexamethonium. Stimulation in the middle cervical ganglion demonstrated that there were foci which activated some cardiac nerves, but not others. Similar foci were located in the rostral pole and along the ventral edge of the stellate ganglion. These results indicate that cells located in discrete regions of thoracic ganglia project to one or more cardiac nerves.

(Supported by the N.S. and N.B. Heart Foundations and the MRC of Canada).

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INTRACELLULAR RECORDINGS FROM THE STELLATE GANGLION (SG) IN THE CAT. Zeljko J. Bosnjak and John P. Kampine. Med. Col. of Wisconsin and VA Medical Ctr., Wood, WI 53193.

Intracellular, in vitro recordings have been obtained from cells of the SG in the kitten. 5% of the cells impaled were inexcitable. They had a high resting membrane potential (up to -80 mV), and preganglionic stimulation of the T_3 ramus gave rise to a slow depolarization. Action potentials in these cells could not be evoked even with direct intracellular depolarizing current (IDC). Excitable SG cells had resting membrane potentials of -55 to -70 mV. They received excitatory synaptic input from preganglionic nerve fibers (T_3) and produced action potentials in response to IDC. Threshold for initiation of an action potential ranged from 15-20 mV above baseline. Prolonged IDCs triggered a phasic or tonic discharge of action potentials in most excitable cells. In 5% of excitable cells spontaneous action potentials were observed without any stimulation. A train of presynaptic stimuli to these cells (1/2 sec)was followed by an after-discharge lasting up to 5 minutes, and the frequency was directly related to the frequency of the initial stimulus train. Excitatory synaptic input was recorded from principal SG cells in response to stimulation of post-ganglionic nerves (ventral and dorsal ansa subclavia and stellate cardiac nerve). More than 70% of the SG cells receive input from all extrinsic nerves studied. This suggests that marked convergence of central and peripheral input occurs on ganglion cells in the SG of the cat.

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SELECTIVE DENERVATION OF THE HEART. W.C.Randall,M.P.Kaye,J.X. Thomas,M.J.Barber. Loyola Univ. Med. Ctr., Maywood, IL 60153.

Total intrapericardial denervation of the canine heart is accomplished in a single operation by surgical transection of all neural projections onto the heart. Through a left (L) pericardiotomy, the L atrium, L sup.pulm.vein, and main pulm. artery (PA) are cleared of neural tissue and the ventrolateral nerve and its branches are cut (Stage I). The fat pad and all nerves are removed from between PA and aorta; the adventitia is circumferentially resected at the root of PA (Stage II). The pericardium is dissected to the superior vena cava (SVC) and cleared from its circumference. Sharp dissection proceeds along SVC to the azygos vein which is cleared, tied, and cut. The surface of the right (R) PA is cleared of nerves and dissection carried along the superior surface of R atrium to its intersection with L atrium (Stage III). Denervation is tested by stimulation of both vagi and stellate ganglia, recording inotropic, chronotropic and dromotropic events before and after each individual step in the procedure. Stage I deletes most parasympathetic and L sympathetic input without interrupting R sympathetic influences. Stage II completes L autonomic denervation but preserves most of the R sympathetic input to SA nodal and atrial areas. Multiple large nerves coursing along the dorsal PA carry inputs from both L and R sympathetics. Stage III completes denervation of AV and SA nodal structures and removes all remaining inotropic influences upon ventricular contractile musculature. Selective denervation of AVN and SAN regions appears feasible. (Sup. NIH Grant HL 08682.)

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CONTROL OF THE CARDIAC RESPONSE TO ACUTE CORONARY OCCLUSION IN DOG. D.C. Randall, J.M. Evans*, B.E. Billman, G.A. Ordway, & C.F. Knapp*. Dept. Physiology and Biophysics, & Wenner-Gren Res. Lab., Univ. of Kentucky, Lexington, KY 40536.

Three basic mechanisms may be involved in the control of cardiac function during coronary occlusion (CO): 1) neural, 2) hormonal (circulating catecholamine) and 3) "intrinsic" (e.g., Frank-Starling law). The response of intact, sedated (Innovar-Vet, .08 cc/kg), chronically instrumented dogs to 5 min. left circumflex CO was tested to delineate the roles of 1-3 above. First, 6 intact (I) and 6 cardiac denervated (D) dogs were studied (5 min. control vs. CO). The major difference between groups was that the occlusion-induced tachycardia was significantly smaller in the denervated dogs [+27 $\pm$ 4 (I) vs. +10 $\pm$ 7/min (D), mean $\pm$ SD]. Changes in d(LVP)/dt were similar [-796 $\pm$ 274 (I) vs. -898 $\pm$ 556 (D) mm Hg/sec]. Changes in stroke volume and mean arterial pressure were also similar. The CO-induced tachycardia was compared in a second group of denervated dogs (n=5) before and after administration of propranolol (1 mg/kg). The slight heart rate increase was similar in both situations. We conclude that: 1) the major role of the cardiac nerves involves modulating changes in the chronotropic state of the heart; 2) changes in d(LVP)/dt result principally from intrinsic phenomena linked to ischemia induced alterations in myocardial performance; 3) changes in circulating catecholamines play only a minor role during acute CO in dog. (Supported by NIH Grant HL 19343 & AFOSR Contract #F44620-74-C-0012).

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RECOVERY OF BARORECEPTOR REFLEX AFTER UNILATERAL VAGOTOMY IN RABBITS. Donald D. Lund, Janine Schmidt*, and Phillip G. Schmid. VA Med. Ctr., Cardiovascular Ctr., Dept. Int. Med., Univ. of Iowa, Iowa City, IA 52240

Biochemical analysis of choline acetyltransferase activity after unilateral and bilateral vagotomy suggest that compensatory sprouting and collateralization occurs after partial denervation (Am. J. Physiol. 236:H620, 1979). Therefore, the time course of functional collateral reinnervation of the SA node provided by the left vagus nerve after a right unilateral vagotomy was studied in rabbits (4 Kg). Reflex heart rate responses to pressor stimuli were studied before and after right vagotomy (V) (N=7) or a sham operation (S) (N=4) in unanesthetized, propranolol treated (2 mg/Kg) animals. The response was quantitated as the slope of a line generated from the R-R prolongation during increases in arterial pressure (ΔRR/ΔBP) produced by bolus injections of phenylephrine (PE) (8 µg/Kg). Sham surgery had no detectable effect on ΔRR/ΔBP responses to PE. In contrast, there was a significant reduction ($P<0.05$) in ΔRR/ΔBP responses to PE after right vagotomy (V=0.9$\pm$0.2 vs S=2.9$\pm$0.9) ($\bar{x}\pm$SE). Responses in the same animals recovered 24 hours after vagotomy (V=1.6$\pm$0.6 vs S=2.1$\pm$0.6) ($P>0.1$). These data suggest that functional collateral reinnervation of the SA node area by the left vagus nerve occurs within 24 hrs after a right unilateral vagotomy. Supported by USPHS Grants HL-24246 & HL-14388.

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EFFECT OF BETA ADRENERGIC BLOCKADE ON THE FREQUENCY-RESPONSE RELATIONSHIP BETWEEN RENIN SECRETION RATE (RSR) AND RENAL NERVE STIMULATION. W. S. Ammons, S. Koyama, H. L. Santiesteban, and J. W. Manning. Emory Univ. Sch. Med.,Atlanta, Ga. 30322

We have recently demonstrated the presence of a frequency-response relationship between RSR and renal nerve activity. This was shown under constant flow as well as constant pressure perfusion of the left kidney of cats. These data gave frequency-response plots that resembled first order expressions, such that RSR increased as frequency increased to reach a maximum at low frequencies of 2-4 Hz. The present experiments were designed to assess the role of the beta adrenergic receptor in renal nerve activation of renin release. The left kidney of α-chloralose anesthetized cats was pump perfused with blood at constant flow or constant pressure. The main renal nerves were stimulated (5-10V, 0.5 msec) at different frequencies for 1 min. Propranolol was administered IV (1.5 mg/Kg) followed by constant infusion (0.5 mg/Kg/hr). At low frequencies (0-2 Hz) at which hemodynamic and filtration parameters did not change, the large increases in RSR, which were present in the control group, were abated. At higher frequencies during which renal vascular resistance increased proportionally, RSR progressively decreased in the beta blocked animals. On the assumption that propranolol blocks the direct neural component of renin release, it can be concluded that during renal nerve activation, an inhibitory component is present and associated with altered intrarenal conditions. (Supported by USPHS NIH Grant HL-16648)

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FUNCTION OF SYMPATHETIC INNERVATION TO HYPERTROPHIED VENTRICLE AFTER PULMONARY ARTERY CONSTRICTION UNDER BASAL AND ACTIVATED CONDITIONS. Phillip G. Schmid, Carol A. Whiteis*, Janine A. Schmidt*, Robert P. Oda*, and Donald D. Lund. VA Med. Ctr., CV Ctr., & Dept. Int. Med., Univ. of Iowa, Iowa City, IA 52240

Biochemical and morphometric data indicate reduced sympathetic innervation in hypertrophied and failing heart chambers. However, little is known about the functional state of the remaining sympathetic innervation. This was investigated in hearts of guinea pigs with sham surgery or pulmonary artery constriction (30 days) with two to three-fold increases in normalized right ventricular weights. The turnover rate constant of norepinephrine, k_{NE}, was determined in each conscious animal under basal resting conditions, (k_{NEa}), and again during one hour of cold stress, 4°C (k_{NEb}). In pressure hypertrophied right ventricles (n=4) k_{NEa} averaged 0.047±0.009 ($\bar{x}$±SE) hr^{-1}; k_{NEb} increased markedly during cold stress to 0.131±0.037 hr^{-1} ($P<0.01$). Corresponding values in sham animals (n=4) were k_{NEa}=0.037±0.005 and k_{NEb}=0.083±0.012 hr^{-1} ($P<0.01$). Therefore, norepinephrine turnover in the residual innervation to the stressed ventricle can increase normally from a basal level which hourly replaces approximately 5% of the neurotransmitter in individual nerve fibers to a level which replaces 13% of the neurotransmitter. Sympathetic innervation to pressure-overloaded heart, although reduced in density, retains the capability to respond to activation. (Supported by a VA Grant, HL-20768, & HL-14388).

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THE EFFECTS OF ANESTHESIA, BODY POSITION AND CENTRAL BLOOD VOLUME ON BARORECEPTOR REFLEX SENSITIVITY IN THE RHESUS MONKEY G.E. Billman, K.K. Teoh*, D.T. Dickey*, H.L. Stone. Dept. of Physiology and Biophysics, University of Oklahoma, Health Science Center, Oklahoma City, Oklahoma 73190

The purpose of this study was to investigate the effects of anesthesia, body position and central blood volume on the systolic arterial pressure R-R interval relationship (an index of baroreceptor reflex sensitivity, BSR). Five male rhesus monkeys (7.0-10.5 kg) were given bolus injections of 4.0 µg/kg phenylephrine during each of the following situations: awake sitting, anesthetized (AN), AN sitting, AN recumbent, AN 90° headdown tilt, and AN after 50% blood volume expansion with normal saline. Anesthesia was introduced with 10 mg/kg ketamine HCl. Beta-receptor blockade (βB) was also performed after anesthesia (1.0 mg/kg propranolol HCl I.V.). R-R interval was plotted against systolic arterial pressure, the slope (BSR index) was determined by linear regression. These data are presented in the table below. An asterisk indicates a significant difference ($P<.05$) from the AN recumbent position (Friedman's two way ANOVA).

AN Recum		AN Sit		Awake Sit		50% Vol		90°Headdown Tilt	
cont	βB	cont	βB	cont	βB	cont	βB	cont	βB
2.62	2.00	2.75	2.19	2.37	--	0.91*	1.35*	1.02*	1.36*

BSR was decreased by 90°headdown tilt and 50% volume expansion while anesthesia tended to increase the slope. These data are consistent with the thesis that central blood volume and BSR are inversely related. Supported by NASA Grant NSG2282.

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ATTENUATION OF BAROREFLEXES DUE TO HALOTHANE. J.L. Seagard, J.H. Donegan*, F.A. Hopp*, and J.P. Kampine. Med. Col. of Wisconsin and Wood VA Medical Ctr., Milwaukee, WI 53193.

Baroreceptor reflexes have been found to be attenuated during halothane (H) anesthesia in patients and in anesthetized dogs. The relative importance of depressant effects of H on carotid sinus baroreceptors, central and peripheral autonomic pathways, and end organ responses were studied to determine the possibility of multiple sites of inhibition of baroreflex function. The baroreflex effects on heart rate initiated by blood pressure changes were examined in conscious and anesthetized (0.75 and 1.5% H) dogs. In addition, carotid sinus afferent activity, cardiac sympathetic efferent activity, and heart rate responses to direct sympathetic and parasympathetic efferent stimulation were examined in the anesthetized dogs. Halothane had no direct effect on carotid sinus afferent activity, but all levels of H significantly attenuated the reflex changes in heart rate produced by the pressure changes. Dose-dependent decreases in cardiac sympathetic efferent activity were produced by H administration. Heart rate changes produced by direct stimulation of cardiac sympathetic efferents were also significantly attenuated at all levels of H while changes due to stimulation of vagal efferents were not significantly blunted until high H levels (1.5%). Therefore H attenuation of the baroreflex appears to have multiple sites of action. At 0.75% H the CNS depressant effects of H appear to predominate, while at 1.5% both central and peripheral effects are evident. (Supported by NIH Grants HL 16511, 06511, 05882, and VA).

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EFFECT OF GLUCAGON-INDUCED CARDIAC ACCELERATION ON THE CHRONOTROPIC RESPONSE TO VAGAL STIMULATION. S.L. Stuesse, M.N. Levy, and H. Zieske*. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Div. of Inves. Med., Mt. Sinai Hospital, Cleveland, OH 44106.

Glucagon (10 µg/kg, i.v.) accelerates the heart independent of sympathetic nervous system stimulation. The effect of glucagon administration on the chronotropic responses to repetitive bursts of vagal stimulation was determined in open-chest, anesthesized dogs. When the cervical vagi were stimulated at constant frequencies, the change in heart rate was not affected by glucagon administration, i.e., no vagolytic effect due to glucagon was apparent.

When the vagi were stimulated intermittenly with one short burst of vagal stimuli delivered each cardiac cycle, the resultant heart period was dependent on the time of vagal stimulus delivery. Glucagon did not uniformly accelerate the cardiac cycle in response to phasic vagal stimulation. Instead glucagon had no effect on the maximum heart rate obtained during a vagal phase response curve, but decreased the minimum heart rate obtained during a given level of vagal stimulation. Thus there was an interaction between glucagon administration and at least one aspect of the vagal phasic response curve.

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Biphasic pressor response to stimulation of the locus coeruleus area in the rat. P. Gauthier and G. Drolet (spon. S. Rossignol). Dép. de physiologie, Université de Montréal, Montréal.

Electrical stimulation of the nucleus locus coeruleus area of the rat anesthetized with α-chloralose evoked a biphasic pressor response persisting for about 90 sec after termination of the stimulus. A tachycardia was observed during the primary phase of the response whereas a bradycardia always accompanied the secondary phase.The cardiovascular response was graded with stimulus intensity and was optimally evoked at high frequencies of stimulation (80-100 Hz). The active sites eliciting the characteristic response were found to be restricted to the dorsal portion of the pontomesencephalic area which includes the nucleus locus coeruleus and the central gray substance. Intravenous dministration of phentolamine (3 mg/kg) considerably decreased the response. Treatment with guanethidine (2 mg/kg I.V.) or 6-hydroxydopamine (100 mg/kg I.V.) selectively abolished the primary phase of the cardiovascular response. Adrenalectomy or adrenal demedullation prevented the secondary phase of the cardiovascular response without affecting the primary phase. The present study suggests that electrical stimulation of the locus coeruleus area in the rat results in the activation of adrenal medullary catecholamine secretions and in some activation of the vasomotor sympathetic neurons. * Supported by the Canadian and Quebec Heart Foundations and MRC (Canada).

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INCREASES IN BLOOD PRESSURE AND HEART RATE AFTER VASOPRESSIN ADMINISTRATION BY MICROINJECTION INTO AREA OF NUCLEUS TRACTUS SOLITARIUS OF RATS. Hideyo Matsuguchi*, Phillip G. Schmid, Frank J. Gordon*, and A. Kim Johnson. VA Med. Ctr., CV Ctr., Depts. Int. Med. & Psych., Univ. of Iowa, Iowa City, IA 52242

Arginine vasopressin (AVP) containing neurons have recently been demonstrated to extend from suprachiasmatic nucleus in the anterior hypothalamus to the nucleus tractus solitarius (NTS). Thus, AVP might have a significant influence on neural control of circulation. This was investigated by monitoring arterial blood pressure (BP) and heart rate (HR) responses to graded injections of AVP (100, 1000, and 10,000 pg) in artificial cerebrospinal fluid (CSF 0.1 µl) into the right NTS of 20 rats anesthetized with urethane. One dose was given to each rat. Injections of artificial CSF (n=12) had no detectable effect on BP and minimal effect on HR (-6.0±1.4 bpm) ($\bar{x}$±SE). AVP injections to NTS produced significant dose related increases in BP of 2.5±2.4, 11.8±0.9, and 20.6±2.8 mmHg and significant graded increases in HR of 10.5±7.6, 22.7±6.0 and 50.0±11.9 bpm. Unlike NTS injection, AVP injected intravenously produced increases in BP only at the highest dose (10,000 pg) and decreased rather than increased HR. Ganglionic blockade with iv chloroisondamine (10 mg/Kg) in 5 rats reduced the BP responses to 10,000 pg of AVP into the NTS to 3.4±1.3 mmHg, and completely eliminated the HR responses. These results suggest that AVP in the region of the nucleus tractus solitarius may serve a significant role in the neural control of circulation. (Supported by VA Grant and HL-25227)

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LONG TERM EFFECT OF RENIN IN THE BRAIN ON DRINKING AND BLOOD PRESSURE. M. Ian Phillips, Laurie Hoffmann and Tadashi Inagami (Spon. B. Schottelius) Physiology Dept., Univ. of Iowa and Univ. of Tennessee, Nashville, Tn.

Renal renin produces angiotensin II when injected in the brain. Purified mouse salivary renin and hog renin were injected into rat brains to test the generality of renin producing angiotensin II in the brain. Rats were prepared with intraventricular (IVT) cannulae and femoral artery catheters. The two renins were injected in a dose of 1 µg/µl volume of saline. Rats were tested for water intake over 15 min and blood pressure (BP) increases.

Two effects were observed: an acute effect and a chronic effect. a) Mouse salivary renin elicited 8.3±4.1 ml water intake with a latency of 80 sec. Hog renin elicited 10.12± 6.9 ml water intake and 24.6 mmHg raise in BP. b) 24 hr intake of water was elevated for days although BP had returned to normal.

Day	0	1	2	3	4	5	6	
Mouse Renin	33±1	88±9.7	105±12	110±17	77±26	75±31	65±12	N=8
Hog Renin	31±1	83±7.8	55±5.2	43±3.4	38±4.6			N=6

The results show that these renins have long term effects which differentially affect the angiotensin II thirst reponse and the blood pressure response.
(Supported by NSF and NIH grants.)

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ATTENUATION ON THE CENTRAL HYPERTONIC NACL PRESSOR RESPONSE BY ANGIOTENSIN II INHIBITION. J.F. STAMLER* AND M.I. PHILLIPS.

In this study we tested the hypothesis that the rise in blood pressure induced by intraventricular (IVT) hypertonic NaCl is mediated or modulated by angiotensin II (AII). We administered 3µl of five different concentrations of NaCl through chronically implanted stainless steel canulae leading to the lateral ventricles in eight adult male Sprague-Dawley rats preceded by 20µg of Saralasin in 2µl of 0.9% NaCl or vehicle alone. At least 15 minutes was allowed for recovery between each dose tested. Mean arterial pressures (MAP) were recorded from femoral Silastic catheters filled with heparinized saline in conscious unrestrained animals. IVT hypertonic NaCl transiently increased the MAP in a concentration dependent manner and Saralasin significantly inhibited the response at each dose (see table).

%NaCl by wt.	0.9%	1.4%	1.9%	2.4%	2.9%
NaCl alone	0	8.25 ±1.7	11.88 ± 3.28	13.88 ± 2.77	18.13 ± 2.41
NaCl + Saralasin	0	1.0 ±1.7	3.38 ± 1.44	8.88 ± 1.08	11.0 ± 0.88
P<		.005	.001	.05	.025

It appears that AII mediates or modulates the pressor response of hypertonic NaCl. Possible mechanisms by which this might occur include 1) modulation of the osmoreceptor cell, 2) modulations of a transmitter distal to the osmoreceptor cell in the effector circuit or 3) AII could be serving as a neurotransmitter. (supported by NSF grant BNS 75-16364 to MIP)

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THE ROLE OF CENTRAL AND PERIPHERAL CHEMORECEPTORS IN THE CARDIOVASCULAR RESPONSE TO DIVING IN DUCKS. David R. Jones, William K. Milsom and Geoffrey R.J. Gabbott† Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 2A9.

Heart rate, blood pressure and hind limb vascular resistance (HLVR) were measured in White Pekin ducks during forced dives of 1-2 min. duration. Using several different experimental approaches, including cross-perfusion between pairs of animals, it was possible to determine the relative roles of the peripheral (carotid body) and central (brain) chemoreceptors and their possible interaction with barostatic reflexes in the cardiovascular adjustments accompanying diving. Our results indicate that stimulation of the carotid bodies by low O_2 and high CO_2 during diving is responsible for 50% of the bradycardia and 25% of the increase in HLVR observed under control conditions. Barostatic reflexes further increase diving bradycardia but oppose the increase in HLVR. Up to 10% of the diving bradycardia and 25% of the increase in HLVR during diving result from stimulation of the central chemoreceptors by blood high in CO_2. These results emphasize the integrated nature of the cardiovascular response to diving arising from simultaneous stimulation of diverse receptor groups.

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RIGHT HEART CATHETERIZATION. B. Wooldridge*, B.R. Williams*, B. Rubal and J.F. Gaugl*. Texas College of Osteopathic Medicine, Ft. Worth, Texas 76107 and Texas Woman's University, Denton, Texas 76204.

The relatively recent introduction of the balloon-tipped, flow directed catheter into the physiology laboratory has facilitated the monitoring of right heart and pulmonary vasculature function. For the undergraduate medical student attempting the procedure for the first time, however, success is often elusive, and the frustration of having the catheter not advance properly distracts from the physiology to be learned in the laboratory experience. This video tape was made to aid the student in his understanding of the concepts and their clinical relevance of the various right side pressures, from the central venous to the pulmonary arterial and wedge. A flow-directed balloon-tipped catheter is followed in its progression from the superior vena cava to the pulmonary artery by visualization of the heart and associated vessels, by the use of diagrams, and by image-intensified fluoroscopy. Pressure changes at the catheter tip are correlated with the location of the catheter. A brief discussion of some factors affecting pulmonary vasculature pressures complete the tape.

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TEACHING INTRODUCTORY PHYSIOLOGY IN A SELF-PACED FORMAT TO LARGE UNDERGRADUATE CLASSES T. Machen, R. Zucker, C. Landau & S. Russell, Physiology-Anatomy Dept., University of California, Berkeley 94720

Physiology 1 is a large (250 students) course for non-majors. We had previously taught P1 using lectures but found that these came to be mere rewordings of the text material (Vander et al, Human Physiology). We were also dissatisfied with the cramming that occurred just before the exams and the competitive nature of the course. We now teach P1 in a self-paced format. Students proceed at their own pace throughout the text which is divided into 10 separate units, i.e. learning objectives, text reading assignment and self-tests (Kluger et al, Work-Book to Accompany Human Physiology). Physiology major tutors answer questions during 20hrs/wk. Mastering a unit is demonstrated by passing a 20 min. quiz at 90% proficiency. If the student fails the exam, s/he can study the unit and take another exam. The student has 3 chances to pass a unit exam before proceeding to the next. Course grade is based on the number of unit exams passed and the grade on a 3 hr. final exam. Despite the increased organizational problems, we are encouraged by the course's flexibility, reduced competition, increased contact between faculty/staff and students and the fact that students are learning on their own. Statistical evaluation shows equal levels of learning in the lecture and self-paced formats.

Supported by NSF CAUSE Project and UCB Committee on Educational Development.

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INNOVATIONS IN TEACHING ON AGING: INTEGRATIVE AND INTERVENTIVE APPROACHES. P.S. Timiras, University of California, Berkeley, CA (94720); L.Z. Feigenbaum, University of California, San Francisco, CA (94143); J. Meites, Michigan State University, Lansing, MI (48824); E.J. Masoro, University of Texas, San Antonio, TX (78284); A. Vernadakis, University of Colorado, Denver, CO (80220) and R.F. Walker, University of Kentucky, Lexington, KY (40506).

As fundamental knowledge accumulates on aging and the aged population increases both in number and life span duration, the establishment of curricula in aging is important for understanding the underlying basic processes and their application to clinical interventions. Although traditionally some aspects of aging have been taught and continue to be offered by specific disciplines, currently an overall emphasis on the aging process, per se, as an identifiable and cohesive body of knowledge is recognized. The methods of integrating such knowledge into existing undergraduate, graduate and medical programs may vary depending on the degree of curricular commitment, the nature of curricular emphasis, faculty availability, student interest, etc. Some of these variables have been tackled by departments of physiology (Masoro, Meites, Timiras) and allied sciences, such as anatomy (Walker), pharmacology and psychiatry (Vernadakis), and medicine (Feigenbaum) to construct programs which, while integrated within the curriculum, still preserve their individuality and prepare the student to pursue basic and applied research in gerontology and geriatrics. The participants are invited to discuss and share their own experiences with the end in mind of formulating guidelines which will permit improvements of the extant programs and will assist in the creation of new ones.

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LEFT MAIN CORONARY ARTERY PRESSURE-FLOW RELATIONSHIPS THROUGHOUT THE CARDIAC CYCLE. W. Robert Taylor*, Baruch Bromberger-Barnea and Robert A. Wise*. The Johns Hopkins University School of Hygiene and Public Health. Baltimore, Maryland 21205

We sought to examine pressure-flow relationships throughout the entire cardiac cycle in a normally beating heart. Using an open chest dog preparation with left coronary artery perfusion pressure maintained independent of systemic pressure, we were able to obtain a pressure-flow curve for any given point in the cardiac cycle. The pressure-flow curve intersects the pressure axis at zero flow (P_{ZF}). We are able to determine P_{ZF} and the slope of the pressure-flow plot at multiple points in the cardiac cycle. P_{ZF} was found to vary from 15-30 mmHg during diastole to near peak left ventricular pressure during systole. The inverse slope of the pressure-flow relationship varied from .55-.70 mmHg/cc/min during diastole to .77-.95 mmHg/cc/min during systole. Both reactive hyperemia and adenosine infusion were found to lower P_{ZF} and the inverse slope at all points in the cardiac cycle. These results are compatible with a vascular waterfall mechanism in which the combination of tissue pressure and vascular tone is the effective downstream pressure. However, they do not exclude the possibility of other mechanisms. (Supported by NIH Grants #HL01799 and HL10342.)

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A MODEL OF THE CORONARY VENOUS SYSTEM. Alan Y.K. Wong,* Gerald A. Klassen, Andrew J. Armour. Dept. of Physiology & Biophysics, Dalhousie University, Halifax, N. S., Canada

The coronary vascular system is characterized by a small blood-volume, high resistance arterial system in which flow is diastolic, a capillary-venule exchange system, and a large blood-volume, low resistance venous system with systolic flow. Models of the arterial system and its collateral vessels have been proposed but the role of the venous system is not well known. Simultaneous measurements were made of intramyocardial pressure (IMP), peripheral (PVP) and central (CVP) coronary venous pressure, as well as phasic coronary sinus outflow. A model of the coronary venous system is proposed to describe the venous pressure-flow relationship. This model consists of an intramyocardial vascular storage region into which the arterial blood flows throughout diastole. During systole, IMP increases squeezing blood from this vascular storage into the epicardial venous system. The vessel resistance to outflow is dependent upon vascular element and IMP. The filling of the storage region and subsequent ejection of blood from it during systole may be likened to filling and ejection of blood from the ventricular chamber. Thus, blocking the inflow will tend to empty the amount of blood in the muscle wall. The data of peak (IMP-PVP) vs peak flow during vagal, right and left stellate stimulation and various inotropic agents conform satisfactorily with the model. (Supported by grants from MRC SDGII and the N. S. Heart Foundation.)

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THE DISTRIBUTION OF MYOCARDIAL BLOOD FLOW DURING PERICARDIAL TAMPONADE. W.J. Keon*, G.C. Taichman*, and G.V. Forester. University of Ottawa Cardiac Unit, Ottawa, Canada. K1Y 4E9

Although coronary flow has been shown to decrease during effusive pericardial tamponade (PT), regional myocardial flow responses have yet to be described. Accordingly, the relative changes in flow (RCF) were measured with microspheres (15μ) in 11 anesthetized dogs at two levels of PT [mean arterial pressures of 84 (PT_1) and 56 (PT_2) mm Hg] and compared to control (132 mm Hg). RCF (%) were:

	Regional	PT_1	PT_2
(a)	Right atrium	-68*	-80*
(b)	Left atrium	-70*	-82*
(c)	Left ventricle (LV)	-49*	-68*
(d)	Septum (S)	-49*	-69*
(e)	Right ventricle (RV)	-44*	-59*
	Transmural		
(f)	LV endo/epi	-11*	-19*
(g)	RV endo/epi	-4	-10*
(h)	S left/right	-4	-11*

At both PT_1 and PT_2, there were significant ($p<0.05$) differences in regional RCF: (a)or(b)>(c)or(d)>(e). Significant differences in transmural RCF were seen only at PT_2 where (f)>(g)or(h). Thus, PT appears to result in a disproportionate decrease in myocardial flow in various parts of the heart.
*significantly different from control

(Supported by Ontario Heart Foundation Grant 5-1.)

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CRITICAL CLOSING, OPENING AND EARLY REPERFUSION IN MYOCARDIAL ISCHEMIA. Colin E. Bayliss*, Richard Baffour* and John Grayson. University of Toronto, Toronto, Ontario, M5S 1A8

The perfused rabbit heart septum model was used to determine the critical closing pressure in normal myocardium and the effect of ischemia at 24°C on the critical opening pressure and on pressure-flow relations during the first few minutes of reperfusion. In 17 septums, closing and opening pressure-flow curves were plotted for the range 4000 to 0 μl/g·min (normal flow = 1000) with interposed ischemic intervals of 5 min (n=10), 30 min (n=2), 60 min (n=3) and 90 min (n=2). Critical closing pressure (mean±SE) was 9.3±0.81 mm Hg. The post-ischemic critical opening pressures were: 10.2±1.34 (5'), 7.8±0.95 (30'), 5.5±1.42 (60') and 8.6±1.35 (90') mm Hg. They were not significantly different ($p>0.05$) from critical closing pressures or related to the ischemic intervals ($r=-0.44$; $p>0.05$). All opening curves were displaced to the right, but within the 95% confidence limits (±2SD), of the closing curve. Critical closing pressure (9.3 mm Hg) agreed with our previous findings in dog hearts (10.4 mm Hg) and with the predictions of the interfacial tension theory (10.5 mm Hg). Critical opening and closing pressures were similar, despite interposed ischemia. Ischemia did not significantly affect pressure-flow relations during the first few minutes of reperfusion.

(Supported by grants from the Ontario Heart Foundation and the Medical Research Council of Canada)

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DYNAMIC CORONARY ARTERY STENOSIS
William P. Santamore, Alfred A. Bove, Rita A. Carey, James F. Spann
Temple University, Philadelphia, PA 19140

Recent clinical and experimental data indicate that changes in arterial vasomotor tone might influence the hemodynamic severity of the stenosis. In 4 closed, chest dogs, we examined the effects of coronary artery vasomotor tone on stenotic resistance (SR) and endocardial blood flow (EBF in ml/100 gm · min) measured with 15μ radioactive microspheres. SR was calculated as the mean pressure gradient across the stenosis divided by the mean blood flow through the stenosis. The mean pressure gradient was calculated as the ascending aortic pressure minus the left anterior descending coronary artery pressure distal to the stenosis. Distal coronary arteriolar vasodilation, induced by adenosine (0.2 mg/kg i.v.) increased SR (1.0±.1 to 7.4±5 mmHg/ml · min^{-1}; $P<.05$) and thereby decreased EBF (54±5 to 30±7, $P<.05$). Proximal coronary artery vasoconstriction, induced by ergonovine (0.8 mg, i.v.), also increased SR (1.0±.1 to 2.7±.9 mmHg/ml · min^{-1}) and thereby decreased EBF (54±5 to 40±6). Thus, changes in vasomotor tone can alter the severity of stenosis which, in turn, can influence blood flow to the myocardium. This mechanism may be important in the pathogenesis of angina pectoris and myocardial infarction.

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INABILITY OF ISOPROTERENOL (ISO)AND PROPRANOLOL(PRO)TO ALTER LATERAL INFARCT SIZE IN DOGS. James M. Downey, R. Douglas Wilkerson,* and Douglas F. Munch*. Depts. of Pharmacology and Physiology, Univ. of South Alabama, Mobile, Alabama 36688

The relationship between the blood flow (BF) pattern immediately after coronary artery occlusion (CAO) and the resulting infarct was studied in dogs treated with ISO (0.5 ug/kg/min. for 2 hrs.), or with PRO (2mg/kg every 6 hrs) after CAO. A 2 mm bead was used to embolize a small coronary artery during closed-chest coronary perfusion with arterial blood. One minute after CAO, radio-labelled microspheres were injected into the perfusate. 24 hours after CAO,dogs were sacrificed after i.v. injection of patent blue violet dye. Hearts were sliced into 4 mm sections and microsphere distribution was visualized by autoradiography of the tissue. Superimposition of developed autoradiographs and tracings of the infarct pattern of stained sections allowed direct comparison of the BF pattern immediately after CAO to the eventual pattern of infarction. In all 8 control dogs, all 6 ISO dogs and all 12 PRO dogs the lateral borders of BF and infarction were superimposable indicating no lateral change in infarct size resulting from treatment. In the control group there was a subepicardial region of the ischemic zone which did not infarct(15.2±2.3% of the total zone). Though ISO did not significantly change the size of this zone, PRO increased it to 35.9±6.5% ($p<0.05$) indicating vertical but not lateral salvage.

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DECREASED AVAILABLE CORONARY VASCULAR RESERVE (CVR) IN BOVINE RIGHT VENTRICULAR (RV) MYOCARDIUM HYPERTROPHIED FROM JUST AFTER BIRTH. M. Manohar, J.C. Thurmon, W.J. Tranquilli, M.D. Devous, Sr., and M.C. Theodorakis. Univ. of Ill., Urbana, IL 61801.

To examine the effects of hypertrophy (H) on CVR in the RV myocardium, hemodynamics and myocardial blood flow (MBF: 15μm microspheres) were studied in 6 normal (N) calves and 7 calves with severe RVH before (Control:C) and during iv infusion (0.2 μg/kg/min) of isoproterenol HCl (I). RVH was produced by banding the main pulmonary artery (within 48 h after birth) 25-30 weeks prior to the study.

	Control		Isoproterenol	
	N	RVH	N	RVH
RV pressure (mmHg)	47±2	127±8*	60±3Δ	154±4*Δ
RV MBF (ml/min/g)	0.69±.04	1.22±0.08*	3.28±0.23Δ	3.76±0.22Δ
RV endo:epi	1.13±.04	1.17±.05	1.10±0.05	0.81±0.04*Δ
RVcvr	150±6	88±5*	25±4Δ	21±5Δ
LV MBF (ml/min/g)	1.12±.04	1.18±.06	3.80±0.08Δ	3.54±.11Δ
LV endo:epi	1.15±0.03	1.23±0.03	0.76±0.05Δ	0.85±0.04Δ
LVcvr	90±5	92±3	22±7Δ	23±4Δ

All data: Mean ± SEM; * N vs. RVH $p<0.01$; Δ C vs I $p<0.01$; cvr= coronary vascular resistance in mmHg/(ml/min/g).

In this model of RVH, RV MBF is markedly increased (77%) while left ventricular (LV) mass and LV MBF remain unaffected. Because RV endo:epi had decreased significantly with I in RVH and because the drop in RV cvr was only 54% of that in normal RV, it is concluded that higher baseline perfusion in RVH occurs at the expense of decreased available CVR.

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DE NOVO DEVELOPMENT OF FUNCTIONAL CORONARY COLLATERALS IN PORCINE MYOCARDIUM. Ronald W. Millard. Dept. Pharmacology and Cell Biophysics, Univ. of Cincinnati Medical Center, Cincinnati, Ohio 45267.

As in human myocardium, the pig native coronary arteries lack functional collaterals. In contrast, the collateral circulation in the dog myocardium is pre-existent. While collaterals develop in man, the dependent myocardium exhibits variable mechanical function. The question remains, can coronary collaterals be induced to supply functionally viable myocardium in a species which lacks a pre-existing functional network? In 6 young domestic pigs (10 kg), a fixed stenosis of the left anterior descending artery (LAD) was made. At 6 wks, when the LAD was completely occluded, LAD peripheral pressure and retrograde flow were >25 mmHg and >7 ml/min, compared with <5 mmHg and <0.5 ml/min in normal hearts. Myocardial blood flow (15 μ spheres) in the LAD region supplied by collaterals was 45 ± 2% of myocardium supplied by native arteries (1.02 ± .04 ml/min/g). Regional myocardial shortening velocity (sonomicrometer) was reduced to 25% of control regions (9 mm/sec). Thus, flow limitation by fixed coronary stenosis coincident with increased myocardial demand stimulates collateral development to functional pig myocardium.
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CAN EXERCISE STIMULATE REVASCULARIZATION IN THE TRANSITION ZONE AROUND A MYOCARDIAL INFARCT? K. Przyklenk* and A.C. Groom, Dept. Biophysics, Univ. Western Ontario, London, Can. N6A 5C1

Between the necrosis and the normal tissue in an infarcted heart lies a *transition zone* of viable, but probably ischemic, fibres. Our goal was to quantitate, on a per fibre basis, the capillary supply to this zone and to determine whether this supply could be improved by exercise. Two variables were used to define the capillary supply: (i) V_f, the number of vessels around each muscle fibre, (ii) F_v, the number of fibres around each vessel. Infarcts were induced in rats by tying the L. coronary artery midway between its origin and the apex of the heart. Five weeks later, when the animals were killed, the capillary supply in the transition was significantly below control values (V_f : 2.34 vs 2.99, $p < .001$; F_v : 2.53 vs 2.74, $p < .001$). To study the effects of exercise, infarcted rats were allowed periods of voluntary running in Wahmann activity cages. Three exercise protocols were used: I: 2 hrs/day, 6 days/wk, for 4 wks; II: 2 hrs/day, 3 days/wk, for 4 wks; III: 2 hrs/day, 6 days/wk, for 2 wks, followed by 2 wks inactivity. For each group mean V_f and F_v in the transition zone were plotted as a function of total distance run. In Groups I and II both variables rose initially to control values and thereafter declined to those found in non-exercised infarcted rats. In Group III no improvement whatever was found. The results suggest that by moderate exercise, if reinforced at least every other day, capillary supply in the transition zone may be restored to normal. (Supported by Ontario Heart Foundation)

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CONTRIBUTION OF MICROSCOPIC, INTRAMURAL ANASTOMOSES TO PERFUSION OF COLLATERALIZED MYOCARDIUM. H. Fred Downey, George J. Crystal* and Fouad A. Bashour. Departments of Physiology and Internal Medicine and Cardiovascular Research Laboratory at Methodist Hospital, University of Texas Health Science Center, Dallas, Texas 75235

Following gradual coronary artery obstruction, epicardial anastomoses are readily visible on the surface of the heart. These macroscopic anastomoses are thought to be the primary source of blood flow for collateral-dependent myocardium. This was tested in 12 dogs by measuring regional myocardial blood flow (MBF) under control conditions and during retrograde diversion of collateral flow (RD). MBF was measured with 9 μ microspheres (Ms) in all dogs and also with 25 μ Ms during RD in 4 of the dogs. Control MBFs in collateralized and in normally-perfused myocardium were similar. RD decreased mean aortic pressure 10%, but did not change heart rate, left atrial pressure, or MBF in normal region. MBF in collateral-dependent myocardium averaged 0.55±0.09 ml/min/g, 67% of control, despite RD and decrease in aortic pressure. 25 μ Ms were preferentially distributed toward the endocardium in the normal region, but were distributed similarly to 9 μ Ms in the collateral-dependent region during RD. We conclude that the voluminous non-divertible collateral flow was supplied by microscopic, intramural anastomoses. A model developed from these observations predicts that, under control conditions (no RD), microscopic pathways must provide approximately 50% of collateral flow.

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MINIATURE RADIATION DETECTOR FOR MEASURING REGIONAL MYOCARDIAL BLOOD FLOW. Ronald P. Karlsberg, Vincent L. Gelezunas,* and Kenneth P. Lyons.* VA Long Beach, UC Irvine, CA 90822

We have developed a radiation detector which is sensitive only to low penetrating radiation. Since low penetrating radiation has limited tissue range, precise spatial resolution is possible. Our portable, miniature solid state device employs surface stabilized glass coated silicon with avalanche internal gain which eliminates the need for probe mounted preamplifiers and enhances sensitivity. In 10 open chest dogs, we determined the reliability and validity of regional myocardial blood flow measurements with this device by repetitive intracoronary injections of 2 mCi of Xenon-133, dissolved in saline, before and after 1 hour of coronary stenosis. In 5 dogs, occlusions of 1 min were produced, and flow measured after release of occlusion. Simultaneous measurements of blood flow with radioactive microspheres in 0.3 gm epicardial (EP) biopsies were obtained. Flow measured with the probe prior to stenosis averaged 1.1±0.2 ml/min/gm (S.D.) and after 1 hour of stenosis ranged from 1.8 in perfused areas to 0.1 ml/min/gm in ischemic areas with similar values for microspheres. Repetitive measurements with the probe in the same area had a coefficient of variation of 9%. Flows obtained with the probe correlated to EP flows (r=.89). Following release of 1 min of occlusion, reactive hyperemia resulted with flows up to 4 ml/min/gm. We conclude, repetitive, reliable and valid in vivo measurements of regional myocardial blood flow in very small volumes can be obtained with this device.

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EFFECTS OF AORTIC PRESSURE AND EXTERNAL WORK ON RATES OF MYOCARDIAL PYRUVATEOXIDATION. R. Bünger, S. Yaffe*, B. Pemanetter* Depts. Physiol., Uniformed Services Univ., Bethesda, MD 20014 (USA) and University of Munich, Germany

We recently showed that ventricular preload (P_v) and norepinephrine (NE) cause work dependent alterations in the rates of myocardial pyruvate decarboxylation (Hoppe Seyler's Z. Physiol. Chem. 358, 1186 (1977), Physiologist 21, 14 (1978), Microcirc. Working Group Eur. Soc. Cardiol., (Springer (1980)). In the present study, work dependence of myocardial pyruvate metabolism was further examined by altering external work via changes in aortic pressure (P_a) at constant P_v (12 cm H_2O). Pyruvate decarboxylation was measured as $^{14}CO_2$-production from [1-14 C] pyruvate in an isolated guinea pig heart perfused with 1 mM pyruvate alone or in combination with 5 mM DL-3-hydroxybutyrate. Stepwise increase in P_a typically produced an increase in external work up to an optimum pressure of 80-100 cm H_2O and then a decrease. In contrast, pyruvate decarboxylation increased with P_a over the entire pressure range (40-120 cm H_2O). Moreover, when external work in presence of NE was adjusted to control work before infusion of NE (by decreasing P_a), $^{14}CO_2$-production did not return to control rate. The findings indicate that rates of pyruvate decarboxylation can be dissociated from work output at high arterial pressure and also during adrenergic stimulation of the heart. Apparently, cardiac hydraulic work is not the only hemodynamic determinant of myocardial pyruvate oxidation. (Supported by USUSH Grant #RO 7638)

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SOURCES OF GLUCOSE RELEASED IN HYPOGLYCAEMIA. C. Gauthier* and G. Hetenyi Jr. Dept. of Physiology, University of Ottawa.

In dogs an increase in the rate of disappearance(Rd) of glucose produced by the infusion of phlorizin(PHL) causes a significant elevation of plasma IRG concentration. This increases hepatic glucose release(Ra), so that the concentration of glucose in plasma decreases only marginally. Infused glucagon increases Ra both by stimulating glycogenolysis and gluconeogenesis. The purpose of the experiments was to establish the relative contributions and time patterns of these two effects of glucagon released in response to a physiological stimulus. In the first series of experiments ^{14}C-U-alanine and ^{3}H-3-glucose were infused: the Ra and Rd of glucose and alanine and the transfer of C-atoms from alanine to glucose were calculated before, during and after a 70 minute infusion of PHL. The metabolic clearance rate(MCR) and Ra of glucose increased together with the transfer of C-atoms during the infusion in both post-absorptive and fasted dogs. In the second series liver glycogen of dogs was pre-labelled with ^{3}H-6-glucose and ^{14}C-1-glucose was infused as tracer. During the infusion of PHL the appearance of ^{3}H in plasma glucose increased.

Conclusion: In response to IRG released during PHL infusion: (1) the increase in Ra is due to an increase in both gluconeogenesis and glucyogenolysis; (2) gluconeogenesis is increased to the same degree in the fasted and post-absorptive dogs; (3) increased gluconeogenesis is achieved by a more efficient conversion of alanine-C to glucose-C. (Supported by the MRC)

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COUNTERPRODUCTIVE INFLUENCE OF GLUCOSE ON HEPATIC GLUCOSE OUTPUT DURING HYPERGLUCAGONEMIA. Diane T. Finegood* and Richard N. Bergman. Univ. of Southern Calif., Los Angeles, Calif., 90033.

Hyperglycemia decreases hepatic glucose production and increases glucose uptake. Controversy exists concerning the relative significance of changes in glycogen phosphorylase, glycogen synthase and glucose-6-phosphatase activities in response to glucose. A 5 compartment computer model of hepatic glycogen metabolism was employed to study the mechanism of the counterproductive influence of glucose during hyperglucagonemia. Specific mechanisms were tested by computer simulation of dynamic changes in net hepatic glucose production and glycogen deposition previously observed in perfused livers. Glucose appears to inhibit glucagon stimulated glucose production by modulating the activity of glucose-6-phosphatase such that substrate cycling between glucose and glucose-6-phosphate is prevented. The decrease in cycling induces an increase in the levels of the intermediates and pushes the glucose toward glycogen. Glucose also inhibits glucagon-stimulated glycogen phosphorylase. Activation of glycogen synthase by glucose has minimal effects in promoting glycogen synthesis. CONCLUSION: Control of glucose-6-phosphatase activity is the primary mechanism of the counterproductive influence of glucose during hyperglucagonemia. Supported by NIH (AM-27619).

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THE DIRECT EFFECTS OF TRIIODOTHYRONINE ON ADIPOCYTE GLUCOSE UTILIZATION AND INSULIN RESPONSE. Vincent A. Rifici* and Murray L. Kaplan. Nutrition Dept., Rutgers University, New Brunswick, NJ 08903.

The action of triiodothyronine (T_3) on the in vitro glucose utilization by adipocytes isolated from the epididymal fat pads of six week old rats was investigated. Cells were treated without or with 10^{-5} or 10^{-8}M T_3 and then incubated for 1 hour without or with 100 uU/ml insulin and U-^{14}C-glucose. Adipocytes incubated with 10^{-5}M T_3 without insulin had decreased incorporation of radiolabel from U-^{14}C-glucose into CO_2, fatty acids, and glyceride-glycerol when compared to untreated cells at media glucose levels of 1.0 mM and higher. There were no changes in glucose utilization with 0.5 mM glucose. The addition of 10^{-8}M T_3 produced no change in glucose conversion by adipocytes. Insulin stimulation of the conversion of U-^{14}C-glucose into $^{14}CO_2$, ^{14}C-fatty acids, and ^{14}C-glyceride-glycerol by adipocytes was lower in cells treated with T_3 compared to cells treated only with insulin. The difference in lipogenesis between the T_3 plus insulin-treated cells and the insulin-treated cells was approximately the same as between basal lipogenesis and T_3 decreased lipogenesis. There was no interactive effect of the two hormones. These data suggest that T_3 has an immediate effect to decrease the glucose utilization by isolated adipocytes. This action of T_3 may be dependent on media glucose concentration. The addition of T_3 to adipocytes does not diminish the insulin responsiveness of these cells.

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GLUCOSE METABOLISM IN RAT HYPOTHALAMUS. Julio M. Martin and Pentti Lautala*, Research Institute, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada.

In vitro glucose oxidation and glucose transport in the rat medial (MH) and lateral (LH) hypothalamic areas were measured. Glucose oxidation was calculated from the conversion of U-^{14}C-glucose to $^{14}CO_2$ and glucose transport from the $^{14}CO_2$ produced from 1-^{14}C-glucose in the presence of phenazine methosulphate and NaF. Increasing glucose in the medium from 1 mM to 20 mM enhanced glucose oxidation two-fold in MH and 40% in LH. Addition of insulin, 100 μU/ml, to the medium decreased glucose oxidation 30% both in MH and LH at both 4 mM and 20 mM glucose. Fasting did not affect glucose oxidation in either of these hypothalamic areas. Glucose transport was not affected by insulin, but was increased significantly when glucose was raised from 0.25 mM to 1.0 mM. Fasting also increased glucose transport in both hypothalamic areas. In conclusion, extracellular glucose concentration seems to be the major regulator of glucose utilization by the rat hypothalamus. Insulin, rather than increasing, seems to decrease glucose oxidation while having no effect on glucose transport. (Supported by a John A. Hartford Foundation Research Grant).

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MEASUREMENTS OF INTRACELLULAR Na^+ AND K^+ DURING INSULIN STIMULATION OF SKELETAL MUSCLE. R.J. Stark, P.D. Read† and J. O'Doherty† Dept. Biol., Purdue Univ. Sch. of Sci. and Dept. Physiol., Indiana Univ. Sch. of Med., Indpls., IN 46223

Measurements of the intracellular Na^+ and K^+ activities (a_{Na}, a_K) and resting membrane potential (E_m) in desheathed rat soleus muscle fibers were made using ion-selective and conventional microelectrodes. These parameters were continuously monitored during insulin stimulation to determine the effect of insulin on a_{Na}, a_K and related changes in E_m. K^+-selective and Na^+-selective (O'Doherty et al. Science 203:1349, 1979) liquid ion-exchanger microelectrodes were used to measure a_K and a_{Na}. The tissues were profused with mammalian Ringer's solution (145 mM Na^+, 5 mM K^+, 2 mM Ca^{++}, 1.5 mM Mg^{++}, 152 mM Cl^- & 2.8 mM glucose) pH 7.4 at 37°C. The profusate was changed to Ringer's solution containing insulin (0.01, 0.1, or 1.0 mU/ml) while maintaining a steady flow rate. The mean ± SEM resting E_m was -77.8 ± 1.3 mV (n-51), a_{Na} was 9.2 ± 0.5 mM (n=37), and a_K was 111.8 ± 6.7 mm (n=17). Continuous measurement before, during, and after the addition of insulin to the profusate (for 4-15 min.), produced no significant change in E_m, a_{Na}, or a_K. These results indicate that the effect of insulin on the plasma membrane and resulting increases in glucose permeability must occur by some mechanism independent of the levels of intracellular Na^+ and K^+. (Supported by NIH AM 26246 and ADA Grants; to RJS a Purdue Research Foundation Faculty XL and Indiana University UROC Grants.)

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PULSATILE PATTERN OF BASAL INSULIN SECRETION IN THE DOG. W.P. Vanhelder,* A. Sirek, O.V. Sirek, K.H. Norwich, Z. Policova,* and T. Vanhelder.* Univ. of Toronto, Toronto M5S 1A8.

Sampling portal blood every 15 minutes by means of indwelling cannulae, we have found evidence that basal insulin secretion in nonanesthetized dogs takes place in 6-7 major secretory episodes over a 24-h period. Reproducible patterns were obtained in four experiments conducted on three normal animals. When frequency of peaks was plotted against the log of insulin concentration, a normal distribution was obtained (lognormal distribution). The means and variances were similar in the three animals: N(3.55, 0.51), N(2.69, 0.81) and N(2.72, 0.88). Low levels of insulin (10-25 μU/ml) alternated with peaks which were occasionally higher than 200 μU/ml. The average duration of a peak was 30 minutes and was independent of ambient glucose levels. The surges observed in the portal circulation seemed to be unrelated to oscillations known to take place in peripheral (posthepatic) insulin concentrations. Surgical hypophysectomy produced inconsistent changes in the pattern of basal insulin secretion of four dogs. The data are in keeping with the notion that the insulin peaks are not solely governed by the hypophysis, implicitly by growth hormone surges, but also by spontaneous repetitive discharges along neuronal pathways functionally related to insulin secretion.

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STUDIES ON INSULIN ABSORPTION FROM DIFFERENT INJECTION SITES IN RATS. W. Zingg, J.M. Smith,* and B.S. Leibel.* Hospital for Sick Children, Toronto, Ontario M5G 1X8

The availability of artificial insulin delivery systems in transplants and mechanical devices, make physiological assessment of the various routes of insulin administration desirable. We have designed a rat model to study the absorption patterns of insulin and the glucose responses, in the peripheral and portal venous circulations and in the thoracic duct simultaneously. Five routes of insulin administration were investigated: intravenous portal (I.V. Po), intravenous peripheral (I.V. Pe), intraperitoneal (I.P.), subcutaneous abdominal (SCA) and subcutaneous leg (SCL). A non-parametric analysis of variance was performed to determine significant differences between administration sites for each sampling site. A respective pattern is seen for each of the routes. I.V. Po and I.V. Pe each resulted in consistent peripheral venous and lymphatic hyperinsulinism with the I.V. Po resulting in a greater fall in glucose concentration. I.P. resulted in a relatively larger amount of insulin appearing in the lymph than I.V. Po and was the only route in which the amount appearing in portal blood was significantly higher than in peripheral blood. The insulin concentration in portal venous blood was in decreasing order: I.V. Pe, I.P. and SCA, SCL. The results suggest that I.P. is the most physiological practical route of insulin administration. (Supported in part by the Medical Research Council Grant No. MA 6542)

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PORTAL AND PERIPHERAL INSULIN AND GLUCAGON LEVELS IN FED VERSUS FASTED RATS WITH REGENERATING LIVERS. Robert P. Cornell. Northeast Missouri State University, Kirksville, MO 63501.

Although insulin and glucagon are putative hepatotrophic factors, increased levels of the two pancreatic hormones together have not been observed in fed rats with regenerating livers. In the present study a partial (67%) hepatectomy (PH) or sham control (SC) consisting of a midline laparotomy with liver manipulation was performed under ether anesthesia. Rats were initially well-fed or fasted for 24 hours prior to surgery with food withheld thereafter. For initially fed rats portal and peripheral insulin were higher at 12 hours after SC (77±7.8 and 36±5.0 μU/ml, n=6) compared to PH (56±4.5 and 28±2.6 μU/ml, n=7). Conversely, portal and peripheral glucagon were higher at 12 hours after PH (1.91±0.315 and 1.05±0.204 ng/ml, n=6) compared to SC (0.53±0.082 and 0.46±0.081 ng/ml, n=7). These fluctuations undoubtedly reflect the differences in liver glycogen and plasma glucose for the two groups of initially fed rats; fed SC=0.26±0.064 mg/dg and 115±4.3 mg/dl (n=6) while PH=0.04±0.007 mg/dg and 95±4.3 mg/dl (n=7). At 24 hr after PH portal and peripheral hyperinsulinemia occurred in initially fasted but not fed rats; fasted SC=39±5.7 and 22±3.2 μU/ml (n=6) while PH=58±6.3 and 29±2.8 μU/ml (n=7). These data suggest that the inability to demonstrate portal and peripheral hyperinsulinemia in initially well-fed rats may be related to the reduction of liver glycogen and, therefore, plasma glucose sustaining reserves in the PH rats compared to the SC group. (Supported by DCWA of St. Louis, MO and NIH Grant AM 22102.)

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TETRAETHYLAMMONIUM MODIFIES GAP JUNCTIONS BETWEEN PANCREATIC B-CELLS. M. Suzanne Sheppard and Paolo Meda*. Institute of Histology, University of Geneva, 1211 Geneva 4, Switzerland.

Gap junctions between the insulin-containing cells (B-cells) of the islets of Langerhans increase during stimulation of insulin secretion (P. Meda et al., J. Cell Biol. 82: 441, 1979) but it is uncertain whether they can also be modified independently of hormone release. Since the K^+ conductance blocker tetraethylammonium (TEA) increases gap junctions in smooth muscle (M.S. Kannan and E.E. Daniel, J. Cell Biol. 78: 338, 1978) and does not alter basal insulin release (J.-C. Henquin, Biochem. Biophys. Res. Commun. 77:551, 1977) we assessed the effects of TEA on B-cell gap junctions. Quantification of gap junctions was performed on freeze-fracture replicas of isolated rat islets incubated for 90 minutes with or without 20 mM TEA at either 2.8 (non-stimulatory) or 6 mM (threshold) glucose. The results show that TEA increased the median size of individual gap junctions ($P<0.01$) as well as the total gap junctional area ($P<0.05$) at both glucose concentrations although gap junction changes were greater at the higher glucose level. As TEA potentiated insulin release in response to 6 mM but not 2.8 mM glucose, these results indicate that B-cell gap junctions may be altered independently of insulin release although greater changes occur when such a release is also elevated. (Supported by the Swiss National Science Foundation no. 3.120.77 and the Canadian M.R.C.)

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GLUCAGON-LIKE IMMUNOREACTIVITY IN RAT HYPOTHALAMUS. Thomas W. Hatton*, N. Kovacevic*, M. Dutczak* and M. Vranic. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario. M5S 1A8.

It has been shown by others that glucagon (IRG) and glucagon-like immunoreactivity (GLI) are detectable in canine brain and GLI (but not IRG) has been demonstrated by immunofluorescence in rat hypothalamus. We report here the extraction and partial characterization of GLI from rat hypothalami (RH). Initially RH were extracted in acid-saline pH 2.8 with 1000 KIU/ml Trasylol (T) or 0.2% benzamidine (B) and large amounts of immunoreactive material were detected by 30K (C-terminally directed) and K4023 (N- and centrally directed) antisera. This material showed an apparent MW of 90,000 daltons by gel filtration on G-150 in 100 mM NH_4HCO_3 pH 8.8 and appeared to displace ^{125}I-IRG from liver membranes. However, whole acid-saline extracts caused significant degradation of ^{125}I-IRG under assay conditions despite the presence of up to 2000 KIU/ml T or 1% B. Degrading activity was not inhibited by 5 uM pepstatin, 10mM EDTA or 1mM phenylmethylsulfonic acid. Therefore, RH were then extracted in acid-alcohol with 1000 KIU/ml T, reconstituted in 2M acetic acid after evaporation and gel filtered on P60 or P30 in 2M acetic acid. Material of 12,000, 9,000 and 3,500MW was detected with K4023 while 30K detected only the 3,500MW material. Extracts did not have any degrading activity, nor was there any non-specific interference. Other brain regions showed no IRG or GLI with acid-alcohol interference. Thus, RH contains substantial GLI but not IRG. The role of these peptides in RH remains to be determined.

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SOLEUS MUSCLE CONTRACTION IN ACUTE ALLOXAN DIABETES. Stephen Paulus* and J. Grossie. Dept. of Physiology, OSU Coll. of Med., Columbus, Ohio 43210.

Fasted rats were rendered diabetic by intravenous treatment with alloxan (50 mg/kg). Two days later Na insulin was administered thrice daily for 6 days. Twitch and tetanic contractile tensions in extensor digitorum longus muscle were significantly reduced 4 days after insulin withdrawal in rats which were severely diabetic (Physiologist 22:48, 1979). Soleus muscle contraction has been studied in order to ascertain differences in response of the two muscle types to severe diabetes. Direct stimulation of normal soleus muscle at 20°C produced an average specific twitch tension of 888±43 gms/cm^2 and a specific tetanic tension of 3195±96 gms/cm^2. On the 6th day of insulin treatment soleus muscle from alloxanized rats showed specific twitch and tetanic tensions of 839±34 and 3337±109 gms/cm^2, respectively. Four days after insulin withdrawal, soleus muscle from moderately diabetic rats generated specific twitch and tetanic tensions of 889±27 and 3379±93 gms/cm^2 respectively. Severely diabetic rats at this time showed average specific twitch and tetanic tensions of 1040±78 and 3567±142 gm/cm^2 respectively, and not significantly different from controls. The normal contraction time (Tc), half relaxation time (T½R) and duration (Dur) of the soleus muscle twitch were: 87±4; 136±9 and 606±30 msec, respectively. By 4 days off insulin severely diabetic rat soleus muscles showed significant increases in these parameters. (Supported by NIH AM23019).

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SPONTANEOUS RELEASE OF ACETYLCHOLINE BY NERVE TERMINALS IN ALLOXAN DIABETIC RAT EXTENSOR MUSCLE. J. Grossie, Dept. of Physiol., Ohio State University, Columbus, Ohio 43210.

Following a 48-hr fast, rats were rendered diabetic with an intravenous injection of alloxan (50 mg/kg) followed by thrice daily treatment with Na-insulin for six days. Control rats were given saline instead of alloxan or insulin. Focal intracellular recordings of minature end plate potentials (mepps) were made in vitro at 20°C. In normal muscle, the arithmetic mean frequency was 1.61±.09/sec (n=40 cells). Mepps were recorded on the sixth day of insulin treatment in alloxanized rat muscles removed 2-6 hours after the last insulin dose and showed a mean frequency of 1.45±.12/sec (n=25). Two days after cessation of insulin treatment, mepps recorded in muscle from moderately diabetic rats showed a slightly reduced frequency of 1.27±.12/sec (n=14) and muscle from severely diabetic rats showed a significantly ($P<.001$) lower frequency of 0.57±.05/sec (n=15). Four days after insulin removal moderately diabetic rats muscle showed an average mepp frequency of 1.25±.09/sec (n=22), significantly ($P<.01$) less than controls. In severely diabetic rat muscle average mepp frequency was 0.47±.05/sec (n=14) and significantly ($P<.001$) less than controls by 3.5-4 days after insulin withdrawal. Average amplitude of control mepps was 0.5±.002 mV and an average resting potential (rmp) of 78±.4 mV. Mepp amplitudes in alloxanized rats varied significantly but were largely explained by differences in rmp and fiber input resistance. (Supported by NIH AM23019).

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ELEVATED PLASMA VASOPRESSIN IN GRAM-NEGATIVE SEPSIS AND SHOCK. M.F. Wilson, D.J. Brackett, P. Tompkins,* L.B. Hinshaw, B.A. Benjamin* and L.T. Archer.* VA Medical Center and Depts. of Medicine and Physiology, Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73104

We reported that in dogs plasma vasopressin (PVP) is elevated to high levels (>500 pg/ml) after endotoxin (LD-100); that it may rise before hypotension and that elevations are sustained. In this study we measured PVP response to E. coli in 6 dogs and 6 baboons. Pentobarbital anesthesia was used, although induction of the baboons was achieved by ketamine. Arterial and venous catheters were placed for continuous monitoring of mean arterial pressure (MAP, mmHg) and heart rate, and for infusion and blood sampling. PVP (pg/ml) was measured by radioimmunoassay at control and up to 5½ hours after beginning a 2 hour E. coli infusion. Control PVP levels were less than 40 pg/ml. The table shows results of E. coli for baboons and dogs.

Hours	C	1	2	3	4	5½	C	½	2
PVP	<40	331	335	205	268	248	<40	672	370
MAP	118	109	67	82	88	96	124	76	71

The data show that massive amounts of vasopressin are released secondary to gram-negative sepsis; that PVP was elevated before hypotension occurred and that the PVP elevation was sustained for the 5½ hour observation period. These high PVP levels may be an important factor in the development of irreversible shock. (Supported by VA Research Service, NIH HL 24590 and Oklahoma Heart Association)

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EFFECTS OF SINOAORTIC DENERVATION ON HYPOTENSION INDUCED BY E. COLI ENDOTOXIN AND BY HYPOVOLEMIA IN CATS. S. Koyama, H. L. Santiesteban, W. S. Ammons and J. W. Manning. Dept. of Physiology, Emory Univ. Sch. of Med., Atlanta, Ga. 30322

Studies were designed to compare the baroreflex buffering capacity to hypotension provoked by E. coli endotoxin and rapid hemorrhage. Within 5 min. after intravenous injection (iv) of 1 mg/kg E. coli endotoxin in alpha-chloralose anesthetized and non-sinoaortic denervated cats, mean blood pressure (MBP) and preganglionic splanchnic nerve (PSN) activity were 75% and 80% of control respectively. There followed a temporary recovery lasting for 2 to 3 min. of MBP and PSN activity to 80% and 90% of control. Finally there was a secondary decay to 45% and 50% of control MBP and PSN activity after 60 min. The temporary recovery of MBP and PSN activity disappeared after sinoaortic denervation, however, the time course of decay in MBP and PSN activity was the same as that in the non-denervated group. Hypotension induced by rapid hemorrhage, 15% blood volume in 30 sec., resulted in a fall in MBP to 90% of control at 5 min. PSN activity was 130% of control level at 5 min. However, in sinoaortic denervated group, MBP and PSN activity induced by hypovolemia were only 65% and 85% of control. These data indicate that arterial baroreceptors do not buffer endotoxic hypotension as effectively as they buffer hypovolemic hypotension. These data support the concept that endotoxin through stimulation of central alpha-adrenergic receptors engage the brain stem descending sympatho-inhibitory pathways as a neural factor in endotoxin shock. (Supported by USPHS NIH Grant HL-16648)

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GRACILIS ARTERIAL ADRENERGIC RESPONSIVENESS DURING HEMORRHAGIC SHOCK. Robert F. Bond, Carol H. Bond*, Lorraine C. Peissner, and Eva S. Manning*. Kirksville College of Osteopathic Medicine, Kirksville, Missouri 63501.

Previous studies suggest gracilis vasculature responds to hypovolemic hypotension by constriction followed by vasodilation (Circ. Shock 4: 327,1977, Circ. Shock 6: 43, 1979). The objectives of the present study were to determine if the effective smooth muscle population and/or adrenergic innervation density varies as the dog moves from reversible to irreversible shock. Gracilis arteries with adventitia intact were removed during various stages of shock; placed in an isolated tissue chamber filled with warm (37°C) Krebs-Henseleit solution and bubbled with 95% O_2 and 5% CO_2. Norepinephrine (NE) dose-response curves and perfiarterial electrical stimulation (ES) frequency-response studies were conducted. The maximum response to NE was unaltered but the maximum responses to ES decreased during both compensatory and decompensatory hypovolemic hypotension. The sensitivity of the innervated α-receptors as measured by the ES necessary to obtain a 50% maximal response (ES_{50}) fell below control during decompensatory hypotension but returned during normovolemic shock. The sensitivity of α-receptors to NE (ED_{50}'s) increased during hypovolemic decompensation and normovolemic shock, but no indication of either α-receptor or smooth muscle desensitization were noted during any stage of shock. (Supported by grants from the AOA and NIH Grant #RR09130).

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MECHANISMS OF NOREPINEPHRINE (NE) DEPLETION IN ENDOTOXIN SHOCK. B. J. Pardini, S. B. Jones and J. P. Filkins. Department of Physiology, Loyola University of Chicago, Maywood, IL 60153.

Endotoxin shock depletes NE in peripheral organs (*Physiologist 22*: 98, 1979) and may underwrite sympathetic nervous system dysfunction. Present experiments employed the spleen as a model for evaluating the mechanism of NE depletion in endotoxicosis. S. enteritidis endotoxin (ET), 15 mg/kg, or saline was administered iv to fasted male Holtzman rats. Spleen NE (SNE) and plasma glucose (PG) were measured at 5 hrs post-injection or near death. ET rats were divided into mild (PG> 40 mg%) and severe (PG<40 mg%) shock groups. Compared to control SNE (1.21±.07 μg/gm), SNE of mild (.79±.10 μg/gm) and severe (.30±.05 μg/gm) ET rats was decreased ($p<.001$). Regression analysis of SNE versus PG for all ET rats yielded $r=.75$ ($p<.01$). To investigate the contribution of impaired reuptake to NE depletion, spleen slices from control and severely shocked rats were incubated with ^{3}HNE (18.3 ng/ml,.1μCi/ml) in oxygenated KRB. ET spleens incubated at 0, 10, 20, and 30 min exhibited 33%, 58%, 51% and 42% decreased net NE retention compared to controls ($p<.05$ at all times). ^{3}HNE/total ^{3}H in ET spleens incubated for 0, 10, and 20 min was reduced 44%, 31% and 20% compared to controls ($p<.05$ at all times) which suggests greater degradation of retained NE in ET spleens. These data indicate NE depletion in ET shock is: 1) related to the severity of shock, and 2) mediated by decreased reuptake and increased degradation of NE. (Supported by NIH Grant HL 08682)

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THE EFFICACY OF NALOXONE (NAL) IN SPLANCHNIC ISCHEMIC SHOCK (SIS). N.J. Gurll, D.G. Reynolds, R.E. Lind* and J.T. Jenkins* Dept. of Surgery, Univ. of Iowa College of Medicine, Iowa City, Iowa 52242.

The opiate receptor antagonist nal improves cardiovascular performance in several models of experimental shock, but its effects in SIS have not been studied. Anesthetized healthy adult mongrel dogs were instrumented to measured mean arterial pressure (MAP), cardiac output (CO), and maximal left ventricular dp/dt (LV dp/dt max). At t=0 we occluded the superior mesenteric artery and either the splenic artery (Group I, n=14) or celiac axis (Group II, n=10) with resultant fall in CO of about 0.5 L/min. At t=2 hr the occlusions were released resulting in decreases of MAP, CO, and LV dp/dt max which were more marked in Group II than in Group I. At t=3 hr half the dogs were treated with nal i.v. 2 mg/kg bolus plus 2 mg/kg·hr infusion, and half got saline in equivalent volumes. Nal increased MAP by 17±4 mmHg ($p<.001$), CO by 0.12±0.02 L/min ($p<.001$), and LV dp/dt max by 239±106 mmHg/sec ($p<.02$) in Group I whereas saline had no effect. Nal was no more effective in Group II than in Group I in restoring MAP, CO, and LV dp/dt max (which increased by 20±4 mmHg, 0.11±0.10 L/min, and 210±20 mm Hg/sec respectively). Scanning EM showed no protection from ischemic damage with nal, and all dogs died within 24 hrs. Nal is only mildly beneficial which is consistent with a minor role for endogenous morphine like substances (endorphins) in SIS. Enkephalins from the gut may be only weak cardiovascular depressants.

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HEPATIC GLYCOLYTIC INTERMEDIATES IN A RAT HEMORRHAGIC SHOCK MODEL. Robert E. Kuttner* and William Schumer. Dept. of Surgery, Univ. of Health Sciences/Chicago Medical School at VA Medical Center, North Chicago, IL 60064.

The final phases of different shock states are frequently accompanied by hypoglycemia. A common view attributes the blood glucose decline to circulatory deficits causing hypoperfusion, tissue hypoxia, and ultimately failure of gluconeogenesis in liver. Since previous work established that in endotoxemia and sepsis the levels of rat hepatic glycolytic intermediates show changes before hypoglycemia can be detected, it was of interest to compare liver metabolites from hypovolemic rats at the same interval. Fasted albino male rats (180-245 g) were bled in 10 minutes of 40% of total blood volume which was estimated as 8% of body weight. The initial mean systemic blood pressure was 40-45 mm Hg. Liver samples obtained by freeze clamp were analyzed. Phosphoenolpyruvate at 3 and 5 h posthemorrhage was 208 and 273 nanomole/g wet tissue respectively. Glucose-6-phosphate was 231 and 271 nmoles. Fructose-1,6-diphosphate was 10 nmole at both times. N=8 for all samples. These values did not differ significantly from controls. Systemic hypotension failed to duplicate the pattern of changes in endotoxemic and septic livers which argues against circulatory deficits as being the key factor in shock metabolic derangements. Dysfunction at a microvascular site as a trigger event is also unsupported as liver lactate was normal.
(Aided by a VA Medical Research Service Grant).

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GLUCOSE METABOLISM IN SEPTIC RATS. Dennis L. Kelleher*, Patricia A. Puinno*, Bing-Chiong Fong*, and Judy A. Spitzer. LSU Medical Center, New Orleans, LA 70112

Glucose metabolism was measured in male rats during septic shock. Acute peritonitis was produced by cecal ligation and puncture. Sham operated rats underwent a laporotomy alone. After 15 hours the rats were anesthetized with pentobarbital and catheters were placed into a carotid artery and jugular vein for blood sampling and infusion. A primed-constant infusion of ^{3}H-6-glucose was begun. Arterial blood samples were drawn at 90, 105, and 120 min following the onset of infusion. The animals were sacrificed and examined for gross signs of peritonitis. Plasma glucose concentration, plasma lactate, blood pH, and hematocrit were determined. Initial MABP did not vary between septic and sham operated rats. During the infusion MABP of septic rats fell progressively, while the MABP of sham rats was stable. Initial heart rates of septic rats were significantly elevated above sham rats and remained elevated. Hematocrits of septic rats were elevated above controls and both groups fell similarly following blood sampling. Blood pH and plasma glucose did not vary between the two groups. Plasma lactate, glucose turnover and metabolic clearance of glucose were significantly elevated in septic rats. The data suggest an accelerated rate of carbohydrate metabolism in septic rats. (Supported in part by NIH Training Grant GM07029 and Navy Contract N-00014-76-C0133)

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PREVENTION OF DEATH IN ENDOTOXIN SHOCK BY FRUCTOSE DIPHOSPHATE ADMINISTRATION. Angel K. Markov*, Don Turner, Nicole Oglethorpe* and Harper K. Hellems. University of Mississippi Medical Center, Jackson, Mississippi 39216

Aerobic metabolism is greatly reduced in endotoxemia and the organism depends largely on energy derived from glycolysis. In late sepsis, glycolysis is rate-limited by acidosis at the phosphofructokinase (PFK) step. Theoretically, administration of fructose-1,6-diphosphate (FDP) should provide substrate that bypasses the metabolic bottleneck. To test this hypothesis, lethal IV doses of E. coli endotoxin were given to 21 dogs. Ten dogs were treated with FDP (FT) and 11 dogs were treated with glucose (GT). Mean arterial pressure of the GT surviving 6 hrs. observation was 35 $\pm$ 7.21% below control, while the FT group returned to control by the 5th hr. ($P < 0.05$). All GT had bloody intestinal fluid loss of 830 $\pm$ 67 ml./6 hrs., while in the FT group no blood was noted and fluid loss was 285 $\pm$ 98 ml./6 hrs. Urinary output of FT dogs was 276 $\pm$ 98 ml./6 hrs. and that of the GT group, 46 $\pm$ 25 ml./6 hrs. ($P < 0.01$). Hemorrhagic necrosis was observed in the stomach and intestine of the GT dogs, while in the FT dogs only vascular congestion was noted. Survival in the FT group was 90% and 18% in GT ($P < 0.01$). Systemic administration of FDP to dogs subjected to lethal doses of endotoxin vastly reduces mortality; prevents circulatory collapse and intestinal hemorrhagic fluid loss; conserves normal urinary output; and protects the mucosa of the stomach and intestine from hemorrhagic necrosis.

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SERUM IMMUNOREACTIVE INSULIN (IRI) AND NONSUPPRESSIBLE INSULIN-LIKE ACTIVITY (NSILA) IN ENDOTOXIN (ET) SHOCK. James P. Filkins. Department of Physiology, Loyola University Medical Center, Maywood, Illinois 60153.

This study compared basal and glucose-provoked IRI and NSILA in serum from fed male rats in either early, mild endotoxicosis-E (1 mg S. enteritidis ET, 3 hrs prior) or late, severe ET shock (5 mg ET at 5 hrs prior). IRI was quantitated using the Phadebas method. NSILA was bioassayed in rat epididymal fat pads (Unit=DPM-$^{14}CO_2$ produced/gram fat/hr x 10^4). In comparison to saline treated controls-C (N=5), mild E (N=15) was associated with a basal hyperglycemia (C=105±8 vs E=161±4 mg/dl) and an increased IRI (22±4 vs 132±9 μU/ml); no differences in NSILA were measured (C=5.1±.62 vs E=4.2±.31 U). Glucose (400 mg iv, 10 min prior) increased IRI in C to 62±6 μU/ml but produced no further increments in E rats - - to 146±12 μU/ml; NSILA was not altered in either the C (5.8±.81U) or the E (3.8±.42 U) rats. Late, severe E was marked by hypoglycemia (C=116±12 vs E=41±8 mg/dl) and a basal IRI hypoinsulinemia (C=36±6 vs E=9.2±3.8 μU/ml); however, NSILA was increased from C=6.2±.54 to E=10.4±.72 U. Glucose provoked an increased IRI (C to 71±12 and E to 138±16 μU/ml) without further increments in NSILA (C=5.4±.61 vs E=11.8±.92 U). These data indicate that early endotoxicosis is associated with hyperinsulinemia due to hyperglycemia and enhanced pancreatic secretion of IRI; in contrast, the hyperinsulinism of late ET shock and progression to hypoglycemia are related to increased NSILA and low IRI. (Sup. by USPHS Grant HL 08682.)

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LOW AND HIGH THRESHOLD FIBERS IN THE VAGI REVISITED. Dale M. Lombardi*and Frank P. Brooks. Dept. of Physiology, University of Pennsylvania, Philadelphia, Pa. 19104.

In 1965 Martinson proposed that there were two populations of efferent vagal fibers supplying the stomach: low threshold, responding to electrical pulse durations of 0.02-0.5 msec and eliciting contraction of smooth muscle, and high threshold, responding at 1-5 mscc with relaxation of smooth muscle and acid secretion. We have reported that phasic antral contractions recorded by extraluminal force transducers and acid secretion both respond optimally to electrical vagal stimulation at 4-5 msec. Using cats anesthetized with chloralose and a balloon filled with 125 ml of saline in the corpus as well as a force transducer on the antrum, we find that at short pulse durations a stimulus bound increase in basal tension independent of phasic contracions does occur. At longer pulse durations there is a post-stimulus fall in basal tension below the original level, and phasic contractions as well as acid secretion. With atropine a purely inhibitory relaxation in basal tone can be seen. Therefore, we confirm Martinson's two threshold fiber populations and find that the high threshold efferents mediate propulsive phasic contracions and acid secretion via cholinergic fibers as well as inhibition of the tone of the corpus muscle via non-cholinergic pathways. Supported by USPHS#RO1AM14563

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ADAPTIVE CONTRACTION AND PYLORIC CLOSURE IN ISOLATED STOMACHS FROM CATS AND RABBITS. K. Schulze-Delrieu, University of Iowa, Iowa City, Iowa 52242.

We sought mechanisms that contribute to the rise of luminal pressures if one fills stomachs from cats and rabbits in vitro. Pressures rose linearly with increasing volumes of intragastric Krebs solution. After several minutes of contractile adaptation, pressures were similar on emptying and on filling the stomachs for a wide range of volumes. Removal of Ca^{++} decreased luminal pressures at volumes from 40 to 260 ml. Stimulation of muscle through excessive K^+ in the Krebs solution increased them (Table).

The function of the pylorus was studied by changing pressures in the distal antrum while measuring pressures in the duodenal bulb and vice versa. In 6 cats the maximal gastroduodenal and duodenogastric pressure gradients at which no transpyloric flow occurred was 20 cm H_2O. Ca^{++} removal decreased the maximal pressure gradient supported by the pylorus and K^+ depolarization increased it. Thus, contraction of muscle in the gastric wall and in the pylorus contributes to the pressure rise that occurs on filling isolated stomachs. The luminal pressures generated are generally below the pressure threshold at which transpyloric flow occurs.

TABLE: Pressures (cm H_2O) in isolated stomachs (at 150 ml).

		Normal Krebs	Ca^{++} free Krebs	High K^+ Krebs
Rabbits	(6)	6.5 ± 0.9	4.4 ± 0.4 p<0.05	11.5 ± 0.1 p<0.05
Cats	(5)	8.9 ± 2.7	4.5 ± 1.2 N.S.	10.10 ± 0.7 p<0.05

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PERIPHERAL PLASMA IMMUNOREACTIVE MOTILIN (IM) CHANGES DO NOT REFLECT VAGALLY STIMULATED IM RELEASE FROM CANINE DUODENUM. J.E.T. Fox, N.S. Track*, E.E. Daniel, J. Howard*. Dept. Neurosciences, McMaster University, Hamilton, L8N 3Z5 and Dept. Clinical Biochemistry, University of Toronto, Toronto, Canada.

Both endogenous and exogenous motilin initiate migrating motor complexes (MMC) but the relationship to peripheral plasma IM levels is unclear. Since increased vagal activity occurs with MMC we investigated whether a, vagal stimulation releases IM into duodenal veins and b, changes in duodenal venous IM are reflected in peripheral IM levels. Duodenal venous and peripheral blood were collected via cannulae in 10 anaesthetized dogs. Cervical vagi were stimulated peripherally at 15V, $5H_z$ and 5 ms. Plasma collected before and during stimulation, was analyzed for IM by radioimmunoassay using antibody GP71. Vagal stimulation increased duodenal IM by 159± 48 pg/ml ($p<0.01$) while peripheral IM sampled simultaneously was only increased by 28± 23 pg/ml (NS). Atropinization blocked this release. These results indicate that vagal stimulation releases duodenal IM but this release is not reflected in peripheral levels. Thus, the increase in vagal activity associated with the MMC may release motilin locally which probably facilitates the MMC onset. The poor correlation of motor activity with peripheral IM is probably due to a damping of the local release by dilution, distribution and clearance of motilin in the periphery. Supported by the Medical Research Council of Canada.

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EFFECTS OF GUT DISTENSION ON AUERBACH'S PLEXUS AND INTESTINAL MUSCLE. S. Yokoyama* (SPON: E. Bozler). Dept. Physiol., Fukushima Medical College, Fukushima, 960 JAPAN

Using peeled longitudinal muscle strip which attached to an intestinal segment of rabbit, effects of gut distension on both neurons in Auerbach's plexus and intestinal muscle were investigated. Distension caused excitatory or inhibitory effect on mechanosensitive neurons. The neurons whose spontaneous discharges showed a frequency increase during muscle contraction and a frequency decrease during muscle relaxation responded to distension with the excitation. The neurons which did not show any correlation in their frequency with muscle contraction and relaxation responded to distension with the inhibition. Additional neurons were found in small numbers whose spontaneous activity did not relate in frequency to muscle contractions and did not change by gut distension. In all cases of distension the excitatory effects on longitudinal muscle strip and intestinal segment were observed and peristaltic movements appeared. It was concluded that gut distension causes excitation of mechanosensitive neurons which regulates contractions of intestinal muscle and produces the peristaltic movement. (Supported by a grant from the Ministry of Education of Japan).

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THE CONTRIBUTION OF THE MUSCULARIS MUCOSA TO THE SEGMENTING ACTIVITY OF THE CANINE SMALL INTESTINE. J.S. Martin and M.F. Tansy. Dept. of Physiol. and Biophys., Temple Univ. Hlth. Sci. Ctr., Philadelphia, PA 19140.

Previous data acquired from intraluminal balloons have shown that the canine muscularis mucosa (MM) alone is capable of producing significant pressure responses to cholinergic and adrenergic stimuli. The purpose of these experiments was to determine whether the pressure events produced by the MM could be associated with significant segmenting activity of both the MM and the intact ilea (IL) of 6 chloralose-urethanized dogs. A segment of MM was decoupled from the tunica muscularis as previously described (Fed. Proc. 37: 373, 1978). Recording ballons were placed in this segment and an adjacent segment of intact ileum. Brodie-type strain gauges were applied circumferentially over the regions of the balloons in both segments and intraluminal pressure responses (IPR) and force events (FE) were recorded simultaneously. Recordings were made during periods of spontaneous motor activity (SMA) as well as after i.a. epinephrine (EPI) and acetylcholine (ACH) infusions. The following data were obtained:

	MM-IPR(cmH_2O)	MM-FE(G)	IL-IPR(cmH_2O)	IL-FE(G)
SMA	1.68± 1.33	2.39± 1.51	1.83± 1.14	1.70± 0.74
EPI	11.36± 5.38	4.00± 1.40	13.00± 4.24	3.40± 1.06
ACH	28.60±22.30	14.50±11.40	24.58±14.40	27.00±19.60

It is concluded that IPR are coincident with FE and the magnitude of both support the concept that the MM mediates a significant portion of canine small bowel segmentation.

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SPONTANEOUS AND STIMULATED CONTRACTILE ACTIVITIES OF CANINE ILEAL MUSCLE DO NOT REQUIRE "SPIKES". Kenton M. Sanders. Eastern Virginia Medical School, Norfolk, VA 23501

It has been reported that contraction of circular muscle of the small bowel is directly dependent upon development of "spike" potentials at the peaks of slow waves. The present study investigated the relationship between slow wave amplitude and contractile amplitude in isolated strips of canine ileal muscle. Segments of ileum, 10-16 cm from the ileocecal junction, were removed from dogs anesthetized with pentobarbital. Strips of muscle (2x6mm) were cut parallel with the circular fibers. The muscles were placed in a recording chamber perfused with Krebs solution. Membrane potential of single circular muscle cells was recorded by standard microelectrode techniques. Mechanical activity of circular fibers was simultaneously measured by a force transducer attached to one end of the muscle strip. Muscles were spontaneously active, producing slow waves at a frequency of 10/min. In 80% of the muscles studied, small phasic contractions followed each slow wave. Spontaneous "spike" potentials were not observed. While maintaining impalements of 3 cells of 3 preparations several doses of acetyl choline (ACh) were administered. ACh (10^{-8}-$3x10^{-7}$ M) caused a dose-dependent increase in the amplitude of the slow waves and in the amplitude of contractions. "Spike" potentials and large contractions were observed when the concentration of ACh exceeded 10^{-6}M. In conclusion, small spontaneous contractions occurred in ileal muscle strips in the absence of "spike" activity. Contractions in response to ACh were related to slow wave amplitude and also occurred in the absence of "spike" activity. (Supported in part by NIH RRO 9028-03.)

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CHRONIC ESTRADIOL EXPOSURE INDUCED HYPERACTIVE GASTROINTESTINAL MOTILITY IN MALE RATS. Larry A. Bruce and Faiz M. Beshudi* Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

An increase in strength of contraction in vitro has been demonstrated in three regional gastrointestinal tissues from male rats which were chronically exposed (4 days) to 17-β estradiol (3 dose levels) when compared to the corresponding regional tissues from male rats not exposed to the steroid. Evaluation of the data with regression analysis illustrates differences in sensitivities among the three regional tissue responses when correlated with three serum 17-β estradiol concentrations; i.e., colonic tissue responses > esophageal tissue responses > antral tissue responses. The 17-β estradiol dose levels administered sc were 75, 300 and 600 μg/kg/day resulting in serum 17-β estradiol concentrations of 3.9, 28.9 and 51.4 ng/ml for each treatment group, respectively. The estradiol concentrations measured (RIA) from the serum of the treated animals were comparable to estrogen concentrations reported in women taking oral contraceptives or during pregnancy suggesting a causal relationship between elevated serum estrogen and gastrointestinal hyperactivity. (Supported in part by NIH Grant #AM-21657).

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VAGAL STIMULATION, 5-HT AND MIGRATORY BEHAVIOUR OF *HYMENOLEPIS DIMINUTA* IN THE RAT INTESTINE. Chi Hin Cho* and David F. Mettrick. Department of Zoology, University of Toronto, Toronto M5S 1A1, Canada.

Stimulation of the efferent vagal nerve (2 msec, 5 V 10 Hz) for up to 60 min in rats infected with ten 11-day-old *Hymenolepis diminuta* induced a time-dependent significant ($P<0.01$) anteriad movement of worm biomass. Ligation of phloric sphincter enhanced the migrational response. Blood 5-HT levels, monitored during vagal stimulation, were significantly elevated after 15 min ($P<0.05$); 5-HT in the intestinal mucosa was significantly decreased while that in the lumen was increased after 30 min ($P<0.05$). Worm tissue levels of 5-HT were not affected. Parenteral, intramuscular and subcutaneous injections of 5-HT (5 mg/Kg BW) all induced a significant anteriad worm migration ($P<0.02$ or lower) and elevated blood ($P<0.01$ or lower) and luminal ($P<0.05$) 5-HT levels for up to 3 hrs following injection. Worm 5-HT levels increased following subcutaneous injection ($P<0.05$). Results indicate vagal stimulation induces a similar worm migration to that following normal host feeding. The migration response following 5-HT administration implicates serotonin as an agent involved in worm migration. However, the extent of the migration was less than that following feeding or vagal stimulation implying that other neurohormonal changes are also involved. (Supported by NSERC, grant A 4667 to DFM)

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EFFECTS OF HYPOXIA ON THE ENERGY METABOLISM AND CONTRACTILE ACTIVITY OF THE ISOLATED, PERFUSED WHELK VENTRICLE. W. Ross Ellington. Univ. S.W. Louisiana, Lafayette, La. 70504.

Molluscan hearts tend to be highly resistant to exposure to hypoxic conditions. Through perfusion of the ventricle of the whelk, Busycon contrarium, under hypoxic conditions (PO_2= 10-14 mm Hg) resulted in rhythmic contractile activity for two hours. Extended perfusion under hypoxia produced a gradual decrease in contractile force as well as irregularity in the occurrence of contractile events. The levels of adenylates and key glycolytic intermediates were determined in neutralized, perchloric acid extracts of ventricular preparations obtained before and during hypoxia. The adenylate energy charge remained constant during the first two hours of hypoxia and showed only a slight decline after four hours (0.92→0.90). Succinate and alanine, both established end products of anaerobic glycolysis in molluscs, accumulated throughout the time course of hypoxia (e.g.,succinate levels increased twenty five fold). During the later stages of hypoxia there was also a decline in arginine phosphate levels. This decrease in the size of the phosphagen pool was accompanied by a small increase in the tissue levels of D-octopine. The results of this study show that the energy demands of the hypoxic whelk ventricle are partially supported by an activation of anaerobic glycolysis which is characterized by the accumulation of multiple end products. (Supported by the American Heart Association, Louisiana Affiliate)

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NEURAL REGULATION OF CYCLIC AMP AND INOTROPIC STATE IN BULLFROG VENTRICULAR MYOCARDIUM. Ronald R. Fiscus* and Steven E. Mayer. Division of Pharmacology, UCSD, La Jolla, CA 92093.

Cyclic AMP (cA) has been proposed to mediate the positive inotropic response in heart elicited by β-adrenergic agonists. Few experiments have directly tested the hypothesis that cA mediates responses induced by stimulation of cardiac sympathetic nerves. In the present study, we stimulated the left vagus nerve (containing both parasympathetic and sympathetic fibers) in bullfrogs and measured cA concentrations and inotropic responses of the ventricles. Hearts were perfused in situ with modified frog-Ringer introduced through the posterior vena cava. The aorta was cannulated for measurement of pressure (AP) and dP/dT. Atropine (10 μM) was infused in some frogs to selectively block parasympathetic action. Concentrations (pmole/mg protein) of cA were: control = 2.72 ± 0.15, vagal stimulation (5 Hz, 1 min) = 3.59 ± 0.38, control with atropine = 4.22 ± 0.30, vagal stimulation (5 Hz and 20 Hz, 1 min) with atropine = 11.9 ± 1.1 and 12.5 ± 2.9, respectively. Atropine alone increased cA, AP, and dP/dT. Vagal stimulation during atropine infusion caused a larger increase in cA, AP and dP/dT. Vagal stimulation without atropine infusion had no effect on cA and decreased AP and dP/dT. Our data are consistent with a role for cA in sympathetic neural regulation of the heart and show an interaction between parasympathetic and sympathetic nerves in regulating myocardial cA. (Supported by grant NHLI 22961 and a fellowship from the California Heart Association.)

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FMRFamide AND ACETYLCHOLINE 'CATCH' CONTRACTURES OF A MOLLUSCAN SMOOTH MUSCLE: DIFFERENT CALCIUM DEPENDENCIES. S.D. Painter, D.A. Price and M.J. Greenberg. Fla. State Univ., Tallahassee, FL 32306

Both acetylcholine (ACh) and the molluscan neuropeptide, phenylalanyl-methionyl-arginyl-phenylalanine amide (FMRFamide), induce catch contractures in the anterior byssus retractor muscle (ABRM) of Geukensia demissa and Mytilus edulis: both are relaxed by 5-hydroxytryptamine. FMRFamide has a lower threshold than ACh, but maximal ACh contractures are more forceful than those of FMRFamide. FMRFamide activity is neither blocked by ACh antagonists, nor potentiated by anti-cholinesterases, suggesting that FMRFamide acts directly on the ABRM rather than indirectly, via neural release of ACh. FMRFamide contractures are less sensitive to the removal of Na from the bathing medium, and less labile in Ca-free medium (with EGTA), than those of ACh. The relatively slow decay of the FMRFamide response in Ca-free medium suggests either that FMRFamide has access to a more slowly exchanging pool of intracellular Ca than ACh, or that the FMRFamide excitation-contraction coupling mechanism is different than that of ACh, and less sensitive to Ca depletion. (Supported by NIH grant HL-09283 to M.J.G.)

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OXYGEN AND CARBON DIOXIDE IN ELECTROPHYSIOLOGICAL RECORDING FROM INSECT MUSCLES. Richard A. Lockshin and Mario Rocco,* Dept. of Biol. Sci., St. John's University, Jamaica, N.Y. 11439

1. Resting potentials of insect muscle are reported to be of the order of -40 to -50 mv. When intersegmental muscles of Manduca sexta and Antheraea polyphemus (Lepidoptera) are fully exposed and the tracheae are removed, we also record values of -45 to -48 mv. If however effort is made both to leave tracheae intact and to prevent submergence of the spiracles in the bath fluid, we record resting potentials ranging as negative as -80 mv, with a mean of -57.5, over periods of at least 5 hrs at room temperature. Thus, in agreement with the earlier findings of Rheuben (J. Physiol. 225: 529 (1972)) insect fibers are often subject to hypoxia during standard dissection procedures. 2. Fibers are frequently found to be in poor condition if the insect is anesthetized with carbon dioxide prior to dissection. Direct exposure of the dissected preparation to the gas irreversibly damages the fiber within five seconds. Depolarization is accompanied by a drop in input capacitance, consistent with rapid swelling of the fiber. No such failure is seen when glycine or other buffers are used. Supported in part by the N.S.F. (PCM 77-15687).

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MORPHOMETRICS, CONDUCTANCE, AND THORACIC FLIGHT TEMPERATURE OF NOCTUID AND GEOMETRID MOTHS. Timothy M. Casey and Barbara Joos,* Rutgers University, New Brunswick, N.J. 08903 and Univ. of Michigan, Ann Arbor, MI. 48109.

Body mass of geometrid moths was lower ($\bar{x}$ = 0.063g±.038S.D. N=50) than that of noctuid moths (0.120g±0.043, N=70 but wing area was similar in the two families yielding wing loadings about twice as high in the noctuids. In both families wing area was independent of body mass while wing loading showed highly signiticant ($P < 0.01$) positive correlation with body mass. Thoracic conductance in both families showed a similar strong inverse relation to body mass. The thoracic temperature excess ($T_{th}-T_a$) of noctuid moths during flight was greater (10.2°C±3.4, N=70) than that exhibited by geometrids (5.6°C ±2.9, N=50). $T_{th}-T_a$ in both families is positively correlated with body mass and wing loading. Higher thoracic temperatures of noctuids during flight is a consequence of greater rates of heat production associated with their higher wing loading, as well as lower thoracic conductance as a result of their larger body mass. Between T_a's of 11 to 22°C, $T_{th}-T_a$ of geometrid moths was similar while $T_{th}-T_a$ of noctuids decreased with increasing T_a. Geometrids were capable of immediate flight at all T_a's. Flight of noctuid moths was routinely preceeded by a bout of pre-flight warm-up. Indications of thermoregulation during flight in noctuid moths but not in geometrid moths is related to their morphology and flight patterns. (Supported by NSF Grant PCM77-16450).

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ENERGETICS OF RUNNING TARANTULAS. Clyde F. Herreid II and Robert J. Full*. SUNY/Buffalo, NY 14260.

Tarantula spiders with an average mass of 12.8g were run at various speeds on a plexiglass enclosed treadmill. Oxygen consumption ($\dot{V}O_2$, ml O_2/g x hr) was monitored by an S-3A Applied Electrochemistry oxygen analyzer during a 30 min rest period followed by a 20 min exercise bout and a 30 min recovery period. A steady state $\dot{V}O_2$ was reached in approximately 14 min independent of velocity. $\dot{V}O_2$ varied in a linear fashion with running velocity where $\dot{V}O_2$=.43 (velocity, km/hr) + .133. The $\dot{V}O_2$ predicted by the above equation at zero running velocity is 3.5 times that of the observed resting $\dot{V}O_2$ of .038 ml O_2/g x hr. Such an elevated rate has been recorded for vertebrates also. A large oxygen debt was observed after exercise which increased with velocity and was considerably greater than 30 min in duration at higher velocities. Invertebrates such as the cockroach (Physiologist 22: 55, 1979) and the land crab (Resp. Physiol., 36: 109-120, 1979) have a minimum cost of transport which is comparable to values of vertebrates. In contrast, the minimum cost of transport for the tarantula (.43 ml O_2/g x km) is only one-fourth the value predicted (1.9 ml O_2/g x km) for a vertebrate of a similar mass. (Supported by NSF grant PCM 79-02890).

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THERMAL DEPENDENCE OF ENDURANCE, OXYGEN CONSUMPTION, AND COST OF LOCOMOTION IN A LIZARD. Henry B. John-Alder* and Albert F. Bennett. Univ. of California, Irvine, CA, 92717

Rates of oxygen consumption ($\dot{V}O2$) of desert iguanas, Dipsosaurus dorsalis, were measured in animals resting or running on a treadmill at speeds up to 1.6 km/h. Body temperature (Tb) was regulated at either 25C or 40C, preferred body temperature (PBT) of this lizard. Sustainable speed and stamina increased greatly with increased Tb: the highest speed sustained for at least 15 min increased from .3 km/h at 25C to .8 km/h at 40C. Between 25C and 40C, $\dot{V}O2rest$ increased from .14 to .32 ml O2/(g·h) and $\dot{V}O2max$ increased from .74 to 2.00 ml O2/(g·h). The speed at which $\dot{V}O2max$ was attained increased from .4 km/h at 25C to .9 km/h at 40C. Net cost of locomotion, given by the slope of $\dot{V}O2$ vs speed, averaged 1.38 ml O2/(g·km) and was not thermally dependent. At any sustainable speed, $\dot{V}O2$ and the total cost of locomotion, given by $\dot{V}O2$/speed, were higher at 40C. There are behavioral and energetic costs and benefits associated with a relatively high PBT. Energetic costs associated with a Tb of 40C are seen in the higher $\dot{V}O2rest$ and total cost of locomotion. Benefits are an expanded aerobic scope and a wider range of sustainable locomotory speeds. (Supported in part by NSF Grant PCM77-24208 and NIH Grant K04 AM00351)

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ELASTIC ENERGY STORAGE BY KANGAROO RATS DURING HOPPING. Andrew A. Biewener*, R. McNeill Alexander*, and Norman C. Heglund*. (SPON: C.R. Taylor). Museum of Comp. Zoology, Harvard Univ., Cambridge, MA 02138.

At high hopping speeds big red kangaroos and wallabies store and recover up to 70% of the decrements in kinetic and potential energy that occur during each step from elastic elements, based on $\dot{V}O_2$ and whole animal external work measurements. This study asked if storage and recovery of energy in elastic elements is equally important in a small hopper. Ground forces acting on the feet of four kangaroo rats (av. mass, 0.098 kg) were measured using a force plate. Limb positions were determined simultaneously using high speed x-ray cinematography. The forces and length changes of extensor muscles of the hind limbs and their tendons were calculated from these measurements and the moment arms of the muscles acting around their joints. No elastic storage and recovery was found in hip or knee extensors over the range of speeds used in the experiments (0.9 - 3.1 $m \cdot sec^{-1}$). A maximum of 16% of the decrements in kinetic and potential energy during each step was calculated to be recovered from stored elastic strain energy in tendons and cross-bridges of ankle extensors (assuming Hookean behavior of these elements). This value was independent of hopping speed. We conclude that the cross-sectional area of the tendons of kangaroo rats is too large to allow them to store significant amounts of strain energy during hopping. (Supported by NRS Training Grant # 5T32GM07117, NIH Grant # AM18140 and NSF Grant # PCM7823319).

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ENERGY METABOLISM OF RUNNING MINKS IN RELATION TO SPEED AND GAIT PATTERN. Terrie M. Williams. Rutgers University, New Brunswick, N.J. 08903. .

Oxygen consumption ($\dot{V}O_2$) and locomotory performance (stride frequency and length, gait analysis) were investigated in the mink (Mustela vison, Schriber) trained to run on a treadmill at speeds from 1 to 7 km/hr. Steady state $\dot{V}O_2$ increased non linearly with increased running speed and was greater at all speeds than predicted for mammals of similar mass. The break in linearity corresponded to a change in gait pattern from walking to bounding. Within each gait pattern $\dot{V}O_2$ showed a linear relation to speed but the slope ($\dot{V}O_2$/speed) was significantly reduced in the bounding gait. This gait transition occurred at a running speed well below the trot/gallop transition point predicted by Heglund et al (1974) (3.5 km/hr as compared to 5.12 km/hr). However, stride frequency at the transition was only 14% lower than predicted values. Below the walk/bound transition stride frequency increased with increased running speed while above it stride frequency remained essentially constant. These results suggest that changes in energy metabolism is closely correlated with changes in stride frequency and gait in the running mink. The relatively high levels of running metabolism may be the consequence of short legs and an elongate body shape. (Supported in part by NSF Grant PCM 77-16450).

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THE INFLUENCE OF FEEDING ECOLOGY ON FUEL UTILIZATION BY FLIGHT MUSCLES OF SOUTH AMERICAN BATS. M.E. Yacoe, J.W. Cummings*, P. Myers*. The University of Michigan, Ann Arbor, MI 48109

The relationship between diet and flight muscle enzyme profile was examined in 10 species of S. American bats of the families Vespertilionidae, Molossidae, and Phyllostomatidae. Animals were grouped by diet as either frugivorous (high carbohydrate) or insectivorous (low carbohydrate, high lipid). Activities of citrate synthase (CS), 3-hydroxyacyl CoA dehydrogenase (HOAD), and hexokinase (HEX) were measured at 25°C. CS activities were generally high in the 10 species (range of means: 144-300 μmoles/g·min). HOAD activities were also high although variability between species was greater (range of means: 25-175 μmoles/g·min). Variation in CS and HOAD can be accounted for by differences in body mass and appear independent of diet. The high CS activities reflect the energy demand of flight. High HOAD values suggest extensive use of fat as the fuel powering flight. Mean HEX activities in the frugivorous species are 2-4 fold higher (6.8-8.3 μmoles/g·min) than in the insectivorous species (1.9-3.7 μmoles/g·min). The greater HEX values ($P<.001$) in frugivorous bats reflect increased reliance on carbohydrate oxidation by their flight muscles. This difference in fuel utilization may be due to the greater access to dietary carbohydrate of frugivorous bats.

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MUSCLES OF NORMAL, DYSTROPHIC AND ALLOPHENIC LITTERMATES. Peter K. Law, Dept. of Neurol., Physiol./Biophys., Univ. of Tennessee Ctr. Hlth. Sci., Memphis, TN 38163

A model was produced consisting of 3 mice in a litter: a normal (129/ReJ-++), a dystrophic (C57BL/6J-$dy^{2J}dy^{2J}$), and an allophene (++↔$dy^{2J}dy^{2J}$). The allophene was synthesized by mixing half-embryos microdissected from normal and dystrophic 4-cell embryos. The uncombined half-embryos developed into the normal and dystrophic littermates. The allophene originated from genetic materials half of which was identical to the normal and the other half to the dystrophic. The allophene showed no physical abnormality. The soleus of the 1-year-old allophene exhibited normal mechanophysiology and fiber resting potentials.

	Normal	Allophene	Dystrophic
Twitch tension (g)	3.7	3.5	1.0
Tetanus Tension (g)	25.5	19.0	7.5
Time-to-peak tension (ms)	17.0	15.0	15.0
Half-relaxation time (ms)	17.5	16.0	19.0
Resting potential (-mV)	72.3	75.5	64.8

Muscle histology and ultrastructure of the allophene demonstrated fiber abnormalities similar to Duchenne carrier. Fiber splitting, size variation, central nuclei, central cores, phagocytosis, and increased endomysial connective tissue that were observed in $>50\%$ of the fibers of the dystrophic soleus, were found in $<5\%$ of the allophene.

(Supported by MDA, NSF PCM 7921008, & NIH RR-05424)

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VISUALIZATION OF SPECIFIC ANGIOTENSIN II BINDING SITES IN THE BRAIN BY FLUORESCENT MICROSCOPY. S.K. Landas*, Dept. of Pathology, M.I. Phillips, J.F. Stamler*, M.K. Raizada*, SPON: G.W. Searle. Dept. of Physiology, Univ. of Iowa, Iowa City, IA 52242

The organum vasculosum of the lamina terminalis (OVLT) has been implicated as the site of receptors mediating central responses of angiotensin II (AII). So far, this has been based on indirect evidence. The results presented here provide the first direct visualization of AII at its site of action by the use of a biologically active fluorescent AII agonist. Fluorescein thiocarbamyl AII (FTC AII) was administered (1µg) with or without an excess of unlabelled AII into the lateral ventricle of adult male Sprague-Dawley rats. Immediately after drinking behavior was observed the rats were decapitated, the brains were rapidly removed and immersed in liquid nitrogen. Cryostat sections (16µ) were taken in the horizontal plane, mounted with buffered glycerol and observed by fluorescence microscopy. The OVLT on the ventricular surface showed unequivically intense fluorescence which was virtually eliminated by an excess of unlabelled AII. Lower intensity fluorescence close to the ependymal surface was seen in the ventral third ventricle but the rest of the brain was dark. This study bridges the gap between physiological response and radioreceptor assay by showing binding and biological activity in vitro. (Supported by NIH and NSF grants to MIP)

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MODIFICATION OF THE CARDIOVASCULAR EFFECTS OF CENTRALLY ADMINISTERED BRADYKININ. Robert E. Lewis* and M. Ian Phillips. University of Iowa, Iowa City, IA 52242.

Intracerebroventricular (IVT) injections of bradykinin (BK) produce an increase in blood pressure whereas injections in the periphery produce a vasodilation. The mechanism of the central pressor response is unknown. Angiotensin II (ANG II) given IVT also produces a pressor response. ANG II converting enzyme (kininase II) degrades BK with high affinity. It was important to determine if bradykinin and angiotensin shared some common mechanism in the brain to increase blood pressure. IVT injections of BK (5 µg) caused an increase in blood pressure that was enhanced by prior injection of Saralasin (P113, 10 µg). Saralasin attenuated the pressor response to IVT ANG II (200 ng).

	BK	P113+BK	ANG II	P113+ANG II
ΔBP (mmHg)	17±4	26±4	20±3	9±2
ΔHR (bpm)	61±19	81±22	-21±9	-3±9

IVT administration of indomethacin (10 µg) also diminished the pressor response to IVT BK (5 µg). IVT Captopril (SQ14,225, 2 µg) potentiated the pressor response to 1 µg BK. The results suggest that the pressor mechanism of BK is not mediated by ANG II, but that ANG II antagonizes the pressor effect of BK. Prostaglandins may play a role in the blood pressure rise induced by IVT BK. (Supported by NIH and NSF grants.)

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LEFT VENTRICULAR RESPONSE TO LARGE VARIATIONS IN PRELOAD DURING AUTONOMIC BLOCKADE. Peter B. Raven, Kevin Klein*, Mark L. Smucker*, Jere H. Mitchell and J.V. Nixon*. Department of Physiology. Texas College of Osteopathic Medicine, Ft. Worth and V.A. Medical Center in Dallas, Texas.

Previous investigations into the left ventricular (LV) response to 5° head-down tilt for 90 min (increased preload-I) and graded lower body negative pressure to -40 mmHg (decreased preload-D) using M-mode echocardiography (E) described the normal "Starling" function curve of the LV. Similar studies were performed during autonomic blockade (B), produced by intravenous atropine (0.04 mg/kg) and propranolol (0.2 mg/kg), on 5 healthy men aged 24 years. Following B, heart rate (HR) increased 58% ($p<0.001$) and diastolic blood pressure (DBP) increased 25% ($p<0.05$). Changes in LV function during I and D prior to and following B were analyzed. Changes during B occurred ($p<0.05$) during D these being average decreases in end diastolic volume of 27 ml, stroke volume of 21 ml, ejection fraction of 10% and systolic blood pressure of 19 mmHg. End systolic volume mean circumferential fiber shortening (VcF), and DBP and HR were unchanged. No significant differences were observed during B under I conditions. These data suggest that D, obtained by LBNP, produces a classic Frank-Starling response while LV function during I requires a functioning autonomic system in order to respond in the classical fashion. Also, it appears that VcF is preload independent. (Supported in part by TCOM Faculty grant 34570 and the Veteran Administration).

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REFLEX RISE IN SYSTEMIC (SX) RESISTANCE FROM INTRACORONARY THIOPENTAL SODIUM (Na Thio). J.A. Estrin,* G.M. Wahler,* A.M. Booth,* C.R. Swayze,* and I.J. Fox.Depts. of Physiology, Anesthesiology, and Surgery, Univ. of Minn., Minneapolis, MN 55455

Na Thio has well known negative inotropic and chronotropic effects and a direct vasodepressor effect, decreasing SX resistance in animals and man after SX administration. We have reported that intracoronary injection of negative inotropic agents (EDTA, verapamil, etc.), reflexly increases the SX resistance by unloading the left ventricular (LV) mechanoreceptors (LV mechanoreceptor reflex). This study was initiated to see if intracoronary Na Thio would produce effects opposite to those noted after SX injection. Pneumonectomized dogs (chloralose-Flaxedil®) were placed on total cardiac bypass with the coronary and SX circulations isolated and perfused separately, the SX at a constant rate so changes in SX pressure reflected changes in SX resistance. Intracoronary injection of 25 mg (N=7) or 75 mg (N=5) Na Thio caused 19±7 and 27±8 % decreases in heart rate ($P<0.05$) and 43±8 and 64±17 % decreases in LV peak dP/dt ($P<0.01$), from control values of 145±11 and 183±10 beats/min and 5±1 and 9±1 mm respectively, for these parameters in the 2 dosage groups, which resulted in maximum increases in SX pressure of 8±3 and 19±5 %over a 5 min period ($P<0.01$), from control values of 70±4 and 84±3 mm Hg. Bilateral high cervical vagotomy abolished the SX pressure rise. In conclusion, intracoronary Na Thio produces a reflex rise in SX resistance which is the opposite of its vasodepressor effect after SX injection.

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INHIBITION OF CAROTID PRESSOR RESPONSE EFFECTED MAINLY BY LEFT AORTIC AFFERENTS IN DOGS. Susan C. Walgenbach,* Anders Melcher* and David E. Donald. Mayo Clinic and Foundation, Rochester, MN 55901

The relative ability of depressor afferents traveling in the left and right cervical vagus nerves to oppose the hypertension induced by bilateral carotid occlusion (BCO) was examined in conscious and anesthetized dogs. In 6 conscious dogs, left vagal section augmented the blood pressure rise due to BCO by 52 mm Hg; in other 6 dogs, right vagal section augmented the BCO response by 6 mm Hg. In 18 anesthetized dogs, cold block of the left vagus increased the pressure rise due to BCO by 28 mm Hg; cold block of the right vagus increased the response by only 6 mm Hg. This data indicates that the inhibition exerted by the vagi is predominantly via the left nerve. The following studies demonstrate that the origin of the vagal inequality resides within the aortic arch. Bilateral section of the aortic nerves eliminated the left vagal dominance in one group of dogs (n=9); bilateral section of the cardiopulmonary afferents in a second group (n=9) did not affect this response. In five dogs on cardiopulmonary bypass with heart and lungs excluded, sudden pressurization of the isolated aortic arch and major intrathoracic arteries from 120 to 220 mm Hg induced a reflex hypotension which was reduced by left but not by right vagal cold block. It is concluded that, in the dog, the left aortic nerve dominates in opposing the increase in arterial pressure induced by BCO. Supported by NIH Grant HL 6143.

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INTERACTION OF THE INFLUENCES OF ARTERIAL BARORECEPTORS AND LEFT ATRIAL RECEPTORS (LAR) ON RENAL BLOOD FLOW (RBF). Fazlul Karim* and Tissa Kappagoda.Division of Cardiology, Dept. of Medicine,University of Alberta, Edmonton, Alberta T6G 2G3

Stimulation of LAR in the dog decreases renal sympathetic nerve activity. However the increases in RBF reported under comparable experimental conditions are small. This study was undertaken to determine whether the magnitude of the changes in RBF could be modified by altering the stimulus to the carotid baroreceptors.

In 8 dogs, anaesthetized with chloralose and artificially ventilated, the LAR were stimulated by stretching two left pulmonary vein-atrial junctions. The RBF was measured at a constant systemic pressure. The LAR were stimulated at low [60.5 ± 0.6 mm Hg (SEM)], medium (90.1 ± 3.2) and high (135 ± 4.3) carotid sinus pressures (CSP). The changes in RBF (ml/100 g kidney weight) are summarised below.

CSP	*Control RBF	Test RBF	Δ RBF	P	
Low	180 ± 10 (SEM)	200 ± 10	20.1 ± 2.4	<0.001	<0.01
Med.	186 ± 17	196 ± 18	10.4 ± 2.2	<0.005	NS
High	204 ± 12	213 ± 14	9.0 ± 4.5	<0.05	NS

*Calculated as the average of initial and final control values.

The largest increases in RBF were observed when the CSP was low. These changes were abolished by either cutting or cooling the cervical vagi. It is concluded that the influence of the LAR on RBF is maximal in conditions where the inhibitory effect of the baroreceptors on sympathetic tone is minimal. (Supported by MRC (Canada) and Alberta Heart Foundation).

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THE IMPORTANCE OF THE SPLEEN IN BLOOD VOLUME SHIFTS OF THE SYSTEMIC VASCULAR BED CAUSED BY THE CAROTID SINUS BARORECEPTOR REFLEX. Artin A. Shoukas, Carol L. MacAnespie* and Martha Connolly Brunner*. The Johns Hopkins Univ. Sch. of Medicine Baltimore, Md. 21205

To quantify the relative importance of the spleen in the carotid sinus baroreceptor reflex control of total blood volume distribution, we studied the reflex control of systemic vascular capacity before and after splenectomy in nine dogs. Venous return was diverted into a reservoir while cardiac output and venous were maintained constant. Intrasinus pressure was lowered or raised between 50 and 200 mmHg which mobilized blood into or out of the reservoir. With the spleen intact blood volume shift amounted to 8.42 ± 1.43 ml/kg and after acute splenectomy the reflex volume shift was attenuated to 5.17 ± .97 ml/kg. In each dog the control (spleen intact) was taken as 100%. After splenectomy the average change in reservoir volume amounted to 68.9% of the control. The spleen's maximum contribution was no more than 32% of the total response. Total systemic vascular compliance before and after splenectomy at the same intrasinus pressure showed no significant difference. However, there was a significant 10% increase in compliance when intrasinus pressure was increased from 50 to 200 mmHg either before or after splenectomy. We conclude that although the spleen contributes to the blood volume mobilization it is not the sole or even major contributor to total systemic blood volume shifts caused by carotid sinus baroreceptor reflex. Supported by PHS HL19039

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STIMULATION OF VAGAL AFFERENT FIBERS CAUSES DESCENDING INHIBITION OF THORACIC SPINORETICULAR NEURONS. Roger Thies and Robert D. Foreman, Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

We have studied with extracellular microelectrodes the discharges of single neurons in the T_1-T_3 segments of the left spinal cord in chloralose-anesthetized cats. Of 9 cells studied, 4 were activated antidromically by contralateral stimulation in the nucleus gigantocellularis, 4 were activated ipsilaterally, and one cell was fired by stimuli to either side. All cells responded to pinching of the skin, especially on the upper left foreleg; 8 fired in response to sympathetic stimuli across the left stellate ganglion; and 6 cells were spontaneously active, at 1-10/sec. Stimulation of the central cut end of the right cervical vagus inhibited either spontaneous or pinch-elicited firing in 3 of 8 cells tested. One neuron was directly excited by left vagal stimulation, with a latency of 16 msec., and one was excited from the right vagus and inhibited from the left. Two of the 3 unaffected cells were tested with only the moderate stimuli used in our previous experiments (10/sec at 10-15 V.); 3 of the 4 inhibited cells required more vigorous stimulation (50/sec at 35 V.) to show inhibition. This descending inhibition by stimulation of a visceral afferent pathway may modulate the transmission of information from the heart to the reticular formation. (Supported in part by NIH Grants HL22732 and 5S07-RR05411).

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REFLEX INCREASE IN HEART RATE (HR) IN THE RAT. Tissa Kappagoda and Susan Kaufman* DEPT. OF MEDICINE, DIVISION OF CARDIOLOGY UNIVERSITY OF ALBERTA, Edmonton, Alberta T6G 2G3

In the dog, stimulation of atrial receptors results in a reflex increase in HR. This reflex has not been demonstrated in any other species. In this investigation the effect of stimulating atrial receptors by stretching the cardiac end of the superior vena cava (SVC) in chloralose anaesthetized rats was examined.

The SVC was stretched 41 times in 16 rats. The mean increase in HR was 24.1 bpm (SEM ± 1.9). The response was abolished by sympathetic blockade [propranolol (P) and bretylium tosylate (B)], cervical vagosympathectomy (VSx) and by the application of lignocaine to the cervical vagi. The effect of the latter was reversible.

	N	Initial response	Post block	Final response	P
P	6	25 ± 5	6 ± 1	-	<0.05
P + B	4	28 ± 5	1 ± 1	-	<0.05
VSx	5	26 ± 4	0 ± .8	-	<0.05
Lig.	4	26 ± 3	-2 ± 4	21 ± 5	<0.05

In five other rats, histological examination of the SVC revealed an abundance of nerve fibres but few classical complex unencapsulated endings. It is concluded that stimulation of the SVC results in a reflex increase in HR whose afferent path is in the vagi and the efferent is in the sympathetic nerves to the heart.

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PRE AND POSTGANGLIONIC CONTRIBUTIONS TO SPLANCHNIC ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RAT. D.Whitehorn, S.M.Morrison*, F.W.Marcoux*. Dept. Physiol., Univ. Vermont, Burlington, VT 05401

The pre-celiac splanchnic nerve of the rat contains both pre-(PREG) and postganglionic(POSTG) fibers. Whole nerve activity is elevated in spontaneously hypertensive rat(SHR) compared with normotensive controls(WKY). We calculated PREG and POSTG activity in anterior splanchnic branches (which have low POSTG content) by measuring whole nerve integrated activity, under chloralose, before and after ganglionic blockade with 25mg/kg hexamethonium(HEX). Baroreceptor reflex was eliminated in the 10 SHR and 10 WKY studied by bilateral deafferentation at the nucleus tractus solitarii(NTS). Activity remaining after HEX was taken as PREG. POSTG activity was estimated by subtracting post-HEX values(PREG) from pre-HEX(PREG+POSTG). Mixed(pre-HEX) spontaneous activity was greater in SHR as expected. Spontaneous POSTG activity was also significantly greater, while PREG levels, although larger on the average in SHR, were not significantly greater than in WKY. Responses evoked by stimulation of posterior hypothalamus were also compared. Mixed and PREG responses were significantly greater in SHR. POSTG evoked activity was larger, but not signficiantly so, in the SHR. The results indicate that both PREG and POSTG fibers contribute to the elevated levels of splanchnic activity in the SHR. (Supported by HL24110).

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INTRACELLULAR ANALYSIS OF NEURAL PATHWAYS WITHIN THE CELIO-RENAL COMMISSURE OF THE FELINE SOLAR PLEXUS. D.L. Decktor* and W.A. Weems, Univ. of TX. Med. School, Houston, TX 77030.

Renal and celiac ganglia are interconnected within the solar plexus via the celio-renal commissure. Studies were designed to characterize the relationship of fiber tracts within the commissure to neurons within the renal ganglia. Solar plexus were superfused in vitro with Krebs solution. Stimulating electrodes were placed on the left splanchnic nerves and the celio-renal commissure. Transmembrane potential of neurons within the renal ganglia was monitored by intrasomatic electrodes. Efferent neural pathways were identified by antidromic activation. Synaptic input generated by stimulation of the commissure was significantly greater than that generated upon left splanchnic stimulation. The increased magnitude of this response may reflect the recruitment of fibers within the commissure which conduct preganglionic input from the contralateral splanchnics, or peripheral inputs traversing ganglionic reflex arcs. This synaptic activation was blocked by hexamethonium (10^{-6}M). No axonal processes extending from the renal ganglia could be demonstrated within the commissure. This finding supports the hypotheses: (1) that neurons within renal ganglia only send axons from the ganglia via renal nerves, and (2) that neurons within renal ganglia receive, via the commissure, presynaptic inputs from fibers of non-splanchnic as well as splanchnic origin. (Supported by NIH Grant HL21351-03.)

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CARDIOVASCULAR CHANGES DURING BRADYKININ INJECTION INTO THE LIVER OF THE DOG. Juliet H. Ashton,* Gary A. Iwamoto,* John C. Longhurst, and Jere H. Mitchell. University of Texas Health Science Center, Dallas, TX 75235

It has been shown previously that injection of capsaicin into a humorally isolated, innervated dog liver (LIV) causes a reflex depressor cardiovascular (CV) response. To study the effect of a physiologically occurring substance on LIV receptors, bradykinin (BK) was injected into the portal vein of 6 splenectomized dogs after the inferior vena cava (IVC) was ligated and cannulated below the right atrium (RA). During LIV injections, contaminated venous return blood was discarded and blood from a separate equilibrated reservoir was pumped into the jugular veins to maintain venous return. RA and IVC pressures remained constant during this period. BK injected into the LIV (avg. dose=16.7±5.5ug/kg, mean±SEM) significantly decreased systolic blood pressure from 108±6 to 95±5 mmHg, diastolic blood pressure from 59±8 to 47±6 mmHg and maximal dP/dt from 2300±146 to 2067±135 mmHg/sec (all $p<.05$). The depressor response began 13±3 seconds after injection of BK. Heart rate did not change significantly. Therefore, BK causes hemodynamic changes similar to those observed during capsaicin injections into LIV. These data further support the hypothesis that the liver may potentially contribute to reflex regulation of the CV system. (Supported in part by NIH training grant HL07360 and HL22669)

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USE OF A LIPID TRACER TO FOLLOW EDEMA FORMATION IN THE LUNG. Gordon R. Neufeld* and Bryan E. Marshall, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104

The ability to measure lung water by indicator dilution has been hampered by the inability to discriminate between changes in blood flow (recruitment) and real changes in water content. We used a lipid soluble tracer to follow fluid accumulation in dogs. C^{14} tagged hexanol was combined with tritiated water and Cr^{51} tagged red blood cells in multiple indicator dilution studies of pulmonary edema. In fourteen dogs of mixed breed, anesthetized and mechanically ventilated, indicator dilution data was collected in a control group and in a group treated with intravenous alloxan (75 mg/Kg) followed by saline (40 ml/kg). At the end of each study the lungs were excised, sectioned, dried and the lipid extracted. The mean water content of the control group was 3.57 ± 0.15 mls/gm of blood free dry lung, while the mean ratio of water to hexanol distribution volume (by indicator dilution) was 2.93 ± 0.62. Thirty minutes following alloxan treatment, in the test group, the mean water to hexanol ratio increased to 12.7 ± 5.9 ($p<.01$) and after 90 minutes was 21.9 ± 5.4. The actual water content of the edematous lungs was 5.85 ± 0.42 mls/gm blood free dry lung. We conclude that the addition of a lipid soluble tracer offers the opportunity to follow edema formation in the lung and may provide information to estimate tissue perfusion or available surface area for exchange in permeability studies. (Supported in part by USPHS NIH Grants HL 23730 and HL 16916).

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VALIDATION OF THERMAL GREEN DYE MEASUREMENTS OF EXTRAVASCULAR LUNG WATER (EVLW) IN THE ISOLATED CANINE LOBE. H.W. Unruh* and L. Oppenheimer*. (Spon: N.R. Anthonisen). Department of Surgery, University of Manitoba, Winnipeg, Canada.

Potential sources of error in measurement of EVLW using heat as diffusible and green dye (G.D.) or 3% NaCl as reference indicators include recirculation, presence of red cells, size of EVLW space, flow rate (Qt) through lungs and heat loss to adjacent structures. To minimize errors, we have tested the thermal green dye technique in isolated canine lobes perfused with nonrecirculating plasma at 38°C. Lobar weights were continuously monitored. We introduced 4 ml boli of plasma stained with G.D. (10 mg/IL) without changing capillary pressure. Measurements of EVLW were obtained at increasing levels of lung hydration at three perfusion rates (approximately 50, 120 and 200 ml/min). Dye dilution estimates of EVLW obtained by multiplying flow (Qt) and the difference in mean transit times of heat and G.D. were compared to direct determinations from the lobar weights. On the average, EVLW increased from 30 ml to 90 ml. At Qt in the 120 ml/min range dye dilution and weight determinations of EVLW correlated well ($r=.995$, $p<.01$), the regression line being no different from identity. No correlation existed however at low or high Qt. Presumably, EVLW calculations using this approach are accurate only when the rate of heat transport in the EVLW space, determined by the velocity of free diffusion of heat is comparable to heat transport in the vascular space determined by Qt. (Supported by MRC of Canada).

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LUNG WATER, VASCULAR PERMEABILITY-SURFACE AREA (PS) AND HEMODYNAMICS VARY WITH LUNG BLOOD FLOW AND MASS. J.R. Snapper*, T.R. Harris, and K.L. Brigham. Pulmonary Circulation Center, Vanderbilt University, Nashville, Tennessee 37232.

The effects of changing lung blood flow and lung mass upon capillary surface area, lung water, and hemodynamics were studied in anesthetized sheep at baseline and increased flow (arteriovenous shunts) before and after first tying off the left lung, followed by tying off the right lower lobe. We measured pulmonary artery (P_{pa}) and left atrial (P_{la}) pressure and injected ^{51}Cr-erythrocytes, ^{125}I-albumin, ^{3}HOH, and ^{14}C-urea into the right atrium, collected timed samples from the aorta and calculated cardiac output (CO), extravascular lung water (EVLW) and ^{14}C-urea PS from time-concentration curves. We also measured extravascular lung water ($EVLW_{pm}$) and dry lung weight (LW_{pm}) separately for ligated and unligated portions of lung postmortem. P_{pa} rose directly with increasing flow per unit lung mass (CO/LW_{pm}) ($r = .78$) while P_{la} stayed constant. Pulmonary vascular resistance ($P_{pa} - P_{la}/CO$) increased with decreasing lung mass (LW_{pm}) ($r = -.78$). ^{14}C-urea PS ($r = .78$) and EVLW ($r = .91$) decreased in direct proportion to the amount of lung excluded from the circulation (LW_{pm}). There was a close correlation between EVLW and $EVLW_{pm}$ ($r = .96$). ^{14}C-urea PS extraction decreased with increasing flow per unit lung mass as expected for a diffusion limited indicator. We conclude that lung vascular pressures (and resistance) depend on flow per unit lung mass; that EVLW and PS accurately reflect the amount of lung perfused. (Supported by NHLBI Grant Number HL 19153.)

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EFFECT OF LUNG MICROVASCULAR INJURY ON PERMEABILITY SURFACE AREA PRODUCT (PS) IN DOGS. M.Zelter*, J.Hoeffel*, A.Lipavsky* (SPON: J.H.Comroe) Cardiovasc. Res. Inst. and Dept. of Medicine Chest Service, SFGH, Univ. of Calif., San Francisco, CA 94110

We measured PS for ^{14}C urea, using ^{51}Cr labeled RBC and ^{125}I albumin as flow indicators, in four groups of 5 anesthetized, ventilated, intact dogs: control, alloxan (80mg/kg), oleic acid (.05g/kg) and glass microemboli (.3g/kg). PS products (ml/min, mean ± S.D.) shown in the table were obtained every hour for 2h baseline and 2h after each intervention. Terminally, we measured extravascular lung water (g/g dry lung).

	Baseline		Intervention		Lung
	1h	2h	3h	4h	Water
Control	8.2±2.2	8.0±1.6	8.2±2.0	7.8±1.4	4.2± .4
Alloxan	5.5±1.2	6.0±1.6	13.5±2.4	15.1±3.7	6.2±1.0
Oleic Acid	8.2±2.2	8.2±2.7	5.4±1.3	3.5±1.4	5.5± .4
Emboli	9.0±3.6	9.0±3.4	6.0±2.6	7.3±3.9	5.0± .5

In each control PS was stable for 4h. In moderate alloxan edema, PS doubled. In moderate oleic acid edema, PS decreased by half. Microemboli edema caused a smaller decrease. Although oleic acid and alloxan edema are both due to increased permeability, at comparable lung water values, PS was much less after oleic acid. We attributed this decrease to a fall in vascular surface area, S, due to obstruction of microvasculature, which may have masked a rise in permeability, P. This hypothesis is supported by the microemboli data. Thus PS may not always detect lung microvascular injury. [Supported in part by Pulmonary Vascular SCOR (HL19155)]

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EFFECT OF BLOOD FLOW ON WHITE BLOOD CELL TRANSIT THROUGH THE PULMONARY CIRCULATION IN DOGS. B.A. Martin,* J.L. Wright,* I. Nicholls,* and J.C. Hogg. UBC Pulmonary Research Laboratory, St. Paul's Hospital, Vancouver, B.C., Canada V6Z 1Y6.

We measured the effect of blood flow on the passage of white blood cells (WBC) through the pulmonary microcirculation in two ways. In the first, artero-venous differences in WBC number were measured under control circumstances and after flow had been decreased by inflating a balloon in the IVC. In the second, we compared the passage of labelled polymorphonuclear cells and red blood cells through the lung using a modification of the indicator dilution technique. When the pulmonary venous WBC count was expressed as a % of the pulmonary arterial WBC count, we found that decreasing pulmonary blood flow caused a change from (mean ± SE) 98.8 ± 1.0 to a minimum of 73.4 ± 7.4 two minutes after the flow was decreased (P <.05). In the single pass experiments we found that recovery of WBCs varied little with blood flow and that the extraction ranged from 83-86% which is consistent with values predicted by Schmid-Schonbein et al. (1) for removal of WBCs in the microcirculation. Taken together, the data suggest that reducing blood flow has little effect on removal of WBCs from the pulmonary microcirculation but does affect the re-entry of WBCs into the circulating pool.

1. Microvascular Research 19: 45-70, 1980.

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ROLE OF FIBRIN IN INCREASED LUNG VASCULAR PERMEABILITY AFTER FAT EMBOLISM IN SHEEP. Philip S. Barie*, Fred L. Minnear*, Asrar B. Malik, (SPON:R.D.Goldfarb). Department of Physiology, Albany Medical College, Albany, NY 12208.

The role of fibrin in the increased permeability after fat embolism (FE) was examined by depleting fibrinogen in 9 sheep with ancrod (0.35 NIH U/kg/day x 3) before placement of acute lung lymph fistulas and left atrial balloon catheters. Four of these animals received 0.2 ml/kg i.v. of a bone marrow suspension we have developed that causes increased permeability (Am. Rev. Resp. Dis. (S) 121:424,1980). Five sheep served as controls. Mean left atrial pressure ($P_{\overline{La}}$) was raised right after FE. Data were taken during steady-state lymph flow ($\dot{Q}$lym) 2 hr after balloon inflation.

	n	$\dot{Q}$lym ml/hr	L/P	$P_{\overline{La}}$ Torr	PVR units
Baseline	5	4.5±0.9	.72±.08	3.5±1.1	4.5±0.3
Balloon		13.0±2.7*	.41±.06*	15.2±1.4*	5.9±0.6*
Baseline	4	5.2±0.8	.60±.07	2.1±0.7	4.0±0.6
FE-Balloon		11.4±1.9*	.46±.05*	12.8±1.0*	5.9±0.6*

L/P=lymph-to-plasma prot. conc. ratio; PVR=pulmonary vascular resistance; *p<.05.

Ultrafiltration after FE with increased $P_{\overline{La}}$ suggests that permeability did not increase after FE in fibrinogen-depleted animals. Thus, fibrin deposition appears to mediate the increased lung vascular permeability seen after fat embolism. (HL-17355, HL-00363, T32-GM-07033)

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ACUTE LUNG SEQUESTRATION OF NEUTROPHILS FOLLOWING RETICULOENDOTHELIAL (RE) BLOCKADE: A ROLE IN LUNG INJURY DURING SEPSIS. M.E.Lanser*, T.M.Saba, M.DeLaughter*, J.Goldman*, Dept. of Physiol., Albany Medical College, Albany, NY 12208.

RE depression following trauma, surgery or particle blockade results in decreased liver and increased lung localization of injected test particles or bacteria (Circ. Shock 7:80A, 1980). The mechanism of increased bacterial lung localization following RE blockade is unknown. We hypothesized that increased lung localization of blood-borne bacteria following RE blockade might be due to their phagocytosis by sequestered neutrophils. The present study examined neutrophil dynamics and their lung localization in rats following RE blockade by particle infusion (gelatinized lipid, 50 mg/100gm body wt). Neutrophil counts decreased ($p<0.05$) 40% from 22,151±3,458/mm^3 to 12,893±2,040/mm^3 15 min after particle infusion and remained depressed for 60 min. In other animals $1x10^7$ ^{51}Cr labelled neutrophils were injected 15 min prior to RES blockade and lung localization measured at 15,30,60 and 120 min thereafter. Lung localization was higher ($p<.05$) at 15 (25.9±1.0 vs 19.6±2.9%) and 30 min (20.1±2.5 vs 14.0±2.1%) in blockaded rats with return to control values thereafter. Thus, RE blockade is associated with lung sequestration of neutrophils capable of phagocytizing blood-borne bacteria. The role of sequestered neutrophils in lung injury during bacteremia warrants investigation. (GM-21447 and T32-GM-07033)

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FORMED ELEMENTS AND LUNG LOCALIZATION OF BLOOD-BORNE PARTICULATES IN SHEEP: IMPORTANCE TO LUNG FLUID BALANCE. G.D. Niehaus*, B.C.Dillon*, T.M.Saba, R.H.Edmonds* and P.T.Schumacker, (Spon:L.Grumbach). Depts. of Physiol. and Anatomy, Albany Medical College, Albany, NY 12208.

The sheep's low opsonic fibronectin (FN) levels and inefficient reticuloendothelial (RE) function is associated with a very high lung localization of blood-borne particulates (Ann. Surg. 191:479,1980). Microparticulate (0.25-1.0μ) localization in the lung and its effect on lung fluid flux was evaluated with the ventilated sheep lung lymph model. The normal distribution of gelatinized colloidal carbon (64 mg/kg) was 21.4±1.8; 2.0±0.7 and 32.7±4.6% injected dose per total organ for liver, spleen and lung, respectively. Prior depletion of opsonic fibronectin depressed RE clearance by liver (15.9±3.3%) and spleen (0.3±0.2%) while lung localization increased to 54.9±12.2%. Electronmicroscopically, carbon uptake (15 min) in the liver was due to phagocytosis by sessile RE cells. In contrast, carbon in the lungs was due to incorporation in platelet aggregates or ingestion by marginated phagocytes. Vascular endothelium appeared normal. Particulate localization in the lung was associated with a sustained 3-fold increase in flow of protein-rich lung lymph. Thus, lung localization of blood-borne particulates in marginated phagocytic cells and/or platelet aggregates may be etiologic in interstitial edema. (GM-21447; T32-GM-07033; HL-07194)

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IMPROVED SURVIVAL FROM LETHAL PULMONARY MICROEMBOLISM SYNDROME (MES) BY ASPIRIN (ASA) PRETREATMENT. B. Kent and P.K. Poddar*. Dept Surg, Bronx V.A. Med. Ctr., Bronx, N.Y. and Depts Surg. and Physiol., Mt Sinai Sch. of Med. New York, N.Y. 10029.

We found previously that prostaglandin E_1 (PGE_1) increases survival in dogs given a lethal dose of microfibrillar collagen (MFC) in the pulmonary artery (PA). PGE_1 causes pulmonary vasodilation and, as does ASA, inhibition of platelet aggregation. To test the role of the latter in survival from MES, we pretreated 7 anesthetized, ventilated dogs with 55mcg/kg ASA before injecting MFC (Avitene, 15-35mg/kg) to increase PA pressure to 300% of baseline. Seven control dogs were given MFC without ASA. Ventilatory support was continued in both groups for 4 hours. While the decrease in PA pressure in the ASA group was not significantly different from the control at the end of 4 hours, the pulmonary vascular resistance (PVR) was significantly lower. Initially PVR decreased similarly in both groups (400% of baseline to 250% in 30 min.), but the control group showed a progressive secondary rise not observed in the ASA group. Within 4 days 7/7 control dogs died while only 2/7 ASA dogs died. The improved survival and inhibition of a secondary rise in PVR were also observed in experiments in which dogs were treated with PGE_1. The platelet aggregation inhibiting effect of PGE_1 rather than pulmonary vasodilation appears to be the key factor in improved survival from MES.
(This project supported by V.A. research funds)

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PULMONARY EDEMA AFTER HCl ASPIRATION IN INTACT DOG LUNG: EFFECT OF ALBUMIN THERAPY. S. Nanjo, J. Bhattacharya and N.C. Staub. Cardiovasc. Res. Inst. and Physiol. Dept., Univ. of California San Francisco, CA 94143

Massive quantities of albumin added to the perfusate of isolated dog lungs prevent edema caused by HCl aspiration (Toung, Ann.Surg.183,1976). In anesthetized, ventilated, open-thorax dogs with continuously weighed left lower lobe (Δwt), we measured lobar pulmonary arterial (Ppa) and left atrial (Pla) pressures, lobar blood flow ($\dot{Q}l$) by Doppler shift, and calculated plasma protein osmotic pressure (Πmv). After a 2h baseline, we instilled 0.1N HCl (2ml/kg) into the lobar bronchus in 3 dogs and followed Δwt for 3h. Terminally, we measured extravascular lung water. In 6 dogs we have 50g albumin (25% solution) i.v. over 20 min, either just before or just after the HCl. In 3 control dogs we instilled .15M NaCl (2ml/kg). The average data are summarized in the table.

Condition	Ppa cmH2O	Pla cmH2O	$\dot{Q}l$ ml/min	Πmv cmH2O	Δwt g/h	lung water g/g dry lobe
Saline	18.5	3.9	193	17.7	2.3	6.0
Acid	19.4	4.5	193	17.2	30.2	8.8
Albumin,Acid	20.7	5.9	307	33.8	42.4	7.8
Acid,Albumin	24.8	5.3	306	33.7	56.3	8.3

Acid aspiration caused severe edema that was not decreased by doubling Πmv. The high Δwt in the albumin treated dogs is partly due to the small rise in Ppa and Pla. Albumin therapy did not prevent or treat the pulmonary edema due to HCl aspiration in these animals. (Supported in part by HL6285 & HL19155)

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LUNG LYMPH FLOW, ($\dot{Q}_L$) VASCULAR PRESSURES, LYMPH PLASMA RATIO (L/P) AND PERCENT SHUNT ($\dot{Q}_s/\dot{Q}_t$) FOLLOWING ACID ASPIRATION IN AWAKE GOATS. R. Winn, B. Nadir*, J. Stothert*, J. Gleisner*, J. Weaver* and J. Hildebrandt. Virginia Mason Research Center and Harborview Medical Center, Seattle, WA 98101.

Intratracheal instillations of 0.1 N HCl (2.5 ml/kg) were given to goats having chronic lung lymph fistulae. Goats were lightly anesthetized during aspiration and recovered from anesthesia within 30 min. We measured $\dot{Q}_L$, pulmonary artery and wedge pressures, L/P ratio and blood gases during air and 100% O_2 breathing. $\dot{Q}_L$ almost doubled within 30 min but returned to baseline by 2 hrs post-instillation and remained at control levels for up to 96 hrs. The L/P ratio and vascular pressures remained relatively constant throughout. $\dot{Q}_s/\dot{Q}_t$ increased from a baseline of 9.4 to mean peak values of 21.9% within 4 hrs, then decreased to an average of 15.5% by 48 hrs. At autopsy the lungs were consolidated and edema foam was found in the airways. The average wet/dry ratio of both lungs was 6.4. Since $\dot{Q}_L$ was only transiently elevated, we may speculate that the increased membrane permeability was masked by diversion of blood flow away from damaged areas. This diversion may result from hypoxic vasoconstriction in regions poorly ventilated due to alveolar instability or fluid. In one goat sodium nitroprusside was infused at 24 hrs and at 48 hrs at a rate sufficient to decrease systemic arterial pressure 20 torr. $\dot{Q}_s/\dot{Q}_t$ increased approx. 16% each time. We conclude that vasoconstriction perhaps hypoxic, directed blood away from unventilated consolidated regions.
(Grant Sup: HL05421, GM07037, HL24163, GM24990, HL22369)

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THE ROLE OF HISTAMINE IN REGIONAL VENTILATION AND PERFUSION AFTER CHEMICAL ASPIRATION. D. J. Zaluzec* and J. B. Pace. Depts. of Physiol. and Medicine, Loyola, Stritch School of Medicine, Maywood, Illinois 60153.

Dogs were chronically instrumented to record pulmonary arterial and lt atrial pressures together with pulmonary blood flow. The dogs were studied 10 days later as a closed-chest preparation. A Carlens tube permitted ventilation and measurement of air flow and tidal volume of rt and lt lungs separately. HCl 0.2N @ 2.3 ml/kg was instilled into the rt bronchus, hemodynamic and blood gas measurements were made every 15 min for 2 hrs following injury in control (C) and in dogs pretreated with chlorpheniramine (CP) 2.3 mg/kg. Chemical aspiration caused a 50% ↑ in pulmonary vascular resistance (PVR) in (C) dogs as compared to a 18% ↑ in (CP) dogs. Peak air flow and tidal volume in the treated lung ↓ 64% and 81% in the (C) group in contrast to a reduction of 41% and 55% respectively in the (CP) group. There was an ↑ in lung water measured as wet to dry (W/D) ratios in injured tissue with higher ratios measured in the (CP) group 10.21 as compared to (C)=8.22. Rt to lt flow redistribution occurred 15 min after injury and persisted for the duration of the study. Regional blood flow in injured lung ↓ 47% in (C) as compared to 12% in (CP) dogs. Arterial O_2 tension in (C) dogs ↓ 46% from control compared to a 57% ↓ in (CP) dogs. The results of this study suggest the overall protective role of localized endogenous histamine release in chemically injured lung. (Supported by USPHS Grant HL 08682.)

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THE TOXIC RESPONSES OF INTRODUCING ENDOGENOUS LUNG LECTIN INTO THE VASCULAR SYSTEM OF THE RAT. Janet T. Powell* and L.D. Davis* (SPON: D.J. Massaro). Univ. of Miami School Med., Miami, FL 33101

Endogenous lung lectin is a β-galactoside binding protein which has been purified to homogeneity from rat lung parenchyma; it cannot be obtained by lung perfusion or lavage. The purified lectin (0.25 mg) was injected intravenously into healthy adult rats. The lectin injected rats all lost weight over a period of 5 days, contrasting with the weight gain evidenced by saline controls. Other effects observed only in lectin injected rats included long periods of hyperventilation, oliguria, hematuria, proteinuria and red cell casts in the urine. The rats received a second intravenous injection of lectin after 7 days, with recrudescence of all the above symptoms. At 15 days the rats were sacrificed with lung and kidney sections taken for pathology. Both lung and kidney sections showed fibrinoid necrosis of small vessels in the lectin injected rats. These results suggest that intravascular injection of lung lectin produces a toxic response. (Supported by NIH grant #HL24261)

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ACTION OF COTTON BRACTS TANNIN ON PLATELETS. Michael S. Rohrbach*, Rebecca A. Rolstad*, Paula Tracy*, and James A. Russell. Mayo Clinic and Foundation, Rochester, MN 55901.

Tannins located in the bracts of cotton plants have been suggested as one of the pharmacologically active agents responsible for the development of byssinosis in textile workers. We have purified cotton bracts tannin using a modification of the method of Taylor and coworkers (Br. J. Ind. Med. (1971) 28:143). This material caused both the aggregation of washed bovine and human platelets and the release of 70-90% of the 5-hydroxytryptamine (5-HT) stored in the platelet dense granules. Maximal release of 5-HT was observed at tannin concentrations of 11 to 44 μg/mL and was essentially complete in 5 minutes. Tannin-induced release of ADP from the platelet dense granules paralleled that of 5-HT. When platelet rich plasma was used in place of washed platelets, the effectiveness of tannin in promoting the release of 5-HT was reduced two orders of magnitude. This decrease was due to a component of the plasma which protected the platelets against the action of tannin. Ammonium sulfate fractionation of platelet poor plasma revealed that the 60-70% fraction contained a compound(s) which protected washed platelets from the action of tannin. We conclude that cotton bracts tannin is capable of causing the release of platelet compounds which could potentially contribute to the pulmonary symptoms associated with byssinosis. (Supported by grants HL-21894 and HL-21584 from NHLBI and MLA-3 and MLA-4 from the Minnesota Lung Association).

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EFFECT OF HYPOXIA ON PHYSIOLOGICAL DEAD SPACE. M.Taxer*, F. Randall*, E.C. DeLand*, and S.F. Sullivan UCLA, Los Angeles, Calif. 90024

The effect of hypoxia on pulmonary vascular resistance is well documented, however, the effect of hypoxia on physiolgical dead space is not known. We studied the effect of hypoxia on physiological dead space (VD/VT) in 12 mongrel dogs anesthetized with halothane. Dogs were ventilated at constant volume, and expired air was collected in a rolling seal spirometer and analyzed by mass spectrometer for mixed expired CO2 (PECO2). Alveolar CO2 (PACO2) was determined from arterial blood gas. Physiological dead space was determined by: VD/VT=((PACO2-PECO2)/PACO2) x100. The animals were ventilated at normocapnia (pH=7.33± .01) with a gas having an FIO2=.5 and VD/VT measured at steady state as determined by nitrogen exchange ratio. This was followed by ventilation at normocapnia (pH=7.35± .01) and an FIO2 of.1. VD/VT was again determined at steady state. VD/VT decreased significantly from 38.9%±1.7% (mean±SEM;n=12) to 35.2%±1.9% as determined by a paired t test: p<.025. Additionally, pulmonary vascular resistance was measured and increased in 10 of the 12 dogs in response to hypoxia. The increase from 280.0±25.8 to 411±56.1 (arbitrary units) was significant (p<.01). It is concluded, then, that in halothane anesthetized dogs, hypoxia decreases physiological dead space and increases pulmonary vascular resistance. (Supported in part by AHA/GLAA grant #554IG3).

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BENEFIT OF ENLARGED FRC IN A HYPERBARIC ENVIRONMENT. H.D. Van Liew and K.R. Murray*. Physiology Dept.,State University of NY at Buffalo, Buffalo, NY 14214.

When an inspired indicator gas becomes mixed with gas in the functional residual capacity (FRC), the resulting mixed concentration (C_m) is $C_m = (C_I V_A)/(V_A+FRC)$, where C_I is inspired concentration and V_A is tidal volume minus dead space. The amount of the indicator that remains in the FRC after the breath is $A_{FRC} = C_mFRC = (C_I V_A FRC)/(V_A+FRC)$. This mass-balance relation shows that FRC is a strong determinant of the mixing efficiency of a breath. An increase of the FRC can have as much effect as an increase of breath size, since V_A and FRC are interchangeable in the equation. We found FRC to be 50% larger than normal in men exercising at 6.75 ATA. The enlarged FRC may have been caused by high expiratory resistance; there is probably a feedback effect on resistance due to widening of airways. Respiratory control may also play a causative role; the men breathed a hyperoxic mixture (O_2 near 20%). During exercise at 6.75 ATA, the subjects were hypercapnic, but they maintained the exercise $\dot{V}O_2$, $\dot{V}CO_2$ and R with less total ventilation and had a more even distribution of ventilation (estimated from compartmental analysis of multiple breath washin data) than during exercise at normal pressure. We suggest that the enlarged FRC is a beneficial response that tends to compensate for high airway resistance and poor diffusive mixing in hyperbaric environments. (Supported in part by NHLBI Grant PO1-HL-14414 and ONR Contract N00014-76-000472.)

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DYNAMIC MECHANICAL PROPERTIES OF LUNG PARENCHYMA. J.C. Smith*, J.P. Butler*, and F.G. Hoppin Jr., Harvard School of Public Health, Boston, MA 02115.

Dynamic parenchymal properties were measured in excised dog lobes during small, constant amplitude sinusoidal volume forcing over a broad range of frequencies (1-40 Hz). Sinusoidal volume changes about various fixed inflation and deflation lung volumes (V_L) were produced by compression of intrapulmonary gas without airflow. Under these conditions measurements of transpulmonary pressure represent the dynamic parenchymal stress. Lung volume changes were computed from Boyle's Law and were 3-4% of mean V_L. The dynamic parenchymal elastance (E) and effective parenchymal viscosity (μ) were determined at each frequency and V_L. Values of E increased with V_L and increased slightly with frequency at any fixed V_L until inertial effects caused a decreasing trend. The inertial effects were greater at lower V_L. At any fixed V_L, values of μ decreased with frequency up to approximately 10 Hz, but μ was nearly constant at higher frequencies. For a given frequency the value of μ in the lower frequency region decreased with decreasing V_L. These results indicate that the dynamic parenchymal elastic and viscous properties depend systematically on lung volume. The frequency dependence of E and μ suggests that the parenchymal dynamic response represents the contribution of multiple parenchymal components with different elastic and viscous properties. (Supported by HL 14580, HL 19170, and HL 07118)

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INFLUENCE OF CONVECTIVE TRANSPORT ON AEROSOL DEPOSITION IN THE LUNG. J.C. Carpin*, D.L. Swift*, and W. Mitzner. Johns Hopkins University, Baltimore, Md. 21205.

Aerosol deposition in the lung is determined by the interaction between convective forces which carry particles along with the tidal air, and the intrinsic motion of the particles which includes settling, diffusion and inertia. To investigate the effect of convective forces alone on particle deposition, deposition measurements were made successively in gases of different kinematic viscosities (air and 80%He-20%O_2) under identical breathing conditions. This enabled us to alter the nature of convective transport in the lung without changing tidal volume or frequency. Intrinsic particle motion in both gases was made the same by choosing a particle size (1.7μm) which had the appropriate slip factor/viscosity ratio. Steady state deposition in human subjects were performed at several breathing rates and tidal volumes in air and then in He-O_2. The deposition in He-O_2 experiments averaged 50% greater than in air for each breathing condition. Since particle residence time and intrinsic particle motion were matched in each case, the differential deposition can be attributed to a change in convective mixing at the tidal-reserve gas interface, or to a change in the surface area of the interface. However, the Reynold's number is lower with He-O_2, and thus parabolic flow is more readily developed and penetrates deeper in the terminal air spaces. This supports the mechanism of increased surface area for particle exchange. Supported by NIH HL-19715, HL-00347.

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EFFECT OF EMETIC AGENT ON SUBMUCOSAL GLAND SECRETION: GASTRO-PULMONARY REFLEX. V.F. German,* I.F. Ueki,* and J.A. Nadel. Cardiovascular Research Inst., UCSF, San Francisco, CA 94143.

We studied the effect of the emetic agent $CuSO_4$ (2% aqueous solution) on submucosal gland secretion in cats, using our microcollection technique for sampling of individual glands. The animal was anesthetized, ventilated mechanically, and paralyzed. The abdomen was opened via a subcostal midline incision, and catheters were inserted near the pylorus and the upper fundus for circulating experimental solutions into the stomach. Both vagi were dissected from the abdominal portion of the esophagus; both celiac ganglia were dissected from the abdominal aorta. Gastric instillation of saline did not affect secretion ($p>0.5$), but instillation of $CuSO_4$ caused the secretory rate to increase from 7.5 ± 2.3 to 25.5 ± 2.9 nl/min (mean ± SE) within 25.7 ± 6.0 min (6 cats, 7 glands; $p<0.01$). The secretory response was not abolished by cooling both abdominal vagi or by atropine sulfate but was abolished by cooling or cutting both celiac ganglia ($p<0.05$) or by IV administration of the α-adrenergic antagonist, phentolamine ($p<0.05$). Electrical stimulation of the central cut end of a splanchnic nerve increased the secretory rate from 10.3 ± 2.2 to 26.1 ± 3.3 nl/min (5 cats, 7 glands; $p<0.05$), an effect that was prevented by administration of phentolamine. Thus, the emetic agent $CuSO_4$ reflexly stimulates submucosal gland secretion; the sensory limb of this pathway is via the splanchnic nerves and celiac ganglia; the efferent limb is via α-adrenergic receptors. (Supported in part by USPHS PPG HL-24136)

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MECHANISMS OF ISOPROTERENOL EFFECTS ON CAROTID BODY CHEMORECEPTORS IN THE CAT. M. Pokorski*, S. Lahiri, and R.O. Davies. Univ. of Pennsylvania Sch. of Med. and Sch. of Vet. Med., Philadelphia, PA 19104.

The responses of carotid body chemoreceptor afferents to intravenous injections of isoproterenol (0.5-1μg) were studied at several steady state levels of PaO_2 and $PaCO_2$ in nine cats which were anesthetized, paralyzed and artificially ventilated at 38°C. Isoproterenol stimulated the chemoreceptor activity; moderate hypoxia and hypercapnia augmented the effect. To distinguish a direct effect from a possible secondary effect originating from increases in venous return and $PaCO_2$, we compared the latency of responses to isoproterenol and hypercapnia before and after carbonic anhydrase inhibition by acetazolamide. After acetazolamide, the latency of response to CO_2 during hyperoxia increased from 3.5 s to 8.0 s whereas that to isoproterenol from 4.7 s to 6.3 s. Thus, the response to CO_2 was delayed more than that to isoproterenol. We conclude that P_{CO2}-H^+ increase is not the cause of the isoproterenol effect. A possible vascular mechanism was excluded by utilizing propanolol which blocked the vascular responses but not the chemoreceptor stimulation. A direct effect of isoproterenol through appropriate receptors in the carotid body is the likely explanation for the stimulation. This effect converges on the mechanisms which bring about O_2 and CO_2-H^+ chemoreception. (Supported in part by grants HL-19737, HL-08899, and HL-09905).

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EFFECT OF DOPAMINE ON HYPOXIC VENTILATORY DRIVE. D.S. Ward* and J.W. Bellville* (SPON: S.A. Ward). Dept. Anesthesiology, Univ. of California, Los Angeles, CA 90024

Dopamine appears to be an inhibitory neurotransmitter in the carotid body in animals and in human beings. Welsh et al. (JCI 61:708, 1978) observed in human subjects that dopamine depressed the hyperpnea due to hypoxia. However, this study may have underestimated the depressant effect since the $P_{ET}CO_2$ was not maintained constant before and during dopamine infusion. Therefore, we investigated the effect in normal human subjects of dopamine on the hyperpnea produced by normocapnic hypoxia ($P_{ET}O_2$ = 53 torr, $P_{ET}CO_2$ = 41 torr). Both $P_{ET}O_2$ and $P_{ET}CO_2$ are continuously controlled to be independent of the subject's ventilation by closed-loop computer control of the inspired gas concentration. Healthy male subjects breathed the hypoxic gas mixture during intravenous saline infusion. After 5 min, dopamine (3 μg/kg/min) was added to the infusion, followed by a 5-min period without dopamine. A short time after instituting the dopamine infusion, the minute ventilation decreased (64% of control, $p < .001$). Ventilation remained depressed at this level throughout the remainder of the infusion period. After the infusion was terminated, ventilation returned toward its prior control level. We conclude that, in man, dopamine appears to be a potent depressant of hypoxic-induced hyperpnea. This depression is sustained throughout the 5-minute infusion providing end-tidal CO_2 and O_2 are held constant.

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TEMPORAL VARIATION OF SPONTANEOUS HUMAN BREATHING PATTERN INTERRELATIONSHIPS. J. Andrew Daubenspeck, Dartmouth Medical School, Hanover, NH 03755.

Changes in the correlation between the spontaneous tidal volume (VT) and inspiratory duration (TI) with time have been demonstrated by Brusil, et al. (J. Appl. Physiol. 48:545,1980) in a human subject during metabolic alkalosis. I have used a piecewise linear regression technique proposed by McGee and Carleton (J. Am. Stat. Assoc. 65(331):1109,1970) to analyze more than thirty 300 breath sequences of VT and TI in 6 subjects during steady-state hyperoxic eucapnia and hypercapnia sufficient to produce minute ventilations of 20-30 LPM. This technique clusters consecutive breaths into statistically consistent regression regimes and can be used to identify changes with time in the interrelationship between VT and TI. Nearly every experiment resulted in more than one statistically distinct regression relationship between VT and TI regardless of which variable was designated to be independent for the regression. Alterations in both regression slope and intercept were noted with time. Some regression regimes encompassed fewer than 20 breaths while others were identified that included more than 125 breaths. The assumption of a fixed, time-invariant relation between these aspects of the breathing pattern is thus not supported by these results in normal subjects at rest or in mild hyperpnea. (Supported by USPHS NIH grants HL00280 and HL19248).

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NO INFLUENCE OF HEMATOCRIT (15 TO 58%) ON RESPIRATORY CONTROL (V_T/P_ACO_2, f/V_T) IN CHEMODENERVATED DECEREBRATE CATS. Herbert L. Borison, Jeffrey Hurst†* and Lawrence E. McCarthy.* Dartmouth Med. Sch., Hanover, NH 03755.

Our purpose in varying the hematocrit was to affect inversely the CO_2 content of the brain as a means of assessing the role of extracellular fluid composition in the control of breathing. Unanesthetized decerebrate cats were chemodenervated by section of the carotid sinus and aortic depressor nerves. Respiration was recorded from a whole body plethysmograph. Endotracheal CO_2 and O_2 were monitored continuously. Steady-state determinations were made of $V_T/\log P_ACO_2$ slope and apneic point and of the f/V_T relationship with pulmonary vagal afferent nerves intact or sectioned, under normoxic and hyperoxic conditions. Hematocrit was reduced or increased by isovolemic exchanges of plasma or packed erythrocytes. No consistent or remarkable changes were detected in central controller function for chemoceptive adjustment of tidal volume and proprioceptive adjustment of frequency over an hematocrit span >40%. These results favor the contention that arterial rather than brain chemical factors are the centrally sensed feedback quantities for respiratory regulation. (†Predoctoral Fellow of the Albert J. Ryan Foundation. Supported in part by PHS Grant NS 04456.)

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Temporal aspects of ventilatory control in awake cats during acclimation to high altitude (5500 m). W.E. Fordyce and S.M. Tenney, Physiol. Dept., Dartmouth Med. Sch., Hanover, NH 03755.

The time courses of several ventilatory variables have been observed in 3 non-purring, awake cats during 48 hr exposures to hypocapnic hypoxia (P_IO_2=73 Torr). Steady state breathing pattern (V_T, T_{TOT}, T_I/T_{TOT}), end-tidal gas pressures and CO_2 response slope (P_ICO_2≤14 Torr), all measured in a plethysmograph, were determined during hypoxic acclimation and as modified by acute normoxia (P_IO_2=146 Torr). On the average, it was found that P_ACO_2 decreased rapidly during the first 30 min of hypoxia to 29 Torr, representing a 23% increase in alveolar ventilation ($\dot{V}_A$) over its sea level value (i.e., control); this was due to a 25% increase in V_T to 38.5 ml from control and an unchanged T_{TOT} (2.5 sec). T_I/T_{TOT} was decreased from .40 to .375 acutely. Over the next 48 hrs, there were slow decreases in P_ACO_2 (to 23 Torr) and T_{TOT} (to 1.8 sec) while T_I/T_{TOT} increased slightly(to .41) and V_T remained fixed at 38.5 ml. During acclimation, acute normoxia resulted in a 20% reduction in $\dot{V}_A$ due to the return of V_T to its control value and an unchanged T_{TOT}. Acute hypoxia also resulted in a doubling of the isoxic CO_2 response slope from its control of 150 ml/min/Torr; over the next 7 hrs of hypoxia, this response slope again doubled and then remained constant. During acute normoxia, the CO_2 response was about twice its control value. Thus, the awake cat appears to acclimate faster to high altitude than does man. (Supported by Parker B. Francis Foundation and PHS Grant NHLBI 02888-23.)

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EFFECT OF T_3 ON BODY TEMPERATURE DURING EXERCISE. Joel M. Stager*, Bruce J. Martin and David Robertshaw. (Spon: R. Elizondo). Physiology Section, Medical Sciences Program, Indiana University, Bloomington, IN 47405.

Although there is evidence that the thyroid hormones influence the level of body temperature during exercise, little is known about the actual mechanisms involved. To determine if this influence is mediated by the known effects of triiodothyronine (T_3) on metabolic rate, we examined the effects of altered T_3 levels on rectal temperature (T_{re}) and metabolic rate ($\dot{V}O_2$) during steady-state exercise in 5 dogs. After control data were collected, the dogs were treated with exogenous T_3 to elevate circulating T_3 levels or treated with propylthiouracil (PTU) to lower the endogenous T_3 levels. When compared to control, during steady-state exercise, T_{re} was found to be significantly increased ($P<0.05$) in the T_3 treated dogs and significantly decreased ($P<0.05$) in the PTU treated dogs. There was also a significant rise in exercise $\dot{V}O_2$ in the T_3 treated dogs ($P<0.05$), while no change was observed in the PTU animals. Because T_3 treatment resulted in an elevation in exercise $\dot{V}O_2$, untreated dogs were run at elevated work loads such that their $\dot{V}O_2$ matched that recorded when pretreated with T_3. T_{re} in these experiments was still significantly lower than after T_3 treatment even though metabolic rates were now equivalent. Since at equal exercise $\dot{V}O_2$, PTU treatment lowered T_{re} and T_3 treatment raised T_{re}, we conclude that the effects of T_3 on body temperature during exercise are mediated through mechanisms other than alterations in metabolic rate.

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MECHANISM OF THE ENHANCED LIPOLYSIS IN ADIPOSE TISSUE OF EXERCISE-TRAINED, COLD-ACCLIMATED AND FOOD-RESTRICTED RATS. J. Lupien*, N. Folléa* and L. Bukowiecki. Dept. of Physiology, Med. Sch., Laval University, Québec, G1K 7P4, Canada.

The effects of exercise-training (11 weeks of daily swimming), cold-acclimation (3 weeks at 5°C) and food-restriction (11 weeks) were studied comparatively in adipocytes isolated from male and female rats. Exercise-training inhibited cell proliferation in parametrial, but not in epididymal adipose tissue, whereas it significantly reduced adipocyte size in both fat depots. Adipocyte capacity for responding lipolytically to epinephrine (10uM) was markedly increased by exercise-training. Enhanced lipolysis was also observed when cells isolated from exercise-trained animals were stimulated by bypassing with dcAMP or theophylline the early metabolic steps associated with hormonal activation of adenylate cyclase. Binding of (-)[^{3}H]dihydroalprenolol to cellular receptor sites was not affected by exercise-training. Cold-acclimation and food-restriction reduced adipocyte size and partially mimicked the effects of exercise-training on adipocyte proliferation and lipolysis. Significantly, cold-exposure (2 days at 5°C) or starvation (2 days) did not increase adipocyte lipolytic capacity. It is therefore concluded that long-term stresses enhance adipocyte responsiveness to lipolytic hormones at metabolic steps distal to stimulus recognition by receptors, possibly at the level of protein kinases or lipases.
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CALCIUM UPTAKE AND CALCIUM/MAGNESIUM INTERACTIONS IN SARCOPLASMIC RETICULUM OF RAT SKELETAL MUSCLE WITH EXHAUSTIVE EXERCISE. W.W. Wright*, A. Bonen*, M.M. Sopper* and A.N.Belcastro* (SPON: L. Bailey). School of Physical Education, Dalhousie Univ., Halifax, N.S., Canada B3H 3J5

Ca++ uptake by sarcoplasmic reticulum (SR) from the gastrocnemius of untrained rats exhausted by treadmill running was examined after 0.5, 5 and 10 minutes incubation in mediums containing four different Ca++/Mg++ concentrations. Both rested controls (C) and exhausted (E) SR showed increased uptake at all time points when Ca++ was increased from 10 to 100 uM ($p\leq0.05$). The addition of 10 mM Mg++ to the lower Ca++ concentration resulted in decreases of from 15.94 to 7.28 and from 14.96 to 4.81 uM Ca++/mg SR protein ($p\leq0.05$) in C and E samples respectively. At 100 uM Ca++ the presence of the Mg++ resulted in a significant depression at 0.5 minutes for C and at all time points for E ($p\leq0.05$). Uptake by both C and E samples was similar with the exception that 10 mM Mg++ added to the mediums resulted in a more dramatic depression in the rate at 0.5 minutes, 10 uM Ca++ (6% vs 54%), and a significant decrease at 5 and 10 minutes, 100 uM Ca++ ($p\leq0.05$), in exhausted as compared to control tissue. This suggests that skeletal muscle SR Ca++ uptake may be more susceptible to control by Mg++ at exhaustion than at rest. (Supported by NSERC grants A-6629 and A-6449).

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THE EFFECT OF CARDIAC DENERVATION ON HEART-RATE ADAPTATIONS TO PHYSICAL TRAINING IN DOGS. G.A. Ordway, J.B. Charles*, D.C. Randall, and D.R. Wekstein. Dept. Physiol. and Biophys., Univ. of Kentucky, Lexington, KY 40536

To determine the effect of cardiac denervation on the development of a training-induced decrease in heart rate at rest and during submaximal exercise, 6 cardiac-denervated (CD) and 6 sham-operated (SO) dogs were exercise trained by means of a 6-week treadmill running program. Gastrocnemius citrate synthase activity increased significantly ($P<0.05$) to the same degree in CD (14±2.4 to 26±3.2 units/mg protein, mean±SEM) and SO (15±1.9 to 28±3.4 units/mg protein) indicating that both groups were equally trained. Resting heart rates (RHR) for SO dogs decreased significantly ($P<0.05$) from 64±4.8 to 51±3.2 bpm from pre- to post-training. CD animals showed no significant change in RHR (95±3.5 to 96±5.3 bpm). Heart-rate responses of SO animals to a standardized, submaximal exercise test decreased significantly ($P<0.05$) from pre- to post-training. However, CD animals displayed no significant decrease in heart-rate responses to the standardized exercise test. Additionally, at pre- and post-training, as heart rates rose in response to exercise-test intensity, they increased to a significantly greater degree ($P<0.05$) in SO dogs compared to CD dogs. The results indicate that in dogs, cardiac denervation prevents the decreases in resting heart rate and heart rate during submaximal exercise normally associated with endurance exercise training. (Supported by HL 19343.)

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Intramuscular Pressure During Isometric Exercise in Fast and Slow Muscle in the Cat. By J.S. Petrofsky, D. Hanpeter*, and C.A. Phillips. Biomedical Engineering Lab, Depts. of Engineering & Physiology, Wright State University, Dayton, Ohio 45435

The relationship between blood pressure, blood flow, and tension during isometric contractions of the cat soleus and medial gastrocnemius muscles was examined here. Isometric contractions were sustained for brief periods of time (between 3 and 15 sec) at tensions of 5, 10, 20, 30, 40, 60, 80, and 100% of the initial strength of 4 cat medial gastrocnemius (fast twitch) and soleus (slow twitch) muscles. During those contractions, the blood pressure was varied between 30 and 400 mmHg through a femoral arterial cannula and the blood flow from the test muscle was assessed by a venous drop counter. In this manner, then, intramuscular pressure could be assessed. The results of the experiments showed at least a 2 fold greater intramuscular pressure in the medial gastrocnemius muscle at all tensions examined; intramuscular pressure during a maximal contraction far exceeding arterial pressure in this muscle but not in the soleus muscle.

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ENERGY COST OF ISOMETRIC CONTRACTIONS IN FAST AND SLOW TWITCH MUSCLES. M. N. Sawka, J. S. Petrofsky and C. A. Phillips. Departments of Physiology and Engineering, Wright State University, Dayton, OH. 45435 and U.S. Army Research Institute of Environmental Medicine, Natick, MA. 01760

The purpose of this study was to compare the energy cost of cat soleus (slow twitch muscle) and medial gastrocnemius (predominantly fast twitch muscle) muscles for isometric contractions. A computer-controlled sequential stimulation system was employed that enabled fused isometric contractions at frequencies of motor unit discharge within the normal physiological range. This allowed submaximal isometric contractions to be maintained at predetermined tensions of 10%, 25%, 50% and 75% of the initial strength of each muscle. For each muscle the gross energy cost of contraction increased with developed tension. When data for both muscles were expressed relative to a uniform muscle mass (100g), contraction time (1 min), and developed tension (1 kg), the total net energy cost of contraction remained fairly constant at each percent tension. However, total net energy cost was greater for gastrocnemius muscle than for soleus muscle at each percent tension studied. In comparison to the soleus muscle, the gastrocnemius muscle consistently had a greater percentage of its total net energy cost provided by anaerobic glycolysis rather than aerobic metabolism. (Supported by NIH Grant #7R01NS16003-01 and Air Force Contract #F33615-78-0501.)

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OXYGEN KINETICS OF STANDARDBRED HORSES DURING EXERCISE. S.A. Barr*, R.M. Glaser, E.L. Fox, R.L. Bartels*, A.A. Gabel* (SPON: P.K. Bajpai). The Ohio State Univ., Columbus, OH 43210 and Wright State Univ. Sch. of Med., Dayton, OH 45435

The purpose of this study was to determine the oxygen kinetics of horses during two submaximal constant load exercises. For this, 10 standardbred horses (mean wt = 466 kg) exercised for 8 min on a motor-driven treadmill at velocities of 86.4 and 259.0 $m \cdot min^{-1}$ at 11% grade. $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by a flow through method where air passed over each horse's nose at a flow rate of 1492 $l \cdot min^{-1}$ (ATPS). Steady-state $\dot{V}O_2$ values were estimated by fitting linear regression equations to the data progressing from the start to the end of exercise. Two lines were fitted--the initial rise and a plateau of $\dot{V}O_2$. The lowest sum of the sums of squares was used to determine the lines best fitted to the data. These two lines were plotted and the intersection was taken as the steady-state $\dot{V}O_2$ value. Time to steady-state $\dot{V}O_2$ was found to be 28 and 56 sec for the low and high velocity exercise, respectively. Mean steady-state values were 14.7 $l \cdot min^{-1}$ (32 $ml \cdot kg^{-1} \cdot min^{-1}$) and 27.7 $l \cdot min^{-1}$ (59 $ml \cdot kg^{-1} \cdot min^{-1}$) for low and high velocity exercise, respectively. In comparison to humans, horses are similar in that they exhibit a longer time to reach steady-state $\dot{V}O_2$ at higher work intensities. In contrast, however, horses appear to achieve steady-state $\dot{V}O_2$ more rapidly. (Supported in part by Horsey, Inc., and the Equine Research Group, The Ohio State Univ. Sch. of Vet. Med.)

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LINEAR DETERMINATION OF THE $\dot{V}O2$ HALF TIME RESPONSE DURING EXERCISE. E.L. Fox, D.T. Kirkendall,* and R.L. Bartels.* Laboratory of Work Physiology School of HPER, The Ohio State University, Columbus, OH 43210

The oxygen consumption ($\dot{V}O2$) ½ time response was determined on 8 female subjects during exercise using a nonlinear equation of the form $\dot{V}O2t = \dot{V}O2ss\ (1 - e^{-kt})$, where $\dot{V}O2t$ = $\dot{V}O2$ at time t of exercise, $\dot{V}O2ss$ = $\dot{V}O2$ at steady state, e = natural log, and k = constant of the curve, and a simple, linear equation of the form $t = b(\dot{V}O2t/\dot{V}O2ss) \pm a$. The slope (b) and intercept (a) of the line, respectively, were determined for each work load and subject using the $\dot{V}O2$ measurements within the initial linear portion of the individual VO2 uptake curves. Two treadmill walks were used ($\bar{X}$ = 38 and 65% VO2 max). For the lower load, the $\bar{X} \pm$ SD for the nonlinear and linear ½ time responses were 21.6 ± 4.8 s and 21.6 ± 4.9 s, respectively, and for the higher load 32.8 ± 5.6 s and 32.6 ± 3.6 s respectively. No significant differences between linear and nonlinear means were found. The linear correlation between determinations was 0.88 ($p<.001$). The SEE was ± 3.4 s with 95% limits of ± 7.2 s. The $\dot{V}O2$ ½ time response for the higher load was significantly greater ($p<.01$) than for the lower load. Linear correlations of 0.84 and 0.76 ($p<.01$) between %VO2 max and linear and nonlinear ½ time responses, respectively, were also found. We conclude that the ½ time response can be accurately determined on a group but not an individual basis from linear equations, and the ½ time response is slower with increasing work loads.

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THE RELATIONSHIP BETWEEN BLOOD FLOW AND FATIGUE IN HUMAN CALF MUSCLE. Douglas S. Campbell* and Daniel Richardson, University of Kentucky College of Medicine. Lexington, Kentucky 40536

These studies compared resting and post exercise blood flow in the calf muscle between rapid fatiguing (RF) and slow fatiguing (SF) male subjects (20 to 30 years of age). Each subject gave a maximal isometric contraction of the calf muscle every 2 seconds for a period of 3 minutes. The RF group had a calf muscle strength decrement of 65% over the 3 minute period as compared to 46% for the SF group. Resting calf muscle blood flow (BF) measured by strain gauge plethysmography, was 2.6 ± 0.4 (SE) ml/min/100 ml for the RF group and 3.9 ± 0.8 for the SF group ($p \leq .05$). BF values representing the relaxation phase of a contraction cycle were measured immediately after the last contraction. These averaged 32.3 ± 12.0 ml/min/100 ml for the RF group and 55.2 ± 7.0 for the SF subjects ($p \leq .10$). These results indicate that the rate of calf muscle fatigue with rhythmic exercise is: 1) related in an inverse manner to resting blood flow; and 2) inversely proportional to the amount of blood flow acquired between contractions. (Supported by Sanders-Brown Research Center on Aging: Grant 6007).

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THE EFFECT OF ISCHEMIA ON POST-EXERCISE HYPEREMIA AND RATE OF FATIGUE IN HUMAN CALF MUSCLE. Hildegard A. Althoff*, Sandra J. Legan, and Daniel Richardson, University of Kentucky College of Medicine, Lexington, Kentucky 40536

This study was designed to examine the effect of total ischemia on: 1) the rate of calf muscle fatigue during rhythmic isometric contractions; and 2) the amount of hyperemia following such contractions. Eight male subjects (20-30 yrs. of age) exerted a maximal isometric contraction of the calf muscle every 2 sec. for a period of 3 min. The contractions were performed under normal blood flow conditions and under ischemia achieved by inflating a pneumatic cuff around the thigh to a pressure of 200 mm Hg. Contraction force was continuously recorded over the 3 min. period. Under normal blood flow conditions, calf muscle strength declined by 46 ± .05% ($\bar{x}$ ± SE) by the end of the 3 min. period. Under the ischemic condition the decrement was 79 ± .05%. Calf muscle blood flow was measured by strain gauge plethysmography before and periodically after the exercise period. Total post-exercise hyperemic blood volumes were increased 4-fold under ischemia as compared to the normal-flow condition. These results support the hypothesis that the rate of calf muscle fatigue during rhythmic isometric exercise is in part determined by the size of the blood flow debt acquired during exercise. (Supported by Sanders-Brown Research Center on Aging: Grant 6007).

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ALPHA-ADRENERGIC BLOCKADE DURING EXERCISE IN DOGS WITH MYOCARDIAL INFARCTION AND LEFT STELLATE GANGLIONECTOMY. P.A. Gwirtz, P.J. Schwartz*, H.L. Stone. Department of Physiology University of Oklahoma, H.S.C., Oklahoma City, Oklahoma 73190

Alpha-adrenergic blockade (α-B) and left stellate ganglionectomy (LSGx) increase coronary blood flow (CF) during exercise in normal dogs by removing an alpha-receptor mediated vasoconstrictor tone which can limit CF. Eight dogs were instrumented to measure left ventricular systolic pressure (LVSP), dP/dt max ('P), heart rate (HR) and CF. They were exercised before (control, C) and after an anterior myocardial infarction (MI) plus LSGx (infarction size = 25±2% of LV). Resting values were similar for LVSP (118±12 mmHg), 'P (3220 ± 587 mmHg/sec) and CF (26±2 cm/sec) under all conditions. α-B increased HR after MI + LSGx (98±9 to 159±16 bpm). At a workload of 6.4 kph, 12% treadmill incline, the changes from resting values for LVSP (31±5 mmHg), 'P (2772±529 mmHg/sec), HR (115±6 bpm) and CF (19±2 cm/sec) were similar in C and MI + LSGx. α-B caused a similar increase in LVSP (33±7 mmHg), but greater increases in 'P (3767±756 mmHg/sec), HR (130±10 bpm) and CF (29±2 cm/sec) in C during exercise. Identical increases were observed after MI + LSGx ('P=3223±478 mmHg/sec; HR = 128±8 bpm; CF = 29±6 cm/sec). In summary, α-blockade resulted in the same coronary blood flow increase during control and after MI and LSGx. This suggests that MI + LSGx does not result in the total elimination of an alpha-adrenergic vasoconstrictor tone. (Supported by HL 18798).

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CHLORPROMAZINE ADMINISTRATION IN RATS: EFFECTS ON THE ABILITY TO EXERCISE IN THE HEAT. Ralph Francesconi and Milton Mager. US Army Res. Inst. Environ. Med., Natick, MA 01760

To identify and quantitate the effects of recurrent chlorpromazine (CPZ) administration on the ability to work in the heat, 2 mg CPZ/day were administered intraperitoneally for 14 days to adult, male rats weighing between 250 and 350 g following completion of CPZ pretreatment. On the fifteenth day these animals were exercised (level treadmill, 9.14 m/min) in the heat (35°C) to hyperthermic exhaustion (Tre = 42.5-43°C). The CPZ-treated rats had significantly ($p < 001$) reduced endurance capacities when compared with saline-treated controls. Increments in rectal temperature (°C/min) while on the treadmill were significantly ($p < 02$) elevated among the CPZ-treated rats. In all animals exercise on the treadmill to hyperthermic exhaustion resulted in significantly ($p < .001$) increased circulating levels of lactate and potassium when these were compared in blood samples taken immediately prior to and subsequent to exercise in the heat, while creatine phosphokinase levels were unaffected. Additionally, lactate levels were significantly ($p < .05$) increased in the post-run blood samples of CPZ-treated rats when compared with the appropriate saline-treated controls. We concluded from these studies that prolonged administration of CPZ in rats reduced their endurance capacity in the heat, increased heat storage while exercising, and exacerbated the effects of heat and exercise on at least one of the pathochemical indices of heat/exercise injury.

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PREINDUCED HYPERTHERMIA IN RATS: EFFECTS ON THE ABILITY TO EXERCISE IN THE HEAT. Milton Mager and Ralph Francesconi. US Army Res. Inst. Environ. Med., Natick, MA 01760

Hyperthermia was induced in a group (n=12) of adult, male rats by intracerebroventricular (ICV) administration of prostaglandin E_1 (PGE_1, 40 µg/20 µl sterile saline). When Tre reached the prescribed temperature (Tre = 39-40°C), the rats were exercised (level treadmill, 9.14 m/min) in the heat (35°C) to hyperthermic exhaustion (Tre = 42.5-43°C). These rats demonstrated significantly ($p<.001$) reduced endurance capacities ($\bar{X}$ = 21.42 min) when compared with a group of saline-treated (20 µl, ICV) normothermic controls ($\bar{X}$ = 32.33 min, n=12). Despite increased vasodilation among the PGE_1-treated animals (ΔTsk/min, $p<.001$) while exercising, no additional thermoregulatory effects were noted as increments in rectal temperature were unaffected. Blood samples taken immediately prior to and subsequent to exercise to hyperthermic exhaustion revealed significant increments in several clinical chemical indices of heat/exercise injury in all animals. For example, in both groups of rats circulating levels of lactate, potassium, and blood-urea nitrogen were significantly increased ($p<.005$) in the post-run blood samples. However, prostaglandin hyperthermia per se had no effects on these parameters either before or after the run. We concluded from these studies that while preinduced hyperthermia markedly attenuated endurance capacity and increased peripheral vasodilation during exercise in the heat, no additional thermoregulatory or pathochemical effects were observed.

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EXERCISE REDUCES UTERINE BLOOD FLOW IN THE PREGNANT PYGMY GOAT. James Metcalfe, A. Roger Hohimer, John Bissonnette and Maralee Lawson*. Univ of Oregon Health Sci Ctr, Portland, Or. 97201

Pygmy goats in the last trimester of pregnancy were trained to walk on an inclined treadmill at rates up to 1.75 MPH and 15° incline. When the animals were well accustomed to the exercise procedure, an electromagnetic flowmeter was placed unilaterally on a middle uterine artery (MUA). Observations were made between 5-21 days after the surgery. The goats were studied while standing quietly on the treadmill and during 5-minute periods of exercise. The average values of MUA blood flow (BF) and maternal heart rate (MHR) during the last 2 min of exercise were expressed as percentage changes from the average values in the 2 min preceding the exercise. In 36 studies on 8 goats, exercising at either 1.50 MPH and 10° incline or 1.75 MPH and 15° incline, MUA BF fell 17 ± 6% (SEM, $p < .05$, $n = 8$) while MHR rose 34 ± 4% above resting levels of 131 ± 5 beats/min. Three animals were studied at several different levels of exercise, ranging from 0.5 MPH and 0° incline to 1.75 MPH and 15° incline. While inter-animal variation was large, each individual showed decreases in MUA BF proportional to the level of exercise. We conclude that during exercise the pregnant Pygmy goat redistributes BF away from the gravid uterus with the reduction in MUA BF being proportional to the intensity of exercise. (Supported in part by USPHS, NIH Grants HD #10034 and HL #05711, and the Oregon Heart Association.)

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MYOCARDIAL PERFORMANCE DURING WHEELCHAIR EXERCISE. S.W. Wilde*, R.M. Glaser, M.N. Sawka, D.S. Miles* and R.J. Durbin*. Wright State University School of Medicine, Dayton, Ohio 45435 and VA Medical Center, Dayton, Ohio 45428

A relatively high incidence of cardiovascular disease is associated with wheelchair confinement. To better assess myocardial performance of wheelchair-dependent individuals, exercise stress testing is desirable. Therefore, the purpose of this study was to apply techniques of impedance cardiography to wheelchair-type exercise. For this, 9 wheelchair-dependent volunteers completed a progressive intensity, discontinuous test on a wheelchair ergometer (WERG) at power outputs (PO) of 60, 120 and 180 kpm·min^{-1}. An impedance cardiogram (ZCG) was recorded for 15 sec immediately following steady state exercise. Stroke volume (SV) calculated from the ZCG was multiplied by the steady state heart rate (HR) to estimate cardiac output ($\dot{Q}$). Values for SV, HR, $\dot{Q}$, Heather Index of contractility and the first derivative of the impedance change were found to increase with PO, while ventricular ejection time and R-Z interval time decreased. The linear relationship between $\dot{Q}$ and oxygen uptake ($\dot{V}O_2$) was found to be: $\dot{Q} = 0.21(\dot{V}O_2) + 2.31$, $r = 0.58$. A similar relationship was found for these variables during WERG exercise when $\dot{Q}$ was determined by CO_2 rebreathing. These data indicate that impedance cardiography may be used for the noninvasive assessment of myocardial performance during wheelchair exercise. (Supported in part by the Medical Research Service of the VA and the VA Prosthetics Center)

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VALIDITY OF POWER OUTPUT DETERMINATION FOR WHEELCHAIR LOCOMOTION. S.R. COLLINS* AND R.M. GLASER. Wright State University School of Medicine, Dayton, Ohio 45435

The purpose of this study was to validate an in-field method of determining power output (PO) requirements for manual wheelchair (WC) locomotion. PO was calculated from the average force required to push a WC and its occupant over level tiled and low pile carpeted surfaces multiplied by the locomotive velocity (3 km·hr^{-1}). Average pushing force was determined by a strain-gauge transducer and an electronic integrator. Ten male volunteers (wt = 73 - 96 kg) were found to require PO levels ranging from 39 to 64 kpm·min^{-1} and 96 to 135 kpm·min^{-1} for WC locomotion on the tiled and carpeted surface, respectively. Subjects then propelled the WC over the test surfaces at 3 km·hr^{-1} while steady state $\dot{V}O_2$, $\dot{V}E$ and HR were monitored. These variables were also monitored while the subjects operated a wheelchair ergometer (WERG) at corresponding PO levels. $\dot{V}O_2$ and $\dot{V}E$ were found to be about 15% higher, and HR about 2% higher for actual WC locomotion. High correlations were found for these variables between WC and WERG operation. Somewhat higher response values for WC locomotion may in part be due to the additional load of steering, and the drag of the gas collection apparatus. The similarity of these responses for both modes of exercise, however, suggests the validity of this method of determining PO requirements for WC locomotion. (Supported in part by the Medical Research Service of the VA and the VA Prosthetics Center)

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CHANGES IN PULMONARY FUNCTION OF WHEELCHAIR ATHLETES FROM EXERCISE TRAINING. D.S. Miles*, M.N. Sawka, R.M. Glaser, S.W. Wilde*, R.J. Durbin*, R.W. Gotshall. Wright State Univ. Sch. of Med., Dayton, OH 45435

The effect of upper body endurance training on pulmonary function was assessed on 8 members of the 1980 national championship wheelchair basketball team. Each subject trained for 8 wk (30min;3/wk) with a wheelchair ergometer (WERG) at a power output (PO) which would elicit 80% of max HR reserve. Measurements were obtained pre- and post-training at rest, during WERG exercise at 60, 120, and 180 kpm, and during maximal exercise. Lung volumes measured by spirometry and FEV at 1, 2, and 3 s were similar pre- and post-training. MEFR increased significantly from 7.4 to 9.7 l·sec^{-1}. MVV_{12} increased slightly from a pre-training value of 181.2 to 187.7 l·min^{-1}. Submaximal exercise $\dot{V}_E$ and $\dot{V}O_2/\dot{V}_E$ values were similar pre- and post-training. Maximal exercise ventilation ($\dot{V}_{Emax}$) increased from 95 to 125 l·min^{-1}. $\dot{V}_{Emax}/MVV_{12}$ x 100 increased significantly from 52% to 69% during maximal exercise. Although lung volumes were not affected by training, these results suggest that upper body endurance training can improve the ventilatory performance of wheelchair athletes. (Supported in part by the Medical Research Service of the Veterans Administration)

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DECREMENTS IN PHYSICAL WORK CAPACITY FOR WHEELCHAIR ACTIVITY WITH ADVANCING AGE. R.M. Glaser, M.N. Sawka, L.L. Laubach*, D.S. Miles*, O. Al-Samkari*, and A.G. Suryaprasad*. Wright State University School of Medicine, Dayton, Ohio 45435 and Veterans Administration Medical Center, Dayton, Ohio 45428.

The purpose of this study was to quantitate the physical work capacity (PWC), peak oxygen uptake (peak $\dot{V}O_2$), and maximal heart rate (HRmax) responses to maximal effort wheelchair ergometer (WERG) exercise for 3 groups (N=6/group) of disabled males: Group I (20-30 yr), Group II (50-60 yr), and Group III (80-90 yr). All groups participated in progressive intensity, discontinuous test protocols on a WERG. For each variable, mean values between Groups I-II, II-III, and I-III were found to be significantly different ($P < 0.01$). PWC, peak $\dot{V}O_2$ and HRmax values were found to decrease with advancing age. These decreases were best described by parabolic models for PWC and HRmax, and by a linear model for peak $\dot{V}O_2$. In comparison to PWC for Group I (506 kpm·min^{-1}), surprisingly low values were found for Groups II (95 kpm·min^{-1}) and III (42 kpm·min^{-1}). When our peak $\dot{V}O_2$ data were combined with other data in the literature for upper body exercise by male disabled individuals, a decrease of 0.19 l·min^{-1} or 2.9 ml·kg^{-1}·min^{-1} per decade of life was found. These data indicate that wheelchair propulsion requires an extraordinarily large proportion of one's PWC with advancing age. This could severely restrict the elderly from performing most wheelchair tasks. (Supported in part by the Medical Research Service of the Veterans Administration)

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AGE-RELATED INCREASE OF PLASMA CATECHOLAMINES IN RESPONSE TO DYNAMIC EXERCISE IN HEALTHY ADULT MEN. Stephen P. Tzankoff*, Jerome L. Fleg*, Arthur H. Norris*, and Edward G. Lakatta. Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224

It is well established that the maximum cardiovascular response to dynamic exercise progressively decreases with advancing age. To determine whether this can be attributed in part to an age-related decline in the secretion of catecholamines, plasma levels of norepinephrine (NE) and epinephrine (E) were determined (radioenzymatic method) along with O_2 consumption (V_{O_2}), heart rate (HR), and systolic blood pressure (SBP) for each work load of a multi-stage treadmill exercise test in 17 male volunteers (ages 26-74; mean = 52.3±3.9). At rest there were no age-related differences in the variables measured: NE averaged 287 and E 28.6 pg/ml. NE and E progressively increased with work load but levels at a given load were higher in the older men. The maximal rate was not age-related, reflecting the health and physical fitness of the older men. However, both HR_{max} and $\dot{V}O_{2max}$ declined with age: HR_{max} (b/min) = 200-(.63·Age), r = .72, $P<.001$; $\dot{V}O_{2max}$ (ml/kg/min) = 45-(.17·Age), r = .50, $P<.05$. NE_{max} increased with age: NE_{max} (pg/ml) = 17+(40·Age), r = .50, $P<.05$. The diminution of cardiovascular performance (HR_{max} and $\dot{V}O_{2max}$) occur in the elderly in spite of higher plasma catecholamine levels in that age group. This suggests an age-related decline in target organ responsivity in the elderly during maximum exercise.

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A COMPARISON BETWEEN PHYSIOLOGICAL CLONUS AND SHIVER OF THE ANKLE. P. A. Iaizzo* and R. S. Pozos* (SPON: L. J. Heller). Sch. of Med., Univ. of Minn.-Duluth, Duluth, MN 55812.

Shiver has been described as a natural amplification of a tremor which is always present (1). A similar conclusion has been drawn for physiological clonus of the ankle produced by fatigue (2). This study was undertaken to investigate if physiological clonus and shiver have similar frequency and amplitude characteristics. To elicit physiological clonus, seven subjects ran on a treadmill until they were fatigued. The subjects were then seated and placed their ankle in a slightly plantar flexed position. This position readily produced an overt involuntary oscillation (2). In the second part of the experiment, the subjects assumed the above mentioned leg position in a $0^{o}C$ environmental chamber until shivering began. In both experimental arrangements, acceleration signals of the knee and electromyograms (EMG's) from the soleus and tibialis anterior were simultaneously recorded on an FM tape recorder and later analyzed on a PDP-12 computer. On comparison of the two sets of data, physiological clonus and shiver shared the same frequency (6-7 Hz) and amplitude characteristics for both the acceleration and EMG. From such data it may be concluded that similar mechanisms may be responsible for both of these oscillations of the ankle.

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BLOOD CALCIUM INCREMENTS FOLLOWING INTENSE PHYSICAL ACTIVITY. Albert F. Bennett and John A. Ruben*. Univ. of California. Irvine, 92717, and Oregon St. Univ., 97331.

Blood pH and plasma calcium concentration were measured before and 10 min after physical activity to exhaustion in 10 species of vertebrates. Blood pH decreased significantly (0.2 to 0.7 pH units) as a result of lactic acid production in the skeletal muscles. Blood calcium levels wre significantly increased after activity in all species with osseous skeletons. Lower vertebrates with well-vascularized skeletons experienced the greatest elevations [trout, 2.3 (rest) to 4.0 (post-active) mM Ca; gar, 2.6 to 4.1; rattlesnake, 2.6 to 4.1]. In the lizard Iguana, blood calcium rose over 50% during 10 min of moderate walking activity and remained elevated for over 30 min after cessation. Fish with poorly-vascularized acellular bone experienced smaller increments (bass, 2.5 to 3.0; crappie, 2.4 to 2.8) and mammals, which have an epithelial bone-tissue fluid barrier, also had smaller increments [rat, 2.6 to 3.0; human, 2.7 to 2.9 (whole blood)]. Vertebrates with cartilaginous skeletons experienced no rise in plasma Ca after activity (lamprey, 2.6 to 2.4, N.S.; dogfish shark, 4.3 to 4.5, N.S.). The most likely source of the increased Ca is dissolution of bone. Intense activity may lead to short-term disturbances in blood Ca homeostasis in many species of vertebrates and a variety of evolutionary adaptations of the vertebrate skeleton serve to lessen this effect. (Supported by NSF Grants DEB 78-10837, PCM 77-242082 & NIH Grant K04 AM00351.)

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IONIZED Ca IN BIOLOGICAL FLUIDS AND BUFFERS. H.G. McKercher*, I.C. Radde, J. Sheepers*, L. Derewlany* and J. Whitten*. Res. Inst.,The Hospital for Sick Children, Toronto, Canada M5G 1X8.

Newer Ca-selective electrodes are capable of measuring the free Ca concentration in both protein-containing and protein-free biological fluids and buffers. Various buffers for in vivo and in vitro experiments contain Ca ion concentrations calculated to be "physiological". Measurements of ionic and total Ca concentrations in such fluids showed that discrepancies existed between expected and observed [Ca]. Krebs-Ringer bicarbonate (KRBB) and phosphate buffers (pH 7.4) consistently showed lower than expected ionized Ca[ICa] at any level of total Ca[TCa] (Table). HEPES, DEA and Tris buffers also produced lowered ICa readings. Addition of bovine serum albumin (BSA) changed the [ICa] profoundly. This phenomenon was pH-dependent. The table gives the % observed/expected [ICa] in the various fluids.

Expected	KRBB	BSA		PO_4 buffer		TRIS/HEPES DEA		
[ICa],mM	pH 7.4	pH 7.4		pH 7.4		25mM/125mM NaCl,pH 7.4		
		2%	6%	10mM	4mM			
0.5	87	--	--	66	82			
1.0	82	50	27	68	83	)98	95	96
2.0	82	57	27	70	85	)		

Total [Ca] may be misleading if used to indicate the concentration of biologically active [Ca] in transport, enzyme and drug experiments. It is thus essential to measure [Ca] in in vivo and in vitro physiological, biochemical and pharmacological systems. (Supported by MRC Canada, MA 1797).

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EFFECT OF PHOSPHORUS ON CALCIUM AND PHOSPHORUS METABOLISM IN MAN. Herta Spencer, Lois Kramer*, Clemontain Norris*, and Dace Osis*. Metabolic Section, Veterans Administration Hospital, Hines, IL 60141

The effect of phosphorus on the calcium balance and on the intestinal absorption of calcium was determined in man. Two intake levels of phosphorus, 800 and 2000 mg per day, were used during different calcium intake levels of 200, 800, 1500, and 2000 mg/day. In order to achieve the 2000 mg phosphorus intake sodium glycerophosphate was added to the constant diet. The diet contained 800 mg phosphorus per day. The intestinal absorption of calcium was determined with oral tracer doses of ^{47}Ca in all study phases. The urinary calcium decreased significantly during the high phosphorus intake, irrespective of the calcium intake, while the stool calcium remained unchanged. ^{47}Ca absorption did not differ significantly in control and high phosphorus studies as was shown by the lack of change of the ^{47}Ca plasma levels and of the fecal ^{47}Ca excretions. Urinary and fecal phosphorus excretions increased significantly during the high phosphorus intake. The increase in urinary phosphorus was greater during the low calcium intake than during higher calcium intakes and the reverse was true for the increase in stool phosphorus. Serum calcium and phosphorus levels remained unchanged during phosphorus supplementation regardless of the calcium intake. (Supported by a research grant from the USDA.)

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CALCITONIN: A PHYSIOLOGICAL REGULATOR OF RENAL CITRATE CLEARANCE AND CIRCULATING CITRATE LEVELS. R. B. Franklin and L. C. Costello, Department of Physiology and Biophysics, Howard University College of Medicine, Washington, D.C. 20059.

In earlier studies we have conclusively demonstrated that administration of porcine calcitonin (CT) produces a marked and consistent hypocitricemic and citraturic effect in rats and dogs. However, even the response with as little as 10 MRC milliunits in rats may be considered as an unphysiological effect. The present study was designed to demonstrate these effects under conditions in which endogenous alterations of CT would occur. We studied the effect of thyroidectomy (TX) on circulating citrate half-time ($T_{\frac{1}{2}}$) in rats. The results demonstrate that removal of the endogenous source of CT by TX markedly increases the citrate $T_{\frac{1}{2}}$. The parathyroids had been transplanted and were functional so that parathyroid hormone was not involved in the response, and the short duration of the TX would rule out any changes in thyroine being involved. In addition, we studied the effect of TX on urinary citrate excretion. The results demonstrate that removal of CT by TX results in a significant decrease in the renal clearance of citrate. Furthermore, the administration of CT resulted in a renal vein hypocitricemia within 15 min. after injection and a concurrent citraturia in the first 30 mins. These results along with earlier investigations continue to support the view that CT may have an important physiological function in the regulation of renal handling of citrate and circulating citrate levels. Supported in part by NIH Grant AM 19049.

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MECHANISMS OF REGULATION OF CANINE ANTRAL GASTRIN RELEASE BY CALCIUM. Richard G. Fiddian-Green* and Arthur I. Vinik* (SPON: Horace W. Davenport). Univ. of Michigan, Ann Arbor, MI 48109

The ability of luminally applied Ca++ to stimulate the release of gastrin from canine antral mucosa in vitro, and the mechanism by which Ca++ stimulates the release of gastrin have been examined in tissues obtained from each of sixty dogs and mounted in Ussing chambers. Luminally applied $CaCl_2$ at pH 7 and at pH 2.5 released gastrin in a dose-related manner, but released more gastrin at pH 2.5 than at pH 7 ($p<0.02$). Gastrin released by $CaCl_2$ at pH 2.5 was reduced by the Ca++ antagonist Verapamil ($p<0.05$) and by displacement of membrane bound Ca++ with Lanthanum Chloride ($p<0.05$) but not by inhibition of Na/K ATPase with Ouabain ($p<0.02$). Release at pH 2.5 in the absence of luminally applied $CaCl_2$ was enhanced by Verapamil ($p<0.02$), Lanthanum Chloride ($p<0.05$), Ouabain ($p<0.02$) and by the Ca++ ionophore A2318 ($p<0.05$). The data suggest that release of gastrin from canine antral mucosa may be triggered by an accumulation of Ca++ within the G cell cytosol. Most of the gastrin was released into the luminal solution. Chromatographic analysis of the released gastrin and complementary recovery experiments suggest that the gastrin released by the serosa is heptadecapeptide gastrin and that released into luminal fluid is a small C-terminal peptide fragment, which may be a degradation product of heptadecapeptide gastrin.

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EFFECTS OF Fe^{++}, Co^{++} and Ni^{++} IONS ON SPIKES ELICITED FROM MYOEPITHELIAL CELLS. M. Anderson. Smith College, Northampton, MA 01063

The myoepithelial cells of the proventriculus of the polychaete worm Syllis spongiphila undergo Ca-spikes. The Ca^{++} channels are unusual in that they also permit passage of Mn^{++} ions. Mn-spikes (elicited in Ca-free, Mn-containing solutions) are of longer duration than Ca-spikes and are not associated with contractions. To test the hypothesis that Mn^{++} ions may pass through Ca^{++} channels because they exhibit a relatively low energy of hydration, the competing effects of 3 other first order transition metal ions (Fe^{++}, Co^{++} and Ni^{++}) were studied on both Ca- and Mn-spikes. Ca-spike (pooled data): The relative effectiveness on reversibly abolishing the Ca-spike (20 animals) and decreasing the maximum rate of rise (2 animals) was Ni > Co ≳ Fe. The pattern of effectiveness of below-blocking concentrations on increasing threshold (20 animals) was Ni > Co ~ Fe. All three cations caused increased durations of Ca-spikes. Mn-spike (pooled data, 5 animals): The relative effectiveness on increasing threshold and decreasing overshoot was Ni > Co > Fe while that on decreasing MRR was Ni > Co ~ Fe. All 3 cations caused decreased durations of Mn-spikes. These data are compatible with the idea that effectiveness in blocking the generation of the spike increases as the energy of hydration of the competing cation increases, and they support the hypothesis that Mn^{++} ions may pass through Ca^{++} channels because they have a relatively low energy of hydration. Supported by USPHS Grant NS12196.

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MICROELECTRODE STUDIES OF INTRACELLULAR Ca REGULATION $[Ca]_i$ DURING INSULIN STIMULATION OF RAT SKELETAL MUSCLE AND ADIPOSE TISSUE. J. O'Doherty¶ P. D. Reed¶ and R. J. Stark, Indiana Univ. Sch. Med. and Purdue Univ. Sch. Sci. Indpls., IN 46223.

The discovery that Ca^{++} binding proteins are multifunctional enzyme-regulators suggests that $[Ca]_i$ regulation may be an important mechanism of insulin action. Ca-selective and open tip microelectrodes were employed to detect any small or transient changes in $[Ca]_i$ that might be induced during hormonal stimulation of "insulin sensitive" tissues. Rat soleus muscle fibers and adipose tissue were profused with oxygenated Ringer's soln. buffered to pH 7.4 at 37°C. Ca- and membrane electrode potentials were continuously measured while the profusate was changed to a Ringer soln. containing (0.01, 0.1 and 1.0 mU/ml) without interrupting the flow. The mean steady-state values determined for E_m and $[Ca]_i$ in muscle and adipose tissue, respectively were -77.8 ± 1.3 mV and 4.9 ± 1.1 x 10^{-7}mM; -46.1 ± 0.9 mV and 4.1 ± 1.2 x 10^{-7}mM. The Ca-selective microelectrodes have been shown to be capable of measuring small hormonally induced changes in $[Ca]_i$ (O'Doherty et al. Science In press). However, insulin stimulation of the above tissues for periods of 4-15 min, produced no significant changes in either E_m or E_{Ca}. These results indicate $[Ca]_i$ is not altered during insulin stimulation and suggest that $[Ca]_i$ regulation may not be involved in the mechanism of insulin action. (Supported by NIH AM 26246 and ADA grants and a Summer Faculty grant to RJS from the Purdue Research Foundation.)

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THE EFFECTS OF PROSTAGLANDIN E_2 AND PROSTAGLANDIN $F_{2\alpha}$ ON SODIUM EFFLUX IN SINGLE BARNACLE MUSCLE CELLS. Edmund Y. Tong, Holly O. Schuur* and Wee-Ling Ooi*. Dept. of Biology, Wheaton College, Norton, MA 02766.

The effects of Prostaglandin E_2 (PGE_2) and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on ^{22}Na efflux in single barnacle muscle cells were studied. At 5 µg/ml, $PGF_{2\alpha}$ produced a diphasic response with an increase in Na efflux of 55%±15(Mean ± S.E.) during the first peak and an increase of 92%±17 during the second peak. PGE_2 also produced a similar effect; however, the estimated values for the two peaks were somewhat lower (56%±14 and 65%±12 respectively) after correcting for the slight stimulation (14%) caused by ethanol (1:4000 V:V) which was present in the stock solution. The enhanced Na efflux seems to be independent of the Na^+-K^+-ATPase since in 10^{-4}M ouabain-pretreated cells, the same diphasic response caused by $PGF_{2\alpha}$ persisted (45%±8 and 71%±16 respectively). On the other hand, in fibers pretreated with digitoxin (10^{-4}M), the $PGF_{2\alpha}$-induced-stimulation was completely abolished. Thus, in addition to its inhibitory action on the Na^+-K^+-ATPase, digitoxin seems to antagonize the effect of $PGF_{2\alpha}$ on Na efflux. Finally, this PG-induced-stimulation of Na efflux appears to be secondary to an increased Ca^{++} influx, since fibers bathed in a Ca^{++}-free medium (containing 10^{-4}M EDTA) showed a complete absence of the $PGF_{2\alpha}$-induced response in the outflow of sodium ions.

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TOWARD AN UNDERSTANDING OF THE MEMBRANE MECHANISM OF ACTION OF ALDOSTERONE: THE BARNACLE MUSCLE FIBER AS A PREPARATION. E. Edward Bittar, Jude Nwoga* and Michael Bárány, Dept. of Physiology, Univ. of Wisconsin at Madison and Dept. of Biochemistry, Univ. of Illinois Medical Center at Chicago.

Previous work with single barnacle muscle fibers led to evidence that these fibers are suitable for studying the inductive mechanism of aldosterone action. This has now been extended to include GTP as a probe of the steroid's membrane mechanism of action. Aldosterone preexposed fibers when injected with $GTPNa_3$ show a transitory rise in the Na efflux which is greater than that seen in unexposed fibers. However, preexposed fibers poisoned with ouabain show in addition a *sustained* response to GTP. Whereas this kinetic response is unaltered by applying actinomycin D overnight or injecting it before GTP, it is markedly reduced by injecting PPi before or after GTP. *Sustained* stimulation is also seen following Gpp(NH)p, $ATPNa_2$ and AMP-PNP injection. In these 4 situations, injection of $MgCl_2$ (but not KCl) almost completely reverses the response. Application of verapamil before GTP or Gpp(NH)p leads to a reduced but *sustained* response, which is largely abolished by injecting EGTA, Fe and Zn. Analysis of preexposed fibers injected with ^{32}P indicates increased labeling of 3 proteins (28K, 40K and 137K). However, fibers injected with GTP fail to show an altered ^{32}P profile. These results support the view that aldosterone acts by stimulating phosphorylation-dephosphorylation reactions and that injected GTP acts by removing internal Mg and trace elements.

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CHARACTERISTICS OF LEUCINE TRANSPORT AT LOW TEMPERATURES IN LIVER IN VIVO. Roger Persell and Audrey E. V. Haschemeyer, Hunter College, City University of New York, New York, NY 10021

Transport systems of liver can be studied by monitoring solute movements during passage of a bolus of fluid injected into the hepatic portal vein. A highly adaptable marine fish, the toadfish, has proven useful for study of temperature effects on transport and protein metabolism in vivo. Leucine transport by liver shows concentrative uptake at normal summer temperature (20°C). Normalized leucine/inulin ratios in liver reach 2.5 within minutes of injection at plasma leucine concentration (0.1 mM). Saturation behavior typical of carrier-mediated processes is observed. Maximal uptake from the 0.1 ml bolus is 0.6 µmole; half-maximal uptake occurs at a dose of 1.5 µmole. Kinetic analysis indicates that 40% of uptake is active transport. At 10°, in contrast, leucine/inulin ratio at 5 min after injection (0.1 mM leucine) was only 1.2. Saturation studies over a dose range of 0.01 to 3 µmole/0.1 ml yielded maximal uptake of 0.2 µmole with half-maximal uptake at 1.0 µmole dose. Although the drop in maximal uptake parallels the direct temperature effect on influx rate constant reported earlier for this system, comparison with data in Antarctic fish at 0°C indicates that this is not the basis for the change in uptake parameters. The results suggest fundamental changes in carrier properties at reduced temperatures in the temperate organism.

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DESTRUCTION OF LIVER MICROSOMAL CALCIUM PUMP ACTIVITY BY CCl_4 AND $BrCCl_3$. Karen Lowrey*, Eric A. Glende, Jr.*, and Richard O. Recknagel. Department of Physiology, Case Western Reserve University, Cleveland, OH. 44106

The capacity of liver microsomes to sequester Ca^{2+} is severely depressed 30 min after CCl_4 administration to rats (JBC 251, 1976, 1197). This effect (our data) is already manifested 5 min after CCl_4 administration, when peroxidation of microsomal lipids is already well advanced. In vitro, microsomes free of Fe^{2+} ions peroxidize minimally at 37° when NADPH and either CCl_4 or $BrCCl_3$ are added. Ca^{2+} pump activity was destroyed when malonic dialdehyde (MDA) production was as little as 0.3 µg per mg microsomal protein. This yield of MDA is much less than that observed with NADPH and Fe^{2+}. Presence of 5 mM ATP during an initial incubation increased subsequently determined Ca^{2+} pump activity as much as 7-fold. In such "ATP-protected" microsomes, Ca^{2+} pump activity was reduced to 3% of control values when lipid peroxidation due to addition of NADPH and $BrCCl_3$ was only 0.6 µg MDA per mg microsomal protein. Glucose-6-phosphatase and aminopyrine demethylase are less severely affected, being reduced to 50% and 25% of control levels, respectively, at the same yield of MDA. The extreme sensitivity of the microsomal Ca^{2+} pump to lipid peroxidation initiated by toxigenic haloalkanes appears to be due to a direct effect of the lipid peroxidation on the pump, associated with some disintegration of microsomal membrane structure. In the NADPH-Fe^{2+} system, loss of Ca^{2+} pump activity is associated with gross disintegration of membrane structure. Work supported by a grant (5-R01-ES01821) from NIEHS, NIH, USPHS.

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TOPOGRAPHICAL DISTRIBUTION OF LECTIN RECEPTORS DURING MYOGENIC CELL FUSION IN VITRO. Brian Herman, Margaret S. Robinson*, and David F. Albertini*. Harvard Medical School, Boston, MA 02115.

Lectin receptors have been implicated in the process of myogenic cell fusion during differentiation in vitro. Using the techniques of resonance energy transfer (RET) and fluorescence microscopy, we have examined the topographical heterogeneity of con A receptors on the myoblast cell surface during the period of maximal fusion. RET studies indicate that (a) con A receptors are initially clustered and undergo dispersion as fusion progresses, and (b) the rate of con A-induced clustering is most rapid at peak periods of cell fusion, when the membrane is most fluid. Pre-fixed 48 hour cultures were labeled with FITC-con A and subsequently processed for indirect immunofluorescent detection of clathrin by primary incubation with affinity purified rabbit anti-clathrin IgG followed by incubation with RITC conjugated goat anti-rabbit IgG. Coincident labeling of con A clusters and clathrin was detected at regions of myoblast cell-cell contact and zones of early fusion. The data suggest that modulation of con A receptor topography during the fusion process, involves components extrinsic to the membrane, possibly those of the clathrin-coated vesicle system.
(Supported in part by NSF grant PCM 79-22781.)

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VOLTAGE-DEPENDENCE OF PHOTOEMF IN BACTERIORHODOPSIN-CONTAINING BILAYER LIPID MEMBRANES IN RESPONSE TO CONTINUOUS ILLUMINATION. Wita Wojtkowski* and Felix T. Hong* (SPON: James A. Sedensky). Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201

Bacteriorhodopsin in the purple membrane of Halobacterium halobium functions as a light-driven proton pump (Oesterhelt and Stoeckenius, 1973). A photovoltage is observed when model membranes that contain oriented bacteriorhodopsin are illuminated by continuous visible light. Analysis of this photoresponse in terms of photoemf and its internal photoconductance (particularly in cases when the photoemf and/or the internal photoconductance is voltage-dependent) is facilitated by use of a null current measurement method (Hong and Mauzerall, 1972). Here we report a voltage-dependent reversal of photoemf in a bacteriorhodopsin-containing bilayer lipid membrane, formed according to the method of Dancshazy and Karvaly (1976). The internal photoconductance, however, has no significant voltage-dependence. (Supported by USPHS. NIH Grant GM #25144)

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FATTY ACID PERMEABILITY OF LIPID BILAYER MEMBRANES. Anne Walter and John Gutknecht. Duke Marine Lab, Beaufort, NC 28516 and Harvard Medical School, Boston, Mass. 02115.

The permeabilities of short-chain monocarboxylic acids were determined for Mueller-Rudin (decane-containing) and Montal-Mueller (solvent-free) planar bilayers. This study was designed to test the effect of solvent on the permeabilities, to resolve discrepancies in previously reported fatty acid permeabilities, and to test the applicability of Overton's rule to very small nonelectrolytes. Permeabilities were determined from 14-C-acid fluxes under conditions where the contribution of unstirred layer effects was eliminated according to the relation $1/J = 1/P^{ul}(HA+A^-) + 1/P^m(HA)$. P^m across egg phosphatidylcholine/decane bilayers for formic, acetic, proprionic, and butyric acids are 110, 66, 260 and 950 um/sec, respectively. The permeabilities of phosphatidlyethanolamine Montal-Mueller membranes are virtually identical. These values are much higher than those previously reported for lipid bilayers and show a selectivity different from many cell and tissue uptake studies. The discrepancies are due to unstirred layer effects. Formic and acetic acids permeate more rapidly than predicted by their hydrocarbon/water partition coefficients, indicating that the membrane is selective for very small nonelectrolytes. We hypothesize that this selectivity is due to deviations from Stokes' law when the molecular size of the solute is smaller than the solvent. (We acknowledge the use of Dr. R. Latorre's laboratory. Supported in part by NIH grant HL12157.)

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THE SODIUM PUMP AS A CURRENT SOURCE: LINEAR THERMODYNAMIC EQUATIONS FOR THE TRANSMEMBRANE POTENTIAL AND ITS TRANSIENT RESPONSES TO CHANGES IN TRANSPORT RATE. Kent M. Chapman. Neurosciences Sect., Brown University, Providence, RI 02912

Linear nonequilibrium thermodynamics indicates the sodium pump may be considered a current source with Na and K transport rates independent of the membrane potential, metabolic power requirements that vary linearly with membrane potential, and thus no unique stoichiometry between transport fluxes and ATP turnover. The role of this current source in establishing the resting ionic concentration ratios and the resting membrane potential has been modelled with linear thermodynamic conductances relating passive and active ion currents and turnover rate to the ionic electrochemical potentials and the metabolic affinity. A nerve cell model with -70 mV resting potential, 1 mS/cm^2 passive membrane conductance, E_{Na}=60, E_K=-90, E_{Cl}=-70 mV, 9.2 kcal/mol ATP hydrolysis affinity and a metabolic reversal potential of -110 mV shows an electrogenic hyperpolarization of 3 mV, resting ATP turnover of 3 pmol/s cm^2, and net power dissipation of 100 nW/cm^2. Its Donnan potential without the pump is -12 mV, while the pump alone without intracellular indiffusable anions generates about -30 mV. Transient responses to step changes in pump rate relax as the inverse of a power series in chemical potentials, rather than exponentially. (Supported in part by USPHS NIH Grants HL-19995 and RR-05664)

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MILK INTAKE, WATER METABOLISM AND GROWTH RATES IN REINDEER CALVES. Jack R. Luick, Robert G. White* and Dan F. Holleman*. Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

Milk intake of reindeer calves allowed free-grazing on shrub tundra in interior Alaska was estimated with the tritiated/deuterated water technique (Holleman et al., J. Dairy Sci. 58:1814-1821, 1975). Over the 25 wk lactation cycle milk intake declined exponentially from 1 l/d (0-3 wk) to 0.2 l/d while water transfer rate increased from 0.5 l/d to 6 l/d. Total body water space remained constant at 66.5% bodyweight. At 0-3 wk of age calf growth rate (130-350 g/d) was correlated with milk intake. Water transfer rate per unit bodyweight-gain in calves older than 4 wk (6.74 l/kg) was twice as high as in the 0-3 wk old calves. The average efficiency of utilization of milk solids for body solids deposition (27-30%) was slightly lower than lambs and dairy calves, however estimates of energy intake to weight gain (29.75 MJ/kg) were similar to domestic livestock. (Supported by AEC Contract AT (45-1)-2229, U.S. Tundra Biome Program (NSF GB-29342), NIH-NIGMS 10402)

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PHYSIOLOGICAL EFFECTS OF VARYING THE DIETARY LEVELS OF LINOLEIC ACID IN THE SPONTANEOUSLY HYPERTENSIVE RAT. Blanche M. Box and Gordon J. Mogenson. Univ. of Western Ontario, London, Ontario, Canada N6A 5C1

Male spontaneously hypertensive rats (SHR) 5 weeks of age were fed three different levels of linoleic acid (LA) in their diet for 19 weeks. High LA (9.5%) was supplied by 17% corn oil, medium LA (4.5%) was supplied by 8% corn oil and low LA (0.17%) by 8% coconut oil. Hypertension developed in all animals with no difference among dietary groups until the rats were 10 weeks of age. Thereafter, the group fed the low LA diet had significantly lower blood pressures than the other two groups. Although no differences in water and food intake were observed during the first 6 weeks, a mild polydipsia developed in all groups during adult life. Water retention as expressed by water intake minus urine volume was augmented in all groups as compared with Wistar Kyoto controls. The group fed the high LA diet excreted significantly less potassium than the other two groups and were hyperkalemic by the end of the experiment. The results suggest that the amount of LA as supplied by vegetable oil in the diet may effect blood pressure and electrolyte balance of SHR. The effects may be mediated through the availability of precursor, LA, for the synthesis of prostaglandins in the renal medulla which are known to be elevated in SHR. (Supported by MRC of Canada)

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EFFECTS OF OXYTOCIN ON REPRODUCTION IN RATS MAINTAINED ON DIFFERENT PROTEIN INTAKE LEVELS. René R. Roth. Department of Zoology, Univ. of Western Ontario, London, Ont. Canada. N6A 5B7

Five groups of female rats, housed in individual cages, were maintained either on a diet devoid of (group I), or supplemented with protein once (gr.II), or twice (gr.III) per week, or on a control diet (gr.IV, V). After 40 days each animal from gr.I-IV was mated. Gr.V was not paired. All the groups were divided into three subgroups each. Four days after mating subgr. A was started on daily injections of 2 USP units of oxytocin, subgr. B was given saline, and subgr.C was left untreated. Gr. V was sacrificed 20 days later. The other animals were sacrificed 30 days after mating. We recorded the number of animals which became pregnant, length of gestation, litter size, embryos in utero, occurrence of pseudopregnancy. The data indicate that: a. oxytocin induced and maintained pseudopregnancy in all groups, inclusive gr. V; b.in protein deficient animals oxytocin increased the percentage of pregnancies and also litter size; c. in the groups with adequate protein intake oxytocin decreased the percentage of pregnancies and also litter size; d. oxytocin lengthened the gestation period slightly in gr.II and shortened it in gr. III and IV; e. the negative effect of protein deficiency upon reproduction appears to be neutralized somewhat by oxytocin; f.oxytocin appears to have a somwhat inhibitory effect on the reproductive process of females maintained on a balanced diet containing adequate amounts of protein. g.hormonal activity appears to be dependent on the nutritional status of the body.

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DORSOMEDIAL HYPOTHALAMIC HYPOPHAGIA: RESPONSE TO WET MASH (WM) AND DRY DIET BEFORE AND AFTER THREE-DAY FAST. Lee L. Bernardis and Larry L. Bellinger, VA Medical Center and SUNY at Buffalo, N.Y. 14215 and Baylor Coll. Dent., Dallas, TX 75246.

Weanling female rats with dorsomedial hypothalamic lesions (DMNL) and sham-operated controls (CON) were given WM, reported to increase growth in rats; other DMNL and CON received powdered lab chow (POW). After a 10-day baseline period (10 DBP) all animals were fasted for 3 days and then refed for 6 days. Food and water intake were corrected for water in the WM. The previously reported lesion effect on body weight and composition (Lee index) were evident during the 10DBP and refeeding. The diet had no effect on these parameters. During the 10DBP both DMNL and CON on WM ate more than the respective groups on POW; this continued throughout refeeding. DMNL rats were hypodipsic as previously reported. Both DMNL and CON-fed WM took in more water than POW fed rats during the 10DBP and on refeeding. Efficiency of food utilization during the 10DBP was greater in the WM-fed DMNL than in the POW-fed DMNL rats but on refeeding it was greater in the latter group. Plasma glucose, total protein and free fatty acids did not differ significantly among the groups but glycerol was higher in the WM-fed DMNL than in the WM-fed CON and POW-fed DMNL. The data confirm and extend previous findings on only slight homeostatic deficits in DMNL rats despite profound hypophagia and reductions in ponderal and linear growth.

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THE EFFECT OF LIVER (L) DENERVATION (D) ON FOOD INTAKE (FI), MEAL SIZE (MS), WATER INTAKE (WI) AND BODY WEIGHT (BW) IN THE RAT. L.L. Bellinger and F.E. Williams. Department of Physiology, Baylor College of Dentistry, Dallas, TX 75246.

The L has been proposed to contain glucoreceptors and have a major role in preabsorptive satiation and longer term FI control. D should by this theory produce hypophagia and alter MS. LD is described in Fed. Proc. 39:500, 1980 except the portal vein was treated with 9% phenol. Rats were kept under a L:D of 12:12h (lights out 1330h). Food was present from 1400-0700h. Water was ad lib. Gr I (D,n=8) and Gr II [Sham (S),n=8] were given chow and FI and WI recorded every 10 min. from 1400-1500h and daily. Gr III (D,n=8) and Gr IV (S,n=8) were given a liquid diet from 1400-1500h and FI was recorded every 3 min; thereafter they were given chow. During 5 days post operation (PO) FI [FI in gm: Gr I, 13.7±0.9,15.9±1.2,18.6±0.9,18.2±1.0,21.2±1.1; Gr II, 13.9±1.3,16.7±1.0,17.9±1.1,18.0±1.4,18.3±2.1; Gr III, 11.6±1.6,13.8±1.2,15.8±1.4,16.8±1.1,16.4±1.8; Gr IV, 9.4±1.5, 13.3±1.3,15.1±1.0,14.0±1.5,15.4±1.7] and WI of the D groups did not differ ($P>0.05$) from S. Initial $\bar{x}$ MS during this 5 day period did not differ ($P>0.05$) between the S&D groups (Gr I, 3.0± 0.3 vs Gr II, 2.9±0.1g; Gr III, 7.9±0.8 vs Gr IV, 9.0±0.7ml). Daily BW did not differ ($P>0.05$) between S&D (0 and final BW in gm: Gr I, 220.8±6.3, 235.6±7.0; Gr II, 218.4±3.2, 231.5±3.8; Gr III, 214.6±7.1, 235.9±7.5; Gr IV, 211.2±11.0, 229.6±10.0). The data questions the role of the L as a major controller of FI (via neural connections) and agrees with human L transplant studies. (Supported in part by BCD research funds.)

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CEREBELLAR MODULATION OF REFLEXLY-INDUCED SWALLOWING. Charles H. Hockman. Schools of Basic Med. Sci. and Clin. Med., Univ. of Illinois, Urbana, IL 61801

In adult cats anesthetized with urethane, trains of low intensity stimuli delivered to discrete points in the fastigial nucleus of the cerebellum (FCb) modulated swallowing induced by stimulation of the superior laryngeal nerve (SLN). The observed effects fell into 3 categories: (1) inhibition of the reflex; (2) facilitation of the reflex; and (3) inhibition of the reflex followed by rebound repetitive swallowing at the offset of both FCb and SLN stimulation. When points were identified that yielded the latter response, a conditioning train of stimuli delivered to the FCb had a facilitatory effect upon reflexly-induced swallowing up to 20 sec after FCb stimulation, an effect observed even with subthreshold SLN stimulation. Since well-organized eating behavior has been reported to FCb stimulation in the unanesthetized, freely-moving cat (cf. J. Martner, Acta physiol. Scand., Suppl., 425, 1975), fastigial neurons may exert influences on regions that normally organize and integrate feeding behavior. Such influences could be exerted via basal forebrain structures which are known to receive projections from the fastigial nucleus. (Supported by a grant from the State of Illinois Department of Mental Health and Developmental Disabilities)

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DO RATS CONTROL SALT INTAKE? B. J. Barber and Melanie Wright*.
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Two rat metabolic cages were connected with a tunnel. Food jars at each end were filled with a "sodium-free" diet. Salt was mixed into the food at one end to bring the concentration to either 1,2,4 or 8% levels. Salt was also added to the water on this end to the same concentration. Levels were changed randomly at one week intervals. A standard rat chow and water were made available for 2 days between level changes. Food and water intake were measured daily from each end. We adopted the working hypothesis that: rats do not behaviorally control salt intake. Thus a hypothetical rat would be expected to eat/drink randomly (equally) from each end of the shuttle box at all salt levels. Daily food and water intakes were divided by two and the expected salt intake calculated from the concentration in use at that time. The actual average (N=40) salt intakes (g/kg body weight) were 0.77 (S.E.=0.08) at 1%, 0.80 (0.10) at 2%, 0.94 (0.11) at 4% and 0.86 (0.16) at 8%. The expected salt intakes for the hypothetical naive rat were 0.62 (0.06) at 1%, 1.11 (0.06) at 2%, 1.93 (0.30) at 4% and 3.83 (0.36) at 8%. The working hypothesis is rejected at the 0.001 level. We conclude that rats behaviorally control salt intake. The data support a high gain for this control system, in fact, the reduction at 8% suggests a nonlinear threshold type control system. (Supported by HL 11678.)

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ELEVATED ENDOCARDIAL HYDROXYPROLINE IN VOLUME OVERLOAD INDUCED CARDIAC HYPERTROPHY

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Pressure overload (POL) & volume overload (VOL), while producing equivalent hypertrophy, yield hearts with different biochemical characteristics. Further, the distribution of stress may be non-homogenous. Hydroxyproline (HP) is elevated in POL; it is not known if HP is altered in VOL. Thus, HP was studied in 5 regions of the myocardium subjected to VOL. VOL was produced by atrial septotomy in 5 adult cats; 5 controls (C) were also studied. After 6 wk hemodynamic parameters were measured, the myocardium excised & disected into RV and LV epi and endocardium & septum. Dried tissue was assayed for hydroxyproline. RV & arterial pressures were normal with no significant ($P > .05$) differences between groups. Pulmonary to systemic blood flow averaged 1.90±.21 in VOL. RV/BW was .83±.05 in VOL; significantly ($P < .05$) greater than C (.68±.04). RV & LV epicardial HP were not significantly different (C: RV=7.64±.52, LV=5.18±.46 VOL: RV=7.64±.52, LV=5.57±20μg/mg). Septal HP was unchanged by VOL (C=3.31±.44, VOL=3.85±.39μg/mg). Conversely VOL endocardial HP was significantly ($P < .05$) elevated above C in RV & LV (RV: C=5.30±.36, VOL=6.33±.18 & LV: C=4.47±.31, VOL=5.55±.38μg/mg). These data show that chronic VOL secondary to atrial septal defect, leading to 22% hypertrophy is associated with significantly elevated RV & LV endocardial HP and normal epicardial & septal HP.

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CONTINUOUS FEEDBACK SYSTEM FOR USE WITH CAT PAPILLARY MUSCLE.

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The recent application of laser diffraction techniques to the study of skeletal and cardiac muscle has allowed for the monitoring and control of sarcomere dynamics during contractions. Strictly isotonic contractions have been difficult to observe due to the compliance of the papillary muscle preparation and the mass, compliance, and friction inherent in the lever and linkage systems. The present system allows for monitoring and/or controlling sarcomere length, whole muscle length, or force in order to create muscle isometric, sarcomere isometric or true isotonic contractions.

The muscle's laser diffraction pattern is sensed by a photopotentiometer for the determination of changing sarcomere length. The muscle is connected between a lever, the fulcrum of which is the armature shaft of a servo motor, and an isometric force transducer. Pre- and after-load are programmed by altering the input current to the lever system via the servo motor's power amplifier. Signal manipulation and gating allow for (a) continuous feedback of changing muscle or sarcomere length, with the elimination of resting length information, to create isometric muscle or sarcomeric contractions at various preloads and (b) gated feedback from the force transducer, with the elimination of resting tension and programmed afterload to allow truly isotonic contractions. This feedback system cancels the mass, compliance, and frictional components inherent in the lever system and mechanical linkages.

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PROLONGED CONTRACTION DURATION IN SENESCENT MYOCARDIUM IS PREVENTED BY EXERCISE.

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Prolonged contraction duration (CD) is a consistant finding in isometric cardiac muscle of senescent (S) compared to adult (A) rats. To determine if exercise (E) would prevent prolonged CD in advanced age, male rats at 3 and 19 mo were run in motorized wheels (.7KMH) for 30 min, 5 X week, for 5 mo and sacrificed with matched controls (C), at age 8 (A) and 24 (S) mo. The moderate E protocol did not alter body or heart weight in either A or S. Isometric left ventricular trabeculae were studied at L_{max}, 24 min^{-1}, 29°C, in Krebs buffer (Ca^{++}=2.5 mM).

	N	Developed Tension (g/mm^2)	Max Rate of Tension ($g/mm^2/sec$)	CD (msec)
A-C	11	2.38±0.34	39.3±4.6	188±7
S-C	9	2.65±0.22	39.7±4.9	232±12
A-E	14	2.66±0.21	43.4±3.4	193±4
S-E	9	3.14±0.47	52.3±8.5	199±5

Developed tension and maximal rate of tension development were not age-related and were not altered by E. CD, while prolonged in S-C relative to A-C, $P < .01$, was unaltered by E in A; E prevented the prolonged CD in S-E, $P < .05$ vs S-C, and A-E or A-C vs S-C were not different. The effect of E to prevent prolongation of CD in S may be mediated by a change in velocity of sarcoplasmic reticulum Ca^{++} sequestration which has been shown to be diminished in S and enhanced by physical conditioning.

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KINETIC STUDIES OF CALCIUM IN INDIVIDUAL HEART SEGMENTS.

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The isolated, perfused rabbit heart was studied with reference to calcium decay and uptake in the individual segments: atria, right ventricle, left ventricle, and septum. For each segment, calcium decay and uptake curves were obtained by direct tissue analysis for calcium. Fitting procedures established single exponential decay and single exponential uptake curves. Equilibrium values of calcium contents, that is pre-washout and post-uptake calcium contents, followed the order: atrial > right ventricular > left ventricular and septal. Except for the atria, the uptake rate constant for each segment was larger than the decay rate constant. While no significant trend could be found for exponential decay rate constants, uptake rate constants followed the order: septal > left ventricular > right ventricular > atrial. (Supported in part by USPHS grants HE 05517 and RR 05407.)

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INOTROPIC ACTIONS OF ISOPROTERENOL IN CAT VENTRICULAR MUSCLE: EFFECTS OF EXTRACELLULAR POTASSIUM.

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Papillary muscles or trabeculae carneae were studied under isometric conditions at 0.2 Hz and 35°. In muscles perfused with a salt solution (PSS) containing 4 mM KCl, 3 nM isoproterenol (ISO) had a positive inotropic effect, increasing force (F), maximum rates of force development (df/dt) and relaxation (-df/dt), and time to peak force (TTP). Higher concentrations of ISO (10-1000nM) further increased F, df/dt, and -df/dt and decreased TTP. The ratio (df/dt)/(-df/dt) was unchanged by ISO. In muscles perfused with 22 mM KCl PSS, ISO had a positive inotropic effect in the same concentration range as in 4 mM KCl, but the increase in F was accompanied by an increase in TTP at all concentrations; df/dt increased less than -df/dt, such that the ratio (df/dt)/(-df/dt) fell to 0.35 (from 1.0) in the presence of 30 nM ISO. The increase in F was equal to that seen in 4 mM KCl, but the increase in df/dt was significantly less. 10 uM phentolamine reduced the positive inotropic effect of 10 nM ISO in 22 mM KCl PSS but did not change the effect on TTP. The results suggest (1) that ISO has a significant alpha-adrenergic agonist action in low concentration in cat ventricular muscle and (2) that the mechanism of excitation-contraction coupling is dichotomous, with intracellular Ca stores of primary importance in 4 mM KCl and extracellular Ca stores of primary importance in 22 mM KCl PSS. (Supported by USPHS grant HL-22685).

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MYOCARDIAL RESPONSES TO THERAPEUTIC ULTRASOUND.

A.J. Mortimer*, O.Z. Roy*, B.J. Trollope*, J.R. Scott*, E.J. Villeneuve*, B. Brezden*, G.V. Forester, G.C. Taichman*, and W.J. Keon*.

Therapeutic ultrasound (US) can be analyzed in terms of its thermal (T) and non-thermal (NT) effects. In studies on isometrically contracting strips of cardiac tissue from rat, cat, and man and isovolumetrically beating whole rabbit hearts, US significantly ($p<0.05$) reduced resting tension (range 4-14%) by NT means while alterations in the systolic mechanics were achieved by T changes. These responses were immediate, reversible, and repeatable and were species independent but directly related to the field intensity. During hypodynamic states induced by hypoxia or acidosis, US exposure significantly improved the systolic tension and decreased resting tension by NT means. Recent studies on the membrane electrical responses to US demonstrate that NT factors were responsible for the observed hyperpolarization while the mechanism for the increased overshoot of the action potential has yet to be identified. In conclusion, US appears to alter a number of cardiac systems. The T component is believed to change the intracellular rates of reaction while the NT factors may be related to streaming and/or mechanical agitation.

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DIMENSIONS OF ISOLATED MYOCYTES. C.H.Greene, D.A.DeBias, D.A. DeBias*,P.A. DeFeo*,S.A.Seamens*, and W.L.Young*. Philadelphia College of Osteopathic Medicine, Philadelphia, 19131.

Retention of structural and biochemical integrity of isolated mammalian ventricular myocytes was evaluated.Dog,rabbit, and rat myocyte suspensions were obtained following enzymatic digestion during retrograde perfusion. Measurements were made on projected positive transparencies of phase-contrast light micrographs(1900X).Morphometric parameters were calculated using the methods of Rakusan et al. Fresh preparations of myocytes were photographed after transfer into a Levy counting chamber. Aliquots of cells were fixed in hyper- and isosmotic buffers to ascertain if fixation was rapid enough to prevent shifts from the intracellular compartment. Samples were embedded and sectioned(1-2μ).Differences bewteen mean lengths, widths,length/width,and volumes were statistically evaluated (Student's t test). Data for "balled" cells were selectively obtained from cells with greatest degree of contraction(smallest l/w ratio). Criteria were reversed for elongated cells. Preliminary results; dimensions of cells prepared in isosmotic buffer were not significantly different from cells prepared in hyperosmotic buffer; fixed,"balled" rat and rabbit cells had similar dimensions(eg $\bar{x}$ length rabbit=37.25μ, $\bar{x}$ length rat= 38.73μ);elongated, fixed rabbit myocytes are significantly longer($p<.01$)than rat cells;cell dimensions of fresh,elongated rabbit myocytes(eg $\bar{x}$ length=186μ) were significantly($p<.001$) longer than fixed,elongated,rabbit myocytes(eg $\bar{x}$ length=165μ); isolated fresh dog myocytes were not significantly larger than rabbit cells(eg $\bar{x}$ length dog=203u).

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STUDIES ON THE OXYGEN CONSUMPTION OF EPIDIDYMAL SPERMATOZOA IN THE RAT. Robert E. Leipheimer* and Robert G. Hart* (SPON: John J. Curry) Dept. of Biology, Slippery Rock State College, Slippery Rock, Pa. 16057

It is well documented that spermatozoan maturation and survival depend on the testicular androgens, but the mechanism is unknown. The effects of castration with and without testosterone replacement on the oxygen consumption of epididymal spermatozoa in the rat were examined. Unilateral or bilateral castrations were performed on adult male rats, and one group of bilaterally castrated animals received daily injections of testosterone proprionate (100ug/100g BW). The animals were killed 14 days after surgery and the epididymis removed. Oxygen consumption was measured for epididymal spermatozoa isolated from the caput, corpus and cauda regions. The average oxygen consumption over a two hour period was recorded and expressed as ul $O_2/10^6$spermatozoa/hour. Spermatozoa were not maintained in the bilaterally castrated group, whereas spermatozoa were maintained in those rats treated with testosterone, emphasizing the importance of testicular androgens for maintenance of spermatozoan viability. There was no difference in oxygen consumption of cauda spermatozoa, indicating that circulating testosterone is capable of spermatozoan maintenance. The oxygen consumption for caput and corpus spermatozoa from the intact side of the unilaterally castrated rats was significantly lower than control. This suggests that some factor (testosterone) entering the epididymis is inhibiting spermatozoan oxygen consumption.

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DEPRESSION OF UTEROPLACENTAL BLOOD FLOW AND FETAL ARTERIAL PO_2 FOLLOWING DRUGS ADMINISTERED TO THE PREGNANT EWE. Merva K.W. Cottle, Garry R. Van Petten* and Peter van Muyden*. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada, T6G 2N8.

Uteroplacental blood flow (UBF) and its effect on fetal oxygenation are critical to fetal well-being. In the unanesthetized, chronically instrumented pregnant ewe and fetus, UBF was monitored in a large branch of the uterine artery using an electromagnetic flow meter and fetal arterial PO_2 was sampled periodically during infusion of drugs to the pregnant ewe. Fetal and maternal arterial blood pressure and heart rate were also measured. Drugs tested in the pregnant ewe were: timolol (β-blocker); promazine (a phenothiazine α-blocker); and phenylephrine (α-agonist). Following administration of each of the above drugs UBF and fetal PO_2 were both depressed, peak 25-64%, and 20-30% reductions, respectively. These decreases were all significant ($p=0.05$). The fetal PO_2 and UBF showed a high correlation. Where cord occlusion (CO), effected by inflation of an implanted umbilical cuff, was imposed after either promazine or timolol the decrease in UBF and fetal PO_2 was even greater than with CO alone. The monitoring of UBF in the pregnant ovine model appears useful in predicting fetal blood PO_2 levels. The results have implications for the use of drug therapy during pregnancy and especially if such drugs are given in addition to possible cord constrictions as could occur during delivery. (Supported in part by MRCC grants to the late Dr. G.R. Van Petten)

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EFFECT OF THE PGI_2 INHIBITOR, TRANYLCYPROMINE, IN UTERINE BLOOD FLOW IN PREGNANT EWES. K.E. Clark*, D.J. Harrington*, and S.J. Stys* (SPON: B.C. Moulton). Dept. of Obstetrics and Gynecology, and Physiology, Univ. of Cinti., Cinti., OH 45267.

Previous reports from our laboratory have shown that prostacyclin (PGI_2) is a potent vasodilator of the uterine vasculature in late term pregnant ewes. PGI_2 has been shown to be important in regulating local hemodynamics in a number of vascular beds. To investigate the role of endogenously produced PGI_2 in regulating uterine blood flow (UBF), the PGI_2 inhibitor, tranylcypromine, was used. Pregnant ewes were chronically instrumented with catheters in the femoral artery, femoral vein, uterine arteries, uterine veins, and with electromagnetic flow probes on each uterine artery. Administration of tranylcypromine directly into the uterine artery led to a dose related increase in uterine vascular resistance (UVR) and a reduction in UBF with a maximal decrease of 40% over control. Mean arterial pressure (MAP) increased significantly and heart rate decreased slightly. However, levels of 6-keto-$PGF_{1\alpha}$, determined by radioimmunoassay, did not change. To investigate the possible mechanism for the observed effects of tranylcypromine, the alpha adrenergic receptor blocking agent, phenoxybenzamine (5 mg/kg) was given and blockade verified. Following alpha blockade, the observed changes in MAP, UBF, and UVR were abolished. These data suggest that the observed vascular effects of tranylcypromine are due to alpha adrenergic stimulation rather than inhibition of prostacyclin synthesis. (Supported in part by USPHS NIH Grant HD11725 and HD13490)

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FETAL-MATERNAL TRANSFER AND CATABOLISM OF OVINE ALPHA-FETOPROTEIN. G.J. Mears*, P.C.W. Lai*, G.R. Van Petten* and F.L. Lorscheider. Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4

The transfer kinetics and catabolism of alpha-fetoprotein (AFP) were determined in chronically cannulated ewes and fetal lambs during the last third of gestation. 1) Pharmacokinetic analyses indicated that a 3-compartment open model described the data after injection of ovine ^{125}I-AFP* into maternal blood (MB), whereas a 2-compartment open model described the data after injection of AFP* into fetal blood (FB) or amniotic fluid (AF). The $t_{\frac{1}{2}}$ of AFP* in maternal plasma (MP), fetal plasma (FP) and AF was 35.8, 16.3 and 42.4 h respectively. 2) Conceptal-maternal transfer of AFP demonstrated that the concentration of AFP* in MP within 6 h was 8X greater when the same dose of AFP* was injected into FB than when it was injected into AF. 3) Fetal-amniotic transfer of AFP showed that the concentration of AFP* within 8 h was 10X greater in AF after AFP* was injected into FB compared to the concentration in FP when AFP* was injected into AF. 4) Maternal-conceptal transfer of AFP was bi-directional. Within the first 4 h after injection of AFP* into MB both the concentration of AFP* and its rate of appearance in FP were significantly higher than that observed in AF. We conclude that the major route of AFP transfer between the conceptus and ewe is transplacental rather than transamniotic, that AFP in AF is a relatively static pool and, that AFP is readily transferred to MP and catabolized. (MRC grant No. MA5292).

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THE EFFECT OF MOLECULAR WEIGHT ON THE ABILITY OF TRITIATED SACCHARIDES TO CROSS THE BLOOD-UTERINE LUMEN BARRIER IN RATS. Ann McRae* and T.G. Kennedy. MRC Group in Reprod. Biol., Depts. of Physiol and Obstet. & Gynaec., Univ. of Western Ont., London, Canada.

The blood-uterine lumen permeability barrier in immature estrogen-treated rats may be selective according to molecular weight (Biol. Reprod. 20: 919-923, 1979). Immature, ovariectomised rats were treated for 3 days with estrogen to induce uterine luminal fluid (ULF) accumulation. Animals with ligated renal pedicles were given single iv injections of either [^{3}H]-mannitol (mol wt 182.2), [^{3}H]-sucrose (mol wt 342.3) or [^{3}H]-inulin (mol wt ≈5200) (specific activity 2.1 Ci/mmol for all substances) and were killed 2h post-injection. Preliminary experiments in which the effects of time of autopsy were investigated, indicated 2h to be appropriate. The volumes of distribution (calculated as injection dose ÷ serum concentration) for [^{3}H]-inulin and [^{3}H]-sucrose were identical (35±1.1 mls), whereas those for [^{3}H]-mannitol were slightly but significantly larger (42±1.4 mls). For [^{3}H]-inulin and [^{3}H]-sucrose the ratios of total radioactivity in ULF to injected dose did not differ significantly ($7.0\pm1.4 \times 10^5$ and $8.7\pm1.8 \times 10^5$, respectively) whereas those for [^{3}H]-mannitol were significantly larger ($29\pm6.3 \times 10^5$). These results indicate that of the radioactive saccharides tested, inulin and sucrose, with the higher molecular weights, were less able to cross the blood-uterine lumen barrier, thus suggesting that this barrier restricts selectively substances according to molecular weight.

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DISTRIBUTION OF BOVINE FETUIN AND ALBUMIN IN FETAL AND MATERNAL PLASMA, ALLANTOIC AND AMNIOTIC FLUIDS DURING DEVELOPMENT. P.C.W. Lai*, L.L. Huang*,, D.E. Panrucker*, R.B. Church* and F.L. Lorscheider. Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4

The specificity and validity of a radioimmunoassay for bovine fetuin (FET) was established by a) parallel dose-response curves of purified FET to various physiological fluids, b) analytical recovery of pure FET (99%), and c) correlation ($r=0.99$) of FET concentrations with those estimated by radialimmunodiffusion (RID) assay. Albumin (ALB) was also measured by RID. FET levels in fetal plasma increased from 10 mg/ml at 4 mo. to 15 mg/ml by 8 mo. gestation, during which time ALB levels remained higher than FET. Neonatal plasma FET levels rapidly declined during the first 14 d postpartum, coincident with a marked reciprocal increase in ALB levels. In contrast, FET concentration in allantoic fluid was significantly higher than that of ALB throughout gestation, with both levels reaching a peak at 7 mo. In amniotic fluid, ALB levels were similar to those in allantoic fluid whereas the levels of FET remained consistently lower than those in allantoic fluid throughout gestation. FET and ALB levels in amniotic fluid peaked at 8 mo. FET levels in maternal plasma declined from 0.7 mg/ml at 1 mo. to 0.4 mg/ml at term. We conclude that 1) at term there is an abrupt shift from FET to increased ALB synthesis by the fetal-neonatal liver, 2) FET appears to be shunted to the allantois whereas ALB is equally concentrated between allantois and amnion, and 3) a maternal systemic immunosuppressive role for FET is unlikely. (MRC grant no. MS-5371)

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IS UTERINE FLUID IMBIBITION OBLIGATORILY COUPLED TO NUCLEAR RECEPTOR-ESTROGEN INTERACTION? P. S. Campbell, R. F. Modlin, and G. A. Newman. The Univ. of Alabama in Huntsville,AL 35899

The early uterotrophic response is presumed to entail a nuclear-mediated event contingent upon receptor-estrogen complex (REC) induction. However, in vivo dexamethasone pretreatment unequally affects the 3 and 24 hr uterine weight response to estradiol, but equally reduces the quantity of nuclear REC present at times corresponding to these two events. Diethylstilbestrol (DES) and estradiol have disparately incongruous potencies upon the early and late uterotrophic responses not paralleled by the quantity of REC retained in the nucleus at the appropriate time. Serial estrogen/antiestrogen injections and measurement of fluid imbibition in relation to receptor availability and nuclear retention were performed to discern the degree to which fluid uptake is rigidly coupled to nuclear localization of REC. Injection of 1 µg estradiol 30 min after an injection of 10 µg DES results in additional fluid uptake by uterus within the percent range of measured "available" cytoplasmic receptor. However, such is without a further increase in the quantity of nuclear REC. The percent increase in water uptake promoted by 1 µg estradiol subsequent to 50 µg of the antiestrogens, CI-628 or nafoxidine, is far in excess of the percentage of "available" cytoplasmic receptor sites and also occurs without elevation of nuclear REC as a result of estradiol injection. Thus, uterine fluid imbibition may be related more to estrogen-stimulated uterine blood flow and/or membrane changes than REC interactions.

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HORMONAL REGULATION OF GONADAL LHRH RECEPTOR LEVELS IN THE RAT. F.A. Lefebvre*, J.J. Reeves, B. Marchetti*, C. Séguin*, P.A. Kelly and F. Labrie, MRC Group in Molecular Endocrinology, Quebec G1V 4G2.

Specific LHRH receptors have been described in rat ovarian homogenate and dissociated Leydig cells. The presence of specific LHRH receptors in gonadal tissue supports previous observations of direct effects of LHRH agonists at the ovarian and testicular level. In order to study the hormonal factors possibly involved in the control of gonadal LHRH receptors, hypophysectomized (hypoX) rats were used. Treatment of hypoX animals with ovine prolactin (oPRL) (30 µg/day for 7 days) produces a 60-80% inhibition of ovarian LHRH receptors. Similarly, hyperprolactinemia induced by pituitary implants in hypoX females inhibits LHRH receptor levels by 50 to 70% after 20 days. The dopamine agonist 2-bromo-ergocryptine (CB-154) can reverse the effect of pituitary implants. In the male, LHRH receptor levels in dissociated Leydig cells are increased by 300 to 500% 2 weeks after hypoX (from 4.0 ± 0.2 to 14.0 ± 0.9 fmoles/mg protein). Treatment of hypoX males with oPRL (200 µg, twice a day), the LHRH analogue [D-Ser(TBU)^6 des-Gly-NH$_2$^{10}] LHRH ethylamide (200 ng/day), or hCG (25 IU/day) inhibits LHRH receptor levels by 45-50%, 60-65% and 65-80%, respectively. The present data show that gonadal LHRH receptors can be modulated by circulating hormones and support the suggestion that these receptors could play a physiological role in the control of gonadal functions in the rat.

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Activation of Fetal Adrenal Response In Vitro After In Vivo Fetal ACTH Infusion. J. Challis, J. Patrick, J. Glickman, B.F. Mitchell, MRC Group Reprod. Bio., Univ. of Western Ontario.

In sheep increased fetal adrenal (FA) cortisol (F) in late pregnancy triggers parturition and fetal organ maturation. The normal stimulation ratio for F (SR; maximum/basal levels of F output) by FA cells in response to ACTH increased from 1.83 on d 130 to 9.56 at term (d 145). To investigate the factors responsible for this change, we implanted maternal and fetal vascular catheters in 8 sheep at d 114-117. Maternal (5 ml) and fetal (2 ml) blood was collected at 8 h intervals between d 125-130. Beginning on d 127, 4 fetuses (Group A) received IV $ACTH_{1-24}$ (10 µg/hr), 4 (Group B) received saline, as continuous infusions until sacrifice 70 h later on d 130. Steroids were determined by RIA.

In Group A fetal F rose from 26.7±8.4 ng/ml at the start of infusion to 132.3±15.9 ng/ml at 48 h. Maternal F was unchanged. Maternal progesterone decreased and estrone increased significantly during the last 8 to 16 h of infusion, as at term. No significant changes in maternal or fetal steroids were found in Group B. At sacrifice mean FA weights were 715±40 mg in Group A and 349±11 mg in Group B (P<0.001). Adrenals were dispersed with collagenase and incubated (4 h) with ACTH (5-5000 pg/ml). Basal F (fg/cell/4 h) rose from 4.2±0.4 in control cells to 200±72 after ACTH. SR in control was 3.1±0.7, after ACTH 16.7±6.1 (P<0.05). We conclude that in late pregnancy there is activation of FA function which can be stimulated at the time of pre-term labour by ACTH infusion for 70 h in vivo.

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ISOLATION, RADIOIMMUNOASSAY AND COMPARTMENTAL DISTRIBUTION OF RAT ACUTE-PHASE α2 MACROGLOBULIN IN PREGNANCY. D.E Panrucker*, P.C.W. Lai* and F.L. Lorscheider. Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4

Rat acute-phase α2 macroglobulin (APα2M) was isolated from serum by gel filtration and negative affinity chromatography. Purity of APα2M was established by immunoelectrophoresis (IEP), gel EP and protein determinations. Goat anti-APα2M was monospecific by IEP, titred at 1.5x10^7 in a double-antibody radioimmunoassay (RIA) and had a Ka of 1.24x10^{11} l/mol by Scatchard plot. Specificity, accuracy and validity of the RIA were established by a) parallel dose-response curves of pure APα2M to various physiological fluids, b) analytical recovery of APα2M in the RIA (103±7.4%) and c) correlation (r=0.99) of APα2M standards by RIA and by rocket IEP. RIA sensitivity was <1ng/ml. The highest levels of APα2M (µg/ml) were in fetal plasma which increased from 860 at 19 d gestation to 2705 at 21 d to labor and remained elevated at 2570 in the neonate at 3 d. Maternal serum APα2M (µg/ml) gradually increased from non-pregnancy levels of 11 to 49 at 9 d pregnancy, 300 at 19 d, a rapid rise at 20 d which peaked during labor at 900 and abruptly declined by 1 d postpartum to 430. Amniotic fluid APα2M (µg/ml) at 11 d was 57 and declined to 10 by 19 d. We conclude that a) sufficient concentration gradient exists from fetus to mother to indicate increased maternal APα2M is of fetal origin, b) compartmental distribution patterns may be influenced by an extra-fetal source of APα2M and c) amniotic fluid levels suggest excretion of APα2M declines as gestation advances. (Supported by Alberta Children's Hospital Foundation)

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EFFECT OF PGE_2 ON CERVICAL COMPLIANCE IN PREGNANT EWES. Stanley J. Stys*, Betsy L. Dresser*, Thomas E. Otte*, and Kenneth E. Clark* (SPON: Bruce C. Moulton). Dept. Ob/Gyn, Univ. of Cinti., Cincinnati, OH 45267.

Cervical compliance increases dramatically at parturition in sheep independent of uterine activity. Recently, in vitro PGE_2 production by the cervix has been shown to increase at parturition. This study investigated the effects of PGE_2 on cervical compliance and uterine blood flow in pregnant ewes. Eight animals were chronically instrumented with pressure balloons within the cervical os and amniotic cavity, an electromagnetic flow probe on a uterine artery, and catheters in maternal cervical os, femoral artery, femoral, uterine, and cervical veins, and fetal hindlimb vein. PGE_2 (10 mg) was administered in a water soluble gel into the cervical os every four hours X3 at least 5 days post surgical preparation (124-142 days gestation). In all eight ewes cervical compliance increased within 8-12 post treatment hours to levels comparable to that seen at spontaneous parturition. Five of the ewes did not progress into labor; compliance in these ewes returned to baseline 24-72 hours after the peak. Uterine blood flow was measured in five ewes during the PGE_2 treatment and demonstrated no significant alterations. Maternal cardiovascular and fetal respiratory parameters were monitored throughout the experiment and remained stable. The present data suggest that PGE_2 may be an important regulator of the biochemical and physical changes which occur in the cervix at parturition. (Supported in part by the Whitaker Foundation)

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COITUS-INDUCED LH RELEASE PATTERNS IN THE CAT ON CONSECUTIVE DAYS OF BREEDING. Donelle Banks* and George Stabenfeldt Dept. of Vet. Med. Repro., Univ. of California, Davis, Ca. 95616

Female cats were permitted to breed twice in the morning on each day of estrus. Estrus ranged from 3 to 7 days in the 11 cycles observed. Plasma samples were collected via the jugular vein immediately precoitus and at 1,2,4,8 and 24 hrs. postcoitus. Luteinizing Hormone (LH) was measured by radioimmunoassay (RIA) using anti-ovine LH serum, ovine LH standards and tracer. There was one dominant LH surge during each estrus ranging from 24-187 ng/ml with a mean of 81±49 ng/ml compared to basal levels of <2 ng/ml. There were additional smaller surges on at least 2 consecutive days. Four of eleven showed LH responses to breeding on every day of estrus. The first LH surge occurred on various days of estrus: 8/11 on Day 1; 2/11 on Day 2; 1/11 on Day 3. The day of ovulation was determined by progesterone measurement of the daily precoital and 8-hr postcoital samples. Daily samples were assayed for estrogen to indicate the level of follicular development at the time of the LH surges.

The results suggest that with 2 matings per day: 1) cats may not be capable of a coitus-induced LH surge on the first day of behavioral estrus; 2) they are capable of multiple LH surges throughout estrus; 3) there is no correlation between the day of the peak LH surge and the no. of days remaining in estrus. (Supported by Animal Protection Institute of America, Sacramento, Ca.)

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MODIFICATION OF EXCITATORY JUNCTION POTENTIAL AMPLITUDE IN SMALL PRESSURIZED MESENTERIC ARTERIES. Elane Zelcer* and Nick Sperelakis. University of Virginia, Charlottesville, Va 22908.

Adenosine (ADO) and angiotensin II (AII) may modulate local blood flow. The effects of these hormones and the neurotransmitter, norepinephrine (NE), were tested on excitatory junction potentials (EJPs) recorded intracellularly from small (100-250 μm) pressurized (40 mm Hg) guinea-pig mesenteric arteries. EJPs (subthreshold for spikes) were evoked with single, 0.5 ms electrical field stimuli at 0.25 Hz; summation of EJPs to trigger spikes and contraction (observed visually) occurred at stimulus frequencies of 8 Hz. EJPs are probably due to release of NE. 10^{-7}M NE did not depolarize but decreased EJP amplitude. Addition of 5 x 10^{-7}M NE produced sustained vasocostriction but transient depolarization, sometimes accompanied by spiking. This suggests that NE may exert a second effect to maintain contraction. Addition of ADO (10^{-5}M) to the superfusate produced a transient hyperpolarization (peak, 10 mV) but decreased EJP amplitude. Summation of EJPs still occurred in ADO (tested following the hyperpolarization), but did not elicit spikes or vasoconstriction. This effect may be due to both decreased EJP amplitude and a direct effect on the muscle (e.g. to depress slow Ca^{2+} channels). AII (10^{-7}M) increased EJP amplitude to single stimuli without changing resting potential or vessel diameter, suggesting that AII may act primarily on the nerve terminal. Modification of the sympathetic input to mesenteric vessels indicates a possible regulatory role of AII and ADO. Supported by NIH Grant # HL- 19242.

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EFFECT OF BEPRIDIL ON CONTRACTIONS AND ACTION POTENTIALS OF VASCULAR SMOOTH MUSCLE (VSM). Suzanne Mras and Nick Sperelakis. Univ. of Virginia, Physiology Dept., Charlottesville, Va. 22908

The effects of a new anti-anginal vasodilator, bepridil (CERM-]978), were examined in rings of rabbit aorta. Since bepridil has a Ca-antagonistic action on cardiac muscle similar to verapamil, the effects of equimolar amounts (10^{-6} and 10^{-5} M) of bepridil and verapamil were compared on VSM contractions (isometric) to norepinephrine (NE) and increased extracellular K^+ concentrations ($[K]_o$). Both bepridil and verapamil significantly decreased the contractures produced by elevated $[K]_o$ (which are presumably due to increased Ca^{++} influx). NE produces contractions probably through both increased Ca^{++} influx and release of sequestered Ca^{++} (possibly from the SR). Both bepridil and verapamil depressed NE-induced responses. However, in low $[Ca]_o$, in which NE produces contractions primarily by release of sequestered Ca^{++}, neither agent had a significant effect. Electrophysiological evidence using cultured reaggregates of rat aortic VSM cells indicated that bepridil (10^{-7} - 10^{-5} M) interfered with Ca^{++} influx, i.e., the Ca^{++}-dependent action potentials, in response to electrical stimulation, were depressed and blocked by bepridil. It is probable that both bepridil and verapamil reduce VSM contractility by decreasing Ca^{++} influx across the sarcolemma in response to elevated $[K]_o$, NE, and electrical stimulation. However, neither agent seems to interfere with NE-induced release of sequestered Ca^{++}. (Supported by NIH grant HL-19242 and by a grant from Wallace Laboratories.)

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PROSTAGLANDINS EXERT DIFFERENTIAL ACTIONS ON ISOLATED CANINE INTRAPULMONARY ARTERIES (IPA) AND VEINS (IPV). Naresh Chand* and Burton M. Altura. SUNY Downstate Med. Ctr., Brooklyn, NY 11203

The sensitivity and contractility of isolated canine IPA & IPV to a variety of prostaglandin (PG) compounds was studied. IPA failed to elicit any measurable (or reproducible) contractile responses to PGA_1, PGA_2, PGB_1, PGD_2, PGE_1, PGE_2 or to $PGF_{1\alpha}$. High concentrations of both PGB_2 (>10^{-7}M) and $PGF_{2\alpha}$(>10^{-6}M), however, elicited concentration-related, but weak, contractile responses, measuring only 5-25% of KCl maximums on IPA. IPA partially contracted by 5-HT exhibited concentration-related relaxations in response to PGE_1; PGE_2, PGA_1 or PGA_2 produced, however, only weak superimposed contractions. In contrast to the IPA, IPV contracted in a concentration-related fashion to all PGs tested, where the contractile sensitivity was (based on EC50's and threshold concentrations): PGB_2>PGB_1>PGD_2>$PGF_{2\alpha}$>PGA_2>> PGA_1> $PGF_{1\alpha}$> PGE_2>PGE_1. In terms of the ability to generate maximum contractile responses on IPV, the PGs were also variable, where PGA_2 and B_2 were the most potent and PGD_2 the least potent. IPV partially contracted by 5-HT exhibited concentration-related relaxations to PGE_1 at low concentrations, followed by secondary contractile responses at higher concentrations. These data are consistent with the notions that there are qualitative and quantitative differences in responsiveness of IPA and IPV to PGs.

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RELAXATION OF VASCULAR SMOOTH MUSCLE BY ANGIOTENSIN II. R.C. Webb and D.F. Bohr. University of Michigan Medical School, Ann Arbor, MI 48109

The effects of angiotensin II on contractile tension were studied in vascular smooth muscle from pigs and rabbits. Helically cut strips of renal and femoral veins were mounted in organ chambers and isometric contractions were recorded. Contraction of the venous strips was induced by application of $3x10^{-8}$ norepinephrine. Subsequent addition of $5x10^{-8}$ angiotensin II caused a triphasic response. 1) there was an initial contraction which was tachyphylactic in both pig and rabbit veins; 2) the contraction was followed by a relaxation below the contraction induced by norepinephrine; and 3) there was a return from the relaxation to the level of the norepinephrine contraction. The duration of the entire response was approximately 4 minutes. The magnitude of the relaxation response varied inversely with the magnitude of the norepinephrine contraction when the contractile state was altered by changing the norepinephrine concentration. Inhibitors of prostaglandin synthesis [indomethacin (5ug/ml) and aspirin (50ug/ml)] attenuated the relaxation but not the contraction in response to angiotensin II. These results suggest that: 1) angiotensin II stimulates the synthesis of prostaglandins in isolated venous smooth muscle; and 2) endogenous prostaglandins modulate the response of venous smooth muscle to angiotensin II. (Supported by NHLBI grant HL-18575 and by a grant-in-aid from the Michigan Heart Association).

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DEVELOPMENT OF MYOGENIC TONE AND NEUROGENIC RELAXATION OF THE PORCINE BASILAR ARTERY. Raymond J. Winquist and David F. Bohr. University of Michigan, Ann Arbor, MI 48109.

Helically-cut strips of the pig basilar artery developed a sustained tonic contraction following application of stretch in vitro. The maintained tone was rapidly inhibited when strips were washed in zero-Ca^{++} saline and was relaxed dose-dependently by sodium nitrite, D-600, papaverine, l-norepinephrine (NE) and isoproterenol. Tone persisted following treatment of strips with phentolamine (10^{-6}M), indomethacin (IN, 3ug/ml), atropine (AT, 10^{-6}M), propranolol (10^{-6}M), tetrodotoxin(TTX, $4x10^{-7}$M) and 6-OH-dopamine (6-OHDA, 300ug/ml). Transmural nerve stimulation(TNS) of vessel strips elicited a frequency-dependent relaxation that was similar in maximum to that produced by sodium nitrite($5x10^{-2}$M). TNS elicited no response following prior incubation with TTX or 6-OHDA but was unaffected after pretreatment with IN or AT, or, concentrations of beta-adrenoceptor antagonists (practolol, timolol, propranolol, 10^{-6}M) which inhibited the relaxation of tone produced by exogenously applied NE. Higher concentrations of propranolol ($2x10^{-5}$M) shifted the frequency-response curve downward and towards the right. The results demonstrate the existence of myogenic tone in the porcine basilar artery which is relaxed following exogenous or endogenous adrenergic stimulation. The results also suggest that neurogenic relaxation may be of importance in regulating cerebral blood flow in the pig. (Supported by NHLBI grants HL 06080 and HL 18575).

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CHANGES IN THE CHARACTERISTIC IMPEDANCE OF THE PULMONARY ARTERY AS A RESULT OF BLOOD VOLUME EXPANSION AND CONTRACTION. C.D. Forcino*, J.P. Dujardin*, D.N. Stone*, S.N. Longenbaker*, L.T. Paul, and H.P. Pieper, Dept. of Physiology, Ohio State University, Columbus, Ohio 43210.

Experiments were performed on anesthetized mongrel dogs to determine the input impedance of the pulmonary circulation. Pressure and flow measurements were made in the main trunk of the pulmonary artery using a Konigsberg transducer and a Biotronex electromagnetic flowmeter, respectively. The input impedance was determined under control conditions, after hemorrhage (-15% of the estimated blood volume), and after volume expansion (+30% of the estimated blood volume with Dextran 70). For each condition the characteristic impedance of the pulmonary artery was calculated by averaging the impedance moduli between 5 Hz and 15 Hz. Values of characteristic impedance increased by 22.43% (±7.88 SE) after hemorrhage and decreased by 16.99% (±5.75 SE) after volume expansion when compared to controls. Time domain analyses, in which the characteristic impedance was calculated as the slope of the pressure-flow relationship during early ejection were performed to obtain values in overlapping pressure ranges. (This research was supported by NIH grant #HL23239).

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MECHANISM OF ACTION OF DOPAMINE ON VASCULAR SMOOTH MUSCLE FROM RABBIT BASILAR ARTERY. David R. Harder, East Tennessee State Univ., College of Medicine, Dept. of Physiology, Johnson City, Tennessee 37614.

This study was designed to help elucidate the mechanisms of action of dopamine on rabbit basilar artery. Electrical recordings were made in isolated basilar arteries by standard techniques using glass microelectrodes filled with 3M KCl. Tension recording was done using rings of artery mounted between tungston hooks connected to a force transducer. The resting membrane potential (Em) in the smooth muscle cells of rabbit basilar artery was -61 + 2 mV. Addition of dopamine depolarized the arterial muscle cells in a dose dependent manner from -56 + 1.8 mV at 5×10^{-7} M to -28 + 1.6 mV at 10^{-4} M. Dopamine also caused a dose-dependent increase in tension with initial increases observed at 5×10^{-7} M reaching a maximum at 10^{-3} M. The maximum dopamine induced increase in tension was 100% of the maximum contraction recorded in 150 mM KCl and about 60% of that induced by 10^{-4} M histamine. This dopamine induced contraction was nearly abolished in Ca^{++} free solution. When Na^{+} was removed to eliminate the inward depolarizing current the maximum dopamine induced contraction was reduced to 10% of that recorded in control solutions containing dopamine. Such findings suggest that dopamine increases Ca^{++} influx by a mechanism involving membrane depolarization. The dopamine induced contraction was also blocked by phentolamine, but was still present after chemical denervation with 6-OH dopamine suggesting that dopamine exerts a direct excitatory action in rabbit basilar artery which involves alpha adrenergic stimulation. Supported by NIH-HL24007.

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HYPOXIA CONTRACTS VASCULAR SMOOTH MUSCLE TREATED WITH TETRAETHYLAMMONIUM. Ivan F. McMurtry and William R. Keatinge*. Univ. of Colo. Hlth. Sci. Ctr., Denver, CO 80262 and London Hosp. Med. Coll., London E1 2AD, England.

Decreased oxygen tension usually has no effect on or reduces tone of vascular smooth muscle in vitro. However, hypoxia elicited contractions of cutaneous veins treated with barium (Vanhoutte, 1975) and of pulmonary artery-parenchyma strips treated with procaine (Lloyd, 1968). Since barium and procaine are believed to suppress membrane K^{+} conductance, we wondered if tetraethylammonium (TEA), a well known inhibitor of outward K^{+} current, would also allow hypoxic contractions. Isometric tension of rings from thoracic aorta of rats and carotid artery of sheep was measured in physiologic salt solution aerated with O_2+5%CO_2 at 37°C. Hypoxia (N_2+5%CO_2) or addition of 1mM NaCN had no effect on resting tension and reduced the active tension induced by K^{+} or norepinephrine. After 1-5 mM TEA, hypoxia caused a transient, rhythmic contraction in 30 of 37 aortic rings. More sustained, less rhythmic, hypoxic contractions occurred in 5 of 6 carotid rings. Contractile effects of hypoxia were mimicked by NaCN. Contractions were inhibited by 10^{-5} M verapamil, but not by 10^{-5}M phentolamine. Depression of K^{+} conductance in some vascular muscle apparently unmasks a direct contractile effect of hypoxia. Vascular smooth muscle treated with inhibitors of K^{+} conductance might be a useful in vitro model of hypoxic pulmonary vasoconstriction. (Supported by NIH Grant HL 14985 and Wellcome Research Travel Grant)

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SALINE MEDIA WHICH REPRODUCE HUMAN PLASMA ULTRAFILTRATE IN CONTENT OF INORGANIC FIXED IONS. William H. Waugh. Depts. of Med. and Physiol., East Carolina Univ., Greenville, NC 27834

Two media have been developed that reproduce, at plasma protein (prot) of 6.7 g/dl, the normal concn of all of the diffusible inorganic fixed ions in normal human ultrafiltrates (UF) at 37°C & pH 7.40. The media are based on: 1) mean concn ratios of inorganic fixed ions in normal human plasma & its UF or equilibrated dialysate as found by Van Leeuwen (1964) & in PO_4 & SO_4 data (Walser, 1961; Swan et al, 1956) as molalities; 2) averaging of means & SD in normal plasma reported with prot values; & 3) calculation of UF composition of the fixed ions by use of linearity between prot concn & molal concn ratios (as shown by Van Leeuwen). Plasma H_2O was equated to 987 g - 0.73 (prot in g/l) & UF H_2O to 993 g/l (after Waugh, 1969). At prot of 6.7±0.4 g/dl, plasma Ca & Mg were 2.41±0.08 & 0.86± 0.06 mM, resp, (4 ref). At prot of 6.7 g/dl, UF of normal plasma had Na 142.3±2.2, K 3.96±0.28, Ca 1.45±0.05, Mg 0.59± 0.04, Cl 114.0±1.9, PO_4 1.11±0.15, & SO_4 0.37±0.07 mmoles/kg H_2O. The media contain Na 141, K 4.0, Ca 1.45, Mg 0.60, Cl 113.4, PO_4 1.10, & SO_4 0.40 mM; HCO_3 for pH 7.40; methylsulfate (CH_3SO_4) (negligible Ca binding); glucose (a normal 1 hr postprandial concn); & mannitol. Osmolalities measure 289 mosm/kg H_2O. The media are made by mixing to mM: NaCl 107.2, KCl 2.9, KH_2PO_4 1.1, $MgSO_4 \cdot 7H_2O$ 0.40, $MgCl_2 \cdot 6H_2O$ 0.20, $CaCl_2 \cdot 2H_2O$ 1.45, glucose 7.5, mannitol 10, & either $NaHCO_3$ 24.6-$NaCH_3SO_4$ 9.2 or $NaHCO_3$ 16.0-$NaCH_3SO_4$ 17.8; & by gassing fully with 5% CO_2 in O_2 or to CO_2 near 23 torr, resp, (open medium).

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COMPARISON OF ^{45}CA UPTAKE CURVES IN KREBS-RINGER AND HEPES BUFFERED PSS. D.L. Davis, D.V. Barag*, and J.M. Price*. Dept. Physiology, University of South Florida, Tampa, Florida.

It has been reported that the buffers used in several commonly used bathing media may alter various tissue response characteristics. Previous reports from our laboratory have shown that ^{45}Ca uptake curves were decreased in HEPES buffered PSS as compared with Krebs-Ringer, although dose response curves to NE and K depolorization were only minimally effected. In the present study the effects on uptake curves of ^{45}Ca of adding phosphate to the HEPES buffered PSS and of omitting phosphate from the Krebs-Ringer PSS were compared. Experiments were conducted with 4 mm rings from dog anterior tibial arteries. After the adventitia was removed the rings were equilibrated for 1 hr in a given PSS (HEPES, HEPES plus phosphate, Krebs-Ringer, or Krebes-Ringer minus phosphate). The rings were then loaded for 5, 15, 30, 60, 90, and 120 m in the same PSS containing 2-3 μCi/ml ^{45}Ca. The ^{45}Ca uptake curves were appreciable lower in HEPES buffered PSS and in Krebs-Ringer minus phosphate. Addition of phosphate to the HEPES buffered PSS increased ^{45}Ca uptake curves to approximate those obtained with Krebs-Ringer PSS. Control uptake curves in HEPES buffered PSS at 37°C and pH of 7.2, 7.4, and 7.6 showed statistically insignificant differences. It is concluded that the absence of phosphate from the HEPES buffered PSS rather than small differences in pH were responsible for the decreased ^{45}Ca uptakes. (Supported in part by a grant from the American Heart Assoc., Florida Affiliate).

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ROLE OF ALPHA-AND BETA-ADRENERGIC RECEPTORS IN SMALL AND LARGE CORONARY ARTERIES. Prasad D.M.V. Turlapaty* and Burton M. Altura. SUNY Downstate Med. Ctr., Brooklyn, NY 11203

Controversy exists with respect to the distribution and activities of alpha-and beta-adrenergic receptors in the regulation of coronary blood flow. Direct, in vitro studies using canine large (1-2mm o.d.) (LCA) and small coronary arteries (0.4-0.8mm o.d.) (SCA) from various regions of the myocardium were examined. Different adrenergic amines (epinephrine: E, norepinephrine: NE, isoproterenol: ISO, phenylephrine: PE) in the presence and absence of phentolamine (PH) or propranolol (PR) were tested for their vasoactivities. In the absence of antagonists, relaxation is induced on resting tone in SCA by all amines except PE, where ISO$\gg$NE$\gg$ E. PE induces contractile responses on SCA. With PR (10^{-6}M), NE, E and PE elicit only contractile responses in both LCA and SCA, where E$>$NE$\gg$PE. PR shifts rightward ISO relaxant concentration-effect curves and enhances the contractile sensitivity of LCA and SCA to the amines. PH treatment did not alter the maximum relaxant effects of ISO, NE or E, or basal tone. PR induced dose-dependent vasospasm. These data indicate that: 1) under physiologic conditions beta-adrenergic (relaxant) responses are dominant, 2) after beta-adrenergic blockade, alpha-adrenergic (contractile) receptors are activated; and 3) there is a clear heterogeneity in distribution and function of adrenergic receptors in SCA and LCA. (Supported by USPHS NIH Grants HL BI 18015 and DA 02339)

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EFFECTS OF GASTROINTESTINAL HORMONES ON RAT PORTAL VEIN. Bruce P. Brown*, Donald Heistad and Sinn Anuras. Department of Medicine, University of Iowa, Iowa City, IA 52242.

Gastrin, glucagon, cholecystokinin (CCK), and secretin are released from the gut into the portal vein in high concentrations. These hormones affect mesenteric arterial smooth muscle. Our study was performed to determine whether they affect the spontaneous rhythmic contractions of longitudinal muscle in the isolated rat portal vein. We recorded isometric portal vein contraction in 45 rats during 15 minute infusions of the following hormones: pentagastrin and glucagon, 10^{-6}, 10^{-7}, 10^{-8}M; cholecystokinin-octapeptide (CCK-OP), 10^{-8}, 10^{-9}, 10^{-10}M; secretin 2×10^{-8}, 2×10^{-9}, 2×10^{-10}M. Pentagastrin did not change amplitude or duration of contractions, but produced small decreases in frequency (10^{-6}M, 2.3 cpm to 2.2 cpm. $p<.01$.) Glucagon did not change frequency or duration. At 10^{-9}M, however, there was a 6.9% decrease in amplitude $p<.005$.) Secretin did not change amplitude or duration, but produced small decreases in frequency (2×10^{-8}M, 2.3 cpm to 2.1 cpm. $p<.01$.) CCK-OP did not change amplitude or duration but produced small decreases in frequency (10^{-8}M, 1.9 cpm to 1.8 cpm. $p<.05$.) Conclusions: Pentagastrin, secretin, and CCK-OP decrease slightly the frequency of spontaneous contraction in the longitudinal muscle of the rat portal vein. These changes are small and probably not physiologically important. Nevertheless, these hormones could have important effects on the portal vein if they affect circular muscle.

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Some Aspects of a Na-Dependent Secretion of Cl in the Turtle Pyloric Antrum. R.G.Gibson,J.M.Kupfer* and William A.Brodsky. Mt. Sinai Sch. of Medicine 10029

Short-circuiting current(I_{sc}) across isolated turtle antrum bathed on both surfaces by identical Na-Ringers(Cl,HCO_3) is 25 to 40 ua/cm^2 with PD 20 to 30 mv(ser.pos.) and transmural resistance 600 to 750 ohms-cm^2. Symmetrical removal of Cl(e.g.SO_4 replacement) reversibly reduces I_{sc} and conductance (G) by 25 and 50 percent respectively. Addition of ouabain($5x10^{-4}$M) to a Na_2SO_4-Ringers preparation (in the presence or absence of HCO_3) irreversibly abolished the PD and I_{sc}, with little or no effect on the conductance;subsequent addition of Cl(20Mm) did not change I_{sc} or PD, but did increase G by 30%. Amiloride($3x10^{-4}$) in the mucosal fluid lowered the PD and I_{sc}, but did not inhibit the subsequent Cl-induced increment of I_{sc}. The evidence suggests that there exists an electro-neutral symport element which carries NaCl as an ion-pair from the nutrient fluid across the basal-lateral membrane(BLM) into the cell. It is assumed that a ouabain-inhibited Na pump in the BLM provides the required driving force for the NaCl flow into the cell via the symport element.

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Na-Dependent, Symport Mediated Secretion of HCO_3 in the Turtle Pyloric Antrum. J.M.Kupfer*,W.A.Brodsky,and R.G.Gibson Mt.Sinai Sch. of Medicine,NY,NY,10029 (Gibson, spon.)

The pyloric antrum of turtles(Pseudemys scripta) contains active transport mechanisms for the reabsorption of Na and secretion of Cl and HCO_3. In contrast to the electrogenicity of the Na pump the HCO_3 pump is apparently part of an electroneutral carrier element that co-transports Na with HCO_3(as an ion pair) from the serosal fluid to the cytoplasmic fluid; this co-transport is driven by the Na pump. The following evidence is presented.(i)Addition of HCO_3 to HCO_3-free, Cl-free Na-Ringers media bathing both surfaces increases the transepithelial PD and I_{sc} without effecting the electrical resistance.(ii)The serosal addition of ouabain blocks the HCO_3 induced effects.(iii)The luminal addition of amiloride, an agent that blocks the passive entry of Na across the apical membrane, does not inhibit the HCO_3 induced increases in PD and I_{sc}.

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INTERACTION OF AMILORIDE, CALCIUM AND CHELATING AGENTS IN RELATION TO SODIUM CONDUCTANCE IN EPITHELIAL TISSUE. Carl L. Thurman and James T. Higgins, Jr., Dept. of Medicine, The Medical College of Ohio, Toledo 43699.

Regulation of sodium transport in epithelial tissues is controlled primarily by apical membrane permeability. Several agents were used to modify the electrical properties of urinary bladders from winter specimens of the Mexican toad, Bufo marinus. The epithelia were mounted in a modified Ussing chamber that affords maximal mucosal-serosal isolation with minimal edge damage (Pflugers Arch 358:44). Under open circuit conditions, we observed transmural potential changes in response to square pulses of current between 1.0 and 11.0 $\mu A/\pi\ cm^2$ with durations of one second. Apically applied amiloride altered transepithelial potential (PD), resistance (R) and short-circuit current (SCC). The median effective dose for inhibition of SCC was found to lie between 1.8 and 4.6 X 10^{-7}M amiloride depending upon the presence of Ca^{++}or calcium chelators. However, when both $Ca^{++}$and a chelator were present stimultaneously in the mucosal medium, the PD and SCC were transiently increased by amiloride concentrations below 10^{-7}M. Under these conditions, R was significantly lower than under comparable circumstances without amiloride. This unanticipated electrical behavior apparently reflects an increase in mucosal to serosal active transport of sodium.(Support by USPHS, NIH Grant HL-07357 and by MCO Nephrology Research Funds).

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EFFECTS OF KCL ON EXCISED CANINE TRACHEAL EPITHELIUM. M.J. Stutts*, R.C. Boucher*, and J.T. Gatzy* (SPON: A.L. Finn). School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514.

The basolateral membranes of the cells of many epithelia are more permeable to K+ than Na+. This permselectivity in some cases contributes to the electrical potential difference (PD) generated by the epithelium. We studied the effects of replacing bathing solution Na+ by K+ on bioelectric properties and solute permeability across canine tracheal posterior membrane mounted in an Ussing chamber. Exposure of the luminal surface to 100 mM/L K+ had no effect. Submucosal K+, 100 mM/L, reduced PD from 31.7 mV, lumen negative, to zero. Short circuit current fell from 66 $\mu a/cm^2$ to zero. The d.c. conductance rose from 2.0 to 6.5 mS/cm^2. The changes were stable for one hour and reversible. Unidirectional permeability coefficients of Na+, K+ and ^{14}C-mannitol were unchanged. Lumen to submucosal Cl^- movement doubled. This increase and the addition of the more permeant K+ accounts for most of the raised G. Replacing bathing solution Cl^- by $SO_4^=$ reduced by 50% the K+ induced increase in G. Depolarization by submucosal but not by luminal K+ indicates that the basolateral membrane of the epithelium is K+ permselective. Further, if mannitol permeability is an index of small hydrophilic solute movement through paracellular paths, then the increase in Cl^-, but not mannitol, K+ or Na+ permeability, suggests the induction of a voltage dependent, cellular Cl^- conductance pathway. (Supported by EPA grant # R-80653901, HL-16674 and HL-22924)

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WATER FLUXES IN THE FERRET TRACHEA IN VITRO. G.M. Loughlin*, M.H. Crowder*, R.L. Boyd* and J.A. Mangos, Dept. of Pediatrics, Univ. of Florida College of Medicine, Gainesville, FL 32610.

A method of stationary perfusion of the ferret trachea was developed in order to study the transepithelial fluxes of fluid. Water fluxes were demonstrated by measuring changes in inulin concentration determined by a colorometric assay sensitive to changes of 0.1 mg/ml. Preliminary studies demonstrated that inulin did not cross the epithelial barrier. Viability of the preparation was assessed at the end of the experiment by trypan blue. In seven tracheas, the flux of water in the unstimulated state was 29 ± 3 (mean ± S.E.) µl/trachea·hour with the net flux out of the lumen. Addition of 10^{-6}M carbachol to the submucosal side reversed the water flux resulting in a net water movement from the bath into the tracheal lumen which ranged between 16 and 107 µl/trachea·hour. Atropine in a concentration of 10^{-5}M blocked the response to carbachol without effect on baseline flux. Measurements of potential difference (PD) were obtained in three additional tracheas. While under unstimulated conditions, PD was 9 millivolts with the lumen negative. Carbachol resulted in a decrease of PD to 4.5 millivolts over 20 minutes. This work demonstrates that in the ferret trachea under basal conditions of stationary perfusion there is a net reabsorption of water from the tracheal lumen. This process may play a role in regulation of the volume of the periciliary fluid. Cholinergic stimulation reverses the net flux and causes a net secretion of water into the trachea. (Supported in part by a grant from the Cystic Fibrosis Foundation.)

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INTRACELLULAR CHLORIDE ACTIVITIES AND ACTIVE Cl SECRETION BY RAT TRACHEA. M.E. Duffey* and M.M. Cloutier* (SPON: S.K. Hong). Depts. of Physiology and Pediatrics, State Univ. of New York at Buffalo, Sch. of Med., Buffalo, N.Y. 14214.

Isolated rat trachea, opened axially into a flat sheet and bathed with a Na-containing Ringers solution, generated a spontaneous transepithelial potential difference of 4.8 mV, serosa positive, and short-circuit current of 62.2 $\mu A/cm^2$. Intracellular ion activity (Cl)c and the electrical potential difference across the mucosal membrane, ψmc, were measured in the tracheal epithelial cells, using Cl-selective and conventional (KCl-filled) microelectrodes. Under these conditions, ψmc=-50 mV and (Cl)c=31 mM. This (Cl)c value is 2.2 times that predicted for an equilibrium (Nernst) distribution across the mucosal membrane and 2.6 times that predicted for equilibrium across the basolateral membrane. When the tissue was then bathed with Na-free Ringers, (Cl)c fell to an equilibrium value and ψmc depolarized significantly. These results provide direct evidence that: (1) Cl is accumulated against its electrochemical gradient by this tissue, and (2) this accumulation is dependent upon the presence of Na. These findings are consistent with a model for Cl secretion by trachea with a Na-coupled basolateral membrane entry step for Cl (Marin, M.G. and M.M. Zaremba, *J. Appl. Physiol.* 47: 598-603, 1979). (Supported in part by SUNY Research Foundation Campus Research Incentive Fund and Cystic Fibrosis Foundation Grant #I150A.)

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ELECTROGENICITY OF Na-K ATPASE PUMP IN IN VITRO FROG STOMACH. T. C. Chu*, G. Carrasquer, M. Schwartz, T. L. Holloman* and W. S. Rehm, University of Louisville, Louisville, Ky. 40292.

Increasing [K] in the nutrient solution usually results in a decrease of positivity of nutrient. This effect is predicted on basis of K and Cl conductance channels in nutrient membrane. However, Davis et al (AJP 209:146, 1965) found, after bathing mucosa on both sides with zero K solutions, that rapidly increasing [K] from zero to 4 mM on nutrient side results in an increase in positivity of nutrient -- an anomalous response. We explain the anomalous response on the assumption of an electrogenic Na-K ATPase pump located in the nutrient membrane in which for 1 cycle more Na is pumped out of cell than K pumped into cell (e.g. 3 Na for 2 K). Normally the effect of Δ[K] via K conductance channels masks the effect on Na-K pump. However, after exposure to zero K solutions with zero H^+ secretory rate, the usual effect of a rapid change from zero to 4 mM K in the nutrient solution on the K channel is temporarily obscured by the predominance of the effect on the Na-K mechanism. In this case, there is a transient increase in the positivity of the nutrient (Na-K pump effect) followed by a decrease (K channel conductance effect). Ouabain (10^{-3} M in nutrient solution) completely eliminates the anomalous response so that only the K channel conductance effect is seen, i.e., the change from zero to 4 mM K makes the nutrient negative. Hence the Na-K pump contributes directly to the PD as well as indirectly via the control of cellular concentrations. (NIH and NSF support).

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CHLORIDE MOVES ACROSS FROG SKIN THROUGH A CONDUCTIVE PARACELLULAR PATHWAY. H. Schoen*, W. Green* and D. Erlij., Dept. of Physiology, SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203

It has been suggested that an important fraction of chloride movement across the frog skin is due to an exchange diffusion mechanism. To further analyze this problem we have carried out measurements of ^{36}Cl flux, electrical resistance, and intracellular potential in amiloride-treated frog skins. We have confirmed that substituting Cl by NO_3 on the apical solution reduces the ^{36}Cl flux from basolateral to apical solutions. This reduction is accompanied by an increase in transepithelial resistance that is directly proportional to the change in isotope flux. Substitution of Cl by NO_3 increased the magnitude of the intracellular potential by 1.0 ± 0.4 mV. These results show that the inhibition of ^{36}Cl flux by NO_3 is due to interference with a conductive movement and not with a typical exchange diffusion and that the permeability of the skin to Cl does not reside in the cells most frequently penetrated with the microelectrode, i.e., it is likely to be in a paracellular route. (Supported by the NY Heart Association)

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INTRACELLULAR K^+ ACTIVITY IN NECTURUS URINARY BLADDER. J.F. Garcia-Diaz* and W. McD. Armstrong, Dept. Physiology, Indiana Univ. Sch. Med., Indianapolis, IN 46223.(SPON:G.A. Tanner).

Transapical p.d. (E_m) and intracellular K activity (a_K^i)were measured with open-tip and K-selective liquid ion-exchanger microelectrodes in isolated Necturus urinary bladders mounted in a perfusion chamber modified from Nagel, J. Physiol. 269: 777, 1977. Care was taken to prevent edge damage. The bathing medium contained 100 mM NaCl and 5.4 mM K^+ at pH 7.2. Microelectrodes were advanced perpendicularly to the luminal surface by a piezo-electric translator in 4-6μm steps. Recordings were fed to a high impedance preamplifier with capacitance neutralization to improve the time response of ion-selective microelectrodes. When impaling with open-tip microelectrodes the preamplifier provided a $0.5 \cdot 10^{-9}$ A current pulse to continously monitor microelectrode resistance. Transepithelial p.d., E_T, ranged from 30 to 80 mV (serosa positive,n=6) and was time dependent. In 46 impalements (6 animals) E_m ranged from -6 to -21 mV (inside negative,mean -11 mV) and a_K^i ranged from 70 to 117 mM (mean 95 mM). Brief (~ 1 min.) luminal exposure to 10^{-5} M amiloride almost completely abolished E_T but hyperpolarized E_m by ca. 40 mV. In all cases, amiloride slightly hyperpolarized (3-12mV) the basolateral membrane potential. Changes in potential recorded by K-sensitive microelectrodes after amiloride application were of the same direction and magnitude as those recorded with open-tip microelectrodes, indicating that a_K^i is not altered by short exposure to the drug. Supported by USPHS AM 12715, HL 23332.

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MEMBRANE AND EPITHELIAL POTENTIALS AND RESISTANCES OF TOAD BLADDER DURING H^+ SECRETION. A.G. Ramsay. Columbia Univ. Collgege of Physicians and Surgeons and the M. I. Bassett Hosp. Cooperstown, N.Y. 13326

Urinary bladder of toad secretes H^+ into mucosal solution in vitro. Mucosal acidification results. If Na^+ transport is stopped, electrical polarity is reversed with mucosa positive to serosa and H^+ secretion can be quantitated with reverse short circuit current. This microelectrode study was undertaken to characterize membrane and epithelial potentials during isolated bladder H^+ secretion, stimulated by 18 mM HCO_3^- ambient solutions gassed with 5% CO_2. Na^+ transport was stopped with mucosal substitution of choline for Na^+ and amiloride addition. This reversed epithelial potential from 26mV mucosa negative to 14 mV mucosa positive. Apical P.D. reversed from 13.7 mV cell positive to mucosa to 16mV cell negative. Basallateral P.D. decreased from 10 to 3.8 mV, but cell remained negative to serosa. Voltage divider ratio increased from 1.90 to 3.54 accompanied by 67% increase in apical resistance from 2181 to 3397 ohm·cm^2. This suggests that apical H^+ conductance is double Na^+ conductance. Shunt resistance doubled to 23,963 ohm·cm^2, a value about half that with 1 mM mucosal Na and no H^+ secretion. Data are consistent with apical electrogenic H^+ pump, significant apical H^+ conductance and an increased paracellular resistance to maintain an epithelial H^+ gradient. (Supported by CRS 137, Walter H.D. Killough Fund and Stephen C. Clark Fund)

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EFFECTS OF CATECHOLAMINES ON ACTIVE Cl^- SECRETION BY THE OPERCULAR EPITHELIUM OF FUNDULUS GRANDIS. Edward Krasny, Jr.* and David H. Evans. Dept. Biol. & Living Resources, RSMAS, and Dept. Biol., Univ. of Miami, Miami, Fla. 33249

The effects of adrenergic-receptor agonist and antagonist agents on the electrical properties and Cl^- fluxes of isolated opercular epithelia from sea water-adapted F. grandis were studied. Epinephrine (EPI) at 10^{-5}M added to the serosal side of the epithelium rapidly decreased the short-circuit current (Isc) by 89.6% which was accounted for by a 100% reduction in the measured net Cl^- secretion rate (n=5). 10^{-5}M phentolamine (PHEN), an α-receptor antagonist, completely blocked the inhibitory response of EPI on the Isc. On the contrary, β-receptor stimulation, either through isoproterenol (10^{-5}M) or EPI + Phen addition, resulted in no significant change in Isc or the net Cl^- flux. Since β-stimulation results in an increased intracellular c-AMP concentration and/or an increased Cl^- secretory rate in several other epithelial types studied, the effects of c-AMP on Isc were studied. Theophylline (10^{-4}M) or dibutyryl-c-AMP (0.1-10mM) had no significant effect on Isc. These results suggest the presence of α-receptors in the chloride cells of the opercular epithelium of F. grandis which mediate the epinephrine-induced decrease in active Cl^- secretion. The evidence also suggests that β-receptors are not present in chloride cells of the opercular epithelium of F. grandis contrary to results found in the opercular epithelium of F. heteroclitus. (Supported by NSF Grant PCM 77-09915).

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Ca++ IONOPHORE A23187 AND CHLORIDE ACTIVITY IN NECTURUS GALLBLADDER. W. McD. Armstrong and A.Diez de los Rios* Dept. Physiology, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

In media containing Ca++, A23187 mimics the net secretory response of isolated mammalian small intestine to adenosine 3' 5'-cyclic monophosphate (cAMP:J.E. Bolton,M. Field. J. Membrane Biol. 1977:35,159). This response involves i)inhibition of coupled apical Na-Cl absorption, ii) stimulation of active Cl^- secretion. In gallbladder only the anti-absorptive effect of cAMP is seen and cAMP abolishes the transmembrane Cl^- electrochemical driving force. Isolated Necturus gallbladders were held in divided chambers between identical oxygenated HCO_3^--free solutions (100 mM Na^+ and Cl^-, 5.4 mM K^+, 1.8 mM Ca^{++}, pH 7.2,23°C). 0.5 μg/ml A23187 in the serosal medium did not affect transepithelial P.D., apical membrane potential(E_m) or intracellular Cl^- activity (a_{Cl}^i). 0.5 μg/ml A23187 in the mucosal medium did not change P.D. (0.1 ± 0.4, SEM, mV: 5 expts). E_m increased ($P<0.05$) from -46 ± 2 to -67 ± 1 mV. a_{Cl}^i decreased ($P < 0.05$) from 19 ± 1 to 12 ± 1 mM but the outwardly-directed Cl^- electrochemical driving force increased from 11 to 20 mV. These results clearly indicate that A23187 does not mimic the anti-absorptive effect of cAMP and suggest that this effect, unlike the secretory response to A23187 seen in the intestine, is not mediated by increased intracellular Ca^{++} concentration. Supported by USPHS AM 12715, HL 23332. A.D.R. was a Fellow of the Ministerio Univ. e Invest., Spain.

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ADRENAL GLOMERULOSA SENSITIVITY TO LONG-TERM INFUSION OF ANGIOTENSIN II IN DOGS BEFORE AND AFTER DIETARY SODIUM RESTRICTION.
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Long-term angiotensin II infusion in conscious dogs results in a marked increase in aldosterone secretion lasting for only a few hours followed by a sustained increase in aldosterone secretion above preinfusion levels but considerably below the levels observed during the acute infusion period. This study was designed to determine whether the adrenal glomerulosa sensitivity to angiotensin II increases during sodium deficiency. Continuous angiotensin II infusion was maintained for two weeks at four rates (5, 10, 15, and 25 ng/kg/min) in 12 dogs before and after dietary sodium restriction (5 mEq Na+/day) for 21 days. Plasma aldosterone concentration was determined daily after the third day of continuous angiotensin II infusion. The following results represent the percent increase in plasma aldosterone concentration above control levels during chronic steady-state conditions in dogs before and after sodium depletion:

	ANGIOTENSIN II (ng/kg/min)			
	5	10	15	25
Sodium-Replete	31%	71%	114%	253%
Sodium-Deplete	37%	73%	129%	267%

Long-term angiotensin II infusion produced similar increases in steady-state levels of plasma aldosterone concentration. These data fail to support the concept of increased adrenal sensitivity to angiotensin II during sodium deficiency.
(Supported by USPHS. NIH Grant HL 09921)

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THE RESPONSE OF AN AGLOMERULAR, ANTARCTIC, TELEOST, DISSOSTICHUS MAWSONI, TO ANGIOTENSIN I AND II.
Jeffrey D. Turner* and Arthur L. DeVries. University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 U.S.A.

A preliminary examination of this aglomerular species revealed a nearly complete absence of a functional Renin-Angiotensin system (RAS). Specimens averaging 19 kg were fitted with dorsal aortic catheters from which blood pressure was monitored and drugs administered. No significant pressor responses were elicited when the decapeptide angiotensin I (AI) was administered in doses as large as 3.0 µg/kg. Further, angiotensin II (AII) only produced a slow rise in average dorsal aortic pressure (ADAP) of 10% and 17% at 1 and 3 µg/kg respectively. Direct AII receptors were blocked by a competitive angiotensin analogue SAR^1ALA^8 sin (Beckman) (10 µg/kg/min) for 5 minutes, and followed by AII 3.0 µg/kg. This resulted in a 43% increase in ADAP. An α adrenergic blockade with Yohimbine (200 µg/kg) prior to a 3.0 µg/kg AII injection, decreased ADAP by 28%. No plasma renin activity was detected, and no elevations in plasma aldosterone, epinephrine and norepinephrine were observed even after AII at 3.0 µg/kg. These results suggest the lack of a functional RAS in these fish. A fact not surprising considering the total absence of the glomerulus and arterial network so intimately related to the RAS in other vertebrates.
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BLOOD COAGULATION ENZYMES IN THE ACTIVATION OF HUMAN PLASMA "PRORENIN".
A.D. Purdon* and D.H. Osmond. Dept. of Physiology, University of Toronto, Toronto, Ontario, Canada. M5S. 1A8.

Factor XII, among others, participates in the cold activation of plasma prorenin (Lancet 1, 1313, 1978). We therefore activated the coagulation system by adding kaolin, 2 mg/ml plasma, for 6 hr. at 4° C. This did not enhance the subsequent plasma renin activity (PRA) determined at 37° C. However, after kaolin at -4° C., 6 hr., PRA rose 10% ($p < 0.005$), indicating that stimulation of coagulation factors activates the renin system. Stimulation by tissue trauma associated with venipuncture enhances PRA by an average of 16% (range 0 - 28%) in blood collected into EDTA, compared with blood collected into protease inhibitors (10 mM benzamidine and 200 µg/ml polybrene), plus EDTA, 12 mM. On the other hand, removal of factors II, VII, IX, and X by absorption onto $BaSO_4$, greatly reduces cold (-4° C., 96 hr.) but not tryptic (1.0 mg/ml plasma) activation of plasma prorenin.

Plasma treatment	PRA (Angiotensin I ng/ml/hr., at 37° C.)		
	Control	After Cold	After Trypsin
- $BaSO_4$	3.1±0.1	18.6±0.5	15.3±0.4
+ $BaSO_4$	3.6±0.1	7.7±0.2	13.0±0.2

Thus, in addition to factor XII, one or all of factors II, VII, IX, and X, participate in cold activation of prorenin, but trypsin in adequate concentration can largely substitute for the missing factors.
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INHIBITION OF ANGIOTENSIN I CONVERTING ENZYME PREVENTS HYPERTENSION DUE TO AORTIC DEPRESSOR NERVE TRANSECTION IN RATS.
R. L. Kline and P. F. Mercer. Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1

We have previously shown that aortic depressor nerve (ADN) transection in rats increases the turnover of norepinephrine in the kidney, and that renal denervation prevents the hypertension due to bilateral cutting of the ADN (Fed. Proc. 39: 962, 1980). To determine whether the renin-angiotensin system is involved in the arterial pressure response to ADN transection, Captopril (C, 50 or 100 mg/kg, p.o. daily) was administered prior to and after bilateral transection of the ADN in Wistar rats. Arterial pressure (tail cuff) and heart rate were measured 3 times a week. Treatment with C for 3-7 days prior to cutting the ADN did not alter the heart rate response, but significantly altered the arterial pressure response. In control animals arterial pressure increased by 25 ± 2 (S.E.) mm Hg on day 1, and remained elevated for 6 days after ADN transection. Arterial pressure in C-treated animals was increased by 15 ± 5 mm Hg on day 1, but returned to normotensive levels within 1 week. Withdrawal of C treatment in ADN-sectioned animals resulted in a rise in arterial pressure, while administration of C to hypertensive, ADN-sectioned animals lowered arterial pressure to normal levels within several days. These data, in conjunction with previous studies involving renal denervation, suggest that renal renin is involved in the arterial pressure response to ADN transection in rats.
(Supported by the Ontario Heart Foundation).

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CAPTOPRIL INHIBITS ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN THE BRAIN.
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Captopril (SQ 14,225, Squibb) is a potent orally-active inhibitor of angiotensin converting enzyme used in the study and treatment of renin-dependent hypertension. It is controversial whether systemic administration of this drug also inhibits the cerebral renin-angiotensin system. We investigated the effect of intracerebroventricular (i.c.v.), subcutaneous (s.c.) and intragastric injections of Captopril on the drinking response of rats injected with angiotensin precursors i.c.v., a response that is blocked when conversion of angiotensin I to II in the brain is inhibited. Captopril injected i.c.v. potently inhibited drinking caused by i.c.v. injections of 10 mUnits hog renin, 10 ng synthetic renin substrate or 10 ng angiotensin I. Inhibition by Captopril was dose-dependent (20 ng to 2 µg) and lasted at least 2 hr after injection of the highest dose. Drinking responses to renin and angiotensin I were significantly reduced also following s.c. injections (5 to 50 mg/kg) or intragastric intubation (10 mg/kg) of Captopril. Neither intragastric, s.c. or i.c.v. injections of the drug inhibited drinking elicited by 10 ng angiotensin II itself. We conclude that Captopril is a potent inhibitor of angiotensin converting enzyme activity in the brain. Contrary to previous reports, Captopril can cross the blood-brain barrier to inhibit angiotensin conversion in the brain when given in doses lower than those commonly used in laboratory studies.
(Supported by the MRC of Canada)

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PROSTAGLANDINS AND RENIN RELEASE IN EXPERIMENTAL RENOVASCULAR HYPERTENSION

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We have examined the hypothesis that prostaglandins play a role in the developmental or maintenance phases of renovascular hypertension. Two 5 mg/kg doses of indomethacin were given to conscious, renal denervated, propranolol treated dogs during the acute and chronic phases of one-kidney (1-KHT) and acute phase of two-kidney (2-KHT) renovascular hypertension. Indomethacin attenuated the elevations in plasma renin activity observed during the acute phase of (1-KHT) and (2-KHT). Plasma renin activity did not return to normal however, and mean arterial blood pressure decreased only slightly. Indomethacin did not lower plasma renin activity or mean arterial blood pressure in chronic (1-KHT) dogs unless plasma renin activity was elevated above the normal level by sodium depletion. Indomethacin failed to alter renal function during either phase of (1-KHT). These results suggest that in the dog renal prostaglandins play only a minor role in the pathogenesis of both acute (1-KHT) and (2-KHT), whereas no evidence was obtained for a role of renal prostaglandins in chronic (1-KHT) except during superimposed sodium depletion. It appears, therefore, that in the dog prostaglandins are involved in renovascular hypertension only under conditions where plasma renin activity is elevated.

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EFFECTS OF CHRONIC SODIUM DEPLETION ON RENAL SYMPATHETIC ACTIVITY IN THE ANESTHETIZED DOG. Shuichi Takishita* and Carlos M. Ferrario, (SPON: K.B. Brosnihan) Cleveland Clinic Research Division, Cleveland, Ohio 44106.

Dietary sodium restriction (LS) alters the point of equilibrium between the renin angiotensin and sympathetic control mechanisms. In the dog one aspect of this interplay involves blunting of the reflex response to carotid sinus hypotension that is reversible by section of vagal afferents (The Physiologist 21: 13, 1978). To obtain more direct evidence, sympathetic nerve activity (SNA) was recorded from the cut end of a renal nerve (RNA) in 16 normal (NS) and 13 LS dogs anesthetized with morphine-pentobarbital. Integrated RNA was measured during changes in mean arterial pressure (MAP) produced by IV infusion of sodium nitroprusside (100 µg/kg/min) or phenylephrine (20µg/kg/min). The classical inverse relationship between MAP and integrated RNA was found before and after bilateral vagotomy (VAGT) in both NS and LS dogs. However, RNA in LS dogs, expressed as % of maximal neural firing was significantly less within the 40 mmHg change when compared to NS dogs. In addition, the critical pressure (point at which RNA ceased) was about 12 mmHg less in LS vs NS dogs ($p < 0.002$). Blunting of sympathetic neural firing in LS dogs was abolished after bilateral VAGT confirming the pronounced buffering effects of either aortic or vagal afferent receptors on SNA in salt depleted dogs. (Supported in part by a grant from NHLBI, #HL-6835).

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DIFFERENTIAL EFFECTS OF DEOXYCORTICOSTERONE ACETATE (DOCA) ON RENAL AND GASTROINTESTINAL HANDLING OF ELECTROLYTES IN PIGS. M.E. Grigorian*, R.J. Grekin, J. Mitchell*, J. Buiteweg*, D.M. Cohen, and D.F. Bohr. University of Michigan, Ann Arbor, MI 48109

Young male pigs eating standard Purina Pig Chow, ad libitum, received approximately 170mEq Na and 290 mEq K per day. Electrolyte intake, urinary and fecal electrolyte output and serum electrolyte levels were determined daily in five DOCA-treated pigs, and in two control pigs. Daily Na and K balances (dietary intake - urinary + fecal output) were calculated. DOCA caused a reduction in urinary Na output from 1.62mEq/kg/day to 0.19mEq/kg/day within 24 hours. Escape from the renal sodium retaining effect of DOCA was complete within 3 days, with urinary Na output returning to pre-DOCA levels. Fecal Na output decreased from 0.86mEq/kg/day to 0.34mEq/kg/day within 24 hours. However, no escape from GI Na retention occured by day 9. Serum Na levels were not detectably changed during this period of time. Urinary K output decreased from 3.34mEq/kg/day to 1.25mEq/kg/day within 24 hours, while fecal K output was not significantly changed. Serum K fell from 4.5mEq/l to 3.0mEq/l by day 6. This hypokalemia occured in the absence of a negative K balance. The control pigs showed no significant changes in electrolyte handling. It is concluded that DOCA has differential effects on renal and gastrointestinal handling of electrolytes and that DOCA induces an intracellular shift of potassium in pigs. DOCA has important extra-renal actions. (Supported by NHLBI grant #HL-18575).

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VENTRICULAR BODY WEIGHT PROFILES IN NORMAL AND CHRONIC ANGIOTENSIN II TREATED RATS DURING EARLY DEVELOPMENT. Maurice S. Holder*; Wilmoth Baker III*; Alvena Smith*; LaVal Cothran, Florida A & M Univ., Tallahassee, Florida

Ventricular Weight-Body Weight ratios (VW/BW) were assessed at different time periods to determine the relationship present in normally developing (ND) and renal hypertensive (RH) mammals during early development. The ND rats showed a consistent decrease in VW/BW with age from 3.47×10^{-3} at birth to 2.24×10^{-3} in sexually mature adults. Conversely, mature rats subjected to chronic angiotensin II (AII) injections at dose levels of (0.25, 0.5, 1.0 and 2.0 µg/Kg) showed a significant ventricular hypertrophy by the 4th week. Maximum hypertrophy was obtained with the 1 Kg/Kg dose and the percent difference from control for VW/BW at 4 weeks was 20.7. Hypertrophy was also present with doses of AII that did not produce sustained elevations in afterload while interruption of (AII) administration reversed the hypertrophy. In (RH) rats, Hypertrophy was equal to that with the 0.5 Kg/Kg dose, and by comparison the AII hypertrophy was greater than that induced with norepinephine. This study shows that ventricular mass per unit body mass decreases with age in normotensive rats during the early stage of development and that this trend can be inhibited with AII. Further, this action of AII is dose dependent and is independent of its pressor action. The results suggest that AII may be involved in both physiological and pathological hypertrophy in mammals.

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REDUCED BLOOD PRESSURE RESPONSE OF SPONTANEOUSLY HYPERTENSIVE RATS TO ARACHIDONIC ACID. Peter Lukacsko*, Edward J. Messina and Gabor Kaley. New York Medical College, Valhalla, NY 10595

Prostaglandins (PG) E_2 and I_2 are potent vasodepressor agents and may contribute to blood pressure regulation. Reduced vascular response to or a deficient capacity to metabolize arachidonic acid (AA) to these PGs may contribute to the development and maintenance of hypertension. The percent change in mean arterial blood pressure (MABP) to intra-arterial (IA) injections of PGE_2 and PGI_2 at doses of 0.01, 0.1, 1.0 and 10.0 nM/100g and to sodium nitroprusside (NaNP) at doses of 0.1, 1.0, 10.0 and 100.0 nM/100g was measured in anesthetized, age-matched, spontaneously hypertensive (SH) and normotensive Wistar (W) and Wistar-Kyoto (WKY) male rats during the 5th, 10th and 20th week of life. There were no significant differences in the decrease of MABP to PGI_2 and NaNP between SH and either W or WKY rats. All age groups of SH rats, however, responded to PGE_2 with greater reductions in MABP than W or WKY rats. The IA injection of AA at doses of 10, 100, 300, 500, 1000 and 3000 nM/100g caused dose-dependent decreases in MABP in all rats. At low doses, responses to AA were significantly reduced in all age groups of SH rats as compared to controls. Similar results were obtained with 20 week old, anti-hypertensive drug treated and 5 week old SH rats (whose blood pressures were in the normal range) indicating that the reduced response of SH rats to AA is not dependent on an elevated MABP and could possibly contribute to the development of hypertension. (Supported by the Westchester Heart Association).

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THE DOG AS A MODEL FOR THE HUMAN "PRORENIN"/RENIN SYSTEM. E.A. Wilczynski* and D.H. Osmond. Dept. of Physiology, Univ. of Toronto, Toronto, Canada. M5S 1A8.

In our quest for an animal model to study hypertension, plasma prorenin/renin was compared in humans and dogs. Prorenin was activated by cold (cryoactivation, 0° C, up to 6 weeks) and trypsin (up to 6 mg trypsin/ml plasma at 22° C, 10 min). After activation, plasmas were incubated at 37°C for determination of plasma renin activity (PRA) by radioimmunoassay, expressed as mean angiotensin I, ng/ml/hr.

	Cryoactivation (days)			Tryptic activation (mg/ml)			
	0	7	35	0	1	3	5
Man (n=3-6)	4.7	10.6	18.5	5.1	18.3	6.6	-
Dog (n=6-12)	1.1	1.1	1.6	1.2	2.1	29.7	49.2

Human plasma has a higher basal PRA, and its prorenin cryoactivates greatly, while the dog's activates minimally. In man, trypsin at 1 mg/ml activates prorenin powerfully, while 3 mg is excessive, and lowers the reading. In contrast, dog plasma requires as much as 6 mg trypsin to reveal the full amount of prorenin, which is much higher than in man. We conclude that, in terms of (1) the absolute and relative quantities of prorenin/renin; (2) the capacity to cryoactivate; and (3) the requirements of trypsin (presumably related to higher levels of endogenous protease inhibitors), the dog's prorenin/renin system differs markedly from man's in the normotensive state. Thus, the dog can serve as a model, but the appropriate technique must be chosen and suitably adapted. (Supported in part by the Ontario Heart Foundation).

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"PROSUBSTRATE" IN PLASMA, AND ERYTHROPOIETIC ACTIVITY IN DIALYSATES OF HUMANS ON CHRONIC PERITONEAL DIALYSIS. (CAPD). D.H. Osmond, A.Y. Loh*, J. Abrams*, M. Selucky*, N. Dombros*, and D.G. Oreopoulos*. Depts. of Physiology and Medicine, University of Toronto, Toronto, Canada. M5S 1A8.

Episodic or steady hypotension occurs in many CAPD patients, possibly due to losses of "pressor agents" into the dialysates. We found such high losses of prorenin, renin, and renin substrate (Clin. Res. 26, 870A, 1978). Concurrently, plasma substrate dropped with time on CAPD from 516 ± 73 to 334 ± 16 ng/ml/hr., in terms of angiotensin I by radioimmunoassay, n=8, $p < 0.025$. However, 424 ng of new trypsin-activatable prosubstrate appeared (758 ± 28 vs. 334 ± 16, $p < 0.001$) suggesting abnormal hepatic release of inactive prosubstrate into plasma, in response to losses of active substrate into dialysate. Considerable erythropoietic (EPO) activity was detected in 14 of 16 patients by injecting concentrated, desalted, dialysate samples subcutaneously into fasted rats (Fried et al PSEBM 94, 237, 1957, Fe^{59} incorporation into erythrocytes). The activity ranged from about 1.5 to 6 EPO units (Connaught) from the equivalent of 100-300 ml dialysate/rat/assay. We postulate that EPO activity is generated in the peritoneal cavity, possibly by alteration of precursors entering from the blood. Some of this activity returns to the circulation, stimulates erythrocyte production sites, and thus produces the improved hematocrit observed in CAPD patients.
Supported by the Ontario Heart Foundation, and the Medical Research Council.

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DECREASE IN TRITIATED WATER PERMEABILITY OF RAT GASTRIC MUCOSA BY TOPICAL 16-16 DIMETHYL PROSTACLANDIN E_2($DMPGE_2$). C.F.Code, S.J.Harrington*,J.H.Steinbach*,J.M.Diamond,CURE,VA Wadsworth Hospital and University of California, Los Angeles, Ca.90073

This study was done to determine if the cytoprotective agent $DMPGE_2$ changes the permeability of gastric mucosa. Water was chosen to estimate permeability because it is inert and moves passively across the mucosa. Tests were done on 8 rats anesthetized with Urethane. The pylorus and esophagus were ligated and 3 ml solution containing 50 mM EPPS, 20 mM Mannitol, 100 mM NaCl and ^3H water at pH8 were placed in the stomach and removed 30 min later. After one or two control tests in each rat 1 µg/ml $DMPGE_2$ was added to instillates used during two more periods. The water permeability was calculated as the ^3H loss during each test period divided by the average ^3H concentration, the time and the surface area of the stomach.

MEAN ± SEM GASTRIC PERMEABILITY (ml hr^{-1} cm^{-2})

Control	$DMPGE_2$ Periods	
Periods	1st	2nd
0.264	0.180*	0.131**
±0.040	±0.017	±0.018

Paired t test with control *P = 0.058; **P = 0.003

Adding $DMPGE_2$ to the test solution reduced the water permeability of the gastric mucosa during the second period of instillation without changing net movement of water. The results support the concept that the gastric cytoprotective action of $DMPGE_2$ is related to the reduced permeability it produces in the mucosa.

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ENHANCEMENT OF ACID SECRETION IN ISOLATED CANINE PARIETAL CELLS BY INDOMETHACIN AND OTHER PROSTAGLANDIN SYNTHESIS INHIBITORS. M.L. Skoglund*, J.G. Gerber*, A.S. Nies*. (SPON: T.J. Burke.) Division of Clinical Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Prostaglandins (PG) are potent inhibitors of histamine stimulated acid secretion, and stimulation of PG synthesis in isolated canine parietal cells by arachidonic acid (AA) also inhibits acid secretion. This effect of AA can be blocked by addition 100 µM indomethacin. To determine if endogenous PG synthesis in the parietal cell modulates acid secretion, we added increasing concentrations of indomethacin to histamine stimulated isolated parietal cells and measured acid secretion by uptake of (^{14}C) aminopyrine. A dose-response curve for histamine (10^{-8}-10^{-4}M) was determined. Concentrations of 1,10, and 100 µM indomethacin were added to histamine stimulated cells to detect any shift of the dose-response curve to the left. Basal and low level (0, 10^{-8}, 10^{-7}M) histamine stimulated acid secretion was enhanced (n=6, $p < 0.05$) in the presence of indomethacin. However, at high levels of histamine (10^{-6}, 10^{-5}, 10^{-4}M), indomethacin did not increase acid secretion above maximal. To assure ourselves that the enhancement of acid secretion was due to PG synthesis inhibition, we repeated the same experiments with 10 µM meclofenamic acid and 30 µM ibuprofen, two other PG synthesis inhibitors. Both drugs enhanced histamine stimulated acid secretion ($p < 0.05$, n=3) with the same pattern as seen with indomethacin. We conclude that endogenous PG synthesis by parietal cells plays an important role in modulating the acid secretory response to low level histamine stimulation.

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SALIVECTOMY INCREASES BILE SALT INDUCED DAMAGE TO THE GASTRIC MUCOSAL BARRIER, K.A. Menkal*, P.G. Dellow and B.L. Tepperman. University of Western Ontario, London, Canada. N6A 5C1.

The salivary glands contain substances which increase the resistance of the gastric mucosal barrier (GMB) to the disruptive actions of ulcerogenic agents (Gastroenterology 79: 1217, 1979). However aside from epidemiologic evidence linking a decrease in salivary secretion to an increase in the incidence of ulcer disease (Scand. J. Gastroenterol. 2:95, 1967) no studies exist demonstrating a direct influence of the salivary glands on the integrity of the GMB. We have studied the effects of salivectomy on bile salt (BS)-induced alterations in ionic fluxes, luminal histamine appearance and mucosal ulceration using urethane anaesthetized rats. In the desalivate group the submandibular-sublingual gland complexes were removed and the parotid ducts were ligated. Sham operated rats served as controls in this study. Experiments were commenced three weeks after surgery. 150 mM HCl was instilled into the stomach and withdrawn at 10 min intervals. BS (5mM) was added to the HCl 40 min after initiation of the experiment and the BS treatment was continued for 60 min.

In the sham operated group (N=5) BS treatment resulted in a significant increase in loss of H+, the appearance of Na+, K+, and histamine in the lumen and the appearance of mucosal lesions. Salivectomy (N=6) significantly exacerbated the ionic fluxes, luminal histamine and ulcer index produced by BS. These results suggest that salivectomy decreases the resistance of the GMB to BS-induced damage. Supported by MRC of Canada.

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STIMULATED SECRETION OF PEPSINOGEN BY ISOLATED FUNDIC GLANDS OF RABBIT GASTRIC MUCOSA. David E. Schafer. Veterans Administration Medical Center, West Haven, CT 06516, and Yale University School of Medicine, New Haven, CT 06510.

As we have previously reported, pepsinogen secretion (PS) by isolated biopsies and/or strips of rabbit fundic mucosa shows the following characteristics: (1) in response to 10^{-4} M acetylcholine (ACH) plus physostigmine (PHY) PS is first evident after about a 45-minute delay; (2) over 2 hr or less, PS is rarely detected in response to ACH at less than 10^{-6}M; (3) inter- and intra-animal variation in both baseline and stimulated PS is substantial; (4) carbachol is usually less effective than ACH-PHY in eliciting PS; (5) there are indications of endogenous ACH release; and (6) removal of calcium from the medium causes only partial inhibition of PS in response to ACH-PHY. For some studies of gastric mucosal secretion the isolated fundic gland preparation of Berglindh et al. has the advantages of reducing intra-animal variation and permitting more direct access of the medium to the basal membranes of epithelial cells. We have now observed PS by isolated fundic glands. With this preparation, variation of PS among replicates is reduced to a few percent. Carbachol and ACH-PHY are about equally effective in eliciting PS; both consistently evoke a clear-cut PS after 10 minutes, the shortest interval so far examined, and up to 1 hr. Thus the isolated fundic gland preparation appears useful for the study of PS by rabbit gastric mucosa. (Supported by Veterans Administration)

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ISOLATED RAT PAROTID ACINAR CELLS: EFFECTS OF CALCIUM LOADING BY THE IONOPHORE A-23187. John A. Mangos, R. Lee Boyd*and Gerald M. Loughlin*, Univ. of Florida College of Medicine, Gainesville, FL 32610

Calcium ions (Ca^{++}) are essential in the stimulus-secretion coupling of exocrine glands. In previous studies from this laboratory it was shown that in isolated rat parotid acinar cells extracellular Ca is necessary for signal transduction from the activated muscarinic cholinergic receptors to the sites of the cellular processes of cGMP synthesis and K efflux (J. Dent. Res., in press, 1980). In this study, acinar cells were loaded with Ca by incubation in high-Ca (5mM) Hanks medium containing the Ca-ionophore A-23187 (10µM) for 1-3 hours. The following functional alterations were observed: a) Ca influx, measured by ^{45}Ca movements from medium into the cells, showed a biphasic pattern with an initial rapid influx phase (3-5 min) and a subsequent slow phase; b) the intracellular [Ca] increased from 3.54±0.51 mM/L at t_0 to 5.14±0.62 mM/L at t_{60min}; c) efflux of ^{22}Na from the cells decreased significantly at t_{60}; d) the intracellular [Na] and [K] changed from 26.2±5.6 and 110.5±10.6 mEq/L respectively at t_0 to 49.6±9.2 and 79.6±6.9 mEq/L respectively at t_{60}; e) the rapid phase influx of Ca was associated with transient K efflux and cGMP accumulation responses by the cells; subsequently, the acetylcholine-induced maximal K efflux and cGMP responses of the cells were markedly decreased; f) the cells began to lose their functional integrity after two hours of incubation. It was concluded that A-23187 induced Ca loading of rat parotid acinar cells initially mimics the effects of muscarinic cholinergic receptor activation but subsequently it disturbs these cellular responses and results in derangement of the ionic homeostasis of the acinar cells.

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IDENTIFICATION OF MUSCARINIC BINDING SITES IN DISPERSED PANCRETIC ACINI BY DIRECT ANTAGONIST BINDING. L. Larose*, G.G. Poirier*, Y. Dumont* and J. Morisset. G.I. Res. Unit, Sherbrooke Univ., Sherbrooke, Quebec, Canada.

The physiological response of the exocrine pancreas to a cholinergic stimulation is known to be mediated by muscarnic receptors. To define the biochemical characteristics of this muscarinic receptor, we have investigated the binding of (^3H) quinuclidinyl benzilate (QNB) and N-(^3H)-methyl scopolamine (NMS) on the rat isolated pancreatic acini. The transformation of the isotherm saturation data for both radiolabeled antagonists showed an apparent homogeneity of the receptor population. Total receptor binding sites were estimated at 2.667 ± 96 fmoles/mg DNA for (^3H)-QNB and at 2.038 ± 81 fmoles/mg DNA for (^3H) NMS. From Scatchard plots, an apparent K_D of 2.7×10^{-10} M was calculated for each ligand. Competition assays of a known muscarinic antagonist (atropine) and two agonists (carbamylcholine and oxotremorine) with both ligands gave ID_{50} comparable to those obtained in other tissues. Hill coefficients close to 1.0 for the antagonists and less than 1.0 for the agonists were also obtained. In this study, we have demonstrated that muscarinic receptor binding sites are present in an isolated pancreatic acini preparation. (Supported by MRC of Canada, Grants MA-7320 and NRC A0415).

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ETHANOL DISRUPTS POTENTIATED AMYLASE SECRETION IN THE EXOCRINE PANCREAS BY INHIBITING SECRETAGOGUE STIMULATED CALCIUM OUTFLUX. Billy W. Long* (SPON: F.P. Brooks.) VA Medical Center and Univ. of Penn. School of Medicine, Philadelphia, PA 19104.

In the guinea pig exocrine pancreas, agents that cause a redistribution of acinar cell calcium and calcium outflux, i.e., carbamylcholine (Carb) and cholecystokinin octapeptide (CCK-OP) as well as agents that increase cellular cyclic AMP, i.e., secretin and vasoactive intestinal peptide (VIP), all stimulate amylase secretion. The combination of any two agents that mediate pancreatic secretion by differing cellular mechanisms produce potentiation of enzyme secretion. Ethanol alters pancreatic secretion in vivo and in vitro. The cellular basis for the effects is unknown. In dispersed microacini from guinea pig pancreas basal amylase release is 2.3±1.2% of total per 30 min. Carb 10 uM or CCK-OP 1 nM each release 11.2±2%, while VIP 10 nM or secretin 1 uM each release 14.9±4%. The potentiated release is 31.9±3%. Ethanol from 80 to 480 mM has no effect on VIP, secretin, or CCK-OP stimulated amylase release. These concentrations of ethanol cause a dose-dependent inhibition of Carb stimulated amylase release, and abolishes the potentiating interaction with VIP or secretin. Ethanol 480 mM caused a 32±3% inhibition of amylase secretion and 60±8% inhibition of $^{45}Ca^{++}$ outflux. These results indicate that ethanol causes a potent and specific inhibition of pancreatic amylase secretion and calcium outflux. This suggests that ethanol alters potentiated pancreatic secretion by disruption of pancreatic cholinergic mechanisms, and stimulated calcium outflux in particular.

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PARALLEL MATURATION OF THE PANCREATIC SECRETORY RESPONSE TO CHOLINERGIC AND THE MUSCARINIC RECEPTOR POPULATION. Y. Dumont*, G.G. Poirier*, L. Larose* and J. Morisset. G.I. Res. Unit, Sherbrooke, Univ. Sherbrooke, Quebec, Canada.

Pancreatic enzyme secretion in response to bethanechol and the concentrations of muscarinic cholinergic receptors were evaluated in 21 day fetal pancreas and in those of rats aged from 1 to 365 days. Enzyme secretion was measured in vitro and the receptor population was evaluated by binding studies of quinuclidinyl benzilate (^{3}H-QNB) on pancreas homogenates. Muscarinic receptors are present in fetal rat pancreas at a concentration of 652 ± 46 fmoles/mg DNA; a maximal concentration of 3605 ± 218 is obtained in 30 day old animals and dropped to 1564 ± 213 when they reached one year. ^{3}H-QNB binding to the receptors exhibited K_Ds between $1 - 2 \times 10^{-10}$ M at all ages. From all data cumulated at different ages, we have plotted the maximal pancreatic secretory response to urecholine (amylase output/mg DNA) against their receptor concentration (fmoles/mg DNA) and obtained a coefficient of correlation of 0.96. This indicates that the maximally cholinergic stimulated enzyme secretion can be related to the total number of muscarinic binding sites per cell. In conclusion, the rat pancreas possesses a population of muscarinic receptors that matures simultaneously with the tissue secretory response to cholinergic stimulation.

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THE PARATHYROID, PARATHOMONE AND PANCREATIC SECRETION. Gur Ben-Ari, Ehud Klein, and David A. Dreiling. The Mount Sinai School of Medicine, Dept. of Surgery, New York, NY 10029.

The effects of parathyroidectomy on steady state secretin induced pancreatic exocrine secretion were studies in a group of dogs. Free flow of pancreatic juice was obtained by direct cannulation of the main pancreatic duct, (the accessory duct being ligated). A gastric canulla prevented entry of gastric acid into the duodenum. Parathyroidectomy caused significant reduction in the secretin induced pancreatic secretion of volume and bicarbonate concentration, and minute change in total protein concentration. These results were not dependent on calcium blood level, and were not affected by calcium administration to the hypocalcemic parathyroidectomized dog. It is suggested that these effects of parathyroidectomy may reflect the direct involvement of parathyroid hormone in the control mechanism of pancreatic exocrine secretion.

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THE ANTITROPHIC EFFECT OF SOMATOSTATIN ON RAT PANCREAS. J. Morisset, T.E. Solomon and A. Lord*. G.I. Res. Unit, Sherbrooke University, Sherbrooke, Quebec and V.A. Wadsworth Hospital, Los Angeles, Ca 90073

This study was undertaken to evaluate the effects of cyclic somatostatin (SS) on pancreatic size and composition in adult rats. Rats were injected S.C. every 8 h for 5 days with 11.33, or 100 µg/kg SS. Pancreatic weight, total pancreatic DNA content and concentrations of amylase, chymotrypsin and soluble protein were measured. The table shows ratio of treatment group values to control; * $P < 0.05$ vs control; $n = 12$ per group.

	Somatostatin µg/kg		
	11	33	100
WT	0.96	0.95	0.94
DNA	0.95	0.90*	0.90*
Amylase/DNA	0.94	0.72*	0.76*
ChTg/DNA	0.85	0.73*	0.68*
Prt/DNA	0.98	0.92	0.86*

SS suppressed pancreatic growth as evidenced by decreases in DNA contents and enzyme concentrations. This study suggests that SS may exercise a constant control on pancreatic growth and size of the acini.

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BILIARY, FECAL AND URINARY EXCRETION OF NATURAL AND SYNTHETIC PROSTAGLANDINS (PG) IN THE RAT. Hugo E. Gallo-Torres, T. Dashman*, C. Witt* and D. Kuhn*. Roche Research Center, Nutley, N.J. 07110

The profiles of excretion of PG of differing biological specificity was investigated in overnight fasted rats. The animals were surgically prepared with biliary fistulae or confined to metabolism cages. The PG (10 µg/kg; 2 to 50 µCi/rat; in 1 ml PEG-400) were administered intragastrically. Excretion data were expressed as a percent of the total administered radioactivity. Biliary excretion of $PGF_{2\alpha}$(I), PGE_2 (II) and PGA_2(III) in the 0-2 h bile was 15, 22 and 12 percent, respectively. Maximum biliary excretion of the antisecretory PG, 11-deoxy-trimethyl PGE_2(IV) (Ro 21-6937), occurred at 45 min. Of the administered dose, 75 and 90% was recovered in the 0-2 h and 0-6 h bile, respectively. Biliary excretion of the antihypertensive PG, 11-deoxy-11-CH_2, 16F-PGE_2(V) (Ro 21-9427), was 46 and 55% in the 0-2 and 0-6 h sample, respectively. Urinary excretion (0-24 h) for PGI_2, IV, II, PGE_1 and V was 10, 19, 23, 28 and 42%. The corresponding fecal excretion was 72, 76, 25, 21 and 43%. Lower total recovery of PGE_1 and PGE_2 (ca. 50%) compared to that of the other PG (85 to 95%) may reflect the loss of metabolizable tritium on carbon 5 and 15 of PGE_1 and PGE_2. These data suggest that the profile of excretion of IV is similar to that of PGI_2, but different from that of V. Biliary excretion is the main route of elimination of IV, thereby diminishing the potential for systemic liability.

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EVIDENCE THAT PICROTOXIN AND SUBSTANCE P INJECTED INTO THE MIDBRAIN INITIATE LOCOMOTOR ACTIVITY VIA MESOLIMBIC DOPAMINERGIC PROJECTIONS TO THE NUCLEUS ACCUMBENS. Douglas L. Jones and Gordon J. Mogenson, Dept. Physiol., Univ. of Western Ontario, London, Ontario, Canada N6A 5C1

The mesolimbic dopaminergic (DA) projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) have been implicated in the initiation of locomotor activity. Since there is evidence of GABAergic and substance P (SP) inputs to the VTA, the GABA antagonist picrotoxin (Ptx), and SP were injected into the VTA to investigate their effects on locomotor activity. Rats were prepared with guide cannulae implanted bilaterally to the VTA and the NAc. Locomotor activity was measured as light-beam interruptions in an activity box. Ptx injected unilaterally into the VTA elicited locomotor activity which was attenuated, but not abolished, by ipsilateral, but not contralateral injections of spiroperidol, the DA antagonist, into the NAc. Locomotor activity initiated by Ptx was not altered by injecting atropine ipsilaterally or contralaterally into the NAc, suggesting that locomotion initiated by Ptx is not mediated through a cholinergic synapse in the NAc. Pretreating the NAc with spiroperidol totally abolished locomotor activity initiated by injecting SP into the VTA, suggesting that SP-induced locomotor activity is mediated entirely by mesolimbic DA projections to the NAc. The results suggest that both GABAergic and SP synapses on mesolimbic DA neurons in the VTA have a functional role in the initiation of locomotor activity. (Supported by the Medical Research Council of Canada)

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HYPOXIA, HYPERCAPNIA AND CATECHOLAMINE RELEASE IN THE CAT CAROTID BODY. R. Fitzgerald, P. Garger*, L. Fechter*, H.Raff* C. Hauer*. The Johns Hopkins Med. Inst., Baltimore, Md. 21205.

Hypoxia (HX) depletes levels of catecholamines (CA) in the carotid body (cb) of several species. HX also stimulates cb neural activity, as does hypercapnia (HC). However, there have been few reports as to the effect of HC on cb CA levels. Cats were anesthetized and had both cbs prepared for excision. In order to attribute depletion to increased release, 150 mg/kg of αmethyl-p-tyrosine - a CA synthesis inhibitor - was given IP. After 1 hour of normoxic (N) conditions the first cb was removed. There followed 0.5 hour of N, HX, or HC; the second cb was then removed. Cb's were immediately stored at -80^0 F, and later analyzed for dopamine (DA), norepinephrine (NE) and epinephrine (E) with standard radioenzymatic methods. Mean values are presented in the table; *= value is significantly ($P < .05$) lower than corresponding control.

Cond.	n	PaO_2 mmHg	$PaCO_2$ mmHg	pHa	DA ng/cb	NE ng/cb	E ng/cb
N#1	3	112	35	7.39	276	1066	398
N#2	3	126	36	7.42	236	846*	379
N	5	107	34	7.34	480	622	212
HX	5	25	37	7.19	259*	434	143*
N	6	107	37	7.41	633	1205	438
HC	6	132	98	7.00	522	985*	358

If synthesis of CA's is equally inhibited, the data suggest differences in cb release of CA's during HX and HC, and the possibility of two receptor mechanisms. Supported by HL 10342.

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EVIDENCE FOR, AND POSSIBLE MECHANISM OF, TOLERANCE TO MORPHINE AT A CHOLINERGIC JUNCTION. Carl Pinsky*(SPON: E. Kroeger). Dept. of Pharmacology & Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E OW3.

Sprague-Dawley albino rats, ca. 120 g, were made dependent on morphine by twice-daily intraperitoneal injections of morphine sulphate in incrementing doses over 15 days; control rats received saline injections correspondingly. Behavioral tolerance developed over the morphine-habituating period. Phrenic nerve-hemidiaphragm preparations were removed from control and experimental animals for measurement of acetylcholine (ACh) release (Mercenaria mercenaria clam heart assay). Morphine sulphate, 10^{-4} to $2x10^{-4}$ g ml^{-1} significantly ($p < 0.01$) reduced stimulated release in control tissues, but did not affect release from nerve terminals in hemidiaphragms from morphine-tolerant rats. Hence, tolerance to the ACh release-impairing effect of morphine has been demonstrated at the singular cholinergic junction in these preparations. Stimulated release from morphine-tolerant tissues in presence of morphine, as distinct from control and drug-free tissues, deviates from a rectilinear relationship between mean release values and inverse square of the coefficient of variation for mean release. Latter result may be explained by an altered relationship between storage and readily-releasable pools of neurotransmitter, along with broad fluctuations in probability of quantal release per impulse (Supported in part by MRC and NMUD/Health and Welfare Canada).

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OPPOSING EFFECTS OF Mg^{2+} AND GTP ON AGONIST-ANTAGONIST INTERACTION WITH CARDIAC MUSCARINIC RECEPTOR: INVOLVEMENT OF -SH GROUPS. Jiann-Wu Wei* and Prakash V. Sulakhe. Physiol., Univ. of Sask., Col. of Med., Saskatoon, Canada, S7N OWO.

The binding of carbachol and atropine to the rat heart atrial muscarinic cholinergic receptor sites was investigated. Mg^{2+}, in low concentrations, increased (4-fold) carbachol binding affinity (K_d) of the receptor site without any effect on K_d for atropine. In contrast, GTP decreased (5-fold) K_d for carbachol, but not for atropine, and the extent of GTP effect depended on the absence or presence of Mg^{2+}. Pretreatment of atrial membranes with N-ethylmaleimide (NEM) altered the carbachol binding curve such that the Hill coefficient (n_H) became very close to 1.0, whereas the corresponding n_H values for control (untreated) or dithiothreitol (DTT)-treated membranes were less than 1.0; NEM or DTT treatments failed to show any effect on atropine binding curve. NEM treatment abolished both Mg^{2+}-induced and GTP-induced alteration in the K_d for carbachol of the receptor site. Monovalent cations in low concentrations did not mimic the effect of Mg^{2+}. Instead, concentration dependent decreases in K_ds for both agonist and antagonist were observed; NEM and DTT treatments failed to alter the monovalent cation effects on carbachol and atropine binding. These observations indicate a likely involvement of -SH groups in the opposing effects of Mg^{2+} and GTP on cardiac muscarinic receptor-agonist interaction. (Supported by Sask. Heart Foundation grant)

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CHOLINERGIC RECEPTORS ON CARP RETINAL GANGLION CELLS:NON-INTERACTION OF SUBSTANCE P AND ACETYLCHOLINE. Randolph D. Glickman* and Alan R. Adolph. Eye Research Institute of Retina Foundation, Boston, MA 02114

The neuropeptide, Substance P (SP) is found in the retinas of many vertebrates. We have reported that SP excites ganglion cells in the carp retina and that acetylcholine (ACh) can also excite many of the same neurons(ARVO, 1980). Mizobe et al. (Brain Res., 1979) and Stallcup and Patrick (PNAS, 1980) have suggested that SP inhibits or desensitizes cholinergic receptors.We have not found SP to have these actions on cholinergic receptors in isolated carp retina. Pulses of ACh were applied to sensitive ganglion cells by microiontophoresis; iontophoretic parameters were adjusted to elicit consistent cellular responses to the ACh. Application of SP to the retina through a nebulizing system (final estimated concentration 10^{-6}M) did not cause decrement in the response to ACh. The ACh effect persisted in the presence of Co^{++}, indicating that ACh directly affects the ganglion cells. Furthermore, SP was occasionally observed to excite cells after the retina was treated with gallamine triethiodide, a nicotinic cholinergic antagonist. SP may excite cholinergic-sensitive cells at a site other than the cholinergic receptor, or via a SP-sensitive interneuron. (Supported by NIH grants T32-EY 07028 and K07-EY00053.)

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DEPOLARIZING ACTION OF GABA ON PERIPHERAL NERVE. Mary E. Morris, Giovanni Di Costanzo*, and Robert Werman. Dept. of Pharmacology, University of Toronto, Toronto Ont., M4T 1E3.

Previous studies have reported that the inhibitory neurotransmitter GABA (γ-aminobutyric acid) increases the excitability of not only central terminals of primary afferent fibres but also peripheral axons (Neurosci. Abs. 4:582,1978; Proc. Can. Fed. Biol. Soc. 22:101,1979). This is consistent with other observations on dorsal root and autonomic ganglia as well as vagus and cervical sympathetic nerves, and suggests a widespread distribution of GABA receptors. In further experiments, using evoked compound action potentials of the isolated, desheathed frog sciatic nerve to assess changes in states of polarization of fibres, depolarizing actions of GABA show slow rates of onset and recovery, some desensitization at higher doses, an EC_{50} of 0.5 mM, greater sensitivity to picrotoxin than bicuculline, and affect both large and small diameter fibres. Relative EC_{50} doses for β-alanine, taurine, and glutamate are $\simeq$ 10 x > GABA; those for muscimol, 3-aminopropane-sulphonic acid, and δ-amino-n-valeric acid are respectively 100, 10, and 3 x < GABA. Low doses of pentobarbital (0.5-5.0 mM) prolong GABA depolarization, while there is depression with higher concentrations. It is concluded that peripheral nerve fibres appear to possess GABA receptors which show considerable resemblance to those located more centrally and will require more precise identification.

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AN INTERACTION OF D-GLUCOSE WITH GABA IN THE POSTERIOR HYPOPHYSIS Ron Mathison*& J.J. Dreifuss (Spon. S. Sheppard) Univ. of Geneva, Geneva, Switzerland.

The amplitude of an antidromically conducted action potential (CAP), measured from the isolated neurohypophysial-infundibular preparation of the rat, was reduced 17.9 ± 0.9% ($\bar{x}$ ± sem, n= 15) upon bath application of 1 mM gamma-amino-butyric acid (GABA). When the normal D-glucose concentration of 10 mM in the perfusing Locke solution was reduced to 1 mM, with concomittant iso-osmotic substitution with either 9 mM sorbitol, 3-methoxyglucose, glucoseamine, L-glucose, sucrose or NaCl, the GABA (1 mM) was potentiated for these respective substitutions by a factor of, 1.89, 1.74, 1.68, 1.60, 1.59 and 1.52. The increase of the GABA effect in 1 mM D-glucose was dependent upon the GABA concentration, was mimicked by muscimol, and was antagonized by bicuculline methylchloride. The D-glucose substitution exhibited a high degree of structural specificity since neither D-galactose, L-arabinose, D-mannose, D-fructose, L-fucose, nor inositol produced an increase in the GABA effect. The results are interpreted to suggest that D-glucose may interact with the neurohypophysial axons to modify the chloride conductance that is activated by GABA, since the rapid onset of the effect apparently excludes the possibility of a direct metabolic effect of the D-glucose substitutions. (Supported in part by Swiss National Science Foundation Grant No. 3.469.79 and Canadian Medical Research Council)

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HIPPOCAMPAL DISINHIBITION AND ENDOGENOUS OPIOIDS. K. Krnjević, R.J. Reiffenstein* and Nicole Ropert*. McGill University, Montreal, Quebec H3G 1Y6

In experiments on rats under urethane, we have confirmed that opioid peptides excite hippocampal pyramidal cells, probably by a process of disinhibition (Zieglgänsberger et al., 1979, Science 205, 415). Repetitive stimulation of various pathways, notably the fimbria, at frequencies of 4-10/s generate a powerful excitation of hippocampal pyramids; intracellular recording has revealed that such repetitive stimulation is accompanied by a sharp diminution in potency of recurrent inhibition (Ben-Ari et al., 1979, Can.J.Physiol. Pharmacol. 57, 1462). One possible explanation for the loss of recurrent inhibition could be inactivation of inhibitory interneurons by endogenous opioids released particularly effectively by stimulation at 4-10/s. This hypothesis was put to the test by making repeated intravenous injections of naloxone (1 mg/kg). As previously reported naloxone very effectively antagonized the excitatory (disinhibitory) action of an opioid (Met-enkephalinamide) applied microiontophoretically into the pyramidal layer of the CA3-CA1 areas; but it neither prevented nor diminished the comparable excitatory (disinhibitory) effect of fimbrial stimulation at 4-10/s. We conclude that endogenous opioids probably do not play a major role in the disinhibition caused by such repetitive stimulation; as suggested by Ben-Ari et al. (1979), this is more likely to be caused by a loss of sensitivity of GABA receptors. (Supported by the Medical Research Council of Canada)

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ALGESIA INDUCED BY INTRATHECAL ADMINISTRATION OF ACTH (4-10) IN THE RAT. K. Yashpal* and J.L. Henry, Dept. Research in Anaesthesia and Dept. Psychiatry, McGill Univ. Montreal H3G 1Y6

Fragments of ACTH bind to opiate receptors *in vitro* and, when given intracerebrally, provoke behavioural changes suggestive of an action on opiate receptors. The abundance of opiate receptors in the spinal cord, and their probable role in gating of transmission in nociceptive pathways prompted the present study to determine the effects on pain threshold of ACTH (4-10) when administered locally to the spinal cord. Male Sprague-Dawley rats were implanted chronically with intrathecal polyethylene catheters by a method similar to that of Yaksh (Physiol. Behav. *17*: 1031, 1976). Pain threshold was measured as the reaction time to tail flick from a noxious radiant heat stimulus. Trials consisted of a number of such stimuli delivered at 5 min intervals. ACTH (4-10) dissolved in artificial CSF was administered in doses of 10 µg in 10 µl, followed by a catheter flush of 10 µl of artificial CSF. This produced a decrease in reaction time to about 50% of the pre-administered value. Reaction time showed only partial recovery after 6 hours, and had returned to control levels after 24 hours. Similar administration of 20 µl of artificial CSF alone had no effect on reaction time. These results support evidence that ACTH fragments interact with opiate receptors and suggest they act as antagonists rather than as agonists.

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ELECTROACUPUNCTURE ANALGESIA IN MICE WITH 200 HZ IS SEROTONERGIC AND WITH 4 HZ IS ENDORPHINERGIC. Bruce Pomeranz* and Richard S.S. Cheng, University of Toronto, Toronto, Ontario M5S 1A1.

This study was designed to test the possible neurochemical mediators of electroacupuncture analgesia (EA). We compared EA produced by needle stimulation at 4 Hz and at 200 Hz. Pain threshold was measured in awake mice using the latency to evoke a squeak response after radiant thermal stimulation of the nose; threshold was compared before and after acupuncture of the first dorsal interosseous muscle of the forepaw using 0.2 Mamps pulses of 0.1 msec duration. The results showed that Naloxone (1 mg/Kg I.P.) completely blocked the 4 Hz EA but had no effect on the 200 Hz EA. This agrees with L. Terenius who showed that 4 Hz releases endorphins into CSF of humans, but 200 Hz does not. Next we showed that manipulations of serotonin affected 200 Hz EA, but had no effect on 4 Hz EA: PCPA or Cinanserin partially blocked EA while 5 HTP enhanced EA. Thus 4 Hz EA may be mediated by endorphins while 200 Hz EA may be partly mediated by serotonin in 2 parallel systems. Supported by NSERC and Ontario Ministry of Health.

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OCULAR EFFECTS OF SUBSTANCE P AND OTHER BIOLOGICALLY ACTIVE POLYPEPTIDES. L. Z. Bito, R. R. Nichols* and R. Siminoff, Dept. Ophthal., College of P & S, Columbia Univ., N.Y. 10032

Seven biologically active peptides and poly-DL-alanine (polyA) were studied in vivo following their intravitreal (i.vit.) injection into rabbit eyes and in vitro on isolated anterior segments of hooded rat and golden hamster eyes. A complete miosis (pupil diameter <2 mm) was observed within 3-4 hrs and was maintained for 6-8 hrs after i.vit. injection of 0.01 µg of physalamine (PA), 10 µg of Substance P (SP), SP-octapeptide (SP8), coherin-B (CB) or 100 µg eledosin (ED), but only a partial miosis after 100 µg α-melanocyte stimulating hormone (α-MSH) or somatostatin (SST). None of these peptides caused any other inflammatory signs (increased intraocular pressure, cells, flare or altered protein or ascorbate levels in aqueous), and retinal function, as determined by the ERG, also remained normal even at supramaximal doses which caused a more rapidly developing (0.5 to 2 hr) and longer lasting (up to 48 hr) maximal pupillary constriction. These peptides also constricted the isolated rat or golden hamster irides, even in the presence of atropine or indomethacin, the relative order of potency being SP8>SP>CB>PA>ED. ED50 for SP8 was =1 µg/ml; polyA or α-MSH (up to 0.2 mg/ml) were ineffective. Thus, the miotic property of SP is shared with many other biologically active peptides. Since none of these peptides produced any other signs of ocular inflammation, they cannot be regarded as the sole mediators of the neurogenic (axon reflex) component of the ocular irritative response. (Supported by EY 00402).

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SOMATOSTATIN (SS) HYPERPOLARIZES HIPPOCAMPAL (HPC) PYRAMIDAL NEURONS IN VITRO. Q.J. Pittman and G.R. Siggins*, Salk Institute, San Diego, CA 92138.

Rat hippocampal slices, 400 µm thick, were perfused over both surfaces with warmed (35°C), oxygenated artificial CSF. The inflow system permitted introduction of drug-containing solutions without interrupting the flow of the perfusate. KCl-filled electrodes were used to penetrate CA1 neurons (n=8) exhibiting stable resting potentials ($\bar{x}$=57 mV, range=44-68 mV) and overshooting action potentials for periods of 1-4h. SS (0.12-1.2 µM) or an active 9 amino acid SS analog (0.1-2 µM) caused reversible, dose-dependent hyperpolarizations in 7 cells. 1 cell did not respond to SS (0.12 µM). A decrease (4-34%) in input resistance (measured by current injection) and reduced numbers of spontaneous and evoked (intracellular injection of depolarizing current) spikes accompanied peptide-induced potential changes. During SS infusion, no consistent changes in the amplitude of EPSP's or of recurrent IPSP's evoked by stratum radiatum stimulation were noted. SS hyperpolarizations persisted in high Mg^{+2} solutions (n=2) in which all synaptic activity was abolished and in cells loaded with Cl^- by passage of negative current (n=3). Our results indicate that SS hyperpolarizes HPC pyramidal cells by a direct, post-synaptic action. The disparity between these findings and the previously reported excitatory effects of SS on HPC could be attributed to the method of drug application or to possible indirect SS effects in vivo. (Supported by MRC Canada and AM 26741.)

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THE EFFECTS OF ALLOMETRY ON OXYGEN CONSUMPTION IN ANOSTRACAN CRUSTACEANS. Richard F. Modlin & P. Samuel Campbell. Univ. of Alabama in Huntsville, AL. 35899.

Metabolism in crustaceans is presumed to follow the "Surface Rule," where it is dependent on surface area and proportional to the 0.67 power of the mass. However, the oxygen consumption in some species of anostracan crustaceans indicates that the "Surface Rule" does not always apply. Gases are exchanged across the epipodites which are located on the limbs of the anostracans. If respiration is to follow the "Surface Rule" as the animal grows, then the total surface area of the epipodites must remain greater than the animal's volume. Heterogonic growth must exist between the epipodites and the rest of the body. Oxygen consumption of Eubranchipus holmani and E. vernalis was measured on a Gilson Differential Respirometer at 8°C. Respiration of all life stages of E. holmani closely approximated the "Surface Rule" (mass to the 0.62 power), while only those individuals of E. vernalis <10 mm followed the "Surface Rule." Oxygen uptake in those >10 mm was proportional to the 0.77 power of the mass. This indicated that metabolism in E. vernalis was dependent primarily on mass or volume rather than surface area. Ratios of the total epipodial surface area to the animals' volume substantiated the above contentions. The results also correlated well with the ecology and the modes of existence of these species. Eubranchipus holmani is planktonic, while E. vernalis tends toward a benthic existence.

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HEMOLYMPH ACID-BASE STATUS OF THE INTERTIDAL CRAB, *CANCER PRODUCTUS* DURING AND FOLLOWING EMERSION. Peter L. deFur and Brian R. McMahon. Univ. of Calgary, Calgary, Alta. T2N 1N4.

Only small (<100 gm) individual *C. productus* occur regularly in the intertidal zone. Acid-base changes resulting from 4 h emersion were investigated in the laboratory and the results compared with those previously reported for large crabs which are essentially subtidal in distribution. Hemolymph pH, carbon dioxide tension (PCO_2) and content ($C{\cdot}CO_2$) plus hemolymph ammonia (NH_4^+) and lactate (L-) were measured prior to, during and following emersion. Both PCO_2 and L- increased progressively during emersion, potentially resulting in a mixed respiratory and metabolic acidosis. The acidosis, however, developed slowly and was significant only at 4 h, suggesting that considerable compensation was occurring. NH_4^+ rose slightly, contributing little to alleviation of the acidosis, but hemolymph $C{\cdot}CO_2$ doubled in 4 h, resulting in a net base excess. The data indicate that this base excess was due to metabolic input of carbonate or bicarbonate to the hemolymph. Upon reimmersion, hemolymph pH, PCO_2, $C{\cdot}CO_2$ and NH_4^+ were rapidly restored to pre-emersion levels, but L- remained elevated for at least 4 h. By 24 h after reimmersion, hemolymph acid-base status had completely returned to levels measured prior to emersion. The more effective acid-base compensation during emersion and the more rapid recovery of initial acid-base status upon reimmersion observed in these small crabs differ markedly from the responses of large (≥ 200 gm) *C. productus*. NSERC Grant #A5762.

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RESPONSES TO ACUTE, SEVERE HYPOXIA IN THE CRAYFISH *ORCONECTES RUSTICUS*. Peter Wilkes, Brian McMahon. Univ. of Calgary, Calgary, Alta, Canada, T2N 1N4

Heart and scaphognathite rates (f_H, $\overline{fsc}$) were measured in *O. rusticus* as ambient PO_2 was lowered from 140 torr to ca. 30 torr over a 20-30 min period, $\overline{fsc}$ began to increase at an ambient PO_2 of 55-80 torr while a significant bradycardia was noted when ambient PO_2 fell below 30 torr. Behavioural responses and associated changes in $\overline{fsc}$ and f_H, hemolymph acid-base and oxygen status were measured during subsequent exposure of 1/2 to 2 h duration to this severe level of hypoxia ($P_{I}O_2$< 30 torr). Behavioural responses involved surfacing and ventilating one or both branchial chambers with air. Aerial ventilation effected a complete release of bradycardia and an immediate and significant decline in $\overline{fsc}$ (53%). Upon termination of aerial ventilation bradycardia was again rapidly reestablished (within 1-2 bts); in contrast however, $\overline{fsc}$ increased more slowly over the subsequent 60 sec. There was no significant change in the hemolymph acid-base status during aerial ventilation. Aerial ventilation had no significant effect on post-branchial PO_2, nevertheless oxygen content doubled indicating increased hemocyanin oxygen binding. It is concluded that aerial ventilation represents a physiologically important method of obtaining extra oxygen during severe hypoxic exposure.

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TOADFISH (OPSANUS TAU) ARE METABOLIC O_2 REGULATORS, NOT CONFORMERS. Gordon R. Ultsch[1], Donald C. Jackson, and Richard Moalli, Div. Biol. & Med., Brown Univ., Providence RI 02912.

Animals are traditionally grouped according to patterns of O_2 uptake ($\dot{V}O_2$) into 2 groups: O_2 conformers, in which $\dot{V}O_2$ is dependent on ambient P_{O_2} (Pm_{O_2}) up to atmospheric P_{O_2}, and O_2 regulators, in which $\dot{V}O_2$ is independent of Pm_{O_2} down to a relatively low Pm_{O_2} designated as the critical P_{O_2} (P_c). The "classic" vertebrate O_2 conformer is the toadfish (Hall, 1929, Am. J. Physiol. 88:212). We have redone his work using a constant-recording flow-through respirometer where Pm_{O_2} was precisely regulated by a gas-water equilibration system with gases supplied from Wosthoff gas-mixing pumps. The results clearly show that Opsanus tau is an O_2 regulator ($\dot{V}O_2$ = 26.0 ± 2.3 µl O_2/g·h, P_c = 29.3 ± 3.5 mmHg, n = 6, data are $\bar{x}$ ± SE) in a manner similar to other fishes. Regulation of $\dot{V}O_2$ is accomplished at least partially by increasing the opercular pumping rate from a variable resting value (12-25 beats/min) to a less variable maximal rate (33-35 beats/min) attained near the P_c and maintained as Pm_{O_2} is lowered. In retrospect, it appears unlikely that any vertebrate possessing both a functional hemoglobin, and an efficient ventilatory mechanism which it can utilize in a normal manner, cannot regulate its $\dot{V}O_2$ over a substantial portion of the normal range of Pm_{O_2}.

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EFFECT OF EFFERENT BRANCHIAL ARTERY PRESSURE, EPINEPHRINE AND ACETYLCHOLINE ON FLOW, RESISTANCE, AND METHYL METHACRYLATE DISTRIBUTION IN THE ISOLATED PERFUSED GILL ARCH OF THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. E.J. Boland* and K.R. Olson. Univ. of Notre Dame and Indiana Univ., School of Medicine - South Bend Center, Notre Dame, IN. 46556.

Catfish gill filaments contain a central filamental vasculature (CFV) that is supplied by prelamellar arteriovenous anastomoses (PAVA) and drained independently of the efferent branchal system (EB). The function and control of blood flow to this network is unknown. Increasing EB pressure by raising EB cannula causes a linear decrease in EB flow with cessation of flow occuring near 40 mm Hg. Increased efferent pressure from 10 to 20 mm Hg, decreased EB flow by 50% and increased CFV flow by 100%. Methacrylate deposition in the CFV was increased at elevated EB pressures (20 vs 0 mm Hg). Epinephrine (10^{-5}M) increased flow in the EB by 142% at 20 mm Hg efferent pressure and decreased flow in the CFV by 82%. Epinephrine produced a transitory increase in whole gill resistance followed by a prolonged decrease. Epinephrine favors methyl methacrylate distribution in the lamellar - EB system and reduces CFV filling. Acetylcholine (10^{-6}M) increased whole arch resistance, decreased EB flow by 74% and increased CFV flow by 200% at 20 mm Hg. Acetylcholine increased methyl methacrylate filling of the CFV system. This data suggests that flow through the CFV can be neural-hormonally regulated to subserve the as yet unknown function(s) of this vasculature. (Supported in part by NSF Grant # 70-23073.

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THE OCULAR OXYGEN CONCENTRATING MECHANISM AND APPEARANCE OF THE RETINAL-SPECIFIC LACTATE DEHYDROGENASE ISOZYME OF THE DEVELOPING FISH. M. B. Fairbanks* (Spon:R. F. Burlington) Central Michigan University, Mt. Pleasant, MI 48859

Among teleosts, appearance of the retinal-specific lactate dehydrogenase ($LDH\text{-}C_4$) isozyme has been tightly coupled to the time of neural retinal differentiation. The significance of this isozyme in fish is unknown. Both physical and chemical characteristics suggest that it supports aerobic metabolism. A summary of the distribution of this enzyme among fish shows that it is highly correlated with the presence of an ocular oxygen concentrating ability. Is appearance of the $LDH\text{-}C_4$ isozyme in the developing fish dependent upon an active ocular oxygen concentrating mechanism? To test this hypothesis a Transidyne oxygen microelectrode was used to measure ocular oxygen tension (Po_2) in medaka (Oryzias latipes) embryos from stage 30 to stage 34 of embryogenesis. Po_2 values were correlated with data on activity of the $LDH\text{-}C_4$ isozyme during the same developmental stages. Comparing ocular Po_2 measurements from embryos in the presence of Diamox, a known inhibitor of the oxygen concentrating mechanism, with control embryos indicates the presence of a functional ocular oxygen concentrating mechanism prior to hatching. This mechanism appears coincident with expression of the $LDH\text{-}C_4$ gene and suggests that presence of the $LDH\text{-}C_4$ isozyme is dependent on and supportive of an aerobic activity. (Supported by Central Mich. University F.R.C.E. Grant #4-22489)

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EFFECTS OF SMALL, RAPID TEMPERATURE CHANGES ON METABOLISM AND SODIUM FLUX IN THE CARP, CYPRINUS CARPIO L. Brenda P. Moffitt, Larry I. Crawshaw and Wilbur H. Sawyer, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Carp, acclimated at 19°C for at least two weeks, were placed in a chamber in a closed circuit system. Metabolic rate (MR), ventilation frequency (f_V) and heart rate (f_H) of the animal and the total Na^+ content of the recirculating water were measured. After stabilization, the fish was subjected to rapid (complete within 5 mins) changes in ambient temperature (T_a) of +2, -2, +4 or -4°C, each lasting about one hour. The T_a was returned to 19°C for an hour between changes. The two degree perturbation was found to alter MR by ±35%, whereas f_V showed only a transitory response, and net sodium flux was unaffected. The ±4° change had a ±60% effect on MR and produced a more lasting change in f_V. No consistent effect on net Na flux was detected. The results suggest that a ±2°C change in T_a presents little physiological challenge to the carp. A 4° change in T_a produces more major physiological effects which continue throughout the hour exposure, but it does not elicit observable stress or agitation, as were seen at higher temperatures. (Supported in part by UPHS Grant 1 RO1 NS15318).

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IMPLICATIONS OF BLOOD VISCOSITY CHANGES WITH TEMPERATURE FOR HEAT EXCHANGE IN TURTLES AND FROGS. B. Lowell Langille. University of Western Ontario, London, Ont. Canada N6A 5C1.

The importance of cutaneous blood flow on thermoregulation in poikilotherms has often been stressed, but rarely are the effects of temperature dependence of blood viscosity considered. We have measured viscosity of blood from turtles and frogs for temperatures from 10°C to 40°C. Turtle blood viscosity fell 39% (3.50 ± 0.16 cP to 2.13 ± 0.10 cP) and frog blood viscosity fell 44% (4.55 ± 0.32 cP to 2.55 ± 0.25 cP) over this temperature range (variations in viscosity at fixed temperature were largely attributable to variation in hematocrit). As a result, rapid decreases in viscosity of blood flowing through the skin (and increases in flow) will occur when turtles and frogs experience a sudden rise in ambient temperature. Reverse changes in skin blood flow occur with cooling. These changes are sufficient to explain different heating and cooling rates. They also explain why, in subsequent experiments, turtles heated faster than they cool regardless of whether ambient temperature was above or below the animals eccritic temperature. It is concluded: (1) that blood viscosity changes can have a large effect on the rate of heat transfer with the environment and (2) heating rates exceeding cooling rates do not prove that thermoregulatory adjustments (e.g., skin vasomotion) are coming into play to keep body temperature close to eccritic. (Supported by the Ontario Heart Foundation)

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Control of Ventilation in an Unrestrained Amphibian, Bufo marinus: CO_2 sensitivity. R.M. Jones and G.R. Stibitz,* Dept. of Physiology, Dartmouth Medical School, Hanover, N.H. 03755.

An "Acoustic Impedance Ventilometer" (AIV) has been developed and used to measure ventilatory parameters in unrestrained and non-instrumented Bufo marinus, and to study aspects of the control of ventilation. The AIV is a Helmholtz resonator (a box with a hole) whose fundamental frequency of resonance is determined by the volume of air enclosed (but not the shape) and the hole characteristics. When the system is driven by a loudspeaker at or near resonance, with an animal inside, the air volume change due to breathing can be measured via a microphone and standard detection circuits as the change in amplitude of the resonating system, or as the phase shift relative to a reference signal. During normoxia steady state ventilatory parameters were: frequency 5.8 ±0.70 lung ventilations/min, tidal volume 5.3 ±0.42 %BW, $\dot{V}_E$ 327 ±53.9 ml BTPS/kg-min (12 trials, 6 animals, 25°C). The steady state ventilatory response to inspired CO_2 was achieved primarily by increased frequency of lung ventilation (8X increase at F_ICO_2 = 0.02). The threshold for response was between F_ICO_2 = 0.01-0.015. Supported by NIH grant HL02888-23.

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ACID-BASE CONTROL IN THE HELLBENDER, CRYPTOBRANCHUS ALLEGANIENSIS. R. Moalli, R.S. Meyers, G. Ultsch and D.C. Jackson. Div. of Biol. and Med., Brown Univ., Providence, RI 02912

The hellbender, a large aquatic salamander, relies on its skin for nearly all of its respiratory gas exchange. In light of recent studies indicating poor control of skin gas exchange in amphibians, we decided to study acid-base changes in this amphibian as a function of temperature. Animals were studied at 5, 15 and 25°C and either with or without access to air at each temperature. Arterial blood gases, ions and lactate concentrations were measured. The relationship between blood H^+ and temperature was also determined in vitro. Access to air had no significant effect on any measured variable. Both H^+ and P_{CO_2} increased with temperature and were the only significant temperature effects. The slope of the in vitro pH/temperature line closely matched the in vivo line ($\Delta pH/\Delta T$ = -0.016). This value is similar to that for other ectotherms. Hellbenders, however, do not alter plasma ionic composition as do gill breathers nor do they actively control arterial P_{CO_2} as do lung breathers; thus thermal adjustments of acid-base status must occur by other means. It is possible to describe the situation in the hellbender by evoking two physical effects of temperature: the Rosenthal effect and the metabolic (Van't Hoff) effect. When temperature increases, pH drops and P_{CO_2} rises (Rosenthal effect) and the production of CO_2 increases (metabolic effect). Increased arterial P_{CO_2} provides a larger gradient for the loss of CO_2 through the skin. Thus, acid-base status can be maintained without active physiological control.

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ACID-BASE CONSEQUENCES OF LONG-TERM APNEIC COLD TORPOR IN TURTLES, CHRYSEMYS PICTA BELLI. D.C. Jackson and G.R. Ultsch. Div. Biol. & Med., Brown Univ., Providence, RI 02912 and Dept. Biology, Univ. of Alabama, University, AL 35486

Turtles were submerged at 3°C without access to air in either N_2-equilibrated water (LO-O_2,10 animals) or air-equilibrated water (HI-O_2,11 animals). Blood samples were taken from indwelling arterial catheters prior to submergence and at weekly or bi-weekly intervals thereafter, and analyzed for pH P_{CO_2}, P_{O_2} and plasma electrolytes (Na^+, K^+, Cl^- and lactate). Control values (mean±S.E.) for both groups: pH-7.97±0.02, P_{CO_2}-12.0±0.44 torr, P_{O_2}-21.0±4.2 torr, lactate-1.7±0.2 mEq/L. Blood pH fell more slowly in the HI-O_2 turtles but survival in this group (41-149 days, mean = 92) tended to be less than in the LO-O_2 group (48-182 days, mean = 120) because of a skin fungus infection. In 2 unaffected HI-O_2 animals, however, control acid-base status continued for 77 days of apnea. Long term survivors (>100 days, n=5 in each group) had the following mean values: LO-O_2 after 84 days; pH-7.37, $PaCO_2$-10.2, PaO_2-0.0, lactate-111. HI-O_2 after 99 days; pH-7.68, $PaCO_2$-10.6, PaO_2-0.5, lactate-53.5. Plasma Na^+ was unchanged at this time in both groups, but Cl^- was significantly lower in both, and K^+ was higher in the LO-O_2 animals. We conclude that turtles can survive for more than 3 months in the absence of O_2 at 3°C and that cutaneous O_2 uptake from air-saturated water can significantly improve the acid-base state of these animals.

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RESPONSES OF SEA TURTLES TO GRADED HYPOXIA. Henry D. Prange and Peter L. Lutz. Indiana Univ. Bloomington, IN 47405 and Univ. of Miami Marine School, Miami, FL 33149.

Immature green (Chelonia mydas) and loggerhead (Caretta caretta sea turtles were subjected to periods of decreased PO_2 in air. They responded with a sharp reduction of oxygen consumption at ambient PO_2's of 2-4 kPa. In most cases, oxygen consumption was maintained at or near control levels until the critical PO_2 was reached. Mean control heart rate for the animals studied was 15 min^{-1}. This rate was maintained down to an ambient PO_2 of 8 kPa below which there was a sharp increase to three times the control rate at P_{crit}. Below P_{crit} there was a precipitous decrease to levels of less than 1 min^{-1}. Respiratory rate (measured in C. mydas only) showed a nearly identical pattern, changing P_{crit} and ceasing at lower PO_2's. Mean venous PO_2 was 1.5 kPa when ambient PO_2 had reached near zero and oxygen consumption had ceased. These data suggest that anaerobic metabolism occurs while there is still oxygen in the blood and that, for the animals in this study, the physiological threshold for the changeover from aerobic to anaerobic metabolism is a PO_2 of about 1.5 kPa. (Supported in part by NSF grants PCM 77-24919 to HDP and PCM 78-09281 to PLL).

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SOMATOSENSORY EVOKED POTENTIALS IN PATIENTS WITH SPINAL CORD INJURY. Donald H. York, Clark Watts* and Casey Lenox*. Dept. of Physiology & Division of Neurosurgery, School of Medicine, Univ. of Missouri, Columbia, Missouri 65212

Previous reports have suggested that the presence of a peroneal evoked response in the early stages following spinal cord trauma may be a useful prognostic sign, indicating recovery of both sensory and motor function in the lower limbs at a later stage. This study involves 48 patients with acute spinal cord trauma who when examined upon admission to the hospital had no reflexes or motor function below the level of lesion. Peroneal evoked responses were undertaken and repeated at intervals during the patients convalescence up to 2 years following the injury. Scalp electrodes placed at the vertex (Cz, 10-20 International System) referenced to link ear lobes were used to record the response to stimulation of the deep peroneal or tibial nerve (6-15mA, 0.1 msec, 1/2 sec). Each individual response was averaged for 100 stimulus repetitions. A minimum of two trials were undertaken on each leg. The results have identified 43 patients, who had normal evoked responses during their hospital course and who were ambulatory at 9 months post injury. However, five additional patients were also identified who also had normal evoked responses, but remained paraplegic. Thus the value of this test as a prognostic indicator for long term recovery of motor function remains questionable. (supported by NIH RR07053)

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EVIDENCE FOR POST-SYNAPTIC INHIBITION OF SENSORY RESPONSES FROM THE NUCLEUS RAPHE MAGNUS. Y. Shah* and J.O. Dostrovsky, Dept. of Physiology, University of Toronto, Toronto, Ontario.

Stimulation of the nucleus raphe magnus (NRM) inhibits sensory responses of neurons in the spinal cord and in the trigeminal subnucleus caudalis. It has been suggested that presynaptic mechanisms may be involved in this inhibition. Here we present evidence that post-synaptic mechanisms are probably also involved. In chloralose anesthetized cats concentric bipolar stimulating electrodes were stereotaxically lowered into the NRM and the nucleus ventralis pars posteromedialis (VPM) of the thalamus. Cells in the subnucleus caudalis responding to either noxious or non-noxious stimulation of the skin were studied using carbon fibre recording electrodes. The cells were either antidromically activated from VPM or were excited by iontophoretically applied glutamate. The effects of NRM stimulation (100 ms train, 500 Hz, 0.1 ms pulses, 130 ms prior to the test stimulus) on such cells were studied. In 5/9 cells, conditioning stimuli resulted in an increased latency of antidromic activation and sometimes a complete block. Conditioning stimuli also blocked the glutamate evoked excitation in 3/3 cells tested. These effects were seen on both nociceptive and non-nociceptive neurons. The observed increases in antidromic latency and the block of glutamate evoked excitation following NRM stimulation suggests a decreased excitability of the cell membrane implying a post-synaptic inhibitory mechanism.

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A COMPUTERIZED STATISTICAL SIMULATION OF THE NORMATIVE SHORT LATENCY AVERAGED AUDITORY EVOKED RESPONSES IN ANAESTHETIZED DOGS. S.J. Whidden, J. Graves*, and R. W. Redding * . Dept. Physiol.,Auburn Univ. , Sch. of Vet. Med.,Auburn,Al.36830,U.S.A

The normative monaural short latency averaged auditory evoked early responses were recorded in thirty mixbreed adult anaesthetized dogs. Ten clicks per second were presented monaurally to right or the left ear,the recordings were amplified, filtered (.32 Hz- 3.2 kHz), and averaged (512). These auditory evoked responses were analyzed statistically and assimilated into different simulations for each population through two computational programs,Continuous Systems Modeling Program (C.S.M.P.) by International Business Machines (I.B.M.) and Statistical Analysis System Program (S.A.S.80) by Statisical Analysis System,Inc.. It has been previously reported from this laboratory that the general population of normative monaural short latency averaged auditory evoked responses were six in number. They occured at a mean latency of 1+.28 milliseconds apart in the canine. This present study was designed to compare the two computational programs C.S.M.P. and S.A.S. 80 for their use in statistical simulation of auditory evoked responses . The results indicate that C.S.M.P. did not produce a true statistical model of the raw wave form because of both latency and amplitude are only mean values and do not represent a "normal response." It was found that the S.A.S.80 program did produce a more realistic picture of the ideal waveforms.

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EMG STUDIES OF ANAL SPHINCTER MUSCLE IN SPINAL CATS. UNMASK OF ACTIVATING INPUT AFTER S2 and S3 DORSAL ROOTS SECTION. Pablo Pacheco* and Bernardo Dubrovsky. U.N.A.M., Mexico and Allan Memorial Institute, McGill University, Montréal, P.Q.

Neurons of deafferented nuclei respond to stimuli which cannot drive them when normally effective stimuli are present. We therefore thought it appropriate to investigate the responsiveness of motoneuron pools in S2-S3 of the spinal cord before and after section of their segmental dorsal roots. In female spinal cats, the EMG was recorded from the left and right halves of the external anal sphincter muscle. With dorsal roots intact, cutaneous and/or proprioceptive stimulation below the region of the thigh did not elicit any detectable EMG response. Stimulation of the upper dorsal skin regions of the hindlimbs elicited ipsilateral responses from the muscle. Perianal, vaginal and rectal distention elicited vigorous bilateral responses. After unilateral section of the S3 dorsal root, skin responses extended to the external surface of the ipsilateral hindlimb, and to their plantar surface. After bilateral S2-S3 dorsal root section, stimulation of perianal and upper dorsal hindlimb regions had no influence on the tonically active EMG. In contrast, large joint movements of the hindlimbs, as well as pressure to the thigh and lower limb musculature became effective stimuli for EMG response. These results suggest that when deafferented of its normal input, CNS neurons become responsive to distantly located sources, and can shift to different sensory modalities (eg. proprioceptive rather than cutaneous).

* P.P. is an MRC Fellow

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INHIBITION OF SPINAL DORSAL HORN UNITS BY PROBING THE VAGINA AND VULVA IN THE SPINAL CAT. James L. Henry, Dept. Research in Anaesthesia, McGill University, Montreal, PQ, H3G 1Y6.

It has been proposed that stimulation of the vagina in the rat induces analgesia probably by an excitation of brainstem noradrenergic neurons which in turn inhibit transmission in nociceptive pathways at the spinal level (Crowly et al. Physiol. Behav. *16*: 483, 1976). The present study used unanaesthetized female cats decerebrated at the intercollicular level under halothane. They were paralysed and ventilated mechanically. Spinal cords were cut at L_1 and segments L_5-L_7 were exposed for recording. Single unit extracellular spikes were recorded at histologically identified sites in laminae I, III, IV and V. Probing the vulva and vagina with a blunt glass probe depressed on-going activity of nociceptive units but failed to affect non-nociceptive units. Responses of wide dynamic range units to noxious and innocuous cutaneous stimuli were both depressed. Mechanical thresholds using von Frey hairs were about 3.6 g/mm^2. Even high intensity stimulation of perivulvar skin failed to alter the activity of the cells studied. Inhibition began within 2 s of stimulation and was fully reversed within 5 s of its termination. Depression could not be blocked by i.v. naloxone or bicuculline. These results demonstrate that probing the vagina and vulva inhibits sensory transmission by a spinal mechanism.

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AMINO ACID EFFECTS ON SINGLE UNITS IN THE LATERAL HORN REGION IN CAT THORACIC CORD. S.B.Backman* and J.L. Henry, Depts. Physiol. and Anaesthesia Research, McGill Univ., Montreal, P.Q.

Little is known about the chemistry of synaptic transmission in sympathetic pathways in the spinal cord. In the present project, amino acid effects were studied on single thoracic units implicated in central sympathetic pathways on the basis of their responses to electrical stimulation of the sympathetic chain and the inferior cardiac nerve. Cats were anaesthetised with chloralose, paralysed and ventilated artificially. Spinal segments T1-T4 were exposed. Single unit extracellular spikes were recorded using the central barrel of multibarrelled micropipettes. Other barrels contained glutamate (Glu, 1 M, pH 7.4), gamma-aminobutyric acid (GABA, 1 M, pH 4.3), glycine (Gly, 1 M, pH 3.5), control (165 mM NaCl) and Pontamine Sky Blue for identification of recording sites. Units included in the results were considered sympathetic on the basis of their location, and all responded antidromically &/or orthodromically to electrical stimulation of preganglionic or primary afferent fibres, respectively. Orthodromic responses were 1-5 spike bursts at 11-23 ms; antidromic responses were usually one spike at 5-31 ms. All units were excited by Glu (30-90nA), the response in some cases being slower than that seen in other parts of the CNS. GABA (20-60nA) and Gly (20-60nA) inhibited both ortho- and antidromic responses. These results demonstrate that spinal units related to autonomic control are excited by glutamate and inhibited by GABA and Gly. (Supported by Canadian MRC, Canadian Heart Foundation and Québec MRC)

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NEUROANATOMICAL METABOLIC MAPPING OF THE AUDITORY PATHWAY USING [^{14}C] DEOXYGLUCOSE IN ANESTHETIZED DOGS. D.R. Kostreva and J.P. Kampine. Dept. of Anesthesiology and Physiology, Med. Col. of Wisconsin and VA Medical Ctr., Wood, WI 53193.

The medullary, and supramedullary auditory pathways were studied in pentobarbital anesthetized mongrel dogs (6-9 kg) using the [^{14}C] Deoxyglucose technique of Sokoloff, et al. (J. Neurochemistry 28:897-916, 1977). The animals were fasted for 2 days and then anesthetized with sodium pentobarbital (35 mg/kg), intubated and placed on positive pressure ventilation. Systemic blood pressure and electrocardiogram were recorded using a Grass polygraph. Auditory stimulation consisted only of ambient room noise, i.e. air conditioner, polygraph, and voice, etc. One mCi of [^{14}C] Deoxyglucose was injected as a single bolus into a femoral vein. After 45 minutes the entire brain was removed, frozen at -40°C and sectioned for autoradiography. Autoradiographs revealed that the auditory pathways had the greatest metabolic activity as indicated by emulsion density. The ventral and dorsal cochlear nuclei, superior olives, trapezoid bodies, lateral lemnisci, inferior colliculi and medial geniculate bodies were all distinctly visible in the autoradiographs as indicated by the marked density of emulsion compared to adjacent structures. This study demonstrates that the auditory pathways remain sensitive to low levels of physiological auditory stimulation during pentobarbital anesthesia. (This study was supported by NIH Young Cardiovascular Investigator Grant HL 21042 and VA Medical Research Service).

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POSITIONAL NYSTAGMUS FROM INGESTION OF ALCOHOL, HEAVY WATER AND GLYCEROL. K.E. Money, J.P. Landolt, A.D. Nicholas and R.P. Cardin. D.C.I.E.M., Downsview, Ontario, Canada M3M 3B9.

In order to elucidate the mechanism of action of alcohol in the inner ear, in initiating positional alcohol nystagmus, fourteen human subjects were tested periodically for positional nystagmus, using electronystagmography, for ten hrs after drinking: i. alcohol (1 ml per kg of body weight), or ii. deuterium oxide (2 ml per kg), or iii. glycerol (1 ml per kg), or iv. alcohol plus deuterium oxide, or v. alcohol plus glycerol. With intervals of at least one week between each, all the five treatments were administered to all the subjects.

Typically the subjects showed vigorous first phase positional nystagmus (fast beats to the lower ear) in the head - lateral positions between ½ hr and 3 hrs after drinking alcohol. Between 3 hrs and 6 hrs after alcohol almost no positional nystagmus was evident, but from 6 hrs to 9 hrs the second phase of alcohol nystagmus was observed in most subjects. An even more vigorous positional nystagmus (fast beats to the upper ear) was observed in the subjects after drinking heavy water, between ½ hr and 5 hrs after ingestion. No reversal or second phase of heavy water nystagmus was observed.

When alcohol and heavy water were ingested together, they cancelled each other's effects, and almost no first phase positional nystagmus resulted. However, the second phase of alcohol nystagmus did appear at the usual time, in spite of the non-occurrence of the first phase.

Glycerol, when given with alcohol, drastically reduced (to zero in half the tests) both the first and second phases of the alcohol nystagmus.

The results are consistent with the view that alcohol nystagmus is caused by a physical effect of alcohol: the creation of a low density area in the endolymph ring of the semicircular canal.

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THALAMOCORTICAL PROJECTION NUCLEI FOR MODALITY-SPECIFIC REGIONS OF SOMATOSENSORY CORTEX - DEMONSTRATION OF DIFFERENT PROJECTIONS FOR AREAS 3a AND 3b OF THE CAT. R.W. Dykes, C.S. Lin* and N. Rehman*. Lab. Microsurgery, Dept. Surgery, McGill, Montreal, Quebec, and Dept. Cell Biology, U. of Texas, Dallas.

Modality-specific regions of somatosensory cortex are correlated with identifiable cytoarchitecture. Area 3a is activated predominantly by afferents from deep tissues while the adjacent area, 3b, receives input from cutaneous receptors. HRP injections limited to these functionally-defined regions were used to test the hypothesis that in cats these cortical areas receive projections from different portions of the thalamus. Craniotomies were performed in mongrel cats anesthetized with sodium pentobarbital. Following electrophysiological mapping of the boundaries, several sites in one of these modality-specific regions were selected for injection of 0.1 µl of 10% HRP (Sigma Grade VI). DAB was used as the chromagen to demonstrate HRP. VPL pars oralis (VPLo) contained HRP-filled cells when the injection was limited to area 3a, while VPL pars caudalis (VPLc) contained HRP-filled cells with injections of area 3b. This separation of HRP-filled cells suggests that VPLo projects to 3a and that it is a separate projection region for deep inputs to cortex. Similarly VPLc projects to 3b and is a separate projection nucleus for cutaneous inputs. Thus, specific somatosensory modalities are served by spatially segregated cell populations in both the cortex and thalamus. (Supported by the Medical Research Council of Canada.)

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DISCRIMINATORY POWER OF PULMONARY FUNCTION TESTS IN CHILDREN

L.David Pengelly, A.T.Kerigan*, and S.R.Leeder*. McMaster Univ. Canada, and University of Newcastle, Australia.

We carried out a survey of respiratory morbidity (RM) and pulmonary function (PF) in 3075 schoolchildren aged 7 to 10 in Hamilton, Canada. RM was assessed by a questionnaire administered to the parents, and PF was measured by the HP 47804A Pulmonary Calculator system. The PF manoeuvres were: FVC, Nitrogen Washout (Multiple breath), and Nitrogen Washout (Single breath). Fourteen measured and 7 indirect indices of PF were obtained from each child. Assuming that a previous history of respiratory disease may reflect as a deficit in pulmonary function, we separated the children into two groups on the basis of a positive or negative response to 6 RM questions. The following 11 indices could discriminate between the groups ($p<0.05$), and are ranked in order of decreasing significance (increasing 'p'): MET(Mid Expiratory Time), FEV_1/FVC, V50/FVC, V50, FEF25-75(MMFR), LSQT, V75, FEF75-85, V75/FVC, PF, IDI(Inspiratory gas Distribution Index). Tests unable to discriminate ($p>0.05$) were: FVC, FEV_1, VC(slow Vital Capacity), RV, FRC, TLC, ADS(Anatomical Dead Space), CV, CV/VC, CC/TLC. Measurements of closing volume and FRC showed considerable variability in this group of children.

We conclude that in the context of a survey sample of this size, if the objective is to find PF deficits associated with a history of RM, tests of the FVC are most discriminatory. (Supported by Environment Ontario and Health&Welfare Canada.)

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THE ROLE OF CENTRAL RESPIRATORY CHEMOSENSORS IN THE CONTROL OF VENTILATION IN THE TURTLE. B.M. Hitzig, J.C. Allen*, and D.C. Jackson. Div. Biol. & Med., Brown U., Prov., R.I. 02912

Adult turtles, Pseudemys scripta elegans, (1-2 Kg) had chronic needle catheters stereotactically implanted in lateral and 4th brain ventricles. After recovery, unanesthetized animals were subjected to either CO_2 breathing (4.5%; n=19), and/or ventricular perfusion with mock cerebrospinal fluid (CSF) with several different bicarbonate ion concentrations ($[HCO_3^-]$), n=17. Continuous recordings of tidal volume (TV), respiratory minute volume (RMV), respiratory frequency (f), and oxygen consumption ($\dot{V}O_2$) were made. Measurements of the ventilation to metabolism ratios ($\dot{V}_E/\dot{V}O_2$) revealed breathing 4.5% CO_2 increased effective $\dot{V}_E$ 7-fold, while perfusion with mock CSF containing reduced $[HCO_3^-]$ (from 32mEq/L (control) to 15 mEq/L) resulted in a 7-fold increase in $\dot{V}_E$. Combining perfusion (30 mEq/L) and CO_2 breathing resulted in an 8-fold increase. Increasing perfusate $[HCO_3^-]$ to 43 mEq/L produced a significant decrease in $\dot{V}_E$ to 3 times control. Examination of TV and f revealed this decrease was due to a significant ($P<.005$) reduction in f, while TV increased. Changes in the $[HCO_3^-]$ of mock CSF always resulted in an alteration in f; CO_2 initially increased only TV. These data show that central respiratory chemosensors (CRC), previously reported by us, are important in the ventilatory response of turtles to inspired CO_2, and that turtle CRC exert their effect by altering f while TV is principally controlled by peripheral receptors. (Supported by NSF PCM78-22333)

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THE EFFECT OF INTRAVENTRICULAR ACETAZOLAMIDE (DIAMOX) ON THE D-C POTENTIAL DIFFERENCE BETWEEN CSF AND BLOOD IN THE DOG. D. G. Davies and S. L. Britton. Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The d-c electrical potential difference between CSF and blood may be important in the regulation of brain extracellular fluid acid-base composition. To elucidate the mechanisms responsible for this potential difference, we studied the effect of intraventricular administration of the carbonic anhydrase inhibitor, acetazolamide (Diamox), on the $\Delta mv/\Delta pHa$ relationships observed during respiratory acidosis and alkalosis in 5 pentobarbital-anesthetized, gallamine-paralyzed dogs (20-25 kg). The potential difference between the cisterna magna and the external jugular vein was measured during perfusion of the ventriculocisternal system with either mock CSF alone or CSF to which Diamox had been added. The results are shown below: (mean ± SE)

	Control	Diamox	Change	% Change
Slope ($\Delta mv/\Delta pHa$)	26.3 (±1.9)	17.6 (±2.1)	-8.7 (±0.7)	34
x-intercept	7.512 (±0.020)	7.626 (±0.037)	0.114 (±0.021)	-

The reduced slope and increased x-intercept could be due to inhibition of carbonic anhydrase-dependent transport of HCO_3^- and/or Na^+ into the CSF. (Supported by USPHS, NIH Grant HL-25984)

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RELATIONSHIP BETWEEN EXPIRATORY AIRFLOW AND GLOTTIC APERTURE DURING QUIET BREATHING. S.J. England* and D. Bartlett, Jr., Department of Physiology, Dartmouth Medical School, Hanover, NH 03755.

In resting human subjects, the vocal cords abduct during inspiration but move toward the midline during expiration, thus increasing respiratory system resistance during the expiratory phase. To assess the importance of these movements in regulating expiratory airflow we have measured breath-by-breath changes in expiratory airflow and glottic aperture during air breathing at rest. Airflow was estimated from an integrated pneumotachograph record or from magnetometer signals. Glottic aperture was determined from motion pictures of the vocal cords taken at 8 frames per second through a fiberoptic laryngoscope. Analysis was limited to breaths which had tidal volume within 10% of the mean value for that experiment. In 22 of 25 experiments on five subjects, airflow during the middle half of the expired volume was positively correlated with the mean glottic width during the same fraction of expiration. This finding suggests that the extent of glottic narrowing during expiration may influence airflow. Although flow is also modified by the balance of activity of inspiratory and expiratory muscles during expiration, it is probable that the vocal cords provide a variable resistance which contributes to the control of expiratory airflow. (Supported in part by NIH Grants HL 19827 and RR 05392 and the Albert J. Ryan Foundation.)

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RELATIONSHIP BETWEEN PERSONALITY CHARACTERISTICS AND RESPIRATORY BEHAVIOR. S.S. Haas*, K. Axen*, H.E. Ehrlichman* and F. Haas. Dept. of Psychology, CUNY Graduate Center, New York, N.Y. 10036; Dept. of Rehabilitation Med., NYU Med. Ctr., New York, N.Y. 10016

Healthy men (M) and women (W), 80 of each, were given the Personality Research Form (PRF) and Eysenck Personality Inventory (EPI). Respiratory pattern was recorded breathing room air and 5% CO_2 in air while supine. Multiple regression of PRF traits on tidal volume (V_T), respiratory frequency (f), minute ventilation ($\dot{V}_E$), and CO_2 response ($\Delta\dot{V}_E/\Delta CO_2$) explained 16-40% of the observed respiratory variance (P range: <.05 - .001). A profile analysis was performed on slow-deep (M: $V_T \geq 1L$, $f \leq 9$/min, n=12; W: $V_T \geq 0.9$, $f \leq 10$, n=10) and rapid-shallow (M: $V_T \leq 0.6$, $f \geq 34$, n=8; W: $V_T \leq 0.5$, $f \geq 35$, n=8) breathers, and on the highest (M=16, W=16) and lowest (M=16, W=16) CO_2 sensitivity groups. Configurations of PRF traits significantly distinguished between these groups. The trait scores of slow-deep breathers and those least sensitive to CO_2 reflect stable, capable, confident individuals who dislike restrictions. Their counterpart groups reflect the opposite personality. On the EPI there was no agreement with published predictions except for a modest relationship for male rapid-shallow breathers and high Neuroticism scores. These findings suggest that individual differences in personality are related to variation in respiratory behavior that is not explained by physiologic and anatomic variables. (Supported in part by RSA 16-P-56801/2/18.)

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EVIDENCE SUPPORTING A ROLE FOR THE CAUDAL PONS IN THE RESPIRATORY PATTERN GENERATOR. Frederick M. Bennett and Walter M. St. John. Dartmouth Medical School, Hanover, N.H. 03755.

Previous work by St. John and colleagues has demonstrated that lesions placed on the midline at the pontomedullary junction greatly alters the pattern of response to P_{aCO_2} in decerebrate cats. Since the data of Vibert et al. suggests that there are few respiratory modulated neurons in this area it was concluded that these lesions interrupted fiber tracts from the rostral pons. To examine this issue, single unit recordings were obtained for 50 neurons located at the midline of the pontomedullary junction in decerebrate vagotomized cats. Activity was tonic in 43 cells. The cycle triggered histogram was used to identify the type of cell; 41 of 43 tonic cells demonstrated respiratory modulation. The predominate type was expiratory, 30 cells; 19 of which were early expiratory. A total of 16 cells had a RMI <.7. This was the cutoff used by Vibert et al. to determine if a cell was respiratory modulated. Increasing P_{aCO_2} increased the RMI of these cells, indicating these cells are respiratory modulated. Cooling this area of the brainstem to 20° C, which should block synaptic transmission but not fiber tracts, in cats with bilateral pneumotaxic lesions produced results similar to placement of midline lesions, i.e. increase in f and a decrease in phrenic "depth" at all P_{aCO_2}. This data is consistent with the caudal pons playing a role in the determination of VT and f. (Supported in part by NIH grants HL20514 and HL00346.)

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EFFECTS OF FREQUENCY OF BREATHING DURING ISOCAPNIC HYPERVENTILATION ON RECOVERY BREATHING PATTERN. B.A.Wilson & B.R.Goslin. School of Human Biology, University of Guelph, Guelph,Ont.Can.

Twelve subjects, niave to the purpose of the study completed 8 one min. hyperventilation(HV) trials at 60% of maximum breathing capacity (MBC).Two trials were run at 4 randomly assigned breathing rates (f); 20,40,60and80 breaths/min.Subjects breathed to a metronome & through a pneumotach which provided the digital display of tidal volume (vt) required to achieve a f x vt value of 60% MBC.During HV, PET CO_2 was maintained by a partial rebreathing system within 2 torr of rest values while PET O_2 averaged 20 torr above rest. Recovery ventilation (V_I), f & vt were calculated in overlapping 10 second periods for 3 mins. Of the 12 subjects 7 showed post-HV hypernea for all trials, none were consistently apnic. At each f of HV there was an initial drop in V_I which averaged 47%, followed by an exponential-like decrease to rest. There was no significant effect of f of HV on the initial drop of V_I across f conditions. The time constants of V_I & vt for recovery to rest for f 80 were signficantly longer than for f 60 and f 40. Orthogonal polynominal analysis identified a significant quadratic element to this relationship. The recovery time for V_I increased progressively more for each increment in f during HV. If neural "reverberation" theory as suggested by others, is used to explain post-HV hypernea, it must contain an element of gain sensitive to f of HV even with a constant total output (%MBC).(Supported in part by NSERC Grant #A7167).

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EFFECT OF GRADED COOLING OF MEDULLARY CHEMORECEPTOR AREAS $I_{(s)}$ ON RESPIRATORY RESPONSE TO CAROTID SINUS NERVE STIMULATION. D.E. Millhorn, F.L. Eldridge and T.G. Waldrop*. Univ. of North Carolina, Chapel Hill, NC 27514.

Respiratory activity can be graded by cooling ventral medullary chemoreceptor areas $I_{(s)}$ (J. Physiol. 287: 191, 1979). We studied the effect of bilateral graded (15 to 40 degrees C) cold block of these areas on the respiratory responses to carotid sinus nerve (CSN) stim in paralyzed, vagotomized and glomectomized cats whose P_{ETCO_2} and body temp were servocontrolled. Inspiratory activity was quantified from peak integrated phrenic activity. In order to avoid the complications of different levels of activity during control, the control level was kept constant near apneic threshold at the different temperatures of area $I_{(s)}$ by manipulation of P_{ETCO_2}. When this was done the respiratory response to identical CSN stim decreased progressively with graded cooling of areas $I_{(s)}$. This decrease was manifested by a reduction in respiratory frequency (f), and the amplitude and rate of rise of phrenic activity. A proportional increase in T_I and T_E accounted for the increase in f. When control level of activity was kept constant at a higher level of activity, the magnitude of the responses were smaller, but still decreased as areas $I_{(s)}$ were cooled. We conclude that the respiratory responses to identical CSN stimuli are affected by the cooling of areas $I_{(s)}$. Thus, peripheral as well as central chemoreceptor input must be processed by areas $I_{(s)}$. (Supported by USPHS Grants HL-17689, NS-11132 and HL-017106)

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THE LONG-LASTING RESPIRATORY INHIBITION FOLLOWING CALF MUSCLE AFFERENT STIMULATION IS CAUSED BY A CENTRAL NEURAL MECHANISM. T.G. Waldrop*, F.L. Eldridge and D.E. Millhorn. Univ. of North Carolina, Chapel Hill, N.C. 27514

In addition to the excitatory response during stimulation and an afterdischarge present following stimulus termination, physical stimulation of calf muscles also produces a long-lasting (>30min) respiratory inhibition (Physiologist 22: 129, 1979). The purpose of the present study was to elucidate the mechanism which is responsible for this inhibitory effect. Anesthetized, paralyzed, vagotomized, glomectomized cats whose airway P_{CO_2} and body temperature were kept constant by servocontrollers were used. Respiratory output was quantified from peak integrated phrenic activity. A prolonged respiratory inhibition, similar to that following physical stimulation of calf muscles, occurred with tibial nerve stimulation (10v, 50Hz, 1ms pulse duration), showing that the long-lasting respiratory inhibition does not result from prolonged activation of muscle receptors. The long-lasting respiratory inhibition failed to occur following calf muscle afferent stimulation in either animals with L_2 spinal transection or in decerebrate cats. We conclude that the prolonged respiratory inhibition which follows muscle stimulation is mediated by a supra-pontine mechanism activated by muscle afferent input and transmitted by spinal pathways. (Supported by USPHS Grants HL-17689, NS-11132 and HL-07106)

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THE RELATION BETWEEN ONCOTIC PRESSURE AND PROTEIN CONCENTRATION FOR SHEEP PLASMA AND LUNG LYMPH. R.J. Roselli, R.E. Parker* and K.L. Brigham Pulmonary Circulation Center, Vanderbilt University, Nashville, TN 37232

The Landis-Pappenheimer equation (Handbook of Physiology-Circulation, 1963) is commonly used to estimate plasma and lymph oncotic pressures (π) from total protein concentration (C_T) in the sheep lung lymph preparation. We measured π and C_T of sheep plasma (127 samples from 12 sheep) and lung lymph (265 samples from 10 sheep) at 25°C. A third order polynominal ($\pi = AC_T + BC_T^2 + CC_T^3$) was fit to the data and the best fit coefficients A(mmHg/(g/dl), B(mmHg/g/dl)^2) and C(mmHg/(g/dl)^3) were found. Best fit plasma coefficients were similar to those found by Landis & Pappenheimer: A=2.20, B=0.081; while the coefficients for lymph were different: A=2.64, B=0.016. The coefficient C was negligible(i.e., 10^{-9} to 10^{-16}) for both sheep lung lymph and plasma. There was considerable variation in best fit coefficients between sheep. The coefficient A, for instance, ranged from 1.83 to 3.07. We therefore fit the same model to the data, with the exception that the coefficient A was determined by imposing van't Hoff's law for ideal mixtures on the linear term of the polynomial. The relative amounts of 8 different sized lymph and plasma proteins were measured using gradient pore gel electrophoresis. Applying this new model we found B=.244 for lymph and .197 for plasma. The coefficient C was again negligible and much less variation in A was found among sheep, averaging 1.80 for lymph and 1.49 for plasma. (Supported in part by USPHS NIH Grants HL22933 and HL19153)

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LUNG FLUID BALANCE IN SHEEP WITH ELEVATED INTRACRANIAL AND LEFT ATRIAL PRESSURES. T. A. Jones*, M. I. Townsley*, B. L. Woods*, J. M. Leurton*, R. C. Stevenson*, K. R. Madden*, and W. J. Weidner. Dept. Animal Physiology, Univ. of Calif., Davis, CA 95616

Elevation of intracranial pressure (ICP) to levels near systemic arterial pressure in sheep has been reported to produce an increase in the flow of protein-rich lymph ($\dot{Q}l$) from the lung. (Bowers et al., AM.REV.RESP.DIS.119, 637-641, 1979; Malik et al., AM.REV.RESP.DIS.119,379, 1979.) The increase in $\dot{Q}l$ has been attributed to a neurally-induced permeability change. It may have also resulted, however, from surface area increases attending the recruitment of additional microvascular beds. In order to specifically rule out surface area increases, the present study (in sheep anesthetized with Na-pentobarbital) sought to recruit all potential filtration beds by increasing left atrial pressure prior to elevating ICP. Under these conditions, increased pulmonary microvascular permeability would be expected to produce an increased flow of protein-rich lymph from the lung. Our results, however, show an increased $\dot{Q}l$ with a concomitant decrease in the ratio of lung lymph to plasma protein concentration which suggests that increased ICP does not increase pulmonary microvascular permeability. These results also suggest that an alteration in pulmonary surface area, not a permeability change is the primary mechanism underlying increases in protein-rich $\dot{Q}l$ during intracranial hypertension. (Supported by CA Affiliate of A.H.A. 79-N-17.)

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EFFECT OF METHYLPREDNISOLONE ON OLEIC ACID INDUCED EDEMA IN THE ISOLATED DOG LUNG. W.F. Hofman and I.C. Ehrhart. Dept. of Physiology, Medical College of Georgia, Augusta, GA 30912

Oleic acid (OA) experimentally induces lung injury similar to that associated with adult respiratory distress syndrome. In this study, lower left lung lobes were ventilated and perfused at constant pressure with heparinized, autologous blood. Lobe weight changes were continuously monitored as an index of edema formation. One group of lobes (n=7) was treated with methylprednisolone (MP; 5.1 mg/g lobe wt.) 1 hr. prior to 1µL/kg OA whereas the untreated group (n=7) received only OA. At ½ hr. intervals over 3 hr. following OA, blood gases were obtained and dynamic lobe compliance (C) was calculated. Pulmonary vascular resistance was partitioned into upstream and downstream components using a venous occlusion technique (Hakim et. al. J. Appl. Physiol. 47(1): 145, 1979). Following OA, both groups of lobes showed a similar reduction in C and venous P_{O_2}. Rate of weight gain was linear in all lobes but weight increased 34% in untreated lobes compared to only 21% in the MP treated lobes over the 3 hr. after OA. Total pulmonary vascular resistance tended to increase in both groups. However, downstream resistance as a proportion of total vascular resistance was increased from 0.32 to 0.44 in the untreated lobes but was unchanged in the MP treated lobes. Thus MP may attenuate the rate of edema formation following lung injury by preventing an increase in downstream vascular resistance. (Supported by Am. Heart Assoc.-Georgia Affiliate, BRSG #5-507-RR05365-19 and NIH #5E03-MB-4106-14).

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COMPARISON OF VENOUS OCCLUSION AND ISOGRAVIMETRIC CAPILLARY PRESSURES IN ISOLATED DOG LUNGS. K. Ryan, P. Kvietys, J. C. Parker and A. E. Taylor. Department of Physiology, Univ. of South Alabama, Mobile, Alabama 36688

In isolated left lower lobes perfused with autologous blood at 37°C, two indirect capillary pressure measurements were compared in the same lobe. Flow through the lobe was controlled by the pressures in arterial (P_a) and venous (P_v) reservoirs and airway pressure was maintained at 3 cmH_2O. The perfused lobes were initially maintained in an isogravimetric Zone III state by adjusting the blood reservoirs until the lung was neither losing or gaining weight. Isogravimetric capillary pressures (P_{ci}) were measured using step decreases in blood flow obtained by lowering the arterial reservoir and elevating the venous reservoir to maintain the lobe in an isogravimetric state. The venous occlusion capillary pressure (P_{co}) were measured by suddenly occluding the venous outflow from the lobe and recording the venous pressure-time curve. Then the inflection point of venous pressure vs. time was used to estimate P_{co}. A regression analysis for the capillary pressures (n=22) produced the following expression:

$$P_{co}=1.04\ P_{ci} - 1.7,\ r = 0.96,$$

over a range of capillary pressures between 3.9 and 14.5 mmHg. Thus, there was excellent agreement between estimates of capillary pressure using either the isogravimetric or venous pressure occlusion methods.

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MECHANICAL PROPERTIES AND CHEMICAL CONTENT OF PULMONARY ARTERIES FROM EXTRALOBAR AND INTRALOBAR SITES. Robert H. Cox. Bockus Res. Inst., Graduate Hosp., Dept. Physiol., Univ. of Pa., Phila., Pa. 19146.

Rings of canine pulmonary arteries were obtained from five sites: the main and left pulmonary arteries, and three intralobar sites. Length-tension data were obtained under conditions of active (145 mM K^+) and passive (0 mM Ca^{+2} and 2 mM EDTA) smooth muscle. Other rings were used to determine connective tissue (CT), water and electrolyte contents. Extracellular water space was determined using ^{60}Co-EDTA as a marker. No significant differences were found in passive mechanics (stress-strain or incremental modulus-pressure curves) between any sites studied. However, collagen (C) and elastin (E) content increased significantly from the main pulmonary artery (MPA) to the smallest intralobar artery (ILA) studied. Also (C + E) and (C/E) both increased from the MPA to ILA. This suggests that differences exist in the organization of the connective tissue matrix at the various pulmonary arterial sites. Maximum active stress (force/area) developed in response to high K^+ increased from the MPA to ILA, as did the optimum stain (L_o). Extracellular water space increased from the MPA to ILA while total water content was not different. Tissue potassium content decreased from MPA to ILA. This suggests that the relative cellular volume decreased from the MPA to the ILA. The larger active stress of small ILA was not the result of a larger relative cell content. (Supported by research grant HL-23779 from NHLBI).

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The Effects of PEEP on Physiologic Dead Space and Alveolar Capillary Perfusion

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Recent experiments in our laboratory have shown a significant increase in $PaCO_2$ with increased end expiratory pressure (PEEP). The present study attempts to define the mechanism by which PEEP increases $PaCO_2$ by measuring the physiologic dead space - tidal volume ratio (V_D/V_T) alveolar capillary flow (ACF) and cardiac output (C.O.) Anesthetized dogs on a mechanical ventilator were subjected to a left thoracotomy. ACF was quantitated using an in vivo microscope fitted to the surface of the superior lung. Catheters were placed for measurement of pulmonary artery (PA) and wedge pressures (PAW) left atrial (LA) and systemic arterial pressure and cardiac output C.O. (thermal dilution). Tidal volume (V_T), physiologic dead space (V_D) and pulmonary shunt were measured. With an open chest, measurements were made at: 1) control (5 cm H_2O PEEP) 2) after increasing PEEP to 15 cm H_2O, 3) after Dextran 70 infusion sufficient to restore C.O. to control levels and 4) after return to control PEEP.

15 cm H_2O increased V_D/V_T, PAP, PAW and LAP. Increasing PEEP reduced ACF, C.O. and pulmonary shunt. Maintaining 15 cm H_2O PEEP and restoring C.O. to control with Dextran 70 restored V_D/V_T to control and improved ACF while PA and LA pressures remained unchanged. We conclude that the elevation in V_D/V_T with PEEP is the result of reduced ACF primarily resulting from PEEP reducing C.O.

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MECHANICAL PROPERTIES OF ISOLATED PULMONARY ARTERIAL VESSELS. J.N. Evans, B. Little,* S. Bronstein* and R. Previti*. Dept. of Physiology & Biophysics, U of Vermont, Burlington, VT 05405

Cylindrical segments of small pulmonary arterial vessels (i.d.=324±23μm) and main branches of the pulmonary artery (i.d. =1.83±.24mm.) were isolated by microdissection from lungs excised from healthy male rabbits. The intact cylindrical segments were mounted on two thin wires which were connected to an isometric force transducer and a microdisplacement device. The passive (T_p) and active (T_A) circumferential tension were determined as a function of internal circumference (C_i). T_p was measured in Ca^{++} free EGTA buffered PSS at increments of C_i. T_A was determined at each C_i by changing solution to PSS where KCl was substituted for NaCl and Ca^{++} elevated to 5 mM. T_p increased as an exponential function of C_i. T_A increased with C_i with a maximum value of 3.71±.63 and 3.64±.8 mN/mm in the large and small vessels, respectively. LaPlace's Law was used to calculate effective pressure-dia. relationships. In the passive condition an increase in pressure from 5 to 10mmHg causes a 40% and 45% increase of dia. in the large and small vessels, respectively. When activated to 20% of maximum tension at a C_i where the pressure equals 15mmHg the curves predict that the large vessels would decrease dia. by 12% while the small vessels by 30%. These results demonstrate that both sets of vessels are quite distensible in the passive condition, but when activated they have the capability to overcome significant transmural pressures. Supported by Grants from NIH 21539 and 14212 (SCOR).

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EFFECT OF DOBUTAMINE ON LUNG VESSELS. W. Macedo*, R. Graham*, and H.S. Goldberg. Sec. of Resp. Dis., University of Manitoba, Winnipeg, Manitoba, Canada.

Canine left lower lobes were isolated and perfused with autologous blood in Zone II of West. Alveolar pressure was kept constant at 5 cm H_2O with 100% O_2. Venous pressure was kept between -5 and -10 cm H_2O. Arterial pressure was varied by step changes in the hydrostatic level of an inflow reservoir. Dobutamine was given as a bolus of 1.0, 4, 8, or 25 mg. The relationship of driving pressure to flow and vascular volume was determined. Driving pressure was raised to about 30 cm H_2O. It was then brought down in steps of 1-5 cm H_2O. In three lobes flow was stopped by clamping the inflow line and arterial pressure was then noted. No significant change in this pressure was seen. The average pulmonary artery pressure at no flow was 8.3 ± .2 cm H_2O. Therefore, at a positive driving pressure of 3.3 cm H_2O there was no flow, suggesting a closing pressure in the pulmonary circulation. The pressure-flow curve was linear from maximal flow to 25% of maximal flow. The slope of this line was unchanged at all doses of dobutamine except 25 mg. At this dose there was an increase in resistance to flow of about 40% ($p < .05$). At all doses in all lobes dobutamine was associated with no significant change in the pressure-volume relationship. (Supported by the Manitoba Heart Foundation).

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PULMONARY AND SYSTEMIC VASODILATION IN RESPONSE TO 6-KETO-PGE_1 IN NEWBORN LAMBS. M.L. Tod* and S. Cassin. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Hepatic metabolism of 6-keto-$PGF_{1\alpha}$, the relatively inactive metabolite of prostacyclin (PGI_2), yields 6-keto-PGE_1 (6-k-PGE_1), a compound exhibiting many of the actions of its parent compound, PGI_2. PGI_2 is a potent pulmonary and systemic vasodilator. Pulmonary and systemic responses to 6-k-PGE_1 were studied in newborn lambs (3-14 days old, 3.2-6.6 kg) under chloralose anesthesia. The left pulmonary artery was pump-perfused at constant flow with inferior vena caval blood. Pulmonary arterial (PAP), left atrial, systemic arterial (SAP) and tracheal pressures, pulmonary flow, heart rate and arterial blood gases and pH were monitored. One-minute intra-pulmonary infusions (n = 14) of 6-k-PGE_1 in doses of 0.078-2.64 μg/kg.min produced dose-dependent decreases in calculated pulmonary vascular resistance of 7-24%, while simultaneously causing decreases of 0-16% in SAP. In three of four lambs, the ratio PAP/SAP decreased following each infusion of 6-k-PGE_1, indicating a greater effectiveness on the pulmonary vasculature than the systemic circulation in normoxic lambs using an open-chest, constant pulmonary flow preparation. These findings are in contrast with the results reported by Lock et al. (Prostaglandins 18:303, 1979), which indicated a relatively greater systemic than pulmonary effect in response to 6-k-PGE_1 in conscious newborn lambs during normoxia. (Supported in part by NIH Grant HL 10834)

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The Effects of Fluid Resuscitation Following Wood Smoke Inhalation. W.R. Clark,* D. Grzyboski, G.F. Nieman, (Spon. D. Fromm) Upstate Medical Center, Dept. of Surgery, Syracuse, N.Y. 13210

Smoke inhalation becomes a much more serious clinical problem when there is an associated cutaneous burn. This study attempts to define the effect of intravenous fluid on the pulmonary response to a standard smoke insult.

Anesthetized dogs were placed on a volume cycled positive pressure ventilator and prepared for hemodynamic measurements. Group I received Ringers Lactate (R.L.) I.V.: 10% of body weight over 2 hours; Group II were exposed to smoke; Group III were exposed to smoke then given R.L. Group I and Group III dogs excreted the fluid load co-incident with a rise in cardiac output (Group I>Group III). Except for a decrease in pulmonary vascular resistance, Group I did not otherwise vary from the initial state. Group II showed a large increase in percent shunt (S) and decrease in static compliance (S.C.) which returned to near normal levels after 5 hours. Group III showed the same initial changes in S. and S.C. without any return towards normal with time. The wet/dry lung weight ratio (W/D) was significantly increased in Group III over Groups I and II. Colloid oncotic pressure was unchanged in Group II; it fell to similar levels in Group I and III but never went below the pulmonary artery wedge pressure.

Previous studies implicate surfactant deactivation as a cause for increased S and reduced S.C. The lung exposed to smoke is vulnerable to pulmonary edema following fluid challenge most likely on the basis of an increase in alveolar surface tension or capillary permeability.

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A METHOD OF CORRECTING FOR THE EFFECT OF CHANGES IN THE WATER CONTENT OF RESIDUAL BLOOD ON NORMAL LUNG WET-TO-DRY WEIGHT RATIO Kermit A. Gaar, Jr. LSU Medical Center, Shreveport, LA 71130

The accuracy of the wet-to-dry lung weight ratio (W/D) is affected by the water content of the residual blood. To study this effect, an equation was formulated empirically. This equation was then verified experimentally using isolated lung lobes. Dogs were anesthetized and heparinized prior to blood collection followed by excision of the left lung. The upper and lower lung lobes were separated and weighed. Isotonic salt solution was added to the blood collected from each dog at the rate of 0.2, 0.4, 0.6, 0.8, or 1.0 ml per ml of blood. A 50 ml portion of this diluted blood was then flushed through the lower lung lobe to replace the normal residual blood with the diluted blood. Following this, the lower lung lobe was homogenized in 150 mls water. Hemoglobin content of the homogenized lung containing diluted blood was determined photometrically using a standard cyanmethemoglobin method. Hemoglobin content of the diluted blood was determined similarly. Using these values, residual blood content of the left lower lung lobe was computed. The upper lung lobe remained intact and served as a control. Both the upper lung lobe and the homogenate were dried and the W/D's determined. Specific gravities and W/D's were determined for both the normal and diluted blood. Using the empirical equation the lower lung W/D was corrected for the effect of dilution of the residual blood. The corrected W/D was then compared with the control lung W/D. Based on this comparison the corrective adjustment in the W/D appeared to adequately account for the effects of variation in water content of residual blood.

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EFFECT OF FLUID VOLUME EXPANSION ON INTRALOBAR INTERDEPENDENCE. J. C. Parker and A. E. Taylor. Dept. of Physiology, University of South Alabama, Mobile, AL 36688

The effects of three successive infusions of Tyrode's solution equal to 5% bodyweight (BW) were studied in 9 intact dogs on positive pressure ventilation. Swan-Ganz balloon catheters (No. 5 or 7) were wedged in small airways of lower lung lobes after ventilation for 15 minutes with pure O_2. Pulmonary artery pressure was recorded with an additional catheter. Residual gas was flushed from the occluded lung segment with 0.5-1.0 ml of Tyrode's solution. The fluid pressure in the segment (Paf), airway pressure (Pao), and esophageal balloon pressure (Peso) were used to calculate an index of interdependence: $K=-\Delta(Paf-Peso)/\Delta(Pao-Peso)$; and a mean segment fluid pressure Paf. The value of K consistently decreased from a pre-infusion value to a minimal value 5-15 min. after termination of the infusion, then recovered toward the pre-infusion value. Mean pre and post infusion K values (means ±SE) were: 1.51±0.35 and 1.10±0.28; 1.29±0.34 and 0.97±0.27; 1.10±0.26 and 0.67±0.14; for successive infusions of 5, 10, and 15% BW, respectively. The group mean Paf values before and after the infusions were: -4.8±2.4 and -3.4±1.7; -8.4±2.7 and -4.2±3.1; -4.2±2.2 and 1.1±2.7 cmH_2O, respectively. These data indicate that the fluid pressures in small, collapsed lung segments are influenced by mechanical stresses transmitted by alveolar wall attachments, and to some extent by the absorptive pressure for fluid.

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AIRWAYS RESPONSE OF YOUNG AND ELDERLY SUBJECTS TO 0.5 ppm SO_2 AND 0.5 ppm O_3. Evelyn Schlenker and Marc Jaeger. Dept. of Physiol., Univ. of Fla., Gainesville, FL. 32610

Ten elderly (73 ± 7.7 years) and 10 young (25.5 ± 4 years) healthy non-smokers were exposed to clean air on day 1 and on day 2, at the same hour of the day, to 0.5 ppm SO_2 0.5 ppm O_3 for one hour. Several lung function tests were performed before, during and up to 3 hours after each exposure. The elderly subjects showed no significant response in Airway resistance (Raw), Functional residual capacity (FRC), Maximum flows at 50% and 25% of vital capacity ($\dot{V}_{50}$, $\dot{V}_{25}$), Forced vital capacity (FVC), Forced Expiratory Volume in one second (FEV_1) or in Mid-maximum expiratory flow rate (MMFR) during or after exposure. On the contrary, when corresponding day 1 values were compared to day 2 values, MMFR decreased significantly in the young subjects from 5.23 → 4.65 L/sec ($P < .001$) during exposure. The young group also showed significant reductions of $\dot{V}_{50}$ (5.3 to 5.1 L/sec ($P < .001$), MMFR (5.35 to 5.00 L/sec, $P < .05$), and FEV_1 (3.8 to 3.6 L, $P < .05$), after exposure when day 1 values are compared to day 2 values. Raw increased from 1.14 ± .36 to 1.98 ± .64 cm H_2O/L/sec ($P < .001$) in 5 of the young subjects and did not change in the remaining 5. Four elderly subjects complained of eye irritation, dry throat during exposure and 2 young subjects had headaches lasting one hour after exposure. These data indicate that the airways of healthy elderly subjects are much less responsive to the combination of SO_2 and O_3 than those of young subjects. (Supported by Francis B. Parker Foundation.)

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VITAL STAINING OF TRACHEAL GANGLIA. M.A. Grillo* and J.A. Nadel (SPON: W.M. Gold). Cardiovasc. Res. Inst., UCSF, S.F., CA 94143

We have developed a method based on neutral red staining for locating tracheal ganglia in living isolated preparations. For staining, tracheal segments 5 cm long are incubated at 21°C in F12 culture medium containing 0.01% dye; alternatively, pledgets soaked in a dye-saline solution are applied topically to the back of the posterior membrane. In either case, the ganglion neurons (including SIF cells) are stained bright red in 20-60 min and are easily seen despite an overlay of unstained collagen. Such neurons pass the trypan blue viability test for at least 5 h and show little or no damage ultrastructurally. Correlated light and electron microscopy suggests that the dye is localized largely in lysosomes and in secretory granules. In dog trachea, the ganglia are distributed all over the muscle surface and occur mainly at the nodal points of the nerve plexus. In ferret trachea, they are metamerically organized along a pair of nerves running its length posteriorly. Because the ganglia are small (0.1-0.7mm, major axis) and thin, their component neurons can be easily counted and measured in whole mounts. Sample counts for rat, ferret and dog ganglia are, respectively, 19, 20, and 108 cells/ganglion with much variation. Cell body sizes for the corresponding species are 27.1 ± 1.3μ, 46.6 ± 1.9μ, and 55.5 ± 4.2μ (major axis; mean ± SE). Thus the method facilitates anatomical study. Coupled with the described properties of the ganglia, it promises to permit electrophysiological and other studies never before done. (Supported in part by USPHS PPG HL-24136)

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CARDIAC LYMPH PLATELETS AS AFFECTED BY BRIEF MYOCARDIAL ISCHEMIA. Lloyd H. Michael, Tedd A. Brandon*, Robert M. Lewis*, Richard R. Miller* and Mark L. Entman. Dept. of Med., Baylor College of Medicine, Houston, Texas 77030

Platelet activity has been implicated in a number of mechanisms leading to cardiac ischemia and to altered vascular permeability. We hypothesized that cardiac lymph may reflect a dynamic state of platelet activity during ischemia, therefore, we examined the movement of labelled platelets into cardiac lymph prior to circumflex coronary artery occlusion (CFX OCC), during CFX OCC and then reperfusion. Washed platelets which had been extracted from platelet-rich dog plasma were labelled (25°C for 24 hrs) with Chromium-51 and infused into the femoral vein of dogs surgically prepared for cardiac lymph collection. ^{51}Cr-platelets were followed in blood and cardiac lymph by gamma counting. CFX OCC ranged from 5-20 mins. in 6 dogs. During CFX OCC, the total number of labelled lymph platelets was not significantly altered in 5 of 6 experiments; however, reperfusion resulted in a platelet decrease in same and slight elevation in 1. With progressive reperfusion for several hrs. ^{51}Cr-platelets decreased in the lymph to a significantly greater degree than in blood. Blood samples had 100-800% greater ^{51}Cr-platelets than did lymph. Thus, CFX OCC for < 20 mins. does not appear to promote platelet activity in cardiac lymph but in fact, may cause a decrease in total number present, possibly through microvascular platelet aggregation as consequence of ischemic period. (Supported by HL17269, HL23161 and Texas Affiliate, American Heart Association)

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VOLUMES OF DISTRIBUTION OF INTACT RABBIT HEARTS. F.Gonzalez*, C.Harris*, and J.B.Bassingthwaighte. Center for Bioengineering University of Washington, Seattle, WA 98195

The distribution of plasma space (V_{pl}), red cell space (V_{rbc}), vascular space (V_{bl}), extracellular fluid space (V_{ecf}), interstitial fluid space (V_{isf}), total water space (V_w) and regional flow in rabbit hearts were determined using the following tracers and techniques: ^{99m}Tc-labeled albumin [V_{pl}], ^{51}Cr-labeled red cells [V_{rbc}], ^{22}NaCl [V_{ecf}], dessication [V_w] and radioactive microspheres [flow]. V_{bl} and V_{isf} were determined from V_{pl}, V_{rbc}, and V_{ecf}. The tracers were injected intravenously into anesthetized male rabbits and allowed to equilibrate in their respective spaces. Following tracer equilibration, the chest was opened and the heart quickly frozen with Freon-22. Microspheres were injected immediately before and after the chest opening, providing a comparison of flow distributions. Each frozen heart was sectioned into 72 pieces and volumes and flows determined for each piece. The mean volumes and average dispersions (S.D.) for 5 rabbits were: V_{pl}=.078±.055 ml/g, V_{rbc}=.042±.028 ml/g, V_{bl}=.120±.083 ml/g, V_{ecf}=.359±.087 ml/g, V_{isf}=.236±.054 ml/g, V_w=.782±.037 ml/g, flow(closed chest)=.633±.366 ml/g/min, and flow(opened chest)=.680±.457 ml/g/min. The relatively large ranges for V_{bl} and V_{pl} reflect that these values represent whole heart pieces composed of both large vessels and capillaries. (Supported by NIH grants HL19135 and HL19139).

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EFFECT OF EXERCISE CONDITIONING ON DIASTOLIC CORONARY RESISTANCE. Isabella Y.S. Liang*, H.L. Stone, Dept. of Physiology University of Oklahoma, H.S.C., Oklahoma City, Oklahoma 73190

Diastolic coronary resistance was determined in five trained conscious dogs. The conditioning regime consisted of treadmill running 5 days/wk for 4-5 weeks. Skeletal muscle aerobic capacity was not elevated. The dogs were instrumented to measure aortic pressure and left circumflex coronary flow velocity during pacing with implanted electrodes. Heart rate was varied from the resting value to 240 bpm before and after adrenergic blockade with propranolol (1 mg/kg), (ßB) or phentolamine (1 mg/kg), (αß). The results were:

		Diastolic Coronary Resistance (mmHg/cm/sec)		
Heart Rate (BPM)		120	180	240
Control	Un-Conditioned	5.37	4.02	3.40
	Conditioned	4.33**	3.04**	2.31**
ßB	Un-Conditioned	6.24	4.62	3.74
	Conditioned	4.98	3.73	3.10
αß	Un-Conditioned	4.39	3.53	2.94
	Conditioned	3.87*	2.39*	1.93*

*P<0.05, **P<0.01 compared to control at the same heart rate. Atrial pacing will result in a similar increase in myocardial oxygen consumption. The difference between diastolic coronary resistance during ßB and αß was increased by 10% following conditioning. These data suggest a change in the neurogenic control of the coronary vasculature by a reduction in sympathetic nervous activity. Supported by Grant #HL 22154

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CORONARY ARTERIAL IMPEDANCE DETERMINED FROM FOURIER ANALYSIS OF PRESSURE AND FLOW WAVE-FORMS IN DOGS. F.W. Heineman*, C.E. Bayliss* and J. Grayson. University of Toronto, Toronto, Canada M5S 1A8

In addition to allowing the numerical description of periodic phenomena such as pulse-waves, Fourier coefficients and their associated phases can be used to determine the input impedances of vascular networks from pressure and flow curves. Aortic pressure (AP) and left anterior descending coronary arterial blood flow (F) were recorded from 10 anesthetized mongrel dogs. The data were digitized at 10 msec intervals and subjected to harmonic analysis. Mean AP and F were used to calculate the LAD resistance. Corresponding harmonic components of the AP and F waves were used to calculate the moduli and phase of the LAD impedance up to a frequency of 20 hertz. The characteristic impedance of the LAD was determined by averaging the impedance moduli over the range of 2-20 hertz. Resistance and characteristic impedance of the LAD (mean±SEM) were 251±30 and 84±13 dynes·sec·cm^{-5}·10^3, respectively. LAD input impedance exhibited a minimum of 46±7 dynes·sec·cm^{-5}·10^3 with an associated phase of +2.3±0.1 radians at approximately the same frequency as the heart rate. The LAD input impedance versus frequency curve derived from the harmonic analysis of AP and F reflects the relationship between coronary blood flow and myocardial contraction in that a minimum vascular impedance occurs during diastole. (Supported by the Ontario Heart Foundation)

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EFFECT OF RIGHT VS LEFT STELLATE STIMULATION ON REGIONAL CORONARY BLOOD FLOW IN THE DOG. L. E. Rinkema, J. X. Thomas, W. C. Randall. Dept.Physiol.,Loyola Univ. Med. Ctr.,Maywood,IL 60153

Sympathetic stimulation produces coronary vasoconstriction when inotropic and chronotropic effects are blocked with beta blockers (Feigl, Vatner, Takenaka, et al.). Regional variations in this flow reduction produced by right (RSS) vs left (LSS) stellate stimulation were delineated in this study. Open chest, pentobarbitalized dogs were given 1 mg/kg propranolol, iv. Hearts were neurally decentralized and paced just above the spontaneous heart rate. Using standard techniques, radiolabeled microspheres (15±2 µm) were injected into the left atrium before and during RSS (N=4) and LSS (N=5). Blood flows in the right (RV) and left (LV) ventricles are shown as percent decreases from RV (.42±.05 ml/min/g) and LV (.78±.08 ml/min/g) controls. LV areas were divided into outer (O) and inner (I) halves. A=anterior free wall, P=posterior free wall.

	RV	%↓ LV base				%↓ LV apex			
		O		I		O		I	
		A	P	A	P	A	P	A	P
RSS	8.2	25.8	8.6	27.2	10.8	23.6	11.7	24.6	12.4
LSS	29.6	13.4	28.8	23.0	22.8	15.4	20.5	18.1	17.0

All ↓ in flow were significantly different (p<.05) from control. RSS produced maximal effects in the anterior LV, while LSS produced a global ↓ in flow with maximal effects in the RV and the outer half of the posterior LV base. (Supported by the Earl M. Bane Charitable Trust Fund.)

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MYOCARDIAL BLOOD FLOW AND OXYGEN CONSUMPTION IN THE DENERVATED CANINE LEFT VENTRICLE. J. X. Thomas, Jr., M. J. Barber and M. F. Oats*. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL. 60153.

Previous reports from this laboratory and others have shown that the chronically denervated dog heart is less susceptible to ischemic injury than either acutely denervated or normal hearts. The purpose of this study was to test the hypothesis that the protective effect of chronic denervation during ischemia is due, in part, to a reduction in oxygen requirements. Coronary arterial-venous O_2 differences (A-Vox Systems), left ventricular (LV) pressure, LV dP/dt, and arterial pressure (AP) were monitored continuously in 10 pentobarbital anesthetized control (C) dogs and in 5 dogs which had undergone an intrapericardial ventricular denervation (D) 2-3 weeks earlier. Myocardial blood flow (MBF) was determined in all dogs using radiolabeled 15µ spheres. Heart rate was held constant in all experiments at 150 by pacing from the right atrium. AP & LV dP/dt were not different in C and D. LV MBF in C was 107.4± 7.23 ml/min/100g and 74.54±7.64 ml/min/100g in D (p<0.01). O_2 difference in C was 13.39±.74 vol % and 12.02±.45 vol % in D (p>0.1). Calculated value of myocardial oxygen consumption was 38.5% less in D than C (p<0.005). These data suggest myocardial utilization of oxygen is reduced in the chronically denervated ventricle, thus contributing to the preservation of function, a smaller infarct, etc. seen during ischemia. (Supported by Schweppe Found. of Chicago and the E. M. Bane Charitable Trust Fund.)

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EFFECT OF TWO STAGE OCCLUSION ON MYOCARDIAL BLOOD FLOW AND FUNCTION. M. F. Oats* and J. X. Thomas, Jr. Department of Physiology, Loyola Univ. Medical Center, Maywood, IL 60153.

Two stage occlusion(TSO), i.e., coronary occlusion preceded by a 15 min period of stenosis, has been used to avoid arrhythmias and fibrillation during occlusion studies. A previous report from this laboratory indicated infarct size was approx. 50% with TSO compared to one stage occlusion (OSO). The purpose of this study was to compare regional and global myocardial function, and myocardial blood flow (MBF) during TSO and OSO. Pentobarbital anesthetized dogs were instrumented to allow continuous measurement of: art. pressure, heart rate, LV pressure, LV dP/dt, and regional segment lengths. The LAD was isolated above the apical branch. MBF was measured at control, 15 min of stenosis, and 15 and 60 min following total occlusion using (15μ) microspheres. OSO dogs underwent the same protocol except the LAD was not stenosed. Following total occlusion, OSO dogs showed increased end systolic and end diastolic lengths with a significant reduction in segment shortening and LV dP/dt. MBF in the core of the ischemic region (MBF<.3ml/min/gm) did not vary from 15-60 min post occlusion. TSO response, function and core blood flows, following occlusion were not different from OSO. These data indicate that TSO provides no protective effect in the core ischemic area with regards to blood flow and regional function, even though infarct size has been significantly reduced during TSO.
(Supported by the Bane Charitable Trust Fund and Chicago Heart Association.)

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ANGIOGRAPHIC STUDIES OF LARGE CORONARY VEINS. E. Hodgson*, G.A. Klassen, J.A. Armour. Dept. of Physiology & Biophysics, Dalhousie University, Halifax, N.S., Canada B3H 4H7

Coronary arterial and venous pressures respond differently to chemical and autonomic stimuli. We investigated angiographic changes in diameter of major coronary veins in response to various stimuli. Single plane angiograms of the coronary veins were obtained by injecting Renografin-76 at a constant flow rate into cannulated epicardial veins. Changes in venous dimensions were recorded on videotape and reoentrograms; results were corrected for changes in ventricular diameter, digitized, and analyzed by computer. Injection of isoproterolol (0.1 mg) or norepinephrine (0.01 mg) resulted in moderate (20-30%) venous narrowing; epinephrine (0.01 mg) produced more (40%) narrowing, with almost complete absence of dye in the epicardial veins during systole. Adenosine (10 mg) caused a biphasic response: marked narrowing (40%) for approximately 15 sec followed by a return to control or greater diameters as the bradycardia decreased. Injection of 2.5 mg/kg of acetylcholine produced marked bradycardia, associated with a 20% increase in venous diameter. Fibrillation of the ventricles resulted in an 80% increase in venous diameter. Thus it is evident that the epicardial veins exhibit significant changes in size, which may be due to either changes in vasomotion or extravascular compression. These observations are evidence for regulation of this portion of the coronary venous system.
(Supported by the MRC & N.S. & N.B. Heart Foundations).

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PARTIAL PURIFICATION OF A TRF INHIBITOR. Jerry Vriend* and Karl M. Knigge. The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

Acetic acid extracts of bovine pineal glands were prepared and filtered through Sephadex G-25. The effects of G-25 fractions were tested on TRF-induced TSH release by dispersed rat pituitary glands in vitro. Highly significant inhibition of TRF-induced TSH release was obtained with material which was retarded on G-25. The ability of this material to inhibit TRF-induced TSH release and to displace ^{3}H-TRF from pituitary tumor cell membranes was interpreted as evidence for a TRF inhibitory factor (TRF-IF) in bovine pineal glands. Evidence for binding to TRF receptors included parallel competition displacement curves with synthetic TRF, data showing that excess synthetic TRF could overcome the inhibition of TRF-induced TSH release, data showing that excess synthetic TRF could overcome the inhibition of ^{3}H-TRF binding by TRF-IF, and finally, data showing copurification of anti-TRF bioactivity (in cell culture) and anti-TRF binding activity (in membrane preparations). Partial purification of TRF-IF was obtained with Sephadex G-10, Sephadex LH-20, and high pressure, reverse phase liquid chromatography. (Supported by grants from NIH, NS 15345 and AM05995)

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ULTRASTRUCTURE OF THE RAT PLACENTA IN ALTERED MATERNAL THYROID STATES. D.M. Van Wynsberghe and Vali Kiaie*. Univ. of Wisconsin, Zoology Dept., Milwaukee, WI 53201.

This study was performed to determine the morphological changes that occur in the rat placenta during maternal hypothyroid and hyperthyroid conditions as compared to the placenta in the euthyroid rat. Placental changes were observed in the euthyroid, hypothyroid, and hyperthyroid rats during early (14-16 days), middle (16-18 days), and late (20 days) stages of pregnancy. Surgical removal of the thyroid gland was performed to induce maternal hypothyroidism. Subcutaneous injections of triiodothyronine every other day prior to and during pregnancy induced maternal hyperthyroidism. Placentas from the euthyroid, hypothyroid and hyperthyroid mothers were removed at the proper gestation ages (early, middle, or late) and prepared for light and electron microscopy. Placentas were fixed in Bouin's solution and stained with hematoxylin and eosin for light microscopy. For electron microscopy, the placentas were double fixed in gluteraldehyde and osmium tetroxide, and stained with uranyl acetate and lead citrate. In each stage of gestation (early, middle, and late), changes were noted in the placental villi, giant cells and general morphology of the deciduace regions of the placentas of the hypothyroid and hyperthyroid rats. The placental changes observed may alter normal placental function, and may therefore contribute to reabsorption of fetuses, miscarriages, or premature births associated with altered thyroid states.

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β-ADRENERGIC RESPONSIVENESS OF HYPOTHYROID, EUTHYROID AND HYPERTHYROID MALE RATS. R.M. Threatte*, C.C. Barney, S.P. Baker* and M.J. Fregly. Depts. of Physiology and Pharmacology, University of Fla. Gainesville, Florida 32610.

The administration of thyroxine (0 to 150 µg/kg b.w., s.c.) to thyroidectomized (Tx) rats was accompanied by a dose-dependent increase in metabolic activity as assessed by rate of oxygen consumption and colonic temperature, relative to euthyroid rats. Increasing doses of thyroxine administered to Tx rats were also associated with increasing concentrations of thyroxine, triiodothyronine and reverse triiodothyronine in serum. The chronotrophic (β_1-adrenergic) response to the administration of the β-adrenergic agonist, isoproterenol (8 µg/kg b.w., s.c.), was related positively to the thyroid status of the rat whereas the dipsogenic (β_2-adrenergic) response to isoproterenol (4,8,25 µg/kg b.w., s.c.) was not altered. Cardiac adrenergic receptor density, as measured by tritiated dihydroalprenolol, was significantly correlated with an increase in total serum triiodothyronine concentration, basal heart rate and the chronotrophic response to administration of isoproterenol. (Supported by Grant AG 815 from the Florida Heart Association, Palm Beach Chapter).

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DIEL CHANGES IN HORMONES AND BLOOD CHARACTERISTICS OF A CANNULATED WHITE-TAILED DEER. Patrick F. Scanlon*, D. F. Gibson,* A. Oelschlaeger,* R. L. Kirkpatrick*, and F. C. Gwazdauskas.* Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (SPON: John J. Ney)

A docile, male white-tailed deer (2.5 y.o.) with an indwelling jugular catheter was bled every 2 hrs on 7 days (each two weeks apart) on 2 of which the deer was given (i/m) xylazine hydrochloride (X). Packed cell volume (PCV), hemoglobin (Hb), serum glucose, blood urea nitrogen (BUN) total serum proteins, corticosteroids, triiodothyronine (T_3) and thyroxine (T_4) were examined from all samples. PCV was relatively stable in the untreated deer but peaked 10-14 hrs after X and declined thereafter. Hb was essentially constant in the untreated deer but varied markedly 2-14 hrs after X. Serum glucose was constant (80-100 mg/dl) in the untreated deer but immediately after X increased markedly to values in excess of 200 mg/dl and remained elevated for 14 hrs. BUN did not fluctuate markedly in the untreated deer but rose at a constant rate to very high concentrations (2 fold increase) after X. Serum proteins fluctuated only slightly in the untreated deer and was markedly elevated for 14 hrs after X following which it declined rapidly. Peak concentrations were greatest after X. Overall diel mean corticosteroids for the untreated and X treated deer were 6.8±0.9 ng/ml, and 15.1±3.5 ng/ml, respectively. X did not effect T_4 or T_3 concentrations.

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CHANGES IN THYROID FUNCTIONS IN OFFSPRING OF ETHANOL FED RATS. S.P. Singh, A.K. Snyder,* S.K. Singh,* and B.N. Premachandra.* Chicago Medical School and VA Med. Center, No. Chicago, IL 60064 and VA Med. Center, St. Louis, MO 63125.

Serum T4 and T3 levels have been shown to be decreased in pups of rats fed ethanol before, during and after pregnancy.

In the present study, rats were fed ethanol during pregnancy only and thyroid functions of their offspring investigated. Pregnant rats were divided into two weight-matched groups and fed Purina lab chow; experimental group received 20% v/v ethanol as the drinking solution and controls received tap water. At 18d of pregnancy, experimental and control rats weighed 299 ± 11.2 and 271 ± 9.7 gm respectively ($p < 0.001$). After delivery, ethanol was replaced with tap water. Only male pups were retained after 2d of age. Mean ± SEM of number of pups/litter were not different; however, pups of ethanol-fed dams weighed significantly less as compared to controls. At 4 wks of age, the data (Mean ± SEM) were:

Animals	T4 µg/dl	FT4 ng/dl	T3 ng/dl	rT3 ng/dl
Controls (10)	3.5 ± 0.59	2.7 ± 0.59	72 ± 18	5.9 ± 1.8
Exp (10)	2.5 ± 0.92	1.5 ± 0.72	48 ± 15	6.0 ± 3.2
p	< 0.02	< 0.001	< 0.01	NS

The data suggest adverse in utero effect of ethanol ingestion on thyroid hormones. (Supported by Veterans Administration)

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ROLE OF NUTRITION IN THE EFFECTS OF CHRONIC ETHANOL INGESTION ON THYROID HORMONES. M.L. Shank,* S.P. Singh, A.K. Snyder,* B.B. Blivaiss and B.N. Premachandra.* Chicago Medical School and VA Med. Center, No. Chicago, IL 60064 and VA Med. Center, St. Louis, MO 63125.

Prolonged ethanol (ETOH) ingestion as drinking solution decreased serum T4 and T3 levels in adult rats (Life Sci. 25: 889, 1979). Since changes in food consumption secondary to ethanol ingestion might have a role in these observed changes, studies were done wherein male adult Sprague-Dawley rats were given 20% or 36% (calorie-wise) ethanol liquid diet and controls received isocaloric (CH_2O substituted for ethanol) liquid diet under pair-feeding technique. Daily total caloric intake of 36% ETOH-fed animals was nearly half (50 ± 2.5 Kcal) as that of 20% ETOH-fed rats (100 ± 3.7 Kcal). After 3 weeks the following data (Mean ± SEM) were observed.

EXP	Gain in B.W. (gm)	T4 (µg/dl)	T3 (ng/dl)	rT3 (ng/dl)
Controls (16)	90 ± 11.8	4.4 ± 0.19	96 ± 6.4	6 ± 0.5
20% ETOH (16)	103 ± 13.0	4.4 ± 0.26	104 ± 6.6	7 ± 0.5
Controls (6)	15 ± 8.4	3.0 ± 0.15	79 ± 5.8	8 ± 1.5
36% ETOH (6)	18 ± 9.8	2.8 ± 0.24	76 ± 5.3	8 ± 0.7

Since serum T4 and T3 were decreased in rats ingesting less food (36% ETOH-fed) and their controls (pair-fed isocaloric control diet), it seems that nutrition plays a role in effects of chronic ETOH ingestion on circulating thyroid hormone levels. (Supported by Veterans Administration)

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POSSIBLE MECHANISMS OF ACUTE vs CHRONIC ADMINISTRATION OF OVARIAN INHIBIN-CONTAINING PREPARATIONS IN THE CONTROL OF FSH SECRETION. Timothy P. Condon* Robert E. Leipheimer* and John J. Curry. (SPON: R.P. Fiorindo) Dept. of Physiology, Ohio State Univ. Columbus, Ohio, 43210.

Acute injection of 1.0ml porcine follicular fluid (PFF) into ovariectomized female rats resulted in a significant decrease in plasma FSH levels relative to both barrow serum (BS) and saline injected controls. Animals treated chronically (0.5 ml twice daily for 3 days) showed plasma FSH levels similar to chronically treated BS controls. Pituitaries from animals acutely treated with PFF showed an increased responsiveness to LHRH as compared to BS controls. Chronically treated animals showed no significant differences. Extracts of hypothalami (HX) from all chronically treated animals (PFF and BS) had similar FSH releasing activities as measured by bioassay; FSH release following addition of HX to pituitary glands in vitro. HX from acutely treated animals had a slight but nonsignificant stimulatory effect of FSH secretion relative to HX from chronically treated animals. However, median eminences (ME) from estrogen-progesterone primed rats incubated with an extract of PFF (PFFX, M.W. 10-30,000 d) showed lower LHRH content than ME's incubated with a similar preparation of BS (BSX). Media from PFFX treated ME's appeared to have a slightly lower LHRH content than ME's treated with BSX and nontreated controls. These data suggest that the observed differences between acute and chronic administration of PFF may be a result of actions at the pituitary and hypothalamic levels.

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SPECIFIC PARATHYROID HORMONE BINDING SITES IN CULTURED CHICK BONE CELLS. N.B. Pliam* K.O. Nyiredy* C.M. Silve* and C.D. Arnaud. Univ. of California and VAMC, San Francisco, CA 94121.

Parathyroid hormone (PTH) has been shown to increase the cAMP content of cultured murine and chick calvaral cells by stimulating adenylate cyclase. To date however, skeletal PTH receptors have not been demonstrated. In the present study we report that PTH binds specifically to chick bone cells in monolayer culture and stimulates cAMP accumulation in a manner that suggests coupling of the two functions. Cells obtained from 18 day chick embryo calvaria were grown to confluence in 35 mm culture dishes. PTH binding was assayed by adding fresh media containing electrolytically labeled, receptor purified [^{125}I]-bPTH (1-34) with or without unlabeled hormone. Cellular radioactivity was measured after washing. The cells specifically bound 3-8% of the label. Nonspecific binding (that which occurred in the presence of 1μg/ml unlabeled hormone) comprised 0-5% of the specific. Steady state occurred after 60 min. Scatchard analysis of displacement revealed a K_d of 3 ng/ml. Insulin glucagon and calcitonin did not compete for binding. Cyclic AMP accumulation showed a dose response curve similar to that describing competitive displacement of the hormone, with half maximal stimulation occurring at 6 ng/ml. bPTH(1-84) proved equipotent with bPTH(1-34) in its abilities to displace the labeled hormone and to stimulate cAMP. The similarity of the doses of PTH causing half maximal stimulation of cAMP and half maximal displacement of binding suggests that the PTH binding sites may be involved in adenylate cyclase activation. (Supported by NIH Grant#AM21614)

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STIMULATION OF GLUCONEOGENESIS IN RAT HEPATOCYTES BY PALMITATE DESPITE SIMULTANEOUS INHIBITION OF PALMITATE OXIDATION. S.A. Blumenthal* (SPON: A.M. Moses). VA Medical Center, Syracuse, N.Y. 13210.

Metabolic studies were done on hepatocytes isolated from 100-200 gm Sprague-Dawley rats after a 48-hour fast. 0.5 mM palmitate (pal) bound to 1% defatted albumin increased glucose (G) formation from 5 mM pyruvate 1.7 times, ketoacid (KA) production 2 times, and the betahydroxybutyrate (BOHB): acetoacetate (AcAc) molar ratio 3-5 times. These increases were all highly significant. Addition of 0.5 mM (+)-octanoylcarnitine (OC) blocked the stimulation of ketogenesis - but not of glucose production - by palmitate. Data from a representative experiment follow (production rates in μmol/mg DNA/30 min).

Exp. Gp.	No.	G Prod.	KA Prod.	BOHB/AcAc
Pyruvate	10	.394±.052	.207±.021	0.28±.05
Pyruvate+Pal	10	.549±.032	.687±.059	1.60±.08
Pyruvate+Pal+OC	10	.553±.050	.249±.026	0.37±.06
Pyruvate+OC	12	.344±.061	.206±.041	0.27±.06

1mM octanoic acid caused a fourfold increase in ketogenesis without affecting basal rates of glucose production from pyruvate. Conclusion: during a fast, hepatic gluconeogenesis is stimulated by intact long-chain fatty acid molecules (perhaps in the form of coenzyme A thioesters) rather than by products of fatty acid oxidation.

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HORMONAL REGULATION OF GLYCOGEN SYNTHASE PHOSPHORYLATION IN SKELETAL MUSCLE. J.L. Chiasson, J.H. Aylward*, H. Shikama* and J.H. Exton. Howard Hughes Medical Institute and Department of Physiology, Vanderbilt University, Nashville, Tenn. 37232

The effects of epinephrine (E) and insulin (I) on the phosphorylation and kinetics of glycogen synthase (GSase) were studied in skeletal muscle. Hindlimbs from fed rats weighing 180-220 g were perfused with Krebs' solution containing 33% aged RBC, 4% BSA and 10 mM D-glucose. Saline (S) with or without E (10^{-7} M) and/or I (1 mU/ml) was infused for 20 min. The treated muscle was then immediately extracted and homogenized in cold buffer containing EDTA, NaF and protease inhibitors. GSase was purified over 2000-fold to near homogeneity with a final specific activity of 40 U/mg of protein. A major step in the purification was the use of a Concanavalin A-sepharose column. Enzyme kinetics did not change during the purification procedure as assessed by GSase activity ratio (-G6P/+G6P) and K_a for glucose-6-P (G6P). The GSase activity ratio was 0.16 in the S group, 0.04 in the E group, 0.16 in the I group and 0.04 in the E + I group. K_a for G6P was 0.15 mM in the S group, 1.0 mM in the E group, 0.19 mM in the I group and 1.2 mM in the E + I group. Chemical phosphate content was 3.1 moles of Pi/85,000 subunit in the S group, 4.9 moles in the E group, 2.6 moles in the I group and 4.7 moles in the E + I group. Our data demonstrate good correlation between kinetics and phosphorylation state of GSase in response to hormonal manipulation. (Supported in part by NIH grant AM 18660.)

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DIFFERENTIAL EFFECTS OF GLUCOCORTICOIDS ON RAT CARDIAC AND SKELETAL MUSCLE GLYCOGEN. James L. Poland and Jerry W. Poland* Med. Coll. of Va., Richmond, Va. 23298

Glycogen recovery in rats following exercise differs in cardiac and skeletal muscles and glucocorticoid dependence has been suggested. The current study was to determine if glucocorticoids would differentially affect glycogen levels in cardiac and skeletal muscles. After dexamethasone administration (400 μgm IP) myocardial glycogen increased from 4.5 to 10.6 mg/g within 6 hours and remained elevated (7.4 mg/g) at 26 hours post-injection. Skeletal muscle glycogen, however, was slower to rise and peaked 17 hours after injection. The increase was from 5.0 to 8.6 mg/g for the soleus; 5.0 to 10.8 mg/g for the RVL; and 4.8 to 13.0 mg/g for the WVL. Concurrently, blood glucose remained stable; liver glycogen increased within 2 hours from 51.9 to 62.7 mg/g and continued to rise, peaking at 17 hours (87.0 mg/g); and plasma FFA levels rose within 2 hours from 299 to 488 μeq/L and remained at this elevated level throughout the 26 hour period. It is concluded that glucocorticoids do differentially affect cardiac and skeletal muscle glycogen. This difference suggests that after exercise, the slow response of skeletal muscle glycogen to glucocorticoids plus the brevity of the elevated plasma corticosterone and FFA levels preclude glycogen supercompensation from readily occurring in skeletal, but not cardiac, muscle. High FFA levels can inhibit glycogenolysis and might be an intermediary for glucocorticoid-induced glycogen changes. (Supported by a grant from the Muscular Dystrophy Association).

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BETA ECDYSONE STIMULATION OF GLUCOSAMINE-6-PHOSPHATE SYNTHESIS IN THE CRAYFISH. J. Ross Stevenson, Kent State Univ., Kent, OH 44242.

Rate of synthesis of glucosamine-6-phosphate (GlcN-6-P) in the epidermis of the crayfish Orconectes is correlated with rate of chitin synthesis, and chitin synthesis can be stimulated by injections of β-ecdysone. Therefore, it was expected that β-ecdysone might also stimulate synthesis of GlcN-6-P, which is an intermediate in the chitin synthetic pathway. Crayfish were paired according to size, sex, and molting stage and one member of each pair was injected with 0.2 μg β-ecdysone/g body weight. After 24 hours, epidermal homogenates from both animals were tested for ability to convert fructose-6-phosphate to GlcN-6-P. Rate of GlcN-6-P formation/μg DNA was always higher in homogenates from the hormone-injected animals.

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FOLATE-DEPENDENT GROWTH OF HUMAN CELL LINE K562 IN VITRO. David Watkins* and Bernard A. Cooper. Dept. of Physiology, McGill University, Montreal, Canada, H3G 1Y6.

K562, an established line of human leukemic cells with erythroid features, has been examined to evaluate its requirement for folate and the effect of folate supplementation on the velocity of cell growth. Cells transferred from replete culture medium (RPMI 1640) to F15 medium containing folic acid (PteGlu) remained in logarithmic growth with a doubling time of about 32 h. Omission of PteGlu from the F15 medium resulted in interruption of accumulation of cells with 1 generation. Doubling time was inversely proportional to folate concentration between 1 and 100 nM folate, 5-$CHOH_4$ PteGlu supporting more rapid growth than comparable concentrations of PteGlu. The majority of intracellular folate was polyglutamate folate, of which about one half was in the form 5-$CH_3H_4PteGlu_n$. The intracellular concentration of folate in cells in active logarithmic growth was within 1 log of that of cells grown in inadequate folate. This suggests that growth rate of K562 is limited by folate and, unlike in human fibroblasts, folate-dependent growth is interrupted without considerable decrease of intracellular folate.

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PAIR-FEEDING REGULAR YELLOW MICE REDUCES THE INSULIN:GROWTH HORMONE RATIO. V. E. Mendel. Dept. Animal Physiology, Univ. of California, Davis, CA 95616

Regular yellow mice (A^{vy}) have been shown to have a larger insulin:growth hormone ratio (I/GH) than their black, lean littermates (C57). The objective of this experiment was to determine whether the ratio is genetically preset. Forty-four C57 and 60 A^{vy} male mice were divided into pens containing 2 or 3 mice. They were fed ad libitum for 2 weeks at which time 54 of the A^{vy} mice were pair-fed to the C57 littermates for 3 wks; the remaining 6 A^{vy} mice were fed ad lib. (AL). Decapitation of 25 C57 and 29 A^{vy} mice occurred at this time for determination of plasma hormones plus body composition. Nineteen C57 and 16 A^{vy} mice were pair-fed for an additional 14 da.; 14 previously pair-fed A^{vy} mice were fed ad lib. (PF + AL). Insulin was significantly greater in the AL A^{vy} mice ($P < 0.05$) than in all groups except the PF + AL group. Both growth hormone and insulin were significantly reduced in A^{vy} mice when pair-fed (C57 vs A^{vy}, $P < 0.05$). The I/GH ratio was similar in all groups except AL A^{vy} ($P < 0.05$). Body fat was significantly greatest in AL A^{vy} ($P < 0.01$) and significantly greater in PF + AL when compared with C57 ($P < 0.05$). Body fat in pair-fed A^{vy} was comparable to that in C57. These data suggest that the hormone ratio does not determine body composition.

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METABOLIC ALTERATIONS ASSOCIATED WITH INCREASED ACTIVITY IN FISH WHITE MUSCLE. William R. Driedzic, Glen McGuire* and Mitchell Hatheway*. Mount Allison University, Sackville, N. B. EOA 3CO.

Time course measurements of glycogen, lactate, creatine phosphate, the adenylates and ammonia contents were made during the transition from rest to various levels of activity in fish (Macrozoarces americanus) white muscle. The muscle was perturbed by direct electrical stimulation resulting in sustained tetanus, 60 contractions/min or 20 contractions/min. The activity was invariably associated with decreases in creatine phosphate followed by increases in lactate levels. Of the metabolites measured creatine phosphate appeared singularly important in the facilitation of glycolytic flux presumably by relief of inhibition of phosphofructokinase. Ammonia levels oscillated indicating that this compound can be fixed in the muscle during the activity period. Under some conditions the work transition was associated with an initial 25% transient increase in ATP content which could not be accounted for by decreases in ADP and AMP levels. It is suggested that an early event in the activation of energy metabolism is a net increase in total adenylates.

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THE SLIMMING DRUG EPHEDRINE DIRECTLY STIMULATES THERMOGENESIS IN BROWN ADIPOCYTES VIA β-ADRENORECEPTORS. L. Jahjah*, N. Folléa* and L. Bukowiecki. (SPON:C. Fortier). Dept. of Physiology, Medical School, Laval University, Québec, C1K 7P4, Canada.

Ephedrine is a sympathomimetic drug that stimulates thermogenesis in man and laboratory animals. Considering that brown adipose tissue is the principal site of catecholamine-induced thermogenesis in homeotherms, we tested whether ephedrine would stimulate brown adipocyte respiration in vitro. Addition of ephedrine (10^{-4}M) to rat brown adipocyte suspensions rapidly stimulated cellular respiration 8 times above basal values, a stable Vmax of 355 nmol O_2/min/10^6 cells being reached in less than 5 min. This value represents 85% of the maximal respiration observed with norepinephrine, the physiological effector of thermogenesis (L. Bukowiecki et al. Am. J. Physiol. 238, (1980), in press. (-)-Ephedrine ($\frac{1}{2}$Vmax=2.10^{-5}M) was more potent than other stereoisomers((+)-Ψ-ephedrine, (-)-Ψ-ephedrine, racephedrine) in enhancing brown adipocyte respiration. β-Adrenergic antagonists (alprenolol and propranolol) were much more effective than α-adrenergic antagonists (phentolamine and phenoxybenzamine) in inhibiting the respiratory effects of ephedrine. It is concluded that ephedrine mimicks the calorigenic action of norepinephrine by directly stimulating brown adipocyte respiration via β-adrenoreceptors. This suggests that the slimming action of ephedrine observed in vivo is mediated by the activation of thermogenesis in brown adipose tissue.

(Supported by the MRC).

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DIVING ENERGETICS AND INTERMEDIARY METABOLISM IN THE HARBOR SEAL, *PHOCA VITULINA*. Randall W. Davis* and Gerald L. Kooyman. Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, California 92093.

The energetics and intermediary metabolism of lactate and glucose were studied in resting and diving harbor seals using a twin tracer infusion method. Catheterized seals were restrained in a specially designed plexiglass domed chamber which limited their movements but required no straps. Primed continuous infusions of ^{14}C-lactate (day 1) and ^{14}C-glucose (2 days later) were made and serial blood samples drawn two hours before and after a ten min dive. Metabolic rate and the production of $^{14}CO_2$ were measured. Dives were performed without struggling by filling the chamber with water. The kinetic relationship between lactate and glucose was determined using appropriate mathematical models for the pre-dive steady state condition and during non-steady state conditions after the dive. Steady state net turnover rates for lactate (60 μmol min^{-1} $kg^{-0.75}$) and glucose (30 μmol min^{-1} $kg^{-0.75}$) were similar to those for other mammals, but oxidation rates were low (less than 20% and 10% for lactate and glucose respectively). Low oxidation rates persist after dives despite large increases in circulating lactate and glucose concentrations. Anaerobic energy production during quiet dives is less than 25% of the non-diving, resting metabolic rate. (Supported by USPHS, NIH Grant No. HLB 23864)

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SEASONAL CHANGES IN METABOLIC CAPACITY AND NOREPINEPHRINE THERMOGENESIS IN THE ALASKAN RED-BACKED VOLE: ENVIRONMENTAL CUES AND ANNUAL DIFFERENCES. Dale D. Feist. Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

Wild red-backed voles (Clethrionomys rutilus) were tested for maximum metabolic rate (M_{max}) and for metabolic response to norepinephrine (M_{NE}) (injection of 2 mg NE·kg^{-1}) in September, November and January. During the same period, voles born and raised in the laboratory were acclimated (for 3.5 mo) in the following groups: 1) +20°C and 24 h light daily (LD 24:0); 2) +20°C and LD 4:20; 3) gradual change from +5°C and LD 14:10 to -5°C and LD 4:20; 4) gradual change from +5°C to -5°C and continuous LD 4:20, and tested for M_{max}. During seasonal acclimatization of wild voles M_{max} increased from 16.23±.61mlO_2·g^{-1}·h^{-1} in Sept. to 20.47±.50 in Nov. to 23.05±1.19 in Jan. and M_{NE} increased from 8.87±.52mlO_2·g^{-1}·h^{-1} to 12.32±1.17 to 14.2±.86. Peak winter M_{max} and M_{NE} in these wild voles were lower than found in a previous winter. In voles acclimated to +20°C and either long or short daily light period M_{max} remained unchanged from Sept. to Jan. In voles acclimated to increasing cold and decreasing or short light period M_{max} increased from 14.40±.64mlO_2·g^{-1}·h^{-1} in Sept. to 20.0 mlO_2·g^{-1}·h^{-1} in Jan. The results indicate that the magnitude of seasonal change of M_{max} and M_{NE} may vary from year to year and suggest that cold is essential to stimulate these seasonal changes in red-backed voles. (Supported in part by USPHS NIEHS Grant #ES00689 and NIGMS Grant #GM10402)

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STEARATE OXIDATION IN THE ALASKAN RED-BACKED VOLE: EFFECTS OF COLD AND NOREPINEPHRINE. Robert G. White* and Dale D. Feist. Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

In order to assess the role of free fatty acids (FFA) in substrate metabolism in cold acclimation, [1-^{14}C] stearate was injected (i.p.) into warm (20°C) and cold (5°C) acclimated voles (Clethrionomys rutilus) subjected to cold or norepinephrine (NE). Stearate irreversible loss (IL) was estimated by isotope dilution while stearate oxidation (SO) was estimated as percent recovery of ^{14}C in expired air. In red-backed voles, cold exposure (-2 to -5°C) or NE injection (s.c., 2 μg/g) elevated the concentration of FFA in plasma by 64% (to 2.8 mM/l). This rise in FFA was associated with a differential stearate metabolism and oxidation in warm vs. cold acclimation. In warm acclimated voles the rise in FFA was associated with a constant IL of stearate (6.0 μM/g·h). However, SO increased from 1.9 to 4.0 μM/g·h such that heat production from stearate was proportioned to metabolic rate. In contrast, in the cold acclimated voles the rise in FFA was assoicated with a 57% increase in IL (to 7.8 μM/g·h) and a 378% increase in SO such that stearate made an increasing contribution to heat production (from 22 to 40%) as metabolic rate increased. The results suggest that during cold or NE induced thermogenesis in cold acclimated voles, stearate is a preferred substrate. (Supported by USPHS Grant #GM10402)

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INCREASED EXCRETION OF WATER, UREA, Na^+ AND K^+ DURING HEAD-DOWN-TILT HYPOKINESIA: A QUESTION OF DIET OR ANTIORTHOSTASIS

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In the rat antiorthostatic hypokinesia (AOH) produces changes characteristic of human bedrest and weightlessness, including diuresis, natriuresis, increased excretion of nitrogen and muscle atrophy. A study was conducted to assess whether these changes were the result of the AOH state or due to dietary influences. Ad libitum food and water intakes of AOH rats were determined. One group was pair fed while receiving water ad libitum and a second group was pair watered while receiving food ad libitum. Pair watering did not result in either a diuresis or a natriuresis. In the pair fed subjects there was neither a natriuresis nor an elevated urinary output of urea N. Pair-watered and pair-fed animals grew at a rate similar to ad libitum fed and watered subjects and at a more rapid rate than AOH rats. Wet weights of heart and gastrocnemius in pair-fed rats were similar to those of ad libitum fed and watered subjects and significantly greater than those of AOH rats. It is concluded that the diuresis, natriuresis, increased urinary output of urea and muscle atrophy are the result of the AOH and are not due to changes in dietary intake. (Supported by NASA grants NSG-2325 and NAS2-10510).

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ACCELERATION RESPONSES OF ENDURANCE TRAINED AND DETRAINED DOGS. John B. Charles*, David C. Randall, and Daniel R. Richardson. Dept. of Physiology, University of Kentucky Lexington, Kentucky 40536

The responses of arterial pressure (AP) and heart rate (HR) in four endurance trained (T) and subsequently detrained (DT) dogs to 2G accelerations were examined. A protocol of successive +2Gz (head-to-tail), -2Gz (tail-to-head) and ±2Gy (side-to-side) loadings were provided by a centrifuge. Measurements were made with the animals reflexive and nonreflexive (alpha and beta adrenergic and cholinergic blockade). Control values at 1G showed no significant differences in AP or HR between the T and DT states in either the reflexive or nonreflexive conditions. At +2Gz there were no differences in AP between the T and DT states in the reflexive condition, although dogs in the T state had a significantly higher HR. The changes in AP and HR at +2Gz in the nonreflexive condition were larger in the T than DT state. At -2Gz in the nonreflexive condition dogs in the T state displayed larger values for HR and AP compared to the DT state. At ±2Gy in the nonreflexive condition the T state showed a higher elevation in HR and AP than the DT state. These results indicate that detraining from an endurance trained state does not diminish the AP response to a +Gz (orthostatic) stress. However, the mechanism of the response is affected, with HR adjustments playing a greater role in the trained animal. (Supported by NIH grant HL 19343 and AFOSR contract #F44620-74-6-0012).

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THE NON-RELIABILITY OF TAIL VEIN HEMATOCRITS IN FASTED, DEHYDRATED OR HEAT-INJURED RATS. C. Kelly* and R.W. Hubbard. US Army Res. Inst. Environ. Med., Natick, MA 01760

Adult male rats (n=65) weighing ~ 500 g were allocated to one of four groups. Microhematocrits were obtained on blood from aortic or tail vein sticks or in-dwelling jugular cannulae.

Group	Aortic Hct	Jugular Hct	Tail Vein Hct
1) Fed (n=20)	43.0 ± 2.1	44.0 ± 2.0	48.0 ± 3.0
2) Fasted (n=20)	46.5 ± 1.7	45.5 ± 2.0	49.5 ± 2.0
3) No Water Fasted (n=20)	46.0 ± 2.0		50.0 ± 1.3
4a) Fed Pre-Heat (n=5)		45.8 ± 0.8	47.8 ± 2.0
4b) Fed Post-Heat (n=5)		49.4 ± 2.8	45.8 ± 1.8
4c) Agonal (n=3)		60.0 ± 8.0	45.3 ± 3.5

There were no significant differences between aortic or jugular hematocrits within either group 1 or group 2. Fasting for 24 h with water (Group 2) or without water (Group 3) increased the aortic hematocrit significantly ($p<.01$) over Group 1. Tail vein hematocrits were significantly higher than aortic hematocrits within groups 1, 2 and 3 ($p<.01$) but did not reflect differences due to fasting. The significant increase ($p<.01$) in the jugluar hematocrit of heated (group 4a vs 4b) or agonal heatstroked rats (group 4a vs 4c) was not apparent in tail vein hematocrits. Thus, tail vein hematocrits appear to be unreliable indicators of plasma volume changes.

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COMPARISON OF EXTREMITY TEMPERATURE CHANGES AFTER LOW BACK HEATING BY DIATHERMY AND ULTRASOUND. B. Rubal, C. Rozier*, L. Reeves*, and J. Parbhoo*. Texas Woman's Univ., Denton, TX 76204

In this study, lower extremity temperature changes induced by the application of therapeutic heat to the low back by diathermy (D) and ultrasound (U) were compared. Cutaneous temperatures from the heel, great toe, mid and lower calf were obtained bilaterally from 10 healthy subjects by telethermometry and liquid crystal thermography. All subjects were submitted to therapeutic heat for 10 minutes at a standard dosage. Mean temperatures (± SE) were compared prior to heating and at 0, 5, 10, and 15 minutes post-heating. Ambient room temperatures ranged from 22-23°C. Pre-therapeutic mid-calf temperatures were the highest and toe temperatures the lowest:

	Diathermy (°C)	Ultrasound (°C)
Mid-Calf	30.3 ± 0.2	30.2 ± 0.3
Lower Calf	29.9 ± 0.1	30.0 ± 0.3
Heel	26.3 ± 0.4	26.0 ± 0.7
Toe	23.6 ± 0.4	25.5 ± 0.7

No difference was noted between the ultrasound and diathermy groups at any post-heating interval. However, all post-heating lower extremity mean temperatures tended to decrease. These data suggest that there is similar change in lower extremity skin temperature after heating the lower back with diathermy and ultrasound.

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ALTITUDE TOLERANCE OF RATS AT VARIOUS AGES. B.A.Rattner and P.D.Altland. NIAMDD, NIH, Bethesda, MD 20205

The altitude tolerance of immature (24-34 days), young adult (130-140 days), and healthy old (600-625 days) ARS/Sprague-Dawley rats was assessed by changes in temperature (T_{re}), weight (BW), plasma enzyme activities, corticosterone (B) concentration, and survival. Groups of rats of each age category were placed adjacent to the chamber (P_B 752 Torr, 102m) or maintained in the hypobaric chamber at a P_B of 349, 321, 281, or 248 Torr (6096, 6706, 7620, or 8230 m) between 0800-1200 h. At the termination of the experimental period, altitude-exposed animals were returned to ground level, T_{re} and BW were determined, and rats were bled by cardiac puncture. The critical threshold for survival was 8230 m for immature rats, and 7620 m for young adults and old rats. Hypothermia directly related to hypoxic severity and elevated plasma B levels ($P<.05$) were charactertistics of immature rats at the survival threshold, although plasma enzyme activities were relatively unchanged. Plasma B and T_{re} were similar at all altitudes in young adult and old rats, but plasma aspartate aminotransferase (PAsAT) and lactic dehydrogenase (PLDH) activities were elevated ($P<.05$) at the survival threshold. Thus, T_{re} and B are important criteria for determining altitude tolerance of immmature rats, however, PAsAT and PLDH activities are more suitable criteria for assessing tolerance in young adult and old rats.

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ENHANCED PULMONARY VASCULAR REACTIVITY IN RATS EXPOSED TO, AND RECOVERING FROM, HIGH ALTITUDE. A. Tucker, K.J. Greenlees*, and N. Migally.* Colo. State Univ., Fort Collins, CO 80523.

Changes in pulmonary vascular (PV) reactivity during the progression and regression of pulmonary hypertension, produced by chronic hypoxia, have not been well characterized. Therefore, we exposed 22 male rats to a simulated altitude (A) of 18,000 ft (380 torr) for various periods up to 42 days. Another 22 rats were exposed to A for 44 days and then allowed to recover (R) at 5,000 ft (632 torr) for various periods up to 42 days. At appropriate times, altitude-exposed and paired control rats (residing at 5,000 ft) were studied using an isolated, perfused lung preparation. PV reactivity was evaluated using hypoxia (HX; 3% O_2), angiotensin II (AII; 0.25, 0.5, and 0.75 µg), and 5-hydroxytryptamine (5HT; 25, 50, and 75 µg). Pulmonary vasoconstriction induced by HX was reduced by Day A-3, remained depressed for the duration of the A exposure, and until Day R-28. Thereafter, HX responses were similar to those of control rats. AII-induced vasoconstriction was enhanced at Day A-7, remained increased through Day A-42, and throughout the entire R period. The potentiation was characterized by both increased sensitivity and reactivity. 5HT-induced vasoconstriction was also increased at Day A-7; primarily due to an increased sensitivity. This potentiation also continued through Day R-42. Thus, high altitude exposure inhibited HX responses, but enhanced the responses to AII and 5HT. This enhanced PV reactivity continued throughout the entire 42 day R period at low altitude. (Supported by NIH Grant HL 25815).

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LUNG DEVELOPMENT IN GUINEA PIGS DURING CHRONIC EXPOSURE TO COLD AND HYPOXIA. Andrew J. Lechner, Margaret J. Grimes*, Lynn Aquin*, and Natalio Banchero. Univ. Colo. Medical School, Denver Colorado 80262.

Weanling male guinea pigs (Cavia porcellus) of initial BW = 207-271 g and age 2-3 weeks were exposed for up to 16 weeks to combined cold (Ta=7°C) and hypoxia (ambient P_{O_2}=85 torr)(BW at sacrifice = 253-932 g). The body growth rate of these animals (C+H GP) was not different from that of control animals (CGP) raised at Ta = 22°C and an ambient P_{O_2} = 133 torr. After 3 weeks of cold and hypoxic exposure and at a mean BW = 400 g, the weight-specific lung volume (V_L, ml/100 g BW) was significantly increased to 3.59 ± 0.29 ($\bar{x}$ ± SEM) in the C+H GP, compared to 2.65 ± 0.16 in the CGP. However, by week 16 of exposure, there were no significant differences in V_L between the C+H GP (2.24 ± 0.08) and the CGP (2.04 ± 0.15). Similar relationships were found between the 2 groups for weight-specific alveolar and pulmonary capillary surface areas. While chronic exposure to both increased oxidative demand and reduced oxygen availability temporarily increased the pulmonary dimensions suitable for gas exchange, normal adult dimensions were ultimately achieved. Furthermore, the similarity of lung growth in cold plus hypoxia to that pattern reported during exposure to cold or hypoxia alone suggests a common and rather nonspecific response mechanism for such 'adaptive growth' in the lung. (Supported by the USPHS, NIH Grant HL-18145).

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CARDIORESPIRATORY RESPONSES TO INTESTINAL INJECTION OF CARBON MONOXIDE. James J. McGrath and Micahel T. Kopetzky. Texas Tech Univ. Health Sci. Ctr., Dept. of Physiol., Lubbock, TX 79430

Experiments were continued to further investigate the mechanism of carbon monoxide (CO) toxicity. Laboratory rats were anesthestized with urethane. A laparectomy was performed and the small intestine isolated according to the method of Poupa et al., (Nature 179:1080, 1957). The pylorus and ileo caecal valve were ligated and air or 100% CO (75 ml/kg) was injected directly into the small intestine. Heart rate and respiratory rate were monitored by impedance pneumography. Carboxyhemoglobin (COHb) concentration was determined spectrophotometrically. One hour after treatment COHb levels reached 72.3±9%. Compared to percent of initial values, respiratory rates were depressed in the CO-injected rats 30 minutes (96.0±8.8% control vs 76.8±8.6¢ experimental) and 60 minutes (93.4±5.0% control vs 53.2±6.0% experimental) after treatment. Heart rates, although somewhat more erratic were only slightly depressed 60 minutes after treatment (98±6.5% control vs 89.4±11.1% experimental). These results indicate that elevated COHb levels produced by intestinal injection of 100% CO produce a severe respiratory depression and have a relatively slight effect upon heart rate.
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EFFECT OF ACUTE AND CHRONIC HYPERCAPNIA ON O_2 TOLERANCE IN RATS J.M. Clark. Inst. Environmental Med., U of PA, Phila., 19104.

Groups of 16-52 rats were exposed to 100% O_2 or to O_2-CO_2 (P_{ICO_2} 60 torr) at pressures of 1.0, 1.5, 2.0, 3.0, and 4.0 ata. Prior to hyperoxic exposure, half of the rats were adapted to hypercapnia (P_{ICO_2} 60 torr) for 5 days. All exposures were continued to 100% mortality except at 1.0 ata where some rats survived for as long as 14 days before sacrifice. Exposure durations for 50% mortality (LD_{50}) in nonadapted (NA) rats at 4.0, 3.0, 2.0, 1.5, and 1.0 ata of O_2 were 6.3, 9.3, 17.2, 27.4, and 76.1 hours, respectively. Corresponding LD_{50} values for NA rats exposed to O_2-CO_2 were 2.0, 2.9, 16.3, 24.8, and 74.8 hours. Survival times of rats exposed to O_2 after CO_2-adaptation were nearly identical to those of NA rats at each pressure. LD_{50} values for adapted rats exposed to O_2-CO_2 at 4.0, 3.0, 2.0, 1.5, and 1.0 ata were 4.1, 7.5, 17.9, 23.6, 65.4 hours, respectively. These data confirm the known acceleration of O_2 intoxication by acute hypercapnia at 4.0 and 3.0 ata, but they show much less prominent effects at 2.0, 1.5, and 1.0 ata. Prior adaptation to chronic hypercapnia clearly has a protective effect in rats exposed to O_2-CO_2 at 4.0 and 3.0 ata. At 2.0, 1.5, and 1.0 ata, where acute hypercapnia has less effect, the effects of adaptation are also less prominent. The observed changes in O_2 tolerance can be explained by cerebral vasodilation with increased brain oxygen tension in acute hypercapnia, and by significant amelioration of this response during chronic hypercapnia. (Supported by NIH Grants HL22259 and HL08899.)

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EFFECT OF HYPERCAPNIA ON VENTILATION IN UNANESTHETIZED RATS IN HYPERBARIC ENVIRONMENTS. K.K. Shida* and Y.C. Lin. Univ. of Hawaii John A. Burns School of Medicine, Honolulu, HI 96822.

A whole-body plethysmographic method was used in this study utilizing a pressure-sensitive, volume-calibrated system for the indirect measurement of tidal volume. Experiments were conducted at atmospheric and at elevated pressures using compressed air or carbon dioxide (CO_2) in air. Respiratory frequency (f_r) and tidal volumes (V_T) were obtained from the pressure trace and minute ventilation (V_E) calculated and averaged over a 30 min. period. When compressed air was used, the inspired CO_2 tension (P_{CO_2}) was less than 2 Torr, and raising the ambient pressure over a range from 1 to 10 ATA caused no significant change in V_E ($P > 0.4$). There was a small, but significant increase in tidal volume ($P < .01$) and decrease in f_r ($P < .01$). However, for a P_{CO_2} of 35 Torr, V_E at 1 ATA was 69% greater than controls in air at 1 ATA. This ventilatory increase due to hypercapnia was only 29% when the ambient pressure was raised to 3 ATA. At a P_{CO_2} of 105 Torr, $\dot{V}_E$ increased 2.3 times at 1 ATA, but only 1.8 times at 3 ATA over their respective controls. The ability to quantify ventilatory parameters in small animals under conditions of elevated ambient pressures will greatly increase the value of the small animal model for determining physiological responses to hyperbaria under a variety of experimental manipulations. (Supported in part by Sea Grant No. NA 79AA-D-00085.)

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EXERCISE INDUCED DYSPNEA AT 46.7 and 65.6 ATA. R. E. Moon*, J.V. Salzano, E.M. Camporesi, B.W. Stolp*. F.G. Hall Environmental Laboratory, Duke University, Durham, N.C. 27710

Three volunteers performed multiple 6 minute bicycle exercises in a dry hyperbaric chamber as a part of the Atlantis deep dive series. Work levels while breathing air at 1 ATA ranged up to each subject's maximum aerobic capacity (1.1 g/L inspired gas density). 15-second MVV was measured for each subject at 1 and 46.7 ATA. At 46.7 ATA the subjects exercised at rates up to 1080 kp.m/min while breathing gas mixtures of 10.1 and 12.3 g/L (5 and 10% N2, 1% O2, balance He). At pressure, when breathing 10% N2, VO2 levels ranged from 72 to 79% of 1 ATA VO2 max; breathing 5% N2 they ranged from 65 to 92%. Arterial blood gases, VE and mean breathing frequency were measured at all exercise levels. In addition, tidal volume, V0.1 (mean flow during initial 100 ms of inspiration) and Vi (mean inspiratory flow) were calculated on a breath-by-breath basis. At constant VE, Vi was consistently lower at pressure than at 1 ATA. Dyspnea was reported only at pressure (2 subjects) and resulted in premature termination of one exercise. PaO2 was usually between 250 and 300 mm Hg (lowest 175). Resting PaCO2 at 46.7 ATA was 3-5 mm Hg above 1 ATA control; further increases (5-25 mm Hg) occurred during exercise. Dyspnea was not consistently related to hypercapnea, acidosis, VE or VE/MVV. The single best indicator of dyspnea was elevation of the ratio V0.1/Vi above 1.0. At 65.6 ATA the subjects breathed 8% N2 (16.1 g/L) and did not report dyspnea while exercising up to 720 kp.m-min (53% of 1 ATA VO2 max). Supported in part by NIH grant: HL07896-17 and MRC of Canada).

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REGULATION OF VENTILATION DURING CHRONIC EUPCAPNIC METABOLIC ACIDOSIS. W.G. Honer* and D.B. Jennings. Dept of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

P_{CO_2} is classically believed to alter $\dot{V}_E$ through changes in central or systemic [H+]. In order to examine this concept we have measured the ventilatory and acid-base responses to alterations in P_{CO_2} in 4 awake dogs during control and during chronic eucapnic metabolic acidosis. Dogs were tube-fed a special diet (Madias et al. J. Clin. Invest. 60: 1393, 1977). After 5-7 days, control measurements were obtained ($PaCO_2 \simeq 35$ torr); the diet was then supplemented with 5.5-7.0 mEq/kg HCl and $PaCO_2$ was maintained within normal limits through inhalation of 3% CO_2 ($PaCO_2 \simeq 33$ torr). Measurements were obtained at 5 and 7 days of acidosis. $\dot{V}_E$ increased in chronic eucapnic metabolic acidosis with an average slope of 0.02 ℓ/min/kg/nm [H+]a. Since cisternal CSF [H+] was unchanged, this modest increase in $\dot{V}_E$ could be attributed to increased peripheral [H+] drive. In contrast, during air control or eucapnic acidosis when arterial or CSF [H+] was increased by inhalation of 5% CO_2 the ventilatory response with respect to [H+] was much steeper (in the order of 0.14 ℓ/min/kg/nm [H+]a). Interestingly, acute hypocapnic acidosis resulted in a profound decrease in $\dot{V}_E$ with no change or an increase in [H+] CSF; [H+]a was unchanged or decreased slightly. In all cases, $\dot{V}_E$ was less than that predicted by the eucapnic ventilatory response to [H+]a. Thus, in acute hypocapnic metabolic acidosis $\dot{V}_E$ was dissociated from changes in arterial or CSF [H+]. (Supported by MRC and the Ontario Thoracic Society).

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ROLE OF ARTERIAL OXYGENATION AND OF THE CAROTID CHEMORECEPTORS IN THE VENTILATORY RESPONSE TO METABOLIC CO_2. E.A. Phillipson, G. Bowes*, E.R. Townsend*, J. Duffin*, and J.D. Cooper*. Departments of Medicine, Surgery, and Physiology, University of Toronto, Toronto, Ontario M5S 1A8

Removal of CO_2 from venous blood of awake sheep (with an extracorporeal membrane lung) at a rate equal to that of metabolic CO_2 production ($\dot{V}_{CO_2}$), results in isocapnic apnea (Fed Proc 39:584, 1980). Similarly, venous CO_2 loading through the membrane lung results in a response of minute volume of ventilation ($\dot{V}_E$) that is essentially isocapnic under normoxic conditions. In the present study we used the same preparation to examine the influence of arterial oxygenation and of the carotid chemoreceptors on the relationship between $\dot{V}_E$ and arterial P_{CO_2} ($\dot{V}_E/Pa_{CO_2}$) during venous CO_2 loading. Studies were performed in 3 intact sheep and in 3 sheep with denervated carotid chemoreceptors (D). In the intact sheep, $\dot{V}_E/Pa_{CO_2}$ was hypercapnic under hyperoxic conditions ($Pa_{O_2} > 200$ torr); isocapnic during normoxia (95-105 torr); and hypocapnic during hypoxia (60-70 torr). In D sheep, $\dot{V}_E/Pa_{CO_2}$ was again hypercapnic under hyperoxic conditions, but in contrast to the intact sheep, did not increase under normoxic conditions, and decreased (ie, was more hypercapnic) under hypoxic conditions. The results indicate that the response of $\dot{V}_E$ to changing $\dot{V}_{CO_2}$ is modulated by arterial oxygenation, and that the isocapnic response during normoxia is dependent upon the peripheral chemoreceptors. (Supported by Grants MA4606, MA5953 from MRC of Canada, Ontario Thoracic Society, Ontario Heart Foundation.)

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EFFECTS OF CHANGES IN CO_2 LOAD WITH BREATHING PATTERN ON VENTILATION IN MAN. Steven M. Lewis and R.D. Tallman, Jr.. Dept. of Biomed. Engineering, USC, Los Angeles, CA. 90007.

The optimal respiratory pattern for a given level of ventilation is a function of the dead space, or the way the inspired CO_2 load changes with TV. We wished to determine whether the respiratory controller is capable of detecting these changes. We studied 2 states: In state 1 the inspired PCO_2 was decreased by 5mmHg for each 10% increase in TV over a baseline value. Inspired O_2 was similarly increased by 5mmHg. In state 2 the inspired CO_2 and O_2 were varied with changes in frequency. Nine naive subjects started the study breathing 4% CO_2, 16% O_2 for 15 minutes. Baseline TV and F were determined from this data. The baseload was set so that if TV and F remained constant during states 1 and 2, the mean inspired CO_2 would also be unchanged. Loadings were applied on a double blind basis. Subjects were studied for 20 min. each under one state, the alternative state and then the original state. The predicted optimal pattern of breathing, a high TV relative to F in load 1 and a low F relative to TV in load 2 was never observed. Mean values were: TV 939, 1970; F 19.6, 20.0; VE 18.5, 21.4; P_ACO_2 40.2, 40.7; P_AO_2 72.9, 69.9 for TV and F loading respectively. The difference in VE was barely significant by paired T test, but was seen in all but one subject. Although these changes are small they suggest that the respiratory controller can sense changes in CO2 loading with respiratory pattern. This work was supported by HL07012 and HL21990.

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GLYCINE, LEUCINE AND LYSINE IN BRAIN AND CEREBROSPINAL FLUID DURING CO_2 NARCOSIS. R. E. Dutton, P. M. Stein* and H. Kazemi. Dept. of Physiology, Albany Med. Col., Albany, NY, 12208, and Med. Services (Pulmonary Unit), Mass. Gen. Hosp., Boston 02114.

Elevation of γ-aminobutyric acid (GABA) and reduction of glutamate has been observed in rat brain during prolonged hypercapnia (Weyne et al., Bull. Eur. Physiopath. Resp. 12:285, 1976). In the present study, amino acids in dog brain and CSF were determined after 1 hour of CO_2 narcosis. Anesthesia was initiated with Na methohexital and was maintained with N_2O and fentanyl. Following tracheostomy and paralysis with succinylcholine, the calverium was prepared for craniotomy. Narcosis was induced with 25-35% CO_2 in O_2 to raise Pa_{CO_2} to 200 mm Hg. At 1 hr cisternal CSF was sampled, $\dot{V}_E$ was measured (36±4 l/min), and craniotomy for sampling brain tissue was performed. The "supercarbia" produced acidosis (pHa=6.66±0.03 and pH_{CSF}=6.70 ± 0.03), and significant elevations of brain glycine (4.3±0.3 to 7.0±0.6 mg/100g), leucine (1.5±0.1 to 3.5±0.3 mg/100g), lysine (2.31±0.35 to 4.65±0.42 mg/100g), and glutamine. Brain glutamate decreased and GABA did not change. Highly significant increases in CSF leucine (0.27±0.04 to 0.46±0.03 mg/100ml), lysine (0.70±0.08 to 1.04±0.08 mg/100ml), and serine were observed. CSF glycine and GABA levels were too low to measure by our ion exchange chromatography technique. These results suggest that the putative neurotransmitters glycine and glutamate may participate in the development of CO_2 narcosis. Leucine and lysine levels in CSF may provide an index of brain glycine and glutamate levels during hypercapnia. (Support: NIH HL-12564)

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CO_2 EFFECTS ON THE V_T vs T_I AND T_E vs T_I RELATIONSHIPS IN DECEREBRATE CATS. T.L. Clanton*, W.T. Lipscomb, and F.J. Clark, Univ. of Nebr. Med. Center, Omaha, NE. 68105

In 12 decerebrate, paralyzed cats changes in inspiratory duration (T_I) and expiratory duration (T_E) were observed from a recording of phrenic nerve activity. Changes in V_T were produced by a servo-respirator which created various sized ramp inflations during inspiration. V_T vs T_I and T_E vs T_I curves were obtained during exposure to $FICO_2$= .04 and compared to preceeding and following control runs in which $ETCO_2$ was maintained at eupneic values. During CO_2 exposure the shape of the V_T vs T_I curve shifted upward such that more V_T was required for any particular value of T_I. Also, the slope of the T_E vs T_I curve was increased during the CO_2 runs. The results suggest that in decerebrate cats exposed to elevated CO_2, the sensitivity of the Breuer Hering inspiratory terminating reflex is reduced and the coupling between T_I and the following T_E is altered.

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CONTROL OF EXPIRATORY FLOW AND EXPIRATORY PAUSE DURING STEADY-STATE AIR AND ELEVATED CO_2 BREATHING IN MAN. Judith Ann Hirsch and Beverly Bishop. Physiology Dept.SUNYAB. Buffalo, NY 14214.

There are two components in the expiratory phase of the respiratory cycle: expiratory flow duration (T_{EF}) and expiratory pause (T_{EP}). The purpose of this study was to define the relative contributions of T_{EF} and T_{EP} to the breath-by-breath volume-timing relationship during altered ventilation. Five adults (22-32 yrs), wore a face mask and breathed air, 1.5, 3 or 5% CO_2 in air from a bag-in-box system while seated. Expiratory pneumotach flow and F_{CO_2} records were analyzed for a five minute control period on air and a 10-20 minute steady-state period on the experimental gas. The breath-by-breath relationship between tidal volume (V_T) and T_{EF} was linear for any of the steady-state conditions and the slope increased linearly from 0.24 ± 0.03 lps on air to 0.47 ± 0.12 lps on 5% CO_2. At higher inspired CO_2 levels, mean T_{EF} was not significantly changed, but mean T_{EP} progressively decreased. The decrease in mean T_{EP} was the major factor in the increase in breathing frequency, while breath-by-breath T_{EF} made the major contribution to the volume-timing relationship. These results show that T_{EF} and T_{EP} during steady-state air and CO_2 breathing are independently controlled. (Supported by NHLBI Grant PO1-HL-14414 and AF Contract #F41609-75-C-0003. JAH is an American Lung Association Fellow.)

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RESPONSE TO INTRAVENOUS AND INTRAPULMONARY CO_2 LOADING IN DECEREBRATE DUCKS. R. D. Tallman, Jr. and F.S. Grodins. Dept. of Biomedical Engineering, U.S.C., Los Angeles, Calif. 90007

The role of intrapulmonary CO_2 receptors (IPC) in ventilatory control was investigated by comparing the arterial blood gas and ventilatory responses to CO_2 loading via the inspired gas and via the venous blood. Adult, male Pekin ducks (2.66 kg. avg. wt.) were decerebrated one week prior to the experiment. Venous CO_2 loading was accomplished with a veno-venous extracorporeal blood circuit including a silicone membrane blood oxygenator. The birds breathed through a chronic tracheostomy. The protocol randomized four states; control (no loading), venous CO_2 loading, inspired CO_2 loading and venous CO_2 unloading. Extracorporeal blood flow was kept constant throughout the experiment. Intravenous and inspired loading both resulted in hypercapnic hyperpnea. For both types of loading as well as unloading, the change in ventilation from control could be expressed as ΔV_E (ml) = $103 \cdot kg \cdot \Delta P_aCO_2$. Ventilatory pattern was different however; inspired loads resulted in slower, deeper breathing than venous loading. Sensitivity to changes in arterial blood gas appears to be the mechanism involved in adjusting ventilation under these conditions. Since inspired and venous CO_2 loading most likely result in different IPC firing patterns, it is concluded the IPC play a minor role in adjusting ventilation to match increases in pulmonary CO_2 load but rather are involved in pattern determination. (This work was supported by HL07012).

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EFFECTS OF PNEUMOTHORAX ON AORTIC CHEMORECEPTOR RESPONSES. S. Lahiri, M. Pokorski*, A. Mokashi*, and E. Mulligan. Department of Physiology and Inst. for Environmental Med., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Responses of aortic body chemoreceptors in the cat to hypercapnia and hypoxia are blunted compared to those of carotid body chemoreceptors (Federation Proc. 38: 1141, 1979). The mechanisms for these differences are not known. Unlike the carotid body, aortic bodies, because of their location in the thoracic cavity, are usually not exposed during experiments. Whether such an exposure would account for the difference in response is not known. We studied steady state responses of aortic chemoreceptors to hypercapnia (3 to 4 levels of $PaCO_2$ at PaO_2> 400 torr) and hypoxia (4 to 5 levels of PaO_2 at a constant $PaCO_2$) before and after bilateral pneumothorax in 10 cats, that were anesthetized, paralyzed and artificially ventilated at 38°C. The extrathoracic left aortic depressor nerve was cut and single or pauci chemoreceptor afferents were studied. Opening of the thorax stimulated the chemoreceptor activity, although arterial blood gases and blood pressure remained the same. Hyperoxia decreased this stimulation. The steady state hypoxic but not the hypercapnic response was augmented. We conclude that the surgical exposure is not the reason for the difference between the aortic and carotid body chemoreceptor response patterns. (Supported in part by NHBLI Grant HL-19737-04).

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EFFECTS OF VAGAL AFFERENTS ON LARYNGEAL RESPONSES TO HYPERCAPNIA AND HYPOXIA. D. Bartlett, Jr. Department of Physiology, Dartmouth Medical School, Hanover, NH 03755.

To clarify the role of vagal afferents in the regulation of the laryngeal airway, the effects of hypercapnia and isocapnic hypoxia on respiratory movements of the vocal cords were studied in anesthetized cats before and after bilateral vagotomy. In vagally intact animals, these stimuli lowered laryngeal airflow resistance by increasing both inspiratory and expiratory vocal cord abduction through activation of the posterior cricoarytenoid (PCA) muscle. Vagotomy produced little change in the laryngeal response to hypercapnia, but led to an increase in expiratory laryngeal resistance during hypoxia owing to a decrease in expiratory PCA activity. Carotid arterial NaCN injection resulted in a similar increase in expiratory laryngeal resistance after vagotomy, suggesting that the response to hypoxia was due to carotid chemoreceptor stimulation. The laryngeal responses to both hypoxia and NaCN were abolished by bilateral carotid sinus nerve section. The results indicate that influences from central and peripheral chemoreceptors have different effects on the activity of motoneurons governing vocal cord movements during expiration. Vagal afferent feedback serves to maintain the patency of the laryngeal airway during hypoxia. (Supported by NIH Grants HL 19827 and RR 05392.)

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THE EFFECTS OF AIRWAY ANESTHESIA ON MAGNITUDE ESTIMATION OF ADDED INSPIRATORY RESISTIVE AND ELASTIC LOADS. N.K. Burki, P.W. Davenport, F. Safdar* and F.W. Zechman. Dept. of Medicine and Dept. of Physiology and Biophysics, University of Kentucky Medical Center, Lexington, KY 40506

The perceived magnitude of an added respiratory load can be expressed by a subject by squeezing a handgrip dynamometer. The relationship between log stimulus and log response (handgrip) is represented by the regression coefficient between these two variables (Steven's Power law). We studied the effect of anesthesia of the airways on this relationship in 5 healthy normal subjects. In each subject handgrip responses to suprathreshold, randomly applied inspiratory resistive loads and to inspiratory elastic loads were measured in the control state. Anesthesia of the upper and lower airways using 4% lidocaine was performed as previously described (Chaudhary and Burki, Clin Sci Mol Med, 1978, 54, 621). Adequacy of anesthesia was tested by the abolition of the cough reflex following inhalation of nebulized 20% citric acid solution. The exponents of the plots between log stimulus and log response (handgrip) for resistive loads (mean = 0.566) and elastic loads (mean = 0.516) did not alter significantly following anesthesia. These results indicate that anesthesia of superficial mucosal receptors in the upper or lower airways does not alter the magnitude estimation of added inspiratory resistive or elastic loads. (Supported in part by NIH Grant No. HL 24412)

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COUGHING TO IRRITATION OF THE TRACHEO-BRONCHIAL TREE AND THE LARYNX AFTER SULPHUR DIOXIDE INHALATION IN RABBITS AND DOGS. G. Sant'Ambrogio, F.B. Sant'Ambrogio and A. Davies. Dept. of Physiology and Biophysics, U.T.M.B., Galveston, Texas 77550.

Sulphur dioxide inhalation (200 p.p.m.) suppresses the inflation apnea in rabbits, but not in dogs and cats. In rabbits it blocks the slowly adapting airways receptors with the rapidly adapting irritant receptors remaining largely unaffected (Davies et al.,1978). We studied cough to mechanical irritation of the extrapulmonary airways and the larynx in 10 anesthetized rabbits and 3 dogs, breathing through a tracheal cannula, before and after inhalation of sulphur dioxide. In rabbits after 5 min of sulphur dioxide inhalation (300 p.p.m.)both inflation apnea and cough to tracheo-bronchial irritation were eliminated. Laryngeal irritation was still eliciting cough, though the response was attenuated. In dogs sulphur dioxide inhalation (300 to 1,000 p.p.m.) did not abolish either the inflation apnea or cough to the tracheo-bronchial irritation. In rabbits cooling of both vagus nerves to 8 centigrades abolished the tracheo-bronchial, but not the laryngeal cough.This indicates that only medullated fibers are involved in the tracheo-bronchial cough. These results seem to suggest a significant role of the slowly adapting airways receptors in the cough reflex from the extrapulmonary airways.
(Davies et al., Respir.Physiol.,34: 83-101.)
Supported by NIH grant RO1-HL 20122.

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A PHYSIOLOGICAL STIMULUS TO UPPER AIRWAY RECEPTORS IN MAN. W.A. Whitelaw and B. McBride*. University of Calgary, Calgary, Alberta, T2N 1N4

Reflex inhibition of breathing in animals caused by stimulation of upper airway receptors with water, chemicals, or touch is usually thought of as part of swallowing or diving reflexes. Human subjects rebreathed into a bag containing 8% carbon dioxide in oxygen until lung and bag gas were mixed. The mouthpiece was then occluded at FRC, and they held their breath with glottis open so that intrathoracic pressure could be measured in the mouth. Involuntary rhythmic contractions of inspiratory muscles produced negative pressure waves that increased in frequency and amplitude through the breath-hold. When gas from the rebreathing circuit was pumped into the nose and out of the mouth at flow rates up to 237 ml/s., both frequency and dp/dt of contractions were reduced. The inhibition was most marked with cold air and high flow rates, and when flow was confined to the inspiratory phase of contractions. It was abolished by upper airway anesthesia. The observations show that upper airway receptors sensitive to stimuli associated with quiet normal breathing can modulate respiratory centre activity. (Supported by the Canadian Thoracic Society and the Hill Foundation).

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EFFECT OF TETRODOTOXIN (TTX) ON RESPONSES OF RAT MESENTERIC VESSELS AND HAMSTER CHEEK POUCH ARTERIOLES TO NEURAL AND NON-NEURAL STIMULI. J.H. Lombard, M.J. Burke*, and W.J. Stekiel. Dept. of Physiol., Med. Coll. Wis., Milwaukee, WI 53226 USA

In low concentrations, TTX appears to specifically block nerves without affecting smooth muscle. To further verify this specificity, we studied the effect of TTX on resting diameters and in vivo responses to neural and non-neural vasoconstrictor influences in small principal arteries and veins of the rat intestinal mesentery and in 3rd and 4th order arterioles of the hamster cheek pouch. Animals were anesthetized and the mesentery or cheek pouch was exteriorized, transilluminated, and suffused with physiological salt solution (PSS). Vessel diameters were measured by image-splitting. Resting diameters and active constrictor responses were measured in the presence and absence of TTX (10 µg/ml) in the PSS. Constrictor stimuli included perivascular nerve stimulation (PVNS) and norepinephrine suffusion (10 µg/ml) in the rat mesentery, and high oxygen suffusion in the cheek pouch. TTX had no effect upon resting diameters of mesenteric arteries or cheek pouch arterioles. Mesenteric veins exhibited a small but significant diameter increase in the presence of TTX. The response to PVNS was completely and reversibly blocked by TTX, but the response of all vessels to non-neural constrictor influences was identical in the presence and absence of TTX. These data indicate that TTX can be used to study active neurogenic tone of in vivo microvessel preparations. (NIH #HL 22972 and GM23594).

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DOES A DELETERIOUS ENVIRONMENT FOR RED CELLS DEVELOP IN THE SPLENIC PULP WHEN ABNORMAL CELLS BECOME TRAPPED? A.C. Groom and S. Nealon*. Department of Biophysics, University of Western Ontario, London, Canada. N6A 5C1.

It is commonly taught that the splenic pulp presents a hostile environment for red cells *on account of* low pH, P_{O_2} and substrate deprivation. We have shown previously (Am. J. Physiol. 231: 1672-1678, 1976; Adv. Exp. Med. Biol. 94: 567-572, 1978) that this is true only when the splenic arterial inflow is completely occluded. However, flow of red cells through the pulp can also be impeded rheologically, following trapping of abnormal cells (J. Lab. Clin. Med. 90: 666-679, 1977) and we have tested the hypothesis that, under these conditions, the availability of O_2 and glucose might be reduced and a decline in pH might occur. When the spleen is drained of blood after tying off the artery, the hematocrit of the outflow rises gradually from 35-40% (arterial) to 75-80%, the last fraction representing a pure sample of blood from the splenic pulp. One hour after intravenous injection of 2.5 x 10^9 heat-treated autologous red cells in anesthetized cats we used the splenic drainage procedure and measured pH, P_{O_2}, and glucose concentrations in samples collected anaerobically from the splenic vein. The values found were not significantly different from those in control spleens, showing that our hypothesis is incorrect. It seems likely that plasma flow through the pulp prevents the development of a deleterious environment even when red cells are themselves immobilized. (Supported by the Medical Research Council of Canada)

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EFFECTS OF METHYLPREDNISOLONE ON THE MICROVASCULATURE OF THE CONTUSED SPINAL CORD. E.D. Means* & D.K. Anderson, VA Medical Center, Cincinnati, Ohio 45220.

Contusion of the feline spinal cord (SC) causes an 80-90% decrease of SC microvascular perfusion (MVP) by 8hrs postinjury. Methylprednisolone (MP) given at 1hr postinjury increases SC MVP to 60% of control at 8hrs. The present study determined the caliber of SC vessels protected by MP. The L2 segment of cats was compressed with 170gms/5min & sacrificed at 8 or 24hrs post injury by perfusion fixation. 5 cats each were injected at 1hr postinjury with 5mg/kg MP or at 1, 8 & 12hrs with 5mg/kg MP & sacrificed at 8 & 24hrs respectively. Controls were uninjured & injured untreated cats. The diameter distribution of SC blood vessels in gray matter was calculated from slides using a camera lucida attachment & digitizer. Histogram plots of vessel diameters were calculated for tissue at the center & both poles of the lesion. Injured untreated cats showed: a) 2 & 3x increase at 8 & 24hrs respectively in 4-6µ diameter vessels; & b) 50% decrease in vessels ranging between 8-35µ at the center & at both poles of the lesion. The # of vessels at the center of the lesion in MP treated cats were the same as injured controls at both 8 & 24hrs, but at the lesion poles the # of vessels in the ranges 4-6µ & 8-10µ were increased above injured controls. These data suggest MP treatment preserves injured SC MVP in the 4-10µ range at the poles of the lesion. We speculate that MP treatment protects variably damaged vessels in tissue perijacent to the lesion perhaps limiting the extent of the lesion. (Supported in part by the VA & the Upjohn Co.)

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EFFECT OF NOREPINEPHRINE ON ARTERIOVENOUS ANASTOMOTIC AND CAPILLARY BLOOD FLOW IN THE ISOLATED CANINE FORELIMB. J.T. O'NEILL*, B.T. SWINDALL*, AND F.J. HADDY. Physiol. Dept. Uniformed Services University, Bethesda, MD 20014

Previous studies from this laboratory (Fed. Proc. 39:273, 1980) suggest that a large proportion of intra-arterially (IA) injected 15µ microspheres bypass the capillaries of the isolated canine forelimb via arteriovenous anastomoses (AVA). Since AVA are well innervated we wondered whether norepinephrine (NE) would also affect AVA flow. The right forelimbs of 6 anesthetized dogs were isolated except for the artery, veins and nerves and were perfused at constant flow (130 ml/min). Radioactive microspheres (RAM) were injected upstream to the infusion pump for evaluation of capillary blood flow and RAM shunt. The brachial and cephalic veins were cannulated for measurement of outflow and withdrawal of samples at a constant rate to evaluate RAM shunt. After a 30 min control period, NE was infused IA for 1 hr. (1 µg/min for 30 min then 2 µg/min for 30 min). RAM was injected just prior to the start and end of the NE infusion. Forelimb perfusion and systemic arterial pressures were measured. NE caused a sustained increase in perfusion pressure. About 30% of cephalic outflow shifted to the brachial vein. Capillary blood flow was increased in both skin and muscle. NE reduced RAM shunt from 59% to 4% of the total injected. This reduction in shunted RAM suggests that NE constricts AVA resulting in an increase in capillary blood flow.

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LYMPH FLOW AND PROTEIN CONCENTRATION IN THE DOG FORELIMB AS AFFECTED BY CAROTID OCCLUSION. Dabney, J., J. Mulcahy*, C. Soika* and D. Dobbins. Dept. Physiol. USUHS Bethesda, MD 20014

Inciarte et al suggested that skin lymph vessels of the dog forelimb constrict in response to norepinephrine (Fed. Proc. 39:273, 1980). The present study tested if carotid occlusion would affect the increased lymph flow and protein concentration induced by local infusion of histamine. In forelimbs perfused at constant flow, lymph flow and its protein concentration were measured. Systemic pressure, forelimb perfusion pressure, and skin small artery and vein pressure were recorded every ten minutes. The protocol was; a control period, 30 minutes of histamine infusion (4 µg base/min), 60 min of histamine and carotid occlusion, and 30 min of histamine alone. Histamine increased lymph flow and protein concentration. Bilateral carotid occlusion increased all pressures for the duration of the occlusion. Lymph protein concentration peaked at 30 minutes of histamine infusion and was not changed during carotid occlusion or after release. Lymph flow was not changed for 20 minutes of occlusion but averaged below the histamine peak after 30 minutes of occlusion. If the catecholamines released by 1 hour of carotid occlusion contract the lymph vessels in the forelimb, the onset is not rapid. Decreases in lymph flow from 30 min of carotid occlusion onward are compatible with active lymphatic contraction, especially since lymph protein concentration is not changed and venous pressure is elevated. Alternately passive compression of lymph vessels by edema could have contributed to a reduced lymph flow.

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A MODEL FOR THE ETIOLOGY OF DIABETIC RETINOPATHY. M. L. Wolbarsht, M. B. Landers, III,* and E. Stefánsson*. Duke Univ. Med. Ctr., Durham, NC 27710

In most forms of diabetes the retinal vessels become leaky, with proliferation at the optic disc and in the mid periphery. This process can be halted by photocoagulation destruction of the photoreceptor layer, allowing choroidal O_2 to reach the inner retina; or by vitrectomy allowing dissolved O_2 in the aqueous to do the same.[1] In both cases the additional O_2 forces the dilated retinal vessels to constrict[1,2] indicating that vascular degeneration results from chronic dilatation. In vivo oxygen electrode measurements in a cat eye show increases in retinal O_2 consumption with increased blood glucose level. We propose a model for diabetic retinopathy in which the high average blood glucose level increases retinal O_2 uptake. The retinal vessels chronically dilate during autoregulation to maintain normal levels of O_2, resulting in leakage and proliferation. The same model will apply to any tissues in which in vivo O_2 consumption is raised by elevated glucose, and O_2 levels are autoregulated. The actual course of the disease, in the eye, as elsewhere, will also follow elevations of average blood pressure as well as average glucose levels.[5] Ref: (1) Wolbarsht, M. L. and Landers, M. B., III, Ophthal. Surg. 11:235, 1980. (2) Koerner, F., et al., Klin. Monats. Augen 172:440, 1978. (3) Craig, F. N. and Beecher, H. K., J. Gen. Physiol. 26:467, 1943. (4) Reed, J. S., et al., Invest. Ophthalmol. Vis. Sci. 19(Suppl):168, 1980. (5) Williamson, J. R. and Kilo, C., New Eng. J. Med. 302:399, 1980.

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CAPILLARY DIAMETERS MEASURED BY A TELEVISION-COMPUTER METHOD. R.G. Safranyos*, C.G. Ellis*, K. Tyml* and A.C. Groom, Dept. Biophysics, Univ. of Western Ontario, London, Canada. N6A 5C1

Present methods for measuring microvascular diameters in vivo have the disadvantage that they are generally subjective, relying on the experimenter to decide the location of the vessel walls. Our goal was to develop a television-computer method to determine capillary diameters objectively. The method is based on the fact that when capillaries are viewed under the microscope, the temporal variation of light intensity within a vessel is greater than that outside, due to the passage of red cells and plasma gaps through the vessel. These temporal variations were determined, at successive points along a TV line perpendicular to the capillary, by sampling the output of a video analyzer (CVI-321) using a Cromemco Z-2D microcomputer. At each point the standard deviation (SD) of light intensity was calculated and the positions of the step-change in SD across the edges of the lumen were used to determine vessel diameter. In this way, we have measured diameters of capillaries at the surface of the sartorious muscle of anesthetized frogs. The capillary diameters ranged from 15 to 29 μm with a measurement accuracy of ± 1 μm. It should be recognised that measurements obtained with this method would not include the thickness of any cell-free plasma layer adjacent to the walls. Subject to this limitation, this technique offers an accurate and objective means of determining microvessel diameters in vivo. (Supported by the Ontario Heart Foundation)

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CORROSION CASTS OF THE MICROVASCULATURE IN HEART AND SKELETAL MUSCLE, STUDIED BY SCANNING ELECTRON MICROSCOPY (S.E.M.). R.F. Potter* and A.C. Groom, Department of Biophysics, University of Western Ontario, London, Canada. N6A 5C1

Studies of microvascular geometry made from microscopic observations of tissues in vivo or after perfusion with a silicone elastomer or India ink are restricted to a two-dimensional field of view. We reasoned that microvascular corrosion casts, if of sufficient rigidity and structural integrity, could yield 3D information when examined suitably under SEM. We have used modified Batson's No. 17 anatomical casting compound to prepare casts of the microvasculature of heart and skeletal muscle in anesthetized rats. After corrosion of the tissue, in 40% KOH at 60°C, each cast was washed thoroughly in distilled water and air dried. Specimens coated with gold palladium were viewed with a Phillips 501 S.E.M. at 30kV. In casts from the L. ventricle the capillary network appeared to follow the syncytial arrangement of the muscle fibres; capillaries were 400-600 μm long, with many anastomoses and characteristic short loops. Casts from skeletal muscle (gastrocnemius and gracilis) show long straight capillaries with fewer branchings than in heart, but in contracted muscle the vessels show a very undulatory conformation. Cross-sectional views show pentagonal cores, 20-60 μm in diameter, created by the anastomotic branches between capillaries. Capillary diameters in μm (mean ± S.D.) were 5.43 ± 2.37 (N = 127), 4.93 ± 1.51 (N = 135) and 4.44 ± 1.39 (N = 135) in L. ventricle, gastrocnemius and gracilis, respectively. (Supported by Ont. Heart Fndn.)

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DIRECT MEASUREMENTS OF CAPILLARY PRESSURES IN RAT INTESTINAL MUSCLE DURING VENOUS PRESSURE ELEVATIONS. Michael J. Davis* & Robert W. Gore. University of Arizona, College of Medicine, Tucson, Arizona. 85724

The analysis of whole organ gravimetric and volumetric data requires information about the numerical relationship between capillary hydrostatic pressure and venous pressure. Indirect estimates (Circ.Res.16:294,1965)indicate that 62% of the increment in venous pressure is transmitted to the capillaries, while others (Acta.Phys.Scand.57:270,1963) have simply assumed a value of 85%. We have made direct measurements of capillary pressures in rat intestinal muscle during venous pressure elevations. The intestinal microcirculation was viewed under the microscope using the Bohlen-Gore preparation(MVR 11: 103,1976). Venous pressure was changed with a snare around the inferior mesenteric vein, and measured with a cannula in an arcade vessel. Capillary pressures were measured with a servo-null transducer. The average from all capillaries studied to date indicate that 77% of the increment in venous pressure is transmitted to the intestinal muscle capillaries. Individual measurements ranged from 65% to 89%. In all cases, capillary pressures followed the changes in venous pressure through the range 10-40 mmHg without any apparent "regulatory" effects on the magnitude or time course of capillary pressure, even though 1st, 2nd and 3rd-order arterioles upstream from the capillaries all showed reflex constrictions (20-50%) during venous pressure elevations. (Supported by NIH Grants HL-13437, HL-17421 & HL-07249).

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EFFECTS OF P_{O_2} CHANGES ON THE AUTOREGULATORY RESPONSES OF INDIVIDUAL SKELETAL MUSCLE ARTERIOLES. R. Morff and H. Granger. Dept. of Medical Physiology, Texas A&M Univ., College Station, TX 77843.

Autoregulatory responses to decreased perfusion pressure were determined for individual arterioles at different branching levels in the cremaster muscle of anesthetized rats (PB, 50 mg/kg IP) suspended in a bath with pH=7.40, and temp.=34°C. Diameters and velocities were recorded using in vivo t.v. microscopy, an image-shearing monitor and cross-corelator. In one group of animals (n=15) the O_2 and CO_2 concentrations in the bath were set so that bath P_{O_2}=68.6±8.0 and P_{CO_2}=36.2±4.1 mmHg. In a second group (n=12) bath gases were set at P_{O_2}= 19.3±5.2 and P_{CO_2}=36.0±4.1 mmHg. A small occluder around the abdominal aorta was used to alter perfusion pressure. The control diameters of 1st (1A) 2nd (2A) and 3rd (3A) order arterioles were not significantly different between the two groups. Vasomotion was routinely observed in 3A vessels in the low O_2 group but not in the high O_2 group. Only the 3A vessels showed significant autoregulation in the high O_2 group, while in the low O_2 group 1A, 2A and 3A vessels all exhibited autoregulatory responses, and the autoregulatory "gain" was greater for all branching levels with low bath P_{O_2}. We conclude that the autoregulatory responses are heterogeneous for the various series elements in the arteriolar tree, and that autoregulation is considerably enhanced in the relatively hypoxic muscle (supported in part by Organized Research Funds from Texas A&M University).

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MICROVASCULATURE IN TURTLE LUNGS. A.P. Farrell and S.S. Sobin Univ. of So. Cal., School of Medicine, Los Angeles, CA 90033

The dimensions and behavior of lung capillaries were examined under a range of static transmural pressures in the red-eared turtle, Pseudemys scripta using a silicone elastomer infusion technique. After perfusion of the lung vasculature with elastomer, the elastomer was allowed to polymerize at a known transmural pressure. Capillary dimensions were measured in histological preparations. The turtle interalveolar capillaries have a high vascular density, with the vascular space to tissue ratio (VSTR) being 88%. The capillaries are also compliant and their cross-sectional dimension increases with transmural pressure; the compliance coefficient (α) is 0.08μm. cm H_2O^{-1}. With such characteristics the turtle interalveolar capillaries can be described in terms of sheet blood flow. Sheet blood flow is applied to high density vascular beds in which sheet thickness is a function of transmural pressure. The present findings have important comparative value since sheet blood flow has previously been applied to the lung interalveolar capillaries in various mammalian species, including humans, and the gill lamellar capillaries of a teleost fish. More important is the striking similarity that exists for the vascular dimensions and behavior of the respiratory capillary sheets in these three vertebrate groups. The vascular densities are all about 90%, and the sheet thickness compliance values lie within the range 0.07 to 0.22 μm.H_2O^{-1}. (Supported in part by AHA/GLAA Res. Award #612 and NIH Grant HL11152)

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ELASTASE ACTIVITY IN THE MEDIA OF CULTURED ENDOTHELIAL CELLS. (T.J. Podor* and Nino Sorgente', School of Medicine, University of Southern California and Estelle Doheny Eye Foundation Los Angeles, Ca. 90033. (Spn:T. Farell). The process of neovascular invasion of tissues is very likely mediated by proteases produced by endothelial cells (EC). We previously demonstrated high levels of neutral proteases and plasminogen activator produced by cultured EC. We now report the presence of elastase in the culture medium of bovine vascular EC. Confluent EC at passage 2 were maintained in RPMI 1640 lacking serum or supplemented with 2-20% fetal bovine serum. The media were collected and dialyzed against H_2O at 4^0. One half of the media were acidified to pH 3.0 to release elastase bound to protease inhibitors; the pH was then adjusted to 8.8 by dialysis against 0.2 M Tris. Elastase activity was assayed in acid treated and untreated media using orcein-impregnated elastin as the substrate and procine pancreatic elastase as a standard (PSEBM 90:323, 1955). Elastase activity was present in all fractions tested and activity levels were consistently higher (2-4X) in the acid-treated media than in the untreated samples. Serum modulated the expression of elastase activity; the highest levels of activity were detected in media containing 5% serum (8 meg.equib/ 2ml of media) and the lowest were in the serum-free media samples (1.2-1.8 meg.equiv.). These results indicate that elastase synthesized by EC may contribute to the invasive process in neovascularization and to the genesis of subendothelial lesions. (Supported in part by NIH grants DE-04074 and EY -03229).

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LEFT VENTRICULAR Na,K-ATPase ACTIVITY IN RATS WITH REDUCED RENAL MASS HYPERTENSION (RRMH) AND SPONTANEOUS HYPERTENSION (SHR). D. Clough*, M. Pamnani, S. Huot*, and F. Haddy. USUHS, Bethesda, MD 20014 (Spon. J. Dabney).

We have previously reported decreased myocardial Na,K-ATPase activity in rats with one-kidney, one clip hypertension (Physiologist 20:18, 1977). We have now extended these studies to RRMH rats and SHR. Rats with reduced renal mass (70-80% removed) were fed a low (.02%) sodium diet and the RRMH rats drank 1% saline for 5 weeks while the controls drank distilled water. In the SHR study, both normotensive Wistar and Wistar Kyoto (WKY) rats were used as controls and the rats were 34 weeks of age. Na,K-ATPase activities of microsomes prepared from left ventricles of hypertensive rats and their paired controls were determined by subtracting Mg-ATPase activity (assayed with ouabain and Na) from total ATPase activity (K and Na). Na,K-ATPase activity (μmoles Pi/mg protein/hr) of RRMH rats was significantly less than that of controls (7.68 and 9.40 respectively, $\bar{d}\pm S\bar{d}$:1.72±0.53, P<.02, N=10) and Mg-ATPase activity was significantly greater (1.93 and 1.11, $\bar{d}\pm S\bar{d}$:0.82± 0.16, P<.001, N=10). On the other hand, there was no significant differences (P>.05) in ATPase activities of SHR compared to Wistar (N=6) or WKY rats (N=6). The results from the RRMH rats provide further evidence for decreased Na,K-ATPase activity in cardiovascular tissue of low renin, presumably volume expanded forms of hypertension. In contrast, decreased Na,K-ATPase activity does not appear to occur in SHR, a genetic form of hypertension.

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EVIDENCE FOR A HUMORAL Na^+ TRANSPORT INHIBITING FACTOR(S) IN ONE-KIDNEY, ONE WRAPPED HYPERTENSIVE DOGS. M. Pamnani, S. Huot*, R. Steffen*, and F. Haddy. USUHS, Bethesda, MD 20014

We have shown that ouabain-sensitive ^{86}Rb uptake a measure of Na^+-K^+ pump activity, is reduced in mesenteric vessels from dogs with one-kidney, one wrapped hypertension (1-WHT) and in tail arteries of rats with one-kidney, one clip, one-kidney-DOCA-salt, and reduced renal mass hypertension. These models of hypertension have low PRA and are presumably volume expanded. Acute volume expansion releases a humoral factor which inhibits Na^+ pump in blood vessels (Physiologist 21:88, 1978). The present study was designed to determine whether the observed suppression of vascular Na^+-K^+ pump activity in 1-WHT is also due to release of a humoral factor. We measured ouabain-sensitive (OS) and insensitive (OI) ^{86}Rb uptake by tail arteries from untouched normotensive rats incubated in supernates prepared from boiled plasma of 1-WHT dogs (N=4) and their paired normotensive (NT) controls. Compared to the OS ^{86}Rb uptake by arteries incubated in supernates from NT animals, the OS uptake in pmoles/mg tissue dry weight by arteries incubated in supernates from 1-WHT dogs was decreased by 34% ($\bar{d}\pm S\bar{d}$:1922±470, P<.01, N=6). OI uptakes were not different. Additionally OS ^{86}Rb uptake by mesenteric arteries and leucocytes from 1-WHT relative to NT dogs was decreased by 38% ($\bar{d}\pm S\bar{d}$:1441±450, P<.05) and 29% ($\bar{d}\pm S\bar{d}$:541l±746, P<.01) respectively. Thus depressed Na^+-K^+ pump activity in the blood vessels and leucocytes of these 1-WHT animals may be due to release of a blood borne Na^+ pump inhibiting factor(s).

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VASCULAR Na^+-K^+ PUMP ACTIVITY AND DEVELOPMENT OF REDUCED RENAL MASS HYPERTENSION (RRMH) IN RATS. S.J. Huot*, M.B. Pamnani, D.L. Clough*and F.J. Haddy. Department of Physiology, USUHS, Bethesda, MD 20014

We have shown that ouabain sensitive ^{86}Rb uptake, a measure of Na^+-K^+ pump activity, is decreased in tail arteries of rats with RRMH. The purpose of the present study was to determine if there is a temporal correlation between the suppression of vascular Na^+-K^+ pump activity and the development of hypertension. Four-fifth nephrectomized male Wistar rats (70-80% renal mass removed) on low sodium diet (0.02% sodium) were divided into control normotensive (NT) and experimental RRMH groups. RRMH rats drank 1% saline and paired NT rats drank distilled water. Systolic blood pressure (SBP) was monitored weekly. At weekly intervals, some RRMH rats and their paired controls were anesthetized and their tail arteries removed to measure ouabain sensitive (OS) and insensitive (OI) ^{86}Rb uptakes. Compared to arteries from NT rats, OS ^{86}Rb uptake (pmoles/mg tissue dry wt) by arteries from RRMH rats was reduced by 19% ($\bar{d}\pm S\bar{d}$:1663±537, P<.025, N=6) and 40% ($\bar{d}\pm S\bar{d}$: 3403±418, P<.005, N=9) at 1 and 5 weeks following nephrectomy respectively. OI ^{86}Rb uptakes were not different. Mean SBP (mmHg) of RRMH rats at 1 and 5 weeks were 143±2 and 167±3 respectively; corresponding values for NT rats were 113±2 and 116±2. These data provides further support for our hypothesis that suppressed Na^+-K^+ pump activity may be causally related to the development of some low renin, presumably volume expanded models of experimental hypertension.

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THE CHRONIC RENAL FUNCTION CURVE IN THE UNTOUCHED KIDNEY OF DOGS WITH RENOVASCULAR HYPERTENSION (RHT). Takuya Amano*, F. Merlin Bumpus and Carlos M. Ferrario, Cleveland Clinic Research Division, Cleveland, Ohio 44106.

We have been able to estimate the effect of chronic renal hypertension (two-kidney one clip) on the renal function curve of the non-clipped kidney of conscious dogs by: 1) surgically diverting the ureter to the dog's flank several weeks beforehand; and 2) measuring the urinary volume, sodium excretion (UNaV), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) at various levels of mean arterial pressure (MAP) (range 80-160 mmHg). Each steady state step change in MAP was produced by infusion of either phenylephrine or nitroprusside on alternate days before and again 3-4 weeks after constriction of the renal artery opposite the diverted kidney. Both before (MAP: 100 ± 1 mmHg) and after hypertension (MAP: 122 ± 1 mmHg) urinary output rose in proportion to each stepwise increase in pressure without concurrent increases in GFR and ERPF. On the other hand, the phenomenon of pressure natriuresis was totally absent when the dogs were normotensive but it occurred after the animals developed renal hypertension. The data indicate that the kidney exposed to high blood pressure in RHT dogs loses the capacity to regulate the urinary excretion of sodium during acute changes in arterial pressure. (Supported in part by an NHLBI grant #HL-6835).

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PLASMA, RBC, AND PHEOCHROMOCYTOMA (P) CATECHOLAMINES (CA) AND B.P. IN NEDH RATS. W.M.Manger, S.Warren*, M.Hulse*, R.Sussman*, M.Forsyth*, P.Turino*, and R.Chute*. N.Y.U. Med. Ctr.,N.Y. 10016

NEDH rats provide a valuable model for studying P since older rats often develop P spontaneously. P can be transplanted in this strain and may be lethal in 3 wks. Even without P about 75% of NEDH rats developed sustained hypertension by 10 wks. of age: mean systolic B.P.s for males and females were respectively 162±4 and 146±4 mmHg. Mean B.P.s gradually increased thereafter but were lower than those in SHR of similar age; heart rates of both strains were similar. Under pentobarbital anesthesia mean plasma dopamine, epinephrine and norepinephrine were respectively 230, 360 and 465 pg/ml. 2 to 3 wks. after P implantation B.P.s frequently ranged from 175 to 200 mmHg and CA were respectively 6350, 1215 and 4240 pg/ml, significantly > in rats without P. Mean tissue CA in P were 90, 5, and 365 ug/g. RBC concentrations of dopamine, epinephrine and norepinephrine in normal NEDH were respectively 600, 60 and 140; RBC CA were markedly elevated in rats with P but much less so than in plasma. Hypophysectomy caused mild hypotension and elevated plasma CA in control NEDH. Hypophysectomy followed by P implantation prevented development of hypertension and prolonged survival time to 3 mos. despite marked increases in plasma CA; P continued to grow and function. Whether a common mechanism is responsible for early development of hypertension and ultimate occurrence of P in some NEDH rats remains to be determined. (Supported partly by the Nat. Hypertension Assoc. and the Pew Memorial Trust)

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VASOPRESSIN AND EPINEPHRINE EFFECTS ON TOTAL VASCULAR COMPLIANCE AND UNSTRESSED VOLUME IN RATS. Nick C. Trippodo and Leigh P. Ziegler.* Ochsner Medical Institutions, New Orleans, La. 70121.

It is unclear whether increased adrenergic stimulation decreases total vascular capacity through a reduction in unstressed vascular volume (Vo) or compliance. And, the effect of vasopressin (V) on vascular capacity is unknown. Total vascular compliance (TVC) and Vo were determined in anesthetized rats by measuring blood volume (BV, 51-Cr-RBC) and mean circulatory filing pressure (MCFP, Physiologist 22:125, 1979) before and after rapid BV changes. TVC was the reciprocal of the slope of the MCFP-BV curve; Vo was obtained by extrapolation. To eliminate reflexes, the spinal cord was cut at T_1-T_2, leaving neural control of respiration intact. Twelve rats received IV infusion of epinephrine (E) at 0.3μg/Kg/min to restore vascular tone; 9 rats received E at 1.5μg/Kg/min to increase adrenergic stimulation; and 12 rats received V (367 IU/mg) at 20 ng/Kg/min plus E at 0.3μg/Kg/min. MCFP, TVC and Vo in the low E (control) group were 8.1±0.3 mmHg (SE), 3.1±0.2 ml/Kg.mmHg and 31.9±1.7 ml/Kg. In the high E group MCFP was increased to 9.9±0.5 mmHg ($p<0.01$) and Vo was decreased to 24.9±1.8 ml/Kg ($p<0.05$), while TVC changed insignificantly (2.6±0.2 ml/Kg.mmHg). In V group MCFP, TVC and Vo were not significantly different from the control group (8.8±0.4 mmHg, 2.8±0.1 ml/Kg-mmHg and 28.5±1.6 ml/Kg), despite arterial pressure increasing greater than in high E group. Thus, under the conditions of this study increased adrenergic stimulation decreases vascular capacity mainly through a reduction in Vo and V had only minimal effects on vascular capacity. Supported in part by NIH HL 22261.

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LENGTH-TENSION PROPERTIES OF ANTERIOR TIBIAL ARTERY (ATA) IN NORMOTENSIVE(ND) AND PAGE HYPERTENSIVE(HD) DOGS. J.M.Price*, E.B.Knauss*, and D.L.Davis. Univ.of South Florida, Dept.of Physiol., College of Medicine, Tampa, Fl. 33612.

Force was measured in the resting state and during electrical stimulation of ATA rings from ND and HD at various circumferences(L). Media thickness was measured on-line with a video analyzer and was higher in HD. L for maximum active force (L_{max}) and L at which resting and active force was first detected (L_o and L_{min} respectively) were used to normalize the length-tension diagram. With L normalized to L_{max} , the HD active stress curve and resting stress curve were lower. With L normalized to L_o or L_{min}, the active stress curve was unchanged whereas the HD resting stress curve was higher. L_{max} was lower in HD but L_o and L_{min} were unchanged. The use of a reference circumference that is changed after hypertension(e.g.L_{max}) will cause a shift in the length-tension curve independently of any change in wall stress with hypertension. The linear regression coefficient for a calculated pressure versus L/L_{min} was higher in HD. Tonic contraction was not present in HD or ND and time to peak force was increased in HD. We conclude that 1) a reference circumference altered by hypertension does not distinguish between length effects and changes related to hypertension and 2) established Page hypertension in the dog is not accompanied by change in the intrinsic contractility of vascular smooth muscle(VSM) but with a decrease in resting distensibility and an increase in tension from VSM due to media hypertrophy.(Supported by NIH #HL21103 and Am. Heart Assoc. [Fla.]).

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ABNORMALITY IN BAROREFLEX CONTROL OF HEART RATE IN PREHYPERTENSIVE DAHL SALT-SENSITIVE RATS. F.J. Gordon*, H. Matsuguchi*, and A.L. Mark. CV Center and Department of Medicine, University of Iowa and VA Hospitals, Iowa City, Iowa 52242.

The Dahl strain of salt-sensitive rats is genetically predisposed to the development of salt-induced and many other forms of experimental hypertension. When maintained on a low salt diet these animals remain normotensive, affording an opportunity to examine possible contributory factors to hypertension in the prehypertensive stage. The present study examined baroreflex changes in heart rate in conjunction with pressor and depressor responses produced by bolus i.v. injections of graded doses of phenylephrine (PE) and nitroglycerin (NG) administered to conscious, prehypertensive sensitive (S) and resistant (R) Dahl rats maintained on a low (0.4%) salt diet. Baroreflex sensitivity was assessed by the slope of the ratio Δ heart rate (HR)/Δ mean arterial pressure (MAP) determined from peak changes in these parameters for several doses of PE and NG. MAP was not significantly different between S and R rats (S=100±4 and R=95±4 mmHg ± SEM). For a given increase in MAP, S rats demonstrated significantly less reflex bradycardia resulting in a decreased slope in baroreflex sensitivity (Δ HR/Δ MAP:S=2.06 ± .13 vs. R=3.05±.15; $p < .001$). In addition, S rats showed greater pressor responses to PE ($p < .001$). Depressor and HR changes during NG were not different between S and R rats. These data demonstrate an abnormality in baroreflex control of HR in prehypertensive S rats and suggest that this may contribute to their augmented pressor responsiveness to PE and their predisposition to hypertension.

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TURNOVER OF NOREPINEPHRINE (NE) IN BRAIN AND PERIPHERAL ORGANS OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE RATS K. P. Patel*, R. L. Kline and P. F. Mercer. Department of Physiol., Univ. of Western Ontario, London, Canada N6A 5C1

Previous studies of NE turnover in SHR have yielded conflicting results, as many studies have used: 1) only one age, 2) either peripheral organs or brain, and 3) either Wistar-Kyoto (WKY) or outbred Wistar (W) rats as controls. We have studied the turnover of NE in two brain regions and three peripheral organs of SHR, WKY, and W rats at 5 and 9 wks of age. NE turnover was determined by measuring tissue [NE] with a fluorescence assay at 0, 4, and 8 hrs after inhibition of tyrosine hydroxylase with α-Methyltyrosine. Differences in NE turnover were inferred by comparing slopes of regression lines calculated for the plot of log [NE] vs time. Arterial pressure of SHR was similar to that of WKY and W rats at 5 wks but averaged 150 mm Hg at 9 wks. There were no significant differences in turnover of NE in any tissue at either age for WKY and W rats. In SHR, NE turnover increased significantly between 5 and 9 wks in hypothalamus and brainstem. NE turnover in kidney and skeletal muscle was significantly greater in SHR when compared to WKY and W rats at 5 wks. At 9 wks NE turnover in kidney and duodenum had decreased while that in muscle remained elevated. These data suggest that NE turnover in peripheral organs is altered prior to the development of hypertension in SHR, and that changes in NE turnover in brain, kidney, and duodenum at 9 wks may be a compensatory response to the elevated pressure. (Supported by Ont. Heart Found.)

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CHEMICAL SENSITIVITY OF VASCULAR SMOOTH MUSCLE (VSM) OF, AND BLOOD PRESSURE OF, F_1, F_2 AND F_3 OFFSPRING RESULTING FROM A CROSS BETWEEN KYOTO WISTAR (WKY) NORMOTENSIVE AND OKAMOTO SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C.R. Triggle and D.A. Corbett. Fac. of Med., Memorial University, St. John's, Newfoundland. A1B 3V6.

Earlier studies report that isolated aortae from SHR, but not from WKY, demonstrate a paradoxical mechanical response to lanthanum ions (La^{3+}) and that a greater part of this La^{3+} response results from an elevation of extracellular H^+. SHR treated from conception with the β adrenergic antagonist, timolol, remained normotensive but still demonstrated the paradoxical mechanical response to raised $[La^{3+}]$ or $[H^+]$. We now report that, expressed as mg tension/mg dry tissue ± S.E.M., the maximum H^+ response in tissues from F_1 rats lies between that of the parents (SHR: 531 ± 61, n = 29; F_1: 407 ± 39, n = 17; WKY: 39 ± 18, n = 18, where n = number of tissues) and is closely related to the diastolic blood pressure (DBP): 187 ± 5, 140 ± 3, 109 ± 5; mmHg ± S.E.M. respectively. Furthermore the H^+ response from F_2 and F_3 offspring (F_2: 273 ± 49, n = 8; F_3: 232 ± 38, n = 9) demonstrates a similar relationship to the DBP (130 ± 5, n = 3; 145 ± 15, n = 3 respectively). The paradoxical chemical sensitivity of VSM from genetically hypertensive rats is thus not secondary to the elevated blood pressure and the magnitude of the sensitivity difference is related to the BP suggesting that a VSM defect may be an important determinant in the initiation and maintenance of raised BP in the SHR. (Supported by MRC(C)).

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RENIN ACTIVITY IN THE MYOCARDIUM AND ITS RELATIONSHIP WITH ATRIAL SPECIFIC GRANULES. L. YUNGE AND M. CANTIN Dept. of Pathology, Univ. of Montreal, Quebec, CANADA.

Ultrastructural radioautography using fucose-^{3}H and leucine-^{3}H has confirmed that specific granules contain glycoproteins (Yunge et al, J. Molec. Cell. Cardiol. 11, 375; 1979: Yunge et al, Cell Tiss. Res. 207, 1, 1980). Since renin is a glycoprotein, the hypothesis that atrial cardiocytes, like juxtaglomerular cells, might manufacture renin and act as stretch receptors was tested. Sodium deficiency produced an increase in renin activity (radioimmunoassay of angiotensin I) and in the number of specific granules while administration of desoxycorticosterone and a high salt diet produced a decrease in both parameters. There was no parallel change in cathepsin D activity. While the maximum renin activity of renal cortex was obtained at near neutral pH, in the atria and ventricle, maximal renin activity was observed at acid pH. After differential and density gradient centrifugation, the bulk of renin activity was found in the high speed supernatant while the specific granule fraction contained very little. Immunocytochemistry at the light and electron microscopic level (using specific renin antibodies and the unlabeled antibody technique of Steinberger) failed to localize renin in specific granules. The observations suggest the presence of renin in the myocardium but its absence in specific granules (Supported by the MRC and the Canadian Heart Foundation).

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ENLARGED FENESTRATIONS IN THE INTERNAL ELASTIC LAMINA OF INTRACRANIAL ARTERIES. Gordon J. Campbell* and Margot R. Roach. Biophysics Dept., Univ. of Western Ontario, London, Canada N6A 5C1.

During histogenesis of intracranial arteries a single internal elastic lamella is formed, as a fenestrated membrane. Analysis of photomicrographs (Scanning Electron Microscope) of the internal elastic lamina from isolated specimens of human intracranial arteries, revealed regions of enlarged fenestrations interspersed among smaller diameter fenestrations. The enlarged fenestrations, which are arranged in clusters or circumferentially-oriented bands, were found almost exclusively within, or adjacent to, the apical region of bifurcations. The mean diameter, number of fenestrations per sq. mm and proportion of the area of internal elastic lamina comprised of fenestrations for the enlarged fenestration group were 7.0 ± 0.34 SEM μm, 2606 ± 284 SEM per sq. mm and 15.0 ± 1.1 SEM percent respectively. The results for the smaller diameter fenestrations found in the straight cylindrical sections of the arteries as well as the apical region of bifurcations were 2.1 ± 0.15 SEM μm, 4720 ± 430 SEM per sq. mm, and 1.8 ± 0.16 SEM percent respectively for the same characteristics. Three possible origins for the formation of enlarged fenestrations are: generation during embryologic coalescence; improper maturation during adolescence; or enlargement of the smaller fenestrations as a consequence of the influence of stresses (hemodynamic or geometrical). (Supported by Medical Research Council of Canada grant no. MT-4290)

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KINETICS OF α-AMINOISOBUTYRIC ACID ACCUMULATION INTO ISOLATED HEPATOCYTES OF THE AMERICAN EEL, ANGUILLA ROSTRATA LESUEUR. Thomas W. Moon. Dept. of Biology, University of Ottawa, Ottawa, Canada. K1N 6N5

The transport of amino acids into tissues is greatly effected by the physiological state of the animal. In a previous study it was shown that at low and presumably physiological levels, alanine is a poor gluconeogenic substrate in hepatocytes isolated from starved American eels (Renaud and Moon, 1980). This study uses the alanine analog α-aminoisobutyric acid (AIB) to probe the "A" or "alanine-preferring" transport system of eel hepatocytes. At 20°C and 20 μM AIB, AIB uptake reached a plateau within 60 min. Flux rate at this plateau was 11 pmoles/hr/10^6 cells, and the distribution ratio was 0.26 (AIB inside/AIB outside, considering water space). Uptake of AIB was linearly related to AIB concentration between 20 μM and 10 mM, with the distribution ratio falling to a value of 0.1 at AIB concentrations above 0.5 mM. Ouabain (2 mM), KCN (2 mM) and iodoacetic acid (2 mM) did not effect the flux or distribution ratio, but a Na-free medium reduced both parameters by approx 2.5-times. It is concluded that if AIB is transported by an "A" system in the eel hepatocyte, the process is passive unlike the mammalian system. (Supported by NSERC of Canada Grant #A6944).

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TURNOVER OF ORNITHINE DECARBOXYLASE IN RAT HEPATOMA CELLS M.L.Pritchard, A.E.Pegg, and L.S.Jefferson, Dept. of Physiology, Penn State Univ., Hershey, PA 17033

In a mutant HTC cell line, HMO_A, ornithine decarboxylase (ODC) turns over at a much slower rate than in the parental cell line (McCann, et al., J. Cell. Physiol. 99: 183, 1979). When the cells were incubated with the protein synthesis inhibitors, cycloheximide (0.18 mM) or puromycin (0.42 mM), HTC ODC activity declined with a $t_{\frac{1}{2}}$ of 13 minutes, while HMO_A ODC activity declined with an apparent $t_{\frac{1}{2}}$ of 9 hours. The two cell types were differentially sensitive to putrescine, the product of the ODC reaction. A 50% reduction of ODC activity in HMO_A cells required an extracellular concentration of putrescine 1000 times higher than was required in the HTC cells. ODC activity in cytosol preparations of HMO_A cells was more resistant than HTC preparations to trypsin digestion when the two were equalized for protein and activity. To determine whether the enzymes differed, ODC was purified by a two-step procedure including a pyridoxamine-phosphate affinity column. HTC and HMO_A ODC were purified to a specific activity 350-fold over that in cytosol. Purified ODC from these cells was found to have similar pH optima profiles, protein band patterns on SDS-polyacrylamide gels, and similar trypsin and temperature sensitivities suggesting the enzymes from the two cell types are identical. Other factors must be responsible for the shorter half life and greater putrescine sensitivity in vivo and the greater trypsin sensitivity of the HTC enzyme in crude extracts. (Supported by AM13499 and CA18138 from NIH)

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POSTBURN TYROSINE RELEASE AND LEUCINE OXIDATION IN RAT SOLEUS. Brian Parr* and Richard Odessey. Department of Physiology, University of Virginia, Charlottesville, Va. 22908

To study the effect of burn injury on muscle metabolism, 50 g rats were anaesthetized and one hind limb was immersed in 90°C water for 3 sec. At 3 days postburn the soleus muscles from burned limbs (B) were 11% smaller than muscles from the contralateral limb (C) or unburned animals (U). Muscles were stretched to approximately rest length and preincubated in Krebs-Ringer bicarbonate buffer containing albumin and 5 mM glucose. A 1 hour incubation in fresh medium followed. There were no significant differences in levels of ATP (3.6 μmol/g) and Cr P(9.8 μmol/g) between U, B and C when incubated in this fashion. Net tyrosine release from B was elevated 35% over C and U. Addition of 0.5 mM leucine decreased tyrosine release by 35% in U and C, but had no effect on B. At 2.5 mM, leucine had no further effect on U and C, but slightly diminished tyrosine release from B by 19%. Leucine oxidation by B was elevated over C and U at both concentrations. Insulin at 100 μU/ml inhibited tyrosine release by 25% in all cases. In conclusion, this mild burn injury stimulates leucine oxidation and decreases tissue sensitivity to leucine's inhibition of net protein breakdown. These findings of altered leucine metabolism suggest that the "protein-sparing" effects of branched chain amino acids may differ in burned and normal tissue. (Supported by BRS 5 S07 RR 05431-18).

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EVALUATION OF METRONIDAZOLE ON WOUND HEALING IN RATS. Edward B. Borden,* Robert J.Sammartano,* Laurence J. Brandt* and Scott J. Boley. Albert Einstein College of Medicine-Montefiore Hospital Medical Center, New York, N.Y. 10467

Metronidazole has proven effective in promoting healing of perineal Crohn's disease but its mechanism of action is not known. One possible mechanism, a direct beneficial effect of Metronidazole on wound healing, was studied in forty-five Sprague-Dawley rats who received a 7 day course of either intraperitoneal Metronidazole, 20 mg/kg/day, or equivalent volumes of intraperitoneal physiologic saline. One day after starting the drug both groups had: 1) full-thickness 1cm skin defects created on the back, and 2) standardized midline celiotomy incisions. The incisions were closed with stainless steel clips 1cm apart for the fascia and continuous silk sutures for skin. All rats were sacrificed 24 hr after the last day of treatment. The breaking strengths of skin and fascia were measured by the method of Crawford et al. The fascial breaking strength was 277gm mean in the Metronidazole group, and 532gm mean in the placebo group ($p < .0001$). There was no difference in the breaking strength of the skin incisions in the two groups nor in the rate of contraction of the skin defects. This study suggests that at least in rats Metronidazole has no beneficial effect on wound healing, but to the contrary may interfere with fascial wound healing. The latter observation may be of importance in view of the increasing perioperative use of Metronidazole as an antimicrobial.

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POLYAMINE METABOLISM IN STIMULATED FIBROBLASTS. Delia R. Bethell* and Anthony E. Pegg. Dept. Physiology, Penn State Univ., Hershey, PA 17033.

Alterations in polyamine metabolism, evidenced by a rapid increase in ornithine decarboxylase (ODC) activity, occurred in 3T3 fibroblasts and their SV-40 transformed counterpart stimulated by addition of fresh serum or subculturing. During the 12 hrs following serum stimulation both cell lines exhibited a rapid increase in ODC activity accompanied by a rise in cellular putrescine (Pu) levels; spermidine (Spd) and spermine (Sp) showed no significant change. In subcultured 3T3 cells, ODC activity and Pu again rose in parallel. Spd and Sp increased to a plateau within 24 hours. When the cells reached density inhibition there was a precipitious decline in cellular polyamine levels, accompanied by a concomitant rise in Pu and Spd in the culture medium. SV3T3 cells showed no rise in Pu corresponding with ODC, but an early increase in Spd. A rise in Pu was not evidenced until Spd was returning to basal levels. There was no change in medium polyamines associated with confluence in the transformed cell line. In serum stimulated confluent cells, alterations in ODC activity were primarily reflected in cellular Pu levels. During a growth cycle there was a much more complex pattern involving all three cellular polyamines. The increased release of Pu and Spd into the medium by the density inhibited 3T3 cells may represent a regulation mechanism lost by the transformed cells. Supported by 1T32HL07223 and CA18138.

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VALINE AND ISOLEUCINE INHIBIT GLUCOSE UPTAKE AND ALANINE RELEASE BY THERMALLY INJURED MUSCLE. J.Turinsky and R.E. Shangraw. Dept. Physiol., Albany Med. Col., Albany, NY 12208.

Thermal injury leads to an increase in glucose utilization and amino acid release by muscle in the burned but not in the unburned region of the body. To evaluate the effect of branched-chain amino acids (BCAA) on these changes, rats were scalded on one hind limb and 3 days later soleus muscles from the burned and unburned limbs of burned rats as well as from controls were incubated in KRB with 10 mM glucose. Valine or isoleucine (2mM) was added as necessary. Without BCAA, burned limb muscles took up 192% more glucose and released 85% more alanine and 155% more tyrosine than controls (all $p<0.001$). The unburned limb muscles did not differ from controls. Valine depressed glucose uptake by the burned limb muscles 19% ($p<0.05$) without affecting the uninjured muscles. Alanine release by all three muscle groups was inhibited about 60% ($p<0.001$). Isoleucine decreased glucose uptake by the burned limb muscles 33% ($p<0.001$) and had only a modest inhibitory effect on that of uninjured muscles. Alanine release by the burned limb muscles was depressed 39% and that by the two uninjured muscle groups by about 70% (all $p<0.001$). Neither treatment affected tyrosine release by any muscle group. The data suggest that thermally injured muscle is more responsive to inhibition of glucose uptake by valine and isoleucine than uninjured muscles. Both amino acids depress alanine release by all muscle groups probably by impairing transaminations rather than net proteolysis. (USPHS GM-22825)

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EFFECTS OF LEUCINE ON THERMALLY-INJURED SKELETAL MUSCLE. R.E. Shangraw and J. Turinsky. Department of Physiology, Albany Medical College, Albany, New York 12208.

The present study tests whether leucine affects the relatively enhanced glucose uptake and amino acid release which are unique to muscle from the burned region. Ether anesthetized rats received a mild scald injury by immersing one hind limb in 90-91.5°C water for 3 sec. Three days later, soleus muscles from burned rats and age-matched controls were incubated in KRB + 10 mM glucose with or without 2 mM leucine for 1 hr. Without leucine, muscles from the burned limb took up 164% more glucose, and released 107% more alanine, 102% more glutamate, 141% more glutamine, and 198% more tyrosine than controls (all $p<0.001$). Muscles from the unburned limb of burned rats did not differ from controls. With the addition of leucine, glucose uptake by control muscles was reduced 24% ($p<0.006$) but the uptake by the burned limb muscles was unaffected. Alanine release by all three muscle groups was diminished about 90% ($p<0.001$). Similarly the releases of glutamate and glutamine were reduced ($p<0.05$). For each of these amino acids, the relatively enhanced release by the burned limb group was eliminated. Tyrosine release was not altered by leucine. The data suggest that leucine provides an alternate energy substrate and that its inhibition of alanine, glutamate and glutamine release by muscle relates to a decrease in transaminations rather than net proteolysis. Thermally injured muscle was more sensitive to leucine's inhibition of amino acid release than uninjured muscle. (GM-22825)

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THE EFFECT OF STARVATION ON LEUCINE KINETICS IN THE CONSCIOUS DOG. N.Abou-Mourad*, P. Williams*, K. Wise*, D. Lacy*, W. Lacy, Departments of Medicine & Surgery, Vanderbilt University, Nashville, TN 37232

While in vitro studies show an increased tissue oxidation of leucine with fasting, in vivo studies show a progressive increase in its plasma concentration. In order to examine the mechanism of this paradoxical response we studied the flux rates of leucine into and out of the plasma compartment using a constant infusion of 4,5,^{3}H(N),L-leucine. Three groups (n=5, each) of conscious dogs were studied at different stages of fasting, 16,48, and 96 hours. With fasting plasma leucine increased from 125±10 to 193±3 μM after 48 h to 218±4 μM after 96 h. The rate of appearance of leucine into the plasma compartment increased by 60% at 48 h of fasting, (from 3.3 to 5.1 μmol/kg/min, ($p<.001$) and then returned to basal post-absorptive levels by 96 h. The clearance of plasma leucine (25.4±0.3ml/kg/min at 16 h) was not significantly changed after 48 h of starvation however by 96 h it had fallen by an average of 40% to a value of 15.6±0.4mg/kg/min ($p<0.01$).

The data suggest that the increase in plasma leucine with fasting is secondary to an initial augmentation in protein breakdown and a later decrease in leucine removal from the plasma compartment. These findings are in contrast to the decrease in protein synthesis without a change in breakdown that we observed with acute insulin deficiency (Diabetes 29,201,1980) This would suggest that the early changes in protein metabolism with fasting cannot be simply due to lower insulin levels.

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PROGRAMMED CELL DEATH IN MUSCLES: LYSOSOMAL CATHEPSINS. Agnes M. Dorsey* and Richard A. Lockshin, Dept. of Biol. Sci., St. John's Univ., Jamaica, N.Y. 11439

During the breakdown of the intersegmental muscles of Manduca sexta (Lepidoptera) the total activity of cathepsin B (hydrolysis of CBZ-ala-arg-arg-4-methoxy-beta-naphthylamide) rises 3-fold, and the activity of cathepsin D (hydrolysis of 3-H acetyl hemoglobin) rises 5-fold. The relative activities, at the highest points, represent 6- and 10-fold increases for B and D, respectively. Although the enzymes are highly active against artificial substrates, they do not reproducibly or rapidly degrade native or iodinated insect actomyosin to identifiable large fragments. Occasionally, a fragment of 70,000 daltons is seen on acrylamide gels after in vitro incubation. Mammalian cathepsin B, however, degrades insect myosin to a fragment 130,000 daltons in size, and mammalian cathepsin D degrades the insect substrate to a 145,000 dalton fragment. (The mammalian enzymes were the gift of John Bird and Richard Brooks, Rutgers.) It is concluded that these enzymes play an important but perhaps not initiating role in the degeneration of the intersegmental muscles. Supported in part by the National Science Foundation. (PCM 77-15687)

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LIVER-MITOCHONDRIAL Sr^{2+} CONTENT IN MICE KEPT ON STABLE Sr^{2+} SUPPLEMENTATION. S.C. Skoryna, S. Inoue* and M. Fuskova*. Gastrointestinal Research Laboratory, McGill University and Medical Research Unit, St. Mary's Hospital Center, Montreal, Quebec, Canada.

Supplemental intake of stable Sr^{2+} was previously reported to be useful in maintaining weight of patients with advanced cancer and in reduction of side effects of chemotherapy. In the present study Sr^{2+} was administered to male adult mice in drinking water ($SrCl_2$ .38%) for a period of 3 months with suitable controls. Sr^{2+}, Ca^{2+} and Mg^{2+} concentration in liver mitochondria, isolated by gradient centrifugation was analyzed by Atomic Absorption Spectroscopy, using HGA. Mitochondrial Sr^{2+} content was found to increase from a mean of .18(±.05) μg/100 mg protein in control animals, to 1.25(±0.1) μg/100 mg protein in the strontium supplementation group. Mitochondrial Ca^{2+} and Mg^{2+} content did not change significantly. Serum Sr^{2+} increased from .005(±.001) mg% to 7.3(±.008) mg%. The increase in mitochondrial Sr^{2+} content in the Sr^{2+} supplementation group results in a marked change of Sr^{2+}/Ca^{2+} ratio. High mitochondrial Sr^{2+} concentration may affect mitochondrial metabolism by preventing excessive uptake of Ca^{2+}.

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ALTERED ACTIVITIES OF CARBOHYDRATE METABOLIZING ENZYMES ASSOCIATED WITH COLD-ACCLIMATION (-1°C) IN THE KILLIFISH (FUNDULUS HETEROCLITUS). Ralph Paxton and Bruce L. Umminger. Univ. of Cincinnati, Cincinnati, OH. 45221.

Killifish acclimated to -1°C demonstrate a hyperglycemic state (500% increase) when compared with 20°C acclimated fish. This study was performed to elucidate the enzymatic mechanisms associated with the altered carbohydrate metabolism seen in cold-acclimated (CA) killifish. Gill hexokinase (HK) was decreased 36% in the CA fish when compared with the warm-acclimated (20°C) fish. There was no change in brain & heart HK with CA. Liver HK, glucose 6-phosphate dehydrogenase (G6PDH), & fructose diphosphatase (FDP) increased by 98%, 68%, and 58%, respectively; while pyruvate kinase (PK), lactate dehydrogenase (LDH), & phosphofructokinase (PFK) showed no change with CA. Muscle G6PDH & PK increased by 41% & 48%, respectively; while PFK decreased by 75% with CA. The results indicate that brain, heart, gill, & muscle do not compensate for glucose metabolism with CA, and may contribute to a reduced glucose utilization in the cold. The muscle also demonstrates a decreased glycolytic capacity. The liver has an increased capacity for phosphorylation of glucose, gluconeogenesis, & the hexose monophosphate shunt. The increase in kidney HK & the decrease in gill HK may play a role not only in the altered carbohydrate metabolism but also in the altered osmoregulatory ability seen in the CA fish. (Supported by NSF grant PCM76-11704.)

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EFFECT OF TEMPERATURE ON INCORPORATION OF UNSATURATED FATTY ACIDS INTO PHOSPHOLIPIDS BY HEPATOCYTES FROM THERMALLY ACCLIMATED RAINBOW TROUT.
J. R. Hazel. Zoology Department, Arizona State University, Tempe, Az. 85281

Hepatocytes from rainbow trout (Salmo gairdneri) acclimated to 5°C or 20°C were prepared by perfusion with collagenase and hyaluronidase. Cell suspensions were incubated at 5°C and 20°C with 75 μM (20 μCi/μmole) 1-^{14}C-labelled oleic (18:1), linoleic (18:2), on linolenic (18:3) acids. Hepatocytes from both acclimation groups incorporated 18:2>18:3>18:1 into total phospholipids at 20°C, however, at 5°C, the order was 18:3>18:2 >18:1. Regardless of assay temperature, hepatocytes from warm-acclimated trout exhibited higher (2-fold at 20°C; 4-fold at 5°C) rates of fatty acid incorporation into phospholipids than hepatocytes from cold-acclimated trout. Warm- and cold-acclimated trout differed in their capacity to incorporate 18:3 into phosphatidylethanolamine (PE). In hepatocytes from warm-acclimated trout the percentages of radioactivity recovered in the various phosphatides was not altered by assay temperature; in hepatocytes from warm-acclimated trout, the percentage of 18:3 incorporated into PE increased 2-3-fold at 5°C relative to 20°C. These results suggest that (1) membrane turnover may be considerably greater in warm-acclimated trout, and that (2) the homeoviscous restructuring of membrane phosphatides may be facilitated by (i) preferred incorporation of 18:3 (and derivatives) and (ii), a channeling of 18:3 into PE at reduced temperatures (Supported by NSF grant PCM 8003454).

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NA-CA EXCHANGE BY RAT MYOMETRIUM PLASMA MEMBRANE VESICLES. A.K. Grover*, C.Y. Kwan* and E.E. Daniel. McMaster University, Health Sciences Centre, Hamilton, Ontario, Canada. L8N 3Z5.

The effect of transmembranous Na-gradients on ATP-independent Ca-uptake by rat myometrium plasma membrane (P.M.) vesicles and release of Ca from preloaded vesicles was studied. When P.M. vesicles loaded with $CaCl_2$ in presence of ATP were diluted in media containing 100 mM NaCl, they lost the stored Ca more readily ($t_{1/2}$ = 6 min) than when diluted in Na-free media ($t_{1/2}$ = 12 min). Conversely when P.M. vesicles preincubated with 100 mM NaCl were diluted 21x in media containing 40 μM $CaCl_2$ and no ATP, the vesicles showed higher Ca-uptake than the vesicles which had not been preincubated with NaCl. The Na-Ca interactions exhibited the following characteristics: (a) the distribution of the Na-dependent Ca-uptake of the various subcellular fractions paralleled the distribution of the P.M. marker enzyme activities, (b) dilution of vesicles into media containing EGTA showed that Na-dependent fraction of Ca-uptake was not instantly released, (c) 4.8 mM NaCl which is equivalent to the final Na concentration after dilution of vesicles, did not influence the Ca-uptake, (d) Na-dependent Ca-uptake was abolished by monensin as well as by A23187 and (e) Li, K, Rb, Cs or sucrose could not replace Na. These characteristics demonstrate that P.M. vesicles of rat myometrium were capable of Na-Ca exchange.
Supported by M.R.C. of Canada.

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COMPARISON OF THE NUMBER OF NA, K-PUMP SITES IN CARDIAC AND SMOOTH MUSCLE. William T. Gerthoffer* and Julius C. Allen* (SPON: C.L. Seidel). Baylor College of Medicine, Houston, TX 77030

Because of the fundamental importance of the Na, K-pump in the physiology and pharmacology of cardiac muscle, cardiac Na, K-ATPase and ^{3}H-ouabain (^{3}H-OU) binding has been widely studied. However, less is known about Na, K-ATPase from smooth muscles. Most studies of smooth muscle Na, K-ATPase report very low activities compared to cardiac and skeletal muscle preparations. The low enzyme activity may be due to problems in isolation of the enzyme or due to a lower concentration of Na, K-pump sites, or both. To test the hypothesis that there are indeed fewer Na, K-pump sites in smooth muscles than in cardiac muscles, we modified a standard ^{3}H-ouabain binding method to make reliable estimates of very low levels of binding in crude membranes from the guinea-pig vas deferens. When compared to binding of ^{3}H-OU in guinea-pig heart homogenates, the data from the vas deferens showed there was higher binding per unit (1μmole Pi/hr) Na, K-ATPase: 6:1, vas deferens; 1:1, heart. However, there are far fewer units of Na, K-ATPase per gram wet tissue in the vas deferens (74 vs 517) and far less ^{3}H-OU binding (13 vs 504 pmole/g wet weight). These data support the hypothesis that the vas deferens contains considerably fewer Na, K-pump sites than does the heart. (Supported by grants HL 05991, HL 24585)

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PROCAINE CONTRACTION OF POTASSIUM-DEPOLARIZED RAT UTERUS FOLLOWING BETA-ADRENERGIC RELAXATION. J.D. Schiff. N. Y. U. Dental Center, New York, NY 10010.

Procaine (10 mM), which normally relaxes potassium-depolarized smooth muscle in vitro, evokes a transient contraction in depolarized rat uterus that has previously been exposed to isoproterenol (100 nM). The contractile response to procaine is blocked by propranolol (10 μM) administered either before or after the isoproterenol. The contraction occurs in calcium-free medium and in the presence of D-600 (1 μM) and is, therefore, the result of procaine-induced release of intracellularly bound calcium ions. The enabling action of isoproterenol is not mimicked by dibutyryl cyclic AMP (0.5-1 mM). These data are consistent with earlier reports that isoproterenol relaxes smooth muscle by intracellular calcium ion sequestration independent of cyclic AMP formation (Schiff & Overweg: Life Sci., 23:2299, 1978) and imply that procaine releases this pool of sequestered calcium. Tetracaine (1 mM) does not duplicate the contractile action of procaine. (Supported by BRSG Grant RR07062, Biomedical Research Support Grant Program, Division of Research Resources, N. I. H.)

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COORDINATION OF METABOLISM AND CONTRACTION IN RAT PORTAL VEIN: ROLE OF PHOSPHORYLASE. Per Hellstrand* and Richard J. Paul. Dept of Physiology, Univ of Cincinnati Coll of Med, OH 45267.

In smooth muscle, actomyosin interaction and glycogen phosphorylase (p'lase) are both reported to be regulated by enzymatic cascades involving Ca^{++}- and cAMP-controlled phosphorylations. As the Ca^{++} sensitivity of both cascades are reportedly similar, it is of interest to determine whether they are separately activatible. P'lase (activity ratio, -/+ 2 mM AMP) and lactate production (J_{lac}, μmol/min·g) were determined under conditions affecting tension (P_o), Ca^{++}, and cAMP (n = 3-8):

	O_2 PSS		Anoxia		Iso + pap		Hyperosm	KCl
	+Ca	-Ca	+Ca	-Ca	+Ca	-Ca	-Ca	+Ca
P'lase	.27	.13	.40	.48	.58	.41	.48	.37
J_{lac}	.12	.10	.51	.71	.66	.45	.30	.14
P_o	↑	-	-	-	-	-	↑	↑↑

(+Ca=2.5mM, -Ca=1mM EGTA, iso+pap=10^{-5}M isoproterenol+10^{-4}M papaverine, hyperosm=PSS+300mM sucrose, KCl=PSS+80mM KCl). P'lase activation in this vascular tissue appears to involve both Ca^{++} and cAMP. With anoxia or cAMP increasing stimuli p'lase and glycolytic flux are activated, but not contraction, both in the presence and absence of $[Ca^{++}]_o$. The metabolic activation and tension development in Ca^{++}-free hyperosmotic medium seems to reflect intracellular Ca^{++} mobilization. As both contraction and p'lase activation are likely to require similar levels of Ca^{++}, additional mechanisms must exist to permit their observed independent activation. Supported by AHA 78-1080, NIH grants HL 23240 and HL 22619. Swedish MRC.

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cAMP MEDIATED MODULATION OF Ca^{2+} DEPENDENT ACTIVATION IN VASCULAR SMOOTH MUSCLE. P. Silver*, M. Holroyde*, R.J. Solaro, and J. DiSalvo. Univ of Cinti. Coll. of Med., Cinti., OH 45267.

Current evidence suggests that the cAMP-cAMP dependent protein kinase (PK) system can directly modulate contractile activity in either gizzard (J.B.C. 253:8347, 1978) or aortic (J. B.C. 254:9951, 1979) smooth muscle by inhibiting the phosphorylation of the myosin light chains (MLC). In this study, we examined the influence of cAMP-PK on the Ca^{2+} requirements for both activation of actin-myosin interactions and phosphorylation of the MLC in aortic native actomyosin (NAM). Incubation of NAM with cAMP and aortic type II PK produced a rightward shift in the relationship between free $[Ca^{2+}]$ and NAM Mg-ATPase activity, doubling the Km for free Ca^{2+} from a control value of 1.1 μM to 2.3 μM. A concomitant shift in the Km of free $[Ca^{2+}]$ for MLC phosphorylation also occurred. However, neither basal nor maximal activities of either parameter were significantly affected by cAMP-PK. Addition of exogenous calmodulin (CM-3μM) to cAMP-PK treated NAM relieved inhibition of MLC phosphorylation in a Ca^{2+} dependent manner. Likewise, depressed superprecipitation of cAMP-PK NAM was increased by Ca^{2+}-CM. These findings suggest that cAMP-PK mediated inhibition of actin-myosin interactions in vascular smooth muscle involves: a) a shift in the Ca^{2+} sensitivity of system and b) Ca^{2+}-CM mediated phosphorylation of the MLC. (Supported by NIH grants HL 20196 and HL 22231.)

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INTRACELLULAR POTASSIUM ACTIVITY IN NORMAL AND DYSTROPHIC MAMMALIAN SKELETAL MUSCLE FIBERS. Harold Silverman*, Milton P. Charlton* and Harold L. Atwood, University of Toronto, Toronto, Ontario.

The K^+ activity of the sarcoplasm and the resting potential were measured in the superficial fibers of the gastrocnemius in normal and dystrophic (dy^{2J}) C57B16J mice using a double-barrel K^+-selective microelectrode. Measurements were made on anesthetized animals with the innervation and blood supply of the fibers intact. Dystrophic fibers showed both significantly reduced average sarcoplasmic K^+ activity and lower average resting potential than normal fibers. Histochemical analysis reveals the superficial fibers of the gastrocnemius to be uniformly fast-twitch, the majority being fast-twitch glycolytic. The dystrophic fibers are also fast-twitch, but show a more oxidative metabolism. This increase in oxidative capacity is correlated with neurally derived overactivity or pseudomyotonia. Since efflux of K^+ is thought to occur during bouts of muscle activity, it is not clear whether the reduction in K^+ reported is due to the overactivity of these fibers or whether the reduction in K^+ might be related to a general membrane defect associated with the dystrophic fiber genome. Current comparison of fast-twitch fibers from normal and dystrophic forelimb muscles (where pseudomyotonia does not occur in the affected animals) should provide insight into this question. Supported by the Muscular Dystrophy Association of Canada.

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MYOSIN ALTERATIONS FOLLOWING COMPENSATORY HYPERTROPHY OF THE PLANTARIS MUSCLE. Earl G. Noble*, Barbara L. Chitovas*, C. David Ianuzzo. Departments of Physical Education and Biology, York University, Toronto, Ont. M3J 1P3, CANADA.

Previously, we have histochemically demonstrated that compensatory hypertrophy of the plantaris muscle is associated with an increase in the proportion of alkaline labile fibres. The purpose of the present study was to ascertain if the histochemical changes are reflected in alterations of the myosin ATPase activity and the myosin light chain composition of hypertrophied muscle. Hypertrophy of the plantaris was induced by myectomy of the synergistic gastrocnemius muscle while the plantaris of the contralateral sham-operated leg served as a control. Following hypertrophy, the plantaris muscle weight was increased by 81% and the alkaline labile fibre population rose from 10% in the control plantaris to 27% in the hypertrophied muscle. The myosin ATPase activity declined from 0.571 to 0.528 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ ($P<.01$) following hypertrophy, when measured at pH 7.0. Electrophoretic patterns demonstrated a shift in the myosin light chain composition of the hypertrophied plantaris that was complementary to the histochemical and biochemical alterations found in this muscle. The results of this investigation suggest that compensatory hypertrophy of the plantaris is accompanied by a change in the myosin composition of the muscle. (Supported by Canada NSERC Grant #0404).

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THYROID HORMONE INDUCED CHANGES IN LDH ACTIVITY AND ISOZYME DISTRIBUTION OF RAT MUSCLE. Luke Nwoye* and W.F.H.M. Mommaerts Dept. of Physiol, UCLA Sch. Med., L.A., CA 90024.

Recent studies have shown significant changes in contraction speed, myosin ATPase activity and light chains of rat soleus muscle following alterations in the thyroid status. The purpose of this study was to determine whether there are corresponding changes in the metabolic profiles of fast and slow muscles. We compared the glycogenolytic capacities of EDL and soleus muscles of thyroidectomized (hypo), euthyroid (Eu) and thyrotoxic(hyper) SD rats. LDH activity (lactate → pyruvate) declined markedly in both muscles of hypothyroid rats, was unchanged in thyrotoxic EDL and increased in thyrotoxic soleus. Agar gel electrophoresis and densitometric analysis showed that the relative contribution of the muscle specific (M) subunits of LDH isozymes increased in both muscles of thyrotoxic, and declinedin hypothyroid rats.

	*LDH Activity U/mg prot. min		% M-subunits in isozymes	
	SOL	EDL	SOL	EDL
EU	(5) 2.3 ± 0.2	6.5 ± 0.9	23.2	61.5
HYPO	(4) 1.3 ± 0.1	3.9 ± 0.6	17.7	38.7
HYPER	(4) 3.5 ± 0.2	6.2 ± 0.3	39.3	81.0

*Mean ± SEM $\times 10^{-3}$

The influence of thyroid hormone is clearly demonstrated by these results and a physiological role in controlling the balance between glycogenolysis and oxidative metabolism in muscle is thereby implied. Supported in part by Muscular Dystrophy Association, Inc.

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ENERGY PRODUCTION OF MAMMALIAN FAST AND SLOW TWITCH MUSCLES IN CALCIUM FREE SOLUTION. J K Barclay Dept. of Physiology, Monash University, Clayton, Victoria, Australia 3168.

This investigation of the effect of calcium free solution on mouse slow twitch soleus (SOL) and fast twitch extensor digitorum longus (EDL) used 500 ms tetanic contractions at 27°C. Determinations of tension (P mN.mm^{-2}) and total heat production (H mJ.g^{-1}) occurred at muscle lengths at and greater than the optimal length for tension development under control conditions and after 25 minutes exposure to low extracellular calcium (Krebs-Henseleit solution minus calcium but containing 2 x 10^{-3} mol.l^{-1} EGTA (0 Ca^{++})). Heat-tension data analysis used linear regression. The equations for SOL were $H = 20.2 + .24P$ (control) and $H = 8.5 + .25P$ (0 Ca^{++}) and for EDL were $H = 45.4 + .65P$ (control) and $H = 0.8 + 1.14P$ (0 Ca^{++}). The intercept was significantly decreased by 0 Ca^{++} in both muscles but only the slope in EDL was affected. The maximum tension decreased by 40% in SOL and by 69% in EDL. Since the intercept represents calcium turnover energy cost, a zero intercept indicates no activation. Thus the EDL response resulted from incomplete fiber excitation. The decreased SOL maximum tension and intercept indicated a muscle limited by calcium availability. Therefore decreased extracellular calcium affected excitation-contraction coupling in SOL and the membrane characteristics of EDL.

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LENGTH-TENSION RELATIONSHIP AND MAMMALIAN DIAPHRAGM MUSCLES IN VITRO. Kevin K. McCully*, Gaspare Farkas*, and John A. Faulkner, Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan, USA, 48109.

Diaphragmatic function has been studied under a variety of physiological conditions, yet the relationship between diaphragmatic function and diaphragm (DPM) muscle length is not well defined. Kim et al.(JAP 41:369-382, 1976), suggested that the DPM of dogs in situ develops tension over a greater range of muscle lengths than do other skeletal muscles. We studied the in vitro length-tension characteristics of small bundles of DPM from rats, cats, rhesus monkeys, dogs, and pigs. Isometric contractile properties were measured in an in vitro bath at 37°C. In each species, the contraction and half relaxation times were consistent with the percentage of Type II fibers found (42-77%). In vitro DPM tension development occurred at lengths 50-140% of the length where active tension was maximal (Lo). The length-tension relationship is not significantly different from results for other skeletal muscles. Passive tension started at 100-105% of Lo and became greater than active tension at lengths between 121-126% of Lo. These data are not different from data in other skeletal muscles. We conclude that the specific functional characteristics of the DPM observed in vivo result from the unique attachments of the DPM and changes in DPM length initiated by activities of the intercostal and abdominal muscles. (Supported by Muscular Dystrophy Association, Inc.).

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CONTRACTILE PROPERTIES OF RAT SOLEUS AND PLANTARIS MUSCLES FOLLOWING PROLONGED RUNNING. Timothy P. White, John A. Faulkner, and John F. Villanacci*. Departments of Physical Education and Physiology, University of Michigan, Ann Arbor, MI. 48109

We tested the hypothesis that long-term running would result in a slowing of the contractile properties of rat skeletal muscle. Female Wistar rats ran at 27 m/min on a 15% slope. Some animals ran for 5 months for up to 2 h/day, while others ran 13 months for up to 1 h/day. Comparisons were with pair-weighted, age-matched sedentary controls. At sacrifice, the soleus (SOL) and plantaris (PLN) muscles were removed and contractile properties were measured at 37°C. The SOL and PLN showed an increased resistance to fatigue. At 13 mo, the PLN of runners developed 76,24 and 14% more tension at 50,80 and 100 Hz stimulation, respectively, than did controls. At a given submaximal frequency of stimulation, slow muscle fibers develop a higher percentage of maximal tension than do fast muscle fibers. These data are consistent with alterations in myofibrillar ATPase activity due to running. We conclude that contractile properties of rat PLN muscle are slowed by long-term endurance running. (Supported by a University of Michigan Faculty Research Grant).

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CONTRACTILE PROPERTIES OF CAT SKELETAL MUSCLES TRANSPLANTED WITH NERVES-INTACT OR WITH NERVES ANASTOMOSED. John A. Faulkner, John M. Markley, Jr.,* and Kevin K. McCully*. Department of Physiology, University of Michigan, Ann Arbor, MI, 48109.

Three to six g extensor digitorum longus (EDL) grafts in cats, in which revascularization and reinnervation occurred spontaneously (standard grafts), regained control values for contraction and relaxation times and for maximum velocity of shortening (V_o) 240 days after transplantation. The maximum tension (P_o) plateaued at 24% of the control value after 190 days (Faulkner et al. Am. J. Physiol. 238:C120-126, 1980). We hypothesized that the reduced function resulted from incomplete reinnervation of muscle fibers. In 10 cats, one EDL was transplanted with nerve-intact and the other with nerve anastomosed. Five animals were sacrificed at 120 days and the remainder at 200 days. Contractile properties were measured and choline acetyltransferase (CA) activity assayed. Since no differences were noted between nerve-intact and nerve anastomosed grafts, data were pooled. Contraction and relaxation times and V_o reached control values by 120 days which was earlier than for standard grafts. After 120 days, the P_o was 40% of control. This P_o was comparable to that of standard grafts 190 to 520 days after transplantation. Compared to standard grafts, the CA activity of the grafts made with nerve repair indicated improved innervation. These data support our hypothesis that reinnervation is critical for the restoration of function in 3 to 6 g autografts. (Supported by Muscular Dystrophy Association, Inc.).

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ORIGIN OF L-PROLINE DURING HYPEROSMOTIC STRESS IN A EURYHALINE, OSMOCONFORMING BIVALVE. S.H. Bishop, D.E. Greenwalt*, and J.M. Burcham*. Iowa State University, Ames, Iowa 50011

The variations in high intracellular free amino acid levels in euryhaline osmoconforming bivalves which occur with changes in the osmotic pressure extracellular media aid in the control of cell volume. Experiments with tissues of the ribbed mussel (Modiolus demissus) indicate that L-pro accumulates rapidly during hyperosmotic challenge. Addition of aminooxyacetic acid (AOA) or L-cycloserine (transaminase inhibitors) blocks L-pro accumulation and causes L-ornithine accumulation. Experiments with gill tissue using ^{14}C-L-arg and ^{14}C-L-orn indicate enhanced conversion to L-pro and L-glu during hyperosmotic stress; there was no conversation of ^{14}C-L-glu to ^{14}C-L-pro in the preparations. AOA blocked ^{14}C-L-arg and ^{14}C-L-orn conversion to L-pro and L-glu. Arginase, an AOA sensitive ornithine aminotransferase, pyrroline-5-carboxylate (P-5-C) reductase, a mitochondrial proline oxidase, and a L-amino acid oxidase which converts L-orn to P-2-C were detected in the tissues of M. demissus. We conclude that L-pro arises from L-arg and not from L-glu. The L-arg is probably released from tissue protein and from phosphoarginine with increased metabolism during hyperosmotic stress. (Supported in part by NSF grant PCM-78-09771).

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CARBONIC ANHYDRASE ACTIVITY IN GILLS AND OTHER TISSUES OF AQUATIC AND TERRESTRIAL DECAPOD CRUSTACEANS. Raymond P. Henry. University of Texas, Marine Science Institute, Port Aransas, Texas 78373

A rapid and sensitive pH-stat method was used to directly assay for dehydration activity of carbonic anhydrase (CA) in crude homogenates of individual gill pairs as well as other tissues of Callinectes sapidus and Gecarcinus lateralis. In Callinectes acclimated to freshwater, where blood Cl is regulated above that in the medium, the posterior 3 pairs of gills have 3 to 7 times the activity per gm fresh wt than the anterior gills (3 to 4x specific activity). This large difference was not seen among gills of Callinectes collected from high salinity water (850 mOsM) where blood Cl is iso-ionic to ambient. In the terrestrial Gecarcinus the majority of CA activity was found in the posterior 2 pairs of gills. These results correlate closely with the branchial distribution of Na-K ATPase and salt transporting cells in both species and strongly implicate CA in the Cl regulatory mechanism. (Supported by NSF grant #PCM 77-24358).

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A NOVEL, SOLUTE INDEPENDANT MECHANISM OF WATER TRANSPORT IN THE DESERT COCKROACH, ARENIVAGA. John Machin and Michael J. O'Donnell*. Univ. of Toronto, Toronto. Ont. M5S 1A1

Adult females and nymphs possess special cuticular structures associated with the mouth, which condense water vapor from the atmosphere and convey it to the gut. At humidities above 81% R.H. (water potential, Ψ = -284 bars) water condenses on to a superficial fluid film which marker dyes and radioactive tracers indicate is rapidly conveyed to the oesophagus and crop (gut $\Psi \approx -25$ bars). Maximum solute concentrations of the order of 500 mM/l (equivalent Ψ = -25 bars) together with the small volume of the absorbing fluid are inconsistent with an uptake mechanism depending on dissolved solutes for water potential reduction. SEM studies suggest that the scale and geometry of spaces between hairs and plates on the absorbing surfaces could take up water from sub-saturated atmospheres by capillary condensation; a mechanism commonly observed in finely porous materials. The unusually high water vapor affinity and water holding capacity of cuticular condensing structures measured with a variety of gravimetric and vapor pressure techniques, supports this conclusion. The proximity of oscillating structures driven by prominent masses of muscle suggests that the considerable work required to extract water of low water potential from the condensing structures comes from muscle contraction. (Supported by Natural Sciences and Engineering Research Council Canada, operating grant A1717).

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EFFECTS OF ADAPTATIONAL SALINITY ON BRANCHIAL Na^+,K^+-,Mg^{++}-and Ca^{++} -ATPase ACTIVITIES IN EURYHALINE GILLICHTHYS MIRABILIS. Byron A. Doneen. Univ. of Michigan, Ann Arbor, MI 48109

G. mirabilis is unusual among euryhaline teleosts by displaying elevated Na^+,K^+ -ATPase activity (5.8 µmole Pi mg^{-1} hr^{-1}; 20°) after 7 days adaptation to freshwater (FW) and reduced enzyme activity following adaptation to sea water (SW) (2.7 µmole Pi mg^{-1} hr^{-1}). However, transfer of SW-adapted fish to hypersaline conditions (150% SW) stimulated branchial Na^+, K^+ -ATPase activity to near the FW-adapted peak level. Animals adapted to a dilute salinity (5% SW) also showed higher gill Na^+,K^+ -ATPase activity than SW-adapted fish, but the time required to elicit maximum activity was greater than after transfer to FW. Branchial Mg^{++} -ATPase activities were rather uniform in FW-, SW- and 150% SW-adapted fish, but this activity was depressed by 50% after more than 3 months in 5% SW. Gill Ca^{++} -ATPase activity was also highest in FW- or 5% SW-adapted Gillichthys (7.2 µmole Pi mg^{-1} hr^{-1}), but lowest in 150% SW-adapted fish (4.1 µmole Pi mg^{-1} hr^{-1}). These results support a model in which branchial chloride cells secrete Na^+ and Ca^{++} ions in SW- or 150% SW-adapted fish, but can absorb these same ions after adaptation to FW. (Supported by Phoenix Project and by NSF Grant PCM 7922985).

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EFFECT OF ACID EXPOSURE ON THE ACID-BASE AND IONIC BALANCE OF TROUT. J. Booth and G. Holeton. Dept. of Zoology, Univ. of Toronto. Toronto, Canada. M5S 1A1.

Rainbow trout were held in a constant pCO_2 pH-stat recirculating system. During exposure to pH 7 for 3 days, blood pH, hematocrit (Hct), and K^+ remained constant. Total plasma CO_2 ($[CO_2]_t$) decreased slightly. Plasma Na^+ and Cl^- increased due to net uptake of these ions from the water. Titration of the water at constant pCO_2 indicated that the trout excreted an excess of base nearly identical to the amount of NH_4^+ appearing in the water. Subsequent exposure of the same fish to pH 4 for 4 days caused an acidemia accompanied by reduced blood $[CO_2]_t$. Na^+ and Cl^- were lost to the water. Hct increased despite repeated blood sampling. The rate of excretion of base increased more than the rate of excretion of NH_4^+. This discrepancy represented an uptake of H^+ by the fish. Following return to pH 7, blood pH and $[CO_2]_t$ returned to normal within 24 hrs. Slower restoration of plasma Na^+ and Cl^- was accomplished by uptake of these ions from the water. Hct fell to levels which would be predicted from the losses due to sampling. Base and NH_4^+ excretion rates returned to normal. The results suggest that (1) NH_4^+ excretion is implicated in the regulation of acid-base balance; (2) acidemia due to acid exposure is accompanied by uptake of H^+ ions from the water; (3) losses of Na^+ and Cl^- due to the acid cause a reduction in the extracellular space; (4) the effects of 4 day exposure to acidic conditions are largely reversible. (This study was funded by NSERC grant #A6367 to G.H.)

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THE INFLUENCE OF LOW ENVIRONMENTAL pH ON CARDIOVASCULAR FUNCTION IN THE RAINBOW TROUT, SALMO GAIRDNERI. C.L. Milligan* and C.M. Wood. McMaster University, Hamilton, Ontario.

Rainbow trout (150-350g) exposed to acidified water (pH 4.0-4.5) for 3 days exhibit a cardiovascular disturbance as indicated by increases in both heart rate and mean arterial blood pressure. The increase in heart rate is due to an endogenous release of adrenaline and not to a withdrawal of cholinergic tone. The rise in blood pressure is associated with increases in hematocrit and whole blood viscosity. These increases are due primarily to a combination of erythrocyte swelling and plasma water loss. The relative contribution of catecholamines to the blood pressure rise will be discussed. (Work supported by NSERC strategic grant to C.M.W.)

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THE MODIFICATION OF RAINBOW TROUT GILL STRUCTURE AND FUNCTION DURING EXPOSURE TO CRUDE PARTICULATE HYDROCARBONS.
Marc E. Duey*, M.P. Wong*, F.R. Engelhardt. University of Ottawa, Ottawa, Ontario, Canada, K1J 7N4

Gill structure and function were found to be useful indicators of environmental stress. Morphological assessment by light, electron and scanning microscopy coupled with the analyses of physiological paramaters were used to evaluate the changes induced by sublethal dosage of Norman Wells and Venezuelan Crude oils. Juvenile rainbow trout (Salmo gairdneri) in fresh water were subjected to 1000 ul/L concentration of weathered oil for 7 days. Exposure regimes resulted in significantly decreased levels of plasma osmolarity, sodium and chloride ions, while calcium concentration was not altered. Hematological examination showed significant decreases in the white blood cell volume indicating a stress response. Mucous secretion increased, this process of coating the tissue traps oil particles. Epithelial cells bulged out and lifted away from the lamella and pilar cell system increasing the diffusional distance to the blood. This in association with some lamellar fusion and evidance of decreased circulation may be responsible for the osmotic and ionic imbalance observed. Chloride cell proliferation and greatly increased apical surface area are believed to be modifications to recruit ions from the medium for the affected fish.

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IMPROVED VISUALIZATION OF APICAL VESICLES IN CHLORIDE CELLS OF FISH GILLS USING AN OSMIUM QUICK-FIX. Timothy J. Bradley. University of California, Irvine, CA 92717

The tubovesicular system (TVS) of fish chloride cells is a vesicle-containing region lying between the tubular reticulum and the apical pit. These vesicles have been implicated in the transport of ions and/or polysaccharides to the apical cell surface. A very rapid shuttling of vesicles is necessary to explain known rates of ion transport. Glutaraldehyde, although an excellent crosslinking agent, cannot rapidly fix such motile systems. Osmium fixatives stop many cell motile systems in less than 1 sec (e.g. ciliary beat). I have therefore employed an osmium quick-fix technique to study the TVS. Gills of seawater adapted Fundulus heteroclitus and Poecilia reticulata were fixed for 20 sec in buffered osmium (1% OsO_4 in Cacodylate, pH 6.0) followed by conventional fixation (glutaraldehyde followed by osmium). Chloride cells were examined in the EM and compared to cells from gills fixed conventionally without a prior quick-fix. Quick-fixation with osmium leads to a striking increase, in both species, in the number of vesicles and vesicle-apical membrane fusion events observed. Control experiments demonstrate that osmium does not act by vesiculating membranes or depolymerizing actin microfilaments. The results suggest the existence of a rapid vesicular shuttle in the chloride cell. The possible role of the shuttle in ion transport will be discussed.
(Supported by NIH grants 5S07 RR05764 and GM27919)

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WATER, ELECTROLYTE AND NITROGEN BALANCE IN FASTING WEANED ELEPHANT SEALPUPS, MIROUNGA ANGUSTIROSTRIS. Daniel P. Costa* and Leo C. Ortiz. Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, Ca., 92093 and Center for Coastal Marine Studies, University of California, Santa Cruz, Ca. 95064.

Elephant seal pups are weaned after a brief nursing period of 28 days, after which they fast without ingesting water for up to 3 months. The mechanisms by which these seals maintain water balance were investigated in this study. Urinary water, electrolyte and urea nitrogen excretion were monitored for 1-5 days in 7 fasting weanling seals. Oxygen consumption and evaporative water loss were simultaneously measured in 9 weanling seals (mean weight 83 kg) for periods of 1-2 hrs with dry air (n=4) and ambient air (n=10, 68% RH, T=18°C). The mean urine production was 177±40 ml/day. Urea excretion averaged 7.3±3.8 gr/day at a mean urea concentration of 0.67±0.18 M. The mean urine osmolarity of 3 animals was 1165 mOsm/s. The mean $\dot{V}_{O_2}$ was 4.39±1.34 $ml O_2$/kg min. Evaporative water loss (EWL) to metabolic water production (MWP) ratio was 0.64±0.15 in ambient air and was 1.03±0.1 in dry air. An EWL/MWP ratio less than 1 allows the seal to make a net gain from metabolically produced water. The net gain in metabolic water is more than sufficient to meet the urinary water loss of fasting weaned elephant seal pups. (Supported by PHS NIH Fellowship #PHS AM 06093-01).

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RENAL ANATOMY OF A JUVENILE OKAPI (OKAPIA JOHNSTONI SCLATER) Noble S. R. Maluf.

This is probably the first description of the renal anatomy of the okapi. The kidney is of the medullary crest type and is divided transversely into six lobes by encroachments of cortex into the medulla. These lobes are demarcated externally by furrows. The cortex is about 85 percent of the renal mass. There are long and short medullary loops. The kidney has about 2.89 x 10^6 glomeruli which form 4.85 percent of the renal cortical mass. The filtering surface of a glomerulus is 0.088 mm^2, making the total filtering surface of one kidney 0.219 square meter. The number of glomeruli per kidney falls into the line which relates number of glomeruli to adult bodily weight and is expressed by log N = 0.59log W + 3.2, where N is number of glomeruli in one kidney and W the adult bodily weight.

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THE EFFECT OF ACUTE DILUTIONAL (DH) AND ISOSMOTIC HYPONATREMIA (IH) ON BRAIN FLUID AND ELECTROLYTE BALANCE. J.E. Melton and E.E. Nattie, Department of Physiology, Dartmouth Medical School, Hanover, NH 03755.

In DH the brain initially swells and then loses K^+ (intracellular) and Na^+ and Cl^- (extracellular) ions which restores brain volume toward normal. This study asks whether the ionic changes are due to hyponatremia or the brain swelling and evaluates CSF ionic content during this stress. Conscious rats were dialyzed with distilled water (DH), mannitol Ringers (IH) or Ringers (control) for 3-6 h. Final plasma [Na^+] was 100-110 mEq/l in DH and IH rats but IH rats had no fall in plasma osmolality. In DH, brain water increased by 10% at 3 h and 5% at 6 h. Brain Na^+, K^+ and Cl^- (per dry wt.) decreased 16%, 4.6% and 27% at 3 h with no further change in Na^+ or K^+ at 6 h but a slight decrease in Cl^-. CSF [Na^+] decreased by 83% of the plasma decrease at 6 h. [Cl^-] decreased proportionally to the fall in plasma [Cl^-] and [K^+] was unchanged. In IH brain water and K^+ were unchanged but Na^+ and Cl^- decreased 13% and 9.5% at 3 h with no further change at 6 h. CSF [Na^+], [K^+] and [Cl^-] were unchanged after 6 h of IH. We conclude that under these conditions 1) brain K^+ loss and CSF ionic changes in DH are related to swelling rather than hyponatremia per se. 2) The loss of tissue Na^+ and Cl^- is, in part, related to hyponatremia independent of brain swelling. (Supported by HL 18351, HL 00364, the Ryan Foundation and RR 05392.)

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REDISTRIBUTION OF INTRACRANIAL FLUID VOLUMES DURING HYPERCAPNIA. M.J. Songer*, W. Dang*, S.R. Heisey, T. Adams and H.K. Fry*. Dept. of Physiology, Michigan State Univ., E. Lansing, MI 48824.

Hypercapnia increases brain blood flow and its blood volume (BBV). Since the brain and its fluid compartments are enclosed in a rigid container, the skull, an increase in BBV necessitates a decrease in the CSF volume and/or an increase in intracranial pressure (ICP). Using anesthetized cats (Dial-urethane, ip, or Na pentobarbital, iv) cerebral ventricles were perfused with an artificial CSF at constant rate, containing inulin and ^{3}H-sucrose from cannulae in the two lateral cerebral ventricles to one in the cisterna magna. Inflow and outflow rates (V_i & V_o, resp.) and concentrations of test molecules (c_i & c_o, resp.) and ICP were measured. While the animal spontaneously breathed air, steady state concentrations of test molecules were established in cisterna magna effluent after which 8% CO_2 in air was breathed. Hypercapnia induction caused small (2-4 cm H_2O) ICP changes lasting 30-90 s while V_o increased transiently (15-20 min) before returning to pre-hypercapnic levels. In addition, c_o's of both test molecules decreased transiently coinciding with the increased V_o. These data are consistent with the hypothesis that hypercapnia-induced increases in BBV occur largely in venous channels surrounding the subarachnoid space, and that CSF contained in this space is displaced through the low resistance cisternal cannula. A poorly mixed compartment, probably the subarachnoid space, contributes to the transient decrease in c_o's of the test molecules.
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MECHANISM OF 2-DEOXY-D-GLUCOSE UPTAKE IN TYPE II GRANULAR PNEUMOCYTES. J.S. Kerr, J. Reicherter,* & A.B. Fisher. Dept. Physiol., Univ. Penn. Sch. Med., Phila., PA 19104 and CMDNJ - Rutgers Medical School, Piscataway, N.J. 08854

Hexose uptake by rat granular pneumocytes (Type II lung epithelial cells) was investigated by incubating isolated cells with 2-deoxy-D-glucose (DOG), a sugar which is phosphorylated but not further metabolized. Cells trypsinized from lungs were isolated in primary culture for 24 hr (A. Fisher, et al, J. Appl. Physiol., in press). Cells attached to flasks were routinely incubated for 60 mins in minimal essential medium containing 5 mM DOG in place of glucose. DOG uptake increased progressively with the time of incubation and approached a plateau of 35-40 nmol/10^6 cells at 60-90 mins. Uptake also increased as a function of external DOG concentration, with a calculated maximal uptake of 52.6 nmol/10^6 cells and a half-maximal uptake at a DOG concentration of 3.1 mM. DOG uptake (5.0mM) was decreased approximately 50% by incubation with 3.3 mM glucose, 0.5 mM ouabain, or 1 mM phlorizin. Lowering the external [Na^+] from 150 to 10 mM decreased DOG uptake by 25%. Free DOG and DOG-phosphate levels were measured before and after precipitation of cell homogenates with $Ba(OH)_2$ and $ZnSO_4$. In 3 experiments, free (non-phosphorylated) intracellular DOG accounted for 21-31% of the total DOG. Calculated intracellular free DOG was 15-20 mM, indicating intracellular accumulation against a concentration gradient. Our findings suggest that DOG is taken up in Type II cells by the Na^+-dependent carrier-mediated process of active transport.

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EARLY PULMONARY METABOLIC CHANGES IN HYPEROXIA. G.H. Gurtner, N. Brennan, M. Peake, S.C. Spielberg, J.T. Sylvester and Y.N. Cha, The Johns Hopkins Medical Institutions & Baltimore City Hospitals, Baltimore, Maryland.

We have previously reported that a cytochrome P-450 mediated reaction, the o-demethylation of p-nitroanisole (PNa) measured in the isolated perfused rabbit lung, was extremely sensitive to damage by hyperoxia (Am.Rev.Resp.Dis. #4, Part 2:319,1980). We have subsequently found, in groups of eight rabbits, that a four hour pre-exposure of the animals to 100% O_2 causes a 44% inhibition of the rate of reaction. In an effort to determine the cause of this acute inhibition, we measured reduced glutathione (GSH) level and the rate of p-nitroanisole metabolism in lung microsomes incubated with excess NADPH. These measurements were carried out in control rabbits and rabbits exposed to four hours of 100% O_2. We found no changes in the rate of microsomal metabolism in the O_2 exposed animals when compared with the control animals but found a substantial depletion of GSH. The results can be explained by the formation of lipid peroxides, the metabolism of which utilizes GSH and causes formation of oxidized glutathione (GSSG). Reduction of GSSG to GSH requires NADPH which is also required for metabolism of PNa. An acute depletion of NADPH could explain the inhibited, in vivo, metabolism which could be returned to normal, in vitro, by the addition of exogenous NADPH. All of these changes precede any alterations in lung compliance or gross appearance and many represent the earliest changes in pulmonary O_2 toxicity. (Supported by PHS Grant #HL10342).

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The Secretion of Pulmonary Surfactant Under Conditions of Reduced Protein Synthesis. Richard J. King and *Helen Martin, Univ. of Texas Health Science Center, San Antonio.

The lipid and protein constituents of pulmonary surfactant are metabolized at similar rates, and may be secreted as a lipid-protein complex. The metabolic requirements of this activity are unknown. The purpose of these experiments was to determine if the synthesis of cellular protein is required for the process of secretion. We carried out our experiments in vitro using primary cultures of adult rat Type II cells. At confluency we added radioactive leucine to the culture medium to study the metabolism of the 35,000 dalton apolipoprotein of surfactant; or radioactive choline to study the metabolism of dipalmitoyl phosphatidylcholine (DPPC). In control cultures leucine labeled the intracellular pool of surfactant apolipoprotein within 15 minutes, and this protein reached peak specific activity within 2 hrs. Cycloheximide at $1x10^{-4}$ M inhibited 90% of the isotopic incorporation within 60 minutes after addition. Choline incorporation into DPPC did not reach a maximum until 6 hours, and was not inhibited by $1x10^{-4}$ M cycloheximide. Cycloheximide did not affect the rate of secretion of DPPC, nor that of the apolipoprotein synthesized before cycloheximide was added. We conclude, therefore, that on-going protein synthesis is not required for the secretion of the constituents of surfactant under conditions in which these components have already been synthesized and (presumably) packaged into lamellar bodies.

Supported by NHLBI Grant HL16725.

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FETAL LUNG MATURATION: ACCELERATION BY MATERNAL FASTING. R.A. Rhoades and D.A. Filler† Department of Physiology, Indiana University School of Medicine, Indianapolis, IN 46223

Preparation of the fetal lung for breathing depends upon the synthesis and secretion of pulmonary surfactant. This investigation examined the influence of dietary restriction on lung maturation. Time-pregnant rats (Sprague-Dawley) were either fed ad libitum or fasted the last 3 days of gestation. Fetal rats were delivered prematurely at 20.5 days (term 22 days) of gestation. Mothers were killed by decapitation and fetuses were rapidly removed. Fetuses were allowed to breathe 3 h (room air) before killed. Only animals that survived for 3 h (pink and breathing) were used for further studies. Neonates (N-48) from pregnant rats fasted 72 h showed a significant decrease in both fetal body weight (20%) and lung weight (30%). Lung protein (mg/g wet wt.) in these animals was unchanged, but lung glycogen (mg/g wet wt.) was decreased (19%). A striking finding was that 69% of the neonates from the fasted rats survived premature breathing while only 20% of the controls survived premature breathing. Moreover, neonate lungs from fasted rats showed a 20% increase in the lecithin/sphingomyelin ratio. Neonate lung slices from fasted mothers incubated 2 h in Krebs bicarbonate buffer also showed a 28% increase in incorporation of [2-^{3}H]glycerol into phospholipids, whereas glycerol incorporation into triacylglycerols was unaffected. These data show that dietary restriction during the latter part of gestation accelerates lung maturation. (Supported by NIH grant HD 10670 and HL K04 317).

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MORPHOLOGY OF FERRET TRACHEAL GLANDS STIMULATED IN VITRO WITH ADRENERGIC AND CHOLINERGIC AGONISTS. C. Basbaum,* I. Ueki,* J. Nadel (SPON: B. Davis). CVRI, UCSF, San Francisco, CA 94143

Electron microscope observations revealed that the autonomic nerve supply to ferret tracheal glands included adrenergic and cholinergic axons. Cholinergic axons greatly outnumbered adrenergic axons. The effect on the glands of adrenergic and cholinergic stimulation was tested by in vitro administration of the α-adrenergic agonist phenylephrine (10^{-5}M) and the cholinergic agonist methacholine (10^{-5}M). The volume density occupied by secretory granules and cytoplasmic vacuoles was assessed. In each of five experiments, randomly selected tracheal rings were incubated with drug agonists while others served as controls. The volume density of secretory granules in unstimulated serous cells was 25% and in mucous cells was 62%. Cytoplasmic vacuoles accounted for less than 1% of the volume of unstimulated cells. Both adrenergic and cholinergic stimulation reduced the volume density of serous granules to 12% (50% depletion) but produced no changes in the volume density of mucous granules. The volume density of cytoplasmic vacuoles increased by 20% in serous cells in response to both drugs. Stimulation effects were blocked by administering the agonists in the presence of the appropriate antagonists. Thus, cholinergic and α-adrenergic stimulation had parallel effects on tracheal gland secretion in the ferret: serous cells were depleted of granules and became vacuolated; mucous cells were unchanged. (Supported by grants from the S.F. Lung Association and USPHS Program Project Grant HL-24136)

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IMMUNOCYTOCHEMICAL LOCALIZATION OF LYSOZYME IN THE FERRET TRACHEA. M. Tom-Moy,* C.B. Basbaum,* and J.A. Nadel (SPON: B. Davis). Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

The trachea secretes a complex mixture of fluids consisting of water, salts, lipids, mucous glycoproteins and other secretory proteins. Among the secretory proteins, IgA, lysozyme and lactoferritin have been described; however, the cellular origin of these secretory proteins has not been morphologically described. The purpose of this study was to identify the cellular localization of lysozyme in the trachea of the ferret. We used the unlabeled antibody enzyme method of Sternberger, an immunocytochemical technique, to localize this enzyme. Specificity controls for the staining included substitution of rabbit antihuman lysozyme (R-AHL) with normal rabbit serum and absorption of R-AHL with 70 μg/ml of human lysozyme. Under the light microscope (LM), lysozyme was localized within the serous-type cells of the ferret submucosal glands. Adjacent sections of the trachea which were stained with routine histological stains confirmed the localization of lysozyme to the serous cells. Immunoreactive lysozyme was absent from the mucous-type cells. LM examination of thick plastic sections revealed that lysozyme was present within the secretory granules of the serous cells. All control sections were negative. These results confirm the specificity of the antiserum for lysozyme and show the cellular localization of lysozyme in the ferret trachea. (Supported in part by USPHS Program Project Grant HL-24136)

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PATTERN OF SALT TRANSPORT IN EXCISED RABBIT TRACHEA. J.D. Davis*, R.C. Boucher*, J.T. Gatzy* and P.A. Bromberg. School of Medicine, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The relative contributions of the surface epithelium and submucosal glands to net Na and Cl transport across airway epithelium has not been determined. In the present study we have measured Na and Cl translocation across the trachea of the rabbit, a species with few submucosal glands. Tracheal segments were mounted in Ussing chambers. Potential difference (PD, mV) and short circuit current (Isc, μamps/cm^2) were measured and d.c. conductance (G, mS/cm^2) calculated. Unidirectional fluxes (J, μeq/cm^2·h) of ^{22}Na and ^{36}Cl were measured under short circuit (SC) and open circuit (OC) conditions. Means of three 15 min. flux intervals for each tissue matched by G, are shown ($-J_{net}$=secretion):

	SHORT CIRCUIT					OPEN CIRCUIT				
	PD	Isc	G	J^{Na}	J^{Cl}	PD	Isc	G	J^{Na}	J^{Cl}
M→S	10.5	71.8	8.0	3.77	4.85	11.2	80.7	8.3	3.33	5.55
S→M	10.0	71.7	7.9	1.75	4.69	11.0	80.7	8.0	1.74	3.90
NET				2.02	.16				1.59	1.65

The sum of the partial ionic conductances approximates G whereas J^{Na+}_{net} accounts for ≈75% of Isc. Submucosally applied acetylcholine (10^{-4}M) induced no change in unidirectional ion fluxes or bioelectric properties under SC conditions. In the rabbit, the surface epithelium actively absorbs Na+\whereas active Cl$^-$ transport was not detected in basal conditions or after cholinergic stimulation. (Support: HL-22924 & 16674)

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BIOELECTRIC PROPERTIES (BEP) AND ION TRANSPORT (IT) IN EXCISED HUMAN BRONCHI. M.R. Knowles*, G.F. Murray*, J.A. Shallal*, J.T. Gatzy*, R.C. Boucher* (SPON: D.W. Powell). University of N.C., School of Medicine, Chapel Hill, N.C. 27514.

We have previously surveyed species and regional patterns of BEP and IT in mammalian airways. In this study epithelial sheets from human segmental bronchi, obtained from 7 patients undergoing lung resection for cancer, were mounted in Ussing chambers. Potential difference (PD, mV) and short circuit current (Isc, μamps/cm^2) were measured, and dc conductance (G, mS/cm^2) calculated. Unidirectional fluxes (J, μeq/hr. cm^2) of ^{22}Na and ^{36}Cl were measured under short circuit (SC) and open circuit (OC) conditions. The means of three 15' flux intervals for 7 tissues, matched by G, are shown ($-J_{net}$=secretion):

	SHORT CIRCUIT					OPEN CIRCUIT				
	J^{Na}	J^{Cl}	PD	Isc	G	J^{Na}	J^{Cl}	PD	Isc	G
M→S	5.73	4.28	6.6	57.3	8.7	4.98	4.52	6.6	62.1	9.4
S→M	3.76	4.41	6.1	54.7	9.0	3.58	4.15	5.9	56.5	9.5
J_{net}	1.97	-.13				1.40	+.37			

J^{Na+}_{net} approximates the measured Isc and the sum of partial ionic conductances ($J^{Na+}_{S\to M} + J^{Cl-}_{M\to S}$) approximates G. The in vitro PDs are similar to in vivo lobar/segmental bronchial PDs in humans ($\bar{x}$=9.8mV, N=7). In basal conditions human bronchi appear to resemble bronchi from other mammalian species in that Na+ is actively absorbed whereas net Cl$^-$ transport cannot be detected.

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TRACHEAL MUCUS IN DOGS EXPOSED TO SO_2:VISCOELASTIC CHANGES DURING EXPOSURE AND RECOVERY. M. King, R. Boileau*, L. Delaunois*, and R.R. Martin. Meakins Christie Lab. McGill Univ. Clinic, Royal Victoria Hosp., Montreal, Canada

Seven dogs were exposed to SO_2 for 14 months (350-650 ppm, 2 hr/day, 3 days/wk, via the nose and mouth). At monthly intervals 3 days post exposure, the dogs were administered xylazine, 2-4 mg/kg i.m., and intubated. Tracheal mucus was collected on a cytology brush and analyzed for elasticity G' and viscosity η' with an oscillating ball microrheometer. The rate at which mucus collected on the brush was taken as an index of mucus flux. No systematic changes in mucus viscoelasticity or flux were observed in the first 7 months of exposure. Then G' and η' fell progressively while the mucus flux increased. Compared with pre-exposure control, mean G' at 1 rad/s decreased by 53%, η' at 1 rad/s decreased by 58% and mucus flux increased by 73%. Three of the dogs were kept for a recovery phase. After 2 months, the viscoelastic changes were, if anything, accentuated, but flux had returned to pre-exposure level. After 12 months, all parameters (G', η' and flux) had returned to original control levels. Supported by Merck Frosst Labs. and MRC of Canada.

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RESPONSE OF MUCOCILIARY CLEARANCE IN MAN TO ACUTE ALCOHOL ADMINISTRATION, D.B. Yeates, P.C. Venizelos and T.R. Gerrity, Section of Environmental Medicine, Department of Medicine, University of Illinois at the Medical Center and West Side Veterans Administration, Chicago, Illinois 60612.

Alcohol consumption has been implicated as a factor contributing to pulmonary disease. High blood levels decrease mucociliary transport in animals. To determine whether alcohol inhibits mucociliary clearance in man, the clearance of deposited radiolabelled particulates from the lung was measured in 12 volunteers on 2 occasions each using a gamma camera. On one study day, immediately after aerosol inhalation the subject ingested ethyl alcohol (0.5 mg/kg), producing blood alcohol levels of 68+7.5 mg/dl after one hour. The initial deposition of aerosol and subsequent retention in the lung over 4.5 hours was measured. A measurement of particle retention was made the following day and subtracted from each measurement to give bronchial retention curves. Aerosol deposition patterns were reproducible within subjects. Average percent retention measurements were not significantly different between the control and alcohol studies but the variance in the curves in the alcohol studies was significantly greater ($p<0.001$). Retention times representing clearance from different parts of the bronchial tree, when compared, suggested an overall discoordination of transport after alcohol administration. Thus, acute alcohol ingestion at levels similar to those achieved during social drinking altered mucociliary clearance. Upon repeated alcohol intake such fluctuations may result in impaired airway clearance and pulmonary function abnormalities.

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THE RELATIONSHIP OF INHALED AEROSOL PENETRATION TO MUCOCILIARY CLEARANCE IN HEALTHY HUMANS. C.S. Garrard*, T.R. Gerrity* and D.B. Yeates, Section of Environmental Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60612

Variations in the depth of penetration of an inhaled aerosol have been invoked to explain differences in mucociliary clearance in the human lung. To confirm and quantitate the effect of aerosol penetration on subsequent mucociliary clearance a monodisperse Tc99m labelled iron oxide aerosol (aerodynamic diameter 8 μm) was inhaled using a standardized breathing pattern, on two separate occasions by 12 healthy human volunteers. Activity within the lung was recorded using a gamma camera. Indices of penetration were derived from the initial deposition pattern profile across a horizontal band of lung (III/II slope ratio)[1] and from the percent activity retained at 24 hr. Intrasubject changes in 24 hr. retention were significantly correlated with the intrasubject changes in the percent activity cleared from the lung at 30, 60, 90 and 120 min ($p<0.05$ to 0.001) and changes in III/II slope ratio with clearance at 30 and 60 min. ($p<0.05$). Results indicate that for a given mucociliary transport rate, changes in aerosol penetration predictably alter clearance rates from the lung. Quantification of the relationship indicates that for each 1% increase in 24 hr. retention there is a 2.8% decrease in clearance at 120 min. This relationship provides a correction factor for changes in whole lung mucociliary clearance rates due to variations in aerosol penetration in the healthy human lung. [1]Physiologist 22 42, 1979

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DOES SLOW REACTING SUBSTANCE OF ANAPHYLAXIS (SRS-A) CONTRIBUTE TO THE DECREASE OF SPECIFIC PULMONARY CONDUCTANCE (SG_L) IN ALLERGIC BRONCHOCONSTRICTION? W.M. Abraham*, A. Wanner, W. Oliver*, Jr., M.M. King*, and L. Yerger*. Div. of Pulmonary Disease, Mount Sinai Medical Center, Miami Beach, FL.

Histamine appears to be the primary mediator responsible for the decrease in SG_L during allergic bronchoconstriction; however, a possible contributing role of SRS-A has thus far not been ruled out. We therefore determined the immediate effect of inhalation challenge with specific antigen on pulmonary flow resistance and thoracic gas volume in five Ascaris suum sensitive sheep. On different occasions separated by 10-14 days, the animals received either an intravenous injection of the H_1-antagonist chlorpheniramine (2 mg/kg) before inhalation challenge, or an intravenous infusion (1 mg/kg/min) of cromolyn or the SRS-A antagonist FPL-55712 before and during inhalation challenge. On two occasions, no pharmacologic agent was administered. Inhalation challenge on these two days decreased SG_L to 38% and 44% of baseline, respectively, indicating a reproducible airway response. The effects of inhalation challenge on SG_L were completely prevented by chlorpheniramine, and blunted by cromolyn (78% of baseline) and FPL-55712 (85% of baseline). We conclude that during allergic bronchoconstriction in sheep 1) the decrease in SG_L is mediated by histamine via H_1-receptors, 2) the airway effects of SRS-A are not reflected by changes in SG_L, 3) systemic cromolyn does not completely block mediator release, and 4) systemic FPL-55712 at the dosages used has cromolyn-like and/or antihistaminic effects. (Supported by NIH grant HL-20989).

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PREDOMINANCE OF POTENTIAL-DEPENDENT CALCIUM CHANNELS IN BOVINE CORONARY ARTERY. P.H. Ratz*, S.F. Flaim & R. Zelis, Cardiology, Pa. State Univ. College of Medicine, Hershey, Pa. 17033

Contraction of vascular smooth muscle can result from activation of receptor-operated (ROC) and/or potential-dependent (PDC) calcium channels. To determine the major channel in bovine coronary, rings were tested during exogenous norepinephrine (NE, 10^{-8}-10^{-5}M) and electrical stimulation at 9 (ES9) and 30 (ES30) volts (2msec duration, 2 Hz). NE induced slight increases in tension (T)(ΔT_{max}=.16±.01gm, ED_{50}=10^{-6}M, n=8). ES9 (NE release from sympathetic nerve terminals) resulted in equal changes (ES9 ΔT=.11±.02 gm, n=19, p>.5 compared to NE ΔT_{max}). Thus, NE-stimulated ROC are few and located at sympathetic nerve terminals. ES30 (activation of PDC) resulted in 10x greater change (ES30 ΔT=1.18±.22 gm, n=8) compared to both NE ΔT_{max} and ES9 ΔT (p<.001). With diltiazem (DZ, 10^{-5}M)(calcium antagonist), ES9 induced a biphasic change:initial increase in T(ΔTpeak=.02±.02 gm, n=5, p<.05 compared to ES9 ΔT) followed by a net dilation ($\Delta T_{plateau}$ = -.07±.03gm, n=8, p<.05 compared to ΔT_{peak}). DZ reduced T during ES30 by 47% (ES30+DZ ΔT = .63±.10 gm, n=8, p<.01 compared to ES30 ΔT). Thus, ROC & PDC both operate in bovine coronary artery. ROC reside near sympathetic nerve terminals and PDC predominate overall. DZ completely inhibits ROC but not PDC, suggesting that the action of DZ in bovine coronary is not solely via calcium influx blockade. (Supported in part by a grant from the York-Adams Chapter of the American Heart Association.)

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EFFECT OF UNSTIRRED FLUID LAYERS ON THE APPARENT OXYGEN SENSITIVITY OF VASCULAR SMOOTH MUSCLE. R.N. Pittman. Dept. of Physiol., Medical College of Virginia, Richmond, VA 23298.

Strips of vascular smooth muscle (VSM) suspended in aerated solutions are widely used to test contractile responses. The assumption is usually made that the solution is well mixed by the movement of gas bubbles. However, under steady state conditions fluid mechanical theory requires the existence of a diffusional boundary layer. Contracted strips usually relax in response to decreases in oxygen tension (PO_2) below some critical value. Since oxygen is consumed by VSM cells, an unstirred layer should cause the PO_2 at the strip surface to be lower than that in the bath and thus lead to an overestimate of the critical PO_2 for contraction. The amount of this error is given by $\Delta p = m\alpha/k'$, where m = oxygen consumption, a = strip half thickness, α = unstirred layer thickness and k' = O_2 diffusion coefficient in the fluid. Values of α ranging from 10 to 200 μm have been found previously in similar situations. Using literature values for m(0.54 x 10^{-2} ml/ (min·g), Coburn et al, Circ. Res. 1979) and k', the error in critical PO_2 for a 200 μm thick strip ranges from 1 to 21 mmHg (α = 10 to 200 μm). For a given level of activation, thin strips are more susceptible than thick strips to agitation by collisions with gas bubbles, and lower bubbling rates generally must be used to ensure stable force recordings. This will tend to increase the contribution of unstirred layers to the apparent oxygen sensitivity of small strips of VSM. (NIH grant HL 25383).

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EFFECT OF OUABAIN ON RELAXATION OF RABBIT CORONARY ARTERY STRIPS BY ADENOSINE. D.H. Foley. College of Osteopathic Medicine and Department of Zoology & Microbiology, Ohio University, Athens, Ohio 45701.

This study was undertaken to determine whether relaxation of artery strips induced by adenosine (Ado) would be altered in the presence of ouabain. Helical strips of left coronary arteries from rabbits were suspended in organ baths containing physiological salt solution maintained at 37°C and bubbled with 95% O_2 + 5% CO_2. Isometric contractions were induced with 10^{-7} - 10^{-6}M acetylcholine; subsequent addition of Ado produced concentration-dependent relaxations. Dose-response curves for Ado (10^{-8} - 10^{-5}M) were obtained in the absence and presence of 10^{-5}M ouabain. Responses to 10^{-8} - 5 x 10^{-6}M Ado were significantly attenuated by ouabain, while responses to 10^{-5}M Ado were not significantly different. The mean ED_{50} values for Ado in the absence and presence of ouabain were 9.6 x 10^{-8}M and 2.9 x 10^{-7}M, respectively (P < 0.005; N = 10). Since the level of pre-Ado tension in the presence of ouabain was adjusted to be equivalent to that in the absence of ouabain, the attenuation of Ado responses cannot be attributed to a greater level of initial tension. These data suggest that a) in these preparations, inhibition of the sarcolemmal Na-K pump by ouabain antagonizes the mechanism of relaxation by 10^{-8} - 5 x 10^{-6}M Ado, and b) vasoconstriction induced by cardiac glycosides in vivo could result in part from inhibition of vasodilation by endogenous Ado. (Supported by Ohio University College of Osteopathic Medicine)

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PARTIAL PURIFICATION OF 3':5'-CYCLIC ADENOSINE MONOPHOSPHATE-DEPENDENT PROTEIN KINASE FROM RAT MESENTERIC ARTERY AND ITS EFFECT UPON CALCIUM UPTAKE BY PLASMA MEMBRANE VESICLES. David M. Kattenburg * and Edwin E. Daniel. Department of Neurosciences, McMaster University, Hamilton, Ont., Can.L8S4J9

3':5'-Cyclic Adenosine Monophosphate (cAMP) and its dependent protein kinase (PK) may control smooth muscle contractility by regulating free intracellular calcium (Ca) concentration. PK has been partially purified from rat mesenteric artery by acid precipitation (pH 5.5) and subsequent DEAE-Cellulose (DC) chromatography. Enzyme elutes from DC at about 220mM salt, and is thus of the type II form. Histone phosphorylational activity is stimulated three-fold by 1uM cAMP (Km_{app} of 2.8*10^{-8}), and has an absolute magnesium requirement with optimal response at 10mM. Free catalytic subunit (CAT) elutes from DC in the presence of 10^{-4}M cAMP. Plasma membrane vesicles from the same preparation actively pump Ca. Vesicles preincubated with CAT in the presence of 2mM ATP exhibit higher Ca uptake levels at two minutes post-preincubation(control: 0.42±0.25; CAT: 2.76±1.27 umoles Ca/ gm protein). This effect is not observed at 0 or 5mM ATP. Boiled CAT exerts no effect, and Sigma PK inhibitor blocks the increase at a concentration (254ug/ml) which inhibits PK activity by 50%. These results suggest a possible role for cAMP-dependent PK in the control of smooth muscle function within a small resistance vessel. (Supported in part by a grant from the Medical Research Council of Canada, and from the Ontario Heart Foundation)

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ORIENTATION OF ELASTIC LAMELLAE AND SMOOTH MUSCLE IN SWINE CAROTID ARTERIES AND ISOLATED MEDIAL TISSUES. J.G. Walmsley* and R.A. Murphy. Dept. of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908

The effect of tissue shortening or arterial constriction on the orientation of smooth muscle (SM) and elastic lamellae was examined. Medial tissue strips were fixed (2% glutaraldehyde) during isometric contractions (10^{-4} M histamine) at lengths (L) defined with respect to the optimal length for force generation (L_0) [Herlihy & Murphy, Circ. Res. 33: 275-283, 1973]. The average orientation of SM cells remained parallel to the long axis of the tissue at all lengths, although the standard deviation increased with shortening. Similar results were obtained when the SM angle was defined with respect to the adjacent elastic lamellae. Tissue shortening was associated with folding of the elastic lamellae, quantified in terms of a waviness index (WI = mid-lamellar shortest distance/total length along folds; Wolinsky & Glagov, Circ. Res. 14: 400-413, 1964). WI was approximately equal to L/L_0 at lengths $\leq L_0$, indicating that WI is a useful estimate of the point on the length-force relationship at which tissues are fixed. In arteries fixed when constricted to various radii against 100 mm Hg pressure, WI was least in the peri-intimal lamellae and increased in peripheral lamina in a fashion dependent on the level of constriction. The variations in WI appear to reflect greater shortening of inner layers of SM. (Supported by NIH Grant 1 P01 HL 19242).

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LENGTH DEPENDENT SENSITIVITY IN VASCULAR SMOOTH MUSCLE E. B. Knauss*, J.M. Price*, D.L. Davis (SPON: C.H. Baker). Univ. of South Florida, Dept. of Physiol., Coll. of Med., Tampa, FL 33612.

Dose-response (D-R) curves were obtained from dog anterior tibial artery rings at various lengths (L) to determine if sensitivity (ED_{50}) to norepinephrine (NE) depends on L. Length for maximum active force (L_{max}) was determined using electrical stimulation before treatment with 6-Hydroxydopamine (Sigma). Culmative doses of NE were then given at 1.0, .85, .70, and 1.15 of L_{max}. Effect of repeating dose-response experiments was tested by obtaining three successive D-R curves at L_{max}. ED_{50} was estimated graphically from a line which connects the responses at two successive doses and passes through one half of the maximum active force for each D-R curve. ED_{50} was lowest at L_{max} (most sensitive) and increased (decrease sensitivity) as L changed from L_{max}. Differences in ED_{50} were significant (P<.05) for 1.0 - .85 L_{max}, 1.0 - .70 L_{max}, and .85 - .70 L_{max}. Although ED_{50} from 1.0 - 1.15 L_{max} decreased, it was not significantly different. There was no significant differences in ED_{50} for repeated D-R curves at L_{max}. We conclude that sensitivity of vascular smooth muscle (VSM) depends on L and the length-sensitivity relation is similar to the length-active tension relation. Length dependence of sensitivity in VSM can affect conclusions on its change in hypertension and may be a mechanism in the autoregulation of blood flow. (Supported by NIH #HL21103 and Am. Heart Assoc. (Fla.)).

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ARTERIAL WALL OXYGEN TENSION AND UTILIZATION IN VIVO. E.W. Kanabus* and D.W. Crawford* (SPON: J.A.Rall). USC School of Medicine, Los Angeles, Ca. 90033.

Mural oxygen tension (PwO_2) was measured with recessed cathode microelectrodes in rabbit femoral arteries in situ. Temperature and external PO_2 of the vessel surface were maintained by suffusion. Oxygen consumption ($\dot{V}O_2$) of the media was calculated graphically by fitting PwO_2 data to a general solution of the steady state diffusion equation in one dimension, assuming uniform $\dot{V}O_2$ following zero order kinetics. $\dot{V}O_2$ calculated from 10 transmural PwO_2 profiles in 5 animals ranged from 1.0 to 9.6 x 10^{-4} ml/ml-sec but varied temporally within individual animals, 10 additional profiles indicating undetectable levels of respiration. PwO_2 profiles showed no discontinuity in oxygen distribution at the wall/lumen interface. With physiologic suffusion (PO_2 = 13-26 Torr) the minimum PwO_2 occurred at the outermost measuring site (100± 8μm from the lumen) and averaged 50 ± 18 Torr and 18 ± 7 Torr below arterial PO_2 (PaO_2) in respiring and non-respiring profiles respectively. The occurrence or magnitude of $\dot{V}O_2$ was apparently unrelated to arterial pressure, anesthesia level, PaO_2 or the imposed blood-suffusion O_2 gradient. Thus under normal conditions of innervation, tethering, and blood perfusion mural respiration may vary appreciably, but precise mechanisms regulating $\dot{V}O_2$ in vivo are as yet unclear. (Supported in part by USPHS. NIH Grants HL14138 and HL18969).

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RHYTHMICITY IN RABBIT FEMORAL ARTERY: A MODEL FOR HUMAN CORONARY SPASM. S.F. Flaim, S.C. Swigart, R. Ginsburg*, and R. Zelis. Divisions of Cardiology: Pa. State Univ., Hershey, Pa. 17033 and Stanford Univ., Stanford, Calif. 94305

Rhythmic contraction of arteries occurs in few tissues, including cutaneous and human coronary arteries. Thus, it may be a common factor underlying certain vasospastic disorders (Raynaud's phenomenon, variant angina). We now report that rabbit femoral artery (RFA) also contracts rhythmically during NE. To characterize this response, rings of midfemoral (MF, n=33) and iliac (IL, n=31) artery were studied during NE alone(10^{-8}-2 x 10^{-5}M), NE+diltiazem (DZ, 10^{-7}, 10^{-6}, 10^{-5}M), and DZ alone. Rhythmicity occurred in MF but not IL in NE alone (10^{-7}-10^{-6}M) (amplitude=78±26 mgm, frequency = .07±.01 sec^{-1}) and was absent in NE+DZ. ED_{50}(x10^{-7}M)(dose NE at ½max response) and maximum tone (T_{max}gm) were obtained for each NE and NE+DZ curve. DZ increased ED_{50} in IL(NE 3.1±1.3, NE+DZ10^{-6} 9.3±1.6, NE+DZ10^{-5} 11.7±1.6, p<.01), increased ED_{50} and decreased T_{max} in MF(ED_{50}: NE 6.7±2.1, NE+DZ 10^{-5} 20.1±8.1, p<.05; T_{max}:NE 1.9±0.2, NE+DZ 10^{-6} 1.1±0.2, NE+DZ 10^{-5} 1.0±0.2, p<.05). DZ had no effect on proximal femoral rings and decreased T_{max} but had no effect on ED_{50} in distal rings. DZ alone reduced tone(basal tone=1 gm)in both regions (-Δtone, mgm)I:DZ10^{-6} 75±37, DZ10^{-5} 166±41,p<.05; MF: DZ 10^{-5} 63±26, p<.05. Since NE+RFA causes rhythmicity and since DZ increased ED_{50} & reduces T_{max} for NE & abolishes rhythmicity, RFA may be a useful model for human coronary spasm. (Supported in part by a grant from the York-Adams Chapter of the American Heart Association.)

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MODERATE COOLING AND RESPONSES TO VARIOUS ALPHA-ADRENERGIC AGONISTS IN CANINE CUTANEOUS VEINS. Paul M. Vanhoutte, W. De Ridder*, T. Verbeuren*, and J. De Mey*, Division of Pharmacology, Faculty of Medicine, University of Antwerp, Wilrijk, Belgium.

We investigated whether or not the increased affinity caused by cooling (Janssens and Vanhoutte, Am. J. Physiol. 234 : H330, 1978) involves specifically one of the postjunctional alpha-adrenergic receptor subtypes of cutaneous veins (Vanhoutte et al., Fed. Proc. 39 : 1070, 1980). Rings of canine saphenous vein were mounted for isometric tension recording; contractions to dopamine, norepinephrine, epinephrine, phenylephrine, methoxamine, clonidine and tramazoline were obtained at 37°C and 24°C. Cooling shifted the dose-response curve to norepinephrine, epinephrine and phenylephrine to the left and that to methoxamine to the right; it did not significantly affect the response to dopamine. Cooling caused a non-parallel depression of the response to clonidine and tramazoline. Similar results were obtained in presence of cocaine, 17-β-estradiol and propranolol. Thus, the augmented response to alpha-adrenergic stimulation caused by moderate cooling is not due to specific facilitation of either the $alpha_1$ or $alpha_2$ component of the action of catecholamines; the final hydroxylation of dopamine is important for the recognition of the alpha-adrenergic signal to contraction, in particular at lower temperatures.

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SMOOTH MUSCLE CELL ISOLATION FROM SMALL ARTERIES OF HYPERTENSIVE RATS. Stephen Falco*, Linda Brann*, William Halpern, Daniel Root*. Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405.

An enzymatic method was devised for the dispersion of viable smooth muscle cells from testicular arteries (500 μm lumen dia.) of hypertensive (SHR) and normotensive (WKY) rats. An artery segment, 5 cm long, was dissected in physiological saline solution (PSS) with 0.2% streptomycin and 1.0 mM EGTA. One wall was cut along the longitudinal axis to allow access of enzyme treatment to the intimal surface. The vessel was incubated sequentially in PSS containing collagenase, soybean trypsin inhibitor (STI), trypsin and a mixture of collagenase, STI and elastase (total time including washes, 3.5 hr). The final supernatant containing dispersed cells was transferred to a culture dish coated with poly-l-lysine. Isolated cells adhered to the dish bottom in 10 min and the solution was replaced with PSS. Using Nomarksi microscope optics, we found that: 1) 95% of the cells excluded trypan blue dye; 2) SHR and WKY cells were 87 μm long and 8 μm wide at the nucleus; 3) 65 mM K^+-PSS shortened cells maximally (20-40%, WKY > SHR) causing surface blebbing and decreased cell volume (~32%); 4) 10^{-4}M norepinephrine contracted the cells. This method offers a new means for obtaining smooth muscle cells of arteries ~500 μm, lumen diameter. Refinement of the dissection technique will allow the examination of single cell characteristics from the smaller resistance arteries of normotensive and hypertensive rats. Supported by NIH HL 17335.

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UNCOUPLING OF METABOLISM AND FORCE DEVELOPMENT IN HYPERTENSIVE AORTA. Charles L. Seidel, Roger Strong* and Susan Cabin*. Baylor College of Medicine, Houston, TX 77030

The purpose of these experiments was to compare the steady state ATP consumption during maintained contraction of thoracic aorta from normotensive (WKY) and hypertensive (SHR) rats. Rings of aorta were mounted in a muscle bath that permitted the simultaneous polarographic determination of O_2 consumption, lactate release and isometric force development. Steady state ATP consumption was calculated from measurements of O_2 and lactate. Basal ATP consumption was 24% higher ($p > 0.05$) in SHR aorta. Upon stimulation with 60 or 120 mM KCl, ATP consumption (μmol/min·g) and force (mN/mm^2) of WKY aorta increased above basal, but the ratio, ATP/force, remained constant (ratio at 60K:54 ± 14; at 120K:64 ± 5). Force was not increased further by 120 mM KCl. In SHR aorta, the ratio was greater relative to WKY aorta at both KCl concentrations and increased with KCl concentration without a further increase in force (ratio at 60K:107 ± 17; at 120K:204 ± 47). These results indicate that the metabolic cost of maintaining KCl induced force is greater in SHR aorta possibly because of force independent, energy requiring processes that are more sensitive to the effects of altered extracellular KCl concentration. Such processes may be membrane ion pumps, the activities of which are thought to be elevated in SHR aorta. (Supported by HL 25349).

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INTERACTIONS OF HYDROSTATIC PRESSURE AND STIMULATION FREQUENCY ON REPOLARIZATION AND MEMBRANE POTENTIAL IN CARDIAC PURKINJE FIBERS. T.J. Doubt and P.M. Hogan. Physiology Dept., State University of NY at Buffalo, Buffalo, NY 14214.

In cardiac muscle increased hydrostatic pressure (HP) increases action potential duration (APD) and decreases resting membrane potential. Increased stimulation frequency has the opposite effect, causing decreased APD and hyperpolarization. In the present study dog Purkinje fiber action potentials were recorded at 1 and 150 ATA during stimulation at 1,2,3 and 4 Hz. Elevated HP caused a uniform upward shift in the APD-diastolic interval relation. For APD measured to -60 mv the shape of the APD-diastolic interval curve was unaffected by pressure. However, for APD measured to full repolarization, increased HP caused the curve to flatten as diastolic interval decreased. Elevated HP attenuated the degree of membrane hyperpolarization caused by rapid stimulation. Further, the hyperpolarization immediately following a reduction in stimulation frequency from 4 to 1 Hz was reduced 45.5 ± 4.5% at 150 ATA. These results suggest that increased HP may inhibit the electrogenic Na+ pump that contributes to repolarization and maintenance of resting membrane potential. (Supported by NHLBI Grant PO1-HL-14414.)

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RIGHT AND LEFT VAGAL STIMULATION ON HEART RATE AND ATRIOVENTRICULAR CONDUCTION IN THE CHICKEN. Jack M. Goldberg, Martha H. Lynn-Johnson*, and Kevin D. Whitelaw*. Univ. Calif., Davis, CA 95616

Chickens were anesthetized with sodium pentobarbital and their chests were opened. The cervical vagi were isolated and decentralized while the right and left cardiac accelerator nerves were left intact. Bipolar epicardial electrograms were recorded from the region of the SA node near the junction of the right anterior cava, near the lower right atrium, the left atrium and right ventricle. In addition a back lead electrocardiogram was also recorded. Supramaximal stimulation of either vagus produced brief periods of arrest (few seconds) followed by bradycardia with initial activation originating from supraventricular sites (70%) or from the ventricle with retrograde atrioventricular conduction (30%). Retrograde atrioventricular conduction may be rate dependent since retrograde conduction was not observed with ventricular pacing. Stimulation of either vagus rarely produced AV block. The negative dromotropic effect during stimulation was minimal, prolonging atrioventricular conduction time during right atrial pacing an average of 17 msec with stimulation of the right cervical vagus and 10 msec with the left cervical vagus. The influence of the vagi on atrioventricular conduction is much less than that found in mammals. Furthermore the negative chronatropic effect is much less than that observed in either mammals or diving birds.

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THE INFLUENCE OF ELECTROTONIC INTERACTIONS ON ACTION POTENTIAL CONFIGURATION IN THE SA NODE. Stephen L. Lipsius. Dept. of Physiology, Loyola Univ. Medical Center, Maywood, IL 60153.

Standard microelectrode technique was used to record action potentials (AP) from the isolated sinoatrial node (SAN) of the guinea pig. Previous work has shown that some SAN action potentials exhibit a two component upstroke: a fast component (FC) followed by a slow component (SC). The present work shows that when the SAN is electrically driven at fast rates the time to peak depolarization of the SC progressively increases. This leads to a progressive increase in AP duration and block of excitation. An early premature excitation can produce a marked delay in onset and peak of SC depolarization. Simultaneous impalement of two cells (<500μ apart) along the periphery of the SAN reveal that the AP upstroke of the cell activated later is coincident with the onset of SC depolarization of the earlier activated cell. TTX (10^{-6}-10^{-5}g/ml) depresses conduction by decreasing FC and produces notching with secondary depolarizations at various times during the earlier activated AP. These potential changes were coincident with the AP upstroke of the cell activated later. The results indicate that the SC of a two component upstroke may result from electrotonic interactions between neighboring cells. Conditions that depress conduction within the SAN can alter action potential configuration through electrotonic interactions. These interactions may be important in block of excitation at the sino-atrial border. (Supported by Chicago Heart Association Grant C80-12.)

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DESENSITIZATION OF VENTRICULAR MUSCARINIC RECEPTORS WITH CONTINUED VAGAL STIMULATION. Jerod M. Loeb. Northwestern Univ. Medical School, Chicago, Illinois 60611.

Recent studies indicate that the cholinergic receptor at the sinus node (SN) can desensitize after prolonged vagal stimulation or iontophoretic acetylcholine. This investigation sought to define whether differences exist between SN and ventricular muscarinic receptors with respect to vagally induced desensitization. Mongrel dogs were anesthetized and instrumented to record arterial pressure, ECG and electrograms from SN, atrium, ventricle, and His bundle. After bilateral vagotomy, supramaximal stimulation of the right cervical vagus (RCVS) for 15 sec resulted in cycle length (CL) increases; maintainance of RCVS for 60 sec resulted in a 2 component decrease in CL. Left cervical vagal stimulation (LCVS) also increased control CL but maintained LCVS did not result in a similar magnitude of receptor desensitization. After induction of complete heart block (CHB) via the introduction of formalin into the AV junctional area, RCVS and LCVS resulted in a progressive increase in ventricular CL. Maximal CL was produced at 10-15 sec and CL then returned toward control following a 2 component time course. Atrial CL was less affected by RCVS and LCVS after induction of CHB and desensitization followed a different time course. These results indicate that atrial and ventricular muscarinic receptors desensitize following prolonged vagal stimulation but show also that the mechanism of desensitization may be different in the atria before and after the induction of CHB. (Supported by USPHS RR-05370)

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EFFECTS OF LIDOCAINE ON THE PROPAGATION OF THE SLOW RESPONSE IN CANINE FALSE TENDONS. Vito Lamanna*, Charles Antzelevitch* and Gordon K. Moe. Masonic Medical Research Laboratory, Utica, NY 13503.

Lidocaine (L) impairs conduction through partially depolarized cardiac tissues. We studied the effect of L (3-5 mg/l) on the conduction of slow responses in dog false tendons superfused with a modified Tyrode's solution (K^+=20 mM, Ca^{++}=9 mM). The conduction velocity was decreased by L and the refractory period prolonged; in many cases block was produced. Similar results were obtained when epinephrine (0.3-0.8 mg/l) was used to elicit the slow response. The membrane resistance (Rm) and the current required to reach threshold (I_{th}) was determined by injection of intracellular current across a sucrose gap. L produced an increase of I_{th} (10-18%) with no detectable change on Rm or membrane potential. When 90% of the Na^+ in the perfusing solution was replaced by choline, L did not change I_{th}. Our results suggest that L impairs the conduction of the slow response by increasing I_{th}, possibly through an effect on the Na^+ background current. (Supported in part by grant #19487, NHLBI; V. Lamanna is a fellow of CONICIT, Venezuela.)

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MYOCARDIAL O_2-SUPPLY IN DOGS FOLLOWING HEMODILUTION WITH BLOOD- AND PLASMA-SUBSTITUTES. G.P. Biro, Dept. of Physiology, Faculty of Health Sciences, University of Ottawa, Ottawa, Canada. K1N 9A9.

The hematocrit was reduced to 20±2% in four groups of anesthetised dogs by isovolemic exchange-transfusions using the following substances: 6% stroma-free hemoglobin solution (Group I, N=9), 6% stroma-free methemoglobin solution (Group II, N=8), 20% Fluosol-DA, a fluorocarbon emulsion, (Group III, N=9) and 6% Dextran 70 (Group IV, N=9). Arterial blood O_2-content was 10ml/dl in Groups II and IV, 13ml/dl in Group I and 15ml/dl in Group III. Whole-blood viscosity and the hemodynamic determinants of myocardial oxygen-consumption were similar in all groups. The average increments in mean myocardial blood flows after one hour of hemodilution, were similar in all groups (90-120%), with similar trans-mural distribution, except in Group III in which endocardial flow-increment was greatest (+169±9%, $p<.05$). The latter group also had the highest coronary sinus blood pO_2: 31±2mmHg; significantly increased from the pre-exchange value. The control levels (20±2mmHg) were maintained in Groups II and IV, while there was a fall to as low as 8±1mmHg ($p<.05$) in Group I. With similar degrees of hemodilution, myocardial O_2-supply appears greatest in Fluosol-diluted animals. The findings suggest that under these conditions, coronary flow may not be regulated primarily to maintain coronary sinus pO_2. (Supported by Ont. Heart Foundation and Med. Res. Council).

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EFFECT OF Ca^{2+} ON THE UTILIZATION OF EXOGENOUS SUBSTRATES BY ISOLATED MYOCYTES FROM ADULT RAT HEARTS. John Montini, Gregory J. Bagby and John J. Spitzer. Dept. of Physiology, LSUMC, New Orleans, La. 70112

This study examined the effect of Ca^{2+} on the utilization of exogenous glucose, lactate and palmitate by freshly isolated noncontracting myocytes from adult rat hearts. A procedure was developed whereby a preparation of isolated myocytes is obtained in which viability is high, yield adequate and the cells are not sensitive to the deleterious effects of Ca^{2+}. A medium containing amino acids was employed and cells were maintained at 37°C in medium supplemented with 1% bovine serum albumin. Viability was between 75 and 85% and fell to 70% after a 60 min incubation period. The following effects of Ca^{2+} on glucose, lactate and palmitate oxidation expressed as nmoles oxidized/min/mg protein were observed (*$p < 0.05$):

ADDITIONS	GLUCOSE	LACTATE	PALMITATE
0.1mM EGTA	0.98±.16	3.71±.61	0.60±.08
NONE	1.36±.24*	4.32±.53*	0.72±.11*
1.6mMCa^{2+} & 0.1mM EGTA	1.46±.22*	4.82±.71*	0.63±.08

The oxidation of glucose and lactate increased 50 and 30% respectively by the addition of Ca^{2+}. A decrease in oxidation rate of all 3 substrates was noted with cells incubating in media containing EGTA. Although Ca^{2+} significantly increased glucose and lactate oxidation, its mechanism of action is presently unknown. (Supported by NIH Grant HL 22101 and NIH Training Grant HL 07098).

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THE RESPONSE OF MYOCARDIAL ATP,CP,LACTIC ACID AND H^+ TO REPER FUSION FOLLOWING TWO HOUR HYPOTHERMIC ARREST OF THE PIG HEART. William Dobbs,R.M. Engelman‡ M.A. Pels‡J.M. Alvarez‡Department of Surgery, U. of Connecticut Health Center,Farmington,CT06032

Eleven 20-25kg pigs were anesthetized and placed on cardiopulmonary bypass to determine the response to 2 hour hypothermic(T=15°C) arrest(A) with a cardioplegic solution containing 35mEq/L K^+,followed by 60 minutes reperfusion(R), with warm oxygenated blood. Tissue samples were obtained for analysis of ATP,CP,and lactic acid(L). A needle pH electrode(MI-408C,Micro electrodes, Inc., Londonderry, N.H.) was inserted into the myocardium for measurement of pH from which (H^+) was calculated. Mean values for ATP,CP,and L(μM/gmwetwt) and (H^+)x10^{-8}M were:

	Prearrest	1hrA	2hrA	15minR	30minR	45minR	60minR
ATP	3.91	3.60	3.40*	3.19*	3.05*	2.94*	3.09*
CP	6.79	2.34*	1.38*	6.56	6.80	7.40	7.52
L	10.18	13.68*	18.31*	12.66	9.21	10.02	13.40
H	3.05	17.00*	28.30*	11.80*	8.35*	6.25*	7.81*
pH	7.52	6.74*	6.54*	6.92*	7.07*	7.20	7.09*

Thus during A, ATP,L, and H changed at constant rates equal to -.026, +4.07, and +14.61μM/gmwetwthr,respectively. CP fellmore quickly during the first hour of A than during the second hour During R the changes in L,H, and CP were immediately reversed, but ATP continued to decline at the same rate as during A.Thus the effect of two hour hypothermic arrest on the enzyme system for maintaining cellular ATP was not immediately reversible by reperfusion and may have been irreversibly damaged. *$p<0.05$ (Supported in part by USPHS.NIH GrantlR01HL 22559-01A2).

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AGE-RELATED RESPONSES TO HEAD-UP TILT AND RETURN TO HORIZONTAL M.A.B.Frey and R.M.Siervogel*, Wright State University School of Medicine, Dayton, OH, 45435.

In 20 men and 21 women aged 18-69 years, systolic (SP) and diastolic (DP) blood pressure, cardiac interval duration (INT), and systolic time intervals were monitored during supine control, 3 min of 70° head-up tilt (T), and 3 min of supine recovery (R). During T, all variables showed sig. ($p<.05$) changes; INT, electromechanical systole (EMS), and left ventricular ejection time (LVET) were reduced, pre-ejection period (PEP) was prolonged, and SP and DP were elevated. At 3-min R, INT and DP had returned to control levels; EMS, LVET, and PEP had returned beyond control levels; and SP remained slightly elevated. At rest, only PEP was correlated with age ($r=.37, p=.02$). It was not, however, correlated with age during T; EMS ($r=.38, p=.02$) and LVET ($r=.42, p=.01$) were, indicating that older subjects tended to have longer EMS and LVET with T. These effects are reflected also in the positive correlation of age with change in INT during T; older subjects experienced less shortening of INT. They also experienced less prolongation in PEP during T ($r=-.36, p=.03$), accompanied at 1-min T by a smaller DP increase ($r=-.38, p=.03$). At 3-min R, EMS was longer in the older subjects ($r=.45, p<.01$), and PEP had resumed its resting age relationship ($r=.41, p=.02$). None of the other variables were correlated with age at 3-min R. These results demonstrate that age affects the ability of the cardiovascular system to adjust to orthostatic stress. This may reflect reduced baroreceptor sensitivity with age. (Supported by NHLBI Grant R01-HL-23194.)

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PHOTOMETRIC MEASUREMENT OF INTRAMYOCARDIAL pH IN GRADED REGIONAL ISCHEMIA. R.J. Young*, G.A. Tait*, G.J. Wilson*, J. Ninomiya*, D.J. Steward* and D.C. MacGregor* (SPON: H. Sonnenberg). University of Toronto, Toronto, Canada.

Continuous measurement of extracellular pH is potentially useful in assessing adequacy of perfusion of the myocardium. A new photometric method of measuring pH has been developed which permits the construction of a small implantable probe (1 mm diam). The colour change of an indicator in a semipermeable chamber is measured through two fiber optic channels.

In this study, changes in pH in response to ischemia were measured, comparing performance of a conventional glass pH needle electrode (0.8 mm diam) with the photometric probe. In 7 dogs the left anterior descending coronary (LAD) artery was cannulated and perfused from the right axillary artery, measuring the flow with an extracorporeal electromagnetic flow probe. The two pH probes were implanted 10 mm deep in the myocardium in the area of distribution of the LAD. Two 20 minute periods of ischemia followed by 30 minutes of recovery were produced by stenosing the perfusion cannula. The first was just sufficient to abolish reactive hyperemia while the second reduced flow to 1/3 of control. Flow was then stopped for one hour. The changes in pH (mean±SEM) during ischemia are shown.

Flow (% of control)	57.6±2.4%	33.3±4.3%	0
Glass (Max pH)	-0.05±0.03	-0.26±0.07	-0.93±0.12
Photo (Max pH)	-0.03±0.02	-0.22±0.07	-0.69±0.20

At critical stenosis there is little change in pH. Further reductions in flow produce progressively greater falls in pH.

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CALCIUM DEPOSITS SUBCUTANEOUSLY IN HANDS (CALCINOSIS CUTIS) AND DICOUMAROL EFFECTS. Henry Brown, Colin Grant*, Edward Gillie* and H. Paul Ehrlich*. Depts. of Surgery, Medicine and Pathology, Harvard Medical School, Boston, MA 02115 and the Veterans Administration Medical Center, Manchester, N.H. 03104.

Crippling, often infected, enlarging, painful calcium hand nodules in an osteoarthritic 50 year old white woman were theorized to be due to calcium chelation with conversion of glutamic acid (Glu) to γ-carboxyglutamic acid (Gla) via vitamin K dependent enzymes. This mechanism not only activates prothrombin in blood clotting, but also is associated with soft tissue calcification and stone formation. Dicoumerol which prevents conversion of Glu to Gla, administered for 3 months to decrease soft tissue calcification gave prothrombin times of the order of 20/12 sec. Very low initial 24 hr urinary calcium excretion, 17 mEq, increased 4 fold to 68 during treatment. It continued to rise after treatment, being 112 to 215 during the next 15 months. Subjective improvement though gradual was striking. It is concluded that low urinary calcium excretion in calcinosis cutis may increase with dicoumarol administration, presumably through vitamin K dependent enzyme mechanisms.

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CHANGES IN THE COMPOSITION OF BONE AND MUSCLE DURING THE ONSET OF CHRONIC METABOLIC ACIDOSIS. John A. Bettice, Department of Physiology, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

Experimental rats were fed a diet of ground rat chow mixed with water and acid (600 mEq H^+/kg food) and were given a 0.3M solution of lysine-HCl to drink. Blood, bone and muscle samples were taken after one, two and four days of acid loading. There were sharp declines in plasma pH (-0.12), partial pressure of carbon dioxide (-8 torr) and bicarbonate concentration (-9.3 mEq/liter) after one day of acidosis, and these parameters remained near the reduced levels during the next three days. Skeletal bicarbonate stores decreased progressively throughout the acidosis (-200 mEq/kg bone on day 4). Intracellular pH within skeletal muscle was reduced only after the fourth day of acidosis (-.06). Sodium content of bone and skeletal muscle were decreased after two days of acidosis (-9 mEq/kg bone; -2 mEq/l icw muscle) but returned to control levels after four days; potassium was progressively released from these tissues throughout the acidosis (-5 mEq/kg bone; -22 mEq/l icw muscle on day 4). The intracellular composition of cardiac ventricle did not change during this acidosis. These results indicate that bone and skeletal muscle are sites for significant amounts of extrarenal buffering during the onset of chronic metabolic acidosis. (Supported by a Young Investigator Award from the National Heart, Lung and Blood Institute HL 19601).

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CALCIFICATION IN CRUSTACEAN CUTICLE: THE FUNDAMENTAL PROCESS. Peter S. B. Digby. Biology Dept, McGill University, Montreal, Quebec, Canada H3A 1B1.

Crustacea precipitate calcium carbonate in their shells. Earlier studies had indicated that electrochemical principles might be implicated in some way. Recent measurements in crabs (Carcinus) and lobsters (Homarus) show the shell to be quite alkaline relative both to its blood and to seawater. Blood is reducing and the shell side of the underlying epithelium shows still stronger reducing activity. Oxidation of the shell by air, oxygen or hydrogen peroxide raises its pH to about 9, at which point calcium carbonate is precipitated from the blood. The experiments suggest that the epithelial cells pass acid, probably as CO_2, to the blood and reducing substances to the uncalcified inner layers of the shell, where they are oxidised with the removal of hydrogen ions to the point at which precipitation of carbonate occurs. These steps resemble closely those apparently involved in the precipitation of carbonate by coralline algae. Interesting parallels occur between such action and that in a piece of steel corroding in seawater, the electronic conduction paths in the biological material being limited to the oxidase complexes. Recognition of such processes suggests that in Crustacea calcification does not necessarily involve such factors as supersaturation of plasma with mineral at normal blood pH, specific nucleating agents or inhibitors of calcification, as are held to be of importance in mammalian bone. (Supported by N.R.C. of Canada)

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URINE AND SODIUM OUTPUT AND GLOMERULAR FILTRATION RATE DURING TREADMILL EXERCISE AND SHOCK-AVOIDANCE IN SALINE-INFUSED DOGS. Alberto Grignolo*, John P. Koepke* and Paul A. Obrist* (SPON: Elliott Mills). University of North Carolina, Chapel Hill, North Carolina 27514.

Urine flow ($\dot{V}$), sodium excretion ($U_{Na}\dot{V}$), inulin clearance (C_{In}), heart rate (HR) and hematocrit (HCT) were measured during 30-min episodes of treadmill exercise at 3 mph or of shock-avoidance in dogs infused with isotonic saline (3-4 ml/min). Exercise led to significant ($p<0.01$) increases in $\dot{V}$ (+33% from 2.03±0.15 ml/min), $U_{Na}\dot{V}$ (+21% from 328±24 μEq/min), C_{In} (+16% from 94±5 ml/min), HR (+56% from 106±3 bpm) and HCT (+14% from 29±1 ml/100 ml); urine and plasma osmolality and free-water clearance did not change. Avoidance caused significant decreases in $\dot{V}$ (-21% from 2.33±0.24 ml/min), $U_{Na}\dot{V}$ (-24% from 317±25 μEq/min) and increases in HCT (+10% from 30±1 ml/100 ml); HR rose moderately (+15% from 121±3 bpm). C_{In}, urine and plasma osmolality and free-water clearance did not change, but fractional water and sodium excretion fell significantly, suggesting a rise in tubular reabsorption. The larger the increase in HR during avoidance, the larger was the decrease in both $\dot{V}$ (rho=-0.43, $p<0.01$) and $U_{Na}\dot{V}$ (rho=-0.57, $p<0.001$). The results show that exercise and avoidance, while eliciting similar cardiac changes, have opposite effects on the urinary output of saline-infused dogs. (Supported by NIH Grant HL-18976).

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CARDIOVASCULAR (CF) AND RENAL FUNCTION (RF) IN SHEEP (S): EFFECTS OF CRUSHING THE LEFT ATRIAL APPENDAGE (LAA). M.B. Zimmerman* and E.H. Blaine (SPON: A. Renzi). Dow Pharmaceutical, Indianapolis, IN 46268, and Merck Institute, West Point, PA 19486.

Recent findings from our laboratory, using a surgical technique to damage the LAA of the heart in S, indicate that these receptors may mediate water ingestion following hypovolemia (Zimmerman et al., Soc. Neurosci. 5:226,1979). The present experiments were designed to examine the effects of damaging the LAA on CF, RF, plasma electrolyte and hormone levels, and plasma (PV) and blood volumes (BV) in conscious S, and to compare the physiologic responses to acute volume expansion before and after surgery. Five female S were studied prior to and 1-week after crushing the LAA. Comparison of the average basal parameters revealed no significant changes in glomerular filtration rate(GFR), renal plasma flow(RPF), blood pressure, PV, BV, or plasma renin activity. Heart rates and plasma K concentrations were elevated, while plasma Na and hematocrits were significantly lowered. Despite the absence of changes in water or food intake, mean body weight decreased by 1.5 kg. These results suggest a reduction in extracellular fluid volume (ECF) and a shift of low-Na, high-K fluid from the rumen to the circulation. Each animal was additionally challenged with an acute i.v. isotonic saline load (10% of body weight) before and after damaging the LAA, and significant differences were observed in the rate of Na and fluid excretion as well as in GFR, RPF, and osmolar clearance/GFR. These experiments suggest that LAA receptors may play a role in the control of ECF.

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URINARY SODIUM EXCRETION FOLLOWING HYPERTONIC SODIUM CHLORIDE INFUSIONS INTO THE INTERNAL CAROTID ARTERY. L. McCullough*, E. Fitzgerald*, S. Gleason* and E. Schneider. Dept. Physiol., U. Tenn. Ctr. Hlth. Sciences, Memphis, TN 38163

We tested, in 16 female dogs, whether Na concentration (conc.) of arterial blood perfusing the central nervous system (CNS) influences urinary Na excretion. Dogs were fed a low Na diet for 3 days; they were then anesthetized with pentobarbital (30 mg/kg), the left common carotid and right external carotid arteries were ligated, and 2 30-min control periods were obtained. Over the next 20 min, 6 dogs (carotid) simultaneously received a 0.4 ml/min infusion of 3.75 M NaCl solution via the right internal carotid artery and a 12.5 ml/min infusion of an isotonic dextrose solution via the saphenous vein; 6 dogs (peripheral) received the NaCl and dextrose solutions via the opposite routes; and 4 dogs (time control) received both infusions via a saphenous vein; 5 additional clearance periods were then obtained. During the infusions, the Na conc. in the jugular vein increased 5.5 ±2.0 mEq/l in the carotid group and decreased 8.2 ± 2.3 mEq/l in the peripheral group. Blood pressure, systemic plasma Na conc. and glomerular filtration rate were not altered in any group. Urine flow and urinary Na excretion increased similarly in all 3 groups. These results suggest that if there is an area within the CNS responding to changes in Na conc. by altering urinary Na excretion, it is not an area perfused by the internal carotid artery. (Supported in part by USPHS. NIH Grant HL-16658)

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EFFECTS OF DIGOXIN AND AMINO SUGAR CARDIAC GLYCOSIDE (ASI-222) ON PLASMA ADH ACTIVITY. Gary B. Guo*, Phillip G. Schmid, Francois M. Abboud. VAH and CV Ctr., Univ. of Iowa, Iowa City, IA, 52242.

Activation of arterial and cardiac baroreceptors by digoxin (D) may result in reflex inhibition of ADH release. Conversely D may cross the blood brain barrier (BBB) and increase ADH release through an action on the CNS. The present studies were performed in conscious dogs to contrast the effect of intravenous (i.v.) D (which crosses the BBB) to the effect of ASI-222 (a semi-synthetic amino sugar digitoxin derivative which does not cross the BBB). Increase in plasma levels of ADH by i.v. D and not by i.v. ASI-222 would suggest that digitalis has a central excitatory effect on ADH release. We measured arterial pressure (AP), hematocrit (Hct), plasma osmolarity (Osm), and ADH before and 2.5, 5, 15, 30 and 50 min after i.v. injections of equimolar doses of D (50 µg/kg, N=6), and ASI-222 (38.5 µg/kg, N=6). ADH remained stable over a period of 50 min after injection of vehicle. Intravenous D increased ADH consistently (within 30-50min, Δ=4.77 ± 1.75 µU/ml) from a control level of 1.31 ± 0.24 µU/ml. In contrast, ASI-222 did not increase ADH in 5 of 6 dogs. Changes in Osm, Hct, and AP could not account for changes in ADH with D. These results indicate that i.v. digoxin increases ADH activity and suggest that this increase occurs primarily through an action on the central nervous system.
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EFFECT OF CHLORPROPAMIDE (CP) ON ADENYLATE CYCLASE (AC) KINETICS AND ATP TURNOVER IN RAT KIDNEY. Arnold M. Moses and Richard Coulson*. Depts. Medicine & Pharmacology, SUNY, Upstate Med. Ctr., VA Med. Ctr., Syracuse, N.Y. 13210.

The injection of the sulfonylurea CP augments the antidiuretic action of AVP and its non-pressor analog, DDAVP, probably by a cyclic AMP-mediated mechanism (Endocrinology 106: 967, 1980). CP action was studied using renal medullary cell membranes of Brattleboro rats in which CP had been injected or added to the assay in vitro. In vivo CP produced an increase in Vmax and a decrease in Km for ATP with DDAVP-activated AC; this is consistent with the role of CP in augmenting vasopressin action. CP in vivo had no effect on the GTP, Gpp(NH)p or F^- stimulated AC activities, suggesting that its site of action is not at the N subunit of AC. CP in vitro decreased the Vmax and increased the Km for ATP. Renal ATP turnover may be altered by CP since kidneys perfused from rats injected with CP could not maintain normal ATP levels following a pressor level of AVP in vitro. Moreover, gluconeogenesis from pyruvate was completely abolished when 10mM CP was in the perfusate. This was accompanied by significant reduction in ATP and GTP levels. Renal gluconeogenesis was not impaired when kidneys from CP-injected rats were perfused, nor was it impaired when 1 mM CP was included in the perfusate. (1 mM is the concentration of plasma CP after in vivo injection).
Conclusions: In vivo CP augments DDAVP-activated AC Vmax, lowers Km for ATP, alters renal ATP turnover.

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EFFECT OF HEMORRHAGE ON PLASMA VASOTOCIN LEVELS IN CONSCIOUS CHICKENS. T. I. Koike, L. R. Pryor* and H. L. Neldon*. Dept. of Physiology and Biophysics, University of Arkansas Medical Sciences Campus, Little Rock, AR 72205.

In order to determine whether volume control of ADH secretion could be demonstrated in the fowl 2 groups of conscious White Leghorn pullets (6 birds per group) were subjected to rapid hemorrhage of 20 and 30% of the estimated blood volume. Blood samples were obtained immediately prior to bleeding and 30 and 60 min subsequent to the start of the bleeding period. The blood removed was re-infused at 60 min and a final sample obtained at 90 min. While hemorrhage resulted in a reduction in blood pressure and an increase in heart rate in both groups no significant changes in plasma immunoreactive vasotocin (AVT) were observed subsequent to bleeding. Plasma AVT prior to bleeding averaged 4.9±3.27 (S.E.) and 4.6±0.31 µU/ml in the 20 and 30% hemorrhage groups, respectively. Hormone levels at 30 and 60 min were 2.6±0.78 and 2.7±0.35 µU/ml (20% hemorrhage) and 5.0±0.60 and 5.4± µU/ml (30% hemorrhage). No changes were observed in plasma osmolality or sodium and potassium concentrations. It is concluded that a reduction in vascular volume is not a stimulus to AVT secretion in the chicken. (Supported in part by GRS 05350 from NIH)

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DRINKING RESPONSES OF HYPERPROLACTINEMIC (HP) RATS. S.Kaufman* (SPON. W. A. Kaufman)Univ. of Alberta, Edmonton, Alberta T6G 2G3

Chronic hyperprolactinemia was induced in inbred Wistar rats by implanting 3 anterior pituitary glands beneath the renal capsule. Over a period of 10 weeks the spontaneous 24 hr intake of the HP rats was consistently greater than that of the matched controls. Water intake during the hours of light was the same for both groups but the dark-time intake of the HP rats (89.6 ± 2.2 ml/kg, n=8) was greater($p<0.005$) than that of the controls (71.8 ± 4.4 ml/kg, n=7). Saline (3%) intake was depressed in the HP rats during both light and dark periods but food intake was the same in both groups. The increased spontaneous 24 hr intake of the HP rats was unaffected by withdrawal of food but was reversibly attenuated to that of the controls by injection of bromocriptine. (2 mg/kg/day). Drinking to AII infusions (0.6 µg/min/kg) was greater ($p<0.05$) in the HP rats (2.4 ± 0.7 ml/kg, n=7) than in the controls (1.0 ± 0.4 ml/kg, n=8). However after 2 M NaCl (5 ml/kg) intake was reduced ($p<0.05$) in the HP rats (24.2 ± 3.3,n=6) compared with the controls (32.9 ± 2.3, n=10). It is concluded that the increased spontaneous intake of HP rats is not secondary to increased food or salt intake and is probably mediated via the pathways of extracellular fluid volume regulation.
(Supported by MRC grant #MA-7085).

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EFFECT OF L-5-HYDROXYTRYPTOPHAN ON WATER INTAKE IN RATS. C.C. Barney, D.C. Kikta*, R.M. Threatte* and M.J. Fregly. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Female rats administered L-5-hydroxytryptophan (5-HTP) (5-25 mg/kg, s.c.) showed a dose dependent increase in water intake during 2 hours following treatment. The increase in water intake induced by 25 mg 5-HTP/kg, s.c. was prevented by the decarboxylase inhibitors benserazide (30 mg/kg, s.c.) and carbidopa (6.5 mg/kg, s.c.). Treatment with the serotonin antagonist, methysergide (3 mg/kg, i.p.), also attenuated the dipsogenic effect of 5-HTP (25 mg/kg, s.c.). Thus, 5-HTP must be converted to serotonin for dipsogenic activity to be observed. In addition, the increase in water intake brought about by administration of 25 mg 5-HTP/kg, s.c. was attenuated by pretreatment with the β-adrenergic antagonist, d,ℓ-propranolol (6 mg/kg, i.p.), the α-adrenergic agonist, clonidine (6.25 µg/kg, i.p.), and the angiotensin converting enzyme inhibitor, captopril (35 mg/kg, i.p.). One hour after administration of 5-HTP (25 mg/kg, s.c.), plasma renin activity of 5-HTP-treated rats was increased above that of saline-treated rats. The same dose of 5-HTP was without effect on mean blood pressure of unanesthetized rats measured by carotid artery cannulation over this same time period. These data indicate that 5-HTP acts to induce an increase in water intake through its conversion to serotonin and subsequent activation of the renin-angiotensin pathway. (Supported by grant HL-14526-08 from the National Heart, Lung and Blood Institute).

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MAINTENANCE OF NORMAL RENAL FUNCTION BY PROSTAGLANDINS IN THE CONSCIOUS DOG. Benjimen R. Walker* and Robert F. Grover. Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262

Conflicting data exist concerning the role of endogenous prostaglandins in normal sodium and water excretion in the conscious dog. Therefore, studies were undertaken to further elucidate the renal effects of prostaglandin synthesis inhibition. Six normovolemic animals with chronic arterial and venous catheters were studied while resting quietly. Meclofenamate (2 $mg \cdot kg^{-1}$ i.v. followed by 2 $mg \cdot kg^{-1} \cdot hr^{-1}$) was administered following control measurements. Parallel time control experiments were also performed on the same animals. The order of the experiments was randomized and at least one week allowed between experiments. Inhibition of renal prostaglandin synthesis was demonstrated by significant reduction in urinary prostaglandin E_2 excretion rate. Urine flow and sodium excretion were reduced by 47% and 41% respectively with meclofenamate treatment. In addition, fractional excretion of sodium was reduced, implying enhanced sodium reabsorption during prostaglandin synthesis inhibition. Renal hemodynamics, glomerular filtration and systemic hemodynamics were unaffected. No changes in renal function were observed during time control experiments. It is concluded that endogenous prostaglandin release may be important in maintaining sodium and water excretion in the conscious dog. (Supported by NIH Grant #HL 14985).

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TUBULAR REABSORBATE IN RENAL LYMPH FOLLOWING ECF EXPANSION. Richard D. Bell. Chicago College of Osteopathic Medicine, Chicago, Illinois 60615.

It is not yet known whether renal lymph is composed entirely of tubular reabsorbate, of a mixture of tubular reabsorbate and blood filtrate or of blood filtrate alone. The present experiments were designed to detect a change in the postulated reabsorbate component of renal lymph during acute ECF expansion. Renal lymph, arterial blood plasma and urine were collected during constant infusion of creatinine in 0.9% saline. Collections were made before (control) and 15 min following ECF expansion (experimental) (Ringer's solution, 10% of body wt.). Renal lymph protein concentration decreased proportionally more than plasma protein concentration, but the lymphatic clearance of protein did not change. In contrast, the changes in lymph creatinine concentration were similar to those of blood plasma, while lymphatic clearances of creatinine were significantly increased. The increase in the lymphatic clearance of creatinine (65% $\pm$ 0.09 S.E.) was adequate to account for the lymph flow increase (67% $\pm$ 0.09 S.E.). Thus, the increase in reanl lymph flow that accompanies ECF expansion appears to be derived from plasma filtrate rather than from creatinine-free reabsorbate. ECF expansion appears to cause an increase in the circulation of plasma filtrate through the renal interstitial compartment with unaltered protein loss. In addition, the proportion of tubular reabsorbate in renal lymph appears to be constant, even during acute ECF expansion. (supported by the Chicago College of Osteopathic Medicine)

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AMOUNTS OF WATER, UREA,Na AND K IN MAMMALIAN RENAL MEDULLA. BODIL SCHMIDT-NIELSEN, BRUCE GRAVES* AND JOANNA ROTH*. MOUNT DESERT ISLAND BIOLOGICAL LABORATORY, SALSBURY COVE, MAINE 04672

Urea and Na concentrations in the mammalian renal medulla are higher in antidiuresis than in diuresis. To determine the actual amounts of solutes and water moving in and out of the renal medulla as urine osmolality increases or decreases we have analyzed the renal medulla (papilla, upper part of inner medulla and outer medulla) of rats and hamsters using a new method which permits accurate determination of water content as well as solutes in the same piece of tissue. Linear regression lines were calculated for the amounts of water (in µl per mg solute free dry tissue) and solutes in these three zones against papillary osmolality (in Osm/ kg H_2O). In both rodents changes in amounts of water and solutes with increasing osmolality of the papilla follow similar patterns: urea increases greatly in all three zones, Na increases much less and only in the papilla, water decreases but primarily in the papilla, and K decreases slightly but significantly in the inner medulla. When the weights of the medullary zones are taken into account it becomes clear that the major changes that occur in the renal medulla with increasing papillary osmolality is a large increase in the amount of urea and a decrease in water content. Changes in Na content are minimal. (Supported by NIH Grant 5 R01 AM15972)

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URATE EXCRETION IN CEBUS MONKEYS AND MONGREL DOGS. Margarita Perez-Gonzalez* and Irwin M Weiner*. (SPON= H. Tepperman). Pharmacology Department, SUNY, Upstate Medical Center. Syracuse. N.Y. 13210.

In some mammals the secretory limb of the bidirectional transport of urate appears to occur via the p-aminohippurate (PAH) mechanism; in others it does not. In animals in which PAH and urate are secreted by separate mechanisms, pyrazinoate (PZ) is a potent inhibitor of urate secretion. The literature in dogs and Cebus is not conclusive. We studied the effects of PAH and PZ on the fractional excretion of endogenous urate (FE_{ur}) in these animals; the concentrations of PZ or PAH in plasma (P_{PZ}, P_{PAH}) were varied over wide ranges. Urate was determined using High Performance Liquid Chromatography (HPLC) with electrochemical detection. PZ and PAH were measured with a new developed method using HPLC which proved to be very specific and sensitive. In Cebus, at low P_{PZ} (1 - 200 µg/ml), FE_{ur} was depressed. At higher P_{PZ}, FE_{ur} increased towards uricosuric levels. PAH at low plasma concentrations (1-20 µg/ml) was slightly uricosuric; FE_{ur} being 1.5 to 2.0 times the control level. At higher P_{PAH}, up to 1000 µg/ml, PAH had no net effect on FE_{ur}. The results in mongrel dogs were analogous except that low P_{PAH} were not uricosuric. We conclude that in Cebus and in the mongrel dog, urate secretion occurs through a mechanism distinct from that which effects the secretion of PAH. (Supported by NIH Grant # 5ROI-AM- 20533-14)

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INTERACTION OF VARIOUS ORGANIC ANIONS IN THE NECTURUS KIDNEY. B.D.S. Khalsa*, J.M. Goldinger*, and S.K. Hong. SUNY at Buffalo, Buffalo, N.Y. 14214.

In the Necturus kidney, the sulfonic dye phenol red is not secreted, and does not inhibit secretion of p-aminohippurate (PAH) (Tanner *et al.*, Amer. J. Physiol. 236:F442, 1979). We studied the renal handling of N-(4-azido-2-nitrophenyl)-2-aminoethyl sulfonate (NAP-taurine), a sulfonic acid analogue of PAH which is secreted by the typical anion transport system in both rabbit and rat kidneys (Stokols *et al.*, Physiologist 22:120, 1979). NAP-taurine was rapidly taken up by the Necturus kidney slice reaching a plateau in about 20 min. The steady state S/M values at 20°C were approximately 3.0 and 2.0 at the medium concentration of 10 and 50 µM, respectively. NAP-taurine accumulation was not affected by metabolic inhibitors, ouabain, removal of sodium, 10 mM PAH, or 0.1 mM probenecid. These findings strongly suggest that NAP-taurine binds to the tissue without being transported into the cell. NAP-taurine partially inhibited PAH accumulation to a maximum of 50% of control at concentrations ranging from 15 to 500 µM, while 1 mM probenecid decreased it to S/M ≈1.0. Efflux of preaccumulated PAH was not affected by 50 µM NAP-taurine, but increased 3 fold with 1 mM probenecid. The results indicate that NAP-taurine affects PAH accumulation by inhibiting uptake only, whereas probenecid affects both uptake and efflux. (Supported by USPHS Grant AM-18918).

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RENAL TUBULAR TRANSPORT OF AMILORIDE BY THE ORGANIC CATION TRANSPORT SYSTEM. Kamel Besseghir* and Barbara Rennick. Pharmacology and Therapeutics, SUNY Medical School, Buffalo, NY 14214

The diuretic amiloride is an organic cation. Its excretion has been shown to exceed GFR in rats and dogs. In this study the mechanism of transport was examined in chickens using the Sperber technique. All of the amiloride which reached the infused kidney was actively transported at infusion rates below 1 x 10^{-8} moles/kg·min. At higher infusion rates the ratio of the transport of amiloride to PAH was less than one. The cationic inhibitors quinine, mepiperphenidol and guanidine produced maximum inhibition of ^{14}C-amiloride transport of 88%, 85% and 56%, respectively. However, the cationic competitor cyanine 863 did not inhibit amiloride transport. Only 80% of the ^{14}C-amiloride was recovered from urine when PAH recovery was nearly 100%. No metabolite of amiloride was found in the urine by low voltage electrophoresis. During the infusion of amiloride, a dose-dependent ipsilateral natriuresis was observed. A maximum ipsilateral antikaliuretic effect and increase in pH were found when 5 x 10^{-9} moles/kg·min of amiloride was reaching the infused kidney. It is concluded that amiloride is secreted by the proximal tubular organic cation transport system.

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TESTOSTERONE STIMULATION OF RENAL ORNITHINE DECARBOXYLASE. K.A. Pass*, J.E. Bintz*, J.J. Postulka*, and H.L. Vallet*. (Spon: D.O. Carpenter). Birth Defects Inst., Div. of Laboratories and Res., N.Y.S. Dept. of Health, Albany, N.Y. 12201

The anabolic effects of testosterone (T) on the kidney resulting in an increase in renal mass have been known for many years. Increased activity of ornithine decarboxylase (ODC) is characteristic of rapidly growing tissues. Renal ODC, as measured by in vitro production of $^{14}CO_2$ from 1-^{14}C ornithine, increased 2000% twelve hours after injection of 300 µg T in adult male mice. Contrary to our earlier results with arginine vasopressin (AVP), chronic stimulation with T resulted in continued high levels of renal ODC. Castration produced a rapid decline in renal ODC levels, at which time (10 days post-gonadectomy) the animals were refractory to AVP stimulation. Mice castrated 120 days previously had normal renal ODC levels and AVP response. Actinomycin, but not cyclohexamide, blocked the T-stimulated increase in renal ODC. 1,3-diamino-Propane (DAP, 22 mg/100g BW), a diamine not normally found in animal tissues, inhibited both basal and T-stimulated kidney ODC. Neither chronic DAP (3 days, bid), chronic T (3 days), nor castration, had any effect on urine specific gravity or serum osmolality (measured by freezing point depression). Although the marked changes in renal ODC caused by T stimulation do not appear to affect the normal functioning of urinary concentrating mechanisms, these results indicate that renal ODC activity is highly sensitive to T stimulation and suggest other roles for T in this metabolically active organ.

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INHIBITION OF TRANSPORT AND METABOLISM BY ARSENATE IN RENAL PROXIMAL TUBULES. P.C. Brazy*, R.S. Balaban*, S.R. Gullans*, and L.J. Mandel. Duke University and VA Medical Centers, Durham, N.C., 27710.

We examined the inhibitory effects of arsenate on transport of sodium, phosphate, glucose and para-aminohippurate (PAH) as well as on oxidative metabolism by proximal convoluted tubules from rabbit kidney. Transport rates were measured with radioisotopes in isolated and perfused tubular segments. Metabolic activity was monitored as oxygen consumption rates and NADH fluorescence in parallel studies with suspensions of cortical tubules. Arsenate (1 mM) in the perfusate reduced fluid absorption (Jv) an index of net sodium transport from 1.24 ± 0.17 to 0.66±0.19 nl/mm·min ($p<0.01$) and phosphate absorption (J-Phos) from 9.93±3.47 to 4.25±1.08 pmol/mm·min ($p<0.01$). Glucose absorption (J-Gluc) was reduced slightly from 66.1± 6.0 to 56.8±4.6 pmol/mm·min ($p<0.05$). PAH secretion was not affected by arsenate. Removal of phosphate from the perfusate had no effect on Jv or J-Gluc. In suspensions of tubules, arsenate increased oxygen consumption rates by 20.5±2.9% and decreased NADH fluorescence by 10.8±1.5%. These effects were concentration-dependent and were magnified in the presence of ouabain. The data indicate that 1) arsenate's dominant effect is to uncouple oxidative phosphorylation, 2) graded uncoupling of oxidative metabolism causes parallel reductions in Jv and J-Phos, and 3) J-Gluc and PAH secretion are less sensitive to partial inhibitions of oxidative metabolism than are Jv and J-Phos. (Supported by USPHS. NIH Grants AM 17195 and AM 26815)

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INTRARENAL VENOUS GLUCOSE LEVELS IN THE DOG: A COMPARISON OF SAMPLING TECHNIQUES. T.V. Peterson, Dept. of Medical Physiology, Texas A&M University College of Medicine, College Station, TX 77843.

A previous study demonstrated that, in the dog, blood samples obtained from the intrarenal veins (interlobular and arcuate) differed in plasma glucose concentration from samples simultaneously obtained from the renal vein (RV). The present study was undertaken to further test the feasibility of the IRV method for studying renal glucose handling. Ten IRV, RV and A samples were simultaneously obtained at 3 minute intervals using two different IRV sampling techniques: one (Group I, n=8) with the IRV catheter being left in place at its highest insertion point and the other (Group II, n=8) with the IRV catheter being randomly moved slightly between each sample. Both groups showed a net renal glucose production. In Group I most of the dogs had mean IRV values greater than RV whereas in Group II most of the dogs had mean IRV values less than or no different from RV. However, in some dogs, the relationships between IRV, RV and A changed abruptly during the experiment. It is concluded that the probability of obtaining an IRV sample which reflects cortical drainage (IRV glucose>RV glucose) is greater with the fixed catheter technique. However, the suitability of the IRV technique or RV-A differences for looking at renal glucose handling during perturbations in glucose homeostasis is questionable because of the abrupt changes which can occur during the control state. (Supported by Organized Research Funds, TAMU College of Medicine)

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COMPARISON OF INHALATION ANESTHETICS IN THE PONY: RESPONSE OF PLASMA CORTISOL AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM. B.Fichtenbaum,* L. Clarke *and H. Garner. Dept. Vet. Med. and Surg., Univ. of Missouri, Columbia, Mo. 65211

Plasma concentrations of renin activity (PRA), aldosterone (PAC) and cortisol (PCC) were measured during experiments involving 6 ponies anesthetized for 2 hrs. with halothane, methoxyflurane, enflurane and isoflurane. Samples were taken prior to anesthesia (control), at 10,40, 70,100,120 min. during anesthesia, and 1 hr. after the pony achieved standing posture (recovery). The plasma endocrine concentrations were determined by radioimmunoassay. The resulting data was subjected to analysis of variance which indicated that 1) PRA and PAC increased significantly ($P<.05$) during anesthesia with halothane, enflurane and isoflurane; similar increases were observed with methoxyflurane but they were not statistically significant; 2) PCC increased significantly ($P<.025$) during anesthesia with all 4 agents; and 3) in the recovery period PAC and PCC remained elevated but PRA decreased below the anesthetic level.

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EFFECT OF HEXAMETHONIUM ON THE RENAL RESPONSE TO ANESTHESIA AND SURGERY. L. Walker* and M. Gellai* (SPON: H. Valtin). Dartmouth Medical School, Hanover, NH 03755.

Our previous studies showed that anesthesia (ANES) and surgery (SURG) in the rat are associated with decreases in glomerular filtration rate (GFR) and PAH clearance (C_{PAH}). In order to evaluate the possible involvement of autonomic mechanisms, the effects of the ganglionic blocker hexamethonium (H) on the renal response to ANES and SURG were examined. In 6 untreated (U) Long Evans rats, variables below were measured in the conscious state, then after induction of ANES with pentobarbital or Inactin, and subsequently after minor surgery. A second group (n=5) was treated similarly, except that H 5mg/kg was administered IV prior to induction of ANES.

			Min after ANES		Min after SURG	
		Conscious	10-40	40-70	10-40	40-70
MABP	U	108	87*	88*	102	101
(mm Hg)	H	116	89*	95*	95*	95*
GFR	U	100	75*	85*	80*	83*
(% Conscious)	H	100	99	119	105	104
C_{PAH}	U	100	75*	83*	64*	60*
(% Conscious)	H	100	80*	88*	78*	78*

*P < 0.05 when compared to Conscious

H did not affect GFR in the absence of ANES. It is concluded that H partially protects against the fall in GFR induced by ANES and SURG, suggesting an autonomic component in the renal response. (Supported by Nat Kid Found Fellowship and NIH)

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TRANSPORT OF OCTANOIC ACID IN RAT RENAL CORTICAL LUMINAL (L) AND ANTILUMINAL (AL) MEMBRANE VESICLES. M.E. Trimble, P.D. Holohan*, P. Williams* and C.R. Ross* Depts. of Physiology and Pharmacology, SUNY Upstate Medical Center and VAH, Syracuse, N.Y. 13210.

Saturation of AL uptake of octanoate (OCT) in the perfused rat kidney (Trimble, AJP 237:F210, '79) was suggestive of facilitated fatty acid transport. Since saturation could also have reflected limitations of metabolism, OCT transport into isolated L and AL membrane vesicles (Kinsella et al, BBA 552: 468, '79) was studied. Production of $^{14}CO_2$ from [1-^{14}C]octanoate by vesicles was insignificant. Binding was estimated to be 35% in AL and 70% in L vesicles with the remainder of the label representing transport into an intravesicular space. Uptake into AL vesicles was more rapid than into L. At 2 min, uptake into AL vesicles was 50% of maximum while uptake into L was only 7%. Due to low initial rates of L uptake, further data were obtained only for AL. Saturation of AL uptake was observed when initial uptake (30 sec) was measured at various OCT concentrations. However, the shape of the curve tended to be sigmoidal rather than parabolic as observed with most other organic ions. V_{max} was 38 ± 14 nmoles/mg protein/min and K_m was 4.2 ± 0.9 mM OCT. Initial AL uptake (1 mM OCT) was inhibited 39% by 5 mM and 80% by 10 mM probenecid (3 membrane preparations). These results which show saturation and inhibition of AL OCT transport are consistent with a carrier-mediated transport process. (Supported by USPHS Grants AM 25695-01A1 and HL 02835 and VA Funds).

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RETROGRADE AXONAL TRANSPORT IS MAGNESIUM DEPENDENT. R. S. Smith and R. E. Snyder*. Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

The retrograde transport of optically detectable particles in myelinated axon isolated from Xenopus laevis was examined distal to a laser lesion. Test solutions diffused from the bathing medium to the axis cylinder via the lesion. All test solutions referred to below allowed unimpaired transport in axons without lesions. A bathing solution of 0.12M potassium glutamate allowed particle transport to continue to within about 10 μm of the lesion for periods in excess of 1 h. Addition of 5 mM EGTA to the solution did not arrest transport, but 1 mM EDTA stopped transport within a few hundred micrometers of the lesion within minutes. The time required for EDTA to stop transport at a given distance from the lesion could be prolonged by preloading the fiber with 10 mM magnesium. EDTA solutions with excess magnesium did not stop transport. The results demonstrate a new experimental approach to the mechanisms of axonal transport and indicate that retrograde particle transport requires ionized magnesium, but not calcium.

(Supported by MRC, Canada)

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QUALITATIVE CHANGES IN ADAPTIVE BEHAVIOUR OF PROPYLTHIOURICAL (PTU)-TREATED RAT. Y. Hoh*[1], V. Havlicek[1], V.C. Sundmark*[1] and L. Van Middlesworth[2]. Dept. of Physiology, U. of Manitoba[1] Wpg., Canada R3E 0W3, and Dept. of Physiology & Biophysics, U. of Tennessee[2], Centre for the Health Science, Memphis, Tenn.

Rats exposed to severe iodine depletiose in fetal and neonatal life exhibit a high incidence of audiogenic seizures. Persistence of seizures into adulthood suggests possible permanent deficits in brain function. Lactating mothers were given 0.02% propylthiourical (PTU) in drinking water for 19 days to produce a temporary hypothyroid condition in the pups. Subjects (Ss) were subsequently weaned to standard laboratory chow and tap water. At 5 months old, Ss were put on 23 hour food deprivation and trained in a 14 pattern maze for food reward. During preliminary training with one designated route the performance of PTU-treated Ss were superior to that of normal controls, with shorter running time ($p < .05$) and less errors ($p < .05$). When an alternate route was introduced, the PTU-treated Ss failed to regain a comparable level of performance after an initial increase in running time and errors. In the open field test the PTU-treated Ss were hyperactive ($p < .01$) and showed little tendency to habituate ($p < .001$). Radioimmunoassay yields a significant increase ($p < .01$) in the hypothalamic β-endophin levels in PTU-treated Ss (6.4 ± 1.3 μg/g tissue Vs. 2.4 ± 0.03 μg/g). Results in behavioural testing suggests the existence of permanent brain dysfunction in Ss which were made temporarily hypothyroid during a critical period time of nervous system development.

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ROLE OF BIOGENIC AMINES (BA) IN BRAIN FREE FATTY ACID (FFA) LIBERATION DURING GLOBAL ISCHEMIA IN RATS. Edwin M. Nemoto and Gerald Shiu.* The Anesthesia and CCM Research Laboratories, Department of Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Brain FFA (i.e., arachidonate, stearate, oleate and palmitate) rapidly increase during global ischemia. We studied the role of BAs and BA release in FFA liberation during ischemia. Albino rats were injected with reserpine 10 mg/kg, IP 12 to 16 hours prior to the studies. Untreated and reserpine rats were decapitated awake, their heads quickly sealed in plastic bags and placed into a 37°C oven for one to 60 min. At the precise duration of ischemia the brains were frozen in liquid N_2. Nonischemic brains were rapidly sampled into liquid N_2. In nonischemic reserpine rats, total FFA, oleate and palmitate were significantly increased compared to untreated controls. Arachidonate and stearate were unchanged. In the first 6 min during ischemia, total FFA was unchanged while oleate and palmitate transiently declined and arachidonate and stearate increased. All increased in untreated rats. After 20 min of ischemia, FFAs were higher in reserpine rats, but similar to controls at 60 min. BA depletion prior to ischemia did not affect arachidonate and stearate liberation, but markedly altered oleate and palmitate. Arachidonate and stearate are thought to arise from phosphatidylinositol and oleate and palmitate from triacylglycerols.

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BARBITURATE DOSE-RESPONSE ATTENUATION OF BRAIN FREE FATTY ACID LIBERATION DURING GLOBAL ISCHEMIA IN RATS. Gerald K. Shiu,* Edwin M. Nemoto and Henry Alexander.* The Anesthesia and CCM Research Laboratories, Department of Anesthesiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We previously reported that pentobarbital 60 mg/kg effectively attenuated liberation of brain free fatty acids (FFA) and most markedly after 10 min of decapitation ischemia (Fed Proc 39(3):407,1980). We now describe the barbiturate dose-response attenuation of brain FFA liberation after 10 min decapitation ischemia in rats. Albino rats were injected IP with pentobarbital 15, 30, 60, 90 and 120 mg/kg and 15 min later decapitated. Barbiturate hypotension and hypoventilation were corrected by thoracic aorta constriction and mechanical ventilation on 100% O_2 in rats anesthetized with 90 and 120 mg/kg. The heads were sealed in plastic bags and incubated at 37°C and the brains frozen in liquid N_2 10 min later. Whole brain palmitate, stearate, oleate and arachidonate were extracted and quantitated by gas-liquid chromatography. Pentobarbital 30 mg/kg significantly reduced FFA liberation compared to awake rats and to the same degree at higher doses. FFA liberation was reduced by 30 to 40% with most dramatic effects on arachidonate and stearate. These results suggest that a barbiturate dose of 1/2 that required for surgical anesthesia may provide maximal therapeutic effects for ischemic brain damage and higher doses would be of no further benefit.

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ALTERED AXONAL TRANSPORT IN INTACT CENTRAL PROCESSES OF PRIMARY SENSORY NEURONS FOLLOWING INJURY TO THE PERIPHERAL PROCESS. M.A. Bisby. Division of Medical Physiology, University of Calgary, Calgary, Alberta T2N 1N4

Injury and subsequent regeneration of peripheral nerves induces changes in the amount and composition of fast transported protein in regenerating axons. Transport was examined in the central, intact, process of primary sensory neurons after injury to the peripheral process to determine whether changes in transport are restricted to the injured process, or affect both processes. The sciatic nerves of rats were crushed bilaterally and 1-35 d later L-(^{3}H) leucine was injected into the L5 dorsal root ganglia (DRG). After 2½ h, nerves, DRG and dorsal roots were removed, sectioned and counted. Activity in the waves of labeled protein transported in the roots and nerves was compared. This ratio did not change from normal at any time after injury. In other animals L-(^{35}S) methionine was injected into DRG 14 d after nerve injury. Transported protein was collected proximal to nerve crushes and root sections, and characterised by SDS electrophoresis. Similar changes in composition occurred in both central and peripheral process. Injury - produced alterations in transport thus affect both central and peripheral processes, even though the central process is not regenerating. (Supported by the Medical Research Council of Canada).

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ROLE OF SYMPATHETIC NERVOUS SYSTEM IN MORPHINE-INDUCED MYDRIASIS IN RAT. Martin C. Wallenstein. New York University, NY, NY 10010.

The effects of three doses morphine (5, 30 & 60 mg/kg) on pupillary diameter (PD) were studied in groups of rats which were normal, neurotransmitter antagonist-pretreated, guanethidine-pretreated or adrenalectomized. The rat's PD was observed from outside a dimly lit chamber through a lens system. Morphine produced dose-dependent increases in PD and exophthalmos. In addition, the PD concurrently underwent fluctuations whose durations were dose-dependent. Alpha-adrenergic antagonists blocked both mydriasis and exophthalmos. However, beta-adrenergic antagonist had no significant effect. This suggested a role for the sympathetic nervous system. Guanethidine-pretreatment only partially blocked the increase in PD and exophthalmos. This indicated that morphine affected another mydriasis-inducing mechanism aside from sympathetic input to the iris. Adrenalectomy also partially inhibited the mydriasis. However, adrenalectomy combined with guanethidine-pretreatment completely blocked morphine-induced mydriasis and exophthalmos. The time scale rules out a role for adrenal steroid hormones. Thus, the results suggested that the mydriasis resulted from a combination of direct sympathetic input and a hormonal effect from the adrenal medulla.

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EFFECT OF ANTIESTROGEN (TAMOXIFEN) ON HYPOTHALAMIC CATECHOLAMINES IN FEMALE RATS. Samarendra N. Baksi, Theresa E. Redington* and Maysie J. Hughes. Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

Estrogen can influence hypothalamic catecholamines, particularly dopamine (DA) concentration, which in turn modulates prolactin secretion. In the present study we investigated the effect of antiestrogen (tamoxifen) treatment on hypothalamic DA and norepinephrine (NE) content in mature female rats. Tamoxifen was injected (SC) at 3 different dose level (10,1.0 and 0.1 mg/kg) daily for 7 and 21 days. Control rats received vehicle (10% ethanol; 1 ml/kg). Each group consisted of 6 rats. DA and NE was estimated by HPLC with electrochemical detection method and is expressed as ng/g of tissue. The uterine wet weight is given as mg/100g. The data (Mean ± SEM) shows that tamoxifen treatment for 7 days does not alter the steady state concentration of DA and NE (not shown) but tamoxifen treatment for 21 days significantly (**p<0.001) increases DA concentration at 0.1 mg dose. NE concentration was significantly (*p< 0.05) reduced at 1.0 mg dose. The antestrogenic (reduced uterine wt) action of tamoxifen was not related to the alterations of DA and NE. (Support: NIH HL 16240).

	21 days of treatment		
	DA	NE	Uterine wt
Control	522± 89	1313±101	244±13
10.0 mg	693±197	1238± 75	98± 4**
1.0 mg	398± 75	1032± 42*	118± 4**
0.1 mg	1354± 52**	1453±129	360± 9**

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EFFECTS OF CATIONS ON THE BINDING OF ^{3}H-SPIPERONE BY SHEEP CAUDATE NUCLEUS. A. P. Carvalho and C. R. Oliveira* and I. Wajda* Department of Zoology and Clínica Neurológica, Univ. Coimbra, Portugal, and Institute for Neurochemistry, Wards Island, New York, 10035.

We studied the effect of monovalent and divalent cations on the interaction between the dopamine antagonist, ^{3}H-spiperone, and the dopamine receptors of sheep caudate nucleus. At 25°C, Zn^{2+}, Cd^{2+} and Ni^{2+} decrease the specific ^{3}H-spiperone binding to caudate homogenate, while increasing the non-specific binding which is observed at the same low concentration of spiperone (2 nM). On the other hand, Ca^{2+}, Mg^{2+} and Mn^{2+} increase the specific binding of ^{3}H-spiperone. The non-specific binding of ^{3}H-spiperone is also increased at the higher concentrations of these ions tested, but there are some significant differences in the low concentration range. The temperature has a strong influence on the effect of monovalent (Na^+, K^+ and Li^+) and divalent (Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+}) cations on the ^{3}H-spiperone binding. Thus, the temperature optimum for ^{3}H-spiperone binding is about 25°C in the absence, but shifts to values above 35°C in the presence of either monovalent (100 mM) or divalent (5.0 mM) cations. In addition, all cations increase the maximum ^{3}H-spiperone binding, and Mn^{2+} is particularly effective in this respect. It is not yet clear how ions cause their effects, but they may be part of a mechanism for regulating receptor activity. (Supported by I.N.I.C., Portuguese Ministry of Education, and NATO Research Grant Nº 1513.)

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EFFECTS OF NALTREXONE ON THE FEEDING BEHAVIOR OF MINIMALLY STRESSED RATS. I. Lang, J.C. Strahlendorf, H.K. Strahlendorf, and C.D. Barnes. Texas Tech Univ. HSC, Lubbock, TX 79430.

Opioid antagonists have been reported to reduce water and food intake, however, the animals were stressed by repeated injection of drugs. These experiments were conducted to test the hypothesis that stress may release endogenous opioids from the brain, which induce an increased feeding behavior which in turn can be blocked by opioid antagonists. Fourteen 4 week old rats were randomly separated into 2 equal sized groups, anesthetized with ether, and implanted subcutaneously with poly (ε-capreolactone) capsules. One group received naltrexone in the capsules, the other group received vehicle. These capsules released drug at a rate of approximately, 250 µg/day for over 8 weeks. Gross metabolic behavior (food intake, water intake, urine output, fecal output, body weight) was monitored for 8 weeks. At intervals of 2 week food deprivation (24 hours) induced feeding was tested over a period of 60 minutes. At the end of the 8th week, the animals were subjected to an analgesic test in order to assess the physiologic effects of naltrexone. Few differences in feeding behavior or intestinal excitability were observed between control and experimental groups, even though the analgesic responses were altered. These results suggest that the inhibitory effect of opioid antagonists on feeding behavior depends on prior stress during drug administration. This stress should be controlled when conducting feeding behavior experiments. Supported in part by HL07289 and Tarbox Parkinson Disease Institute.

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IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF NEUROPEPTIDES IN THE RAT BRAIN. C. Pelletier, R. Leclerc* and J. Guy*, MRC Group in Mol. Endocrinol., CHUL, Quebec, Canada.

During the last few years, peptides, known to be present in the pituitary and gastro-intestinal tract, have been found in the central nervous system. In order to clearly identify the structures as well as the subcellular organelles involved in the synthesis and/or storage of these peptides, we performed an immunoelectron microscopic localization of several of these peptides, such as enkephalins, ACTH, α-MSH, β-endorphin, substance P and VIP in different regions of the rat brain. For this purpose, we used both the pre- and post-embedding staining techniques involving use of specific antibodies and the peroxidase-antiperoxidase complex. For all the peptides so far studied, a general pattern of immunostaining was observed. In neuronal cell bodies as well as in axon fibers and terminals, the staining was mostly concentrated in large dense core vesicles (60-100 nm in diameter). In dendrites, the reaction was rather diffuse without any specific association with organelles. In terminals, the small clear vesicles were free of reaction product. Many but not all the positive terminals were observed forming synaptic contact with other unstained neuronal elements, especially dendrites. These ultrastructural observations suggest that neuropeptides can not only be involved in classical neurotransmission but can also be released outside the synaptic junction to act as local hormones.

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EFFECT OF HYPOPHYSECTOMY AND CHRONIC STRESS ON IMMUNOREACTIVE SOMATOSTATIN (IRS) IN THE RAT HYPOTHALAMIC AND EXTRAHYPOTHALAMIC BRAIN. N. Kato*, M. West* and V. Havlicek, Dept. of Physiology, U. of Manitoba, Winnipeg, Canada, R3E 0W3.

It has been suggested that somatostatin (SRIF) is responsible for the inhibition of growth hormone (GH) secretion induced by stress and that GH exerts a positive feedback effect on hypothalamic SRIF. We studied the effects of chronic stress on the content of immunoreactive SRIF (IRS) measured by double antibody RIA in the hypothalamus, thalamus, and amygdala of Long Island rats (males, 200-250 gm) separated into 4 groups; sham hypox (SH); sham hypox-stressed (SS); hypox (H); hypox-stressed (HS). Stress consisted of placing the rats on a warm plate at 50° for 2 min. 4 times daily for 7 days. Hypox decreased IRS in all 3 brain regions. Chronic stress increased IRS in the hypox groups, but did not change IRS in the sham hypox group.

Brain Region	IRS-content (ng/gm tissue) mean ± S.E.M.			
	SH (6)	SS (6)	H (10)	HS (13)
Thalamus	272 ± 41	198 ± 20	156 ± 12[a]	281 ± 33[b]
Hypothalamus	804 ± 92	673 ± 82	395 ± 41[a]	560 ± 56[c]
Amygdala	785 ± 92	641 ± 107	414 ± 47[a]	703 ± 77[b]

a SH:H significant at $p < 0.01$; b H:HS significant at $p < 0.01$; c H:HS significant at $p < 0.05$

The data suggest that SRIF is not only modulated by GH but also may have an independent primary role in the CNS in stress related conditions. (Supported by MRC of Canada.)

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LOCALIZATION OF ENKEPHALIN-LIKE IMMUNOREACTIVITY WITHIN THE HUMAN BRAIN AND SPINAL CORD. J.T. Quinlan and M.I. Phillips, Dept. of Physiology, Univ. of Iowa, Iowa City, Iowa 52242

Enkephalin-like immunoreactivity has been detected in human brain and CSF by radioimmunoassay, but there have been no previous reports of the distribution of enkephalin-like immunoreactive structures within the human brain and spinal cord.

Samples of human brain and spinal cord were collected at autopsy from a series of patients who had met a sudden, cardiorespiratory demise. The tissues were fixed in formalin, with post-mortem intervals less than six hours. Vibratome sections, 100µ, were incubated with either rabbit anti-leucine enkephalin or anti-methionine enkephalin, at a 1:1000 dilution for 24° at 4°C. The peroxidase/anti-peroxidase method was used.

Typical immunoreactive neuronal fibers with varicosities were found in hypothalamus, basal ganglia, amygdala, brainstem and cord, and cerebellum. Greatest staining density was in hypothalamus, infundibulum and median eminence.

The discrete localization of enkephalin-immunoreactive neuronal fibers within the human brain and spinal cord, with wide variation in the density of these fibers, may reflect the functional role of enkephalins in human CNS. (Supported by NSF and NIMH grants.)

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THEORETICALLY PREDICTED TETANIC HYPERPOLARIZATION BY SODIUM LOADING OF A SYSTEM IN WHICH ACTIVE TRANSPORT IS LINKED TO FRANKENHAEUSER-HUXLEY KINETICS. G.M. Schoepfle and J.T. Tarvin*. Univ. of Alabama Med. Ctr., Birmingham, AL 35294

The Frankenhaeuser-Huxley excitation equations for the Xenopus node are coupled to an active transport current term Ip such that Ip = gp (V - Ep) where V is membrane potential and Ep is an emf which is both sodium and ATP dependent (Schoepfle & Tarvin, The Physiologist, 22:113, 1970). The pump conductance gp is set at a level which insures that for single shock excitation there exists net sodium accumulation within the node throughout an interval sufficient to obtain a return to normal of leak conductance factors m. h and n. Analysis of subsequent repetitive activity involves iterative numerical solutions of the differential equations which indicate that a quasi-steady state is quickly attained, with the result that successive voltage-time patterns appear identical. Internal sodium concentration at the end of any inter-spike interval is now adjusted to a level such that throughout the interspike interval the time integral of total sodium current vanishes. The required increase in internal sodium concentration is sufficient to establish tetanic hyperpolarization. Sodium loading to a comparable extent induces infinitesimal tetanic hyperpolarization when gp vanishes. Resting membrane potential is negligibly affected by setting gp equal to zero. This is due to the stabilizing effect of the relatively high Frankenhaeuser-Huxley non specific leak conductance. NIH Support.

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SLOW CHOLINERGIC (MUSCARINIC) DEPOLARIZATION ASSOCIATED WITH A CONDUCTANCE DECREASE IN FRESHLY ISOLATED SMOOTH MUSCLE CELLS. John V. Walsh, Jr. and Joshua J. Singer*. University of Massachusetts Medical School, Worcester, MA 01605

The direct action of a transmitter on the smooth muscle cell membrane is difficult to assess in tissue preparations because of possible additional actions on neural elements in the tissue and because of the shunting effect of the syncytium on conductance changes caused by focal transmitter application. To avoid these difficulties, intracellular recordings were made from single smooth muscle cells enzymatically dissociated from the stomach muscularis of Bufo marinus. Acetylcholine (ACh), briefly applied either iontophoretically or in a known concentration by pressure ejection from a micropipette, caused a slow depolarization (sometimes giving rise to action potentials) which had a latency on the order of hundreds of milliseconds, required several seconds to reach peak amplitude and lasted well over a minute after termination of the application. This depolarization was accompanied by a conductance decrease even though these cells normally always show outward-going rectification. These results were also obtained at low $[Na^+]_o$ i.e. 12mM, or in the presence of 45mM TEA (consistent with the failure of TEA to affect "M-current" in sympathetic neurons). These initial findings, not previously reported in smooth muscle, suggest that a decrease in K^+ (or possibly Cl^-) conductance is responsible for the depolarizing ACh potentials. SUPPORTED BY NSF PCM-7904938, NIH HL14523, & March of Dimes.

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THE EFFECT OF METABOLIC INHIBITION ON DESENSITIZATION AT THE NEUROMUSCULAR JUNCTION OF THE FROG. T.J. Chesnut* and C. Edwards (SPON: H. Tedeschi). Dept. of Biol. Sci. and Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222.

The depolarization of the muscle end-plate membrane induced by continual application of cholinergic agonist decreases with time (desensitization). It has been proposed that accumulation of intracellular calcium (Ca_i) may decrease the response. To learn more about the possible involvement of Ca_i, the effects of 0.25 mM dinitrophenol (DNP) and 0.1 mM 1-fluro,2,4-dinitrobenzene (FDNB) on the desensitization of frog sartorius muscles to carbachol have been followed under voltage clamp. Both desensitization time constant and depth were measured. Recovery was measured by application of carbachol 5 minutes after termination of the desensitizing dose. Desensitization was deeper (90.3 ± 3.47% vs. 73.6 ± 5.15% for control) and the time constant was shorter (112 ± 17.0 sec. vs. 262 ± 50.0 sec. for control) during metabolic inhibition. Recovery was also less (61.8 ± 5.53% vs. 98.6 ± 9.07% for control). Desensitization curves occasionally exhibited two time constants. Metabolic inhibition did not significantly affect the fast time constant, but it did reduce the slow time constant (200 ± 37.0 sec. vs. 378 ± 53.4 sec. for control). The data are consistent with the hypothesis that elevation of free Ca_i due to influx through the end-plate channel augments desensitization and that recovery is partially dependent on the ability of the muscle cell to buffer this calcium load. (Supported by NIH, NS 07681).

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EXTRACELLULAR AND TRANSMEMBRANE CURRENT FLOWS DURING THE STRYCHNINE SPIKE. A. L. Towe, M. D. Mann† and G. W. Harding*. Univ. of Wash. Sch. Med., Seattle, WA 98195 and †Univ. Nebr. Sch. Med., Omaha, NE 68105.

The primary response evoked in lateral postcruciate cortex by stimulation of the contralateral forepaw in chloralosed and immobilized cats was studied before and after topical application of 2% strychnine sulfate solution. Recordings were taken at 0.1 mm intervals in depth through cortex and were assembled into depth-time potential field surfaces, $\phi(z,t)$. Calculation of extracellular $[J(z,t)]$ and transmembrane $[i_m(z,t)]$ currents was performed in a one-dimensional analysis, with conductivity taken as constant through depth and time. Strychnine enhanced and extended the normal pattern of current flow, especially in the upper half of the cortex. This increased current could be resolved into two components, one ascribable to enhanced EPSPs and the other to paroxysmal depolarization shifts that develop on neurons in the upper layers of the cortex. A model for the production of convulsive activity is proposed wherein enhanced EPSPs are produced by antidromic activity originating in layer I on the thalamocortical fibers that project both into layer I and layer IV and deep III. These EPSPs are sufficiently large to evoke paroxysmal depolarization shifts, which are viewed as calcium spikes and which produce most of the current flow seen during the strychnine spike. Such intense synaptic input may normally be prevented by recurrent inhibition in the thalamus. (Supported by USPHS grants NS00396 and NS05136 from NINCDS.)

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PARADOXICAL EFFECTS OF LITHIUM ON FIELD POTENTIALS OF DENTATE GRANULE CELLS IN SLICES OF RAT HIPPOCAMPUS. Brian A. MacVicar*, Greg Weir*, Kim Riexinger* and F. Edward Dudek*. Dept. Zool. and Erindale College, Univ. Toronto, Mississauga, Ont. L5L 1C6. (SPON: S.S.Tobe)

Although lithium (Li^+) is widely used in treatment of manic depression and is toxic at higher concentrations, relatively little is known about its effects on the electrophysiological properties of neurons in the mammalian brain. The effects of Li^+ on evoked field potentials of dentate granule cells were studied in vitro with rat hippocampal slices. Li^+ (substituted for choline) reduced the amplitude of the antidromic population spike in a dose-dependent manner; 10 mM Li^+ had little or no effect, whereas 40 mM strongly reduced the response within 15 min. A paradoxical effect was observed at 25 mM Li^+: the antidromic spike was reduced by ~50%, but a second population spike frequently appeared. This Li^+-induced spike was reversibly blocked by Co^{2+}, which suggests that it was caused by Ca^{2+} influx. Li^+ (25 mM) also consistently reduced the population EPSP to perforant path stimulation, although occasionally a population spike was also induced. These paradoxical effects of Li^+ on neurons of the mammalian brain can be reconciled with previous observations on simple neuronal systems and may be involved in the therapeutic or toxic effects of Li^+ under clinical conditions. (Supported by NSERC grant A0395 and Banting and Atkinson Foundations.)

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VOLTAGE DEPENDENT DRUG BLOCKADE OF THE CHANNELS OPENED BY L-GLUTAMATE AT THE CRAYFISH NEUROMUSCULAR JUNCTION. Michael S. Dekin* and Richard P. Shank* (SPON: C. Edwards). Dept. of Physiology, Temple Univ. Sch. Med., Philadelphia, Pa. 19146

Various drugs were studied to gain information about the channels opened by L-glutamate (L-glut) at the crayfish neuromuscular junction. Ionic currents were measured using a conventional two microelectrode voltage clamp. Reversal potentials (E_r) were determined either by interpolation or by extrapolation after the I-V relationship was linearized with 200 µg/ml Concanavalin A for 1 hr. (Dudel, J. Physiol. Paris, 75, 1979). Both methods gave similar results. Values of E_r range between 0 and +10 mV. d-Tubocurarine (d-TC), local anesthetics and barbiturates all affect the dose-response relationship of iontophoretically applied L-glut vs. synaptic current in an uncompetitive manner. The neurally evoked EPSC is also depressed. The slope of the dose-response curve, input resistance (.2-.5 MΩ) and E_r are not changed. The block is voltage sensitive, increasing with hyperpolarization. At -80 mV the apparent dissociation constant (K_D) for d-TC is 5×10^{-3} M while at -150 mV the K_D decreases to 2.5×10^{-3} M. At all membrane potentials the relative potency of these drugs follows the sequence: barbiturates ≥ local anesthetics > d-TC. These drugs are known to block non-selective cation channels such as those opened by acetylcholine at the vertebrate endplate. Thus, the results suggest that the L-glut induced channels may also be non-selective for cations. (Supported by NIH grants NS #13979 and NS #07681)

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INFLUENCE OF CITRAL CONCENTRATION AND AIR FLOW RATE ON THE ELECTROANTENNOGRAM OF THE FEMALE MOSQUITO, *Aedes aegypti*.

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Department of Biophysics, University of Western Ontario, London, Ontario, Canada. N6A 5C1

The electroantennogram (EAG) can be used to study the interaction of olfactory factors important to the location of prey by mosquitoes such as the concentrations of attractants and repellents and the relative motion of the air. Pulses of citral were used to stimulate the isolated antenna in a continuous flow olfactometer at concentrations between 6 x 10^{-8} and 2 x 10^{-6} mol/ℓ at mean velocities between 17.5 and 175 cm/s. The mean EAG amplitude for 59 measurements on 9 antennae was 142 ± 11 μV (±S.E.M.) for 4.8 x 10^{-7} mol/ℓ citral at an air velocity of 70 cm/s. For rectangular stimulus pulses the rise and fall of the EAG was exponential with time constants of approximately 5 and 20 s. Analysis using multiple linear regression of the EAG amplitude was done for direct, inverse and log functions of concentration and flow. The largest correlation coefficient obtained for multiple correlation was 0.57 using 1/amplitude versus 1/concentration and 1/flow which supports the hypothesis that the EAG and olfactory transduction involve reactions analagous to simple enzyme kinetics. This also demonstrates that the flow and concentration are not entirely independent.

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CEREBRAL BLOOD FLOW (CBF) RESPONSE TO VENOUS PRESSURE ELEVATION. Elizabeth M. Wagner* and Richard J. Traystman. Johns Hopkins Medical Institutions, Baltimore, MD 21205.

The effect of venous pressure elevation on CBF was measured under conditions of constant arterial blood pressure and cerebrospinal fluid pressure (atmospheric). CBF was measured using the cerebral venous outflow (CVO) and radiolabeled microsphere (RM) techniques simultaneously. Cerebral venous outflow pressure was measured and referenced at the outflow port of the confluence of the sinuses. In 6 pentobarbital anesthetized, ventilated dogs, CVO remained unchanged from control (18.7±3.1 ml/min) as cerebral venous outflow pressure was elevated from -15 to -8 mmHg. As cerebral venous outflow pressure was further elevated to 10.0±3.0 mmHg, there was a continuous decrease in CVO to 7.8±2.4 ml/min. However, simultaneous measurement of CBF with RM showed no change in total or regional CBF. These experiments indicate that cerebral venous outflow pressure elevation opens intracranial venous anastomotic channels and diverts CVO from confluence drainage to other cerebral venous drainage channels. This effectively prevents large intracranial venous pressure increases from occurring. Further experiments were carried out in 4 dogs in which venous anastomotic drainage was prevented by raising superior vena cava pressure (downstream cephalic pressure) from control (0 mmHg) to 15 and 25 mmHg. CBF (RM) decreased from control (28.0±4.2) to 25.1±3.0 and 21.4±1.7 ml/min/100g respectively. These data suggest that the autoregulatory function of the brain is compromised with elevated venous pressure. Supported by: HL-10342 and HL-07199.

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THE DILATATORY CAPACITY OF THE PIAL ARTERIOLES DURING GRADED HYPOTENSION. U.I. Tuor* and J.K. Farrar*(SPON: I.C. MacDonald) University of Western Ontario, London, Canada. N6A 5C1

The cerebral arterioles continue to dilate at pressures below the lower limit of autoregulation but this dilatation is insufficient to maintain cerebral blood flow at normal levels. We have examined the reserve dilatatory capacity of pial arterioles during graded hemorrhagic hypotension in 10 rabbits using hypercapnia ($PaCO_2$=60 mm Hg) as a superimposed dilatatory stimulus. Mean arterial blood pressure (MABP) was reduced in 10-20 mm Hg increments and pial arteriolar caliber measured at normocapnia ($PaCO_2$=40 mm Hg) and hypercapnia at each pressure level using an image splitting technique. Under normocapnic conditions the arterioles dilated as MABP was reduced; reached maximal dilatation at 35 mm Hg; and collapsed progressively as MABP was reduced further. Hypercapnia produced an increase in vessel caliber superimposed on that resulting from MABP reductions. This CO_2 response was present at all pressure levels above 35 mm Hg and maximal vessel caliber during hypercapnia was observed at a MABP of 45 mm Hg. The dilatatory response to both hypotension and to hypercapnia was dependent on arteriolar size and was greatest in the smallest vessels (<50μm). The lower limit of autoregulation to hemorrhagic hypotension in rabbit is 65 mm Hg(approx). Thus there is a considerable dilatatory reserve at pressures below the lower limit of autoregulation but the mechanisms mediating "autoregulatory vasodilation" are not capable of evoking this reserve capacity. (Supported by the Ontario Heart Foundation)

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ANESTHESIA EFFECTS ON CEREBRAL BLOOD FLOW IN THE CAT. J.C. Hartman*, T. Adams, S.R. Heisey, M.A. Steinmetz and H.K. Fry*. Dept. of Physiol., Mich. State Univ., E. Lansing, MI.

Local cerebral blood flow (CBF) was measured continuously in the cortex (3 mm below brain surface) using a thermodynamic technique (A.J.P. 238:682-696, 1980) while anesthetized (iv Na Pentobarbital), adult cats breathed spontaneously either room air or an 8% CO_2 in-air mixture (hypercapnia). Anesthetic level was held at either plane 1 (light anesth.; LA; N=4) or plane 2 (moderate anesth.; MA; N=4) of Stage III using a maintained infusion technique. During initial air breathing, CBF ($cc \cdot min^{-1} \cdot cc^{-1}$) was 0.46 ± .080 in LA cats and 0.68 ± .034 in MA cats. After induction of hypercapnia, CBF increased rapidly to 1.52 ± .264 in LA cats and 2.04 ± .138 in MA cats, then declined to steady state levels of 0.74 ± .081 and 0.76 ± .052 in LA and MA cats, respectively. During subsequent room air breathing, CBF decreased to 0.47 ± .075 and 0.61 ± .034 in LA and MA cats, resp. These data demonstrate that LA cats maintain CBF lower than MA cats throughout both air breathing periods. During hypercapnia, maximum CBF is higher in MA cats, but steady state CBF's following the peak are not different. These data suggest that anesthetic depth may have been an important, unsuspected variable in earlier studies in which different methods for measuring CBF were evaluated using room air and CO_2 breathing cats. Our continuous measurements of CBF also reveal its transients in all test phases. (Res. supported by BRSG funds, COM, Mich. Heart Assoc. Grant 15584 and by NIH grant NS 13565.)

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EFFECTS OF HEMORRHAGE ON REGIONAL CEREBRAL BLOOD FLOW. R.Y.Z. Chen*, F.C. Fan*, G.B. Schuessler*, and S. Chien. Columbia U. Coll. of P&S, New York, N.Y., 10032

The effects of graded hemorrhage on regional cerebral blood flow (rCBF) were studied with 15 μ microspheres in 9 pentobarbitalized dogs under controlled ventilation (P_aCO_2 = 40 mmHg). Changes in mean arterial pressure (MAP), cardiac output (C.O.) and mean cerebral blood flow (mCBF) from control values are (mean ± S.E.M.):

Volume bled =	11 ml/kg	22 ml/kg	33 ml/kg	45 ml/kg
MAP	- 6± 2%	-16± 3%	- 31± 6%	-59± 5%
C.O.	- 7±10%	-28± 7%	- 36± 6%	-62± 5%
mCBF	+13± 4%	+ 4± 6%	+ 26±13%	-38± 9%
mCBF/C.O.	+28±11%	+51±12%	+104±27%	+84±32%

The ratio of rCBF per unit brain tissue weight to C.O. per unit body weight is listed below according to the rank order in the control state (C). Also listed are % changes after 45 ml/kg hemorrhage (Δ%).

	Caud. Nucl.	Thal-amus	Cort. Gray	Cere-bellum	Hypo-thal.	Pons	Med. Oblong.	Cort. White	Corp. Call.
C	8.01	5.25	4.94	4.66	4.41	3.78	2.83	2.66	2.02
Δ%	+ 50	+143	+101	+113	+154	+148	+190	+ 49	+ 52

The results indicate that rCBF is non-homogeneous and that the responses of rCBF to hemorrhage show considerable regional variations, being most prominent in regions where the neurons regulating cardiovascular activity are located. (Supported by NHLBI research grant HL12738 and NRSA grant HL-07114).

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OCULAR AND CEREBRAL PERFUSION FOLLOWING BILATERAL/UNILATERAL CAROTID OCCLUSION. Raymond Connolly*, Ellen Keough, Karen Ramberg*, Lloyd Wilcox, Jr., and Max Ramenofsky*. Tufts New England Medical Center, Boston, MA 02111.

Great difficulty exists in creating animal models for human cerebral and/or ocular ischemia by occlusion of one or both carotid arteries.We have studied blood flow to the brain and eye in a number of animals using Ru^{103} labeled microspheres and the Reference Sample Method.In baboons (Papio anubis) complete bilateral carotid occlusion does not alter blood flow (control 0.361 ml/min/g,occluded 0.336 ml/min/g, t=0.218,p=.41).In dogs there was no effect of unilateral carotid occlusion on the ocular blood flow (control 0.321 ml/min/g,occluded eye 0.303 ml/min/g,t=.232,p=.41).Similar results were obtained in monkeys (Macaca assamensis) (control 0.498 ml/min/g,occluded eye 0.515 ml/min/g,t=.174,p=.43).This lack of effect is probably due to collateral flow pathways. This theory is supported by data obtained with neonatal pigs. Unilateral occlusion causes a reduction in ocular blood flow (control 0.486 ml/min/g,occluded eye 0.302 ml/min/g,t=5.0, p<.001) and a less pronounced reduction in cerebral flow (control 0.688 ml/min/g,occluded hemisphere 0.566 ml/min/g, t=1.51,p<.10).Neonates have poorly developed collateral flow pathways compared with adult animals.These data are of interest in terms of producing cerebral ischemia models as well as in light of the increasing use of fetal and neonatal disease models.(Supported in part by NIH 1-T32-EY07045;National Society to Prevent Blindness 20697).

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^{11}C-IODOANTIPYRINE FOR THE MEASUREMENT OF LOCAL CEREBRAL BLOOD FLOW BY POSITRON EMISSION TOMOGRAPHY: VALIDATION AND SYNTHESIS. MD Ginsberg, AH Lockwood,* RD Finn,* R Busto,* JA Campbell,* TE Boothe,* and B Djermouni.* University of Miami and Mt. Sinai Medical Center, Miami and Miami Beach, FL, 33101.

Positron emission tomography and an in vivo autoradiographic paradigm can be used to make repeated local cerebral blood flow (lCBF) measurements in man. Iodoantipyrine (IAP) is a suitable lCBF tracer and has been synthesized with a ^{11}C label ($T_{½}$ 20.4 min). $^{11}CH_3I$, prepared from $^{11}CO_2$, was used to methylate 3-methyl-1-phenyl-2-pyrazolin-5-one to form ^{11}C-antipyrine. Following silica gel column chromatography and iodination, HPLC confirmed ^{11}C-IAP purity to be >99.9%. ^{11}C-IAP was injected as a bolus into the carotid artery of N_2O-anesthetized rats, and its cranial clearance was monitored by collimated probes in a coincidence circuit. Isotopic clearance was multiexponential. No shunt peak was present; thus, single-pass extraction of the tracer was complete. In other rats, hemispheral lCBF was measured by indicator fractionation following the jugular venous bolus injection of a mixture of ^{11}C-IAP and ^{14}C-IAP. Mean arterial pCO_2 was 31.1 torr. CBF values using ^{11}C-IAP (1.15 ± 0.09 ml gm^{-1} min^{-1}) were in excellent agreement with CBF using the commercially produced ^{14}C-IAP (1.16 ± 0.11) and with reported values. These results establish the feasibility of measuring lCBF with ^{11}C-IAP in the human brain by emission tomography. (Supported by USPHS Grants NS 15639, NS 15883, and NS 05820. MDG is an Established Investigator of the American Heart Association.)

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GEOMETRY OF MEDIAL DEFECTS IN HUMAN INTRACRANIAL ARTERIES. Peter B. Canham and Philip Mok*. Biophysics Department, The University of Western Ontario, London, Canada N6A 5C1.

Medial defects are regions of the tunica media with diminished or absent vascular smooth muscle[1]. We embarked on this research, in part because of the similarity of location of medial defects and the occurrence of saccular aneurysms at the apex of branching major brain arteries. Arterial branches from adult human autopsy specimens were fixed at 100 mm Hg transmural pressure either as separate segments of vessels or as part of a whole brain fixation using 6% glutaraldehyde and post fixation with 10% formalin. Longitudinal serial sections were cut in a plane parallel to the plane of the bifurcation. We analyzed 14 bifurcations from 5 autopsies. The thickness of the tunica media was measured on photomicrographs at several positions near the apex for each of several serial sections of each bifurcation. We used computer graphics to reconstruct the 3D geometry. The medial defects are equivalent to a groove with parallel sides and width of 0.02 to 0.1 mm extending completely through the apical region. Medial defects were also found at the converging site of the vertebral arteries leading into the basilar artery and to a lesser extent at lateral angles. Our interpretation is that the defect is more associated with the local geometry and blood pressure than to forces relating directly to blood flow. (Research supported by the Ontario Heart Foundation and the Todgham Medical Fund)

[1]Stehbens, W.E. (1959), J. Pathol. 78: 179-185.

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CORTICAL Pco_2 AND FOCAL CEREBRAL ISCHEMIA. Paul J. Feustel and John W. Severinghaus. CVRI, Univ. of Calif., San Fran., CA 94143

With a 10 min occlusion of the circulation to the brain, lactic acid should convert all brain HCO_3 to CO_2 and raise P_tco_2 (tissue) to about 400 torr. We have measured cortical P_tco_2 after MCAO (middle cerebral artery occlusion) both as an index of ischemia and to investigate possible tissue gas bubble formation. Six cats were anesthetized with halothane/N_2O (0.8%/70%) and mechanically ventilated. Local cortical blood flow (LCBF) was measured by H_2 clearance. A surface CO_2 electrode was placed in a location where previous experiments had shown rapid O_2 depletion with middle cerebral artery occlusion (MCAO). Control cortical P_tco_2 was 39.8±6.0 torr (Mean±S.D.) while P_aco_2 (arterial) was 30.0±6.0 torr. P_tco_2 following MCAO increased within one minute and from 2 to 4 minutes rose at 21.1±10.7 torr/min, reaching 124.0±51.4 torr at eight minutes post-MCAO. LCBF fell from 82.2±29.8 ml/min/100g to zero with MCAO. When MCAO was released after 8 min, P_tco_2 fell rapidly below control to 34.1±2.5 torr (P_aco_2=31.2±2.0 torr) reflecting a hyperemia (LCBF=203±71 ml/min/100g), and then slowly rose toward normal over a thirty minute period as CBF fell to control levels (96±50 ml/min/100g). After sacrifice (KCl IV) P_tco_2 rose further at a slower rate. Thus, although P_tco_2 rises at a rapid rate initially, it remains lower than would be expected from the complete liberation of CO_2 from HCO_3. The increase in P_tco_2 may be limited by its diffusion to adjacent perfused tissue. (Supported by HL06285)

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FUNCTIONAL AND METABOLIC POST-BIRTH DEVELOPMENT OF THE RAT LEFT VENTRICLE. Russell T. Dowell. Dept. of Physiology, Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190

Heart adaptive responses of young and adult animals differ qualitatively and quantitatively. To understand these adaptations, the developmental characteristics of the left ventricle (LV) were defined. Litters of 8, male, Sprague-Dawley rats were selected. Rats were weaned at 21 days of age and studied 3,5,7, and 9 wks post-birth. LV function was measured in situ. After functional study, the heart was excised, the LV dissected, weighed, and assayed for marker enzymes. LV weight (mg) increased 5-fold over the 6 wk period (146±5 to 802±21) with the largest increase (184%) between 3 and 5 wks. LV pressure (mmHg) increased from 73±6 at 3 wks to 102±6 at 9 wks. Maximum dP/dt (mmHg/sec) increased progressively over the same period from 3850±390 to 6160±480. Enzyme activities related to glycolytic (PFK) and anaerobic (LDH) metabolisms were not altered. In contrast, aerobic metabolism was enhanced during development. Citrate synthase activity in LV homogenate (μmole/gm/min) increased from 101±3 at 3 wks to 138±2 at 9 wks. Combined influences of enhanced aerobic enzyme activity and LV growth show the preferential accumulation of mitochondria in post-birth heart development. Thus, an enhancement in post-birth LV function is accompanied by comparable increases in aerobic metabolic capacity. (Supported by NIH grants HL 23025 and HD 13127)

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CHANGES IN NEONATAL CEREBRAL CIRCULATION DURING ASPHYXIA: EVIDENCE OF SYMPATHETIC MEDIATION. M.J. Hernández, R.A. Hawkins and R.W. Brennan*. Hershey Medical Center, Hershey, PA 17033

Recently we reported that asphyxia produces major changes in regional cerebral blood flow (rCBF) in newborn dogs. In those studies rCBF decreased in forebrain by 64%, and increased by 65% in hindbrain. In the present study, we investigated a possible sympathetic mechanism responsible for these changes. An autoradiographic technique, employing ^{14}C-iodoantipyrine, was used to measure rCBF in dogs 1-7 days of age under the following conditions: (1) normoxia, (2) after 5 minutes of asphyxia, and (3) after 5 minutes of asphyxia in dogs pre-treated with the α-blocker, phenoxybenzamine (PBZ, 200ug/kg). In the control group, conditions of normoxia (pO_2= 95 ± 4 Torr), normocarbia (pCO_2= 39.2 ± 2.6 Torr), and normotension (MABP= 61 ± 3 Torr) prevailed. In both asphyxiated groups, arterial pO_2 was reduced to about 5 Torr, and pCO_2 elevated to between 70 and 110 Torr. In addition to rCBF, regional cerebrovascular resistance (rCVR) was calculated. Mean (± SE) forebrain rCVR was 3.03 ± 0.22 $Torr/ml^{-1}\cdot min^{-1}$. 100 gm in the normoxic dogs, and increased in the non-treated asphyxiated dogs to 5.61 ± 0.50 $Torr/ml^{-1}\cdot min^{-1}$. 100 gm ($P<0.001$). However in the PBZ-treated dogs, these increases were prevented and forebrain rCVR (2.76 ± 0.29 $Torr/ml^{-1}\cdot min^{-1}$. 100 gm) was not significantly different in this group from that of the control normoxic dogs. Therefore it appears that forebrain vasoconstriction during asphyxia in newborn dogs is largely mediated through the sympathetic nervous system. (Supported by NIH grant NSHD 15791).

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BLOOD PRESSURES, INTERSTITIAL FLUID PRESSURE AND PLASMA COLLOID OSMOTIC PRESSURE IN THE UNANESTHETIZED FETAL GUINEA PIG. Debra F. Anderson*, Kent L. Thornburg and J. Job Faber, Dept. of Physiology, School of Medicine, University of Oregon Health Sciences Center, Portland, OR 97201.

This study was performed to measure the blood pressure and interstitial fluid hydrostatic pressure in the awake fetal guinea pig. The plasma colloid osmotic pressure was measured acutely in the anesthetized fetus. The vitelline vessels were catheterized and blood pressures measured 3 to 28 hours after surgery. The vitelline artery pressure was 30.1 ± 1.4 mm Hg (SEM; n = 16) and the vitelline vein pressure was 3.9 ± 0.5 mm Hg (n = 8). Fetal heart rate was 143 ± 15 bpm (n = 14). None of these varied with the time elapsed since surgery. In addition, a polyethylene capsule attached to a catheter was implanted subcutaneously in the fetus for the measurement of interstitial fluid pressure. Pressures were measured 3 hours to 5 days after surgery and were equal to -0.18 ± 0.29 mm Hg (n = 17). There was no relationship between the measured pressure and the duration of implantation of the capsules. All of these pressures have been corrected for intrauterine pressure. Finally, the plasma colloid osmotic pressure was measured in 47 fetuses carried by 25 sows. The oncometer used was a modification of that of Prather et al. and used an Amicon Um-10 membrane. The mean colloid osmotic pressure was 18.6 ± 0.6 mm Hg. There was a positive correlation between fetal weight and colloid osmotic pressure.
Supported by HD12017.

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EFFECTS OF FETAL EXPOSURE TO CARBON MONOXIDE IN THE RAT. D. Penney, M. Baylerian*, J. Thill*, S. Yedavally*, & C. Fanning*, Dept. of Physiol., Wayne St Univ. Med. Sch., Detroit, MI 48201

Increasing concern for harm to the fetus by CO is being given to air pollution and cigarette smoking. Pregnant rats were exposed to 200 ppm CO for the last 17 days of gestation. Body weights (BW) of treated fetuses were absolutely smaller and wts. of both ventricles (2V) absolutely larger than controls on each of the 4 days before birth and at birth. 2V/BW of treated fetuses was about 28% larger during this time. Placental wt. of this group was larger than controls and the difference increased as birth approached. Fetal cardiomegaly was not due to edema, as water content was not altered. At birth, hemoglobin conc., hematocrit and red cell count were mildly depressed in the treated animals, and red cell indices suggested an immature erythrocyte population. The number of fetuses/female and live young/litter of exposed animals were smaller, but not significantly. 2V lactate dehydrogenase (LDH) M subunit content was elevated in the treated fetuses, while there were no differences in LDH activity. 2V cytochrome c content increased steadily during the final 4 days of fetal development; conc. was depressed only at birth in the exposed animals. Both 2V DNA and hydroxyproline conc. are depressed at birth by CO exposure, while DNA content/2V is increased and hydroxyproline content is decreased. The CO-exposed pregnant females displayed no decrement in BW or polycythemia at parturition. While fetal cardiomegaly later regresses (Tox. Appl. Pharm., 53, 271, 1980), is the heart then normal? (NIH grant HL-22859-02).

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Ontogenic Characterization of Erythrocyte Plasmalemmae from Normal and Dystrophic Chickens. I: Phospholipid content Mark Kester*, Richard Dodge*, and C.A. Privitera. Department of Biological Sciences, SUNY, Buffalo, New york 14260.

Significant differences are observed in the phospholipid (PLP) content of nucleated erythrocyte plasmalemmae between age matched dystrophic (line 413) and normal (line 412) chickens. The plasmalemmae preparation purity is evaluated by marker enzymes. Lipids from the membrane preparation are separated by two dimensional thin layer chromatography. Our system separates clearly phosphatidyl (p) choline (PC), p-ethanolamine (PE), p-serine (PS), p-inositol (PI), sphingomyelin (SP), phosphatidic acid (PA), lysophosphatidylcholine (LPC), and neutral lipids. Data are expressed as percent of total PLP and as micromoles PLP phosphorous per microgram total lipid spotted. The dystrophic chicken erythrocyte has increased levels of PS and decreased levels of PE compared to age matched controls. Significant differences at the .005 confidence level are observed for PS at day 40 and 54 ex ovo and PE at day 27 (.01 C.L.), 33 (.05 C.L.) and 54 (.005 C.L.) ex ovo. These data are correlated with the onset of dystrophic symptoms as expressed through flip testing and the emergence of an unknown, distinct, Ninhydrin positive. Rhodamine 6-G positive PLP located between PE and PS on dystrophic plates only. These structural changes are being related to functional changes as data from ^{51}Chromium erythrocyte half-life experiments and osmotic fragility studies suggest other defects associated with the dystrophic chicken erythrocyte. (Supported in part by Research Foundation SUNY/Buffalo Grant #150-H079H).

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CHANGES IN CELL SURFACE HYDROPHOBICITY AND CELL-MEDIUM INTERFACIAL FREE ENERGY FOLLOWING EXPOSURE TO STEROIDS, A-23187, ZYMOSAN AND ASCARIS ANTIGEN. S. Schürch*, N.A.M. Paterson* and D.J.L. McIver* (SPON: S.H. Song). Depts. Biophysics, Medicine, Pharmacology, Univ. Western Ontario, London, Can. N6A 5C1

We have used contact angles on cell layers of a series of polymeric droplets in aqueous 2-phase and 3-phase systems of dextran, poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) to determine the cell-medium interfacial free energy. Test droplets of the denser dextran-rich and PVA-rich phases were formed on cells in the PEG-rich phase as the bathing medium. Measured interfacial tensions between the phases combined with Good's interaction parameter allowed us to determine the cell-medium interfacial energy for a particular polymer system. Interfacial free energies for human erythrocytes and neutrophils, and for pig pulmonary macrophages relative to an aqueous bathing solution from 4% PEG were between 0.2 and 1.0 $\mu J.m^{-2}$. The values are in excellent agreement with previous results obtained by the approach of the limiting (critical) interfacial tension for spreading (Zisman, Good and Girifalco). Exposure of neutrophils to steroids and stimulation of the macrophages with the calcium ionophore A-23187 (1 µM) and zymosan caused a more than 20% change in the interfacial free energy and substantially increased hydrophilicity, while ascaris antigen increased macrophage surface hydrophobicity. Exposure to these agents probably alters the hydration at the cell surface. (Supported by the Medical Research Council)

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ACTIVE TRANSPORT INDUCTION IN A VERTEBRATE CELL. Terry W. Pearson, Patrice M. Lilly,* and Samuel B. Horowitz.* Cellular Physiology Lab., Michigan Cancer Fdn., Detroit, MI 48201.

Analysis of nuclear and cytoplasmic uptake, using cryomicrodissection, demonstrates that *Rana pipiens* oocytes engage in both facilitated permeation and the active transport of α-aminoisobutyric acid (AIB). Facilitated permeation is constitutive, while active transport is substrate-induced following an induction period of ~20 h. The substrate-dependent active transport induction was examined by use of cycloheximide, known to block protein synthesis. Cells were incubated in ^{3}H-AIB-Ringers (20°C) in absence and presence of cycloheximide (0.05 mM) and analyzed at various times for Na^+, K^+, water, and ^{3}H-AIB content. Incubation with cycloheximide has no effect on cellular AIB content during the period of facilitated transport. The agent exerts its effect by inhibiting the onset of active transport, suggesting that substrate-induction of AIB transport, like enzyme induction, involves the synthesis of new protein. The decrease in AIB uptake is not associated with alterations in cellular cation (Na^+ and K^+) or water concentrations. These findings demonstrate that substrate-induced active transport, known in bacteria and yeast, occurs in the higher eukaryotes as well. (Supported by NIH grants GM 19548 and HD 12512 and an institutional grant from the United Foundation of Greater Detroit.)

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ZINC VERSUS CALCIUM MODULATION OF THE LYMPHOCYTE PLASMA MEMBRANE NA,K-ATPASE. G.B. Segel*, A.H. Lichtman*, W. Simon* and M.A. Lichtman, University of Rochester School of Medicine, Rochester, New York 14642

We explored the effect of divalent cations on the Na,K-ATPase in lymphocyte membrane vesicles. In the presence of background ionized calcium of 4 µM (and total Cu and Zn of 2 µM and 2.5 µM), Na,K-ATPase activity was 25% of maximum. EGTA (5 µM) increased Na,K-ATPase activity, which reached maximal activity at 25 µM (ionized calcium of 1.0 µM). Histidine, in place of EGTA, also activated the Na,K-ATPase, which reached its maximal activity at 0.25 mM histidine. When calcium was added to the ATPase assay fully activated by histidine, no inhibition was observed at an ionized calcium as high as 30 µM. Since histidine binds Cu and Zn avidly but does not bind Ca (confirmed by the Ca electrode), the binding of a cation other than Ca must explain the activation of the Na,K-ATPase by EGTA. Chelation of Cu with triazine and bathocuproine did not enhance or inhibit the Na,K-ATPase activity. The addition of 40 µM Zn to the Na,K-ATPase assay in the presence of 50 µM histidine caused maximal Na,K-ATPase inhibition. The calculated free Zn of 3 µM under these conditions is similar to the total Zn (2.5 µM) measured in the absence of chelator, when ATPase is similarly inhibited. These data show that chelation of very low concentrations of divalent cations enhance the catalytic rate of the lymphocyte Na,K-ATPase, and that Zn may be the principal cation that modulates the plasma membrane ATPase under the conditions of these studies.

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Na^+/K^+ INTERACTIONS IN THE CONTROL OF INHIBITION OF DOG KIDNEY NaK-ATPase BY VANADATE. Patricia M. Hudgins* & Guy H. Bond. Kirksville College of Osteopathic Med., Kirksville, MO 63501

Inhibition of NaK-ATPase by vanadate is strikingly dependent on the relative concentrations of Mg^{2+}, K^+ and Na^+. These cations act by influencing vanadate binding. We have used the apparent dissociation constant for vanadate (Kv) as a measure of vanadate binding. Kv decreased as a function of $[K^+]$, and the decrease was dependent on the fixed $[Na^+]$. With 144 mM Na^+, Kv decreased from 9 µM (at 2 mM K^+) to 0.6 µM (at 10 mM K^+). With 104 mM Na^+, however, the corresponding values were 2.5 and 0.4 µM. This result suggests that Na^+ and K^+ compete for a site at which K^+ promotes, and Na^+ antagonizes vanadate binding. Kv also decreased as a function of $[Mg^{2+}]$, but the percent decrease was largely independent of $[Na^+]$. Therefore, Na^+ does not appear to compete with Mg^{2+}. When $[Na^+]$ increased from 34 to 144 mM, Kv increased sharply with 4mM K^+ (from 0.2 to 3 µM). The increase was much less with 20 mM K^+ (from 0.2 to 0.6 µM). Kv was a sigmoid function of $[Na^+]$, suggesting that more than one Na^+ binds to displace K^+. When NaK-ATPase activity was studied as a function of $[Na^+]$ in the presence of vanadate, two classes of Na^+ sites were seen: high-affinity activation sites and low-affinity sites at which Na^+ reversed inhibition by displacing K^+. There are apparently two classes of K-sites as well: high-affinity activation sites and sites of lower affinity at which K^+ facilitates vanadate binding. (Supported by a grant from the American Heart Assoc., Missouri Affiliate).

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ALTERATIONS IN RED BLOOD CELL (RBC) Na^+-K^+ CONTENTS DURING ATP DEPLETION. M.G. Clemens, I.H. Chaudry and A.E. Baue. Yale University, New Haven, CT 06510

Normal cellular electrolyte balance is maintained by a combination of membrane permeability barriers and metabolically dependent pumps. This study investigates the effect of ATP depletion on Na^+ and K^+ balance in RBC's. RBC's were collected from healthy human volunteers. ATP depletion was accomplished by incubation at 37°C in substrate-free imidazole buffer for up to 18 hrs, and its repletion by the addition of 10 mM glucose and 10 mM adenosine. The cells started to accumulate Na^+ after 8-10 hrs of substrate-free incubation. At this time cellular ATP had fallen to <0.1 µmole/ml packed cells. K^+ depletion was not evident until 10-13 hrs substrate-free incubation. Addition of substrates after ATP depletion resulted in an increase in cellular ATP to >0.3 µmoles/ml within 2 hrs and to about 50% of control at 6 hrs. Lowering of cell Na^+ was evident at 2 hrs of regeneration and complete at 6 hrs. K^+, however, remained lowered even at 6 hrs of regeneration. Inhibition of the N^+-K^+ pump with 20 µM ouabain resulted in both a progressive increase in Na^+ and loss of K^+ that was detectable at 3-5 hrs. The complex relationship between Na^+ and K^+ changes in ATP depletion in contrast to ouabain poisoning suggests that altered RBC electrolyte balance in depressed metabolic states is the result of energy dependent changes in membrane permeability as well as dysfunction of the Na^+ pump. (Supported by USPHS Grant 5 RO1 HL 19673 05 and Army Contract DAMD-17-76-C-6026.)

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EFFECTS OF FREE NH_2-GROUPS OF GASTRIC MICROSOMAL MEMBRANES ON THE FUNCTION OF K^+- STIMULATED ATPase. Tushar K. Ray, Zhou Meng Ai* and Parimal C. Sen*. Dept. of Surgery, SUNY Upstate Medical Center, Syracuse, NY 13210.

The effects of free NH_2-groups associated with various protein and lipid components of gastric microsomes on the function of the K^+-stimulated ATPase were studied using the NH_2-reactive probes, TNBS, FDNB and MDPF. Phosphatidyl ethanolamine (PE) reacts with TNBS and FDNB to yield stable products such as TNB-PE and DNB-PE. The extent of labeling with TNBS was considerably enhanced in presence of K^+ and valinomycin and was 70% (pH 8.0) of the total PE compared to 31% in their absence. The labeling with FDNB was nearly equal to that with TNBS in presence of K^+ and valinomycin. The K^+-stimulated ATPase associated with the gastric microsomes was inhibited (90%) by 2 mM TNBS. This TNBS inhibition was protected (50%) when the microsomes were preincubated with 2 mM ATP together with or prior to TNBS treatment. Besides PE, TNBS also reacted with the free NH_2-groups of microsomal proteins. Formation of this protein-TNB, but not the TNB-PE was greatly reduced in microsomes pretreated with ATP suggesting direct interaction of TNBS with some critical NH_2-group of the enzyme as the mechanism of ATPase inhibition. Similar mechanisms of protection of the K^+-ATPase by ATP from MDPF inhibition was observed. Intravesicular accumulation of H^+ by both MDPF and TNBS-treated and untreated vesicles revealed effects which correlate well with the effects of the agent on K^+-ATPase. (Supported by USPHS, AM 25544 and AM 00623)

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The Interaction of a cAMP-dependent Protein Kinase with Sodium-Potassium Adenosine Triphosphatase (Na-K ATPase) in Rat Synaptosomal Membranes. R.B. Lingham* and A.K. Sen, Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Rat brain synaptosomal membranes were prepared and treated with either NaCl or cAMP plus NaCl as described by Corbin et al. (J. Biol. Chem. 1977, 252: 3854-3861). The Na-K ATPase activity in the cAMP plus NaCl-treated membranes was significantly higher ($p<0.001$) than the same activity present in the NaCl-treated membranes. The increase in Na-K ATPase activity was accompanied by a decrease in membrane-bound cAMP-dependent protein kinase activity which was significantly lower ($p<0.001$) than the activity present in the control membranes. The soluble extract from the membranes treated with cAMP plus NaCl displayed protein kinase activity which was cAMP-independent. A cAMP-dependent protein kinase inhibitor increased the Na-K ATPase activity of the NaCl-treated membranes but not the activity of the cAMP plus NaCl-treated membranes. The results indicate that washing the membranes with cAMP plus NaCl results in the dissociation and removal of the catalytic subunit of a cAMP-dependent protein kinase from the membrane. These findings support the tenet that the Na-K ATPase enzyme system present in rat brain synaptosomal membranes may be modulated by a membrane-bound cAMP-dependent protein kinase (supported by Medical Research Council of Canada MT-2485).

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REGULATION OF CALCIUM-DEPENDENT POTASSIUM EFFLUX IN HUMAN RED BLOOD CELLS. Gordon A. Plishker. Dept. of Neurology, Baylor College of Medicine, Houston, Texas 77030

Reductions in Ca-dependent K efflux correlate with an association of a soluble protein with the plasma membrane. The exposure of intact cells to 1 mM Cd during a 30 min. pretreatment with iodoacetic acid [IAA] produces a 50% reduction in Ca-dependent K efflux during a subsequent incubation in a Cd-free solution containing IAA and $CaCl_2$. Scans of the SDS polyacrylamide gels from a membrane fraction of cells pretreated with Cd revealed more than a two-fold increase in levels of a 25,000 dalton protein. A comparison of the membrane and soluble fractions revealed the protein band in both fractions but predominantly in the soluble fraction. White ghosts did not contain this protein band. Differences in K efflux were also shown to correlate with a difference in the degree of [^{3}H]-IAA labeling of this 25,000 dalton protein. The greatest K losses were observed in cells pretreated with IAA, washed and then incubated in media that did not contain IAA. The addition of IAA reduced the K loss by 50%. The [^{3}H]-IAA labeling of the 25,000 dalton protein during the pretreatment was 20% of the value found when [^{3}H]-IAA was present during both the pre-incubation and incubation phases. Results suggest that the Ca sensitivity of the K efflux process can be reduced by exposure to Cd and/or IAA. Correlated with this inhibition of K efflux is an alteration of a soluble protein that interacts with the plasma membrane. (Supported by the MDA and NIH Grant #GO-8705).

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EVIDENCE FOR CHLORIDE ACTIVATED PASSIVE K+ MOVEMENTS IN OYSTER TOAD FISH ERYTHROCYTES DURING VOLUME REGULATORY SHRINKAGE. P.K. Lauf, and B.E. Theg*, Dept. Physiology, Duke Univ. Med.School, Durham,N.C. 27710,& Duke Univ.Marine Lab. Beaufort,N.C. 28516.

Recently we proposed that a Cl^- dependent K^+ transport system, activated in low K^+ sheep red cells by N-ethylmaleimide (Biochim.Biophys.Res.Commun. 92:1422,1980)and perhaps responsible for the development of the low K^+ steady state cells,may be of ancient origin. Testing this hypothesis we studied the anion dependency of the volume regulatory shrinkage (VRS) in red cells of oyster toad fish (OPSANUS TAU). Cells were pre-equilibrated in either Tris-buffered (pH 8) NaCl, $NaNO_3$ or choline-Cl media, iso-osmotic with toad fish plasma (310 mOsm/L) and containing 10^{-4}M ouabain. Upon exposure to the same media, however, diluted to 210 mOsm/L (hypotonic), cellular water contents rose instantaneously from about 2.9 to 4 Kg H_2O/Kg dry cell solids indicating an almost 40% volume expansion due to water influx. About 1 hr later the water content of cells kept in hypotonic NaCl or choline-Cl media had returned to approximately original values. However, cells kept in hypotonic $NaNO_3$ did not volume regulate. Ion analyses of cells and supernatants revealed that in Cl^- media a large part of the ouabain insensitive K^+ flux was accompanied by net Cl^- loss. This was in spite of hyperpolarization of the membrane (calc. from the Cl^-_i/Cl^-_o ratio),which opposes outward movement of K^+ ions. Since K^+ movement and hence VRS were suppressed in NO_3^- media, VRS in toad fish red cells apparently occurs via KCl^- extrusion mediated by Cl^-activated K^+transport.(Marine Biomed.Center ESO 1908 supp.)

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PURIFICATION AND DETECTION OF A UNIQUE MITOCHONDRIAL CREATINE KINASE. Robert Roberts. Washington University, St. Louis, Missouri 63110

The role of mitochondrial creatine kinase (CK_m) in energy storage and transport, and whether CK_m is different from cytosolic CK isoenzymes (MM,MB,BB) remain controversial since CK_m has not been purified and with 2 subunits (M and B), only 3 isoenzymes should exist. We previously isolated CK_m but have now purified CK_m and characterized its unique properties. CK_m was liberated from dog heart mitochondria with 83 mM, Na_2PO_4, pH 7.4 and fractionated on Sephacryl S-200 with further purification on CM Sephadex C-50 (Barbital, pH 7.8). The product exhibited a single cathodic electrophoretic band without MM, MB or BB CK having a specific activity of 320 IU/mg. A single protein band was observed on SDS gels with a mol wt of 42,000. Amino acid analysis showed it to be composed of a subunit which is distinctly different from that of the M or B polypeptide. MM and BB CK hybridized to produce MB after freezing and thawing but CK did not hybridize with MM or BB CK. CK_m has a half-life of 20 min compared to 145 min for MM CK. Antibody to CK_m produced a single precipitant line to CK_m on agar but no reaction to BB, MB or MM and exhibited no binding to ^{125}I-BB or -MM CK. Unlabelled CK_m but not MM or BB displaced ^{125}I-CK_m binding to CK_m antiserum. Thus, CK_m is an immunologically and biochemically distinct isoenzyme. Use of a radioimmunoassay should help to elucidate the metabolic role of mitochondrial creatine kinase.

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REGULATION OF CARDIAC MYOFIBRIL ADENOSINE TRIPHOSPHATASE DURING DEVELOPMENT. A.N. Belcastro*, M. Sopper*, A. Bonen*, M. Low*, (SPON: L. Bailey) School of Physical Education and Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

Myofibril ATPase activity of cardiac muscle decreased during development. The ATPase activity at 10 days (0.519 ± 0.018 umoles Pi/mg/min) was greater than 21 and 51 day activities (0.274 ± 0.016 and 0.299 ± 0.029 umoles Pi/mg/min) ($P< 0.05$). Following pre-incubation in a cAMP-protein kinase medium, the growth pattern for cardiac myofibril ATPase was not altered. At 10 days, the cAMP-dependent activity was 13% greater compared to the control ATPase value ($p< 0.05$). No effect was observed for 21 and 51 day ATPase activities ($p> 0.05$). Increasing the ionic strength of the incubation mediums resulted in a decrease in ATPase activities for all age groups ($p <0.05$). The cAMP-dependent ATPase activities at 10 and 21 days were greater than the control values at similar ionic strengths ($p< 0.05$). The cardiac myofibril ATPase activities in the presence of EGTA were depressed during development with relative activities of 42%, 30% and 23% observed for 10, 21 and 51 day rats ($p< 0.05$). The cAMP-dependent, EGTA sensitive ATPase activities were higher at 10 and 21 days ($p< 0.05$). The results suggest that a cAMP-dependent regulation of cardiac myofibril ATPase activity may be occurring during development. (Supported by NSERC grants A-6629 and A-6449).

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HEAT AND TENSION IN RESTED AND PACED TWITCHES OF RAT PAPILLARY MUSCLE. Norman R. Alpert, Robert P. Goulette*, and Louis A. Mulieri. Dept. of Physiol. & Biophys., Univ. of Vermont, Burlington, VT 05405.

We compared rested (5 min interval), single twitches with paced (5 sec interval), single twitches of left ventricular papillary muscles (2.2±0.5mg weight, 0.45±0.09mm area, mean± SE) from 191±29g rats. We used deposited film, bismuth-antimony thermopiles (Mulieri et al., Am. J. Physiol. 233, C146-C156, 1977) with high thermal resolution and long-term stability to allow initial and recovery heat measurements in single twitches without averaging. The rested isometric twitch is mechanically indistinguishable (within 2-5%) from the paced twitch (Krebs-Ringer solution, 21°C) and has a peak tension of 6.7±0.6g/mm^2, a maximum rate of tension development of 39±5g/mm^2·sec, a time to peak tension of 0.29±0.01 sec, and a twitch vs. resting tension ratio of 5±0.04. Initial heat is 64% larger and is evolved at a 22% greater rate in rested twitches than in paced twitches. Recovery heat appears as a secondary rise in heat output starting 4.4±.24 sec after stimulation. The ratio of recovery heat to initial heat is 1.3±0.1 in a rested twitch and 0.9±0.1 (Bugnard's method) in a paced twitch. Increasing Ca^{++} from 2.5 to 11mM in the presence of 5mM caffeine causes a 75-100% increase in both initial and recovery heat while the twitch tension is reduced by 20-40%. These results show that heat evolution is not tightly coupled to isometric tension and the paced twitch is myothermically more economical than the rested twitch. Supported by PHS #R01 17592.

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SOME ELECTROPHYSIOLOGICAL CHARACTERISTICS OF SPHEROIDAL RE-AGGREGATES OF EMBRYONIC CHICK HEART. J. V. Milligan, N. G. Giddins* and H. J. Mitchell*. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Dispersed cells from embryonic chick heart will re-aggregate when cultured in "non-defined" Medium 818A to form solid spheroids, 50 to 500 μm in diameter. These spheroids exhibit normal cardiac electrical activity and are theoretically ideal for voltage-clamp studies but they have not been used extensively because of problems with the medium. We have used a commercial medium (Waymouth MD705, GIBCO) supplemented with 10% Fetal Calf Serum (GIBCO), 4% Chick Embryo extract and 1 μg/ml Insulin (SIGMA). We compared the size, number and electrophysiological characteristics of aggregates produced in this medium to those produced in Medium 818A (kindly supplied by Dr. R. L. DeHaan) and found only minor differences. Double penetrations, with two micropipettes, showed that the surface of 10% of the aggregates was isopotential, a property required for voltage clamp studies. Spontaneous potentials recorded whilst the bathing media was reduced to 20% [Na^+] showed a decrease in dV/dt indicating that Na^+ channels were present. No movement of the spheroidal aggregates was seen although any rod-like aggregates showed vigorous movement. Since it is now possible to produce these aggregates easily with our "almost-defined" culture medium, experiments to relate ionic currents to mechanical activity can be attempted. (Supported by Queen's University and in part by MRC.)

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MECHANICAL AND ELECTRICAL PROPERTIES OF DEVELOPING SWINE MYOCARDIUM. Z.J.Penefsky, N.M.Buckley, C.Barry* and A.L.Sorenson.* Mt.Sinai Sch.of Med., and Albert Einstein Coll.of Med., New York, N.Y. 10029 and 10461.

Responses of myocardium from 12 piglets aged 18 hours to 9 days were studied in moderator bands from hearts excised after animals were stunned and exsanguinated. Muscles were equilibrated for 60-90 minutes after being mounted in the myograph. Isometric tension and transmembrane potentials were recorded by conventional methods. The first derivative of the tension curve was used to evaluate mechanical responses by the model active state (Penefsky, Pp.239-251 in: Research in Physiology, eds. Kao, et al, Aulo Gaggi Publ.,1971). Stretching a muscle between initial and optimal length increased contractile tension and the time-course of contraction and relaxation at all ages. Stretch relaxation and post-extrasystolic potentiation were present in all muscles. The force-frequency relationship was negative at birth but positive by the first postnatal week. Increasing $[Ca^{++}]_o$ from 2.7 to 5.4 mM increased contractile force and velocity of contraction and relaxation at all ages, but shortened action potential duration only after 2 days of age. At elevated $[Ca^{++}]_o$, the force-frequency relationship was positive at all ages. These results suggest that the membrane processes responsible for generating ionic currents and maintaining contractile force continue to mature postnatally in swine. (Supported in part by USPHS. NIH Grant HL-15444).

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MATURATIONAL CHANGES IN CANINE VENTRICULAR MUSCLE. I Park*, D Driscoll*, L Michael. Lillie Frank Abercrombie Section of Cardiology, Dept. of Pediatrics and Section of Cardiovascular Science, Dept. of Medicine, Baylor College of Medicine, Houston, Texas 77030

We evaluated postnatal changes in isolated canine ventricular muscle by measuring: 1) active and passive length-tension curves 2) maximum active tension (max-AT), time to peak tension (TPT), and relaxation time (RXT) of isometric contraction and 3) the effect of paired stimulation (PS), isoproterenol (Iso) and Calcium (Ca). Muscles were obtained from 4 groups (G) with ages of: (1) 3 days (N=11); (2) 15 days (N=6); (3) 37 days (N=6); (4) adult (N=6). The muscle strips were suspended in a standard isometric myograph at 29°C and 15 stimuli/minute; the response to PS, Iso & Ca was measured at Lmax. AT of G 4 was greater than G 1; AT of G 2 and 3 were similar and intermediate between G 1 and G 4. PT of G 4 was less than G 1, 2 and 3. TPT was identical in G 1, 2 & 3 and was shorter than G 4. In contrast, RXT was shortest in G 1 and was progressively longer in older age groups. Adult muscles developed greatest max AT in response to PS. However, muscles from G 1 and 2 developed greatest max AT in response to Ca. In G 3, max AT-PS was equal to max AT-Ca. In summary, G 1, 2 and 3 had greater PT and less AT than G4; max force development was achieved by PS in G 4, by PS or Ca in G 2 & 3, and by Ca in G 1. This study further demonstrates maturational changes in myocardial contractile properties and their response to PS, Iso and Ca. (Supported by HL 22309, 23161, 17269)

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REGRESSION OF CARDIAC HYPERPLASIA IN YOUNG RATS. Karel Rakusan and Borivoj Korecky, Dept. of Physiology, University of Ottawa, Ottawa, Ontario.

Cardiac hyperplasia was produced by constricting the abdominal aorta in 12-days old male rats. The left ventricular weight increased by 73% at 22 days and by 136% at 35 days of age when compared to their control littermates. The formation of new myocytes (hyperplasia) could account for the major part of the above increase while smaller portion was due to an increased size of the existing cells (hypertrophy). The average size and the total number of myocytes were obtained by two independent approaches: a) from the direct measurements of the cell dimension in three major layers of the left ventricular wall by quantitative morphology; b) from the chemical determination of total DNA and subsequent calculation of muscle cell DNA. The ratio of muscle to nonmuscle nuclei was determined separately and the DNA per nucleus and the number of nuclei per cell were assumed to be constant. When the aortic bands were removed at 22 and 35 days of age and the animals were sacrificed 2, 3 or 6 weeks later, no significant differences were found in the left ventricular weight, the size of an average myocyte and the total number of myocytes per heart as compared to control littermates. The results may be interpreted either as a reduction in the number of myocytes during regression of cardiac hyperplasia or as an increase in the number of cardiac myocytes in normal hearts even after 22 days of age.

(Supported by Ontario Heart Foundation and MRC of Canada).

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A NEW METHOD OF MEASURING THE ISOMETRIC TENSION OF CULTURED HEART MUSCLE STRANDS. Michael O. Toll* and Arunachalem Chockalingam*[1] (SPON: E.H. Wood). Faculties of Engineering and Medicine[1], Memorial University of Newfoundland, St. John's, NFLD, Canada A1B 3X5

Cardiac cell suspensions (1.5×10^5 cells ml^{-1}) were obtained from the hearts of 2-3 day old Sprague-Dawley rats using mechanical and trypsin disaggregation techniques. Before culturing, agar coated glass cover slips (4x4 cm) had thin strips of palladium metal (100 μm wide, 2 cm long) vacuum deposited over the agar. Glass microspheres (100 μm dia.) were fixed to the palladium at 3 mm intervals using silicone grease. This grease was also used to seal the prepared cover slips to the bottom of stainless steel rings (3 cm o.d.) to form the culture chambers. About 3 ml of the cell suspension fluid filled the chambers and additional cover slips sealed the tops prior to incubation at 37°C for 6-8 days. By then, the cells had differentially grown along the palladium lines and had attached themselves to the glass microspheres to form strands. Glass suction micropipettes were used to secure the microspheres for strand suspension. One micropipette was used as the fixed support while the other was fixed to a hollow stainless steel cantilever beam of a variable capacitance force transducer. This transducer had the following characteristics: resonant frequency 120 Hz, sensitivity 0.021 mV/μg, compliance 7×10^{-4} μm/μg. With this system noise-free isometric tension records have been made directly from cultured cardiac strands. (Canadian Heart Foundation Supported)

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LENGTH-TENSION AND FORCE-FREQUENCY PROPERTIES OF CULTURED HEART MUSCLE STRANDS. Arunachalem Chockalingam*, Michael O. Toll*[1] and Theordore B. Hoekman* (SPON: E.H. Wood). Faculties of Medicine and Engineering[1], Memorial University of Newfoundland, St. John's, NFLD, Canada A1B 3V6

The length-tension (LT) and force-frequency (FF) properties of cultured rat heart strands were studied over a range of temperatures. The strands beat spontaneously and when connected to the force transducer this rate increased exponentially from 69 to 90 min^{-1} when the temperature was raised from 15 to 37°C. During both spontaneous and electrically stimulated contractions, strand LT curves were found to be qualitatively similar to that of isolated heart muscle. However, the length range for active tension (AT) development was ±1% of the optimum length for peak AT, suggesting that substantial amounts of non-contractile tissue are connected in series with active cardiac cells. When electrically stimulated the FF curves showed positive inotropic response up to about 90 min^{-1}; at higher frequencies contractility decreased in a manner similar to that seen in isolated rat cardiac muscle. The frequency where the FF curve peaked (90-105 min^{-1}) was directly related to temperature. For a given contraction rate (80-240 min^{-1}) the greatest isometric tension occurred at 20-25°C. Since the physiological properties were reproducible and qualitatively similar to those of intact cardiac muscle, cultured strands appear to provide a useful cellular model for the study of myocardial contractility. (Canadian Heart Foundation supported)

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MYOCARDIAL O_2 SUPPLY. Eugene A. Lentini. Department of Physiology/Pharmacology, Philadelphia College of Osteopathic Medicine, Philadelphia, Pennsylvania 19131.

Previous experiments have led to the determination of the myocardial diffusion coefficient of O_2 ($D'O_2$). The data was calculated from the rat's trabeculae carneae stimulated at 1/sec. and recording the isometric developed tension utilizing standard electronic techniques. The value calculated from the experimental data for the $D'O_2$ was $1.41 \times 10^{-5} \pm 0.21 \times 10^{-5}$ cm^2/min atm which did not significantly differ from that of skeletal muscle. The basic model was further developed to allow the determination of an extracellular activation oxygen pressure (PiO_2). The diffusion distance calculated from Krogh's formula was not in agreement with the model system data. Additional calculations revealed that approximately 2.5 fold increase in oxygen supply is required when myocardial hypertrophy is present. This assumes that the capillary to fiber ratio remains constant. The data indicate that the cellular membrane and associated constituents offer a significant hindrance to the diffusion of oxygen into the cell.

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A SOMATIC COMPONENT OF EXPERIMENTAL MYOCARDIAL INFARCTION. D.A. DeBias, C.H. Greene*, D. Heilig*, A. Nicholas*. Philadelphia College of Osteopathic Medicine, Philadelphia, Pennsylvania. 19131.

Many treatment modalities employed by osteopathic physicians are predicated upon clinical observations that palpatable, paraspinal, soft tissue, changes are associated with visceral disease. These changes occur at the same level of segmental innervation as the involved viscera. If conduits of reciprocal communication do exist between the musculoskeletal system and other systems of the body, it should be feasible to delineate the constituents of the somatic component to the specific cardiomyopathy of chronic MI in an animal model. It is therefore our objective to demonstrate and quantitate, via EM and standard morphometric analyses, a somatic component to experimental MI. Significant changes in skeletal muscle cytoarchitecture have been found in the infarcted animals: (1) sarcomere length is significantly reduced (MI=4.04 μm vs C=4.85 μm, $p < .01$), (2) number of sarcomeres per test area significantly increased (MI=2.34×10^{-10} μm^{-2} vs C=1.94×10^{-10} μm^{-2}, $p < 0.01$), and (3) the area occupied by sarcomeres per test area is significantly decreased (MI=0.11 $\mu m^2/\mu m^2$ vs C=0.13 $\mu m^2/\mu m^2$, $p < 0.02$). Other analyses in progress include mitochondrial, ribosomal, and myofilament densities; other involved tissues include skin, fat, and fascia.

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PULMONARY VENTILATION AND THE DETERMINATION OF LACTATE THRESHOLD DURING INCREMENTAL-LOAD WORK. R. Donald Hagan and Larry R. Gettman.* Inst. for Aerobics Res., Dallas, Tx 75230

It was the purpose of the present investigation to illustrate the use of exponential equations to describe the relationships between exercise pulmonary ventilation ($\dot{V}_E$) and oxygen uptake ($\dot{V}O_2$) and $\dot{V}_E$ and carbon dioxide ($\dot{V}CO_2$), to describe the use of differential calculus to calculate the lactate threshold and to examine the relationship between the lactate threshold and maximal aerobic power ($\dot{V}O_2$ max). During incremental-load work, $\dot{V}_E$ increased exponentially in relation to elevations in $\dot{V}O_2$ and $\dot{V}CO_2$ for each subject. The R^2 values for the exponential equations relating $\dot{V}_E$ to $\dot{V}O_2$ and $\dot{V}_E$ to $\dot{V}CO_2$ were R^2 = 0.993 for the majority of the subjects and R^2 = 0.90 for the group (n=45). Since exponential equations do not possess a point of inflection, the onset of lactic acidosis induced by incremental-load work cannot be identified by changes in exercise pulmonary ventilation. However, differentiation of the $\dot{V}_E$ to $\dot{V}O_2$ exponential equation gives the minimum slope of the equation and corresponds to the lowest ventilatory equivalent for oxygen, and hence the $\dot{V}O_2$ at the lactate threshold ($\dot{V}O_2$-LT). In our subjects, $\dot{V}O_2$ max ($\bar{X} \pm S.D._1$) was 3.85 + 0.69 L·min^{-1}, and $\dot{V}O_2$-LT was 1.75 ± 0.32 L·min^{-1}. The $\dot{V}O_2$-LT was 45.7 ± 0.06% $\dot{V}O_2$ max (range 36 to 63% $\dot{V}O_2$ max). The correlation coefficient between $\dot{V}O_2$-LT and $\dot{V}O_2$ max was 0.80 while the value between $\dot{V}O_2$-LT and % $\dot{V}O_2$ max was only 0.35.

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ALTERATIONS IN ANAEROBIC THRESHOLD CONSEQUENT TO SMOKE EXPOSURE IN FIREFIGHTERS. S.N. Koyal, R.C. Jung*, J.G. Mohler and C.R. Collier. LAC-USC Medical Center, Los Angeles, CA 90033

The chronic effects of acute inhalation of smoke from fires results in loss of lung function, but its effect on the anaerobic threshold (AT) is not known. It was the purpose of this investigation to determine the effects of chronic smoke exposure on AT in firefighters. Nine young men in physical training of the Los Angeles City Fire Department with no history of cardiopulmonary disease were tested. Each completed a series of pulmonary function tests followed by an incremental treadmill exercise to exhaustion to determine $\dot{V}O_2$Max. Lactate (LA) and other cardiorespiratory variables were measured at rest and at each work rate after the 1st and 2nd year of firefighting. The $\dot{V}O_2$ at AT was calculated by interpolation assuming that LA is an exponential function of $\dot{V}O_2$ and that AT is reached when LA = 18 mg% (Fed.Proc. 37:3, 1978). The mean values of $\dot{V}O_2$ and % of $\dot{V}O_2$Max (F_{Max}) at AT after 1st and 2nd years were as follows: $\dot{V}O_2$=2.00 L/min ± 0.37 and 1.58+0.38, F_{Max}=52%+12 and 42%+12. The mean changes of $\dot{V}O_2$ and F_{Max} were -0.42 L/min +0.20 (P<.0002) and -9.9%+8.3 (P<.007). From these observations, we conclude the reduction in AT may be due to smoke exposure and alterations in $\dot{V}O_2$Max and AT may be caused by changes in systems other than the lung.

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BLOOD GAS RESPONSE TO EXERCISE IN THE DUCK DURING ISOTHERMIA. J. P. Kiley*, W. D. Kuhlmann*, and M. R. Fedde. Dept. of Anatomy and Physiology, Kansas State Univ., Manhattan, KS 66506

Ducks running at ambient temperatures (25°C) hyperventilate (arterial PCO_2 decreases about 5 torr) but body temperature increases as much as 2°C. To determine the importance of hyperthermia on exercise hyperpnea, ten adult White Pekin ducks were exercised on a treadmill (3° incline) at an ambient temperature of -5°C for 20 minutes at a speed of 1.47 km/hr. The brachial artery, right ventricle, and left ulnar vein were cannulated under local anesthesia. A microthermister was advanced to within 2 cm of the heart, for the measurement of blood temperature. At an ambient temperature of -5°C ducks maintained body temperature constant throughout exercise. Heart rate, systolic and diastolic blood pressure significantly increased during exercise. Arterial PCO_2 decreased approximately 3 torr during running while mixed venous PCO_2 increased initially before returning to control values. Changes in arterial pH were small, although a significant decrease was observed during exercise. Mixed venous pH markedly decreased at the onset of exercise, reaching a steady value for the remainder of exercise. Mixed venous PO_2, arterial and mixed venous plasma [HCO_3^-] all decreased during running, while lactic acid levels increased approximately 75% over control values. We conclude that ducks hyperventilate during exercise even though body temperature remains constant. Hyperthermia, therefore does not appear to play a major role in eliciting the hyperpnea of exercise in the duck.

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EXERCISE TRAINING EFFECTS ON SKELETAL MUSCLE ENZYMES AND HEART RATE IN PATIENTS WITH CHRONIC LUNG DISEASE. B.A. Kendregan* and M.J. Belman* (SPON: C. Mittman). City of Hope National Medical Center, Duarte CA 91010.

Impaired ventilatory muscle mechanics limits exercise capacity in symptomatic patients with chronic obstruction pulmonary disease (COPD). Exercise training can therefore be done only at relatively low steady-state levels. In order to evaluate if objective training changes develop, we performed closed skeletal muscle biopsies from the arms and legs of COPD patients before and after a 6 week training program. Of the 15 subjects, 8 trained their arms and 7 trained their legs. The mean FEV_1 and MVV for the arm trained group was 1.12 ± 0.36 liters and 58 ± 23 l/min; for the leg group the mean FEV_1 was 1.00 ± 1.4 liters and the MVV 47 ± 5 l/min. While in general there was improved submaximal endurance for the trained limbs as tested by a steady-state submaximal cycle endurance test, skeletal muscle enzymes, including citrate synthase, 3 hydroxyacyl CoA dehydrogenase and pyruvate kinase did not change significantly in the exercised limbs. In addition, comparison of the heart rates at comparable submaximal oxygen consumptions did not show a significant change after training for both the exercised and non-exercised limbs. The results suggest that 1) subjects with this degree of air flow obstruction are incapable of exercising at an intensity high enough to generate characteristic training responses; 2) other mechanisms must be sought to explain the increased exercise endurance in these subjects.

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VENTILATORY MUSCLE ENDURANCE IN ATHLETES AND NON-ATHLETES. Bruce J. Martin and Joel M. Stager*. Physiology Section, Med. Sci. Program, Indiana Univ., Bloomington, IN 47405

Do the ventilatory muscles of normal persons become fatigued during standard forms of strenuous endurance exercise? If so, then one of the effects of the intense training performed by endurance athletes should be increased ventilatory muscle endurance. To investigate this possibility, 8 endurance athletes and 8 non-athletes were compared in studies of both short- and long-term maximal ventilation. The two groups were matched for age, body size, and vital capacity. While athletes and non-athletes had similar short-term maximal ventilation (12-sec MVV), the athletes displayed significantly greater ventilatory endurance on two long-term breathing tests. In the first, 80% of the MVV was sustained until exhaustion; endurance times averaged 11 minutes in the athletes and 3 min in the non-athletes (P <0.01). In the second, ventilation was voluntarily incremented 30 l/min every 4 minutes. Before exhaustion, athletes reached a ventilation that was a greater fraction of their MVV (75% vs. 67%, P <0.01) than did non-athletes. Although the energy cost ($\dot{V}O_2$) of submaximal levels of ventilation was identical in the two groups, athletes reached a significantly higher peak $\dot{V}O_2$ during this incremental test. These characteristics of endurance athletes resemble those of persons who have undergone specific endurance training of the ventilatory muscles, suggesting that ventilatory muscle fatigue may occur in normal persons during strenuous endurance exercise.

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INFLUENCE OF TRAINING ON THE MAXIMUM OXYGEN CONSUMPTION OF HYPOPHYSECTOMIZED RATS. J.G. Edwards*, K.A. Rowlett*, and C.M. Tipton. Ex. Sci. Prog., U. of IA., Iowa City, IA. 52242

The importance of the pituitary hormones for an increase in $\dot{V}O_{2max}$ with training is unclear. With the aid of an enclosed rat treadmill (JAP 47:1278, 1979), this problem was investigated with male Sprague-Dawley rats that were assigned to a hypophysectomized (HYPX) or a sham HYPX group at 50-60 days of age. $\dot{V}O_{2max}$ was measured 10-19 days after surgery and it was 10% lower than the sham HYPX values. After a 40 day transition period, a 16-17 week exercise program was initiated and results were as follows: $\bar{X}$, SE, ml·min^{-1}·kg^{-1} body weight or fat free weight; *significance at 0.05 level between NT and T HYPX groups.

GROUP	N	START $\dot{V}O_{2max}$ (BW)	END $\dot{V}O_{2max}$ (BW)	END $\dot{V}O_{2max}$(FFW)	END MAX HR
SHAM HYPX	11	55.7 ± 3	54.1 ± 1		545 ± 11
HYPX NT	16	35.5 ± 3	32.3 ± 1	36.5 ± 2	330 ± 5
HYPX T	14	34.5 ± 1	35.5 ± 1*	40.6 ± 1*	330 ± 6

Training was also associated with longer run times, higher soleus muscle cytochrome oxidase activity, higher plantaris and red vastus muscle glycogen levels, and a reduction in food consumption. These composite findings suggested that chronic exercise can cause metabolic adaptations without the presence of pituitary hormones and that tissue changes could be responsible for an increase in $\dot{V}O_{2max}$. (Supported in part by HL-21245-03, and GM-7045-02).

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TRAINING INFLUENCES ON MYOCARDIAL AND CARDIOVASCULAR PARAMETERS OF HYPOPHYSECTOMIZED RATS. T. G. Bedford*, D. D. Lund, J. G. Edwards*, R. D. Matthes*, and C. M. Tipton. Ex. Sci. Prog. and Cardiovasc. Center, U. of Iowa, Iowa City, IA 52242.

To evaluate the combined effects of chronic exercise and pituitary hormones on cardiovascular performance, male hypophysectomized (HYPX) rats were exercised for 16-17 weeks. Changes in $\dot{V}O_{2max}$, run time, cytochrome oxidase activity, and anesthetized resting heart rates before LBNP indicated they were trained (T). Hearts from 8 T and 8 nontrained (NT) animals were measured for choline acetyltransferase (CAT), a marker of parasympathetic innervation, and for binding of (^{3}H) quinuclidinyl benzilate (QNB) to denote the density of muscarinic receptors. Right atrium (RA) results ($\bar{X}$, SE, *significance at 0.05 level) for CAT were: NT=6.9±0.7, T=9.6±0.7* nmol product, hr^{-1}, mg $protein^{-1}$, and for QNB were: NT=449±12, T=322±46* fmol QNB bound, mg $protein^{-1}$, respectively. Data from other heart regions were not significantly different; however, unlike normal T animals, conditions of lower body negative pressure had no significant influence on the cardiovascular reflexes of the T HYPX group. These data indicate that training causes adaptations in the absence of pituitary hormones and suggests that increased parasympathetic activity to the RA may be associated with a decrease in receptor density in this region.

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INCREASED RESPONSE OF SMALL ARTERIOLES TO β_2-ADRENERGIC STIMULATION FOLLOWING EXERCISE TRAINING. D.L. Wiegman, P.D. Harris, F.N. Miller and I.G. Joshua*, Microcirculatory Systems Research Group, Dalton Research Center, University of Missouri, Columbia, MO 65211

Sprague-Dawley rats (281±5g;$\bar{X}$±SE) were exercised 7 days/week for 6 weeks by swimming 1 hr/day with weights (5% of body weight) attached to their tails while control rats (277±6g) remained sedentary. Animals were anesthetized with urethane (800 mg/kg) and α-chloralose (60 mg/kg). The cremaster muscle with intact circulation and innervation was suspended in a tissue bath. The responses (changes in diameter) of third order arterioles to increasing concentrations of topically applied terbutaline (a β_2-adrenergic receptor agonist) were measured via television microscopy. A concentration-dependent dilation was obtained. Sensitivity to terbutaline is expressed as a pD_2 value ($-\log ED_{50}$). (*=P<.05)

Group	N	Predrug Diameter(μm)	Maximal Diameter(μm)	Sensitivity (pD_2)
Sedentary	7	15±2	25±2	5.9±.2
Exercised	11	15±1	34±2 *	6.5±.1 *

Exercise training increased arteriolar sensitivity and the maximal dilation to β_2-adrenergic stimulation. Nitroprusside gave no further dilation in either sedentary or exercised rats. Thus, the increased maximal diameters suggest a structural change in the arterioles of exercise trained rats. (Funded by USPHS Grant HL 21901.)

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CARDIAC RESPONSE TO EXERCISE DURING BETA-ADRENERGIC BLOCKADE. D.R. Knight, Gerald Todd, H.L. Stone. Department of Physiology University of Oklahoma, H.S.C., Oklahoma City, Oklahoma 73190

Selective adrenergic blockade was investigated to determine the role of heart rate (HR) and left ventricular contractility (dP/dt) on the change in coronary blood flow velocity (CBF) and myocardial oxygen consumption (MVO_2) after exercise. Dogs were instrumented to measure left ventricular pressure or aortic pressure by a solid state transducer and left circumflex flow velocity by a Doppler flow transducer. Coronary venous blood samples were obtained by a catheter placed in the coronary sinus and HR was measured by telemetry. The dogs were exercised on a treadmill at submaximal workloads before and after the administration of Atenolol (1mg/kg I.V.). Measurements were taken before beginning exercise and at various workloads. At the highest workload of 6.4 kpm and 16% grade, the HR increased by 132±9 bpm and dP/dt increased by 3339±561 mmHg/sec. The CBF increased by 28±4 cm/sec and the MVO_2 increased by 4.94±.16 mlO_2/cm/sec. After selective beta-blockade and at the same workload the HR increased by 84±11 bpm and dP/dt increased by 1600±400 mmHg/sec when compared to control. MVO_2 was increased by 3.47±.69 mlO_2/cm/sec. CBF increased by 25.5±4 cm/sec. Although HR, dP/dt and MVO_2 were less during exercise with selective beta-blockade the reduction in CBF was not as great as expected. This may indicate an incomplete blockade or a direct activation of other beta-adrenergic receptors by circulating catecholamines. (Supported by Grant #22154)

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CHANGES IN ARTERIAL WALL PERMEABILITY TO ^{125}I-ALBUMIN IN THE TREADMILL EXERCISED COCKEREL. M.D. Ezekowitz*, J.L. Kelley*, S.W. Herren*, D.E. Parker*, M.A. Morgan*, H.L. Stone, University of Oklahoma Health Sciences Center,Oklahoma City,OK 73190

The arterial endothelial is believed to provide the major barrier to the ingress of macromolecules into the wall. Permeability of this barrier was measured in 5 pairs (1 exercise; 1 control) of age matched, normotensive adult cockerels. Each pair received the same amount of isotope, from the same batch prepared the day of the experiment, and containing < 1.5% free iodide. The birds were exercised at 2 mph at 0° elevation for 2 minutes after receiving a bolus injection of the isotope (50 μCi). Non-exercised controls were treated in other respects similar to exercised group. After obtaining blood samples, animals were sacrificed with an overdose of Nembutol. The aorta was dissected and divided into 8 segments from the aortic valve to the trifurcation. The permeability of each segment was calculated as follows:

$$\frac{\text{Activity in the Wall}}{\text{surface area} \times \text{mean blood activity} \times \text{experimental time}}$$

The % free ^{125}I in the plasma was 1.07 ± 0.02 and the wall was 6.55 ± 3.43. Mean permeability was greater in the exercise group in 4 out of 5 of matched pairs. The average difference of these 4 pairs was 7.8636×10^{-9} $ml/cm^2/sec$. The single reversal was 1.7298×10^{-9} $ml/cm^2/sec$. We conclude that (1) Arterial wall permeability probably increases with treadmill exercise; (2) The distribution of permeability along the length of the aorta is not altered with exercise.

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EFFECTS OF REPETITIVE EXERCISE ON THE RESPONSE TO PHENYLEPHRINE IN AORTA-CONSTRICTED RATS. E.M. HASSER* and R. T. DOWELL. UNIV. OF OKLA. HSC, OKLA. CITY, OK 73190

This study examined the effects of repetitive exercise on the response to phenylephrine in aorta-constricted rats (AC). Female rats were subjected to sham operation (S) or AC. After 5 wk, AC rats either remained sedentary, or were subjected to a treadmill exercise program (EX) for 6 wk. Rats were then anesthetized for hemodynamic study. Phenylephrine (PE) was infused IV. AC resulted in elevated LV wt. and LVP in AC (178±6 mmHg) and AC+EX (166±4) as compared to S (134±4). Mean femoral arterial pressure (MAP) was comparable to S in AC and AC+EX. Cardiac index (CI) and stroke index (SI) were reduced in AC animals, and EX raised these values, but not to the level of S. Resistance (TPR) was elevated in AC and AC+EX. With PE, MAP increased to a higher peak level in AC (211±9mmHg) and AC+EX (215±5) than in S (172±7). This MAP is associated with a greater increase in TPR and peak TPR (16.3±3.2mmHg/ml/min) in AC. AC+EX exhibited decreased TPR (8.8±1.7) from AC, but not to the same level as S (4.2±0.7). At comparable MAP, AC+EX had lower TPR and higher CI than AC. There were no differences in LV dP/dt. In S, as MAP increased with PE, CI decreased due to decreased SI and HR. AC show a small decrease in CI and SI, with increased HR. AC+EX decrease CI, SI and HR, but not to the same degree as S. Thus AC and AC+EX show enhanced MAP responses to PE. AC show greater TPR response, low CI, and no HR reduction. AC+EX results in lower TPR, higher CI and decreased HR. Supported by HL 23025, HD 13127, HL 07430

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INCREASED CARDIOVASCULAR RESPONSES TO VOLUNTARY STATIC EXERCISE AFTER NEUROMUSCULAR BLOCKADE (NMB) IN BABOONS. S.F. Hobbs*, L.B. Rowell, and O.A. Smith. Univ. of Wash., Seattle, Wa. 98195

When an NMB is applied during static exercise increased neural activity is required to recruit additional motor units in order to hold a constant load. To determine whether the cardiovascular responses are also increased by NMB at any given constant load, baboons were trained to hold loads to their voluntary limit for a food reward. An electromagnetic flow probe was implanted on the subclavian artery of the working arm. Catheters were implanted in the axillary artery of the working arm and a femoral artery and vein. The experimental protocol was: a) heavy load (4.0-5.5 kg) contractions, b) light load 1.0-1.5 kg) contractions with NMB, c) light load contractions as NMB regressed and d) light load contractions (no NMB control). Gallamine was infused in the working arm for NMB. Each contraction was followed by 3.5 min rest. Compared to control NMB elevated mean arterial pressure (MAP), heart rate (HR) and subclavian blood flow, and decreased endurance time. Heavy load contractions and light load contractions with NMB and with similar endurance times yielded similar MAP and HR. The augmented responses with NMB cannot be explained by reduced arm blood flow (ischemia), increased tension or a systemic effect of the blocking agent. The results suggest that a "central command" signal related to the extent of motor unit activation is an important input to cardiovascular centers during exercise. (Supported by NIH Grants HL-16910, RR-00166, and 5T32-HL07090.)

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ALVEOLAR CO_2-VAGAL INTERACTIONS AND COLLATERAL FLOW RESISTANCE DETERMINED WITH A WEDGED CATHETER. L.E. Olson and N.E. Robinson. Michigan State Univ., E. Lansing, MI 48824

The interaction between the vagus nerve and alveolar CO_2 tension in determining the resistance of airways less than 5 mm diameter was studied at FRC in 7 anesthetized (chloralose-urethane), paralyzed (succinylcholine) mongrel dogs artificially ventilated with room air. End tidal CO_2 was maintained at 5%. A fiberoptic bronchoscope (5 mm OD) isolated a sublobar lung segment by obstructing an upper lobe airway. 0, 5, and 14% CO_2 in O_2 entered the segment thru a flowmeter (V) and the bronchoscope suction port, and left via collateral airways. Segment pressure (P_s) was recorded from a catheter within the suction port and airway opening pressure (P_{ao}) recorded at the trachea. Steady state resistance of airways distal to the bronchoscope tip ($R_{ss}=P_s-P_{ao}/\dot{V}$) was determined for each gas mixture with the vagus nerve ipsilateral to the isolated segment intact, cut and electrically stimulated (3ms, 30Hz, 5-7v). R_{ss} was unaffected by vagal sectioning. Vagal stimulation increased R_{ss} for each gas. R_{ss} was highest with 0% CO_2. Factorial analysis revealed a significant interaction between the effect of the vagus and alveolar CO_2. The difference between R_{ss} cut and R_{ss} control did not change with CO_2. The difference between R_{ss} cut and R_{ss} stim was greatest with 0% CO_2. We conclude that segment hypocapnia constricts small/collateral airways and potentiates the bronchoconstrictor effect of vagal stimulation. (USPHS #HL-17768)

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MECHANICAL PROPERTIES OF THE IMMATURE LUNG. A. Kumar*, C. Doyle*, B.C. Clutario* and E.M. Scarpelli. Pediatric Pulmonary Division, Albert Einstein Coll. of Med., Bronx, New York 10461.

Volume-pressure curves were plotted for 22 immature rabbit fetuses (27 days gestation) during the first air inflation-deflation of the lungs from their normal liquid-filled fetal state. Prior removal of the anterior thorax (pentobarbital anesthesia) permitted simultaneous observation of anatomic correlates by stereomicroscopy. Inflation to 25 cm H_2O (P25) was characterized by progressive aeration of conducting airways in a pattern of axial or monopodial branching. The deflation curve from P25 to P0 retraced inflation, i.e., there was neither pressure nor time-dependent hysteresis. Inflation from P25 to P40 produced aeration of terminal lung units (TLU): Recruitment of TLU, which was both pressure and time-dependent (observations up to 2 min), was marked by apparent bubble formation with rapid coalescence of adjacent bubbles and preferential filling of distal (subpleural) TLU before central TLU. When pressure was lowered to P30, volume tended to increase with respect to volume at P40 (negative compliance). This was a function of both TLU recruitment and enlargement of previously aerated TLU. During deflation from P30 to P5-10, fetal pulmonary fluid, which had been displaced to the periphery of TLU during inflation, tended to refill TLU. The difference between deflation and inflation curves in this range (hysteresis) was due to air retained in TLU. Air remaining at P0 was entrapped in TLU by liquid locks in proximal airways. (Supported in part by NIH HL 07060).

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TOPOGRAPHY OF ESOPHAGEAL PRESSURE CHANGES DURING BREATHING. Kevin Hist*, J. Andrew Daubenspeck, Robert E. Nye, Peter Anderson* and John Erkkinen*, Dartmouth-Hitchcock Medical Center, Hanover, N.H. 03755

While local static pressures have been found to be uniform within the mid-esophageal region, little is known about the distribution of dynamic pressure changes in this region during tidal breathing. We are investigating the topography of these esophageal pressure changes and the influence of lung volume and posture upon this distribution in normal subjects. A triple pressure transducer catheter is used to map the intra-esophageal pressure changes with each breath. Transducers are located 5 cm. apart along the catheter, which is positioned with reference to the lower esophageal sphincter and then withdrawn to the desired location. Standard spirometric techniques are used to monitor tidal and lung volumes. Observations to date have shown that maximal simultaneous pressure changes in the mid-esophagus may vary 100% or more among three locations spanning a 10 cm. range. A single 10 cm. esophageal ballon would detect the greatest pressure change within this region and would be less influenced by the large topographical variation in pressure change we have recorded. We conclude: 1) Breathing related pressure changes within the mid-esophagus are not uniformly distributed; 2) Care must be exercised when interpreting pressure changes measured at discrete locations within the mid-esophagus; 3) The conventional 10 cm. esophageal balloon may be more useful than discrete techniques in measuring maximal mid-esophageal pressure changes. (Supported in part by USPHS NIH grants HL19248 and HL00280 and by a Phoenix Mutual Life Insurance Company Medical Scientist Scholarship.

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CONTRIBUTION OF VISCERAL PLEURA TO THE ELASTIC RECOIL OF ISOLATED SHEEP LUNGS. Joseph Engelberg and C. C. Tussey*. Dept. Physiol. & Biophysics, Univ. of Kentucky Med. Ctr., Lexington, Ky. 40536

A strip of lung tissue, about 1 cm wide x 3 mm thick, was excised from the lung surface; just beneath it a second strip, about 1 cm wide x 1 cm thick, was likewise excised. The length-tension relation was determined for each strip. The length-tension of the pleura alone was deduced, using data obtained for the second strip, by subtracting from the length-tension relation of the first strip the estimated contribution of lung tissue adhering to the pleura.

(1) At low lung volumes the elastic modulus of the pleura is some 50x greater than that of the lung tissue beneath it. (2) The elastic energy of recoil stored in the pleura appears to be on the order of 10% of the total recoil energy of the lungs. (3) At higher lung volumes the pleura does not appear to limit the expansion of the lungs. (Supported by Tobacco and Health Research Institute.)

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"CHOKE POINT" MOVEMENT DURING FORCED VITAL CAPACITY (VC) DEFLATION IN DOGS. S. Mink* and L.D.H. Wood*. (Spon: E. Faridy). Sec. of Resp. Dis., University of Manitoba, Winnipeg, Canada.

We used the retrograde catheter to locate the airway site of flow limitation ("choke point", CP) at 4 lung volumes (V) in 7 open chested dogs. By moving the catheter along the airway, three different sites representing the CP loci of the four lung volumes were found. Mean (± SD) lateral airway pressure (P) at these loci and volumes are tabulated (*denotes the value at CP loci) with the corresponding values of maximum expiratory flow ($\dot{V}$max) and lung recoil (Pel).

Volume (%VC)	70 ± 3	52 ± 3	31 ± 9	18 ± 1
Pel (cmH_2O)	9.7 ± 1	6.2 ± 1	3.9 ± 1	2.1 ± 1
$\dot{V}$max (lps)	9.3 ± 2	7.7 ± 2	5.8 ± 2	2.9 ± 1
P trachea (cmH_2O)	-7.6 ± 3*	-8.0 ± 3*	< -30	< -30
P lobar bronchus	2.6 ± 3	0.5 ± 3	< -30	-4.5 ± 2*
P 4.3 mm bronchus	5.1 ± 2	2.5 ± 1	-0.4 ± 1*	-1.1 ± 1

CP moved peripherally about the knee of the $\dot{V}$max-V curve (31% VC), and then mouthward with further lung deflation. For similar values of P, the 4.3 mm bronchus represented the CP loci at 31% VC but not at 18% VC. According to "Starling resistor" theory, this was due to decreased stability of the peripheral airway at 31% VC. Alternatively, both CP loci and P* were a consequence of factors determining flow limitation where gas velocity first equaled bronchial wave speed. (Supported by MRC of Canada).

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PARADOXICAL ADRENERGIC RESPONSE OF AIRWAYS IN RATS. R. Lee Boyd* and M. J. Fregly, Depts. Pediatrics and Physiology, Univ. of Florida College of Medicine, Gainesville, FL 32610

Adrenergic response in the airways of anesthetized (35 $mg \cdot kg^{-1}$ sodium pentobarbital, i.p.) female Sprague Dawley rats (0.2 - 0.35 kg) was studied by plethysmographic measurement of functional residual capacity (FRC, ml), airway resistance (Raw, $cmH_2O \cdot ml^{-1} \cdot sec^{-1}$) and specific airway conductance (SGaw, $ml \cdot sec \cdot cmH_2O^{-1} \cdot ml^{-1}$). Measurements were made before and 15min after stimulation with sympathomimetic agonists or antagonists as shown below:

AGENT	ISOPROTERENOL	PHENTOLAMINE	PROPRANOLOL
DOSAGE	$10\mu g \cdot kg^{-1}$ s.c.	$5mg \cdot kg^{-1}$ s.c.	$6mg \cdot kg^{-1}$ i.p.
NO OF RATS	6	5	9
% CHANGE FRC	+35.2	+15.0	+0.2
% CHANGE Raw	+33.8	+99.1	-13.6
% CHANGE SGaw	-42.8	-47.5	+17.2

The increased Raw and decreased SGaw indicate bronchoconstriction after β-adrenergic stimulation (isoproterenol) or α-adrenergic blockade (phentolamine). The increased FRC under these conditions suggests air trapping at end tidal expiration or compensatory elevation of lung volume in response to the increased resistance to air flow. The reduction in Raw and increases in SGaw without change in FRC after β-adrenergic blockade (propranolol) suggest that dilatory control of the airways is maintained by the α-adrenergic system. In contrast, the results with isoproterenol suggest that the β-adrenergic system may be responsible for bronchoconstriction in female rats. These results suggest that catecholaminergic control of smooth muscle of rat airways differs from that of other mammals tested to date. (Supported by HL-14526-08 grant).

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EVIDENCE OF CONTINUED FLOW LIMITED EXPIRATION AT RESIDUAL VOLUME (RV). Webster, P.D.*, University of Toronto. (SPON: Alison Froese) Toronto, Ontario, Canada. M4N 3M5

Based on the work of Leith and Mead (JAP 23:251), Davis et al (JAP 49:695) attempted to measure the volume of gas trapped behind closed airways at RV. They were troubled by evidence of some continued airflow in their older subjects. This is consistant with our finding that mechanical occlusion at the mouth at RV resulted in a slowly rising mouth pressure in these subjects that could maintain positive intrathoracic pressure (Amer.Rev.Resp.Dis. 120:417). To further define this phenomenon we measured airflow with a device sensitive to very small flows after the subject had apparently completed a forced vital capacity expiration. In subjects that could not maintain positive intrathoracic pressures small inspiratory and expiratory flows occurred without obvious pattern verifying patency of the airways and absence of positive intrathoracic pressure. In all subjects that could generate continued expiratory pressures continued expiratory flow was measurable. Not only did expiration continue but flow decreased with decreasing lung volume in a manner similar to the full flow volume curve. This suggests that the flow limitation active throughout the forced expiration remains in effect as long as expiratory effort continues.

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CHARACTERIZATION OF METHACHOLINE BRONCHOCONSTRICTION BY RESPIRATORY IMPEDANCE SPECTRA. D.V. Skirball*, E.C. Deal, Jr. J.G. Horowitz*, S.G. Kelsen, E.H. Chester*. Cleveland V.A. Medical Center, Departments of Medicine and Biomedical Engineering, C.W.R.U., Cleveland, Ohio 44106.

We have used frequency spectra of the total respiratory impedance (Z), obtained from forced oscillation techniques, to characterize alterations in mechanical abnormalities induced by methacholine. The Real (ReZ) and Imaginary (ImZ) parts of Z from 3 to 30 Hertz were computed from sinusoidal data. Forced oscillations, plethysmography, and spirometry were performed on 7 asthmatics before and after each of several provocations at progressive doses. At the maximal dose administered methacholine resulted in an increase in airway resistance (Raw) from 1.84 ± 1.4 ($\bar{x}$ ± SD), to 9.7 ± 4.1 $cmH_2O/L/sec$. The impedance spectra had corresponding changes in both position and shape. ReZ was increased throughout the frequency range examined, however it decreased with increasing frequencies: at 3 Hz ReZ increased from 3.6 ± 1.0 to 9.3 ± 4.7, whereas at 30 Hz it only increased from 2.9 ± 0.9 to 3.4 ± 0.7. In the spectra of ImZ, the resonant frequency increased. To characterize the spectra, various indices and parameters based on one and two compartment models were examined and compared. Results show that impedance spectra can be used to assess bronchial reactivity to methacholine and that multicompartment models are required to describe the frequency dependence of the observed spectra. (Supported by grants from the Veterans Administration and the Northern Ohio Lung Association.)

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HISTAMINE AEROSOL-INDUCED BRONCHOCONSTRICTION IN DOGS: ROLE OF VAGAL TONE OR REFLEXES. M. Yanta, S. Loring, R. Ingram, J. Drazen. Harvard School of Public Health and Depts. of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston, MA 02115.

There are conflicting reports regarding the role of the vagus nerve in histamine aerosol-induced bronchoconstriction. Some investigators report data consistent with vagovagal reflexes while others have suggested only a minor role for vagal tone in mediating this response. We measured airway resistance (RL) in 9 anesthetized, paralyzed, mechanically-ventilated mongrel dogs with the cervical vagi intact, then sectioned, and then peripherally stimulated at two intensities during exposure to aerosols of increasing histamine concentrations. If the role of the vagus were through constant efferent tone interacting with the bronchoconstrictor agent, dose response curves would not intersect. If vagal efferent activity in proportion to bronchoconstrictor dose (i.e., vagovagal reflexes) predominated, the slope of the dose response curve would be steeper before than after vagal section, with or without stimulation. 3 dogs showed responses indicating additive interaction, 4 had responses indicating vagal reflex activity, and 2 dogs had intermediate patterns. We concluded that in a population of dogs, vagal mechanisms interacting with histamine-induced bronchoconstriction may vary from additive interaction of the constrictor agent with constant efferent tone to vagal reflexes. (Supported by NIH grants HL 19170, 07010, 22920, and 00549).

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CHANGES IN THE RELATIVE CONTRIBUTIONS OF LARGE AND SMALL AIRWAYS TO RESISTANCE AT FLOW LIMITATION IN HEALTHY SUBJECTS. K.P. Strohl*, C.F. O'Cain*, R.H. Ingram, Jr., M.A. Yanta*, E.R. McFadden, Jr. Departments of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston, MA 02115.

To determine the relationship between changes in density dependence of maximal expiratory flow and changes in the predominant site of bronchoconstriction, we altered the pattern of inhalation of a methacholine aerosol to achieve deposition either centrally (by short choppy breaths) or peripherally (by slow deep breaths). In six subjects we recorded PEFV maneuvers on air and on 80% helium-20% oxygen (He/O_2) before and after each pattern, using concentrations of methacholine and numbers of inhalations designed to give equivalent degrees of bronchoconstriction as assessed by decreases in maximal flow (Vmax) on air. Vmax at 30% VC on air fell 27 ± 6% (mean ± SEM) from control values following slow deep breaths, and 26 ± 5% following short choppy breaths. Vmax on He/O_2 also fell following both inhalation patterns. Density dependence following slow deep breaths decreased from 1.44 ± 0.05 to 1.27 ± 0.04, and increased (1.40 ± 0.04 to 1.53 ± 0.07) following short choppy breaths. In three subjects, inhalation of radionuclide-labeled aerosol confirmed that the slow deep pattern was associated with a diffuse, more peripheral deposition, while the short choppy pattern led to central deposition. We conclude that changes in density dependence reflect the predominant sites of obstruction following acute aerosol challenges. (Grants HL 16463 and HL 07010)

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BRONCHIAL-ARTERIAL INTERDEPENDENCE IN ISOLATED DOG LUNG: ANALYSIS AND MEASUREMENTS. Stephen J. Lai-Fook and Michael J. Kallok. Mayo Clinic and Foundation, Rochester, MN 55901.

We modelled the bronchus and artery as adjoining cylindrical tubes in an elastic continuum. A finite element solution of stress and strain was computed for reductions in arterial diameter from the uniform state. Nonuniform tensile radial stress (S) had a maximum value in the region adjacent to the bronchial-arterial joint equal to 3 times mean periarterial S. Peribronchial S fell to zero away from joint. Bronchial cross-section became oval, elongated along common center line while artery elongated at right angles to this line. To verify these shape changes, we measured orthogonal diameters (D1,D2) of bronchus and artery, lined with tantalum, from x-rays taken with x-ray source directed along the common center line (D1) and at right angles (D2) to it. At transpulmonary pressure (Ptp) of 4 cm H_2O, mean bronchial D2/D1 in 5 lobes increased from 0.89 to 1.20 as arterial pressure (Pa) decreased from 30 to -10 cm H_2O while arterial D2/D1 decreased from 1.11 to 0.49. Bronchial cross-section area (D2xD1) did not change. Similar results were obtained at a Ptp of 25, but D2/D1 changes were smaller. Measurements confirm model's predictions. Peribronchovascular interstitial fluid pressure may be nonuniform with a maximum of 3 times mean periarterial pressure. Any changes in bronchial or arterial diameter may reduce resulting ventilation/perfusion mismatch. (Supported by HL 21584 from NHLBI.)

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COUGH MECHANICS DURING EXPIRATORY MUSCLE WEAKNESS FROM PROGRESSIVE CURARIZATION. N.S. Arora* and T.J. Gal*. (SPON: D.F. Rochester). Univ. of Virginia, Charlottesville, VA 22908

We studied 6 healthy nonsmoking males (mean age 25) in the supine position. Maximum static inspiratory and expiratory pressure (PImax, PEmax), cough pleural pressures (PplC), cough flow rates ($\dot{V}c$) and cough volume (Vc) were measured in control state and after each of 4 incremental 0.05 mg/kg i.v. doses of d-tubocuraraine (dTc). PplC (CmH_2O), $\dot{V}c$ (L/sec) and Vc (L) were measured during 3 bursts of cough starting from total lung capacity (TLC) and functional residual capacity (FRC). Control values at FRC (mean ± SEM) were: PImax 149±10 CmH_2O and PEmax 145±15 CmH_2O. With the last dose of dTc, values were 42% and 17% respectively (P<0.001). Data for the first cough bursts at TLC and FRC are tabulated; (+) indicates significant difference (P<0.001) from control.

	COUGH FROM TLC			COUGH FROM FRC		
Condition	PplC	$\dot{V}c$	Vc	PplC	$\dot{V}c$	Vc
Control	136±17	8.3±0.6	74±3	95±13	2.0±0.3	31±1.3
dTc2	113±14+	8.2±0.4	76±3	84±12+	1.9±0.5	33±1.1
dTc4	65±10+	7.3±0.3	69±3	43±4 +	2.1±0.4	33±1.3

These results show that $\dot{V}c$ and Vc did not decrease significantly even when PplC fell to less than half of control. Since PplC is reduced, cough-induced dynamic compression of large airways is diminished. If so, flow velocity would be reduced. We conclude that this may be the major effect of expiratory muscle weakness on cough dynamics. (Supported by NIH-NHLBI Grant # HL 22022).

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COMPUTER SIMULATION OF REBREATHING MEASUREMENTS OF LUNG TISSUE VOLUME. Stanley A. Wilkins, Jr.* and Mitchell Friedman, Univ. of North Carolina School of Medicine, Chapel Hill, N.C., 27514.

Inert gas rebreathing (RB) methods are useful in measuring combined pulmonary tissue and capillary blood volume (VTPC) as well as pulmonary capillary blood flow ($\dot{Q}c$), diffusing capacity (D_L) and FRC. We previously validated an algorithm for measuring VTPC in normal dogs and dogs with oleic acid induced lung injury (JAP 48:66, 1980). We have developed a model to evaluate the effects of maldistribution of ventilation ($\dot{V}_A$), $\dot{Q}c$, D_L and FRC on RB estimates of VTPC. The model, containing 2 alveolar compartments, simulated a lung that rebreathed a mixture of 10% He, 0.3% CO, and 1.0% C_2H_2 in a stepwise manner through a dead space from a reservoir in a closed system. CO and C_2H_2 uptake in each compartment was assumed to follow a single exponential. The model predictions of RB parameters were similar to those obtained in dogs in whom focal, defined $\dot{V}_A/\dot{Q}c$ imbalances had been created. Using the model, we then calculated the effects of graded maldistributions of $\dot{Q}c$, $\dot{V}_A$, VTPC, D_L and FRC on the model estimate of VTPC. Maldistribution of $\dot{Q}c$ or VTPC produced negligible errors in computed VTPC. Moderate maldistribution of FRC, D_L, or $\dot{V}_A$ (% distributions in 2 compartments between 50:50 and 30:70) resulted in less than 15% underestimate of VTPC. Greater degrees of maldistribution of these parameters resulted in larger errors in computed VTPC. Thus, RB estimates of VTPC are predicted to be reasonably accurate in moderate states of maldistribution of $\dot{V}_A$, $\dot{Q}c$, D_L, VTPC or FRC. (Supported in part by NIH grant HL-21168 and EPA R-805184)

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RELATIONSHIP BETWEEN OLEIC ACID DOSAGE AND EDEMA IN THE ISOLATED CANINE LUNG PERFUSED AT CONSTANT PRESSURE. Ina C. Ehrhart and Wendell F. Hofman. Medical College of Georgia, Augusta, Georgia 30912.

Pulmonary edema was induced by administering oleic acid to ventilated and blood perfused lung lobes. Lobes were treated with 1 (n=6) or 45 µL/kg body weight (n=6) oleic acid or saline (n=7). Lobe weight increased linearly over 1-3 h following oleic acid. Slopes of regression lines relating weight to time indicated a more rapid rate of weight gain at the higher oleic acid dosage. Total lobe weight gain was greater in the 45 than in the 1 µL/kg group (0.60 ± 0.10 versus 0.31 ± 0.07 g/g initial lobe wt) and greater in the acid treated lobes than in the controls (0.13 ± 0.05 g/g initial lobe wt). Pulmonary vascular resistance increased 82% after 45 µL/kg oleic acid but was unchanged after 1 µL/kg oleic acid or saline. The decrease in PaO_2 was greater in the 45 µL/kg group than in the controls, 47 vs. 22 Torr. High vascular pressures, increased flow velocities in patent vessels and fibrin deposition are not essential for oleic acid associated edema since weight increased at constant pressure perfusion with heparinized blood. The relationship between oleic acid dosage and weight gain suggests that oleic acid increases permeability by affecting the vascular endothelium either directly or through biochemical intermediates. (Supported by Am. Heart Asso.-Georgia Affiliate, BRSG #5-507-RR05365-19 and NIH #5E03-MB-4106-14).

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EFFECT OF DOPAMINE (DOP) AND DOBUTAMINE (DOB) ON PULMONARY PERFUSION AND GAS EXCHANGE IN UNILOBAR PULMONARY EDEMA IN DOGS. R.B. Light*, J. Ali*, and L.D.H. Wood*. (Spon: A. Naimark). Sec. of Resp. Dis., University of Manitoba, Winnipeg, Canada.

Both DOP and DOB have been reported to increase intrapulmonary shunt (Qs/Qt) in low pressure pulmonary edema. To determine whether this effect was due to redistribution of blood flow towards edematous lung units or to the associated increase in cardiac output (Qt) we studied 19 dogs with oleic acid (OA) pulmonary edema localized to one lower lobe (E). The dogs were anaesthetized, ventilated with 0_2 and had catheters placed in the pulmonary artery and both lower lobe pulmonary veins. Lobar shunt (Qs/Q_L) in E, lobar distribution of Qt by radionuclide labelled microspheres (Q_L/Qt) and Qt were measured 2 hours after OA. The dogs were then randomly assigned to one of 4 groups: DOP (10 µg/kg/min), DOB (20 µg/kg/min) or saline (S) were infused, or a systemic a-v fistula (F) was opened. After 20 minutes, Qt and Qs/Q_L in E had increased by 50% in DOP, DOB and F, but neither changed in S. Because Q_L/Qt in E did not change in any group, we conclude that increased shunt is not due to redistribution of Qt toward edematous lung units by pulmonary vasoactive effects of DOP, DOB or by the associated increase in Qt. Conceivably, increased Qt reduced transit time of blood through edematous lung, aggravating diffusion limitation of 0_2 exchange which is interpreted as increased shunt. (Supported by MRC of Canada).

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EFFECT OF DOPAMINE (DOP) AND DOBUTAMINE (DOB) ON PULMONARY 0_2 EXCHANGE IN CANINE OLEIC ACID (OA) PULMONARY CAPILLARY LEAK (PCL). K. Duke*, F.W. Santman*, and L.D.H. Wood*. (Spon: V. Chernick). Sec. of Resp. Dis., Univ. of Man., Winnipeg.

In patients with PCL, vasoactive agents may be used to maintain cardiac output (CO) when pulmonary intravascular pressures (Piv) are lowered to reduce PCL. However, increased CO on DOP is associated with increased pulmonary shunt (Qs/Qt) and decreased PaO_2. To determine whether similar effects occurred on DOB, we studied 13 dogs ventilated with 0_2 2 hours after PCL was induced with OA (C1). Measurements were repeated during a 30 minute infusion (10 µg/kg/min) of DOP or DOB, and again 30 minutes after the drugs were discontinued (C2). The mean (±SD) results were (*denotes difference from C1 and C2):

	C1	DOP	C2	C1	DOB	C2
CO(lpm)	4.2±.5	5.6±1.7*	3.9±.4	3.1±1.1	4.8±1.2*	2.7±0.7
Qs/Qt(%)	25±8	37±12*	25±10	34±16	52±15*	39±17
PaO_2(mmHg)	327±109	240±128*	261±152	225±151	133±125*	151±167

DOB and DOP caused similar increases in CO and Qs/Qt. To determine whether increased Qs/Qt was due to increased CO or to pulmonary vasoactive effects of DOP and DOB, we studied a third group of dogs in which CO was increased by opening a systemic arterio-venous fistula. Because Qs/Qt increased with CO in the same proportion as on DOP and DOB, we conclude that these agents increase Qs/Qt by their effects on CO. Accordingly adequate CO can be maintained at reduced Piv with DOP or DOB without causing additional shunt due to their pulmonary vasoactivity. (Supported by Manitoba & Dutch Heart Foundations).

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RELEASE OF ANGIOTENSIN CONVERTING ENZYME BY THE LUNG AFTER PSEUDOMONAS BACTEREMIA IN SHEEP. A.B. Gorin, G. Hasagawa*, M. Hollinger* Internal Medicine, U.C. Davis, Davis, CA 95616

We studied release of angiotensin converting enzyme (ACE) by the lung after acute endothelial injury. In 8 adult sheep with chronic lung lymph fistulas, we measured lymph flow, and both ACE activity and total protein content in lymph and plasma before and during 24 hours after an infusion of live Pseudomonas organisms. Under baseline conditions, plasma ACE was 4.93±.43 u/ml (mean±sem). The [L]/[P] ratio for ACE (MW= 145,000) was .93±.18 , compared to a ratio of .79±.08 for albumin (mean±sd). Predicted [L]/[P] ratio for a 145,000 dalton molecule was .51 (estimated from polyacrylamide gradient gel electrophoresis of matched lymph and plasma samples). The lung secreted 11.1 u/hr ACE into lung lymph during the baseline period. After Pseudomonas infusion pulmonary vascular clearance for total protein more than doubled (baseline, 3.1±.6 ml/hr: 2 hours post infusion, 6.7±1.2 ml/hr; mean±sem, $P<.05$). Release of ACE into lung lymph doubled after acute lung injury and equaled 22.3±13.8 u/hr (mean±sem) at 4 hours after onset of the infusion. We did not see a rise in plasma content after acute lung injury. Failure to detect a rise in plasma content might result from dilution in the large vascular pool, or rapid catabolism of the ACE at a site distant from lung. (Supported by NIH grant EH&S #ES02093-02).

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ROLE OF ANGIOTENSIN II (AII) IN MODULATING PULMONARY ADRENERGIC FUNCTION, Daniel F. Ventura*, and Robert J. Porcelli, VAMC, Northport, N.Y. and S.U.N.Y. at Stony Brook, N.Y. 11790.

The participation of the adrenergic, histamine and serotonergic receptor systems in the pulmonary pressor response to AII was studied in the isolated perfused cat lung. α-adrenergic blockade with dibenamine (1.01µM) reduced the control AII vasoconstrictor response (0.2µM) from 68.4±13.2% to 46.6 ±9.6% ($p<.05$). ß-adrenergic blockade with propranolol (1.2 µM) increased the control vasoconstrictor response to AII from 49.0±10.2% to 90.2±13.3% ($p<.001$). These effects were unique for the adrenergic system since neither H_1 or H_2, nor 5-HT receptor blockade produced significant alteration in the AII pressor response. To determine whether AII released catecholamines from the lung as a part of its vasoconstrictor response, an isolated saline-perfused lobe preparation, completely separate from systemic circulation, was used. AII raised lobar norepinephrine output in this preparation by 39.0±10% ($p<.05$) from 129.0±27 pg/ml to 181±41 pg/ml. These data demonstrate that: 1). AII has both a direct myotropic effect on pulmonary vascular smooth muscle and an indirect effect involving lobar catecholamine release; 2). histamine and 5-HT receptor activation do not participate in the pulmonary pressor response to AII, and; 3). AII levels may modulate pulmonary adrenergic neuronal activity. (Supported in part by: Veteran's Administration, NHLBI RO1-HL-23210 and the Nassau-Suffolk Lung Association).

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RELATION BETWEEN PULMONARY SEROTONERGIC (5HT) RECEPTORS AND PULMONARY VASCULAR TONE AND RESPONSIVENESS, R.J. Porcelli, G. S. Cohen*, P.R. Villano*, and E.H. Bergofsky, VAMC, Northport, N.Y., and S.U.N.Y. at Stony Brook, N.Y. 11790.

The relation between the pulmonary vascular serotonin receptors and pulmonary vascular responsiveness was studied in an isolated perfused cat lung. 2-D-Bromo LSD (Bol, 1.1μM) was a highly specific antagonist, decreasing the control vasoconstrictor response to 5-HT (.11μM) from 80.2±12.9% to 43.1± 12.6% ($p<.05$) while having no antagonism of histamine (.18μM), norepinephrine (.12μM), angiotensin (.02μM), hypoxia (8% O_2) and hypercapnia (10% CO_2-Air). Cyproheptadine (Cypro, .11μM) inhibited the vasoconstrictor response not only to serotonin (43.4±8.3% to 6.87±2.7%, $p<.05$) but also to histamine (71.2± 14.8% to -14.3±2.8%, $p<.05$). Like Bol, Cypro had no effect on the responses to other agents used in this study, however, each reduced baseline vascular resistance by 7.4% (Bol) and 3.9% (Cypro), $p<.05$. The vasodilation observed with histamine was not blocked by either metiamide or propranolol. These data demonstrate: 1). that specific pulmonary serotonergic receptors exist and share some important characteristics with the histamine receptor system; 2). circulating serotonin may contribute to pulmonary vascular tone, and; 3). histamine has a pulmonary vasodilator effect not mediated by H_2 or β-adrenergic receptors. (Supported in part by: Veteran's Administration, James S. Mountain Fund, and NHLBI-R01-HL-23210).

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PULMONARY EDEMA FOLLOWING OLEIC ACID OR COLLOIDAL VOLUME OVERLOAD IN THE DOG. R.C. Schaeffer, Jr., R.W. Carlson, S. Valdes* and M.H. Weil. USC Institute for Critical Care Medicine, Los Angeles, CA and Mount Carmel Mercy Hospital, Detroit, MI 48235

Pulmonary edema was studied in dogs (N=28, 19-28.4 kg) after oleic acid (OA, 50 μl/kg) or volume overload (30 min, 75 ml/kg) with dextran 70 (DEX, $\bar{x}$ m.w.=70,000) or hydroxyethyl starch (HES, $\bar{x}$ m.w.=450,000) to a pulmonary microvascular pressure (P_{mv}) of 40 mm Hg. Extravascular lung water was estimated by the measurement of lung thermal volume (LTV, thermal-conductivity) at 0, 1 and 2 hrs after OA or fluids, and lung wet/dry weight (W/D) was measured at 2 hrs. OA produced a progressive rise in LTV, a decline in cardiac index (CI) and P_{mv}, as well as hemoconcentration and hypovolemia. DEX and HES produced a marked increase in CI, P_{mv} and oncotic pressure (π) with hemodilution. LTV and W/D were strikingly elevated ($p<0.001$) after OA or DEX, but not after HES (p=ns). Hemorrhagic edema fluid (EF,Hct=2%) sampled from the airway after OA or DEX contained equivalent protein concentration and π as plasma (P). After DEX the concentration of DEX in EF and P was similar. However, a small amount of high m.w. DEX was excluded from EF. These findings confirm that low pressure pulmonary edema after OA is associated with hypovolemia and marked changes in alveolar-capillary permeability to protein. The EF findings after DEX are reminiscent of severe permeability pulmonary edema. The failure of HES to induce these changes may be explained by size-limited stretched pores.

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COMPARISON OF SALINE AND HOMOLOGOUS PLASMA INFUSION ON SHEEP FLUID AND PROTEIN TRANSPORT. W.A. Nylander*, R.J. Roselli, J.B. Tribble*, K.L. Brigham, J.W. Hammon*. Dept. of Thoracic and Cardiac Surgery and Pulmonary Circulation Center, Vanderbilt University, Nashville, TN 37232

The effects of homologous plasma bolus (PB) and saline bolus (SB) [20 cc/kg/30 min.] on lung transvascular protein and fluid movement were compared in chronically instrumented unanesthetized sheep. Aortic, pulmonary arterial and left atrial pressures were monitored. Lung lymph flow (Q_l), plasma oncotic pressure (π_p) and lymph oncotic pressure (π_l) were measured. Protein clearance (PC), protein flux (PF), pulmonary microvascular pressure (P_{mv}), and oncotic gradient ($\Delta\pi=\pi_p-\pi_l$) were calculated. The multiple indicator dilution technique was used to calculate cardiac output (CO) and extravascular lung water volume (EVLWV). Pre and post infusion steady state values were compared. PB significantly increased ($p<0.05$) Q_l(29.5%), PF(35.0%) and PC (23.6%). SB also significantly increased Q_l(37.5%), PF(16.9%) and PC(26.1%). PB and SB minimally changed EVLWV and CO. PB increased P_{mv} (8.4%) which produced a dilution of lymph protein and an increase in $\Delta\pi$(15%). Plasma oncotic pressure rose during PB and fell during SB. However, lung fluid balance was similar because there was a compensatory change in π_l. (Supported in part by USPHS NIH Grant Nos. HL24641, HL22933 and HL19153.)

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ULTRASTRUCTURE OF CANINE LUNG IN ALTERED MICROVASCULAR PERMEABILITY INDUCED BY HEMORRHAGIC SHOCK AND REINFUSION. R.P. Michel*, J.C. Hogg, (SPON: M. McGregor). Lyman Duff Lab., McGill University, Montreal, P.Q., Canada H3A 2B4

Previously we found that hemorrhagic shock, with or without reinfusion, increased microvascular permeability, since lung lymph flow and lymph/plasma protein ratios increased, and that lung wet/dry weight ratios were higher with reinfusion. Light microscopy revealed increased granulocytes in the reinfused group. To determine if endothelial or alveolar epithelial lesions caused the permeability change, 378 electron micrographs from 3 groups of 7 dogs each were graded blindly on a 0-4 scale for intravascular granulocytes and platelets, interstitial edema and granulocytes, endothelial and epithelial blebs and pores. We found the reinfused group had more intravascular granulocytes (1.54, $p<0.01$) than the shock or control (1.08) groups, and more interstitial edema (0.53, $p<0.05$) than controls (0.23). We conclude the altered microvascular permeability, increased wet/dry weight ratios, and interstitial edema seen by electron microscopy may be mediated by intravascular granulocytes, and occur without visible damage to the endothelium or epithelium. (Supported by MRC of Canada Grant #MA-6474).

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LUNG VASCULAR PERMEABILITY IN SHEEP: CORRELATION OF LUNG LYMPH WITH ELECTRON MICROSCOPIC (EM) DATA. J. Hobson* and K. Brigham. Vanderbilt University, Nashville, Tennessee 37232.

While collecting lung lymph and measuring vascular pressures in sheep, we infused dextran (MW=40,000) and correlated lung lymph/plasma (L/P) concentrations with interstitial dextran seen on EM of lung biopsies taken serially. Dextran leaked from lung capillaries. L/P dextran concentrations reached a maximum of 0.68 by 3 hrs. after dextran infusion and dextran was visible in alveolar interstitium. In other animals, we infused histamine phosphate (4ug/kg/min) to increase lung capillary permeability before infusing dextran. In these animals, L/P dextran concentration reached 0.85 and more dextran was present in the alveolar interstitium than in the control animals. Dextran was present in endothelial vesicles by 1 hr. after infusion in both groups and could also be seen in endothelial junctions, but we could not determine the principal mode of transport. Histamine treated, but not control, animals had occasional open junctions in lung capillaries. Dextran distribution in the interstitium was not homegeneous and interstitial/intravascular concentrations appeared lower on EM than L/P concentrations. We conclude that EM tracer measurements of lung capillary permeability agree qualitatively with lung lymph measurements. Interstitial tracer concentrations appear lower on EM than in lung lymph, probably because of an excluded volume. Histamine increases lung capillary permeability to macromolecules judged both by lymph and EM techniques. (Supported by Grant No. HL 19153.)

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EFFECTS OF CATECHOLAMINES ON LUNG FLUID AND PROTEIN EXCHANGE IN SHEEP. Fred L. Minnear*, Philip S. Barie*, Asrar B. Malik, Department of Physiology, Albany Medical College, Albany, NY 12208.

Anesthetized sheep were prepared with a lung lymph fistula to study the effects of epinephrine (E) or norepinephrine (NE) infusion on lung fluid and protein exchange. Steady-state lymph flow ($\dot{Q}$lym) was attained at baseline and 2 hr after iv infusion of E or NE (45 μg/kg/min). Steady-state data are shown below:

	n	$\dot{Q}$lym (ml/hr)	L/P	$\bar{P_{pa}}$ (mm Hg)	PVR (mm Hg/l/min)
Baseline	6	8.4±1.8	.68±.08	17± .8	5.9± .08
E		10.0±1.8	.68±.06	23± .9*	12.1± 1.2*
Baseline	11	9.2±1.5	.78±.03	16±1.5	6.5± .08
NE		11.5±2.0*	.74±.04*	22±1.8*	9.3± 1.7*

L/P=lymph-to-plasma protein concentration ratio; $\bar{P_{pa}}$ = mean pulmonary artery pressure; PVR=pulmonary vascular resistance . *=values are significant ($p<.05$) using the paired t-test. $\dot{Q}$lym and L/P did not change in animals given E, despite an increase in $\bar{P_{pa}}$, probably due to an increase in PVR proximal to the site of fluid exchange. However, a similar increase in $\bar{P_{pa}}$ following infusion of NE caused a small but significant increase in fluid filtration due to increased microvascular pressure. The results indicate that lung vascular permeability to proteins does not increase after either E or NE infusion. (HL-17355, HL-00363, T32-GM-07033)

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CENTRAL HEMODYNAMICS OF THE SPONTANEOUSLY HYPERTENSIVE RAT COMPARED TO A NORMOTENSIVE CONTROL. David L. Scott* and Jean-Pierre L. Dujardin*. (SPON: H.P. Pieper). Dept. of Physiology The Ohio State University, Columbus, Ohio 43210.

Total peripheral outflow and total arterial compliance were both measured as a function of arterial pressure by means of a method not previously used in rats. Slow oscillations in arterial volume and pressure were induced by means of an external piston pump (period=3 sec, stroke volume=1.09 ml), connected to the arterial system via the carotid artery. Arterial pressure was monitored in the abdominal aorta through a right femoral artery catheterization. During pump operation (duration=10 sec) arterial pressure and pump displacement were recorded on a digital oscilloscope. Using a curve fitting technique, the diastolic periods of these recordings could be used to characterize the arterial system. In all rats (age 20 weeks), the total peripheral outflow became zero at a positive pressure, the intercept pressure P_i. In the normotensive (WKY) rats, P_i was equal to 37.9±2.3 (SE) mmHg. In the spontaneously hypertensive rats (SHR), P_i was significantly larger, namely 69.0±5.6 (SE) mmHg. Total arterial compliance in the SHR was less than in the WKY when determined at their own respective mean arterial pressure. At any given pressure, however, compliance was greater in the SHR than in the WKY. This last finding supports the hypothesis that hypertension causes structural changes in the arterial wall which are adaptive in nature. (This project was supported by The Central Ohio Heart Chapter of AHA.)

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ALTERATIONS IN LEFT VENTRICULAR PUMPING ABILITY FOLLOWING DIETARY SODIUM (Na) RESTRICTION IN RATS. Michelle M. Spech* and Carlos M. Ferrario. Cleveland Clinic Research Division, Cleveland, Ohio 44106.

The ventricular pumping ability of Sprague-Dawley rats (14-19 weeks old) subjected to 4-6 weeks of dietary Na depletion was characterized in order to establish the involvement of the heart in the pathophysiology of the salt depleted state. Following anesthesia with methoxyflurane, Na depleted rats were tachycardic, and their stroke volume (0.09 + 0.01 ml) and cardiac output (37.6 + 6.1 ml/min) were significantly less than those recorded in the control group (0.19 + 0.01 ml and 64.1 + 6.2 ml/min, $p < 0.001$). The mean arterial and left ventricular end-diastolic (EDP) pressures were not different from those in normal animals. In addition, both hemoconcentration and a tenfold increase in plasma renin activity were present. Peak cardiac pumping ability, expressed as the maximum stroke volume (SV) and cardiac output (CO) reached during the preload stress, was essentially the same in both groups. However, the relationships of SV and CO to EDP during the ascending limb of the function curve were displaced to the right of the control at EDPs between 1 and 8 mm Hg.

The data indicate that maintenance of cardiac performance in the Na depleted rats requires a compensatory increase in the left ventricular filling pressure, a paradoxical finding in view of the hypovolemic state. (Supported in part by an NHLBI grant #HL-6835).

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LEFT VENTRICULAR HYPERTROPHY IN DOCA HYPERTENSIVE PIGS. John Mitchell*and David F. Bohr. University of Michigan, Department of Physiology, Ann Arbor, MI 48109

Young, male pigs were chronically instrumented to monitor mean arterial pressure(MAP) and cardiac output(CO). Following recovery, test animals were implanted with 100mg/kg deoxycorticosterone acetate(DOCA) and control animals received similar DOCA-free implants. At termination(2-88 days post-implant) the heart was removed and the left ventricle and septum dissected out. For each heart, a left ventricular index(LVI) was determined by dividing the weight of the left ventricle(grams) by the weight of the animal(kg.). Hypertensive pigs(n=13) showed a significantly greater LVI(3.38±.13 gms./kg) than normotensive pigs(n=9;LVI=2.55±.17). There was a positive linear relationship(r^2=.22;$p<.05$) between LVI and change in heart work (ΔMAP from pre-implant level x days since implant). Eight of the DOCA and 3 of the control pigs received a sodium restricted diet for 3 weeks. In 5 of these DOCA-treated pigs the pressure returned to normal and the LVI was 3.02±.22; in 3 the pressure did not fall and the LVI was 3.66+.12. Both groups differed from the control pigs placed on a similar diet (LVI=2.44±.37). These results suggest that 1) ventricular hypertrophy occurs in DOCA hypertensive pigs as a function of magnitude and duration of the pressure elevation, and 2) a low sodium diet can, when decreasing the MAP, decrease the magnitude of the hypertrophy. (Supported by NHLBI grant #HL-18575).

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TREATMENT OF NEONATAL SPONTANEOUSLY HYPERTENSIVE RATS (SHR) WITH INDOMETHACIN OF SPIRONOLACTONE: EFFECTS ON BLOOD PRESSURE DEVELOPMENT. M.M. Mullins* and R.M. Cook* (SPON: S.N. Kolmen). Wright State University, Dayton OH 45435

We reported elevated serum aldosterone concentrations and depressed pulmonary prostaglandin (PG) degradation (suggesting elevated arterial levels of PG) in 10-day-old SHR (Mullins, Kleinman and Russell, Fed. Proc.38:1258,1979). This study examines the effects of therapeutic-level doses of spironolactone (SP) or indomethacin (IN) on blood pressure (BP) in the maturing SHR. Groups of SHR and normotensive Wistar-Kyoto (WKY) were injected subcutaneously with IN or SP, 15μg/10gm body weight, daily at ages 1-10 days. BP (tail cuff) was measured weekly between 6 and 12 weeks of age. BP (x̄±sem in table) of IN-treated SHR was significantly lower than vehicle (V)-treated

Drug/Age	6	7	8	9	10	11	12
IN	90.7	101.9	126.9	154.3	151.3	150.6	153.8
	±3.4	±3.5	±2.7	±4.0	±3.2	±3.8	±3.2
SP	100.0	121.3	132.1	165.0	163.5	144.1	177.1
	+2.9	±6.8	±4.7	±4.8	±5.2	±5.4	±12.5
V	105.0	138.1	140.0	194.3	171.4	223.1	201.8
	±3.8	±5.2	±6.2	±7.1	±7.1	±8.2	±8.7

at all ages. BP of SP-treated SHR was also lower, but not always significantly so. Neither drug had an effect on WKY BP (not shown). These data suggest that elevated systemic PG concentration in neonatal SHR may play a role in the initiation of hypertension in that strain. (Supported in part by Miami Valley Chapter, AHA and Wright State U. Basic Sci. Res. Grant)

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ALTERATIONS IN ARTERIAL PRESSURE URINE FLOW AND PROTEIN EXCRETION FOLLOWING INFUSION OF HUMAN TOXEMIC PLACENTAL EXTRACT IN RATS. Silvana Briancesch*i and John J. Curry. Department of Physiology, Ohio State University, Columbus, Ohio, 43210.

Spontaneous hypertensive disease of pregnancy (SHDP), also termed toxemia of pregnancy or preeclampsia/eclampsia develops after the 24th week of gestation in affected humans. The symptoms of the disease include an increase in systolic and diastolic blood pressure, proteinuria, and a decrease in plasma volume. Term placentas were obtained from SHDP patients and from patients undergoing normal pregnancies and acid extracts prepared, lyophilized and reconstituted in 0.9% NaCl at a concentration of 0.1 gm lyophilizate/ml. One femoral artery and vein were catherized for measurement of arterial pressure and for infusion of test material respectively. Urine was collected for determination of volume and protein content. Rats receiving toxemic placental extract (TPE) showed a progressive increase in arterial pressure above animals receiving normal saline. Animals receiving TPE also showed a pronounced increase in urine flow and urinary protein excretion. These findings are consistent with the increase in systolic pressure protienuria and hypovolemia seen in SHDP and suggest that the placenta might be the source of the agent or agents responsible for eliciting the disease. Since NPE had a slight effect upon these measures the results also suggest that these agents are produced to at least some degree in all pregnancies. (Supported by American Heart Assoc., Central Ohio Chapter Grant #79-12.)

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DEVELOPMENT OF TACHYPHYLAXIS TO ANGIOTENSIN II IN NORMOTENSIVE AND DOCA HYPERTENSIVE RENAL VASCULATURES. David M. Cohen and David F. Bohr. University of Michigan Medical School, Ann Arbor, MI 48109

The rate of tachyphylaxis development to angiotensin II (AII) was examined in kidneys from 4 normotensive(N) and 3 DOCA hypertensive (D) rats. Kidneys were isolated and perfused with a physiological salt solution (PSS) under constant flow conditions. Renal vascular resistance changes were assessed in response to high, but sub-maximal intra-arterial doses (0.1 μg, N; 0.01 μg, D) of AII. Doses were repeated every 6 minutes and results are expressed as % of initial response.

Rat	PSS					PSS and D-600				
	% of initial response									
N	100	89	77	69	59	100	92	76	66	53
D	100	100	98	98	99	100	80	67	38	28
	0	6	12	18	24	0	6	12	18	24
	minutes from initial response									

Kidneys from N rats developed tachyphylaxis whereas those from D rats did not. When kidneys were perfused with PSS and D-600, a calcium antagonist, marked tachyphylaxis developed in D rats, but did not alter the existing tachyphylaxis in N rats. (This study was supported by NHLBI grant #HL-18575).

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FURTHER STUDIES ON DEPRESSOR ACTION OF KIDNEYS TRANSPLANTED ACUTELY INTO HYPERTENSIVE RATS. Tanenao Eto*, William M. Fraser* and Sibley W. Hoobler. Cleveland Clinic, Cleveland, OH 44106

The BP is lowered acutely by normal kidney transplants into rats with various forms of hypertension such as DOCA-salt; 1 kidney-1 clip; SHR. It is not lowered in hypertension induced by renin administration to the recipient rat. The depressor function of the normal kidney appears to be blunted by perfusion of the kidney with angiotensin II in a subpressor dose. When the kidney of a normal Wistar rat is transplanted into an SHR the mean fall in BP at 45 min is -42 ± 8.SEM mmHg (n=6); when both the donor rat and the SHR recipient are pretreated with sufficient indomethacin to inhibit prostaglandin synthesis, the BP reduction was comparable: -50 ± 4.SEM mmHg (n=6). It is therefore unlikely that prostaglandin is the mediator of the depressor effect. From these experiments and previous work on the subject it is evident that the normal kidney, when exposed to hypertension, releases an unknown vasodepressor which is not related to the renin angiotensin system and in fact may be inhibited by it.

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THE EFFECT OF NEONATAL OVARECTOMY ON BLOOD PRESSURE (BP) AND MYOCARDIAL BETA RECEPTORS AND ADENYLATE CYCLASE (AC) IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). S.G. Iams, S.J. Blumenthal* and M.M. McConnaughey*, Depts. Physiol. and Pharmacol., East Carolina Univ., Greenville, NC 27834

We have previously reported that the development of hypertension could be inhibited by gonadectomy of 30 day old male and female SHRs, (J. Lab. Clin. Med. 90:997, 77 and 94:608, 79). Sex steroids have been reported to cause hypertension and alter the responsiveness of vascular tissue to adrenergic stimulation. Changes in beta receptors and/or myocardial AC might explain the BP retardation seen in the gonadectomized SHR. SHRs, ovariectomized (OVX) at 3 to 5 days, used in this study, had a significantly ($P<.05$) lower BP at age 30 and 90 days. There was no change in the apparent number of beta-adrenergic receptors as assessed by [^{3}H]-dihydroalprenolol (DHA) binding, or basal AC activity. However, the % stimulation of AC by 1μM isoproterenol (ISO) was significantly ($P<.05$) lower in the OVX rats.

AGE (days)	30		90	
	Sham	OVX	Sham	OVX
B.P.	123±2(9)	110±2(9)*	178±2(8)	162±5(5)*
DHA Bound (fmol/mg)	45±5(7)	52±5(8)	32±1(8)	35±3(5)
% ISO stim AC	69±7(9)	52±6(9)*	63±6(8)	31±6(5)*

*Significantly less than Sham

The results suggest that the lower BP seen in the OVX SHR was not caused by altered numbers of beta receptors or basal AC activity; however, changes in AC stimulation might play a role.

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VASOPRESSIN IN THE NEW ZEALAND GENETICALLY HYPERTENSIVE (NZGH) AND NORMOTENSIVE (NZNR) RAT. J. T. Crofton, C. M. Allen*, L. Share, P. G. Baer and B. C. Wang. Univ. of Tenn. Center for the Health Sciences, Memphis, TN 38163

To determine if vasopressin is involved in either the development or maintenance of hypertension in NZGH rats, 24-h urinary excretion of vasopressin ($U_{ADH}V$), as an index of vasopressin secretion, was measured weekly for 8 weeks, starting when both NZGH and NZNR were 4 weeks old. While $U_{ADH}V$ increased progressively with time in both groups ($p < 0.01$), no differences were found between the two groups. Systolic blood pressure (SBP) increased from 87 ± 4 to 131 ± 6 mm Hg ($p < 0.01$) in the NZNR and then plateaued, whereas it rose progressively from 105 ± 4 to 184 ± 5 mm Hg in the NZGH ($p < 0.01$). SBP was significantly different ($p < 0.001$) between the two groups at each of the 8 weekly measurements. Between the 10th and the 12th week of the experiment, the pressor responsiveness to bolus i.v. injections of vasopressin and angiotensin II was measured in conscious NZGH and NZNR rats. Response of the NZGH to graded i.v. injections of both hormones was nearly twice that seen in the NZNR. In view of the increased responsiveness of the NZGH rat to pressor agents, vasopressin could be a pathogenetic factor in this form of hypertension, even though its secretion rate is not elevated. (Supported by USPHS Gr HL-19209 and USPHS Gr HL-12990)

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INFLUENCE OF CONVERTING ENZYME INHIBITION AND UNCLIPPING ON BILATERAL RENAL RESPONSES IN GOLDBLATT HYPERTENSIVE RATS. W. C. Huang*, D.W. Ploth, P.D. Bell, D. Jirakulsomchok*, and L.G. Navar. Univ. of Alabama Med. Ctr., Birmingham, AL 35294.

Studies were performed to evaluate the effects of combinations of converting enzyme inhibitor (CEI) infusion (3 mg/kg·hr) and unclipping on the renal function of each kidney of two-kidney, one clip (0.25 mm clip for 3-4 wks) Goldblatt hypertensive (GH) rats. Unclipping of 5 GH rats 1 hr before CEI resulted in fall of BP from 152 ± 5 to 109 ± 7 mmHg and decreases in GFR (25%), urine flow (V, 38%), and sodium excretion ($U_{Na}V$, 34%) for contralateral kidneys and increases in these indices (51%, 63%, and 1114%, respectively) for the unclipped kidneys. Superimposed CEI infusion resulted in further decrease in BP (Δ8 mmHg) and significant increases in all indices of renal function of both kidneys. Infusion of CEI for 1 hr before unclipping of 5 GH rats resulted in decreases of BP from 157 ± 7 to 136 ± 3 mmHg and increases of GFR (43%), V (96%) and $U_{Na}V$ (105%) for contralateral kidneys and decreases of these indices (47%, 41%, and 45%, respectively) for clipped kidneys. Unclipping during CEI infusion resulted in decreases of BP from 129 ± 11 to 121 ± 4 and to 109 ± 8 mmHg at 30 min and at 1 hr. Increases in GFR (27%), V (187%), and $U_{Na}V$ (3209 %) for the unclipped kidneys and decreases of these indices for the contralateral kidneys were observed. These data indicate that unclipping and CEI do not result in identical renal effects and suggest that mechanism(s) in addition to reduced angiotensin II may be involved in these responses.

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COMPARATIVE EVALUATION OF FUNCTIONAL CHANGES OF VASCULAR SMOOTH MUSCLE (VSM) OF THE SHR AND DOCA HYPERTENSIVE (HT) RAT. J.A. Wren*, R. Watkins*, and I.W.F. Davidson, Bowman Gray Sch. Med., Winston-Salem, N.C. 27103 (Supp. in part by N.C. Heart A.)

Contraction velocity analysis assesses the phasic and tonic contributions (Watkins and Davidson, E.J.P. 62:177, 1980) to total developed tension of VSM. We used this method to quantitate the functional changes that occur in SHR and DOCA HT. Male, SD rats were unilat. nephx. and injected with DOCA (3mg, SC, biweekly) plus saline for 12 wks. BW were 391g control (C) and 387g DOCA. The BW of male SHR were 380g and WKY (C) 392g. Aortic helical strips, in KR bicarb. 37C, were contracted with NE ($1X10^{-5}$M). Velocity of tension development (T,mg) was fitted to: $T=Ae^{-\alpha t}+Be^{-\beta t}$, where the terms reflect phasic and tonic component contribution, respectively. (*p<.01)

	N	Tm,mg	A,mg/min	α,min^{-1}	B,mg/min	β,min^{-1}	%phasic	%tonic
WKY	10	1073	3671	9.15	155	0.22	36.2	63.8
SHR	9	506*	3289	6.53*	41*	0.25	70.8*	29.2*
C	26	776	3285	9.00	119	0.29	48.8	51.2
DOCA	20	635*	3005	8.13*	72*	0.27	61.2*	38.4*

Tmax was decreased in both HT models. Contribution of the tonic component to the contraction was decreased and the phasic increased in both SHR and DOCA HT. The significant decrease in the phasic rate constant, α, indicates that Ca^{++} influx and availability to the contractile elements is decreased. These changes in function, observed in both forms of HT, indicate a relative inability of VSM to develop maximal tension.

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EFFECT OF CHRONIC TREATMENT WITH CAPTOPRIL ON REACTIVITY OF AORTIC RINGS FROM NORMOTENSIVE AND HYPERTENSIVE RATS. D.C. Kikta* and M.J. Fregly (SPON: C.C. Barney). Department of Physiology, University of Florida, Gainesville, FL 32610.

The effect of chronic treatment with the antihypertensive agent, captopril (C), a converting enzyme inhibitor, on reactivity of aortic rings from 4 groups of rats was studied: 1) normotensive, untreated rats (NT), 2) normotensive, C-treated (48 mg C/kg b.w./day for 5 wk) rats (NT-C), 3) renal hypertensive (renal encapsulation for 5 wk), untreated rats (HT), and 4) hypertensive, C-treated rats (HT-C). Contractile responses to angiotensin I (A-I) (10^{-10} to 10^{-6} M) did not differ statistically among the 4 groups; although responses of the HT-C group to A-I tended to be less than those of the other 3 groups. Contractile responses of all 4 groups of rings to A-I were attenuated following addition of C (2 X 10^{-4} M) to the bath for 30 min. These data indicate that C was washed out of the NT-C and HT-C rings during the 2 hr premeasurement equilibration period since C had to be added to the bath in order to alter the response to A-I. Chronic treatment with C significantly attenuated contractile responses to KCl (20 to 100 mM), norepinephrine (10^{-9} to 10^{-5} M), and phenylephrine (10^{-8} to 10^{-4} M). Therefore, chronic administration of C appears to alter vascular smooth muscle such that its responsiveness to α-adrenergic agonists, in the absence of C, is depressed while responsiveness to A-I is not changed. (Supported by Grant AG308 from the American Heart Assn., Florida Affiliate).

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CONTRIBUTION OF ADRENERGIC NERVE ENDINGS TO POTASSIUM RESPONSE IN SHR RESISTANCE ARTERIES. Clifford W. Whall and David F. Bohr. University of Michigan, Ann Arbor, MI 48109

An increase in extracellular potassium ion(K) concentration causes contraction of isolated blood vessel segments, both by direct depolarization of the smooth muscle and by the release of norepinephrine(NE) from adrenergic nerve endings. We have determined the contribution of adrenergic nerve endings to this K response in isolated mesenteric resistance arteries (≈150μm ID) from spontaneously hypertensive(SHR) and normotensive(WKY) rats. Vessels were mounted as described previously (Blood Vessels 17:1-5,1980), and contractile force(mN/mm vessel length) determined in response to 124mM K depolarizing solution in the absence and presence of the α blocker phentolamine(PTN;$5x10^{-7}$M). SHR vessels(n=10) developed a greater contractile force than did those from WKY(n=7) in the absence of PTN. Alpha-blockade produced a greater reduction in SHR (47%) than in WKY (35%) vessels. These results suggest that: 1) a large component of K induced contraction in both SHR and WKY is due to NE released from nerve endings in the vessel wall; and 2) there is an enhanced adrenergic component of the response to elevated K in SHR. (Supported by Michigan Heart Association #34152 and NHLBI grant #HL-18575).

Contractile Force

	SHR	WKY	
K	3.0±.2	2.0±.3	*
K+PTN	1.6±.1	1.3±.2	NS
	*	*	

(*=n<.05

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DIETARY SODIUM INDUCED HYPERTENSION AND CATECHOLAMINE EXCRETION IN THE SPRAGUE-DAWLEY RAT. Harold D. Battarbee, J.W. Dailey*, Laurel McNatt*, and G.E. Farrar*.Depts. of Physiology and Pharmacology, LA State Univ. Med. Ctr., Shreveport, LA 71130.

Male rats(255±10g) were divided into 3 groups and fed diets containing: 0.21 mEqNa and 0.23 mEqK/g (Control Diet), 0.91 mEqNa and 0.24 mEqK/g (High Na Diet), 0.98 mEqNa and 0.35 mEqK/g (High Na+K Diet). Systolic blood pressures (SBP) and 24 hr urinary norepinephrine(NE) excretion were measured weekly for 4 wks.Control SBP did not differ significantly from 123±1 mmHg (SE) over the period. High Na SBP rose to 146±3 mmHg, 150±4 mmHg, 152±4 mmHg and 150±3 mmHg for dietary wks 1,2,3 and 4 respectively(p<.001). High Na+K SBP rose to 146±3mmHg, 152±4 mmHg, 153±3 mmHg and 147±3 mmHg for wks 1,2,3 and 4 (p<.001). Animals were selected from High Na Forming 2 subgroups-those with SBP ≤ 150 mmHg and those with SBP ≥ 170 mmHg. NE was suppressed -15.7±8.5% in SBP ≤ 150 mmHg and -32±3% (p<.01) in SBP ≥ 170 mmHg by the end of the 1st wk. High Na+K NE did not suppress despite a Na intake and SBP similar to that of High Na. NE of SBP ≥ 170 mmHg (+8.4±10%) differed from SBP ≤ 150 mmHg (-18±7%) at wk 3 (p<.05). NE at the 2nd and 4th wks were not different. The results indicate that acute dietary sodium loading suppresses NE and elevates SBP. A dietary K supplement prevents this suppression even though SBP is elevated. More prolonged dietary Na loading is accompanied by a return of NE to control levels despite a continued elevation of SBP. This results in an inappropriately high NE rate for the level of blood pressure observed. (Supported in part by a grant from the American Heart Association of Louisiana, Inc.)

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ATTENUATION OF THE CENTRAL ACTIONS OF ANGIOTENSIN II (AII) IN SODIUM DEPLETION (SD). Julianna E. Szilagyi and Carlos M. Ferrario. Cleveland Clinic Research Division, Cleveland, OH

We have shown that activation of the opiate system in the brain is necessary for the expression of the central actions of AII at the level of the area postrema. Since SD is accompanied by elevated plasma AII and blunted vasoconstrictor responses to intravenous (iv) AII, we evaluated the effects of SD on the interaction between the central action of AII and the opiate receptors. Mongrel dogs (N = 15) were placed on a normal (40 mEq Na/day; NS) or SD (4 mEq Na/day) diet for 21 days. All dogs were anesthetized with a mixture of morphine (2 mg/kg im) and α-chloralose (66 mg/kg iv). Dose-response (DR) curves to AII given either iv or into the vertebral arteries (vtb) were compared before and after naloxone (0.8 mg iv; Nx) administration. Nx given to NS dogs resulted in a significant parallel shift of the DR curve to AII (vtb) to the right of control. DR curves to AII in SD dogs (vtb and iv) significantly shifted to the right of the NS controls. The iv and vtb curves were indistinguishable and also identical to that obtained in NS dogs (vtb) after Nx. Nx did not alter the SD DR curves. These data indicate that 1) SD abolishes the expression of the sympathetically mediated pressor response to central AII; 2) the absence of changes in responses to vtb AII after blockade with Nx may suggest a concurrent desensitization of the opiate mediated effect after SD. (Supported by NHLBI #HL-6835 and HANEO Fellowship).

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EFFECTS OF CAPTOPRIL ON PULMONARY ANGIOTENSIN II FORMATION. Peter C. Houck, Mary Fiksen-Olsen*, Steven L. Britton, and J. Carlos Romero, Dept. Physiology, Mayo Clinic, Rochester, MN.

Experiments were performed to examine the effect of small doses of Captopril on the pulmonary formation of angiotensin II (AII). Angiotensin I (AI) was infused into the vena cava at a dose which maintained mean arterial blood pressure 20 mmHg above baseline. Blood samples were drawn from the pulmonary artery and left ventricle for measurement of AII by radioimmunoassay. Arterial minus venous AII concentration represents pulmonary AII formation. After mean arterial blood pressure returned to baseline the lowest of three doses of Captopril (0.005, 0.01, 0.02 mg/kg) was administered as an intravenous bolus. Ten minutes later the same dose of AI was infused which originally produced a 20 mmHg increase in blood pressure. This procedure was repeated for the two remaining doses of Captopril. These increasing doses of Captopril produced an increasing blockade of the pressor response to the AI infusion. This reduction in the pressor response was paralleled by a progressive inhibition of pulmonary AII formation. These results show that Captopril inhibits pulmonary formation of AII. These data suggest that part of the decrease in the pressor response to an AI infusion following Captopril is due to a decreased formation of AII in the pulmonary circulation. These data do not, however, exclude the possibility that peripheral formation of AII participates in the pressor response to an AI infusion. (Supported by NIH grant HL 16496)

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REFLEX INHIBITION OF RENIN SECRETION AND INDUCED RENAL VASODILATION FOLLOWING ACUTE LEFT CIRCUMFLEX OCCLUSION IN DOGS. Avi Livnat* and John E. Zehr. Department of Physiology, University of Illinois, Urbana, Illinois 61801.

We examined the reflex effect of brief coronary artery occlusion (CAO) on renal blood flow (RBF) and renin secretion (RS). Studies were conducted in chloralose-anesthetized dogs maintained on a salt-free diet for at least 3 days. A snare was placed around the left circumflex artery near it's origin. The left renal artery and vein were exposed via flank incision, a flow probe was placed around the artery and a curved needle inserted into the vein for collection of renal venous blood. Values of BP, RBF, and RS were obtained for a 30 min control period, 4 min after the completion of a 10 ml/kg hemorrhage, immediately following and 6 min after CAO. The CAO consisted of two 1 min occlusions separated by a 1 min interval. The results indicate that CAO reflexively inhibits the RS response to nonhypotensive hemorrhage and prevents renal vasoconstriction (P<0.05, n=6). Both effects were completely abolished by vagotomy (n=4). Renal denervation also abolished the response (n=3). No response was observed during identical time-controls (n=3). The response was more pronounced in dogs with carotid sinus denervation even though CAO resulted in marked reductions in BP (20%) under these conditions. These results demonstrate the presence of a cardio-renal reflex which can be activated by myocardial hypoxia and which acutely suppresses RS. This response is more pronounced in the absence of CS buffering. (Supported by HL 15307 and the Illinois Heart Association.)

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BLOOD KININS IN NORMAL SUBJECTS AND IN PATIENTS WITH CONGENITAL DEFICIENCY IN PLASMA PREKALLIKREIN AND KININOGEN. A new method for its determination. Alfonso G. Scicli*, Theodor Mindroiu* and Oscar A. Carretero (SPON: M. Webster). Henry Ford Hospital, Detroit, MI 48202.

Circulating kinins may be important in the regulation of blood flow and blood pressure. Blood kinins have been measured in normal subjects, however, the concentrations reported varied from 0.1 to 5 ng of bradykinin (bk) per ml of blood. We have developed a new method to measure blood kinins. Blood (≤6 ml) is collected in less than 10 sec directly into 25 ml of ethanol. Kinins are further purified by extracting lipids with ether and by removing kininogen and other interfering substances by chromatography on QAE Sephadex and BioRex 70 then kinins are measured by RIA. Recovery is 50%. In 17 normal subjects (random samples) bk concentration after correcting for recovery was 28±3.6 pg/ml of blood, while in 4 other subjects bk was below the limit of sensitivity (11 pg/ml). Bk in three patients deficient in plasma prekallikrein (Fletcher trait) was 16, 21 and 31 pg/ml. One patient deficient in high molecular weight (HMW) kininogen (Fitzgerald trait) had 26 pg/ml while another which was deficient in both low and HMW kininogen (William trait) had no detectable bk. These results indicate that bk levels in normal subjects are much lower than those previously reported. Further, bk are in the normal range in patients deficient in either plasma prekallikrein or its substrate. This suggests that renal and/or other glandular kallikrein generate at least part of the circulating kinins.

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HORMONAL CONTRIBUTIONS TO MYOCARDIAL HYPERTROPHY IN RATS.
Caroline O. Oyedeji*, Karam F. A. Soliman and Maurice S. Holder*, Florida A&M University, School of Pharmacy, Tallahassee, Florida 32307

The effects of angiotensin II (A-II), thyroxine, adrenal medullectomy, norepinephrine (NE) and exercise on myocardial hypertrophy produced by these methods were assessed by using ventricular weight to body weight ratios (VW/BW). The highest value was obtained with exercise (23.4%). Hyperthyroidism by the addition of thyroxine (2 mg/liter) in drinking water showed a 15.6% hypertrophy, A-II 13.8% and NE 9.5%. These responses were not aged related. Decline of the hypertrophy effects of these drugs was accomplished with propranolol in all cases except in A-II treated animals, however, it was not totally abolished. Adrenal medullectomy by itself had no hypertrophic or atrophic effects on the heart while atrophy occurred with thyroidectomy. Treatment with Angiotensin II for a fixed period of time and then stopping for the same period resulted in a reversal of hypertrophy. The results confirms pervious evidence which has shown that NE might be involved in the hypertrophic process of the heart and other hormones may bring about equal or greater degrees of hypertrophy. Moreover, the results show that angiotensin II appears to stimulate hypertrophy independently of NE or thyroxine.
(Supported by a FAMU Institutional Grant, NIH Grant #RR08111 and NASA Grant NSG 2183)

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CARDIOVASCULAR RESPONSES TO PARTIAL OBSTRUCTION AT VARIOUS POINTS IN THE INTRATHORACIC CIRCULATION OF CONSCIOUS DOGS.
K.L. Goetz, H.D. Schultz*, W.D. Sundet*, and D.C. Fater.
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Partial occlusion at various sites along the intrathoracic portion of the circulation has been attempted by Henry et al. (Circ. Res. 4:85, 1956) in anesthetized dogs, but similar experiments on conscious dogs have not been reported. We now describe the hemodynamic effects elicited by partial occlusion of the mitral orifice (MO), pulmonary veins, pulmonary artery, tricuspid orifice (TO), and inferior vena cava in conscious dogs. In another abstract (H.D. Schultz et al.) we describe the renal responses produced by these maneuvers. The most dramatic cardiovascular responses were elicited by obstructing the circulation at the MO. Obstruction sufficient to produce an increase in left atrial pressure of nearly 10 mm Hg caused increases in pulmonary arterial and aortic pressures and a decrease in central venous pressure. HR increased, SV decreased, and cardiac output was constant or increased slightly. TPR tended to increase. In contrast, partial occlusion at the other sites produced quite different effects. For example, the only other site at which occlusion elicited an increase in HR was the TO, and the response was considerably smaller than that elicited by mitral occlusion. These results demonstrate that the reflex circulatory responses elicited by acute partial obstruction of the intrathoracic circulation vary with the site of obstruction. (Supported by NIH grant HL13623).

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RENAL RESPONSES TO PARTIAL OBSTRUCTION AT VARIOUS POINTS IN THE INTRATHORACIC CIRCULATION OF CONSCIOUS DOGS.
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St. Luke's Hospital and Foundation, Kansas City, MO 64111

Henry et al. (Circ. Res. 4:85, 1956) partially occluded various sites within the intrathoracic circulation and reported that a diuresis occurred in anesthetized dogs only when the left atrium was included in the area engorged by the occlusion. This report to our knowledge has not been confirmed. We now describe related studies in the conscious dog. Partial occlusions were produced at the mitral orifice, pulmonary veins, pulmonary artery, tricuspid orifice, and inferior vena cava (IVC). Hemodynamic variables were measured and are reported in another abstract (K.L. Goetz et al.). UV and $U_{Na}V$ increased and Uosm decreased during a 30 min obstruction of the mitral orifice and returned towards control values afterward. No changes in these variables occurred during occlusion of the pulmonary veins, pulmonary artery, or tricuspid orifice. A slight decrease in UV and $U_{Na}V$ occurred during IVC occlusion. PRA decreased with mitral obstruction and increased with IVC occlusion. UV and $U_{Na}V$ correlated with changes in left atrial pressure, but no consistent relationship could be detected for the other hemodynamic variables. These results are consonant with the suggestion of Henry et al. that the diuresis elicited by increasing left atrial pressure with a balloon is reflexly elicited from left atrial receptors. (Supported by NIH grant HL13623).

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AN IMPLANTABLE TELEMETRIC DEVICE FOR *IN VIVO* PRESSURE MONITORING.
David Fleming, John Bettice, Albert Leung* and Wen Ko*. Engineering Design Center, Case Western Reserve University, Cleveland, Ohio 44106

We have designed, developed and fabricated a small, fully implantable telemetric device for the continuous, long-term monitoring of intracranial pressure (ICP) or arterial blood pressure (BP) in freely moving animals. An *in vivo* testing program to demonstrate the stability, accuracy and biocompatibility of the implant device is underway. The regimen of testing involves comparison of simultaneous ICP measurements made with an externally placed, commercially available transducer connected to the implant system via a transcutaneous connector. Tests of several units have shown that the implant device is capable of long-term, *in vivo* operation which provides accurate and rapid measurement of pressure changes with some drift in baseline measurement when implanted for an eleven week period. The unit has also been used for BP monitoring. A vapor driven implantable pump filled with heparinized saline is used in conjunction with the implant to provide a clot-free pathway from the transducer to the artery. Initial, acute studies have demonstrated the practicality of using the unit for the continuous, long-term monitoring of BP in freely moving animals without the risk of transcutaneous catheters.
(Jointly supported by NIH grants GM 14267 and RR 00857)

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MEASUREMENT OF EVANS BLUE DYE IN BLOOD FOR THE DETERMINATION OF BLOOD VOLUME IN RHESUS MONKEYS.
C. T. Liu, R. P. Sanders*, and M. J. Griffin*, U.S.Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD 21701.

Evans blue dye is used for the determination of plasma volume. Only a limited number of indirect blood volume measurements may be made in small or hypovolemic animals. A new technique was established for blood volume determinations, without net blood loss. A baseline signal was first established with arterial blood from 4-10-kg, unsedated, chair-restrained monkeys. Blood was withdrawn into a heparin-wetted pump syringe and returned i.v.. Evans blue (2.26 mg) was injected into the femoral vein, and 6 minutes later arterial blood was again withdrawn by syringe pump at a rate of 6 ml/min through a sterile cuvette-densitometer, monitored at 630 nm. After the signal was recorded, blood was returned i.v. Dye concentration was determined from a calibration curve of Evans blue (0-12.5 µg/ml) in arterial blood. Similar curves were also obtained when dye was mixed in blood with different hematocrits and from different animals (baseline set by corresponding blood blank). Since a factor (1.35 ± 0.01) was derived to convert saline to blood values (pen deflection) at any given concentration, a saline standard curve could also be used as a substitute for blood in calibration. Mean rhesus monkey blood volumes (n = 14) were shown to be 71.4 ± 8.9 (SD) ml/kg, agreeing with published data (J. Med. Primatol. 5:336, 1976). The new technique is suitable for the determination of blood volume in monkeys or small animals.

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AN IMPLANTABLE MULTICHANNEL BIOTELEMETRY SYSTEM FOR PRESSURE, ECG, AND CARDIAC OUTPUT MEASUREMENTS IN THE ADULT YUCATAN MINIATURE BOAR.
J.M. Terris and R.C. Simmonds*. Dept. of Physiology, Uniformed Services Univ., Bethesda, MD 20014

A completely implantable, 250g multichannel biotelemetry system (Konigsberg Instr., Pasadena, CA) is in use in the adult Yucatan miniature boar (150-250 lbs). The system sensors include implantable pressure transducers for central venous (6mm) and arterial (7mm) pressures, 2 ECG leads, and a 22mm ID electromagnetic flow probe (Zepeda Instr., Seattle, WA) for cardiac output determination. Sensor leads and flowmeter module are connected to a hermetically sealed mainframe package (5.2x2.2 x1.1cm) containing the power converter, signal conditioner, clock generator, and multiplexer. Power is provided by Ni-Cad batteries rechargeable by inductive coupling from an external power source. Incorporated into the power pack is an 88-108 MHz oscillator and transmitting antenna, inductive pickup coil for battery recharging, and an externally controlled RF switch for turning the transmitter on & off and controlling the flow channel mode. Completing the system are a receiver and demodulator that convert the signal to the original waveforms. The flowmeter module, mainframe module and sensors are implanted in the thorax; Ni-Cad batteries, transmitting antenna, and inductive pickup coil are subcutaneous. Current chronic studies are determining the full potential of this system. It has so far proven to be a convenient method for obtaining pressure, ECG, and cardiac output measurements in our adult pig model, and precludes having to remove the animals from their pens.

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BLOCKADE OF THE PRESSOR ACTIVITY OF EXOGENOUS ANGIOTENSINS BY ANGIOTENSIN ANTAGONISTS IN THE FRESHWATER TURTLE.
G. A. Stephens. Univ. of Delaware, Newark, DE 19711

The pharmacological characteristics of the renin angiotensin system were studied in a primitive vertebrate, the red-eared pond turtle, Pseudemys scripta elegans. Both angiotensin II-amide (AII) and angiotensin I (AI) injected intravenously at doses of 0.1, 0.5 and 1.0 µg/kg produced dose dependent increases in arterial blood pressure in conscious turtles. The AI pressor responses averaged 70% of the AII response at the same dosage. Infusion of the angiotensin II analogue [Sar^1, Ala^{8}] - AII (10 µg/kg/min) reduced the pressor responses to AI and AII by an average of 68% and 80%, respectively. The converting enzyme inhibitor SQ 20881 (1 mg/kg bolus + 0.5 mg/kg/hr) did not alter the response to AII but reduced the response to AI by 76%. Neither [Sar^1, Ala^{8}] - AII nor SQ 20881 altered the pressor response to norepinephrine (3 µg/kg). The alpha adrenergic antagonist phentolamine (0.5 mg/kg bolus + 1 mg/kg/hr) decreased the pressor response to norepinephrine by 77% and decreased the responses to AI and AII by 39% and 50%, respectively. The results suggest that an angiotensin converting enzyme may exist in the turtle similar to that found in mammals and that the pressor response to angiotension in the turtle may be partially due to catecholamines. (Supported by Univ. of Del. Res. Foundation)

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CARDIOPULMONARY STABILITY OF THREE CONSTANT-DOSE CHLORALOSE ANESTHETIC MAINTENANCE REGIMES. C. Buchanan*, B. Rubal, B. McNichols*, and J. Lucas*. Texas Woman's Univ., Denton, TX

In this study, cardiopulmonary stability (CPS) of three constant-dose, twelve hour, chloralose anesthetic maintenance regimes were assessed. Fifteen dogs were premedicated with 5 mg/kg morphine and anesthetized with α-chloralose (C). All dogs were given 28 mg/kg C each hour after initial induction with 80 mg/kg C. Group I(G-I) dogs were maintained by constant infusion of C and allowed to breathe spontaneously; group II(G-II) dogs were constantly infused with C and ventilated; group III(G-III) dogs were maintained by the hourly bolus of C and allowed to breathe spontaneously. Heart rate (HR), arterial diastolic pressure (DP), pulse pressure (PP), % change max dP/dt and arterial blood gases were compared at hourly intervals. In general, differences ($P < 0.05$) were found in the CPS within the first six hours, and consistent trends noted throughout the 12-hour anesthetic period. HR, DP, % change max dP/dt and PCO_2 were greatest in G-I but severe hypoxemia and acidemia were present. In the ventilated group, blood gases were optimal, HR was the lowest and PP was the greatest. Dogs in G-III were acidemic, hypoxemic and had the lowest DP and PP but demonstrated the least alteration in % change max dP/dt. The mean HR and DP in this experiment ranged from 49-102 bpm and 53-86 mmHg, respectively. These data suggest that there is a difference in the CPS of long term constant-dose chloralose anesthetic maintenance regimes. (Supported by AHA, Texas Affiliate #41068.)

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EVIDENCE THAT LIPID A IS THE ENDOTOXIN MOIETY WITH HYPOGLYCEMIC AND INSULIN-LIKE ACTIVITIES. L. Witek-Janusek and J. P. Filkins. Depts. of Physiology and Maternal Child Health Nursing, Loyola University of Chicago, Maywood, IL. 60153.

Systemic endotoxemia induces profound, progressive hypoglycemia (Circ. Shock 5: 347-355, 1978). The present study evaluated the moiety of endotoxin, i.e., lipid A, carbohydrate (CHO), or protein that is responsible for hypoglycemic activity (HA) in vivo and insulin-like activity (ILA) in vitro. HA was evaluated using the lead-sensitized rat (PSEBM 134: 610-612, 1970). ILA was quantitated using glucose oxidation in the rat epididymal fat pad (Circ. Shock 5: 317-323, 1978).

Endotoxin Preparation	Composition			Activity	
	Protein	CHO	Lipid A	HA	ILA
S. enteritidis, Boivin(SeB)	+	+	+	+	+
S. enteritidis, Westphal(SeW)	-	+	+	+	+
SeB + Alkaline hydrolysis	+	+	-	-	-
S. minnesota Re 595 (Sm)	-	±	+	+	+

Thus, the following findings suggest lipid A is responsible for the HA and ILA of endotoxin: positive HA and ILA using preparations with intact lipid A but no protein (SeW) or containing no protein and little CHO (i.e., the heptose-deficient mutant strain, Sm) and loss of HA and ILA following alteration of the lipid A structure by mild alkaline hydrolysis (0.25 N NaOH for 60 min at 100°C) of SeB. Therefore, the lipid A moiety of endotoxin appears to play a central role in its HA and ILA. (Supported by NIH Grant HL 08682.)

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INFLUENCE OF HEMODYNAMIC RESPONSES ON PLASMA VASOPRESSIN LEVELS DURING ENDOTOXIN SHOCK. D.J. Brackett, P. Tompkins* and M.F. Wilson. VA Medical Center and Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

The purpose of this study was to correlate plasma vasopressin (PVP) levels with hemodynamic responses during endotoxin shock. Two groups of 8 dogs each were infused with either endotoxin (LD-100) or saline. After pentobarbital anesthesia, the femoral vessels were catheterized for infusion and for measurement of cardiac output, aortic pressure, and left ventricular pressure. Respiration parameters, pH, pO_2, pCO_2, and hemoglobin were also measured. PVP was measured by radioimmunoassay. Control PVP levels were always less than 40 pg/ml and remained at this level in the saline infusion group for the entire 4 hr. observation period. In the endotoxin infusion group PVP levels were elevated within 30 min. and remained elevated for at least 4 hr. Increased PVP levels were observed in a portion of the animals before the development of hypotension. When severe hypotension (less than 40 mmHg) was momentarily produced PVP reached levels greater than 1100 pg/ml. Both responses are consistent with reports that vasopressin is released in response to hypovolemia as well as hypotension. Superior mesenteric artery constriction and cardiac dysfunction have been reported at PVP levels observed in this study. (Supported by VA Research Service and Oklahoma Heart Association.)

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IN VIVO SKELETAL MUSCLE INSULIN RESISTANCE DURING E. COLI ENDOTOXIN SHOCK IN THE DOG. Richard M. Raymond*, James M. Harkema* and Thomas E. Emerson, Jr. Depts. of Physiology and Surgery, Mich. State Univ., East Lansing, MI 48824

The action of insulin in stimulating glucose uptake by an isolated, innervated, constantly perfused gracilis muscle preparation was studied during 0.5 mg/kg intravenous E. coli endotoxin administration. Mongrel dogs of both sexes (20 ± 2 kg) were anesthetized with sodium pentobarbital (30 mg/kg),heparinized (10,000 units) and allowed to breathe spontaneously.Close intra-arterial infusion of insulin (25-100 mU/ml/min) to the gracilis muscle prior to endotoxin administration resulted in a 177% increase in muscle glucose uptake with no change in muscle oxygen uptake or blood gases. During insulin infusion, cross-matched blood was returned to the dog in place of the gracilis venous outflow. This was done to prevent muscle perfusate insulin from reaching the systemic circulation. Hourly insulin infusions to the gracilis muscle at 1, 2, 3 & 4 hours after endotoxin administration failed to stimulate muscle glucose uptake. Arterial glucose concentration increased initially following endotoxin, then at the third and fourth hour was below control. Arterial blood pressure decreased to approximately 40-50 mmHg throughout the experiment after endotoxin administration. These data provide direct evidence that an in vivo skeletal muscle preparation, perfused at constant inflow becomes resistant to insulin in promoting glucose uptake following endotoxin shock in the dog, and occurs without any local muscle metabolic alterations. (NIH GM26394)

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STEROID/ANTIBIOTIC TREATMENT FOLLOWING SUSTAINED HYPOTENSION RESULTING FROM LD_{100} E. COLI INFUSION. B.K. Beller-Todd*, L.T. Archer*, B.A. Benjamin*, D.J. Flournoy*, S.D. Kosanke*, R.B. Passey*, and L.B. Hinshaw. VA Medical Center and Univ. of Oklahoma Health Sci. Ctr., Oklahoma City, OK 73104.

Early, aggressive therapy with methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS) significantly increases survival of baboons given LD_{100} E. coli. This study was designed to determine if baboons would survive if initiation of MPSS and GS was postponed until sustained hypotension had occurred. All baboons were given 2-hr infusions of LD_{100} E. coli and monitored for 12 hr: Group I (N=8) received saline instead of treatment and Group II (N=4) received MPSS/GS 4 hr after the initiation of E. coli, at which time each animal had been hypotensive at least 3 hr. Three of 4 treated baboons survived while all untreated animals died. WBC and mature neutrophil concentrations were decreased in all animals throughout the observation period, but immature neutrophil numbers were greater by 8 and 12 hr in the treated compared with nontreated group ($p<0.05$). By 12 hr, E. coli blood concentrations were an order of magnitude lower in treated than nontreated baboons. Histopathologic lesions that consistently characterized all nonsurvivors were adrenal cortical and medullary congestion and hemorrhage, congestion of liver sinusoidal spaces and central veins, and excessive numbers of neutrophils in the hepatic sinusoids. Data show that survival is increased with MPSS/GS even after sustained hypotension. (Supported by VAMC and NIH HL24590.)

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ERYTHROCYTE STROMA-INDUCED RETICULOENDOTHELIAL SYSTEM DEPRESSION AND INCREASED SUSCEPTIBILITY TO INFECTION. G.J. Grover, D.J. Loegering, Department of Physiology, Albany Medical College, Albany, NY 12208.

Previous studies from this laboratory demonstrated that injection of hemolyzed blood or erythrocyte stroma depressed reticuloendothelial system (RES) phagocytic function and increased susceptibility to endotoxin shock. This study was done to determine if RES depression after phagocytosis of erythrocyte stroma or foreign particulates will increase susceptibility to bacterial infection in rats. Stroma from hemolyzed rat erythrocytes contained 112 mg/ml Lowry protein and 95 mg/ml hemoglobin. Phagocytic index, determined from the blood clearance of fixed sheep red blood cells, was decreased 32.4% ($p<.05$) and 36.2% ($p<.05$) after i.v. injection of 0.75 and 0.5 ml/100g stroma, respectively, but was unchanged with 0.3ml/100 g. Infection was induced by cecal ligation and puncture with stroma injection 1 hr after cecal ligation. Stroma, at 0.3 ml/100g had no effect on time to death, but time to death was decreased with 0.5 ($p<.05$) and 0.75 ml/100g ($p<.001$). Hemoglobin (100 mg/100g) had no effect on time to death. RES blockade with gelatinized lipid emulsion (50 mg/100g) and colloidal carbon (32 mg/100g) also decreased time to death following cecal ligation. Thus, RES depression with erythrocyte stroma was associated with increased susceptibility to infection. This data suggests that hemolysis and phagocytosis of erythrocyte debris after thermal injury may contribute to increased susceptibility to infection. (GM-26102)

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THE EFFECT OF ACTH AND HISTAMINE ON THE CIRCADIAN RHYTHM OF CORTICOSTERONE SECRETION FROM THE ADRENAL GLAND IN VITRO. Fanny L. Edwards* and Karam F. A. Soliman, School of Pharmacy, Florida A&M University, Tallahassee, FL 32307

The circadian variation in the in vitro secretion of corticosterone from the adrenal gland in response to adrenocorticotropine hormone (ACTH), and histamine was assessed in control and adrenal medullectomized Sprague-Dawley male rats. All animals were adapted for a minimum period of ten timed 12 hours dark-12 hour light ilumination cycle before use in these experiments. Drugs were added to the media at 1200 hr., 1800 hr., 2400 hr., and 0600 hr., and the glands were incubated for 1 hr. Results of these experiments show that the secretions of corticosterone from the adrenal gland vary diurnally when ACTH is added to the incubation media of intact and adrenal medullectomized animals. The peak occurred at 2400 hr. and the trough at 1200 hr. However when histamine or the drug vehicle were added to the incubation media of control and adrenal medullectomized animals, there was no significant diurnal variations in corticosterone secretions. Grant No. NIH-DRR-RR0811108.

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ACTH INDUCED ADRENAL Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THIOURACIL FED MALE MICE. Lee A. Meserve and Shu-mei Ting.* Dept. Biological Sciences, Bowling Green State University, Bowling Green, OH 43403

Effects of thiouracil induced hypothyroidism on adrenal Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) activity and its response to exogenous adrenocorticotropin (ACTH) was tested in male Swiss-Webster mice. Pregnant mice were fed Lab Blox Mash with or without 0.25% thiouracil throughout pregnancy and lactation, and offspring received the same diet until the end of experiment. Four month old euthyroid and hypothyroid male mice were decapitated after either: rapid removal from the cage, s.c. physiological saline once daily for 4 days; or s.c. ACTH (2IU) once daily for 4 days. Adrenal pairs were excised, weighed, and homogenized in glycine buffer for determination of 3β-HSD activity. Euthyroid mice evidenced significant increases in adrenal 3β-HSD activity concentration (92%) and total activity (120%) in response to ACTH, but not to saline injection. Thiouracil feeding increased unstimulated activity concentration, but not total activity. Saline injection did not increase enzyme activity of thiouracil fed mice, but ACTH administration increased activity concentration to that in ACTH treated euthyroid mice, and significantly increased total activity (104%). Results suggest the adrenal steroidogenic enzyme 3β-HSD is active in thiouracil fed mice and its activity increases in response to ACTH as in euthyroid animals. (Supported in part by a Bowling Green State University Faculty Research Grant.)

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ACTH and Corticosteroid (11OHCS) Responses to Hypoglycemia in Conscious Dogs. Maureen K. Wood*, Jeanette Shinsako*, and Mary F. Dallman. Dept. of Physiol., UCSF, San Francisco, CA 94143.

Study of factors that modulate the activity of the adrenocortical system require use of reproducible stimuli for which the quantitative relation between stimulus intensity and response is known. We have investigated insulin-induced hypoglycemia in conscious dogs deprived of food for 17h. 5 dogs were injected iv with saline, .01, .05, .10, .25, or .50 U insulin/kg BW. Doses were administered in random order with at least 72h between experiments. Frequent venous blood samples were drawn during 60 min for measurement of plasma glucose, ACTH, and 11OHCS. Plasma glucose, ACTH, and 11OHCS responses were insulin-dose dependent ($p < .01$). .25 and .50 U/kg produced equivalent maximum responses. A rapid 11OHCS (5-15 min) response occurred following decreases in glucose of only 15-20 mg/dl. The log of the peak ACTH concentration (at 28-34 min) was linearly related to the nadir in plasma glucose concentration ($r^2=.70$, $p < .001$). ACTH levels remained elevated if glucose remained below 40 mg/dl after 35 min, and returned to control values if glucose returned to within 10 mg/dl of its control concentrations.
CONCLUSIONS: The pituitary-adrenocortical system predictably responds to small, acute decreases in plasma glucose. The overall responses are determined by both the magnitude and duration of the fall in plasma glucose. (Supported by USPHS grants AM06704, AM07625, and NASA NCA2-OR665-806).

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Adrenal Responses are Initiated by Unmeasurable Increases in Plasma ACTH Concentration. Charles E. Wood*, Jeanette Shinsako*, and Mary F. Dallman. Dept. of Physiol., Univ. of Calif., San Francisco, CA 94143.

We have previously reported that 15 ml/kg hemorrhage (HEM) increased plasma ACTH 11 pg/ml and corticosteroids (11OHCS) 2.7 ug/dl, and that during hypovolemia, there was an apparent dissociation of ACTH and 11OHCS ($r^2=.06$). The purpose of this study was to test for HEM-induced shift in adrenal sensitivity to ACTH. Conscious dogs with chronic catheters were pretreated with dexamethasone (4 mg), 4 hrs before the experiment. Each dog underwent 2 experiments(±HEM at onset of 30 min ACTH infusion), 4 days apart; the order of experiments was randomized. Infusions of 5, 10, and 20 ng ACTH/min (N=4/dose) raised 11OHCS to levels linearly related to the log of the infusion rate. HEM did not change the magnitude of the 11OHCS response to ACTH. Cortisol half-disappearance time, and therefore adrenal gain, was not changed by HEM. Infusion of 5 ng ACTH/min elevated 11OHCS 3 ug/dl, while theoretically increasing ACTH 6-12 pg/ml in steady-state; the first rise in ACTH causing the first rise in 11OHCS (app. 3.5 pg/ml) would be unmeasurable leading to an apparent dissociation of ACTH and 11OHCS (assuming ACTH MCR=35 ml/kg/min, and lag of 3 min: Wood, et al, Fed. Proc. 39: 1072, 1980). Because the adrenals are normally exquisitely sensitive to ACTH, it is likely that increases in 11OHCS during 15 ml/kg HEM are caused by unmeasurable increases in ACTH. (Supported by USPHS grant AM06704 and NASA NCA2-OR665-806)

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RADIOIMMUNOASSAY OF CORTISOL AND 11-DEOXYCORTISOL IN POST-SPAWNED ATLANTIC SALMON, SALMO SALAR. Melvin Weisbart and D. Kenneth Jenkins*. St. Francis Xavier Univ., Antigonish, N.S. B2G 1C0

Attempts at developing radioimmunoassays (RIA) for cortisol and 11-deoxycortisol have met with success for cortisol and failure for 11-deoxycortisol. The cortisol RIA was validated by analysing each of six samples of blood by three different methods: double isotope derivative assay (DIDA) and RIA with and without chromatographic purification of the plasma extract. The RIA without chromatography gave significantly lower plasma cortisol concentrations ($P<0.01$) than the DIDA: the correlation coefficient was 80.3%. The RIA with chromatography tended to give lower values than the DIDA but higher values than the non-chromatographic RIA. The interassay coefficient of variation was 9.12% (n=8) whereas the intraassay coefficient of variation was 7.85% (n=6). The average water blank value per RIA tube was 3.18 ± 0.41 pg (n=14). 11-Deoxycortisol, which was definitively identified in the plasma of Atlantic salmon by DIDA, did not interfere with the cortisol RIA. Attempts to validate an RIA for 11-deoxycortisol by comparing each of six samples of blood by three different methods as outlined for the cortisol RIA met with failure. Very high and variable water blanks after thin-layer chromatography and failure to obtain satisfactory accuracy with the non-chromatographic RIA prevented the development of a satisfactory RIA for 11-deoxycortisol. (Supported by NSERC #A0781 and UCR #612.)

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COMPARATIVE EFFECTS OF PROLACTIN SUPPRESSION AND ESTROGEN ANTAGONISM ON THE GENESIS OF γ-IRRADIATION INDUCED MAMMARY TUMORS IN FEMALE SPRAGUE-DAWLEY RATS. Margaret Goodrich-Smith*, Carolyn K. Brown* and Clifford W. Welsch. Dept. of Anatomy, Michigan State University, East Lansing, Michigan 48824.

333 female Sprague-Dawley rats were divided into 8 groups (41-42 rats/group) and subsequently given a single exposure to 400 R of total body ^{137}Cs γ-irradiation at 59 days of age. Prolactin secretion was suppressed in 2 groups of rats by daily s.c. injections of 2-bromo-α-ergocryptine (CB-154) (400 µg/100 g b.wt.) from 29-89 days of age (Series 1) or from 89-149 days of age (Series 2), prior to the onset of palpable mammary tumors (MT). The estrogen antagonist Tamoxifen was injected s.c. daily (50-80 µg/100 g b.wt.) to 2 groups of rats during the time spans indicated above for CB-154. 4 groups of rats were injected with the diluent and served as controls. All rats were palpated bi-weekly for MT and sacrificed 11 months after γ-irradiation. Total number of palpable MT which developed in each group and significance levels were:

Series 1, controls, 43; CB-154 treated, 46. $P>0.05$
Series 2, controls, 58; CB-154 treated, 42. $P<0.05$
Series 1, controls, 51; Tamoxifen treated, 36. $P<0.05$
Series 2, controls, 51; Tamoxifen treated, 25. $P<0.05$

These results provide evidence that estrogens are important in both the initiating and early promotion phases of γ-irradiation induced murine mammary tumorigenesis whereas prolactin appears to be important only in the early promotion phase of this neoplastic process. (Supported by NIH contract N01-CB-74090)

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ANDROGEN-RELATED HEPATIC TUMOR IN FANCONI'S ANEMIA (FA). R.P. Spencer, M.K. Karimeddini*, J.J. Quinn*, A.J. Altman*, J.J. Sziklas*. University of Connecticut Health Center, Farmington, Connecticut 06032

The most frequent sex hormone-related tumors are those of breast (estrogen) and prostate (androgen). However, sex hormone associated hepatic tumors also occur and present opportunities to explore the time course and growth rates. FA is a syndrome of pancytopenia (aplastic anemia) in association with other congenital malformations such as skeletal and skin changes. Anabolic androgen steroids (AAS) benefit the aplastic anemia and have been used in therapy. A 9 year old girl with FA had been on oxymetholon (an AAS) for 6 years. Radiocolloid liver scan revealed hepatic tumors. These had blood supply principally from the hepatic artery, and possessed a large blood pool. The tumors did not concentrate Ga-67 citrate. The tumors progressed further when the patient was changed to another AAS, DecaDurabolin®. Discontinuance of AAS was followed by marked regression of the lesions, so that a liver scan 18 months later revealed nearly absent tumor. Over the initial period of 161 days of study while on AAS, the tumors grew an average of 0.08 grams/day. During 385 days of regression, the mean tumor loss was 0.03 grams/day (likely an underestimate). Such readily studied androgen associated tumors might be utilized to determine the effects of anti-androgens or other therapy, or of varying dosage schedules. (Supported by USPHS CA 17802 from National Cancer Institute).

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LH AUGMENTS THE STIMULATORY EFFECT OF FSH ON ESTROGEN (E) SYNTHESIS BY THE RAT TESTIS. David K. Pomerantz, MRC Group in Reproductive Biology, Univ. Western Ontario, London, Canada

Injection of infant rats with FSH stimulates the aromatization of androgens to E by the testis. There is a poor understanding of the mechanisms underlying this process. The following experiments tested whether the availability of androgen substrate might influence the response of the testis to FSH. Six hr after injection of 12-day-old rats the testes were removed and methanolic extracts prepared for purification by Sephadex LH-20 chromatography. Testosterone and E were measured by RIA. A standard, submaximal dose of FSH (200 ng/g S-13) increased E from 109 ± 16 to 397 ± 76 pg/g testis ($P < 0.01$). Injection of hCG at 1 or 2 iu/g significantly elevated the concentration of testosterone in the testes, but had no significant effect upon E concentration. Combined injection of the standard FSH dose with 1 or 2 iu of hCG elicited E concentrations of 791 ± 86 and 728 ± 88 pg/g respectively. Injection of 90 ug/g of testosterone alone increased E to 220 ± 19 pg/g ($P < 0.05$). When this same dose of testosterone was combined with the standard dose of FSH an E concentration of 403 ± 31 pg/g was elicited. These data show that the amount of E formed after in vivo treatment with FSH can be increased by simultaneous treatment with hCG. Presumably this effect is due in part to the ability of hCG to elevate intratesticular testosterone levels. The results suggest that when FSH alone is injected the amount of E formed is limited by the availability of aromatizable androgen substrates. (Supported by MRC)

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NUCLEAR PROGESTIN RECEPTOR IN CERVIX OF WOMEN. C.A. Pinkert*, M.A. Lorincz* and J.A. Holt. Dept Ob/Gyne, U.C. School of Medicine, Chicago, IL 60637

In studying the endocrine biology of the cervix we have partially characterized progestin receptor (PRn) in glandular cervix. PRn in the 800 x g peliet was extracted with 0.5 M KCl, adsorbed onto hydroxylapatite followed by incubation with 5 nM 3H-R5020 for 18h at 4C and then washed twice with Tween 80-containing buffer. Bound 3H-R5020 was subsequently eluted with ethanol and counted. With these assay procedures human serum had no detectable binding.

Nuclear binding of 3H-R5020 was heat labile and readily inhibitable with 100 fold excess molar progesterone (90%), but not with diethylstilbestrol (3%), testosterone (-6%) or cortisol (5%). Cervix PRn in follicular phase specimens (n=6) was 930 ± 144 fmol/g wet wt or 66 ± 13 fmol/mg soluble protein; luteal phase specimens (n=6) had 995 ± 148 and 84 and ± 13, respectively ($P > 0.5$). Follicular and luteal phase cervical cytosols had 2989 ± 812 and 1861 ± 188 fmol/g wet wt available progestin receptor, respectively. These observations suggest that high luteal phase concentrations of serum progestin cause apparent translocation of cytoplasmic PR to the nucleus where rapid processing prevents accumulation. (Supported in part by: Mothers' Aid Fund of Chicago Lying-In Hospital, CA-14599-06-311, PO1-CA-27476-01 and ACS IL DIV 78-63 & 79-52.)

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ANDROGENIC RESPONSE TO BILATERAL AND UNILATERAL CASTRATION IN MICROTUS OCHROGASTER. J.C. Agee* R.E. Falvo and B.A. Shepherd*. Southern Illinois University, Carbondale, IL 62901

The effects on peripheral testosterone (T) concentrations and seminal vesicle/coagulating gland weights (SAG) were observed in 70 sexually mature Microtus ochrogaster which were either bilaterally or unilaterally orchidectomized. Animals were necropsied at intervals up to 35 days post-surgery, serum T concentrations determined by RIA, and SAG weights recorded. In the bilaterally orchidectomized voles, serum T concentrations declined to near baseline levels by 24 hrs. post-surgery. By day 5 the SAG weights declined to 15% of intact testicular controls. In the hemicastrated voles, by day 1 serum T concentrations declined to 53% of intact controls, but by day 5 serum T concentrations had returned to near those of the controls. By day 5 the SAG weights had declined to 50% of intact controls and remained at this level until the end of the study. No significant change in testicular weight was observed following hemicastration. While these data suggest that compensatory testicular hypersecretion does occur following hemicastration, compensatory testicular hypertrophy was not observed in this study.

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DSP, DSO, AND NATURE OF THE ANDROGENS ENTERING AND LEAVING THE EPIDIDYMIS DAILY IN THE CONSCIOUS GOAT. B. G. Brown*, B. M. Wickwire*, W. H. Hay*, B. W. Gray*, and V. K. Ganjam. School of Veterinary Medicine, Auburn University, AL 36849.

A technique for simultaneous rete testis and contralateral vas deferens cannulation was perfected in the domestic goat. Rete testis fluid (RTF) volume collected in 24 hrs ranged from 2 to 24.0 ml (n=35). Mean (± S.E.) RTF flow-days were 5.0 ± 1.3. A majority of the goats (80%) showed a peak-flow of RTF by Day 2 post-cannulation. A significant ($P<0.01$) decline from peak-flow (56.8 ± 4.5%) resulted in dramatic cessation of RTF flow next day. Cauda epididymal flows continued from 15 to 39 days. Daily sperm output (DSO) as realized in cauda epididymal fluids (CEF) and daily sperm production (DSP) established in RTF were $1.5 \pm 0.14 \times 10^9$ and $1.21 \pm 0.13 \times 10^9$, respectively. Correlation between volume of RTF (total mls collected/24 hrs) and DSP (1×10^9 sperm/day) was significant ($r=0.92$; $P<0.01$; $y=0.18x - 0.56$). Quantitation of testosterone (T) and dihydrotestosterone (DHT) in RTF and CEF were achieved by RIA following fractionation on high pressure liquid chromatography (Methanol: Water, 60:40; 1800 psi and C_{18} µBondapak column with 1.0 ml/min flow-rates). In contrast to high concentrations of testosterone (30-40 ng/ml) DHT was negligible in RTF. On the other hand CEF contained low T and relatively higher DHT.

It therefore appears that in the goat DHT might play a major role in epididymal sperm maturation.

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RECEPTOR-LIKE STEROID BINDING COMPONENTS IN THE HUMAN PLACENTA
Muazaz A. Younes*, Norman Huang*, Norma F. Besch* and Paige K. Besch. Dept. Ob/Gyn, Baylor Coll. Med., Houston, Tx. 77030.

Steroid binding studies were performed in placental cytosol prepared by ultracentrifugation followed by 35% $(NH_4)_2SO_4$ precipitation, overnight incubation at 4°C and charcoal separation of bound from free. In agreement with our previous findings (Amer. J. Obstet. Gynecol. 136, 1003, 1980), a high affinity, low capacity binding component for androgens was found in term human placentas. A K_D of 4.45 ± 1.25 X 10^{-9}M was found for testosterone (N=20) with a binding capacity (Q) of 314 ± 260 fm/mg protein. In 26 placentas, 17α-methyl-androst-1,9,11-triene-17βol,3-one (R1881), showed similar characteristics (K_D= 2.73 ± 1.25 X 10^{-9}M; Q= 157 ± 73 fm/mg protein). In addition, the presence of a binder for Estrone (E_1) was demonstrated in 3 of the 7 placentas studied (K_D= 8.37 ± 1.01 X 10^{-9}M; Q= 35 ± 18 fm/mg protein). Neither progesterone nor 17α,21-dimethyl-19-nor-preg-4,9-diene-3,20-dione (R5020) bound to the cytosol under the described conditions. However, in temperature-exchange assays, specific binding was shown for progesterone in 3 of the 8 placentas studied and in 5 of the 8 for R5020. Placentas from 18, 20, 30 and 34 week gestations gave K_Ds for the androgens and E_1 similar to those found for the term placenta; the Qs, however, increased with length of gestation. The possible role of these macromolecules as steroid receptors in the human placenta will be discussed.
(Supported in part by St. Luke's Episcopal Hospital).

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THE RENAL EXCRETION OF CREATININE AND URINARY ESTRIOL IN FETAL HEALTH ASSESSMENT.
William C. Foster and Robert A. Donato*. LAB OF Clin. Chem., Jeanes Hosp., Phila., Pa. 19111.

The measurement of urinary estriol is a reliable means of assessing fetal viability. Variation in urine output can create questionable results. Daily urinary creatinine has been accepted as relatively constant and estriol results are expressed as mg. estriol per gm. creatinine, or the estriol/creatinine ratio. It has been reported that impaired renal function may affect urinary estriol excretion which would affect the estriol/creatinine ratio. Therefore, the renal excretion of creatinine was studied in 56 subjects with a range of 0.2 to 2.04 mg/ml, the low values being related to renal impairment, and showing a ten-fold range. The estriol/creatinine range ranged only from 13.0 to 18.0, with a mean of 15.0. Urinary estriol was measured by the method of Foster and creatinine determined on a Beckman Creatinine Analyzer.

1. Fed. Proc. 33 (3); 267 (1974).

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THE RELATION OF STRUCTURAL CHANGES IN THE CORPORA ALLATA OF THE COCKROACH, DIPLOPTERA PUNCTATA TO INCREASES IN JUVENILE HORMONE BIOSYNTHESIS.
C.M. Szibbo and S.S. Tobe. Dept. of Zool., Univ. of Toronto, Toronto, Ont. M5S 1A1.

In the adult female of the viviparous cockroach, Diploptera punctata, increases in the rate of synthesis of the gonadotrophic hormone, juvenile hormone (JH) during oocyte development are accompanied by structural and cellular changes in the JH-producing paired endocrine glands, the corpora allata (CA). A period of mitosis and cell division in the CA precedes maximal JH synthesis; peak cell number per gland and peak gland volume is correlated with peak JH synthesis. Following extirpation of one corpus allatum, the remaining gland produces JH at rates similar to that of a pair of CA, although the volume and cell number per gland is no greater than that of normal CA. Although cell division in the CA may be involved with the normal increase in JH synthesis associated with the gonotrophic cycle, the proliferation of hormone-producing cells does not appear to be the main compensatory mechanism in JH synthesis following unilateral allatectomy.
(Supported in part by NSERC A9408.)

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ACETATE METABOLISM IN LUNG SLICES OF CONTROL AND ESSENTIAL FATTY ACID (EFA) DEFICIENT RATS. J.A. Balint, D.A. Beeler*, and E.C. Kyriakides*. Departments of Medicine and Biochemistry, Albany Medical College, Albany NY 12208

Our previous studies demonstrated enhanced Δ9 desaturation of exogenous palmitate and stearate by lung slices from EFA deficient rats, reversible in vitro by linoleate. To examine differences in metabolism of endogenous and exogenous palmitate we incubated lung slices from control and EFA deficient rats in KRB buffer pH 7.2 (O'Neill and Tierney,AJP,1974) with 1 mM 1-^{14}C-acetate, 0.25 mM 1-^{14}C-octanoate, 1 mM 1-^{14}C-palmitate or 1-^{14}C-stearate for 1 or 2 H and determined the % of ^{14}C in monounsaturated FA by argentation chromatography of methyl esters of phospholipids (PL) with the following results at 1 H: - (expressed as % of ^{14}C in PLFA mean ± SEM).

	n	Acetate	Octanoate	Palmitate	Stearate
Control	3	3.3 ± .22	5.6 ± .05	2.1 ± .12	12.4 ± .30
EFA Def.	3	12.0 ± .75	11.6 ± .85	4.5 ± .56	27.2 ± .32

Results at 2 H showed similar differences. The data confirm our previous findings of enhanced desaturation of palmitate and stearate in EFA deficient lung with great desaturation of stearate. However, they also indicate that newly synthesized palmitate, whether from acetate or octanoate is more extensively desaturated both in control and EFA deficient lung slices, than exogenous palmitate. These results suggest preferential utilization of endogenous palmitate at the CoA ester stage. (Supported by Grant HL-15273).

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THE INFLUENCE OF PHENOBARBITAL ON ACETATE INCORPORATION INTO STEROLS. S. A. Ostrove*, Dept. of Biochem. Reg., Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065; E. J. Flynn*, Dept. of Pharm., College of Medicine & Dentistry of New Jersey, Newark, NJ 07102; and J. C. Hall Dept. of Zool. & Physiol., Rutgers The State University, Newark, NJ 07102

Phenobarbital (PB) has been shown to influence the rate of growth of mouse fibroblasts, LM cells, in culture as well as their rate of sterol biosynthesis. The effect of PB on these parameters is dose dependent. As the concentration of PB increases, the growth of cells is slowed as seen by an increased doubling time. However, the amount of protein/cell increases, becoming 2-5 times as great as the control cells at the 2 mM level of PB. This causes an apparent increase in the size of the cells. Cells treated with 2 mM PB maintain their normal morphology for a longer period of time as compared to the untreated cells which become round after 7 days. Incorporation of acetate into sterols and fatty acids is seen to be biphasic. On days 0 to 2 there is less incorporation of acetate into desmosterol or fatty acids in the treated cells, whereas on days 3 thru 5 incorporation is equal or greater than in control cells. The synthesis of lanosterol is lower on days 0 and 1 but is increased on days 2 thru 5. It has also generally been observed that as the cells approach confluency their rate of lipid synthesis decreases. This decline is slower in the treated cells. Thus, in addition to altering the uptake of acetate, it appears that PB exerts its influence on sterol biosynthesis at a point beyond the branch for fatty acids due to the difference between lanosterol and desmosterol synthesis.

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INFLUENCE OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ ON CANINE ARTERIAL *IN VITRO* LIPID BIOSYNTHESIS FROM U^{14}C-GLUCOSE. B. H. Perlmutter and M. E. Soulsby*. Dept. Physiology & Biophysics, Univ. Ark. Med. Sci., Little Rock, AR 72205.

This study was designed to investigate the influence of exogenous prostaglandin on *in vitro* incorporation of U^{14}C-glucose into canine aortic lipid. Aortae were excised from sodium pentobarbital-anesthetized dogs, stripped of their adventitial layer and incubated four hours in the presence of labeled substrate alone or labeled substrate plus prostaglandin. The tissue was subsequently homogenized and the lipid phase extracted. Thin layer chromatography was used to separate individual lipid subfractions. Incorporated ^{14}C was then measured by liquid scintillation. Both PGE_2 and $PGF_{2\alpha}$ (0.10-0.025 μ /ml) decreased ($p<0.01$) 14C label incorporation into triglycerides and increased ($p<0.01$) ^{14}C label incorporation into phospholipid, while not significantly altering total 14C label incorporation. Studies conducted on the intimal and medial layers separately failed to demonstrate a difference in the extent to which ^{14}C was incorporated into these two tissue layers. Tissue "blanks" performed following destruction of enzymatic activity failed to demonstrate any significant background uptake of ^{14}C. Therefore, the *in vitro* effect of both prostaglandins PGE_2 and $PGF_{2\alpha}$ on aortic wall lipid synthesis from glucose is to favor the synthesis of phospholipid at the expense of triglycerides. (Supported in part by a Grant-in-Aid of the American Heart Association)

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EFFECTS OF AGING AND DIETARY CARNITINE MANIPULATION ON MYOCARDIAL CARNITINE CONCENTRATION, PALMITATE OXIDATION, AND LIPID DEPOSITION. Laurel A. Traeger* and D.W. Edington* (SPON: Timothy P. White). Univ. of Michigan, Ann Arbor, MI 48109.

Carnitine concentration was measured in hearts frozen in situ and (1-^{14}C) palmitate oxidation rate measured in whole heart homogenates from 6.5 and 18 month old rats. In 18 mo hearts short and medium chain acylcarnitine concentration was 50% lower than 6.5 mo hearts, while concentrations of free and total carnitine and long chain acylcarnitine were not different between age groups. Palmitate oxidation rates were not different between ages; addition of 1 mM L-carnitine to the incubation medium produced 5 fold increases in palmitate oxidation in both groups. We conclude 1) endogenous carnitine is sufficient to support palmitate oxidation in 18 mo hearts, and 2) the metabolic pathway from palmitate to CO_2 retains maximal functional capacity in 18 mo hearts. In another experiment 6.5 and 18 mo rats were fed either carnitine-free (CF6.5, CF18) or 2% DL-carnitine-supplemented (CS6.5, CS18) diets. In CF18 hearts myocardial carnitine concentration was 15% lower, palmitate oxidation rate 77% higher, carnitine palmitoyltransferase activity 39% higher, and lipid deposition (electron microscopy) 55% higher than CS18 hearts. In 6.5 mo hearts there were no differences between dietary groups in all variables. These results suggest that in the aging myocardium there is a sensitivity of the entire fatty acid metabolic pathway to small changes in carnitine concentration. (Supported by a Rackham Graduate School Dissertation Grant)

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INFLUENCE OF PROSTAGLANDINS E_2 and $F_{2\alpha}$ ON CANINE ARTERIAL *IN VITRO* LIPID BIOSYNTHESIS FROM ^{14}C-ACETATE. M. E. Soulsby* and B. H. Perlmutter. Dept. Physiology & Biophysics, Univ. Arkansas for Medical Sciences, Little Rock, AR 72205

This study was designed to investigate the influence of prostaglandin on *in vitro* incorporation of ^{14}C acetate into canine aortic lipid. Aortae were excised from pentobarbital-anesthetized dogs, stripped of their adventitial layer and incubated four hours in the presence of labeled substrate alone or labeled substrate plus prostaglandin. The tissue was subsequently homogenized and the lipid phase extracted. Thin layer chromatography was used to separate lipid subfractions. Incorporated ^{14}C was measured by liquid scintillation. PGE_2 (0.10-0.05 μg/ml) decreased ($p<0.01$) ^{14}C incorporation into total lipid, as a result of decreased ($p<0.01$) ^{14}C phospholipid. Other lipid subfractions were not affected. $PGF_{2\alpha}$ (0.10-0.05 μg/ml) increased ($p<0.01$) ^{14}C incorporation into total lipid, as a result of an increase ($p<0.01$) ^{14}C in phospholipid, triglyceride and FFA. Other subfractions were not affected. Studies conducted on intimal and medial layers separately failed to alter the extent to which ^{14}C was incorporated into these tissue layers. Tissue "blanks" performed following destruction of enzymatic activity failed to demonstrate any significant background uptake of ^{14}C. Therefore, the *in vitro* effect of PGE_2 is to decrease aortic wall lipid synthesis from acetate, while the effect of $PGF_{2\alpha}$ is to increase aortic lipid synthesis from acetate. (Support in part by a Grant-in-Aid of the American Heart Association).

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INHIBITION OF MEDIUM AND SHORT-CHAIN FATTY ACID OXIDATION IN RAT HEART MITOCHONDRIA BY DICHLOROACETATE. K.C. Man* and J.T. Brosnan*. (SPON: M.E. Brosnan). Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland.

Dichloroacetate (DCA), an activator of pyruvate dehydrogenase, inhibits fatty acid oxidation by muscle from normal, starved and diabetic rats. In this work we have attempted to elucidate the mechanism of its inhibition. DCA (1-10mM) markedly decreased medium and short-chain fatty acid oxidation (O_2 uptake and release of $^{14}CO_2$ from labeled fatty acids) by heart mitochondria; the oxidation of long chain-fatty acid, however, was unaffected by the compound. The oxidation of short and medium-chain acylcarnithines was unaffected by DCA. No effects of DCA were evident on fatty acid oxidation by liver mitochondria. Further studies showed that DCA directly inhibits the activity of mitochondrial acyl CoA synthases. Thus the effect of DCA on the oxidation of short and medium-chain fatty acids in these mitochondria is direct and not due to its effects on pyruvate dehydrogenase. (Supported by MRC and CHF).

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CYCLIC NUCLEOTIDE ACTIVATION OF A CARDIAC TRACYLGLYCEROL LIPASE. Warren K. Palmer* and Lawrence B. Oscai. Univ. of Illinois at Chicago Circle, Chicago, IL. 60680

Perfusion of the isolated rat heart with 1 mg/ml dibutyryl cyclic AMP (Bt_2 cyclic AMP) increased intracellular triacylglycerol (TG) lipase activity 92%. At the same time, cardiac levels of TG were reduced 51% and tissue free fatty acids (FFA) were increased 2.4-fold. Perfusion of the heart with 0.0025 mg/ml Bt_2 cyclic AMP resulted in a 71% reduction in endogenous lipase activity. Under these conditions TG increased 2.1-fold above control and FFA decreased 74%. Perfusion with varying amounts of Bt_2 cyclic AMP elicited a high negative correlation between intracellular lipase activity and heart TG content ($r=-0.94$) and a high positive relationship between endogenous lipase activity and tissue FFA content ($r=0.97$). Homogenization of the myocardium in the presence of 300 uM cyclic AMP increased intracellular enzyme activity from 73 units to 141 units while homogenization in the presence of 0.001 uM exogenous cyclic AMP decreased activity to 22 units. Addition of purified protein kinase catalytic subunit to heart homogenate increased endogenous lipase activity 78% and FFA 1.5-fold. The enzyme activity had characteristics similar to lipoprotein lipase (LPL), i.e. sensitive to salt inhibition and required serum for activity. These data suggest that LPL may play a role as an intracellular regulator of cardiac TG. (Supported in part by USPHS. NIH Grants AM # 17357, HD # 10987, and KO4-AM00216)

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SPARE β-ADRENORECEPTORS IN RAT BROWN ADIPOSE TISSUE. A. Paradis*, N. Folléa* and L. Bukowiecki. Dept. of Physiology, Medical School, Laval University, Québec, G1K 7P4, Canada.

The radiolabelled agonist (±)[^{3}H] hydroxybenzylisoproterenol (HBI) has been used to quantitatively correlate receptor occupancy by catecholamines with the principal physiological function of brown adipose tissue, heat production. Binding of HBI to intact brown adipocytes was rapid (2-3min), stable (during at least 20 min), reversible (by an excess of isoproterenol) and saturable. It displayed the affinity ($\frac{1}{2}$Bmax=32nM), specificity and stereoselectivity expected of binding to adenylate cyclase-coupled β-adrenoreceptors. β-Adrenergic agonists competed for receptor occupancy with an order of potency typical for the β_1 subtype of adrenergic receptors: (-)-isoproterenol >> (-)norepinephrine ≈ (-)epinephrine >> corresponding (+) stereoisomers. Polarographic respiratory measurements disclosed that HBI stimulated cellular oxygen consumption 10-12 times above basal values with high affinity ($\frac{1}{2}$Vmax=4nM). Maximal respiratory rates (410 nmol O_2/min/10^6 cells) were observed 3 min after addition of HBI to adipocytes suspensions. Occupancy by HBI of less than 15% of total available adrenoreceptors was sufficient for promoting maximal respiration. It is concluded a) that catecholamines control thermogenesis in brown adipose tissue via adrenoreceptors of the β_1 subtype and b) that the high sensitivity of brown adipose tissue for catecholamines can be explained by the presence of a large reservoir of adrenoreceptors ("spare" receptors). (Supported by the MRC).

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RELATIONSHIP BETWEEN THE KETONE BODIES ACETOACETATE (AcAc) AND β-HYDROXYBUTYRATE (βOHB) IN VIVO. Susan E.H.Hall, G.J.Hetenyi, Jr., Department of Physiology, University of Ottawa, Ont. Can.

^{14}C-3-Na-AcAc was infused after an overnight fast i.v. to 6 normal subjects, 5 insulin dependent diabetic patients on diagnosis, 6 obese patients before and after 1-2 wk. starvation, and 4 rats. 4 other rats received ^{14}C-2-Na acetate. Specific activity (SA) in blood of AcAc and βOHB was followed. The turnover rate of AcAc (RT_{AcAc}) was calculated as

$$\frac{\text{rate of infusion dpm/min}}{\text{SA. plateau dpm/mmol}}$$

(MCRAcAc) as $\frac{RT(AcAc)}{\text{concentration}}$. In rats when ^{14}CAcAc was infused the ratio $\frac{SA\beta OHB}{SAAcAc}$ was 0.30±0.07 similar to that in people, compared with 0.76±0.09 when ^{14}C-2-Na acetate was the tracer, hence the exchange between ^{14}C-3-AcAc in blood and liver is very small. Only RT_{AcAc} and not total ketone RT can be calculated using ^{14}C-AcAc as tracer. RT_{AcAc} (μmol/min.m^2) was lower in normal subjects (46.1±6.2) than diabetics (260.1±78.8) or the obese before (158.5±24.9), or after (540.4±60.4) starvation. AcAc concentration in blood and RT_{AcAc} were exponentially related. MCR_{AcAc} (1/min.m^2) normally 1.31±0.08 was 0.670±0.11 in diabetics. It fell markedly from 0.987±0.09 to 0.459±0.03 in starvation. Normally, at rest, AcAc metabolism could account for approximately 10% of oxygen consumption and 33% in the obese. During starvation all energy requirements could be supplied by AcAc. Supported by MRC (Canada).

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THE ROLE OF INTESTINAL LIPOPROTEINS IN TRIGLYCERIDE TRANSPORT. Yih-Fu Shiau. VA Hospital and U. of Penn., Phila., PA 19104

During luminal infusion of different concentrations of ^{3}H labelled oleic acid (OA), partition of the re-esterified OA into intestinal lipoproteins was studied. Intestinal lymph was collected at a steady state and lipoproteins were fractionated by gradient ultracentrifugation. Total lipids were extracted, triglyceride (TG) separated by TLC and the incorporation of ^{3}H measured. Data are expressed as μmoles/hr.

Amount of OA infused	9.2	46.9	73.9
% ^{3}H recovered from the lymph	70	82	85
OA incorporated into lymph lipids	5.7	28.3	60.3
OA incorporated into lymph TG	4.8	25.3	54.8
partition of lymph TG into lipoproteins	(μmoles/hr)		
Chylomicron (CM)	1.8	19.7	47.5
Very low density lipoproteins (VLDL)	2.2	4.9	6.5
Low density lipoprotein (LDL)	0.7	0.6	0.7
High density lipoprotein (HDL)	0.1	0.1	0.1

These data show (a) increasing luminal OA concentration enhances incorporation of intestinal TG into both CM and VLDL, (b) at a low concentration of luminal OA, VLDL carries the majority of intestinal TG, (c) at high concentration of luminal OA, CM become the most important particles carrying intestinal TG, and (d) TG contents in other lipoproteins, LDL and HDL, are independent of luminal OA concentration. (Supported by Veterans Administration Medical Research Grant MRIS-7864)

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DISTRIBUTION OF TOCOPHEROL IN HUMAN PLASMA LIPOPROTEINS. Willy A. Behrens*, James N. Thompson* and René Madère* (SPON: K.J. Kako). Bureau of Nutritional Sciences, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2.

Lipoproteins were removed from human plasma at density of 1.225 by ultracentrifugation. Three classes of lipoproteins were separated by 4% Agarose-column chromatography: very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). A highly sensitive HPLC method with fluorometric detector was used to estimate α-tocopherol in plasma and column eluates. Plasma tocopherol was not significantly different in males ($n = 6$) and females ($n = 4$), and it was found primarily in lipoproteins. The distribution of α-tocopherol in males was: VLDL, 8%; LDL, 59%; and HDL, 33%. In contrast the distribution in females was: VLDL, 2%; LDL, 40%; HDL, 58%. Protein distribution in males was: VLDL, 4%; LDL, 37%; and HDL, 59% and in females: VLDL, 2%; LDL, 23%; and HDL, 75%. The α-tocopherol concentration (expressed as μg α-tocopherol/mg protein) in lipoproteins was also different. The values were in males: VLDL, 7.0; LDL, 4.3; and HDL, 1.5; females: VLDL, 3.7; LDL, 5.2; and HDL, 2.3. These data suggest that the different distribution of α-tocopherol in plasma lipoproteins in males and females reflects not only different levels of lipoprotein but also a different binding capacity of the proteins for the vitamin.

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ROLE OF METABOLIC FACTORS IN THE CHANGES IN MOUSE SKIN TUMORIGENESIS MEDIATED BY TEMPERATURE ACCLIMATION.
A.G. Hakaim*, J.F. O'Connell*, J.F. Pitt*, S.E. Weisbrode* and H.S. Weiss. Dept's of Physiology & Pathobiology, Ohio State University, Columbus, Ohio 43210.

Skin tumorigenesis is accelerated in C3H male mice acclimated to cool Ta and inhibited by acclimation to warm Ta. For example, BaP in toluene, applied repetitively to the clipped back in wkly dose of 0.2 mg in 0.08 ml, resulted in mean tumor appearance time of 17.4 ± 0.9, 21.8 ± 0.8 and 24.3 ± 0.8 wks for cool (16C), normal (23C) and warm (32C), respectively. To try to separate local skin from systemic mechanisms in the Ta-cancer effect, we measured in toluene treated mice the following: in skin - temperature (Ts), oxygen uptake (QO_2) and hyperplasia; systemic - rectal temperature (Tr), food intake and liver and muscle QO_2. The following changes ($P \leq 0.05$) were noted: In comparison to 23C, Ts was 6% lower in 16C and 6% higher in 32C. Skin QO_2 was 119% higher in 16C but not different at 32C. Skin thickness was increased 60% in 16C, but not altered by 32C. Tr and muscle QO_2 were unchanged by 16C and 32C. Food intake was 50% higher in 16C and 30% lower in 32C. Liver QO_2 was increased 165% in 16C and decreased 60% in 32C. Except for Ts, metabolic changes generally indicate increases in 16C and decreases in 32C. Compared to normal 23C, changes in cool Ta are more consistent and pronounced than in warm Ta. Increased skin QO_2 and hyperplasia are opposite those expected from decreased Ts, suggesting that systemic factors may be more important than local factors in the Ta-cancer effect.

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ENZYMICALLY-MODIFIED TUMOR CELL MEMBRANE: ALTERATION OF THE SPECIFICITY AND POTENCY OF THE ANTIBODIES PRODUCED BY THE HYBRID CLONES.
Anwar A. Hakim, Univ. of Ill. Med. Ctr. Chicago. Ill. 60680.

The transition of a normal cell to a malignant one is accompanied by changes which include reduced requirement for serum growth factors, agglutination by lectins, and altered membrane glycoproteins. There is a correlation between one or more of these properties and the malignant state of the cell. If malignancy is defined as the capacity of cells to produce a growing tumor in a suitable host, the present studies report suppression of malignancy in hybrids with no changes in the cellular properties associated with the parent cells. Human skin fibroblasts synthesize and secrete a growth promoting factor (Hakim, Experientia 34, 1515, 1978). Human skin fibroblasts were fused with spleen cells of BALB/c mice pre-immunized with human mammary carcinoma (HMCC) or malignant melanoma(HMMC) cells before and after treatment with vibrio cholera neuraminidase(VCN). The cells were fused in MEM containing 50% polyethylene glycol (PEG) and immediately separated into various clones. Supernatants from these hybrid cloned cell cultures were examined for antibodies to the tumor cell surface. Immunization with VCN-treated tumor cells gave spleen cells which if fused with HSF produced hybrid clones whose antibody products recognize determinants shared by similar cell types to the immunogen and also cross reacted with other neoplastic types. Such increased potency of the antibodies is the result of the unmasking of potentially-immunogenic sites. The immune state of the parent cells influence the morphological state of the hybrid cells.

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B CELL MATURATION FACTOR IN MEDIA FROM BURSAL CULTURES.
Hygie Reynolds* and Paul Nathan. Univ. of Cinti., College of Medicine and Shriners Burns Institute, Cincinnati, Ohio 45219

Extracts of bursal tissue induce immature B cells to express a mature B cell surface antigen (Brand, Gilmour and Goldstein, Science 193:319, 1976). The present study sought such a B cell maturation factor in media from bursal cell cultures. Monolayer cultures were prepared from chick embryonic bursal tissue. Immature B cells as targets for the assay were obtained from chicken bone marrow separated on a discontinuous bovine serum albumin density gradient. Lymphocytes were harvested from the top two layers and incubated 4 hrs in either bursal culture media or control media not contacted by a culture. A cytotoxic assay for cell surface immunoglobulins (Ig) was used to detect the induced mature B cells. The results were expressed as percent dead cells. Culture media samples were collected every 3 days from day 4 to day 25. The mean of samples from 4 cultures was calculated for each of the 8 sampling days. The mean ± SEM of these means for bursal cultures is 7.3 ± 1.0 percent and for chick kidney control cultures was -10.2 ± 2.0 percent. The bursal mean is significantly greater than zero ($p < 0.0005$); the kidney mean is significantly less than zero ($p < 0.001$). We conclude (1) bursal cultures release a factor which induces a small percentage of immature B cells in bone marrow to express surface Ig similar to that seen in mature B cells (2) this factor is not evident in media from kidney cell cultures (3) a factor inhibiting the assay reaction appears to be present in the media from kidney cultures.

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EFFECT OF CAROTID BODY DENERVATION ON THE AROUSAL RESPONSE TO HYPOXIA IN SLEEPING DOGS. G. Bowes*, E.R. Townsend*, and E.A. Phillipson. Department of Medicine, University of Toronto, Toronto, Ontario M5S 1A8

Arousal is a major component of the response to hypoxia during sleep, but the role of the carotid bodies in mediating this response is uncertain. We therefore studied the arousal and ventilatory responses to hypoxia during sleep in 3 trained dogs, before and 1-4 weeks following carotid body denervation (CBD). During the studies the dogs breathed through a cuffed endotracheal tube inserted via a chronic tracheostomy. Isocapnic progressive hypoxia was induced by a rebreathing technique and arterial O_2 saturation (Sa_{O_2}) was measured with an ear oximeter. Sleep stage was determined by electroencephalographic and behavioral criteria. Following CBD, ventilatory responses during wakefulness, slow-wave sleep (SWS), and rapid-eye-movement sleep (REMS) were < 10% of control values. Prior to CBD, arousal during hypoxia occurred at a Sa_{O_2} of 78.5 ± 1.4% during SWS, and 69 ± 1% during REMS. Following CBD, arousal failed to occur during progressive desaturation to 60% in SWS and 50% in REMS, at which levels hypoxia was arbitrarily terminated. In a few studies where greater degrees of hypoxia were allowed to develop, the dogs occasionally passed directly from sleep into coma, requiring active resuscitation to regain normal consciousness. The results indicate a critical role for the carotid chemoreceptors in mediating the arousal response to hypoxia during sleep. (Supported by Grant MA4606 from the M.R.C. of Canada.)

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HUMAN VENTILATORY RESPONSES TO INSPIRATORY RESISTIVE LOADING DURING WAKEFULNESS AND SLOW WAVE SLEEP. C. Iber*, A. Bersenbrugge*, J. Skatrud*, J. Dempsey. Univ. of Wisconsin, Madison, WI 53706

The tidal volume (VT), ventilatory (VE), and mouth occlusion pressure (P_{100}) response to repetitive inspiratory resistive (17 cm H_2O/L/sec) loading were assessed during wakefulness (AW) and slow wave sleep (SWS) in 6 naive male subjects during eupneic breathing using an airtight mask. The AW response to loading showed 1) increase in P_{100} (31±3%) on the second loaded breath in 4 of 5 subjects 2) increase in VT (18±20% first loaded breath, 16±3% maintained for five loaded breaths) in 5 of 6 subjects 3) decrease in VE and frequency over 5 minutes of loading. During SWS the response to loading included 1) no change in P_{100} on the second loaded breath in 4 of 4 subjects 2) decrease in VT (-31±4% first loaded breath, -28±2% maintained for five loaded breaths) 3) decrease in VE and mild desaturation (-2%)(but no change in frequency) until arousal (0.5 to 3 min) in two subjects. These results show that SWS abolishes the augmented inspiratory activity (P_{100}) in response to resistive loading and that frequency and VT responses to loading may be significantly modified by SWS. (Supported by V.A. Medical Research)

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CORRECTION OF CHRONIC CO_2 RETENTION AWAKE AND ASLEEP BY NORMALIZING PLASMA [HCO_3] WITH ACETAZOLAMIDE. J. Skatrud*, J. Dempsey and C. Iber*. University of Wisconsin and William S. Middleton Veterans Hospital, Madison, Wisconsin 53705.

This study compared the relative effectiveness of normalization of plasma [HCO_3] with acetazolamide (ACET) versus medroxyprogesterone acetate (MPA) in correcting chronic CO_2 retention in 14 awake patients with COPD during random application of 1) Placebo 2) Placebo 3) MPA 20 mg po tid for four weeks and 4) ACET 250 mg po bid for 10 days. Five of these patients aged 60±8 years and weighing 87±15 kg (FEV_1=0.88± 0.31 l) were also studied asleep. Awake. Mean decrease in $PaCO_2$ was 6±5 mm Hg during MPA with 10 of 14 patients showing a response compared to 4±4 mm Hg with only 5 of 14 showing a response to ACET despite a decrease in pHa and pHcsf (7.40± 0.01 to 7.32±0.01 and 7.30±0.01 to 7.27±0.01 respectively). Sleep. Arterial blood gases during placebo NREM sleep (n=5) were pHa 7.38±0.04, $PaCO_2$ 57±4, and PaO_2 47±7 mm Hg compared to awake pHa 7.39±0.05, $PaCO_2$ 54±4 and PaO_2 50±9 mm Hg. ACET did not lower $PaCO_2$ asleep in 3 of 5 patients despite a decrease in pHa to 7.31±0.04 (range 7.26-7.37). MPA decreased $PaCO_2$ 8±2 mm Hg (range 6-11 mm Hg) and increased PaO_2 7±5 mm Hg (range 2-15 mm Hg) in all 5 patients despite a slight increase in pHa to 7.40±0.03. Patients who did not respond to ACET during sleep similarly did not respond during wakefulness. These patients are an example of a "responsive" control system which is relatively insensitive to the normally potent feedback between [H+] and alveolar ventilation.

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RELATIONSHIP OF GENIOGLOSSAL AND DIAPHRAGMATIC EMGs TO ARTERIAL OXYGEN DESATURATION IN NORMAL HUMANS AND PATIENTS WITH OBSTRUCTIVE SLEEP APNEA. E. Ünal*, T.D. O'Connor* and M. Lopata* (Spon: R.V. Lourenço) University of Illinois Hospital and VA West Side Medical Center, Chicago, IL. 60680.

In order to study the effects of hypoxia on upper airway (UA) and respiratory muscle (RM) activity, genioglossal EMG (EMG_{ge}) and diaphragmatic EMG (EMG_{di}) quantitated as peak moving average were studied in 5 supine volunteers during isocapneic hypoxia and in 6 patients with obstructive sleep apnea (OA) during nocturnal sleep. During isocapneic hypoxia both EMG_{ge} and EMG_{di} increased linearly with decreasing Sa_{O_2} (r=0.82±0.11 and 0.89±0.09, respectively). At sleep onset, in all patients synchronous cyclic changes in the amplitude of both EMGs were observed which became more pronounced as sleep progressed and OA occurred at the nadir of the cycle resulting in cyclic episodes of desaturation. EMG_{ge} and EMG_{di} showed an inversely linear relationship with changes in Sa_{O_2} (r=0.70±0.19 and 0.77±0.15, respectively) with the exception of first two post-apneic breaths. The slopes of both EMGs vs Sa_{O_2} were significantly lower during REM sleep than in NREM sleep. EMG_{ge} vs EMG_{di} relationship was linear in the normal subjects (0.86±0.08) and in patients with OA (0.91±0.07) with the exception of first two post-apneic breaths. In conclusion, hypoxia causes proportional increases in UA and RM activity in awake normal humans and in patients with OA, contributing to the termination of OAs in the latter (Supported by ALA grant #2443930338).

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THE EFFECT OF CHRONIC HYPOXIA ON BREATHING PATTERN IN WAKEFULNESS AND SLEEP. A. Berssenbrugge*, J. Dempsey, J. Skatrud*, and C. Iber*. Univ. of Wisconsin, Madison, WI 53706

The effect of hypoxia on breathing pattern was examined in 3 healthy males for 4 consecutive nights at BP=450 mm Hg. The most apparent effect of hypoxia on pattern was the occurrence of periodic breathing (PB); defined by cyclical changes in V_t interspersed with apneic periods (5-18 sec). No PB or apneas occurred at sea level. Sleep during short-term hypoxia (2-24 hrs) caused PB and apnea particularly in light sleep. (Table). PB was associated with marked desaturations (59-64% Minimum Sa_{O_2}). PB and apnea were greatly decreased in all sleep stages with acute normoxia (Sa_{O_2}>90%) as well as with acclimitization (12 vs 96 hrs at PB 450). Hypocapnic alkalosis was evident both awake (Pa_{CO_2} 31.7 mm Hg) and asleep (Pa_{CO_2} 33.3 mm Hg) (2-24 hrs). Hypocapnia during sleep persisted during acute normoxia and intensified with acclimitization.

SLEEP STAGE→		AWAKE	I		II		III/IV	
P_{IO_2} (mm Hg)→		84	84	>150	84	>150	84	>150
PER. BREATHING	12-24 HR	16%	70%	4%	76%	22%	24%	--
(% TIME)	84-96 HR	0%	45%	0%	46%	3%	9%	0%
APNEA	12-24 HR	13	137	58	136	47	40	--
(#/HR)	84-96 HR	0	36	4	29	6	1	0

PB reflects instability in ventilatory control. Our results show that sleep accentuates this instability that occurs with acute hypoxia. Stabilization of the control system is a critical facet in the time dependent ventilatory acclimitization that occurs with chronic hypoxia. (supp. by NIH, VA, USARMDC).

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VENTILATION IN TREADMILL WALKING AND ARM CRANKING IN FEMALE SWIMMERS: RELATIONSHIP TO CO_2 RESPONSIVENESS. G.J.F. Heigenhauser,* N.B. Oldridge,* and N.L. Jones. Dept. of Medicine, McMaster Univ., Hamilton, Ontario. L8S 4J9

We examined the relationship of the ventilatory response to carbon dioxide ($\Delta\dot{V}I/\Delta PCO_2$) during rebreathing at rest to the ventilatory response to metabolic carbon dioxide production ($\Delta\dot{V}E/\Delta\dot{V}CO_2$) during arm cranking and treadmill walking in 24 young female swimmers: 8 synchronized swimmers (SS), 8 competitive speed swimmers (CS) and 8 recreational swimmers (RS). $\Delta\dot{V}I/\Delta PCO_2$ (ℓ.torr^{-1}) was similar in all groups: 1.48 ± .33, 2.04 ± .13 and 1.87 ± .18 for SS, CS, and RS respectively. $\Delta\dot{V}E/\Delta\dot{V}CO_2$ (ℓBTPS.ℓSTPD^{-1}) of 25.3 ± 1.0, 25.9 ± 1.4 and 24.6 ± 1.1 observed during treadmill walking for the SS, CS and RS respectively was similar for all groups. However, during arm cranking, $\Delta\dot{V}E/\Delta\dot{V}CO_2$ of 27.5 ± .9 for SS and 27.6 ± 1.1 for CS were significantly lower than 30.8 ± 1.5 for RS. We did not find a significant relationship between $\Delta\dot{V}I/\Delta PCO_2$ and $\Delta\dot{V}E/\Delta\dot{V}CO_2$ for treadmill walking (r = 0.11) or arm cranking (r = 0.15). We conclude that the ventilatory response to metabolic carbon dioxide production during either treadmill walking or arm cranking in these subjects was not related to the ventilatory response to carbon dioxide during rebreathing at rest but was lower in trained individuals when exercising with trained limbs.
(Supported by Health and Welfare Canada, and Ontario Heart Foundation).

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RESPIRATORY AND STEPPING FREQUENCIES IN CONSCIOUS EXERCISING CATS. Steve Iscoe, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

The incidence of entrainment between respiratory and stepping frequencies has been investigated in exercising cats. Stainless steel wire microelectrodes were surgically implanted in the right hemidiaphragm (D), quadriceps (Q), and hamstring (H) muscles of six cats. Electromyograms were recorded while the cats were at rest or walking on a treadmill at speeds between 0.31 and 1.67 m/s (3.74 mph). Autocorrelation of D activity showed cats to have 2 respiratory patterns: regular (peaks in the autocorrelogram) or irregular (flat autocorrelogram). Autocorrelation of Q or H activity revealed stepping frequency. Crosscorrelation of D and Q or H activity revealed the presence of entrainment between respiration and stepping. In 4 cats, entrainment was either very weak or absent but the other 2 cats clearly showed entrainment of the two activities, even in the absence of a regular respiratory rhythm. In conclusion, at speeds up to 1.67 m/s, respiratory frequency is not tightly controlled in cats; however, the occurrence of entrainment in some instances clearly indicates the existence of neural circuitry linking these two pattern generators.
(Supported by the Botterell Foundation and the MRC of Canada).

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NO EFFECT OF NALOXONE ON HYPOXIC VENTILATORY DEPRESSION IN ADULTS. S. Kagawa*, M.J. Stafford, T.B. Waggener*, and J.W. Severinghaus. CVRI, University of Calif., San Francisco, CA 94143

We tested the hypothesis that CNS hypoxic ventilatory depression is mediated by endorphine release in 4 healthy adult male subjects. Subjects were given FIO_2=0.3 for 10 minutes followed by isocapnic hypoxia (end tidal P_{O_2}=45 mmHg; end tidal P_{CO_2}=resting level). During hypoxia ventilation initially increased then stabilized at a lower plateau (Weil, JV., C.W. Zwillich, Chest 70:1 Suppl., p.124, 1976). Naloxone (1.2 mg) was then injected intravenously. Ventilation was observed for 40 minutes after naloxone. With hypoxia, 3 of 4 subjects initially increased ventilation to 384±51% (mean±S.D.) of resting value, then stabilized at 248±27% of resting value within 19 minutes. One of four subjects initially increased ventilation to 155±8% of resting value then stabilized at 105±16% of resting value. Blunted peripheral chemoreceptor activity was suggested in this subject. None of the subjects significantly increased ventilation after injection of 1.2 mg of naloxone. The results from 1 subject given 10 mg of naloxone also showed no significant change in ventilation during hypoxic depression. From these preliminary results, we conclude that hypoxic CNS ventilatory depression in adults is not mediated by endorphine release.

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EFFECT OF EPIDURAL MORPHINE ON CONTROL OF BREATHING IN MAN. D.D. Doblar,* R.J. Reynolds,* J.D. Baskoff,* S.M. Muldoon and R.L. Watson.* Anesthesia and Operative Service, Walter Reed Army Medical Center, Washington, D.C. 20012.

Considerable interest has developed in the profound analgee n resulting from the epidural administration of a single 10 mgm dose of morphine. Since the effects on respiratory drive of epidural morphine are unknown, the ventilatory (VI) and airway occlusion pressure (P100) responses to CO_2, using the CO_2 rebreathing technique, were determined. Patients selecting epidural anesthesia for lower extremity procedures were studied preoperatively and then postoperatively with a T10 or lower local anesthetic level. After a 10 mgm dose of preservative-free morphine (A.H. Robins) was given, the CO_2 responses were repeated one hour and six hours post-morphine. There was no effect of the local anesthetic alone on the VI or P100 vs CO_2 response slopes. When compared with the postoperative slopes the VI vs CO_2 responses were blunted an average of 27% at one hour and 34% at six hours post-morphine. The P100 vs CO_2 response slopes were blunted 36% at one hour and 33% at six hours when compared to the postoperative values. The P100 values were decreased 26% at one hour and 40% at six hours post-morphine respectively when compared to the postoperative values. Two of the ten patients demonstrated no blunting at either time of study. From these data we conclude that respiratory drive as measured by the VI and P100 vs CO_2 response slopes is blunted both at one and six hours post-morphine injection. Supported by USUHS Grant R08000 and GM25926

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HYPERMETABOLISM AND VENTILATORY RESPONSIVENESS IN CATS. Merrill Adams, George Swanson and John V. Weil*. CVP Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262.

Ventilation is tightly coupled to the metabolic demands of the body. Previous studies have demonstrated that alterations in the metabolic rate ($\dot{V}O_2$) lead to changes in the ventilatory responses to hypoxia and hypercapnia (Weil et al., 1972; Zwillich et al., 1975; Zwillich, Sahn and Weil, 1977). The mechanism of this coupling remains unclear. The relationship between metabolism and ventilation was examined in anesthetized cats rendered hypermetabolic by an infusion of 2,4-dinitrophenol (2,4-DNP). The ventilatory responses to progressive isocapnic hypoxia and to hypercapnia were measured before and after a dose of 2,4-DNP which doubled metabolic rate ($\dot{V}O_2$). Ventilation increased in direct proportion to metabolism such that arterial P_{CO_2} remained unchanged compared to control values. Body temperature did not change. Results suggest that the hypoxic ventilatory response curve is shifted upward and to the left and that the hypercapnic response curve was shifted upward with no apparent change in slope. These findings are similar to those observed during moderate exercise, and after protein and carbohydrate ingestion. This animal model may prove useful for investigating the relationship between metabolism and ventilatory responsiveness. (Supported by NIH Grant HL 14985).

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ALTERATION OF RESPIRATORY RATE DURING SODIUM FLUORIDE INFUSION. Richard J. Sinclair. Texas College of Osteopathic Medicine, Ft. Worth, Texas 76107.

Experiments were conducted to determine if the increased respiratory rate observed during sodium fluoride (NaF) infusion was due to a direct effect of fluoride (F) on respiratory control centers in contact with cerebrospinal fluid (CSF). Five anesthetized, mongrel dogs were administered 0.1 mg/kg/min NaF for two 30 min periods following two 30 min control periods. Two 30 min recovery periods followed cessation of the NaF infusion. CSF was sampled via a needle puncture of the Cisterna Magna for determination of pH, P_{CO_2}, F^-, Na^+, K^+, Cl^-, and HCO_3^-. Arterial plasma was concurrently sampled for similar determinations. Blood pressure, heart rate and respiratory rate were continuously monitored. Increased respiratory rate is more closely related to CSF F^- than that in the arterial plasma. The ratio of arterial plasma F^- to CSF F^- is slow to approach unity, indicating a blood-CSF barrier to F^-. As F^- increases in the CSF, respiratory rate increases. Plasma and CSF pH and P_{CO_2} are unchanged by NaF infusion. Plasma HCO_3^- falls significantly ($p<0.05$) from 20.1 mEq/l before NaF to 15.6 mEq/l after recovery. Plasma K^+ significantly increases from 3.33 mEq/l to 4.54 mEq/l after recovery. This suggests a metabolic acidosis which may result from alteration of renal bicarbonate reabsorption. The increased respiratory rate may arise from a direct effect of F^- on respiratory control centers, or is a compensation to metabolic acidosis. (Supported by an AOA Research grant).

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DOPAMINE AND CAROTID BODY FUNCTION IN THE NEWBORN LAMB. Dennis E. Mayock*, Robert D. Guthrie*, David E. Woodrum*, Univ. of Washington, Seattle, WA 98195 SPON: W. Alan Hodson

Previous studies have demonstrated that carotid body (CB) function in the lamb is deficient during the first day when compared with 2 wks. following birth (Belenky et al, JAP, 47(5)927-930,1979). Dopamine (DA) inhibits CB function in adults. If the Day 1 CB response is mediated by DA,then the inhibitory effect of DA should be diminished when compared with a mature animal. $\dot{V}_E$,V_T and T_{tot} were measured in tracheotomized animals during quiet regular breathing in room air and for 20 seconds after inhalation of 100% N_2. Response changes induced by IV bolus injection of DA 10ug/Kg or saline control were assessed before and after carotid body denervation (CBD).

(age of lamb)	% change from control ventilation ($\dot{V}_E$/Kg) DA infusion	N_2 inhalation	N_2 + DA
2 wk old	-50.5±12.2*	+224.6±74.4*	+46.9±85.6+
1 day old	-18.0±10.7+	+77.8±31.1+	+12.6±10.0+

* $p<.001$ + NS

CBD abolished the DA and anoxic responses. Control ventilation decreased 28.8% from preCBD controls in the 2 week old animals ($p<.02$). No change was seen in the one day old lambs. Thus, CB function of the 1 day old lamb - both in terms of response to acute hypoxia and effect on baseline ventilation - differs from the more mature 2 week old animal. We suggest the differences relate to maturational changes in CB dopamine content. (Grants HD 10356 & MCT-000955)

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CAROTID SINUS NERVE AND CENTRAL RESPIRATORY DRIVE IN NEWBORNS. Edward E. Lawson* and Walker A. Long* (SPON: David E. Millhorn) Univ of N Carolina, Chapel Hill, NC 27514

The origin of the biphasic respiratory response to hypoxia (hyperpnea followed by hypoventilation) in premature humans is unknown. To study this respiratory pattern without confounding blood gas changes, we used anesthetized, paralyzed, and ventilated(FiO_2 1.0) newborn animals(7 piglets 4-14d; 3 lambs, 5h-8d). End-tidal CO_2 was maintained constant by an electronic servocontroller. Respiratory output(RO) was recorded following integration of the phrenic nerve activity. Hypoxic excitation of the carotid body was stimulated by trains of electrical stimuli to the carotid sinus nerve(CSN). In each of the animals RO increased during CSN stimulation. In addition, following stimulus offset all 10 animals demonstrated a prolonged respiratory afterdischarge before returning to the prestimulus control values; findings similar to previous results in adult cats (Eldridge JAP 37:723, 1974). RO of all lambs remained increased as long as CSN stimulation was applied confirming the persistent respiratory response to hypoxia seen in lambs and adult sheep. However, in 6 of 7 piglets RO peaked shortly after onset of CSN stimulation and then, despite continued stimulation, gradually returned toward the control value. Since O_2 and CO_2 changes did not occur, the mechanism of this biphasic response must be neural in origin. These findings indicate that hypoxic neural depression is not an obligate component of the biphasic respiratory response to hypoxia. (Supported by NIH grant HD-13280 and United Cerebral Palsy)

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PROGRESSIVE BREATHING RESPONSES WITH AIRWAY OCCLUSION IN FETAL LAMB IN UTERO. Immanuela R. Moss and Emile M. Scarpelli. Pediatric Pulmonary Division, Albert Einstein College of Medicine, Bronx, New York 10461.

Intratracheal pressure (Pmax) and respiratory drive (dP/dt) from the occluded, liquid filled trachea of term fetal lambs in utero were measured for each breath during the onset of fetal breathing. Progressive breathing responses at the onset of fetal breathing were observed (1) during spontaneous breathing, (2) during sciatic nerve stimulation, (3) following naloxone administration and (4) during Fetal CO_2 Tests (Moss and Scarpelli, J. Appl. Physiol. 47:527, 1979). These responses were characterized by linear increase of both Pmax and dP/dt for 6.8 ± 0.4 breaths ($\bar{x}$ ± SEM) over 5-8 seconds, following which these parameters became stable. The rate of rise of Pmax and dP/dt was lowest during spontaneous breathing, and increased incrementally in the presence of sciatic stimulation, hypercapnia and naloxone. Mechanical factors could not account for these responses in the liquid filled lung, nor did appreciable chemical changes occur during this period. These results suggest that progressive breathing responses at the onset of fetal breathing may stem from gradual recruitment of central respiratory neurons, and that the rate of rise of such recruitment depends on facilitation by somatosensory and chemical stimulation and on release from natural (endorphin) inhibition. (Supported by NIH HL 00688 and HL 23995).

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THE PATTERN OF BREATHING OF INFANTS IN THE FIRST 90 MINUTES AFTER CESAREAN SECTION DELIVERY. J.T. Fisher, J.P. Mortola, B. Smith*, G. Fox* and S. Weeks* Depts. of Physiology and Anaesthesia, McGill Univ., Montreal, Canada H3G 1Y6.

Newborn infants delivered at term by cesarean section (CS) were studied at 10 and 90 minutes (min) after birth. Respiratory timing, volume and mouth pressure were measured by a pneumotachograph and a pressure transducer connected to a face mask applied to the infant's face. Occlusion of the mask at end expiration (functional residual capacity, FRC) or at end inspiration was also performed. The general respiratory pattern following CS delivery is extremely variable with frequent sighs and a tendency to maintain lung volume above end expiration. The least variable respiratory component was inspiratory time (T_I). In most cases tidal volume (V_T) at 90 min was less than the 10 min value. T_I had a tendency to increase from 10 to 90 min while T_E decreased. Respiratory frequency was unaltered. Mouth occlusion pressure at 0.1 sec after an inspiratory effort from FRC was 4.4 cmH_2O independant of time after delivery. Occlusion at FRC resulted in a prolongation of T_{I_O} to 190% of the control value (T_{I_C}). The apnea (T_{E_O}) following occlusion at end inspiration was 155% of the control expiratory time (T_E). This suggests phasic vagal feedback may have a stronger effect than tonic feedback at birth.

Supported by MRC Canada.

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THE FIRST BREATH OF INFANTS DELIVERED WITH CESAREAN SECTION. J.P. Mortola, J.T. Fisher, B. Smith*, G. Fox* and S. Weeks*. Dept. of Physiology and Dept. of Anaesthesia, McGill Univ., Montreal H3G 1Y6 Canada.

We have studied the pattern of breathing in 8 full term infants delivered with cesarean section. Immediately after delivery a face mask connected to a pneumotachograph was positioned and the first breaths recorded with the umbilical cord still intact. The respiratory airflow was recorded on tape for future play back during which the airflow offset of the respiratory integrator was carefully adjusted until the drift was reduced to a minimum. Babies were healthy with mean body weight of 3.6 kg, ± .3 S.D. and APGAR index of 9 or 10. The mean tidal volume ($\bar{V}_T$) of the first six breaths varied between 20 and 33 ml (mean of all babies 27 ± 5). V_T of the first breath (Br_1) was similar or more frequently larger than the following breaths. The mean breathing frequency (f) of the first six breaths of all the infants was 51 ± 19 breaths/min; f of Br_1 was generally smaller than in the following breaths due to a long expiratory time (T_E 5.33 ± 5.02 sec). In 5 of the infants the inspiratory time was also longer than in the following breaths. The mean increase in end expiratory volume after six breaths was 18.4 ± 11.5 ml, corresponding to a mean increase of 3.1 ± 1.9 ml/breath. However, the amount of air left in the lung at the end of Br_1 was 15.8 ± 17.2 ml (range 1.3 - 44.6 ml) indicating that the largest increase in end expiratory volume occurs usually with Br_1.

(Supported by MRC Canada).

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ROLE OF GLUCOSE IN THE CONTROL OF FETAL BREATHING MOVEMENTS. Bryan S. Richardson*, John M. Bissonnette and A. Roger Hohimer. Univ. of Oregon Health Sciences Ctr., Portland, OR 97201.

Fetal lambs were prepared by placement of catheters in the trachea, axillary artery and amniotic fluid. Experiments were carried out at least 3 days later in unanesthetized animals. In 10 experiments on 7 animals an I.V. bolus of 25 gm glucose (G) to fasted mothers resulted in an increase in fetal plasma G from 11.3 ± 1.0 mg/dl (SEM) to 67.3 ± 3.6, 48.8 ± 3.7, 26.4 ± 3.6 mg/dl at 15, 60 and 120 min, respectively. Fetal axillary artery pH decreased from 7.401 ± 0.009 to 7.364 ± 0.010 at 60 min ($P < 0.01$) and 7.381 ± 0.010 at 120 min and pCO_2 increased from 45.9 ± 0.9 to 48.0 ± 1.7 and 47.0 ± 1.4 at 60 and 120 min, respectively. The percent time fetal breathing movements (FBM) increased from 20.3 ± 5.4% during the 150-min control period to 30.7% ± 5.2% during the 150 min following the G bolus ($P < 0.05$). Peak FBM incidence, 45.2 ± 9.2%, was noted from 60-75 min after the G bolus. In 16 experiments on 8 non-fasted animals studied over a similar time of day, the mean fetal plasma G was 19.0 ± 1.0 mg/dl and the mean percent FBM was 47.3 ± 5.2%. In 26 observational periods on 15 animals both fasted and non-fasted a significant relationship existed between fetal plasma G level and incidence of FBM; % FBM = 2.65 × plasma G (mg/dl) - 6.02; $r = 0.63$ ($P < 0.01$). We conclude that G is an important determinant of FBM and that G stimulation of FBM in the fasted animal may act either directly or by acutely altering pCO2 and/or pH. (Supported by MRC of Canada and by HD 10034, HD 11251 and HL 05711 of USPHS.)

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EFFECTS OF ACUTE HYPOXEMIA ON CENTRAL RESPIRATORY DRIVE IN THE NEONATAL PRIMATE. William LaFramboise*, Robert D. Guthrie*, David E. Woodrum*, University of Washington, Seattle, WA 98195. SPON: W. Alan Hodson

Premature human and monkey infants have a biphasic ventilatory response to hypoxemia during the first week of life - an initial increase in minute ventilation ($\dot{V}_E$) followed by a return to baseline levels. To determine whether the inability to sustain hyperventilation during hypoxemia was due to central hypoxic depression,airway occlusion pressure (central respiratory drive) was determined in eight tracheostomized, premature M. nemestrina (.85 of gestation) during NREM sleep at 2 and 21 days of age in room air and after 1 & 5 minutes of hypoxemia (PaO_2 ≈ 25 Torr). $\dot{V}_E$/Kg, tidal volume (V_T/Kg) and inspiratory occlusion pressures at 200 msec ($P_{0.2}$) increased with a concomitant fall in $PaCO_2$ and T_I/T_{tot} at 1 minute of hypoxemia on day 2. By 5 minutes, $\dot{V}_E$/Kg fell and $PaCO_2$ returned to baseline levels while V_T/Kg fell below control ventilation values.Despite the decreases in $\dot{V}_E$/Kg and V_T/Kg, $P_{0.2}$ remained elevated throughout the stimulus. Frequency was elevated and T_I/T_{tot} was less than control after 5 minutes on day 2. Both $\dot{V}_E$/Kg and $P_{0.2}$ showed a sustained increase during the 5 minutes of hypoxemia on day 21. The persistent elevation in $P_{0.2}$ despite a drop in $\dot{V}_E$/Kg at 5 minutes on day 2 suggests that a change in the effector, i.e. increased airway resistance and/or decreased respiratory compliance, mediates the return of ventilation to baseline levels. (Supported by grants HD 10356 and MCT-000955)

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ROLE OF CSF $[H^+]$ IN THE CONTROL OF FETAL BREATHING MOVEMENTS. John M. Bissonnette, A. Roger Hohimer and Bryan S. Richardson* Dept. of Obstetrics & Gynecology, University of Oregon Health Sciences Center, Portland, OR 97201.

Fetal lambs were prepared by placement of catheters in the trachea, axillary artery, amniotic fluid and lateral cerebral ventricle and foramen magnum. Experiments were performed 3-7 days later in unanesthetized animals. The fetal CSF was perfused with artificial CSF whose $[HCO_3^-]$ varied from 7.5 to 35 mEq/l. The ventriculocisternal flow rate was 123 µl/min and a new steady state for cisternal CSF $[HCO_3^-]$ was achieved in 60-90 min. Cisternal $[H^+]$ was regulated over a range from 38 to 73 nEq/l and the effect on the incidence and depth of fetal breathing movements, measured from tracheal pressure deflections, examined. At cisternal $[H^+]$ of 44.9 ± 0.8, 53.4 ± 0.8, and 71.8 ± 1.0 nEq/l (SEM), the time during which fetal breathing movements were present was, respectively, 15.3 ± 5.9, 36.9 ± 13.2 and 44.0 ± 11.3 percent of the 60-min observation period. At these three levels of cisternal $[H^+]$ the average depth of net intrathoracic pressure change was 7.2 ± 2.2, 13.1 ± 1.8 and 16.5 ± 2.5 torr. The central fetal CSF perfusion caused no significant changes in arterial blood gases, pH, mean arterial pressure or heart rate. We conclude that central $[H^+]$ is an important determinant of the incidence and depth of fetal breathing movements.

Supported by HD 10034, HD 11251, HL 05711 from USPHS and by MRC of Canada.

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BRAIN AND CSF ACID-BASE REGULATION AND VENTILATION DURING HYPERCAPNIA IN THE NEWBORN DOG. Eugene E. Nattie and William H. Edwards*. Departments of Physiology & Maternal and Child Health, Dartmouth Medical School, Hanover, NH 03755.

We studied central nervous system (CNS) ionic composition, acid-base regulation and ventilation in the lightly anesthetized newborn puppy during 3 h of hypercapnia (F_ICO_2 = .08). Compared to adult dog, puppy blood and cerebrospinal fluid (CSF) acid-base and electrolyte values were similar, brain tissue water (89.1% vs 76%) and the corrected choride space (41% vs 22%) were greater, brain tissue Na^+ was higher and K^+, lower. Tissue $[HCO_3^-]$, estimated from in vitro CO_2 titration curves, was higher in puppy but estimated cell $[HCO_3^-]$ values, correcting for the large ecf space, were similar to adult dog values. During hypercapnia, CSF $[HCO_3^-]$ increased 2.0 mmol/l ($P < .01$) by 15 mins, and 6.2 mmol/l ($P < .01$) by 3 h. The quantity, CSF $[Na^+] - [Cl^-]$, increased stoichiometrically as CSF $[HCO_3^-]$ increased. By 3 h, CSF pH did not differ significantly from control values and quantitatively, CSF pH regulation in puppy was superior to that observed in adults. In brain tissue, at 3 h, $[Na^+]$ and $[HCO_3^-]$ were increased ($P < .05$). V_E, stimulated by CO_2 exposure, tended to decrease as CNS acid-base regulation occurred. CNS acid-base regulation in hypercapnia is quite good in the newborn puppy and ionic exchange mechanisms between CNS and blood involving Na^+ and Cl^- are important in this regulation. (Supported by HL 18351 and RCDA HL 00364.)

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PERMEABILITY OF INTESTINAL CAPILLARIES TO SMALL SOLUTES
M. A. Perry*, D. N. Granger, N. A. Mortillaro and A.E.Taylor.
Dept. of Physiology, University of South Alabama, Mobile.

The permeability of the capillaries in the cat small intestine was studied with the double indicator dilution technique. A rapid injection of a mixture of a vascular tracer (^{125}I-gamma globulin) and two diffusible tracers was made into the artery supplying an isolated loop of the small intestine. Venous samples were collected at one second intervals for 20 to 30 seconds from a cannula in the vein draining the segment. The extraction of individual samples collected before the peak of the vascular tracer curve were constant. A mean of values on this initial plateau was used to estimate extraction. In 10 experiments on five cats, the mean extraction for raffinose was 0.67 and for inulin was 0.44. Permeability-surface area products (PS) for raffinose ranged from 11 to 51 ml/min/100g and for inulin the range was 6 to 24 ml/min/100g. PS for both diffusible tracers increased with increasing flow through the tissue indicating flow limitation of these molecules even with blood flows which were three times resting levels. The ratio of the permeabilities for the diffusible tracers was, in all instances, less than the ratio of their free diffusion coefficients. The data indicates a high permeability of intestinal capillaries to small lipid insoluble solutes. Neither raffinose nor inulin is restricted by the capillary wall. (Supported NHLBI 15680)

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SUPEROXIDE RADICALS AND INTESTINAL ISCHEMIA. D.N.Granger
J. M. McCord*, G. Rutili* and A. E. Taylor. Depts. of Physiology and Biochemistry, University of South Alabama Mobile.

The permeability of intestinal capillaries was assessed under control conditions and after regional ischemia using the relationship between the lymph to plasma protein concentration ratio (L/p) and lymph flow. Lymph flow and L/p was determined at venous pressures of 0, 10, 20 and 30 mmHg. The osmotic reflection coefficient (σ_d) was estimated using σ_d=1-L/p when L/p is filtration rate independent. One hour of regional ischemia in autoperfused segments of cat ileum caused a dramatic increase in capillary permeability (σ_d was reduced from a normal value 0.92 to 0.59). Pretreatment with either benadryl + cimetidine, indomethacin or methylprednisolone did not significantly alter the permeability increase induced by regional ischemia. Pretreatment with superoxide dismutase (SOD), an oxide radical scavenger enzyme, significantly attenuated the capillary permeability change induced by regional ischemia (σ_d=0.74) With both kidneys ligated, SOD pretreatment more effectively prevented the ischemia-induced increase in capillary permeability (σ_d=0.86). These findings indicate that superoxide radicals are responsible, at least in part, for the increased capillary permeability in the ischemic bowel. (Supported by NHLBI 15680)

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INTRINSIC REGULATION OF GASTRIC BLOOD FLOW AND OXYGEN UPTAKE. L. Holm-Rutili*, M. A. Perry* and D. N. Granger
(Spon. J. M. Downey) Dept. of Physiology, University of South Alabama, Mobile.

Autoregulation of blood flow is generally absent in sympathetically innervated stomach preparations. The purpose of this study was to determine if the ability of the stomach to autoregulate blood flow and oxygen uptake is altered by sympathetic denervation. Blood flow, oxygen extraction, local arterial pressure and venous pressure were continuously monitored in sympathetically innervated and denervated autoperfused dog stomach preparations. As perfusion pressure was reduced in increments from 120 to 20 mmHg in innervated preparations, blood flow, and oxygen uptake decreased while oxygen extraction and vascular resistance increased. Reductions in perfusion pressure in denervated preparations resulted in a decrease in blood flow, oxygen uptake and vascular resistance while O_2 extraction increased. The ability of the stomach to regulate blood flow and O_2 uptake was significantly improved following denervation. However, oxygen uptake in denervated stomachs were generally higher than innervated stomachs. Autoregulation of gastric blood flow therefore appears to be influenced by sympathetic tone and/or oxygen uptake (Supported by NHLBI 15680).

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ROLE OF BILE SALTS IN THE BILE-INDUCED ILEAL HYPEREMIA. P. R. Kvietys* and D. N. Granger. Dept. of Physiology, University of South Alabama, Mobile, AL 36688.

Intraluminal placement of diluted gallbladder bile increases blood flow to the ileum, but does not alter blood flow to the jejunum. Since the ileum is the major site of bile salt absorption, we designed experiments to assess whether bile salts are involved in the bile-induced hyperemia in the ileum. In an autoperfused in situ preparation of the canine terminal ileum, we measured arterial and venous pressures, blood flow, and arteriovenous oxygen difference. The effects of luminal placement of the following solutions on ileal hemodynamics and oxygenation were determined: 1) gallbladder bile (20%), 2) cholestyramine treated gallbladder bile (20%), 3) cholestyramine alone, or 4) a mixture of three bile-salts (taurocholate, 13.5 mM; taurodeoxycholate, 4.2 mM; taurochenodeoxycholate, 2.7 mM). Diluted gallbladder bile doubled ileal blood flow and significantly increased ileal oxygen uptake. Cholestyramine treated bile or cholestyramine alone did not significantly alter ileal blood flow or oxygenation. The mixture of bile salts increased ileal blood flow and oxygen uptake to the same extent as seen with diluted gallbladder bile. The results of these studies indicate that the bile salts play a major role in the bile-induced hyperemia in the ileum. (Supported by NHLBI 15680).

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Dependence of Intestinal Absorptive Hyperemia on Mucosal Oxygenation. H. Glenn Bohlen. Dept. Physiology, Indiana University Medical School, Indianapolis, Indiana 46223

Intestinal glucose absorption decreases villus PO_2 in rats from a normal of 14-17 mm Hg to 4-7 mm Hg. The decline in tissue PO_2 may contribute to the near doubling of intestinal blood flow during glucose exposure. This possibility was investigated using an in vivo preparation of the rat small intestine. The suffusate PO_2 over the muscularis was held at a constant 40-45 mm Hg as the mucosal suffusate PO_2 was changed in random order to 5-10 mm Hg, 40-45 mm Hg and 70-75 mm Hg. At rest, mucosal tissue PO_2 increased only 4-5 mm Hg as the suffusate PO_2 increased from 5-10 mm Hg up to 70-75 mm Hg; intestinal blood flow decreased by approximately one-half. During glucose exposure (100 mg%), mucosal tissue PO_2 was 5-7 mm Hg, blood flow was nearly doubled relative to the resting state for each suffusate PO_2. However, actual blood flows at high suffusate PO_2 were about half that at the lowest suffusate PO_2. At rest, lowering the mucosal suffusate PO_2 to 5-10 mm Hg decreased the villus tissue PO_2 to within 2-3 mm Hg of that during glucose exposure. However, blood flow increased less than 30% of that during glucose exposure at a suffusate PO_2 of 5-10 mm Hg. The data indicate that oxygen availability to the mucosa is inversely related to the magnitude of blood flow at rest and during absorption. However, oxygen of itself causes only about one-third of the absorptive hyperemia response. Supported by NIH Grant HL 20605.

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COMPARISON OF VASCULAR AND METABOLIC EFFECTS OF HIGH PROTEIN (P), FAT (F), AND CARBOHYDRATE (C) DIETS AND ETHANOL IN THE CANINE JEJUNUM. H. Siregar* and C.C. Chou. Depts. of Physiology and Med., Michigan State Univ., East Lansing, MI 48824.

The vascular and metabolic effects of digested products of high protein (HP)(64% P, 22% C, 8% F), high fat (HF)(45% F, 29% C, 18% P), and high carbohydrate (HC)(68% C, 8% F, 18% P) (United States Biochemical Co.) diets in the jejunal lumen were compared by alternately placing a mixture of each of these solutions with 10% gallbladder bile into the lumens of the two adjacent jejunal segments, while measuring local venous outflow and oxygen consumption ($\dot{V}O_2$). In addition, the effects of gin (10% dilutent of 90 proof Beefeater^R gin, containing 4.5% ethanol) and 4.5% ethanol solution were also compared. The percent changes in blood flow and $\dot{V}O_2$ from control (normal saline in the lumen) were:

	HC	HP	HF	Gin	Ethanol
N	18	18	18	8	8
Flow	16.4*	21.0*	24.7*	+14.9*	+16.2*
$\dot{V}O_2$	+12.8*	+17.1*	+11.1*	+ 0.1	- 2.0

* significant change from control.

The HF-induced hyperemia was significantly greater than that induced by HP, which was greater than that induced by HC. However, the increases in $\dot{V}O_2$ for all three diets were the same. Gin and ethanol increased flow to the same extent and neither altered $\dot{V}O_2$. (Supported by Grant HL-15231 from NHLI).

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CARDIOTOXIC FACTORS RELEASED FROM THE CANINE JEJUNUM. R.P. Pittman, J.T. Senko*, R. Nyhof*, and C.C. Chou. Dept. of Physiology, Michigan State University, E. Lansing, MI 48824

Plasma samples from dogs subjected to irreversible hemorrhagic shock were assayed for cardiotoxic substances in the Langendorff guinea pig heart preparation. Blood samples were obtained from the femoral artery and jejunal vein of hemorrhaged animals (Wiggers model, 35 mmHg for 3 h then retransfusion of shed blood) and sham hemorrhaged dogs. The guinea pig heart bioassay was a sensitive assay detecting cardiodepressant activities in 1:10 dilutions of shocked plasma diluted with Krebs-Ringer-bicarbonate perfusate. Depressive activities were not found in either arterial plasma (+0.07±5.0 cardiodepressant units, CDU) or the jejunal venous plasma (-9.0±3.0 CDU) before hemorrhage. Significant increases of CDU were observed in the jejunal venous plasma (+20.0+7.0 CDU, $p < 0.01$) but were not observed in the arterial plasma (+10.0± 4.0 CDU) after the hemorrhage procedure. Sham hemorrhaged dogs did not show significant changes of CDU throughout the experiment. Cardiotoxins as detected by the Langendorff guinea pig heart bioassay are released into the canine circulatory system during hemorrhage and the intestine may be one of the sources of these toxins. (Supported in part by Michigan Heart Association Grant)

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EFFECT OF ENDOGENOUS GASTRIN ON GASTRIC SECRETION AND BLOOD FLOW. M.J. Volkert*, S.S. Shirazi*, and J.D. Coon* (SPON: D.G. Reynolds). Univ. of Iowa, Iowa City, IA 52242.

Gastrin is known to increase acid secretion, but there is a controversy regarding its effect on gastric blood flow. We studied the relationship of gastric acid secretion and blood flow by using an antral transposition model (8 dogs). This model produces hyperacidity due to increased gastrin levels. Five dogs with antrectomy were used as control. Gastric secretion was measured at base line and after inhibition with 300 mg of Cimetidine before and after operation. Fundic mucosal blood flow (FMBF) was measured by using γ labeled microspheres. The results are expressed as mEg/hr for H+ and ml/min/100g tissue for blood flow.

	Antral Trans. 8 dogs		Antrectomy 5 dogs	
	H+	FMBF	H+	FMBF
Preop base line	1.1±0.4		0.5±0.3	
Cimetidine	0.3±0.1*		0.2±0.1	
Postop base line	5.8±2.0*	98±19	0.4±0.2	120±21
Cimetidine	0.6±0.3	110±23	0.1±0.05*	130±34

*indicated P value at least <0.05 when compared to controls

These results indicate that 1) antral transposition increases gastric acidity, 2) this increase in gastric acidity is not associated with an increase in blood flow, and 3) Cimetidine decreases the hyperacidity due to increased gastrin, but this decrease in acidity is not associated with a decrease in blood flow. We conclude there is no linear relationship between gastric acid secretion and gastric blood flow.

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SPLANCHNIC VASCULAR RESPONSES TO HEATING ARE MODIFIED BY PLASMA RENIN ACTIVITY IN MAN. P. Escourrou*, P.R. Freund*, and L.B. Rowell, Univ. of Wash. School of Medicine, Seattle, Wa. 98195

To determine the role of the renin-angiotensin system in heat-induced splanchnic vasoconstriction, 5 normal men (21-32 yr) were heated in water-perfused suits for 40-50 min in two separate experiments. Experiment 1: Heating caused (values are compared with measurements made during a 30-min normothermic control period): i) 90 ± 50% (mean ± SD) rise in splanchnic vascular resistance (SVR); ii) plasma renin activity (PRA) (radioimmuno-assay) increased from 111 ± 56 ng/100 ml·3 hr to 253 ± 130; iii) forearm vascular resistance (FVR)(venous occlusion plethysmography) fell to 15 ± 4% of control value; iv) heart rate (HR) and rectal temperature (T_r) rose 49 ± 10 bpm and .65 ± .22°C. Experiment 2: The objective was to block renin release by intravenous infusion of propranolol (P)(1 mg/min) during the first 10 min of heating (10-mg dose). After P heating caused i) 64 ± 25% increase in SVR; ii) PRA increased from 77 ± 33 to 154 ± 103; iii) FVR fell to 12 ± 4% of control value; and iv) HR and T_r rose 29 ± 7 bpm and .64 ± .27°C. Although PRA increases due to heating were not uniformly blocked by P (and in one case PRA actually rose after P infusion) the changes in SVR still parallel the changes in PRA responses regardless of direction (r=0.81). P had no effect on FVR, a measure of skin vasodilation. We conclude that the renin-angiotensin system contributes to part of the splanchnic vasoconstriction during heating. (Supported in part by NHLBI Grant HL-16910)

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EFFECTS OF FEEDING ON GUT O_2 CONSUMPTION AND BLOOD FLOW IN CONSCIOUS NEWBORN LAMBS. Daniel I. Edelstone* and Ian R. Holzman* (SPON: P.M. Taylor). Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, PA 15213

In 7 lambs (ages 7-10 d) catheters were placed in the descending aorta, portal vein, and left atrium. Two to six d later we determined blood flow(Q) and O_2 delivery(DO_2) to the gut and O_2 consumption(VO_2) and O_2 extraction($VO_2/DO_2 \cdot 100$) by the gut in the conscious lambs during fasting and at 1,2, 3,4, and 6 h postprandially. O_2 contents of arterial(A) and portal venous (PV) blood were measured with a Lex-O_2-Con analyzer, and blood flow was quantified with the radioactive microsphere technique. VO_2 was calculated with the Fick principle. The table indicates the mean results (*=p<0.05 when compared with fasting values; analysis of variance).

Gut:	Fasting	1h	2h	3h	4h	6h
DO_2(ml O_2/min/kg)	5.6	6.7*	6.0	5.5	5.3	5.2
VO_2(ml O_2/min/kg)	1.2	2.0*	1.7*	1.5*	1.2	1.2
O_2 extraction (%)	22	32*	31*	28*	24	23
A-PV(ml O_2/dl blood)	2.1	2.8*	2.9*	2.7*	2.2	2.1
Q (ml/min/kg)	55	71*	61	57	52	57

Our results show that VO_2 by the neonatal gut increases 1-3h postprandially. The increased O_2 requirement of the gastrointestinal tract is met both by a greater gut DO_2 and by an increased gut oxygen extraction.

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LIVER BLOOD FLOW AND O_2 CONSUMPTION BEFORE AND AFTER FEEDING IN CONSCIOUS NEWBORN LAMBS. Ian R. Holzman* and Daniel I. Edelstone* (SPON:P.M. Taylor). Magee-Womens Hospital, University of Pittsburgh, PA 15213

We determined hepatic arterial(Q_{HA}) and portal venous(Q_{PV}) blood flows and calculated liver O_2 delivery(DO_2), O_2 consumption(VO_2), and O_2 extraction($VO_2/DO_2 \cdot 100$) in 7 chronically catheterized conscious newborn lambs. Studies were done with the lambs fasted and at 1,2,3,4, and 6h postprandially. We measured O_2 contents with a Lex-O_2-Con analyzer, blood flows with the radioactive microsphere technique, and calculated VO_2 with the Fick principle. The table indicates the mean results (*=p<0.05 when compared with fasting values; analysis of variance).

Liver:	Fasting	1h	2h	3h	4h	6h
DO_2(ml O_2/min/kg)	5.8	6.0	5.7	5.3	5.4	5.3
VO_2(ml O_2/min/kg)	2.3	2.4	1.9	1.5*	2.0	2.2
O_2 extraction (%)	44	42	35	31*	39	44
Q_{HA}(ml/min/kg)	6	7	7	6	6	8
Q_{PV}(ml/min/kg)	66	81*	71	65	62	65

Our results show that the neonatal liver, as compared with the adult liver, maintains a high VO_2 during both fasting and fed states except for a transient decrease 3h postprandially. This decreased VO_2 is associated with a decreased O_2 extraction rather than with a change in DO_2. The rise in Q_{PV} at 1h reflects an increased gut blood flow secondary to feeding.

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JEJUNAL K_f MEASURED BY THE INTEGRATED DIFFERENCE BETWEEN INFLOW AND OUTFLOW. Bruce L. Johns*, Tom D. Bennett* and Carl F. Rothe. Indiana University, Indianapolis, IN 46223.

Temporal separation of vascular volume and transcapillary exchange events is important for proper estimation of the capillary filtration coefficient, K_f. In 8 isolated, pump-perfused segments of canine small intestine, fast (<0.5 sec) changes in venous pressure (ΔPv) of 10 and 20 mmHg were used. Vascular volume was obtained by a 1-min infusion of indocyanine green dye during control and at 3 min following a pressure increase or return to control. Tissue monitoring of Cr-51 tagged RBCs provided an independent estimate of vascular volume changes. The integrated difference between tissue inflow (by arterial pump tachometer) and outflow (by venous flow-probe) (IDF) was used to assess vascular and transcapillary events for 5-min periods of each ΔPv. The data were digitally recorded at 1-sec intervals and fitted (by non-linear least squares regression) to an equation having 2 exponential terms and a linear rate term. The vascular volume change by dye, tagged RBCs, and the fast exponential of the IDF were almost identical and had a time-constant (τ, by IDF) of 2-3 sec. The pressure increase and return to control showed the same volume changes. The 2nd exponential of the IDF had τ's of 25-30 sec. The time derivative of the 2nd exponential at t=0 gave estimates of 0.58 and 1.17 *ml/min-100 gm mmHg for* K_f at 10 and 20 mmHg ΔPv, respectively. These values for K_f are 5-10 times larger than values typically reported from whole tissue studies. (Supported by USPHS Grant HL-07723.)

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REPRODUCTIVE FUNCTION AND BRAIN SEROTONIN. S.L. Jones*, D.B. Hudson*, and P.S. Timiras. University of California, Berkeley, CA 94720

The periodic release of pituitary hormones regulating reproductive function is purported to be influenced by serotonergic neurons. Concomitant alterations in regional brain serotonin (5-HT) levels, circulating pituitary hormone (FSH, LH, TSH) levels, estrous cyclicity and end-organ (ovary) size, under a variety of conditions (e.g. lighting, drugs, nutrition, age), would suggest functional relationships. Para-chlorophenylalanine (PCPA) which interferes with 5-HT synthesis, was injected chronically for 15 to 90 days, commencing at weaning, and its effects were studied at periodic intervals. PCPA reduces 5-HT levels in all brain regions, from 50% in the hypothalamus to 90% in the pons-medulla. This decrease is also age-dependent, the greater decrease occurring at later ages. In the treated animals, body and adrenal weights were unaffected, vaginal opening appeared to occur at a later age, and the ovary did not undergo the expected size increase which normally follows puberty. Delay in ovarian development is further supported by significantly low levels of gonadotropins. In contrast, TSH levels are markedly increased (300%) in the PCPA animals, with a marked decrease in serum T3 and T4 levels. It is concluded that adequate levels of brain 5-HT determine the normal timetable of pituitary-gonadal and pituitary-thyroid development. (Supported by grant NIH AG0043 and U.C.B. President's Undergraduate Fellowship).

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EFFECT OF PINEALECTOMY ON THE LEVEL OF MELATONIN IN THE RETINA OF RATS. P.L. Tang*, S.F. Pang* and H.S. Yu*. (SPON: T.M. Wong). Department of Physiology, University of Hong Kong, Hong Kong.

The effect of pinealectomy on the melatonin (N-acetyl-5-methoxytryptamine) level in the retina of rats was studied. Male Sprague-Dawley rats (333±4g) were kept in a temperature-controlled room at 23±3°C, and were adapted to a 12L:12D photoperiod, lights on at 0600h. Food and water were available ad lib. After 2 weeks, half of them were pinealectomized. One week after the operation, all the rats were decapitated at 4-hour intervals around the clock, starting at 1200h. Retinas were dissected out and homogenised in 0.2 N perchloric acid. Melatonin was then extracted by chloroform at pH≥10 and quantified by radioimmunoassay. In the control rats, there is a diurnal rhythm of melatonin in the retina with the highest level at 2400h and the lowest at 1600h. Pinealectomy does not change melatonin levels in the retina in all the time periods studied and the diurnal rhythm of melatonin in the retina persists in the pinealectomized rats. Thus, there is no compensatory effect observed following pinealectomy in our short term experiment. Moreover, our finding suggests that the retina in rats may synthesize melatonin from its own tissues and/or through uptake of melatonin from other extrapineal sources. Blinding and tissue-culture is a possible clue to this assertion. (Supported by Hong Kong University Research Grant 335/034/9940 and 334/034 0269).

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RELEASE OF N-ACETYLSEROTONIN BY THE PINEAL AND RETINA OF RATS IN VITRO AND THE EFFECT OF NOREPINEPHRINE. P.H. Chow, S.F. Pang*, H.S. Yu*. Dept. of Anatomy and Physiology, University of Hong Kong, Hong Kong.

N-acetylserotonin, the precursor of melatonin, has been demonstrated in the rat serum with high concentrations from mid-dark to the early light period and low concentrations from mid-light to the early dark period. The diurnal rhythm demonstrated in the rat serum is similar to the rhythms of N-acetylserotonin in the pineal and the retina of rats. Thus, it has been suggested that N-acetylserotonin may be secreted by the pineal and the retina. In this study, the release of N-acetylserotonin by the pineal and the retina in vitro with and without norepinephrine was investigated. Male rats were adapted in a photoperiod of 12L/12D. After 2 weeks, they were sacrified. Pineals and retinas were incubated at 37°C in TC199 containing 10% fetal calf serum and gentamicin (40µg/ml), and a gas tension of 3% CO_2 and 97% air. Each 3 cm plastic culture dish certain either 2 pineals or 4 retinas in 1.5 ml of medium. After the initial 2 hr period, the medium was discarded and renewed. Subsequently, medium was collected at 4-hour intervals. N-acetylserotonin was extracted from both tissues and medium and determined by RIA. Preliminary experiments indicate that NAS was released by both pineals and retinas. Moreover, norepinephrine (1×10^{-4} M) was found to increase the release of N-acetylserotonin by 300% in retinas and 40% in pineals. (Supported by the Univ. of Hong Kong Research Grant 335/034/9940 and 334/034/0269)

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DISSOCIATION OF THE NEURAL COMPONENTS GOVERNING THE LIGHT-DARK (L/D) ENTRAINMENT OF PINEAL ACTIVITY AND DRINKING BEHAVIOR FROM THAT OF EPISODIC GROWTH HORMONE (GH) SECRETION IN THE RAT. W.J. Millard*, S.M. Reppert*, S.M. Sagar* and J.B. Martin, Mass. Gen. Hosp., Boston, MA 02114

Most circadian rhythms in the rodent are cued to the 24-hr day by environmental lighting. Since the neonatal administration of monosodium glutamate (MSG) causes severe retinal damage and lowers plasma GH levels in the adult, we determined whether L/D entrainment of the GH rhythm, as well as circadian rhythms in pineal activity and drinking behavior, was lost in MSG-treated rats. Neonatal rats were injected with MSG (4 mg/g b.w., sc), and controls 10% NaCl. At 10 days of life half of the control animals were blinded by orbital enucleation. All animals were raised in diurnal lighting (L/D 12:12). Adult male rats were fitted with intra-atrial catheters and sampled over 6 hrs for plasma GH at both "lights-on" and "lights-off". Pineal activity was determined by measuring N-acetyltransferase at mid-dark, mid-light, and mid-dark after a 30 minute light pulse. GH profile analysis showed that pulses occurred randomly in relation to the L/D cycle in both blind and MSG animals. In contrast, normal L/D entrainment of water intake and pineal activity was preserved in the MSG rat. These data suggest that, in MSG rats 1) L/D entrainment of circadian rhythms, presumably generated in the suprachiasmatic nucleus (SCN), remain intact whereas, 2) neural mechanisms which regulate L/D entrainment of pulsatile GH secretion are disrupted. The latter likely occurs at the level of the arcuate nucleus. Supported by USPHS Grant #1-RO1-AM26252-01.

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EFFECTS OF SUDDEN EXTENSION OF THE DARK OR LIGHT PERIOD ON THE RETINAL MELATONIN LEVEL IN GUINEA PIGS. H.S. Yu*, S.F. Pang* and P.L. Tang*. (SPON: A.C.L. Hsieh). Dept. of Physiology, University of Hong Kong, Hong Kong.

To study the effects of sudden extension of the dark (D) or light (L) period on retinal melatonin (Mel) level, 2 experiments were conducted on male pigmented guinea pigs (445±17g) adapted for 2 weeks to a photoperiod of 12L:12D with lights on at 0600h. In experiment 1, the experimental animals (E) were subjected to an extended D period (lights remained off after 0600h) on the day of sacrifice. The E were decapitated in D and the control animals (C) in L at 1200h. The retinas were dissected out. Mel was extracted by chloroform and quantified by RIA. The retinal Mel level per pair of retinas in the E (386±64 pg; N=8) was significantly higher ($P<0.05$) than that of the C (266±16 pg). In experiment 2, the E were exposed to an extended L period (lights remained on after 1800h). At 2400h, the C were decapitated in D and the E in L. The retinal Mel level in the E (241±17 pg; N=8) was significantly lower ($P<0.05$) than that of the C (307±27 pg; N=8). Thus, D increases while L decreases the retinal Mel level. These correlate well with the hypothesis that depolarized photoreceptors in D synthesize and secrete more Mel while hyperpolarized ones in L synthesize and secrete less Mel. Since Mel aggregates pigmented cells in guinea pig retinas, L and/or D regulation of retinal Mel level seems to be an adaptation of animals to D or L environment. (Supported by the University of Hong Kong Research Grant 335/034/9940 and 334/034/0269).

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EXISTENCE OF GLUCOCORTICOID BINDING IN THE STRIATUM: MECHANISM FOR INCREASED CHOLINE UPTAKE. Barbara B. Turner and Charles H. DeFiore*. Depts. of Biology & Psych., VPI & SU, Blacksburg, VA 24061

Corticosterone is selectively bound by distinct areas of the rodent brain, in particular, the hippocampus (HC). Autoradiographic data show no specific binding of corticosterone in much of the brain, including the striatum (caudate-putamen, C-P). However, cats pretreated with glucocorticoids show a selective increase in high-affinity choline accumulation in the C-P (Riker et al., Molec. Pharm. 16:886, 1979). We wished to demonstrate the existence of cytoplasmic receptors for glucocorticoids in the C-P. Sprague-Dawley male rats, previously adrenalectomized, were anesthetized and perfused. The C-P and the HC (for comparison) were dissected out, homogenized, and aliquots from the resultant cytosols were incubated with varying concentrations of 3-H corticosterone. The 3-H corticosterone bound to receptor protein was separated from free steroid by passage through LH-20 columns. Our results indicate that the C-P has a maximal binding capacity of 167±14 femtomols/mg protein compared to 343 femtomols/mg protein for the HC. The apparent K_d for the two tissues is similar: 2.1X 10-9 M for the C-P, and 1.5X10-9 M for the HC. These data 1) provide evidence that cytoplasmic receptors for glucocorticoids do exist in at least some "non-target" areas of the brain and 2) provide a physiological mechanism by which glucocorticoids may modulate aceylcholine function in the C-P. Supported by NIH Biomedical Research Support Grant #2 SO7 RR 07095-13.

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ROLE OF ADRENAL NERVES IN MEDIATING INHIBITION OF STRESS-INDUCED ACTH SECRETION. Errol B. DeSouza* and Glen R. Van Loon* (Spon: O. Sirek) Depts of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada M5S IA8

Compensatory adrenal growth in response to unilateral adrenalectomy appears to be mediated in part by a nerve pathway from adrenals. In this study we examined the possibility that adrenal nerves modulate the ACTH response to stress. In adult male rats, a 2 min restraint stress increased plasma concentration of ACTH (35±5 to 386±67 pg/ml; p<0.005) and corticosterone (7±2 to 41±4 µg/dl; p<0.001) at 5 and 15 min respectively. A similar stress in bilaterally adrenalectomized rats increased plasma ACTH from 536±62 to 1114±166 pg/ml; p<0.025). Adrenal denervation was produced by removal of the adrenals and immediate replacement in the adrenal beds with subsequent revascularization. In these animals, basal plasma ACTH was increased (183±45 pg/ml; p<0.025) above that of sham-operated rats, but was less than that of adrenalectomized rats (p<0.005). Also, basal plasma corticosterone in these adrenal-denervated rats was increased (13±2 µg/dl; p<0.05) compared to sham-operated controls (7±2 µg/dl). Plasma ACTH response (183±45 to 1241±253 pg/ml) to restraint stress in these adrenal-denervated rats was potentiated, in spite of a plasma corticosterone response from 13±2 to 26±6 µg/dl.
CONCLUSION: These data suggest that in addition to inhibition by glucocorticoid there exists an inhibitory mechanism mediated by adrenal nerves which inhibits the plasma ACTH response to stress. (Supported by MRC MA-5183)

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ADRENAL DENERVATION BLOCKS THE EPINEPHRINE RESPONSE TO INTRACEREBRAL β-ENDORPHIN. Nathan M. Appel*, Doris Ho*, Errol B. De Souza* and Glen R. Van Loon* (Spon: O. Sirek) Depts. of Physiology and Medicine, University of Toronto, Toronto, Canada

The importance of endorphins, the generic term for endogenous opioid peptides, appears well established in neuroendocrine regulation. Endorphins alter secretion of a number of pituitary hormones. In this study we examined the effect of endorphin on a nonpituitary hormone, epinephrine. Intracisternal administration of synthetic human β-endorphin (7.25 nmoles) produced a major, prolonged increase in plasma epinephrine in unanesthetized, freely-moving rats bearing a chronic intraarterial cannula when compared with the response to intracisternal saline vehicle. Prior intraarterial administration of naloxone (1.1 µmoles/kg) blocked this β-endorphin-induced increase in plasma epinephrine, supporting mediation by opioid receptors. Since plasma epinephrine is derived almost entirely from adrenal medulla, it appears that β-endorphin increases plasma epinephrine secretion from adrenal medulla. Rats with bilateral adrenal gland denervation showed a significant plasma corticosterone response to 2 min restraint stress, but failed to show a plasma epinephrine response to that stress. Furthermore intracisternal β-endorphin failed to increase plasma epinephrine in adrenal-denervated rats.
CONCLUSION: Intracerebral endorphins increase plasma epinephrine concentration by increasing central sympathetic outflow to adrenal medulla.
(Supported by MRC MA-7000)

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ESTROGENIC MODIFICATION OF ADRENERGIC BUT NOT BENZODIAZEPINE OR OPIATE RECEPTOR BINDING IN FEMALE RAT BRAIN. M. Wilkinson, H. Herdon[1]* and Dale Grovestine*. Dept. Physiol. & Biophys., Dalhousie U., Halifax, NS, Canada B3H 4H7 and [1]Dept. Ob/Gyn., St. George's Hospital Med. School, London, UK

We previously reported that in the rat circulating estrogens (E) affect the numbers of brain α- and β-adrenergic receptors (αR and βR) (Brain Res., 167 195 & 168 652). We have extended this work to include [^{3}H]-flunitrazepam (FNZ), [^{3}H]-dihydromorphine (DHM) and [^{3}H]-enkephalinamide (ED) binding in brain tissue from adult female rats. In a variety of hormonal manipulations (eg. OVX + E; OVX + E + PROG.; OVX + E capsules) no differences were observed in FNZ, DHM or ED binding to whole brain membranes consequent to E treatment. Further studies in vitro revealed that the potent estrogen stilbestrol (DES; 50µM) when incubated with immature female hypothalami (H) caused highly significant inverse changes in αR and βR (by Scatchard analysis). Thus, at 4h, αR are reduced by 30% whilst βR increase by 60%. In adult OVX H similar changes are seen (αR: -25%; βR; +140%). Under the same conditions (immature H) FNZ and DHM binding are increased by <20%. No changes are seen in αR, βR or FNZ in cerebral cortex incubations. This in vitro preparation may be a useful method with which to study steroid-neurotransmitter interactions in the brain (supported by MRC Canada, MA-7131).

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PORCINE FOLLICULAR FLUID AND ESTRADIOL: EFFECTS ON PERI-OVULATORY GONADOTROPIN RELEASE. Michael E. Rush,* Oladapo A. Ashiru,* and Charles A. Blake. University of Nebraska Medical Center, Omaha, NE 68105

In rats with atrial cannulae, injection of either 0.1 or 0.5 ml of the inhibin-containing material, porcine follicular fluid (pFF), at 1000, 1300 and 1530h proestrus (P) completely blocked the increases in plasma FSH and LH on P observed in controls, but a small rise in plasma FSH occurred during the morning of estrus (E). PFF administered at 1000, 1300, 1530, 1800, 2100, and 2400h P completely blocked the FSH rise on E. When the 1000h injection was omitted, a partial elevation in plasma FSH was observed throughout P and E and a proestrous LH surge occurred. Silastic implants of estradiol-17β (E_2) given at 1300h P had no effect on LH or FSH during P or E, nor did the E_2 prevent the small rise in plasma FSH seen on the morning of E in rats given pFF up to 1530h P. Constant-rate (50 ng/h) iv infusion of LHRH on P in pFF-injected rats did not elevate plasma FSH. It did elevate plasma LH partially as compared to that of phenobarbital-blocked controls. These data suggest that decreasing E_2 titers on P either alone or in conjunction with decreasing inhibin titers play no major role in the selective release of pituitary FSH during E but support the view that decreasing inhibin titers could be involved. These data are also the first to show pFF to block the LH surge, by an action exerted, at least in part, at the pituitary to prevent LHRH-induced LH release. (Supported by the NIH grant nos. HD11011 and HD07097.).

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LHRH AND ANTI-LHRH ACTIVITY IN VENTRICULAR CEREBROSPINAL FLUID OF CHILDREN WITH HYDROCEPHALUS. Karl M. Knigge and Shirley A. Joseph. The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

Cerebrospinal fluid (CSF) of the lateral or third cerebral ventricles was collected during and/or after surgery on 38 children with various types of hydrocephalus. The volumes of CSF collected ranged from 2.5 to 1,660 ml. Two to 10 ml. samples were lyophilized, extracted with absolute methanol and the supernatant taken to dryness. LHRH in these extracts was measured by RIA. LHRH and anti-LHRH activity was measured using monolayers of dispersed rat anterior pituitary cells. Radioimmunoassayable LHRH was present in 17 of 38 samples, ranging in concentration from 21 to 1,127 pg/ml. In bioassay, 5 of the 17 samples exhibited no LH releasing activity. Instead they inhibited or depressed the LH-releasing action of a challenging dose of 1 ng. of synthetic LHRH. The inhibitory effect could be overcome by increasing the dose of LHRH. This anti-LHRH like activity was found only in the CSF of children with congenital hydrocephalus.
(Supported by Ford Foundation 780-0616 and NSF-PCM 78-16123.)

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IDENTIFICATION OF PUTATIVE CRF NEURONS IN THE GOLDFISH HYPOTHALAMUS. James Fryer and Leonard Maler*, Univ. of Ottawa, Ottawa, Ont., K1N 9A9.

The nucleus preopticus (NPO) of the goldfish hypothalamus is composed of magnocellular (MC) and parvocellular (PC) neurons which directly innervate the pars distalis and neurointermediate lobe. After a systemic injection of horseradish peroxidase (HRP), HRP reaction products can be visualized in NPO perikarya following the retrograde transport of HRP from axon terminals in the pituitary. In rostral PC, but not MC neurons, the uptake of HRP was enhanced following activation of the hypothalamo-corticotroph axis by stress or by injection of the adrenocortical inhibitor, metopirone. Light microscopy revealed a significant increase in nuclear area of rostral PC neurons after 5 days of metopirone administration. The ultrastructural appearance of the majority of these PC neurons revealed a lobulated nucleus and a reduction in the number of neurosecretory granules indicative of enhanced secretory activity. Some of the PC neurons retained a rounded nucleus, and contained neurosecretory granules which were significantly smaller than metopirone-responsive PC neurons. These observations suggest that these metopirone-responsive PC neurons qualify as CRF (corticotropin-releasing factor) neurons.
(Supported by MRC).

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DENSE-AS-ONE-GOES ANALYZED PHYSIOLOGIC MONITORING OF AGING GAUGES COST-EFFECTIVELY PATHOGNOMIC TEMPERATURE AND OTHER RHYTHM ALTERATIONS INCLUDING FEVER. H. Levine, E. Halberg,and F. Halberg. UConn, Farmington,Ct. and Univ. of Minnesota, Minneapolis, Mn. 55455, Univ. of L'Aquila,Italy

Automatic ambulatory recording was carried out mostly at 6 or 12 minute intervals for spans ranging from days to months and information was stored in solid-state devices. Human aging was gauged by rectal and axillary temperature, motor activity and pulse leading to the construction of time-qualified 90, 90 tolerance intervals (Chronodesms-Experientia 34:713-716,1978) and the cosinor-derivation of rhythm characteristics (Physiology Teacher 1:1-11,1972). Data before, during, and after ambulatory surgery and a nosocomial infection analyzed by the fit of a 24-hour cosine curve to 24-hour data intervals displaced in 6-hour increments revealed a rhythm alteration within 6 hours after surgery for rectal and axillary temperature and only with a lag for wrist activity: an acrophase (Ø) change precedes fever and deserves exploration whenever hospital infections threaten (Am. J. Epidemiology 111:5,1980). On a day with afebrile laryngitis rhythm-alteration rendered the same temperature value (within the Chronodesm at one time) predictably too high or too low only a few hours earlier or later - i.e., this temperature value within the physiologic range in the absence of fever was pathognomic.

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REPLICATED FREQUENCY OF LIGHTING REGIMEN SHIFTS AND AGING IN THE FACE FLY REVEAL CIRCADIAN-CIRCASEPTAN INTERACTION. D.K. Hayes*, F. Halberg and T. Teslow* (SPON: H.C. Cecil). Livestock Insects Lab, SEA-AR, U.S. Dept. Agric., Beltsville, Md. and Univ. Minn, Minneapolis, Minn. 55455

At about monthly intervals a set (S) consisting of newly emerged adults of the face fly, Musca autumnalis were introduced into 12 jars (G numbered 1-12) each in a separate light-tight compartment at 26° ± 1.3°C and exposed to a spectrum of lighting regimens: G1, continuous light (LL); G2, fixed regimen of light daily for 16 hr, LD 16:8; G3 through G12, LD 16:8 shifted (ΔΦLD) by -90° (6 hr lengthening of a single D span) at intervals that differed by 1 day among the 10 G's; the interval between successive shifts varied from 2 days from G3 to 11 days for G-12. The proportions of flies dead at about 50% mortality in any one S reveal significant effects of the frequency of lighting regimen shifts. With truncation nearest 50%, namely 51, 50, 40, 47, 52, and 57%, corresponding χ^2 values for intra-S differences were 23.5, 72.7, 176.9, 10.5, 164.7, and 122.6 and corresponding P-values were <.01, <.005, <.005, <.05, and <.005. The data suggest circaseptan interaction that may be optimized by manipulating the frequency of schedule shifts. Life span shortening and lengthening are both of broad interest whether ones goal is fighting insect pests or modeling with proper comparative physiologic qualifications, optimization of the human life span.

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EVIDENCE FOR MULTIOSCILLATORY CONTROL OF THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN THE RAT. Phyllis W. Cheung* and C.E.McCormack. The Chicago Med. School, Chicago, Il. 60612.

While studying the relationship between circadian rhythms of wheel-running activity and estrous cyclicity in female rats (Charles River, CD) exposed to dim continuous light (2 or 20 lux), we observed that the active portion (alpha) of the activity rhythm of 3 out of 8 rats spontaneously split into two components. One component had a period of 22.9-23.4 hr and thus this component was advancing, while the other component had a period of 24.7-25.5 hr and thus was delaying. The two components continued to move towards each other for 5-10 days until they rejoined. In 2 rats, alpha split and rejoined repeatedly. Vaginal smears indicated that the estrous cycle became irregular during splitting in one rat which previously had regular 4 day estrous cycles. In this rat, when the two components rejoined, regular 4 day cycles resumed. The other 2 rats were in persistent vaginal estrus even before alpha split. These results indicate that in the rat, the rhythm controlling regular estrous cyclicity is normally coupled to the locomotor activity rhythm and is dependent on the integrity of the circadian system. On the other hand, circadian patterns of free-running locomotor activity are not dependent on regular estrous cyclicity (Biol. Reprod 19:425). Our results also demonstrate that in the rat, as in the hamster (J.Comp.Physiol A, 106:333), the normal circadian rhythm of locomotor activity is controlled by at least two oscillators. (Supported by IR01-HD-13131-01.)

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TEMPERATURE DEPENDENCE OF THE HAMSTER CIRCADIAN PACEMAKER. Finley P. Gibbs. University of Rochester, Rochester, NY 14642

Blinded male hamsters were maintained in running wheel cages and their activity rhythms recorded. After several weeks hamsters were made hypothermic by anesthetizing with ether, wetting of the fur with alcohol, and immersion in ice and water. Colonic temperatures as measured by mercury thermometer were lowered to between 10°C and 20°C for six hours. The animals were then warmed with a heat lamp and hair dryer and replaced in their home wheels. Inspection of the activity records revealed phase delays following most of the experiments. Using the phase delays to calculate the rate at which the clock was running during the hypothermic bout, Q_{10}'s were determined. The Q_{10} for the hamster circadian pacemaker depended on the temperature to which it was lowered. At 10°C it was 1.13(1.05-1.25)(±1SD), at 12°C it was 1.11(1.02-1.23), at 16 it was 1.06(0.97-1.18), and at 20°C it was 1.03(0.96-1.13). It was concluded that the hamster circadian pacemaker is better temperature compensated than that of the rat which in similar experiments demonstrated Q_{10}'s in the range of 1.2 to 1.4. This finding is consistent with other physiological data on hamsters which indicate that normal function is better preserved at lower temperature when compared to rats.

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INCIDENCE OF APNEA DURING THE REM AND NON-REM STAGES OF SLEEP IN ADULT DOGS. Susan DeMesquita* and Eugene Aserinsky, School of Medicine, Marshall Univ., Huntington, WV 25701

A high incidence of sleep apnea has been reported to occur in REM sleep (R) or in 'transient' stages (T) which are basically portions of record unclassifiable by conventional methods. In this study, R and Non-REM (NR) segments were selected to minimize the duration of T, and all apneas ≥ 4.0 sec were measured. In addition, NR segments were identified as either following R or a long (34.3±9.9 min.) waking (W) section. The results tabulated below (mean ± S.E.) indicate a preponderance of apneas for those NR segments following R pds. The total number of apneas for all other sleep states including the REM pd was sig. (p<.01) lower. The highest concentration of apneas (i.e., apneas/min.) would appear to occur in the so-called T state separating R from the succeeding NR. This T was defined as starting with the last rapid eye movement and ending with the stir that invariably terminated a REM pd. However, except for the stir, this T pd is not distinguishable from an ocularly quiescent portion within the REM pd. In all probability the apneas were associated with the stir rather than the T.

SLEEP SEGMENT	DURATION (min)	TOTAL APNEAS/SEGMENT
NR (after W)	15.9±2.0	0.39±.12
NR (after R)	18.9±1.8	2.33±.36
R	6.4±0.4	0.50±.18
T (between W & NR)	1.9±0.4	0.44±.14
T (between R & NR)	1.0±0.3	0.39±.15

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CIRCADIAN AND CIRCANNUAL PROFILE TESTS OF HUMAN BLOOD PRESSURE YIELD CHRONODESMS AND TENSOPSIES. Erna Halberg*, Julie Halberg*, Nancy Rowe*, Jong Lee*, Howard Levine* and Franz Halberg.Univs. of Minnesota, Mpls. 55455, USA and of L'Aquila 67100, Italy.

Individualized chronodesms (time-specified 90,90 tolerance intervals) (Experientia 34:713, 1978) for systolic and diastolic blood pressure and pulse were established cost-effectively in clinical health and disease. By Roche Arteriosonde (Hoffman LaRoche, Cranbury, NJ) or preferably, with pulse, by an instrument from Nippon-Colin (Komaki, Japan), the blood pressure of women in 3 age groups, a) adolescent, b) young adult, c) post-menopausal, in health or disease, was monitored about every 10' during at least 24 hrs, in the absence of stimulation other than a diurnal activity-nocturnal rest schedule (on a research ward, hospital routine, or in the home)--with replications at intervals ranging from days to years. In health, maximal and minimal blood pressure differed in groups a, b and c by an average of 43, 40 and 42 Torr diastolic and by 47, 43 and 52 Torr systolic, respectively. As percentage of 24-hr minimum, the 3 groups averaged changes of 57, 53 and 63 systolic and of 102, 85 and 86 diastolic, respectively. Chronodesms for circadian time-specified blood-pressure profiles (tensopsies) contained in many, though not all cases the anticipated proportion of the distribution of retest values. The temporal characteristics at intermodulating frequencies and interacting age trends quantify health and risk, screen for disease and, when need be, guide treatment timed according to rhythms.

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BIOSTATIONARITY--BIOERGODICITY AND COST-EFFECTIVE, CONVENIENT MINIMAL PHYSIOLOGIC SAMPLING REQUIREMENTS IN ROOMS WITH STAGGERED ILLUMINATION. Franz Halberg, Franca Carandente*, Erna Halberg* and Jung-Keun Lee*. Univ. of Minn, Mpls., MN, 55455, Univ. of L'Aquila, Italy, FDA, Washington, D.C., 20204, USA

Within a specifiable range for a specifiably homogeneous population, biostationarity means consistency of temporal and other characteristics of a biologic process and bioergodicity means an individual and population consistency of a process characterized by biostationarity. When a bioergodic-biostationary model holds in physiologic experiments, similar if not identical individuals may be studied at one time to describe the behavior of an idealized individual as a function of time, e.g., for rectal temperature and serum corticosterone. 42 adult female Sprague-Dawley rats were singly-housed in 6 different rooms at 24±1°C and 50% relative humidity with food pellets and water freely available, for >2 weeks in 6 rooms with lights being automatically turned on and off daily 4½ hours later in each room, i.e., on in room 1, 2 ... 6 at 06^{00}, 10^{30}, 15^{00}, 19^{30}, 00^{00} and 04^{30} and off 12 hours later. Rectal temperature from all animals was measured in rapid sequence within 1 hour. Data were coded with time as hours after lights on. A circadian rhythm was demonstrated ($P<.05$) in each of 2 studies (1½ mos. apart), with as few as 2-4 animals from each room. Rhythm characteristics estimated with small numbers of rats were similar to those of all animals investigated in these studies and earlier (Halberg et al., Space Life Sci. 2, 437-471, 1971).

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EFFECTS OF DOPAMINE (DOP) AND DOBUTAMINE (DOB) ON CARDIAC OUTPUT AND LEFT VENTRICULAR MECHANICS IN CANINE OLEIC ACID (OA) PULMONARY CAPILLARY LEAK (PCL). F.W. Santman*, R.M. Prewitt*, and L.D.H. Wood*. (Spon: H. Friesen). Dept. of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

In patients with PCL, vasoactive agents may be used to maintain cardiac output (CO) when pulmonary intravascular pressures (Piv) are lowered to reduce PCL. To describe the mechanisms of cardiovascular action of DOP and DOB, we measured CO and left ventricular (LV) end-diastolic (ED) and end-systolic (ES) volumes (V) by radionuclide angiography, and the corresponding pressures (P) by catheter-tipped transducers in 12 dogs in 5 sequential conditions: 1) 2 hours after OA; 2) after plasma volume expansion; 3) during infusion of either DOP or DOB (10 µg/kg/min); 4) after plasma volume reduction; and 5) off DOP or DOB. Mean CO, stroke volume (SV), and ESP increased, and EDP, EDV and ESV decreased on DOP and DOB, indicating that both drugs were effective in maintaining stroke volume at reduced Piv in canine PCL by increasing LV contractility. As in normal dogs (Fed. Proc. 39: 708, 1980) DOP did not alter diastolic V-P curves, but DOB reduced EDP at the same EDV indicating increased diastolic compliance or unstressed volume. Furthermore, DOP increased and DOB decreased systemic vascular resistance, which may account for the greater reduction in ESV on DOB. Both drugs increased CO without altering right atrial pressure, suggesting that they increased mean circulatory pressure or decreased resistance to venous return. (Supported by Manitoba and Dutch Heart Foundations and MRC of Canada).

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MYOCARDIAL FAILURE IN LETHAL E. COLI-INDUCED SHOCK. L.T. Archer*, B.A. Benjamin*, E.K. Beller-Todd*, D.J. Brackett*, M.F. Wilson, and L.B. Hinshaw. VA Medical Center and Univ. of Oklahoma Health Sci. Ctr., Oklahoma City, OK 73104.

Past studies have documented myocardial dysfunction in canine endotoxin shock. The purpose of this study was to determine the effect of lethal E. coli-induced shock on the myocardium. E. coli (LD_{100}) or saline was infused for 30 min into a small heart donor dog (wt range 6-9 kg). Experiments were conducted on isolated working left ventricles supplied by blood from a large support dog (wt range 23-33 kg). Myocardial performance was evaluated 3-7 hr after onset of E. coli or saline infusion under conditions of controlled mean aortic pressure and cardiac output. Results were as follows:

Mean aortic pressure (mmHg)		100	50	150	100
LVEDP (mmHg)	Control	2.4	2.3	4.8	2.8
	E. coli	7.6	6.8	14.5	9.6
	p value	<.01	<.005	<.005	<.001
-dP/dt (mmHg/sec)	Control	2699	1344	3819	2735
	E. coli	1666	901	2141	1422
	p value	<.001	<.01	< .02	<.005

Myocardial efficiency and power were depressed, O_2 uptake was elevated and coronary blood flow was unchanged in E. coli-treated compared with control hearts. Data support the presence of heart dysfunction in gram-negative septic shock. (Supported by VAMC and NIH HL24590 and HL07430.)

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CARDIAC BETA BLOCKADE AND THE EFFECTS OF OPIATE ANTAGONISTS IN ENDOTOXIN-TREATED RABBITS. Gary F. Merrill and Helen E.Mozolic. Department of Physiology, Rutgers University, New Brunswick, NJ 08903.

Opiate receptor blockade by naloxone or naltrexone improves cardiac performance in lethal endotoxin and hypovolemic shock. Neither bilateral cervical vagotomy nor stellate ganglionectomy interfere with these beneficial effects in hypovolemic shock. We have examined the influence of cardiac beta blockade on naltrexone treatment of endotoxin shock in rabbits. Thirty six anesthetized New Zealand white rabbits (2.4±0.4 kg) were treated as follows: Group A, E. coli endotoxin (0.5 mg/kg i.v.); Group B, endotoxin plus naltrexone (20 mg/kg i.v.) simultaneously; Group C, endotoxin, naltrexone and propranolol (2 mg/kg i.v.) simultaneously. Cardiac output ($ml.min^{-1}.kg^{-1}$) left ventricular dp/dt max ($mmHg.sec^{-1} \times 10^3$), mean systemic arterial pressure, Pa (mmHg) and peripheral resistance were monitored for 120 minutes. Dp/dt max in Group A decreased significantly ($P < 0.05$) from 2.0±0.1 to 1.2±0.2 two hours after the endotoxin challenge. No significant differences were seen in either Groups B or C. Cardiac output varied considerably both within and amongst the three groups. Systemic arterial pressure was 99.6±1.0, 66.4±2.6 ($P < 0.05$); 82.4±2.5, 60.0±2.6 ($P < 0.05$) and 83.0±0.8, 69.6±2.8 at time 0 and 120 minutes after the challenge in Groups A, B and C respectively. We conclude that cardiac beta receptors are not involved in mediating the beneficial effects of naltrexone in endotoxin shock. NJAES grant #18130.

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MATERNAL HEMODYNAMICS DURING GUINEA PIG PREGNANCY. A. Roger Hohimer, Mark J. Morton* and James Metcalfe. Univ of Oregon Health Sciences Center, Portland, Oregon 97201

We investigated the maternal cardiovascular adjustments to pregnancy in the guinea pig. Near-term (59-65 days) pregnant (P) and nonpregnant (NP) guinea pigs matched for pre-pregnant weight were prepared with carotid artery and right ventricular (RV) catheters. Three to 5 days later, the animals were restrained and studied in a chamber which allowed measurement of oxygen consumption ($\dot{V}O_2$). Multiple measurements of $\dot{V}O_2$, mean arterial blood pressure (MABP), heart rate (HR) and right ventricular end-diastolic pressure (RVEDP) were made over a 1-hr period. Single RV and arterial blood samples were taken for O_2 content. Body weights were 816 ± 35 g (SEM) for the 9 NP animals and 1214 ± 33 for the 18 P animals. $\dot{V}O_2$ was 12.9 ± .6 ml O_2/min in NP vs 17.1 ± .6 in P animals. Neither HR nor MABP were significantly different, 323 ± 8 b/min and 69 ± 2 mm Hg in NP and 309 ± 6 b/min and 71 ± 1 mm Hg in P animals. Arteriovenous O_2 content difference was 6.3 ± .4 ml O_2/100 ml blood in NP vs 7.2 ± .3 in P animals. The Fick cardiac outputs (CO) were 219 ± 21 ml/min vs 250 ± 14 ml/min in NP and P sows, respectively. RVEDP was the same in both groups, 1.0 ± .5 and 1.0 ± .6. We conclude that guinea pig CO is increased at the end of pregnancy but O_2 extraction also increases. This increased CO is not due to increased HR. The increased stroke volume cannot be explained by an elevated cardiac filling pressure. (Supported in part by USPHS, NIH grants HD #10034, HL #05711, and the Oregon Heart Association)

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CHANGES IN MYOCARDIAL CONTRACTILITY WITH GRADED SYSTEMIC HYPOXEMIA AS EVALUATED BY POSTEXTRASYSTOLIC POTENTIATION. R.M. Lust, Jr.*, L.O. Lutherer, M.W. Cooper*, and M.E. Gardner* Texas Tech University, Health Sciences Center, Departments of Physiology and Medicine, Lubbock, Tx. 79430

Studies completed in our laboratory have demonstrated the validity of the use of postextrasystolic potentiation (PESP) in the measurement of inherent cardiac contractility, but only under certain, well-defined conditions. Being able to accurately measure cardiac reserve has enabled us to investigate areas where information regarding myocardial function has previously been based primarily on indirect evidence. In vivo myocardial function during systemic hypoxemia is not well understood, particularly due to an inability to measure contractility directly. In our studies, anesthetized dogs instrumented for standard hemodynamic parameters and echocardiographic recordings were placed on hypoxic gas mixtures equivalent to 11.7% and 13.3% O_2 at sea level (balance N_2) administered via the respirator for 15 minutes with and without propranolol blockade. Results indicate that near 13.3% O_2 there is an increase in contractility which appears to be catecholamine based. At 11.7% O_2 contractile reserve is reduced and there appears to be direct depression of the myocardium in the abscence of catecholamines. The suggestion is made that 1) there is a hypoxic threshold for catecholamine release, 2) there is a hypoxic threshold for direct effects on the myocardium, 3) the thresholds are different for the two effects and 4) the nature of the two actions are opposite in influence.

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INFLUENCE OF RESPIRATION ON STROKE VOLUME AND CARDIAC OUTPUT DETERMINATION BY IMPEDANCE CARDIOGRAPHY. B.Doerr*, M.A.B.Frey, and D.S.Miles*. Wright State University, Dayton, OH 45435

Impedance cardiography provides a noninvasive beat-to-beat determination of stroke volume (SV). Calculation of SV depends on measurements of amplitude of the derivative of thoracic impedance change (dZ/dt) and ejection time (T) from the impedance cardiogram (IC). The conventional method of calculating SV has been to use only tracings in end-expiration (End-E) which rest exactly on a designated baseline (BL). This severely limits the number of usable beats, since respiration (R) shifts the IC from its BL. This study investigated the influence of R on SV calculated independent of the BL in 7 women (20-44 yr) seated at rest. T, dZ/dt (from notch in the upstroke to peak deflection regardless of BL drift), HR, SV, and cardiac output (CO) were determined in specific phases of 3 continuous R cycles from the following beats: 1. all; 2. all inspiratory (I); 3. I on BL; 4. all E; 5. E on BL; 6. all End-E; 7. End-E on BL (conventional). ANOVA demonstrated no difference ($p<0.05$) among phases for T, dZ/dt or SV. HR during phases 5 and 7 was slower and during 2 faster than the other phases. CO, however, did not differ by phase. Thus, for seated subjects, BL shifts of the IC due to R have minimal influence on SV, provided dZ/dt and T are calculated as described. Increasing the number of data points by using beats from all R phases should provide greater reliability in CO determination by impedance. (Supported in part by American Heart Association, Miami Valley Chapter.)

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REYNOLDS STRESSES NEAR A STENOTIC AORTIC VALVE.
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The specific feature of turbulent flow that appears likely to be damaging to platelets and red cells is the Reynolds stresses (TRE). Such stresses are a measure of the shear stresses within the turbulent eddies, and are calculated as the fluid density times the product of simultaneously measured fluctuations of two mutually perpendicular velocities. In this study, we quantified TRE in the region of a surgically excised human stenotic (0.2 cm^2) aortic valve in vitro. TRE was measured with a two channel laser Doppler anemometer in a flow system in which the valve was mounted. The fluid was glycerin and saline (viscosity, .04 Poise and density, 1.1 gm/ml). At a pump rate of 73 beats/min, and stroke volume (SV) of 54 ml, the peak pressure gradient (ΔP) was 165 mm Hg. The root-mean-square value of TRE throughout ejection was 1800 dynes/cm^2 and during mid-ejection it was 2500 dynes/cm^2. At a SV of 73 ml, peak ΔP was 200 mm Hg, root-mean-square TRE throughout ejection was 2800 and during mid-ejection it was 3500 dynes/cm^2. Transiently T_{RE} reached 20,000 dynes/cm^2. T_{RE} was higher than shear stress at the wall (110 dynes/cm^2 at a SV of 54 ml and 140 dynes/cm^2 at a SV of 73 ml). These observations suggest that TRE in the region of a stenotic aortic valve may be sufficiently high to activate platelets and may explain the increased platelet reactivity observed in such patients. This increased platelet reactivity may contribute to the deposition of platelets on stenotic aortic valves.

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COMPARISON OF STI HEART RATE REGRESSION FORMULAE FROM TWO EXPERIMENTAL PROTOCOLS. B. McNichols*, B. Rubal, C. Buchanan*, J. Lucas*, K. Barnard*, and L. Lee* (SPON: A. Waldo). Texas Woman's Univ., Denton, TX 76204

Although systolic time intervals (STI) are often used to assess cardiac performance, they are influenced by heart rate (HR) and loading factors. In this study, HR regression formulae from two different experimental regimes were compared in 10 chloralose anesthetized dogs. HR changes in 5 dogs were induced by electrical stimulation of the right vagus following vagal ligation; 5 dogs underwent a left thoracotomy and atrial pacing after beta blockade with propranolol. Statistical differences ($p < 0.01$) were found when HR regression formulae for the electromechanical systole (EMS), the left ventricular ejection time (LVET) and the pre-ejection period (PEP) were compared between the two groups:

(Paced)	EMS = -0.592 (HR) + 339 msec.	(r = -0.747)
	LVET = -0.430 (HR) + 220 msec.	(r = -0.739)
	PEP = -0.024 (HR) + 104 msec.	(r = -0.062)
(Vagal)	EMS = -0.924 (HR) + 366 msec.	(r = -0.871)
	LVET = -0.979 (HR) + 285 msec.	(r = -0.859)
	PEP = 0.057 (HR) + 80 msec.	(r = 0.170)

These data suggest that although STI heart rate regression formulae provide a predictive reference for evaluating heart performance, they should not be applied indiscriminately to different experimental protocols.

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MYOCARDIAL RESPONSES TO ALPHA ADRENOCEPTOR STIMULATION WITH METHOXAMINE IN LAMBS. John C. Lee, R. Fripp* and S.E. Downing. Yale University, School of Medicine, New Haven, Ct. 06510

This study was undertaken to evaluate the effects of methoxamine on left ventricular performance, coronary flow (CF) and myocardial oxygen metabolism ($M\dot{V}O_2$) in lambs. Measurement of LV dP/dt max, LVEDP, CF and $M\dot{V}O_2$ were made in 9 lambs using a hemodynamically controlled preparation described previously (Amer. J. Phys. 244:1381, 1973). External cardiac work and heart rate were held constant. They were given atropine (1 mg), TEA (100 mg) and practolol (4 mg/kg) to provide autonomic blockade. Responses to progressive doses of methoxamine (0.4 to 6.0 mg/kg) were recorded. Dose-related increases in LV dP/dt max and decreases in LVEDP were observed. CF, myocardial O_2 extraction and $M\dot{V}O_2$ did not change significantly. These positive inotropic responses were eliminated by giving phentolamine (10 mg). The positive inotropic action of methoxamine was also demonstrated in four additional animals by obtaining ventricular function curves. Initially, the mean stroke volume at LVEDP 10 cm H_2O (SV_{10}) was 3.96 ml (±0.06 SE). After methoxamine, this value increased to 4.80 ml (±0.13 SE) ($p<0.005$). These observations support the hypothesis that alpha-adrenergic receptors are present and mediate a substantial positive inotropic action on neonatal lamb myocardium.

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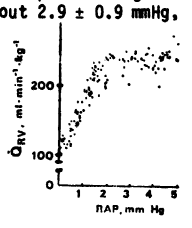
RIGHT VENTRICULAR FUNCTION IN FETAL LAMBS. Kent L. Thornburg and Mark J. Morton.* Dept. of Physiology and Medicine, Univ. of Oregon Health Sciences Center, Portland, OR 97201.

We assessed the importance of preload (P) and afterload (A) to fetal RV output (QRV). Under sterile conditions, vinyl catheters were placed in 5 fetal lambs in the right atrium, carotid artery, left cephalic vein, left brachial artery and thoracic cavity; an electromagnetic flow sensor was placed on the main pulmonary artery. After 5.7 (± 0.4 SEM) postoperative days, blocking doses of propranolol and atropine were administered and blood withdrawn into a syringe until right ventricular output was 1/3 control and/or mean transmural right atrial pressure (RAP) fell to 0.5 mmHg. Blood and Ringers were reinfused to raise RAP. Blood gases and hematocrits were monitored. Cardiac function curves (QRV vs. RAP) were constructed by computer sampling at 5 sec intervals. All curves were similar in shape and showed a steep ascending limb which flattens into a plateau at RAP of about 2.9 ± 0.9 mmHg, a RAP close to control (2.9 ± 0.5) mmHg where ventricular output was (252 ± 118 $ml \cdot min \cdot kg^{-1}$). We also conclude that in fetal lambs with blockade at RAP < 3 mmHg, P is the more important determinant of RV output; above 3 mmHg A was the important determinant of output as determined by pharmacologic manipulation of arterial pressure. (Supported by Oregon Heart Association and HD 10034 from USPHS.)



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THE FACTORS INFLUENCING THE RECOVERY OF ISOMETRIC EXERCISE. Donald E. Stafford* and Jerrold S. Petrofsky. Biomedical Engineering Laboratory, Departments of Physiology and Engineering, Wright State University, Dayton, OH 45435

The purpose of this study was to evaluate some of the factors influencing the recovery of a muscle following a fatiguing isometric contraction. Five male volunteers between the ages of 19 and 24 years served as subjects in these experiments. Two sequential isometric contractions at tensions of 25%, 40%, or 70% of the maximum strength (MVC) of the hand grip muscles were maintained to fatigue; the time interval between the contractions was 3, 7, or 20 minutes. During the recovery interval, non-fatiguing contractions of 0%, 5% or 10% MVC were performed. The results showed that, as expected, the time interval greatly influenced the recovery of the muscle after a fatiguing isometric contraction. Much to our surprise, sustaining a non-fatiguing contraction during the recovery interval also dramatically reduced recovery. (Supported by Air Force Contract No. F33615-78-C-0501.)

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EFFECT OF DYNAMIC EXERCISE ON THE RECOVERY FROM FATIGUING ISOMETRIC EXERCISE. P.V. Fiore*, J.S. Petrofsky and R.M. Glaser (SPON: C.A. Phillips). Wright State University School of Medicine, Dayton, OH 45435

The purpose of this study was to evaluate the effect of dynamic exercise on the recovery from fatiguing isometric exercise. College age males performed fatiguing isometric contractions at a tension of either 25, 40 or 70% of their maximum voluntary strength of their quadriceps. Each contraction was followed by a recovery period of either 1, 3, 10 or 20 min. During the recovery period, subjects either rested (control) or performed dynamic exercise on a bicycle ergometer at a load which elicited either 25, 50 or 75% of max $\dot{V}O_2$. The extent of recovery was determined by comparing the endurance time for a subsequent fatiguing contraction at the same tension. Only one combination of isometric tension-recovery time-dynamic exercise load was performed in a 24 hr period. For the 3 isometric tensions tested, there was about 80% recovery of endurance when resting during the 20 min recovery period. The extent of recovery was exponentially related to recovery time. Dynamic exercise at 25 or 50% max $\dot{V}O_2$ had little effect on endurance of subsequent isometric contractions. In contrast, dynamic exercise at 70% max $\dot{V}O_2$ reduced isometric recovery. (Supported by USAF contract no F33615-78-C-0501)

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POST EXERCISE HYPEREMIA IN CONDITIONED RUNNERS AND UNCONDITIONED SUBJECTS FOLLOWING RHYTHMIC CALF MUSCLE CONTRACTIONS AT MATCHED PERCENTAGES OF MAXIMAL STRENGTH. Daniel Richardson and Douglas Campbell*. University of Kentucky Col. of Medicine. Lexington, KY 40536.

These studies compared post exercise hyperemia in the calf muscle between conditioned distance runners and unconditioned male subjects following rhythmic isometric contractions. The force per contraction ranged from 7.5 to 30 percent of each subject's calf muscle MVC (maximum voluntary contraction). Contraction frequency ranged from 20 to 80 contractions per min. (cpm). Average MVC was 502±16 (SE) pounds for the unconditioned subjects, and 447±29 pounds for the runners. Calf muscle blood flow was measured by strain gauge plethysmography. Resting flows were statistically the same for both groups and averaged 3.8±0.4 (SE) ml/min/100ml. Following 30% MVC contractions at 50 and 80 cpm (intensities which simulate running) post exercise hyperemic volume averaged 23.8±5.0 (SE) and 47.0 ±8.0 ml/100ml. respectively for the unconditioned group. Respective values for the runners were 14.7±2.7 and 25.4±3.2 ml/100ml. At lower intensities hyperemic volumes of the 2 groups were similar. This suggests that during rhythmic calf muscle exercise conditioned runners either have a lower blood flow and/or accumulate a lower flow debt when exercise is at intensities with which conditioning occurs. (Supported by Univ. of Ky. Res. Found. and Col. of Med. Basic Res. Sup. Grant).

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Impact of Helmet Weighting on Static and Dynamic Fatigue of Neck Muscles. C.A. Phillips and J.S. Petrofsky. Biomedical Engineering and Biomechanics Laboratory, Wright State University, Dayton, Ohio 45435

Helicopter pilots often experience neck muscle fatigue, especially with helmets weighted with avionics. The purpose of this study was to quantitate the extent of fatigue of the neck muscles (sternocleidomastoid on trapezius, part 1) during lateral rotation and dorsiflexion with helmets of various weights and centers of mass. Muscle utilization and fatigue were quantified by amplitude and time domain analysis of the surface electromyogram (EMG) during dorsiflexion and lateral rotation of the head. The static component of exercise was quantified by performing a fatiguing isometric contraction after the dynamic exercise. The results of these experiments showed that helmets of any weight had a significant increase in the static and dynamic component of neck muscle fatigue. The implication of weight and center-of-mass of helmet design with respect to neck muscle utilization and fatigue will be discussed.

This work was supported by Army Contract No. DAMD17-80-C-0089 and Air Force Contract No. F33615-78-C-0501.

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THE METABOLIC RESPONSE TO POSTPRANDIAL EXERCISE IN MAN. B. Zinman,* J.D. Nelson*and E.B. Marliss. University of Toronto, Toronto, Ontario M5S 1A8

An elegantly coordinated sequence of fuels is mobilized from endogenous stores to supply the energy requirements of exercising muscle in the postabsorptive state. To determine how such a sequence may be altered by prior meal consumption, healthy adults (n=7) were studied with breakfast alone (B), breakfast followed 30 min later by 45 min of cycle ergometer exercise at 50% $\dot{V}O_2$ max (B+E), and with the same exercise but no prior breakfast (E). The expected increments in glycemia and insulin (IRI) observed in B were abruptly curtailed with onset of exercise (B+E), returning to premeal levels (80±3mg/dl, 0.4±0.1ng/ml). In the postexercise period small and transient increments occurred (Δ=33±6mg/dl, 0.81±0.15ng/ml). In E, glycemia remained constant (90±6mg/dl) and insulin fell. Successively higher peak blood lactate levels occurred after B (1351±195), E (1790±260) and B+E (2592±836μM). Whereas plasma free fatty acids fell after B, they rose significantly with E (to 1000±147μM) and were intermediate with B+E. Corresponding differences in respiratory quotient (RQ) were of maintenance of values near unity (0.95±0.08) for B+E in contrast with initial rise with progressive fall to 0.84±0.03 in E. Thus the metabolic and hormonal responses to exercise are distinctly altered by prior meal ingestion. The principal fuel for muscle would appear to be carbohydrate, whereas in E early glucose utilization gives way to later fatty acid predominance as fuel. (Supported in part by MRC MA-5767.)

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HORMONAL AND METABOLIC RESPONSES OF FIT YOUNG MEN TO DAILY PROLONGED EXERCISE. B.H. Sabiston and M.W. Radomski. Defence and Civil Institute of Environmental Medicine. Downsview, Ont.

Hormonal and metabolic responses were studied in 21 fit young soldiers (VO_2 max: 59 ml/kg.min) who marched for six days, 35 km/day, at a speed of 6 km/hr. All subjects worked at 35-40% VO_2 max. Blood samples were drawn at the end of the daily march and at the same time during control and recovery periods, the weeks before and after the march, respectively. Triglycerides decreased progressively throughout the march, remaining low until the second day of recovery. Cholesterol was unchanged. Free fatty acids were elevated on the first day, remained high throughout the march and returned to control levels on the first day of recovery. Glucose decreased with exercise but returned promptly during recovery. Insulin and growth hormone were not affected by exercise. Total thyroxine did not change during the march but free thyroxine decreased steadily. Cortisol was markedly elevated throughout the march and required at least two days without exercise before control levels were re-established. Blood urea nitrogen and urinary total nitrogen were significantly elevated. The results show that moderate and non-fatiguing exercise, when prolonged and repeated daily, can nevertheless evoke a stress hormone response and elicit cumulative metabolic changes despite adequate rest and caloric repletion. Duration of exercise is clearly an important determinant of the precise metabolic adjustments to submaximal work.

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CARDIO-RESPIRATORY AND METABOLIC RESPONSES OF ELITE CANADIAN OARSMEN TO EXERCISE ON A ROWING ERGOMETER.

D.C. McKenzie, E.C. Rhodes, and D.R. Stirling, Dept. of Sport Science, University of British Columbia, and Dept. of Kinesiology, Simon Fraser University, Vancouver, B.C.

Seven elite male oarsmen were studied during 5 min. 45 sec. of maximal effort designed to simulate the standard 2000 meter race in an 8-oared shell. Ventilation and oxygen uptake were assessed using an open circuit method. Heart rate (HR) was monitored by direct bipolar ECG. Blood was taken for serum lactate (LA) estimation at one minute intervals, via an indwelling catheter. Anthropometric data indicated a mean age of 23 yrs., ht. 193 cm., wt. 92 kg., and percent body fat of 9.0%. Pulmonary ventilation exceeding 200 l/min. was common with a mean pulm. vent. of 198 l/min. (BTPS). The mean maximal aerobic capacity was 6.26 l/min. standardized to 68.64 ml/kg - min. The aerobic uptake after one minute was 5.40 l/min. which rose steadily to the maximum value. HR increased to a mean maximum of 182 bpm but was 93% of this value after 1 minute. Excess CO_2 values showed a rapid and sustained increase during the initial period of work indicating that the athletes were performing above their anaerobic threshold. The mean minute values of LA were 5.05, 8.48, 10.47, 11.87, 13.23 and 14.68 mmol/l which reflects the high degree of anaerobic metabolism which must be sustained throughout the entire event.

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EFFECTS OF SLEEP DEPRIVATION ON EXERCISE. Gary M. Gaddis* and Bruce J. Martin. Medical Sciences Program, Indiana University, Bloomington, IN 47405.

While much work has been done investigating effects of exercise on subsequent sleep, little is known of the effects of sleep deprivation on subsequent exercise. To investigate whether sleep loss influences exercise, the metabolic, ventilatory, and perceptual responses to exercise after sleep deprivation were measured. Six subjects performed cycle ergometer exercise daily in two separate three-day series. Subjects performed control and experimental series in random order. In both series daily exercise involved 8 minutes each at work loads preset near 25%, 50%, and 75% of estimated maximal oxygen uptake ($\dot{V}O_{2max}$). In the experimental series, subjects were 30 hours sleep deprived before the ride on day two. Differences due to sleep loss were most apparent at 75% $\dot{V}O_{2max}$. At this level, immediately after sleep deprivation, significant increases in perceived exertion ($P<0.01$), ventilation ($\dot{V}_E$) ($P<0.01$), O_2 uptake ($\dot{V}O_2$) ($P<0.05$) and CO_2 production ($\dot{V}CO_2$) ($P<0.05$) were observed. Despite ad libitum sleep the night after post-deprivation exercise, significant increases at the 75% $\dot{V}O_{2max}$ work load in $\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$ persisted on day 3 (all $P<0.05$). The results indicate that there are physiological effects of sleep loss on exercise, and these effects outlast perceptual effects. (Supported by NIH Grant 5 SO7 RR5571)

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ALTERATIONS IN GLOMERULAR SIALIC ACID (SA) ASSOCIATED WITH EXERCISE PROTEINURIA (EP). Edward J. Zambraski, J. Goldstein,* C. Lakas,* M. Shepard,* and M. Bober.* Dept. of Physiology, Rutgers Univ., New Brunswick, NJ 08903.

The exact mechanism of EP remains to be determined, although EP appears to be of glomerular origin (Kid. Int. 16: 385, 1979). Certain models proteinuria have been associated with decreases in glomerular polyanionic character (GAC) or SA. To determine if EP was due to changes in GAC or SA, these parameters were measured in resting and treadmill exercised dogs (n=5) and rats (n=23). In dogs, to obtain tissue, animals were first unilaterally nephrectomized then run to exhaustion after recovery. For rat studies littermates were assigned to control or exercise groups. Renal cortical SA was measured using the thiobarbituric assay and colloidal iron staining (CIS) was used to asses GAC. CIS was rated 0 to 3 (0 - no uptake; 3 maximum). Values are mean ± SEM. († $P < .05$).

	Dogs		Rats	
	Rest	Exercise	Rest	Exercise
SA (μ mole/gm Cortex)	2.71±.09	2.83±.14	2.77±.07	†3.03±.09
CIS	1.94±.63	1.39±.28	2.31±.20	†1.22±.39

At 3, 6 and 24 hours post-exercise values for GAC in rats were 1.23±.68, 1.83±.34 and 0.96±.14 respectively. By 3 hours post-exercise SA had returned to control levels. These data suggest that exercise may alter SA metabolism and that EP may be due to a decrease in GAC.

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INFLUENCE OF DIET AND EXHAUSTION ON TISSUE LEVELS OF GLYCOGEN IN NONTRAINED RATS. L.I. Vailas*, K.A. Rowlett*, P.L. Kershner*, A.C. Vailas*, and C.M. Tipton. Lipid Research Center and Exercise Science Program, University of Iowa, Iowa City, IA. 52242

Previously, (Ia. Acad. Scie., 60, 1979), we reported that nontrained rats eating a mixed diet composed of 58% carbohydrates (CHO), 16% proteins (PRO), and 26% fat (F) for three days after exhaustion failed to normalize liver glycogen levels even though the muscle values were within baseline values. Similar results occurred when the 24 hr. post exhaustion diet was 54% PRO and 46% F. After standardizing a treadmill test for exhaustion using $\dot{V}O_{2max}$, changes in rectal temperature, and blood alterations, we repeated the study and found 24 hrs. after exhaustion that a mixed diet or one containing 82% CHO did not result in normal liver levels even though these diets were associated with expected muscle glycogen values. Associated with both experiments were a 30% reduction in the total amount of calories consumed. These results reinforced the published findings from "trained" animals by Fell et al., (AJP 238:R328, 1980), and suggested that 1) post exhaustion diets should consider fructose as a possible carbohydrate source, 2) the total carbohydrate calories available for the post exhaustion period be elevated, 3) nontrained populations be used to elucidate the responsible mechanisms. (Supported in part by funds from GM-7045-02, and Iowa Corn Producers Association).

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EFFECTS OF DICHLOROACETATE (DCA) ON LACTIC ACID (LA) AND GLUCOSE (GLU) TURNOVER RATES IN RESTING AND EXERCISING DOGS. Charles D. Ciccone*, Edward J. Zambraski and Gary F. Merrill. Dept. of Physiology, Rutgers Univ., New Brunswick, NJ 08903

Previous studies have shown that DCA reduces the lactacidemia associated with exercise in dogs (J. Appl. Physiol. 48: 427, 1980). Four dogs were constantly infused with ^{14}C LA and ^{3}H GLU while at rest and during treadmill exercise to evaluate the effects of DCA on LA and GLU turnover rates. Rates of appearance (Ra) and disappearance (Rd) ($mg \cdot min^{-1} \cdot Kg^{-1}$) were calculated. Two dogs were tested at prolonged-moderate work loads pre- and post-DCA administration, and two dogs were exercised only post-DCA. Values are mean ± SEM.

	Rest (n=4)	Exercise (n=2)	DCA Exercise (n=4)
Heart Rate	129±7	197±3	198±7
Ra LA	52.8±12.1	213.0±26.3	188.0±51.4
Rd LA	49.4±13.8	198.0±91.6	253.8±130.2
LA (mg/dl)	14.3±1.4	61.5±14.5	61.8±17.9
Ra GLU	100.5±28.3	506.7±46.4	295.0±68.7
Rd GLU	106.7±37.6	447.3±66.7	304.6±67.6
GLU (mg/dl)	103.3±16.6	103.0±22.0	102.6±15.0

These data indicate that DCA influences LA metabolism during exercise by altering LA, Ra and Rd, although Rd seemed more affected. DCA also influenced GLU, Ra and Rd, but absolute values of GLU remained constant. Since DCA activates pyruvate dehydrogenase, these data suggest that this enzyme may be important in regulating LA metabolism during exercise. (Supported in part by NJ Heart Assoc. Grant # 27-5127).

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PULSE INJECTION, ^{13}C-TRACER STUDIES OF LACTATE METABOLISM IN HUMANS DURING REST AND TWO LEVELS OF EXERCISE. George A. Brooks, Robert S. Mazzeo, Dale A. Schoeller, Peter D. Klein, and Thomas F. Budinger. Exercise Physiology Laboratory, University of California, Berkeley 94720.

Two young, healthy males were studied at rest and during easy and hard bouts of continuous exercise. Easy exercise was 2 hrs. of work below the blood lactate inflection point (LIP). Hard exercise was 45 min. of work above the LIP. After 20 min. of rest, easy or hard exercise subjects received an IV injection of 100 mg DL-[2,3-^{13}C]-sodium lactate. At rest $\dot{V}O_2$ approximated 0.3 l./min, blood lactate was 0.75-1.0mM, and 10.5-16% of the injected dose (calculated as a percentage of the L isomer) was collected as $^{13}CO_2$ within 2 hrs. During easy exercise $\dot{V}O_2$ approximated 1.75 l./min, blood lactate was 1-2 mM, and 86-88% of the dose was collected as $^{13}CO_2$ within 2 hrs. During heavy exercise $\dot{V}O_2$ approximated 2.5-3.0 l./min, blood lactate was 4-8 mM, and 49.4-53.2% of the dose injected was collected as $^{13}CO_2$ within 45 min of injection during hard exercise. Cumulative recovery of tracer as CO_2 after 45 min of hard exercise was the same as during a similar point of easy exercise. Oxidation appears to be the major metabolic end point of lactate during rest and exercise. (Supported by NIH Grand AM19577).

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WATER LOSS AND SHELL PORE GEOMETRY IN EGGS OF THE WEDGE-TAILED SHEARWATER. C. V. Paganelli, R. A. Ackerman, G. C. Whittow, and T. N. Pettit*. Univ. of Hawaii at Manoa, Honolulu, HI 96822, and State Univ. of New York at Buffalo, Buffalo, N.Y. 14214

Eggs of the Wedge-tailed Shearwater (Puffinus pacificus chlororhynchus) (SW) are similar in size and mass to those of the domestic chicken and lose a similar proportion of their initial mass as water vapor during the course of incubation. However, the SW incubates its single egg 52 days, 2.5 times longer than the chicken. Daily loss during incubation, primarily by diffusion through pores in the shell, occurs about 2.2 times faster in chicken eggs than in SW eggs. Shell water vapor conductances (G) in chicken and SW eggs are 14.4 and 6.1 mg day^{-1} $torr^{-1}$, respectively. To determine whether the lower G of SW eggs results from fewer pores, smaller pores, or some combination of these factors we performed pore counts (n) on the shells of both species. For chicken and SW eggs the values of n are about 12,400 and 3,700 per egg, respectively. Pore radii computed from Fick's first law, using measured values of G and shell thickness, are 7.6 and 8.0 μm for chicken and SW, respectively. Thus pore radii are nearly the same in the two species, but SW eggs have fewer pores than chicken eggs, thus lessening water loss rate over the relatively long SW incubation period.

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INCUBATION WATER LOSS IN EGGS OF LAYSAN ALBATROSS *(DIOMEDEA IMMUTABILIS)* AND BLACK-FOOTED ALBATROSS *(DIOMEDEA NIGRIPES)*. G.S. Grant*, C.V. Paganelli, T.N. Pettit*, G.C. Whittow and H. Rahn. Univ. of Hawaii, Honolulu, HI 96822 and State Univ. of NY at Buffalo, Buffalo, NY 14214.

During natural incubation the typical avian egg loses about 15% of its initial mass as water vapor. Is this true even in the 300 g eggs of the Laysan (LA) and Black-footed Albatross (BFA) which incubate continuously for 65 days, about 1.6 times longer than other species with eggs of similar size? Periodic measurements of LA and BFA egg mass were made at Midway Atoll in the Central Pacific. The mean daily mass loss ($\dot{M}_{H_2O}$) for 98 LA eggs was 674 mg·day^{-1} and for 75 BFA eggs was 707 mg·day^{-1}. Thus, in 65 days these eggs will lose 15% of their initial mass. $\dot{M}_{H_2O}$ is governed by shell water vapor conductance (G) and vapor pressure within the egg (P_A) and within the nest (P_N): $\dot{M}_{H_2O} = G (P_A - P_N)$. G for LA and BFA eggs (32 mg·day^{-1}) is nearly 20% lower than one finds in eggs of similar size but with much shorter incubation periods. Nest vapor pressure measured by hygrometry falls within the range reported for other species (17.3-19.1 torr). Egg temperature (34°C) and hence water vapor pressure within the egg (40 torr) are significantly lower than in other birds. Thus the 15% mass loss is achieved by reducing both conductance and egg temperature, but not nest vapor pressure. (Supported in part by NSF Grant PCM 76-12351 to GCW.)

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CORRELATION BETWEEN EGG SHELL POROSITY AND AMBIENT HUMIDITY OF NESTING HABITAT IN RATITES. Craig Patrick Black, Geoffrey F. Birchard, and Gregory K. Snyder. Dept. of EPO Biology, Univ. of Colo., Boulder, CO 80309.

Diffusive water vapor loss through the egg shell equivalent to about 16% of fresh egg weight is a nearly universal characteristic of bird eggs; significant deviation in either direction adversely affects hatchability. Walsberg's (Am. Zool., 1980. In press) theoretical analysis of factors affecting egg water loss suggests that nesting habitat ambient humidity is the primary determinant of egg water loss (and thereby shell porosity). To test this hypothesis, we compared shell conductance in eggs from several ratite species, a group with close taxonomic affinities which nests in a variety of habitats. After adjusting shell conductance values for differences due to egg size and incubation period length, we found that shell conductance values for the Rhea (Rhea americana), Emu (Dromiceius novae-hollandiae), and Ostrich (Struthio camelus), all of which nest in dry savannah or desert habitat, were lower than that for the Australian Cassowary (Casuarius casuarius), a jungle nesting species, by 10.0%, 16.8%, and 11.7%, respectively. These results support the hypothesis that shell conductance increases with increasing nest habitat ambient humidity.

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OXYGEN EQUILIBRIUM CURVES (O2EC) FOR CHICKEN WHOLE BLOOD AND SEPARATED ISOHEMOGLOBINS: EFFECT OF VARYING ORGANIC PHOSPHATE CONCENTRATION ON O2EC SHAPE. L.A. Maginniss and R.B. Reeves. Dept. of Physiology, Sch. of Med., SUNY, Buffalo, N.Y. 14214.

Whole blood O2EC from several avian species including chicken develop a plasma Ca dependent shoulder at the foot of an otherwise sigmoid curve. Shoulder magnitude is intensified with repeated O2EC runs on the same blood sample. Oxygen transport properties of separated chicken isoHb's provide evidence for the origin of this O2EC shape-change phenomenon. Chicken isoHb's were separated on DE-52 cellulose and stripped by ion-exchange chromatography. Continuous O2EC for isoHb solutions (.26 mM Hb4 in 100 mM KCl, pH 7.5) were generated at 25°C by thin film dual wavelength spectrophotometry (430-453 nm) and electrode oximetry. In the absence of organic phosphate, Hb 1 (pI 7.7), Hb 2 (7.0) and Hb 1 + Hb 2 mixture (3:1) revealed high affinity (P50 = 2-3 torr) sigmoid curves. Additions of 1-3 mM inositol hexaphosphate (IHP) to the three isoHb solutions maximally increased P50 to 15-27 torr, but had no apparent effect on sigmoid curve shape. Intermediate IHP concentrations, however, altered curve shape dramatically; addition of .1-.2 mM IHP to the Hb solutions induced non-sigmoid O2EC shapes similar to those observed in whole blood. Our results suggest that small changes in intraerythrocyte organic phosphate concentration, by an as yet unknown mechanism, may be responsible for anomalous curve shapes obtained with avian whole blood in vitro. (Supported in part from NIH Grant PO1-HL-14414.)

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BLOOD OXYGEN EQUILIBRIA IN THE HOUSE SPARROW (Passer domesticus). R. B. Reeves and L. A. Maginniss. Dept. of Physiology, School of Medicine, SUNY, Buffalo, N. Y. 14214

Oxygen equilibrium data from blood of small Passerine birds are scant. We measured continuous dynamic isocapnic oxygen equilibrium curves (O2EC) on whole blood sampled by heart puncture. O2EC were generated by dual wavelength (430-453 nm) spectrophotometry and electrode oximetry on thin blood films. Blood pH was estimated from film CO2 tension and blood buffer line. Isoelectric focusing showed a two-hemoglobin system in all animals (18) examined; the fast Hb (pI = 7.44) was 24% of total, the slow Hb (pI = 8.96), 76%. Acid-base status at Tb = 41 deg C. was pH 7.49 ±.02 and PCO2 30.1 ± 3.5 torr. Hematocrit was 44.3% in 16 animals of 25 g body weight.

T	curves/ animals	pH	P50 torr	Δlog P50/ΔpH
35	14/4	7.59	24.8 ± 2.2	-.60 ± .03
41	23/7	7.50	38.1 ± 0.9	-.46 ± .06
45	9/3	7.44	42.7 ± 1.6	-.46 ± .01

Hill plot was non-linear at all temperatures. CO2 Bohr effect was not saturation dependent. Sparrow O2EC was significantly different from shape of man's Hb A curve (i.e. less steep). (Supported in part from NIH Grant PO1-HL-14414.)

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CAN AVIAN INTRAPULMONARY CHEMORECEPTORS DETECT CHANGES IN MIXED VENOUS PCO_2 AND INITIATE APPROPRIATE BREATHING RESPONSES? M.R. Fedde, J.P. Kiley*, F.L. Powell and P. Scheid. Dept. Physiology, Max Planck Inst. exp. Med., D-3400 Göttingen, FRG

The importance of intrapulmonary chemoreceptors (IPC) in the control of breathing was tested in anesthetized, spontaneously breathing White Pekin ducks with vascular delay loops inserted in both brachiocephalic arteries so that PCO_2 changes at the carotid bodies and brain were delayed by 30 to 60 sec. Blood equilibrated with air (low PCO_2), 85% CO_2 in O_2 (high PCO_2), or blood withdrawn from the right atrium (mixed venous PCO_2) was infused for 30 sec at a rate of 100 ml·min^{-1} into the right ventricle while simultaneously withdrawing a like amount from the right atrium. Infusing mixed venous blood produced no change in tidal volume (V_T), respiratory frequency (f), or end-expired CO_2 concentration ($F_E'CO_2$). Infusing blood with low PCO_2 induced simultaneous reduction in V_T, f, and $F_E'CO_2$ within 4 sec after the infusion began; infusing blood with high PCO_2 induced an increase in V_T, f, and $F_E'CO_2$ within 4 sec. These responses occurred long before the stimulus could have reached the carotid bodies and brain and suggest that other CO_2 receptors, notably IPC, can detect PCO_2 changes in mixed venous blood and induce appropriate changes in ventilation.

802

FACTORS DETERMINING THE LEVELS OF RESPIRATORY GASES IN CAUDAL AIR SACS OF BIRDS. P. Scheid, J.R. Torre-Bueno, and J. Piiper. Dept. Physiol., Max Planck Inst. Exp. Med., D-3400 Göttingen, W. Germany.

In caudal air sacs of birds the concentration of CO_2 is substantially higher, and that of O_2 lower, than in inspired air. Re-inspiration of dead space gas, an inevitable mechanism in tidal breathing, contributes significantly but cannot quantitatively explain the difference between air sac and inspired concentrations. Three other mechanisms have been dismissed on experimental grounds: (1) gas exchange across air sac walls, (2) recirculation of lung gas ("Hazelhoff loop"), and (3) preferential inspiration of the initial portion of the tidal volume (dead space) into caudal air sacs. Two further mechanisms remain to explain the difference: (1) Gas exchange in parabronchi of the neopulmo. Although there is experimental evidence for a significant contribution by this factor, it does not appear to be quantitatively sufficient. (2) Incomplete gas mixing in caudal air sacs (stratification). Experiments in ducks with bolus injections suggest that mixing of the dead space and fresh gas fractions in the gas inspired into caudal air sacs is incomplete. In steady state, air sac residential gas is thus expected to equilibrate with the re-inspired dead space gas layer which is higher in CO_2 and lower in O_2 than inspired fresh air.

803

AORTIC COMPLIANCE AND PULSE-WAVE VELOCITY IN CHICKENS AND DUCKS. J. M. Ploucha* and R. K. Ringer, Michigan State Univ., East Lansing, MI 48824

Previous researchers have suggested birds possess an inelastic arterial tree. The present study establishes the pressure-volume and pressure-radius relationships of thoracic and abdominal aortic segments of chickens and mallard ducks. Each segment was affixed to rigid tubing such that length could not change. The segment was inflated by constant flow to a pressure of 300 mm Hg by an infusion/withdrawal pump. Aortic external diameter was measured subsequently at 50 mm Hg increments. The diameter increased rapidly with small pressure increments up to 150 mm Hg, above which little change in diameter occurred. The pressure-volume curves for the thoracic segments were sigmoidal with greatest compliance in the physiological pressure range. The abdominal segments were significantly less compliant than thoracic segments. Incremental pulse-wave velocities in the physiological pressure range, calculated from the pressure-volume curves, were very similiar to those of mammals, i.e. 6-10 m/sec. The pulse-wave velocities of the abdominal segments of both species were significantly higher than those of the thoracic segments. Turkey aortic pulse-wave velocities were calculated from pressure-volume curves (Speckmann and Ringer, 1966. Can. J. Physiol. Pharmacol. 44:901-907) as 8-12 m/sec in the physiological pressure range. Thus, it appears that the pulse-wave velocities of turkeys, ducks, and chickens are similiar to mammals.

804

STRUCTURE/FUNCTION CORRELATIONS IN LUNG EVOLUTION. Steven F. Perry. Univ. of Oldenburg, 2900 Oldenburg, Fed.Rep. Germany

Vertebrate lung structure can be expressed graphically by plotting "degree to heterogeneity of internal partitioning" against structural type: unicameral, paucicameral, multicameral. A large oxygen diffusion capacity (D_{MtO2}) and a low minute work of breathing ($\dot{w}$) occur simultaneously in heterogeneously partitioned lungs. The low $\dot{w}$ is attributed to high compliance of the thin-walled, membranous regions. Multicameral structure makes possible a large ratio of respiratory surface area to parenchymal volume without long diffusion distances within the parenchyma. Heterogeneously partitioned, multicameral lungs thus provide the possibility both for a large, easily ventilatible surface area and for a low $\dot{w}$. The avian lung-air sac system may have evolved from this lung type. In the rat D_{MtO2} is 5 times less than in a pigeon of similar body weight and $\dot{w}$ is 10 times greater. The mammalian lung may owe its success to the effecient breathing mechanism. Preliminary morphometric comparison of multicameral, heterogeneously partitioned lungs (Varanus exanthematicus) and unicameral, homogeneously partitioned lungs (Tupinambis nigropunctatus) show that the capability for sustained aerobic activity in the former must be due to differences in lung architecture, since D_{MtO2} in both species is the same (1.9 ml/min.kg.torr) for specimens of ca. 500g. (Supported in part by DFG grants Du 50/3 and Du 50/4 to H.-R. Duncker)

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CARDIOVASCULAR TOLERANCE TO SEVERE HYPOXIA IN HIBERNATORS VS NON-HIBERNATORS. S. B. Jones and B. J. Pardini, Dept. of Physiology, Loyola University of Chicago, Maywood, IL 60153.

Hibernating species are known to tolerate severe hypoxia but respiratory failure has usually been the criterion for death. Limitations of cardiovascular function during severe hypoxia in hibernators have not been described. The object of this study was to assess cardiovascular function with controlled hypoxic respiration. Mean arterial pressure (MAP in mmHg) heart rate (HR) and rectal temperatures (Tre) were monitored in pentobarbital-anesthetized animals respired initially on room air and then made hypoxic for 4 hrs with 5% O_2 95% N_2. Arterial oxygen tension after 15 min of hypoxia was <30 torr in all groups. With initial Tre=37 C, all rats died within 45 min. Criterion for death was MAP <5. Response of hamsters (Mesocricetus auratus) was of two types. Animals either failed rapidly (death within 60 min) or survived the hypoxic challenge (MAP=62±8 at 4 hrs). Control experiments of normoxic ventilation did not cause MAP failure after 4 hrs in either rats (124±9) or hamsters (83±5). With initial hypothermia (Tre=30 C) all hamsters survived hypoxia (MAP=68±13 at 4 hrs) whereas all rats died within 3 hrs. With initial Tre=30 C, ground squirrels (Spermophilus tridecemlineatus) all survived in hypoxia (MAP=54±8 at 4 hrs). A similar response was observed in the woodchuck (Marmota monax) whose MAP=80±12 after 4 hrs of hypoxia. These data suggest that cardiovascular function in severe hypoxia: 1) is better maintained in hibernating species and 2) is enhanced by initial hypothermia. (Sup. NIH HL 08682.)

806

PANTING IN DOGS: PATH OF AIR FLOW IN RESPONSE TO HEAT AND EXERCISE. Marcia B. Goldberg*, Vaughan Langman* and C. R. Taylor. Mus.of Comp.Zool., Harvard Univ., Cambridge, MA 02138

Panting is the major avenue of evaporative cooling in dogs exposed to heat and/or exercise. We found modulation of evaporation was achieved by varying the paths of airflow during inhalation and exhalation. The direction of airflow through the nose and mouth was determined by measuring pressure changes and temperature at the openings of one nostril and the mouth in three dogs (av. weight 22 kg). Rates of oxygen consumption and respiratory evaporation were measured simultaneously. Three patterns of panting were observed as the demand for respiratory evaporation increased: I-- inhalation and exhalation through nose; II-- inhalation through nose, exhalation through nose and mouth; and III-- inhalation through nose and mouth, exhalation through nose and mouth. Pattern I was observed in resting dogs when ambient temperature was below 26° and when animals ran at slow speeds in the cold (eg.$<$ 0.8 $m \cdot s^{-1}$ at 10°C). Patterns II and III were observed when dogs rested quietly at ambient temperatures above 30° and during exercise except when dogs ran slowly at very low temperatures. Patterns II and III rarely occurred independently for long periods of time, instead there was normally a continual oscillation between the two. Pattern III seemed to be used more as temperature and/or speed were increased and there was a weak correlation (r^2 = 0.63) between rate of respiratory evaporation and the percentage of time pattern III was utilized. (NSF grant #PCM 7823319)

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DIVING METABOLISM IN MARINE MAMMALS. Michael Castellini* (Spon: F. White). Scripps Institution of Oceanography Physiological Research Laboratory, La Jolla, CA. 92093

Marine mammals possess an exceptional capacity for breathold diving. In an effort to assess possible biochemical adaptations to potential low oxygen stress during these dives, studies are in progress comparing key enzymes in energy producing pathways from a wide range of marine and terrestrial mammals. The enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) have been measured in a variety of tissues. Contrary to present hypotheses concerning anaerobic capacity in diving mammals, muscle LDH and PK activity levels exhibited no relationship to diving ability. Brain enzyme activities were constant across all species examined. Heart levels showed no consistent patterns but were highest in the two longest divers. Liver and kidney enzymes showed a small elevation in marine forms but did not correlate with breathold capacity. We conclude that predictions correlating diving abilities in marine mammals with enhanced tissue glycolytic potential are, except possibly for heart tissue, incorrect. These data support new evidence suggesting that marine mammals may power most of their natural dives using aerobic mechanisms.
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INFLUENCE OF VARYING INTRATHORACIC PRESSURE ON PULMONARY CAPILLARY BLOOD FLOW AS MEASURED BY SOLUBLE INERT GAS UPTAKE.
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To illustrate the effects of varying intrathoracic pressure on pulmonary capillary blood flow ($\dot{Q}p$) we measured $\dot{Q}p$ in six healthy males by soluble inert gas uptake. As previously described we measured lung volumes, argon, acetylene and freon concentrations during exhalation of a previously inhaled VC breath of the test gases. $\dot{Q}p$ is then calculated for each 2% decrement in the lung volume during the exhalation. We varied intrathoracic pressure prior to exhalation by varying the inspiratory flow rates and with Mueller maneuvers. Mouth pressures during the Mueller maneuvers were controlled at -20, -40, or -60 mmHg and maintained for 5 secs. Initial $\dot{Q}p$ results were obtained 2 secs. after the release of the negative intrathoracic pressure. The results revealed a slight trend to increasing $\dot{Q}p$ with increasing negative pressures but the differences were not statistically significant. These results suggest that though cardiac output may be augmented immediately after the release of a Mueller maneuver; hemodynamics return to baseline within 2-3 secs. in normal individuals.

(supported by the Ontario Heart Foundation).

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EFFECTS OF BRAIN OR CAROTID BODY HYPOXIA ON PULMONARY HEMODYNAMICS.
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Brain or carotid bodies of systemically normoxemic dogs were perfused with hypoxic blood (PO_2 25) from an extracorporeal lung. The brain was perfused at constant pressure; carotid bodies were perfused at constant flow, but pressure was maintained constant by adjusting outflow resistance. Five minutes of brain hypoxia increased blood flow to the head but caused no change in cardiac output, mean arterial pressure, pulmonary perfusion pressure, pulmonary artery pulse pressure, systemic vascular resistance, and pulmonary vascular resistance. Prior to vagotomy carotid body hypoxia did not alter any pulmonary parameter measured; following vagotomy carotid body hypoxia increased pulmonary vascular resistance. Carotid body hypoxia decreased cardiac output and increased systemic vascular resistance, both before and after vagotomy; mean arterial pressure was increased only after vagotomy. Post-vagotomy, carotid body hypoxia-hypercapnia (PO_2 20; PCO_2 72) increased cardiac output, mean arterial pressure, systemic vascular resistance, pulmonary vascular resistance, pulmonary perfusion pressure, and pulmonary artery pulse pressure. These studies fail to indicate that short term brain hypoxia has any influence on pulmonary hemodynamics but suggest that alteration of pulmonary hemodynamics can result reflexly from carotid body hypoxia or hypoxia-hypercapnia. (Supported by NIH Grant #1F32HL05923-01 and BRSG from Coll. Vet. Med.).

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EVIDENCE FOR HYPOXIC CONSTRICTION OF ALVEOLAR VESSELS.
W. Mitzner, J.T.Sylvester, and Y.K. Ngeow*. The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.

We have previously shown that hypoxia (PO_2=50mmHg) results in a parallel shift of the pulmonary artery pressure (Ppa) vs flow (F) curve to higher Ppa (Physiologist 22:89,1979). Such behavior is most simply interpreted using a Starling resistor circulatory model where hypoxia increases the critical pressure. In the present study we attempted to determine if the locus of this critical pressure might be in alveolar vessels. If it were, an increase in alveolar pressure (Palv) should add directly to the hypoxic increase in critical pressure,whereas, if it were only in extraalveolar vessels, the increase in lung volume should pull on these vessels and decrease the critical pressure. Families of Ppa-F curves were obtained in isolated blood perfused pig lungs at several Palv between 0 and 25 mm Hg. In the control state, for Palv greater than 10 mmHg, increases in Palv caused parallel shifts in the Ppa-F curves, with the shift in each curve (i.e.,the increase in Ppa) being nearly equal to the increase in Palv. Hypoxia resulted in a shift in the whole family of curves to higher pressures, such that the increase in Palv still resulted in a further parallel shift in the Ppa-F curve. Thus hypoxia can act in a manner similar to an increase in Palv. Furthermore, for Palv greater than 10 mmHg, increases in Palv act additively to the effect of hypoxia. These results suggest that hypoxia acts directly on alveolar vessels to cause an effective increase in tone. Supported by NIH grants HL-00347 and -10342.

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EFFECT OF HYPOXIA ON ALVEOLAR-CAPILLARY MORPHOLOGY.
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Kapanci and Costabella (Fed. Proc. 38:964, 1979) reported that alveolar wall morphology can be altered by ventilation with 8% O_2. The effect of hypoxia on capillary morphology was examined by removing the left ribcage of a dog and cannulating the left lower lobe. The lobe was ventilated with 100% N_2 and the remainder of the lung with 100% O_2. After 15 min, both the lobe and the rest of the lung were inflated to 15 cm H_2O and rapidly frozen. Three samples from the lobe and two from the lung were obtained and processed for TEM (J. Appl. Physiol. 45:325, 1978). Two blocks were examined from each sample and 5 fields randomly photographed from each. The depths of alveolar wall pleats and capillary folds were measured. The alveolar surface area not involved in pleats or folds was measured by planimetry and divided by the total alveolar surface area present in the field to give the free alveolar surface area. Results to date show that pleat depth increases from 2.6 to 4.79 μm during N_2 breathing. Fold depth increases on N_2 from 1.75 μm to 2.92 μm. Pleat and folds often appeared to be associated with pericytes or contractile interstitial cells. Free alveolar surface area decreased from 91.5% on O_2 to 70.9% on N_2. The results suggest that hypoxia may exert a direct effect upon alveolar-capillary morphology. Folds may increase the vascular resistance of the capillaries. This may in part explain the redistribution of blood flow which occurs during hypoxic vasoconstriction. (Supported by NIH HL 21943.)

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BLUNTED HYPOXIC PRESSOR RESPONSE IN THE ISOLATED HAMSTER LUNG.
Norbert F. Voelkel*, Benjimen R. Walker*, Ivan F. McMurtry and Merrill Adams. CVP Research Laboratory, University of Colorado Health Sciences Center, Denver, Colorado 80262.

Many mammals demonstrate a marked increase in pulmonary arterial pressure (PAP) in response to hypoxia. However, recent studies have shown a blunted hypoxic pressor response in isolated lungs from rats exposed to altitude, while the pressor response to angiotensin II (AII) was augmented. Diminished sensitivity of the pulmonary vasculature to hypoxia may be an advantageous adaptation to chronic hypoxia. Fossorial rodents, such as the hamster, are regularly exposed to hypoxic and hypercapnic conditions while in their natural environment. We were therefore interested in the pulmonary vascular responsiveness to hypoxia (F_{IO_2}=0%,3%,10%) in a species genetically adapted to fossorial behavior. Isolated perfused lungs from five normal hamsters and six normal rats were studied. The hypoxic pressor response was blunted in the hamster when compared to the rat, while the response to AII was greater.

	ΔPAP (mmHg)			
	10% O_2	3% O_2	0% O_2	0.5μg AII
Rat n=6	3.83±0.69	18.8±1.6	--	17.3±1.0
Hamster n=5	0	4.9±1.0	8.8±0.1	20.9±1.3

The responses seen in the hamster are similar to those of rats exposed chronically to high altitude, and probably represent a successful adaptation genetically selected in this species to cope with a hypoxic environment. (Supported by NIH Grant HL 14985).

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THE EFFECT OF SINOAORTIC DENERVATION AND HEMORRHAGE ON PULMONARY BLOOD FLOW DURING UNILATERAL HYPOXIA IN DOGS.
William B. Strawn* and Michael G. Levitzky. LSU Medical Center, New Orleans, La. 70112.

Seven dogs anesthetized with 30mg/kg pentobarbital had their left and right lungs separately ventilated following tracheotomies and placement of Carlens endotracheal tubes. Following sternotomy, blood flow was monitored by electromagnetic flow probes on the main (Q_T) and left (Q_L) pulmonary arteries. Catheters were placed in the left atria and femoral arteries. Swan-Ganz catheters were placed in the main pulmonary arteries via external jugular veins. During bilateral ventilation with 100% O_2, Q_L was 41.4 ± 6.1% of Q_T. When the left lung was ventilated with 100% N_2 and the right lung with 100% O_2, PaO_2 was 63 ± 4.1 torr and Q_L fell to 30.7 ± 2.2% of Q_T. When 15% of estimated blood volume was rapidly withdrawn, PaO_2 fell to 50 ± 3.3 torr and Q_L rose to 41.4 ± 3.5% of Q_T. Three dogs which had undergone sinoaortic denervation prior to these experiments showed similar decreases in PaO_2 and Q_L with unilateral hypoxia, and also showed increased blood flow to the hypoxic lung during hemorrhage. Thus, the increased blood flow to the unilaterally hypoxic lung during hemorrhage does not seem to be mediated by the arterial baroreceptors or chemoreceptors. (Supported by NHLBI Grant 22641, NIH Cardiovascular Training Grant HL 07098, and American Heart Association-La., Inc.)

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THE EFFECTS OF HISTAMINE ON THE PRESSURE-FLOW RELATIONSHIPS OF THE PULMONARY AND SYSTEMIC VASCULAR BEDS IN INTACT DOGS. Mario Seoane* and Howard S. Goldberg. Sec. of Resp. Dis., University of Manitoba, Winnipeg, Manitoba, Canada R3E 0Z3.

Controversy exists on the effects of histamine on the pulmonary vasculature. Both, vasoconstriction and vasodilatation have been reported. Systemic effects of histamine are dilator and less controversial. In intact anaesthetized dogs, histamine phosphate, 10 µg/kg/min i.v. was given as a continuous infusion (group A, n=6), or as a 10 µg/kg bolus at different values of cardiac output (CO) (group B, n=5). CO and pulmonary artery, pulmonary wedge and aortic pressures were measured. The normal pressure-flow relationships were obtained by altering the CO. Driving pressure for pulmonary blood flow was computed taking into account the proportions of Zones II and III of West. Pressure-flow relations after histamine were compared to normal. In the pulmonary circulation, although a conventional calculation of pulmonary vascular resistance showed a significant increase, there was no difference from a normal pressure-flow relationship. In the systemic circulation, a distinct difference was observed. This difference showed two components. An early direct effect of the drug on systemic arterioles producing a fall in arterial pressure with no change in CO, and a later decrease in CO caused by a reduction in venous return. We conclude there is no effect of histamine on the normal pressure-flow relationship of the pulmonary vasculature while there are simultaneous effects in the systemic circulation. (Supported by MRC of Canada).

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EFFECTS OF DIFFERENTIAL POSITIVE END-EXPIRATORY PRESSURE (PEEP) ON LOBAR PERFUSION AND O_2 EXCHANGE IN PNEUMONIA. L.D.H. Wood*, R.B. Light*, and S. Mink*. (Spon: V. Chernick). Sec. of Resp. Dis., University of Manitoba, Winnipeg, Canada.

We previously reported that Peep had little beneficial effect in improving gas exchange in canine left lower lobe (LLL) pneumonia (Physiol. 22: 89, 1979). This was because while Peep improved lobar gas exchange, it also increased relative perfusion (Q_L) to the diseased lobe. We therefore studied 6 dogs with LLL pneumonia in which each lung was ventilated separately with O_2, and Peep was applied only to the left lung. Measurements of pulmonary shunt (Qs/Qt%), arterial Pa_{O_2}, and hemodynamics were made before (C1), after (C2), and during (P) 12 cm H_2O Peep. Changes in Q_{LLL} were determined by radiolabelled microspheres and expressed as a percent of cardiac output. The mean (±SD) results were: (*denotes $p < .01$)

	C1	P	C2
Pa_{O_2}	310 ± 86	532 ± 58*	337 ± 84
Qs/Qt%	29 ± 5	12 ± 5*	26 ± 5
Q_{LLL}%	26 ± 5	22 ± 5	22 ± 5

We conclude that unilateral Peep increased Pa_{O_2} and reduced Qs/Qt because it improved regional gas exchange without diverting blood flow toward the pneumonia lobe. Furthermore, these results indicate the mechanism of the increased Q_{LLL} in our original study was compression of alveolar vessels in the normal lung. (Supported by MRC of Canada and the Canadian Lung Association).

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HEMODYNAMIC SIGNIFICANCE OF RIGHT VENTRICULAR EJECTION FRACTION IN PULMONARY HYPERTENSION. Milena L. Lewis, and Lynn C. Christianson*. VA Medical Center, N.Y., N.Y. 10010

The hemodynamic implication of a reduced RVEF in patients with lung disease is unclear since EF has not been measured simultaneously with other indices of right heart performance. We have measured RVEF by an external isotope counting technique during right heart catheterization, in 48 patients with chronic lung disease, on 53 occasions. Normal RVEF by our method is 0.56 ± 0.06(SD), the same as published values. Pertinent parameters in the patients were: CI 3.4 ± 0.6 L/min /m^2; $\bar{P}_{PA}$ 28 ± 14 torr; Pa_{O2} 63 ± 16 torr; S_{aO2} 89 ± 6%; FEV_1 1.4 ± 0.8L. Average RVEF for the group was 0.44 ± 0.09; it was below the normal range (< 0.45) in 26 of 53 studies. No significant correlation was observed between RVEF and blood flow, stroke work, nor degree of ventilatory impairment. A highly significant correlation was found only between RVEF and afterload, as expressed by $\bar{P}_{PA}$ (r=0.74) or by diastolic gradient (DG) (r=0.79). Of the 13 patients with DG ≤6 torr, only one had a reduced RVEF; in 28 patients with DG between 6 and 16 torr RVEF was equally divided between normal and reduced (range 0.60 to 0.35). Only when DG exceeded 16 torr did all patients have a reduced RVEF. We conclude that RVEF, in this type of patient, is principally an index of ventricular afterload, and cannot be used as an indicator of myocardial dysfunction.

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STIMULATION OF CARDIOVASCULAR INHIBITORY REFLEXES ARISING FROM THE LUNG BY HYPERINFLATION ARACHIDONIC ACID, AND CAPSAICIN. Sharon S. Cassidy. University of Texas Southwestern Medical School, Dallas, TX 75235

Hyperinflation, Capsaicin (Cap) and certain prostaglandins are known to stimulate pulmonary C-fiber receptors. Lung hyperinflation (HI) also causes a reflexly mediated fall in blood pressure (BP) and heart rate (HR). The purpose of this study was to determine whether the Cap and arachidonic acid (AA) cause reflexly mediated decreases in HR and BP. In 14 dogs, pulmonary blood flow was diverted to the right lung by ligating and cannulating the left pulmonary artery (LPA). Left pulmonary veins were cannulated and diverted to reservoirs. Response of the HR and BP was measured during HI to 30 cm H20 and during rapid 4 ml injections of AA or Cap into the LPA before and after left cervical vagotomy (LVX). Maximum BP response occurred 15 s following HI and Cap, and 90 s following AA.

	Before LVX				After LVX		
	Initial			Final			
	HI	Cap	AA	HI	HI	Cap	AA
HR(%Δ±SD)	-33±12	-36±6	-24±9	-29±14	-4±3	-2±2	+3±10
BP(%Δ±SD)	-30±14	-21±11	-36±13	-22±8	-2±3	-2±2	-3±9

Cap and AA stimulate neural receptors in the lung to cause reflex bradycardia and hypertension the afferent limb of which travels with the ipsilateral vagus. Hyperinflation may stimulate these receptors by releasing of an AA metabolite.

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LUNG INTERSTITIAL FLUID REDISTRIBUTION INFLUENCES TIME COURSE OF TRANSVASCULAR FLUID FLUX. L. Oppenheimer* and H.S. Goldberg. (Spon: N.R. Anthonisen). Univ. of Manitoba, Winnipeg, Canada.

We measured transudation directly in isolated lobes perfused with autologous plasma stained with indocyanine green. Following step changes in capillary pressure, transudation (T) was calculated from the changes in outflow relative to the inflow concentration of albumin ($\Delta C/\Delta t$): $T=Q\,C(t)\,dt$ where Q is constant and represents flow of perfusate and t time. The slopes and 0 time intercepts of the transudation curves correlated well with the slow phases of weight gain (r=.89 and .92 respectively), the regression lines being no different from identity. If transudation represents flux of water across the membrane into a finite compartment, the transudation curve should be a single exponential. In all cases however, a fast (80 x faster on average) and a slow phase were obtained. The slow phase coincided with the slow phase of weight gain. Presumably the fast phase of transudation represents flux into a relatively non-compliant perimicrovascular space followed by movement to a much larger, compliant downstream interstitium. To test this hypothesis, we repeated our experiments in lobes at different levels of hydration in an effort to change the perivascular space. As hydration increased the fast and slow phases of transudation progressively blended and transudation became a monophasic curve reflecting the sum of membrane and tissue conductance and the combined compliances of perimicrovascular and downstream interstitium. (Supported by MRC of Canada).

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THE INCREASE IN LUNG VASCULAR PERMEABILITY AFTER PULMONARY EMBOLIZATION WITH DIFFERENT-SIZED MICROEMBOLI. A.Johnson*, P.H.Neumann*, N.B.Gertzberg*, A.B. Malik. Albany Medical College, Albany, NY 12208.

We examined if the size of microemboli influences the increase in lung vascular permeability after embolization. Embolization was induced in anesthetized sheep by injecting glass beads 100 or 500 µm diam. into the right atrium to attain similar increases in pulmonary artery pressure ($\bar{P}_{pa}$). Lung lymph flows ($\dot{Q}$lym), lymph-to-plasma protein ratios (L/P) and lymph protein clearances ($\dot{Q}$lym x L/P = C_L) were determined. The steady-state baseline (BL) and post-embolization (PE) values were:

	100 µm (n=6)		500 µm (n=4)	
(*p<0.05)	BL	PE	BL	PE
$\dot{Q}$lym (ml/hr)	6.4 ±0.9	12.4 ±1.9*	3.6 ±0.4	6.5 ±0.8*
L/P	0.71±0.03	0.74±0.02	0.57±0.09	0.58±0.11
C_L (ml/hr)	4.4 ±0.5	9.3 ±1.7*	2.0 ±0.43	3.8 ±0.95*
$\bar{P}_{pa}$ (mm Hg)	16.9 ±1.2	41.3 ±2.9*	11.8 ±1.0	37.9 ±3.4*

Embolization with 100 or 500 µm beads produced the same increases in $\dot{Q}$lym (97±22 vs 84±14%) and C_L (109±29 vs 87±10%) from BL. Therefore, the increase in permeability was independent of size of the microemboli. Since the larger emboli were expected to occlude a greater fraction of the bed and cause smaller increases in C_L than the smaller emboli, the site of increased permeability may not be in the occluded vessels. (HL-17355, HL-00363, HL-07194)

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ROLE OF OXYGEN AND CARBON DIOXIDE IN EXERCISE HYPEREMIA. David E. Mohrman. Univ. of Minnesota, Duluth, MN 55812.

The role that changes in muscle O_2 and CO_2 levels play in increasing skeletal muscle blood flow during exercise is unclear. In this study, dennervated dog gastrocnemius/flexor digitorum superficialis muscles were perfused at constant pressure (125 mmHg) with blood whose gas composition could be varied with a membrane oxygenator. Average results (± SE) from several situations are indicated below. With normal arterial gas composition, muscle exercise at 8 twitches/sec. caused muscle blood flow ($\dot{Q}$) to increase by 5x over its resting value while venous blood oxygen tension (PO_{2v}) fell to 28 mmHg and venous carbon dioxide tension (PCO_{2v}) rose to 51 mmHg. Note however that using the oxygenator to reduce the PO_{2v} of a resting muscle to 27 mmHg or to increase the PCO_{2v} to 60 mmHg caused only a modest increase in $\dot{Q}$.

Arterial blood	Exercise state	(n)	PO_{2v} mmHg	PCO_{2v} mmHg	$\dot{Q}$ ml/100g/min
Normal	rest	(13)	76 ± 4	38 ± 1	25 ± 3
	8 Hz	(13)	28 ± 1	51 ± 1	123 ± 7
Low O_2	rest	(7)	27 ± 2	40 ± 2	31 ± 5
High CO_2	rest	(6)	87 ± 9	60 ± 2	32 ± 7

These data indicate that reduced O_2 levels and increased CO_2 levels in themselves fall far short of accounting for the increased blood flow which accompanies muscle exercise. (Supported in part by USPHS NIH Grant HL22975.)

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ACETATE RELEASE DURING EXERCISE HYPEREMIA. R.P. Steffen*, J.E McKenzie*, and F.J. Haddy, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20014

We have previously reported that intraarterial infusion of Na Acetate produces vasodilation in the dog forelimb (AJP 203: 125, 1962) and that this vasodilation occurs mainly at the small vessel level (Physiologist 21:115, 1978). We have also shown that femoral venous acetate concentration increases during treadmill exercise in the conscious dog and therefore suggested that acetate may play a role in exercise hyperemia (Fed. Proc. 39:270, 1980). In the present study, arterial and venous whole blood acetate concentrations were measured in 7 collateral free gracilis muscles before and during twitch contraction at two rates (6V,0.3 msec, 1 and 4HZ, 10 min each). Contractions at 1HZ increased $\dot{V}O_2$ from 1 to 25 ml/min/100g, venous outflow from 9 to 18 ml/min/100g, acetate output from 430 to 1612 n moles/min/100g and venous acetate concentration from 223 to 296 n moles/ml. Contractions at 4HZ increased $\dot{V}O_2$ from 2 to 84 ml/min/100g, venous outflow from 17 to 44 ml/min/100g, and acetate output from 280 to 2788 n moles/min/100g. Venous acetate concentration did not change significantly. Acetate output and vascular resistance correlated significantly ($r=-0.87$, $p<.01$). Intraarterial infusion of isosmotic Na acetate in quiescent gracilis muscle perfused at constant flow (n=3) reduced perfusion pressure with calculated increases in arterial whole blood acetate as low as 129 n mole/ml. The possible role of acetate in exercise hyperemia deserves continued study.

822

THE EFFECTS OF HYPEROXIA DURING ACUTE ANEMIA. C.K. Chapler, S.M. Cain and W.N. Stainsby, Depts of Physiology, Queen's Univ., Kingston, Ont. K7L 3N6; Univ. of Alabama in Birmingham, Alabama 35294 and Univ. of Florida, Gainesville, Florida 32610.

We have previously suggested that chemoreceptors contribute to the circulatory and metabolic changes observed during acute anemia. In this study, anesthetized paralyzed dogs were ventilated on room air, then on 100% O_2 and again on room air. This sequence was repeated after the hematocrit (Hct) was reduced to 15% by isovolemic hemodilution with dextran. Cardiac output (Q_T), limb and total oxygen uptake ($\dot{V}_{O_2}$) and limb venous flow (Q_L) were determined. In 10 animals the hindlimb was denervated (D) by sciatic and femoral nerve section and in another group of 10, limb innervation remained intact (I). At normal Hct limb resistance (R_L) increased ($p < 0.01$) by the 3rd min of hyperoxia in the D group while no change occurred in the I group. In addition, no change in limb $\dot{V}_{O_2}$, Q_T or heart rate occurred at normal Hct in either group while whole body $\dot{V}_{O_2}$ declined 7% in the D group. Hyperoxia during anemia resulted in an increase in R_L by 2 min in the D group while both groups decreased heart rate ($p < 0.01$), whole body $\dot{V}_{O_2}$ ($p < 0.01$) and limb $\dot{V}_{O_2}$ ($p < 0.01$). These patterns were reversed when the anemic animals were ventilated with room air. The changes in R_L in the D group during hyperoxia were consistent with a direct effect of high PaO_2 on the peripheral vasculature. The heart rate responses coupled with the body and limb $\dot{V}_{O_2}$ data during hyperoxia suggest that chemoreceptors also contributed to the compensatory responses in anemia. (Supported by the MRC of Canada and NHLBI 14693).

823

EFFECTS OF HIGH DOSES OF AN H_2-RECEPTOR AGONIST ON FORELIMB VASCULAR PRESSURES AND SKIN LYMPH. David E. Dobbins, Connie Y. Soika*, John E. Mulcahy*, and Joe M. Dabney. Dept. Physiol USUHS, Bethesda, Maryland 20014

We have previously reported that the local infusion of the H_1 and H_2 histamine receptor agonists 2(2-pyridyl) ethylamine and 4-methylhistamine respectively, in a dose range of 8-40 µg/min fails to mimic histamine's actions on capillary permeability but that higher doses of the H_1-receptor agonist do increase lymph flow and protein concentration. We now report the effects of high doses of 4-methylhistamine on vascular pressures and skin lymph flow, protein concentration and protein transport in the canine forelimb perfused at constant flow. Infusion of 4-methylhistamine at 40, 80, 200 and 400 µg/min for twenty minutes at each infusion rate resulted in a significant decrease in mean systemic arterial pressure, forelimb perfusion pressure and skin small artery pressure at all infusion rates. Lymph flow and protein transport were significantly decreased as a result of the infusion while lymph protein concentration remained unchanged. Since previous work has implicated the H_2-receptor in histamine-mediated increases in capillary permeability, failure of 4-methylhistamine to significantly increase capillary permeability may be due in part to increased circulating levels of catecholamines as a result of systemic hypotension. Thus, this H_2-receptor agonist would appear to be a better mimic of histamine's vascular actions than on microvascular permeability.

824

REGULATION OF VENOUS VASOACTIVITY IN CONSCIOUS DOGS. Thomas H. Hintze and Stephen F. Vatner, Departments of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, Boston, MA 02115

Venous capacitance is considered to be one of the fundamental mechanisms involved in cardiovascular regulation. This aspect of circulatory control has not been examined extensively utilizing direct measurements of venous caliber in conscious animals due to lack of appropriate techniques. In order to study this problem in conscious animals, 10 dogs were instrumented with ultrasonic crystals on opposing surfaces of an ileal vein to measure its diameter. Catheters were placed in the ileal vein and aorta for pressure measurements. One week after recovery from operation, direct and continuous measurements of venous diameter and pressure were recorded. Both norepinephrine (1 µg/kg) and epinephrine (1 µg/kg) iv reduced venous diameter (control = 5.17 ± 0.43 mm) by 11.5 ± 3.4%* and 7.4 ± 2.2%* respectively, while venous pressure (control = 9.6 ± 1.2 mmHg) rose by 3.5 ± 0.9* and 3.8 ± 1.5* mmHg respectively. Stimulation of the carotid chemoreflex increased mean arterial pressure by 52.3 ± 8.6* mmHg (control = 111 ± 4.5 mmHg), and ileal venous pressure by 3.7 ± 0.7* mmHg, while decreasing ileal venous diameter by 7.2 ± 2.4%*. Thus, these techniques permit the assessment of venous dimensions by direct measurements in conscious animals and demonstrate ileal venous vasoconstriction in response to adrenergic stimulation by both humoral and neural mechanisms.

* significant change, $p<0.05$

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INHIBITION OF NOREPINEPHRINE RELEASE FROM ADRENERGIC NERVE ENDINGS IN HUMAN SAPHENOUS VEINS BY ACETYLCHOLINE. N.J. Rusch*, D.K. Rorie*, J.T. Shepherd and G.M. Tyce* Mayo Fdn., Rochester, MN 55901.

These studies examined whether inhibition of evoked release of norepinephrine (NE) by acetylcholine (ACH) could be demonstrated in human saphenous vein. Veins, removed from cadavers 2-12 h after death, were incubated in L-[7-^{3}H]NE ($1x10^{-6}$M) then mounted for superfusion and isometric tension recording. Superfusate was collected continuously before and during electric stimulation (ES; 10V, 2msec, 8Hz). Total ^{3}H was determined in the superfusate and in the tissue after study, and the fractional loss of radioactivity from the tissue was calculated. [^{3}H]NE and its metabolites were separated in superfusate and in vein extracts by column chromatography. The fractional loss of radioactivity elicited by ES was decreased ± 30% when ACH ($2.7x10^{-6}$M) was present in the superfusate. This decrease was reversed by atropine (10^{-8}M), and to a lesser extent by hexamethonium ($3x10^{-4}$M). Decreases in isometric tension occurred concurrent with the decreases in total ^{3}H overflow, although the magnitude of the tension changes frequently did not parallel changes in overflow of total ^{3}H. The metabolites in superfusate and in vein extracts indicated that the degradative enzymes monoamine oxidase and catechol methyltransferase were still active, and in addition, that neuronal and extraneuronal uptakes were operative. These results suggest the presence of inhibitory cholinergic receptors on noradrenergic neurons innervating human cutaneous veins. (Supported by grants HL 23217, HL 5883 and NS 9143.)

826

α-ADRENERGIC AND METABOLIC CONTROL OF FOREARM BLOOD FLOW DURING INTERMITTENT HAND-GRIP CONTRACTIONS. C.A. Williams and A.R. Lind. St. Louis University, St. Louis, MO 63104

We reported that fatiguing intermittent isometric hand-grip contractions at 60% MVC (held for 4 sec with 8 sec of rest between each contraction) induced increases in mean blood pressure to 150-160 mmHg but only half-maximal levels of forearm blood flow, to 20-22 ml.min^{-1}.100 ml^{-1}. It was proposed that pre-capillary sphincters and smaller arterioles were removed from active vasoconstriction by the action of locally released metabolites but that larger arterioles and small arteries remained under the influence of sympathetic constriction. When exercise was performed by 5 volunteer subjects after infusing 0.5 mg phentolamine into the forearm via the brachilal artery, blood flow attained maximal levels, 38 ml.min^{-1}.100 ml^{-1} as fatigue occurred. Infusion of 50 μg phenylephrine before phentolamine prevented this increase in flow. During intermittent contractions, total catecholamine levels were measured in venous samples from the exercising forearm. These changed from 45 pg/ml at rest to levels of 987 pg/ml at fatigue. Plasma K^+ concentration increased from resting values of 3.5 mEq/L to values of 4.5 mEq/L at fatigue. In contrast, there was a continuous increase in the amount of ATP seen in the venous plasma as exercise continued with the lowest levels at rest and the highest levels at fatigue. (Supported, in part, by NIH Training Grant HL 07050 and Air Force Grant AFOSR-76-3084.

827

EFFECTS OF NOREPINEPHRINE AND α-BLOCK ON O_2 UPTAKE AND BLOOD FLOW IN DOG HINDLIMB. S.M. Cain and C.K. Chapler. Depts. of Physiol., Univ. of Alabama in Birmingham 35294 and Queen's Univ., Kingston, Ontario K7L 3N6.

Norepinephrine (NE) may increase regional tissue O_2 demand at the same time that it restricts O_2 transport by vasoconstriction. To see if any potential increase in O_2 uptake ($\dot{V}O_2$) was throttled by restricted blood supply in canine hindlimb, NE was infused at 1μg/kg·min intravenously (IV) and intra-arterially (IA) into anesthetized dogs. $\dot{V}O_2$ was measured for the hindlimb (less the paw) and the whole animal for a 20 min control, 20 min of NE infusion IV or IA, and 20 min recovery. The sequence was repeated for the other route. The order was reversed for each experiment. The experiments were repeated in a second group of animals given 3mg/kg phenoxybenzamine (α-bl). With IV infusion, cardiac output tended to increase in both groups while limb blood flow showed little change. With IA infusion, cardiac output again tended to increase in both groups. In the α-bl group, limb blood flow showed little change with IA infusion but it was decreased by 40% in the no block group. There were no significant changes in limb $\dot{V}O_2$ in either group with either route of infusion in spite of a modest but significant increase in total body $\dot{V}O_2$ in both groups with both IV and IA NE. We concluded that the calorigenic effect of NE was neither well marked in the limb nor was it attenuated there by blood flow restriction. Skeletal muscle of the anesthetized, paralyzed dog was not the principal site of NE's calorigenic action. (NHLBI Grant #HL14693)

828

IMPORTANCE OF NEURONAL REUPTAKE OF NOREPINEPHRINE IN HUMAN SAPHENOUS VEINS. Walter J. Janssens* and Raymond H. Verhaeghe*.(SPON P.M.Vanhoutte) Catholic Univ. Leuven, Thrombosis and vascular research; B3000 Leuven, Belgium.

The response of vascular smooth muscles to adrenergic nerve stimulation or exogenous norepinephrine is greatly influenced by neuronal and extraneuronal uptake of the catecholamine. The present experiments were designed to determine the relative importance of the two removal mechanisms in the human saphenous vein. Preparations were incubated in Krebs-Ringer containing ^{3}H-norepinephrine and the tissue uptake of the catecholamine was measured. Cocaine inhibited the tissue uptake, whereas hydrocortisone had no effect. In preparations mounted for isometric tension recording cocaine but not hydrocortisone potentiated the response to exogenous norepinephrine. Other preparations were mounted for superfusion with Krebs-Ringer after incubation with ^{3}H-norepinephrine. Electrical stimulation caused an increase of tritiated neurotransmitter and its metabolites,both of neuronal and extraneuronal origin,in the superfusate. Cocaine augmented this release.The present experiments indicate that in the human saphenous vein1) neuronal and extraneuronal uptake remove the neurotransmitter 2) neuronal reuptake is far more important.

829

COMPARISON OF HEMODYNAMIC EFFECTS OF MOLSIDOMINE (SIN 10) AND SIN 1. Volker B.Fiedler* and Rolf-Eberhard Nitz* (SPON: M.I. Barnhart), Dept.Pharmacology, Cassella AG, Frankfurt, F.R.G.

The hemodynamic effects of SIN 10 (N-carboxy-3-morpholino-sydnonimine-ethylester) and of its metabolite, SIN 1 (3-morpholino-sydnonimine) were studied in dogs. Both compounds were given i.v. and i.d. in identical doses: .01,.025,.05,and .10 mg/kg. 4 animals were used per dose level. We measured afterload (AoP,BP,TPR), preload (LVEDP,PAP,venous return), and heart performance (LVP,dP/dt,HR).

Hypotension occurred after administration: a short-lasting response after SIN 1 (3-5 min) but longer-lasting after SIN10 (over 30 min). Up to 0.05 mg/kg no changes in TPR were calculated but a reduction in resistance occurred after 0.10 mg/kg. HR increased after SIN 1, and dP/dt was augmented. SIN 10 had no significant effects on heart performance. A marked reduction in venous return, LVEDP, and PAP indicated an increasing venous capacity after both compounds.

The rapid onset of hemodynamic effects separated SIN 1 from SIN 10. SIN 1 reached the maximum effects shortly after administration while SIN 10 had the maximum effects about after 30 min. A comparable resorption of both agents led, finally, to the same reduction in pre- and afterload, and duration of action was identical. It is assumed that the rate of release of SIN 1 brings about different hemodynamic effects. SIN 10 may be regarded as a "predrug" of SIN 1, and the delayed release of this active agent explains the slow fall in pre- and afterload without changes in HR and dP/dt after SIN 10.

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THE EFFECTS OF H_1 AND H_2 BLOCKERS ON HYPEROSMOLAR VASODILATION. Michael R. Pinsky*, Philip Smith*, Eugene Bleeker and B. Bromberger-Barnea. The Johns Hopkins Medical Institutions, Baltimore, Md. 21205

The intravascular injection of a bolus of hyperosmolar solution is known to produce peripheral vascular dilation. The mechanism for this dilation is not fully understood. Mediator release, such as histamine, has been suggested as a possible cause. We therefore studied the possible role of histamine in mediating hyperosmolar vasodilation. We injected 20cc 10% NaCl or 50cc 25% Manitol into either right-heart bypass preparations with constant pulmonary inflow or intact dogs. Hyperosmolar vasodilation could not be blocked by H_1 (Benedryl 1 mg/kg) and/or H_2 (Cimetidine 10 mg/kg) blockers. Furthermore, serum histamine was not elevated in either mixed venous or arterial blood during hyperosmolar vasodilation. However, vasodilation produced to a similar degree by histamine injection caused elevation of serum histamine levels measurable by our assay method. We conclude that hyperosmolar vasodilation is not primarily mediated by histamine.
(Supported by NIH Grant HL-10342)

831

THE EFFECTS OF HYPERCARBIA ON CEREBRAL BLOOD FLOW IN NEWBORN LAMBS. A.A. Rosenberg*, M.D. Jones, Jr.*, R.A. Molteni*, M.A. Simmons* and R.J. Traystman. The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Eleven unanesthetized lambs were studied at 3-11 days of age to define the effect of hypercarbia on cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$). CBF was measured using radioactive microspheres. $CMRO_2$ was the product of CBF and the difference in O_2 content between arterial and sagittal sinus blood. Mean $CMRO_2$ was 6.17±.28 (SE)ml/100g/min and was unchanged as Pa_{CO_2} varied from 35-60mmHg. In contrast, CBF rose 5.42±.63ml/100g/min/mmHg change in Pa_{CO_2}. These values for $CMRO_2$ and $\Delta CBF/\Delta Pa_{CO_2}$ differ from those in a previous study (Am J Phys 235:H162, 1978) of the fetal sheep in utero (4.09±.29ml/100g/min and 2.68±1.09ml/100g/min/mmHg) respectively. The value of $\Delta CBF/\Delta Pa_{CO_2}$ in the newborn is twice that of the fetus; however, the difference between fetus and newborn largely disappears if one compares the effects of Pa_{CO_2} on CBF per unit $CMRO_2$ ($CBF/CMRO_2$). $CBF/CMRO_2$ rose .68±.093ml/ml O_2 consumption/mmHg in lambs, compared to 0.54±.09ml/ml O_2 consumption/mmHg in the fetus. Data from the literature in newborns and adults of other species with varying $CMRO_2$ show a wide range in $\Delta CBF/\Delta Pa_{CO_2}$; however, recalculation of these data shows the responses of $CBF/CMRO_2$ to Pa_{CO_2} are very similar to that in the fetal and newborn lamb. It is probable that differences in $\Delta CBF/\Delta Pa_{CO_2}$ reflect differences in $CMRO_2$ among fetuses, newborns, and adults.
(Supported by HD 13830)

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CESSATION OF SHIVERING DURING SHOWER REWARMING OF HYPOTHERMIC SUBJECTS. Brooks, C.* Kuehn, L. and Livingstone, S. Defence & Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada M3M 3B9

A pilot experiment was conducted in which the efficacy of a hot shower was examined in the rewarming of six acutely hypothermic young male Caucasian volunteers. Of particular interest was the point at which massive shivering of the subject ceased. The hypothermia was induced in each lightly-clothed subject by immersion to the neck in a deep tank of water at 5°C in a room of 20°C air temperature. The subjects experienced a mean rectal temperature decrease of 1.63 ± 0.23 °C (S.D.), a mean body temperature decrease of 5.30 ± 1.08 °C with a concomitant metabolic rate of 1.99 ± 0.38 $\frac{kcal}{m^2 min}$ and total heat loss of 80.5 ± 14.6 kcals over a duration of 30.3 ± 7.7 min. Despite massive shivering of the subjects during and immediately after the exposure, during an immediate application of warm shower water (at 43°C) to the back of the head the shivering ceased abruptly and transiently in two of the subjects, a diminution of shivering occurred in two other subjects while two subjects continued to shiver. All remained in the shower for periods over an hour in which their rectal temperature at first fell in significant afterdrops of 1.05 ± 0.31 °C before returning to thermalneutral values during which no shivering occurred; subjective thermal comfort was experienced throughout the rewarming. This experiment demonstrated potential dangers to hypothermic subjects during shower rewarming since the cessation of shivering is usually taken as an index of the return of the hypothermic subject to near-thermoneutral conditions.

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DIAZEPAM AND BODY TEMPERATURE CHANGES IN MAN DURING COLD EXPOSURE. Sheilagh M. Martin, Biology Dept. Mt. St. Vincent University, Halifax, Nova Scotia B3M 2J6

The use of drugs and alcohol by those exposed to cold or cold/wet environments has raised the question of whether the thermoregulatory response to cold may be decreased by the use of those agents. There is a particular concern over the easy access to and widespread use of the benzodiazepines, particularly diazepam. This study was undertaken to investigate the effects of an acute dose of diazepam on the thermoregulatory ability of man during a short-term cold exposure. Eleven subjects, 8 women and 3 men, were randomly assigned to the two experiments. They were exposed for 1 hr. in a cold room (12.23 ± 0.16°C) after 0.5 hr. at ambient temperature, and in a second experiment were exposed to cold (11.93 ± 0.19°C) 1 hr. after ingesting 10mg of diazepam. The following measurements were made: heart rate, skin temperature (6 sites), rectal temperature and metabolic rate. Blood samples (10ml) were taken before drug ingestion, 1 hr. after the drug ingestion and following the cold exposure. The plasma levels of diazepam were ascertained by gas chromatographic procedures involving electron-capture detection. The plasma level of diazepam was 323.00 ± 40.8 ng/ml before the cold exposure. The results show that there was a consistently significant difference ($p<0.05$) in the rectal temperature at the times measured in the two experiments. None of the other measurements showed a significant difference. It would appear that a high plasma concentration of diazepam (higher than 180 ng/ml required for sedation) does impair the normal thermoregulatory process when exposed to cold. The precise mechanism for this response remains to be elucidated. (Supported by the NSERC).

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THE PHYSIOLOGICAL RESPONSE TO SUDDEN COLD WATER IMMERSION AFTER VIGOROUS ACTIVITY. McDonald, A.* Goode, R.C., Livingstone, S., Duffin, J.* Bowen, C.* and Kuehn, L. Departments of Physiology and Physical Education, University of Toronto, and the Defence & Civil Institute of Environmental Medicine, Downsview, Ontario M3M 3B9

Subjects were immersed in the Craig whole body water calorimeter (head exposed; Craig and Dvorak, 1976) at 35°C and 19°C for 45-60 minutes after a period of rest or 30 minutes of exercise (treadmill running at 80% maximum heart rate). The rates of decline of rectal temperature were 0.039 and 0.019 °C/min for cold (exercised and resting), and 0.012 and 0.0072 °C/min for warm (exercised and resting) subjects respectively. VO_2 was similar in exercised and resting subjects; it increased slightly in warm and 3 fold in cold over pre-immersion values. Heat loss was 8.19 and 8.28 kcal/min for cold (exercised and resting) and 1.98 and 0.99 kcal/min for warm (exercised and resting) subjects respectively. Ventilation volume did not change during warm exposures but increased markedly during cold exposures, reaching up to 60 l/min after 55 minutes of immersion. Hand grip strength decreased linearly in cold immersion for exercised and resting subjects at approximately 0.5%/min. It appears that vigorous pre-immersion exercise may shorten survival time in cold water due to an increased cooling rate.

Craig, A. and Dvorak, M. (1976). Heat Exchange Between Man and the Water Environment. Proceedings of the Fifth Symposium on Underwater Physiology. Edited by C. Lombertsen, FASEB, Bethesda, Maryland, pp 765-773.

835

THE EFFECT OF ALCOHOL ON MAN'S ABILITY TO ADAPT TO MILD EXERCISE IN +5 TO -15°C ENVIRONMENT. T. Graham, Human Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Alcohol (Alc) ingestion has been associated with hypoglycemia and hypothermia. Studies concerning cold stress have often neglected the former and failed to demonstrate the latter. This project examined the effect of Alc on Man's response to a mild-moderate cold stress. Male volunteers (3 groups of 6) were tested once with 2.5 ml of 40% Alc/kg, 1:2 in water and lemon and once with a placebo (the same volume of water and lemon). The subject wore a sweat suit and, after ingestion, performed intermittent, bicycle work (40% $\dot{V}O_2max$) with a 20 min work - 10 min rest pattern for 3 hr in air at +5, -5, or -15°C. The peak blood Alc (11.87 ± 0.82 $mM \cdot l^{-1}$) occurred at 87.4 ± 7.5 min; there was no temp. effect. Based on pulmonary $\dot{V}_{O_2}$ RQ and blood glucose data, there was no temp. or Alc effect on metabolism. Mean skin temp (T_{SK}) was colder ($p \leq 0.05$) at -15°C throughout the 3 hr. From 45-105 min T_{SK} was progressively colder ($p \leq 0.05$) in +5, -5 and -15° temp. Alc resulted in colder T_{SK} from 45-135 min. During the third hour core temp (T_C) was colder ($p \leq 0.05$) at -15°C. With Alc, T_C was colder ($p \leq 0.05$) from 25-105 min. Mean body temp was colder ($p \leq 0.05$) at -15°C from 45-180 min. It was also colder ($p \leq 0.05$) with Alc for the first 135 min There were no significant Alc-temp interactions, thus Alc had a greater effect at the lower temp. Alc had no apparent metabolic effect but resulted in greater decreases in body temperatures during the time when blood Alc levels were highest. (Supported in part, by the Distilled Spirits Council of United States, Inc.)

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MODULATION OF MAXIMUM THERMOGENESIS BY FEEDING. Lawrence C.H. Wang. Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

The present study investigated the availability of substrates as a limiting factor in acute cold exposure. Using a self-control design, maximum thermogenesis was measured in male Sprague-Dawley rats at -10°C under HeO_2 (21% oxygen, balance helium) following a single modification of overnight feeding regime to alter endogenous substrate profile prior to cold exposure. Overnight fasting resulted in the lowest total heat production (9949 ± 189 cal/120 min, $\bar{X}$ ± S.E., n=11), maximum rate of thermogenesis (1320 ± 23 cal/15 min) and final body temperature (31.0 ± 0.3°C). These values were significantly lower than those found after overnight rationing (10630 ± 231; 1386 ± 29; 32.8 ± 0.5, respectively) or ad libitum feeding (10821 ± 224; 1395 ± 27; 33.5 ± 0.3). Rats fasted overnight but fed intragastrically a 5 ml substrate mixture (3 kcal/ml) 60 min prior to cold exposure resulted in the highest values for all parameters (11553 ± 207; 1488 ± 23; 33.7 ± 0.4). The increases were not due to the specific dynamic action of food (dietary-induced thermogenesis). Since the level of maximum thermogenesis and cold tolerance can be modulated by feeding, it is evident that substrate availability limits thermogenesis in severe cold independent of respiratory-cardiovascular functions and cellular oxidative capabilities. (Supported by Canada NSERC Grant A6455)

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BEHAVIORAL THERMOREGULATION AND SLEEP DISTRIBUTION DURING ENTRY INTO HIBERNATION IN THE MARMOT. V. M. Miller* and F. E. South. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19711

Entry into hibernation in the marmot (M. flaviventris) consists of repeated bouts of slow wave sleep (SWS) and activity. The decreased thermoregulatory sensitivity of SWS and the periods of complex activity act to regulate the rate at which body temperature declines. Ten entries at a $T_{amb.}$=5°C were observed in 7 marmots. Brain temperature (T_B) was monitored continuously along with cortical EEG, EOG, and EKG. Behavior was observed closely by remote video. EEG records were scored using conventional criteria for states of arousal over the range of T_B=35-25°C. The mean total recording time (TRT) was 316±32 min (SEM). The awake state occupied 27±4% of TRT. Behavioral patterns of nest building and postural adjustments were observed in all animals at T_B=32-30°C and 27-25°C. These activity bouts of 1-20 min duration constituted 1-13% of TRT. SWS occupied greater percentages of total sleep time as T_B decreased: 82±2% at T_B=35-30°C and 93±2% at T_B=29-25°C ($p<0.05$). This increase in SWS represented a concomitant decrease in the number of SWS bouts with an increase in length of each bout. The distributions of SWS and activity in the marmot during entry into hibernation demonstrate that entry is a complex process rather than merely an extension of SWS. (Supported by UDRF and DIMER)

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STUDIES ON THE CONTROL OF BLOOD PRESSURE IN THE HIBERNATING MARMOT. William J. Ray,* Marvin L. Zatzman and Patrick D. Harris. Dept. of Physiology, University of Missouri Med. Center and Dalton Research Center. Columbia, Mo. 65212

Studies were conducted with Marmota Flaviventris of both sexes containing: chronic arterial catheters, impedance electrodes, renal arterial flow probes and iliac arterial flow probes. Renal involvement in the control of arterial blood pressure was examined by means of two experimental forcings: (1) Furosemide (F) infusion of 0.01 mg/min following a prime of 0.5 mg/kg, (2) a non pressor norepinephrine (NE) bolus of 2.7 X 10^{-5} mg/kg. The following continuous measurements were obtained: heart rate, electrocardiogram, aortic blood pressure, cardiac output (normothermic animals only), renal blood flow (RBF), and iliac blood flow (IBF). Plasma for plasma renin activity (PRA) and, in some animals plasma glucose and electrolytes was sampled at intervals for post forcing analysis. RBF and IBF flow patterns indicate changes in peripheral vascular response to both NE and F between the hibernating and normothermic animal. PRA response to forcing appears to be similar in both hibernating and normothermic animals. (Supported by USPH. NIH Grant # HL-17847)

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CORONARY CIRCULATION IN HIBERNATING, COLD-ACCLIMATED, AND NORMOTHERMIC HAMSTERS. James T. White*, Larry W. Krieger*, Mark A. Young* and Gary F. Merrill. Department of Physiology, Rutgers University, New Brunswick, NJ 08903.

Coronary vascular responses to global ischemia, adenosine (250µg) and alterations in perfusion pressure were compared in isolated, spontaneously rhythmic hearts (n=22) of hibernating (H), cold-acclimated (CA) (nonhibernating), and normothermic (N) Syrian golden hamsters (125±3.8 gm) perfused at 38 and 9°C. No differences in heart rates or in the vascular responses to adenosine amongst groups were seen at either 38 or 9°C. At 38°C the magnitudes of reactive vasodilation upon release of inflow occlusion (30 sec.) were similar in all three groups. At 9°C the response was abolished in H and CA hearts and noticeably reduced in N hearts although a significant ($p < 0.05$) response was still observed in the latter group. At 38°C all three groups displayed classical flow auto-regulation when pressure was elevated from 80 to 120 mmHg and subsequently lowered to 60mmHg. The greatest transient increase in flow at the elevated pressure occurred in H hearts. At 9°C steady state flow rates at 80 mmHg were equal to those at 38°C in all groups. Changes in perfusion pressure were accompanied by steady state flow rates not significantly different from transient responses at corresponding pressures. We conclude that the responses to adenosine were largely temperature dependent, while differences in autoregulatory and reactive hypermic responses were largely temperature independent. Supported in part by grant #18130, NJAES.

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INDUCTION OF SUMMER HIBERNATION AND SUPPRESSION OF FEEDING IN THE WOODCHUCK BY INFUSION OF HIBERNATING WOODCHUCK BLOOD TRIGGER. W. A. Spurrier and P. R. Oeltgen*. Loyola University of Chicago, Maywood, IL. 60153 and VA Medical Center, Univ. of Kentucky, Lexington, KY. 40506.

During the summer months, when woodchucks (Marmota monax) do not hibernate, 12 were infused intravenously with hibernating woodchuck blood, designated Hibernation Induction Trigger (HIT), and caged in a 5°C dark room. Summer hibernation occurred in 2 of 3 infused with 4 ml of HIT plasma; 3 of 5 infused with 7 ml of HIT plasma dialysate, 4 of 4 infused with 30 mg of HIT albumin separated by gel chromatography. None of the 7 controls infused with equal amounts of non-hibernating woodchuck plasma, or dialysate, hibernated. After receiving HIT, daily food intake decreased 45% to 80% over several weeks prior to hibernation. Only 0 to 10% suppression of feeding occurred after receiving non-hibernating plasma. Other collaborators [1]using the identical HIT samples found that the intravenous and cerebroventricular infusion of the HIT albumin fraction into primates suppressed long term food intake 32%-68%. Thus plasma from hibernating woodchucks contains a constituent(s) having a metabolic effect, which induces hibernation and may be an anorectic substance.

[1]R. B. Meeker, et al. *Physiologist 22, 4: 86, 1979.*

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SENSITIVITY TO STIMULATION OF REWARDING HYPOTHALAMIC SITES DURING HIBERNATION IN THE 13-LINED GROUND SQUIRREL. *William P. Clarke* and William C. Hartner. Northeastern University, Boston, MA 02115*

The sensitivity of the brain of hibernating 13-lined ground squirrels to electrical stimulation was examined. Ten adult male and female squirrels were each implanted with a bipolar stimulating electrode in the area of the medial forebrain bundle in the lateral hypothalamus and a stainless steel thermocouple reentry tube in the anterior midbrain. Stimuli consisted of 0.3 second trains of monophasic, square waves at 200 Hz and 0.2 msec duration applied for one minute periods. In euthermic ground squirrels, this stimulation was rewarding as indicated by the occurance of self-stimulation behavior. Stimulation during hibernation at ambient temperatures between 8° and 14° C elicited cardioaccelleration, a rise in brain temperature (T_{br}) and, infrequently, electromyographic activity. The threshold stimulus intensity for the production of cardioaccelleration was variable and not correlated with T_{br} or the time spent in hibernation. This variable sensitivity to CNS stimulation may be a reflection of spontaneous fluctuations in the hibernating squirrel's level of arousal during hibernation.

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BEHAVIORAL AND METABOLIC ASPECTS OF LOW TEMPERATURE TORPOR IN THE BROWN BULLHEAD, ICTALURUS NEBULOSUS. Larry I. Crawshaw, Daniel E. Lemons* and Marsha Parmer*. Depts. of Rehabilitation Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Oxygen uptake was obtained from brown bullheads acclimated for three weeks or longer at 8 temperatures (3-17°C at 2°C intervals). At the lower temperatures, oxygen uptake ($ml\ O_2 \cdot hr^{-1} \cdot kg^{-.77}$) was stable and very low ($\bar{x}$±1S.E.M.): At 3°, 1.27±0.27; at 5°, 2.09±0.74 and at 7°, 3.82±0.71. At higher temperatures, oxygen uptake became higher and more variable (at 9°C, 7.20±1.30). Spontaneous activity was measured at the same 8 temperatures in an annular activity tank. Activity was lowest at 3°C, but did not show major changes from 5-13°C. Activity was increased at 15° and 17°C. To evalauate possible changes in central thermoregulatory mechanisms, bullheads acclimatized to 8-10°C in early winter were placed in a thermal gradient (8-22°C). The fish selected temperatures near 22°C. After several hr. the gradient was slowly cooled over a period of 6 hr. and the temperature range lowered to 6-12°C. At this time the bullheads appeared to become torpid and always did so in the warm end of the gradient. At very low temperatures, brown bullheads become relatively unreactive and exhibit very low rates of oxygen uptake. During slow cooling there is no major readjustment in thermoregulatory behavior. (Supported in part by USPHS NIH Grant 1 R01 NS15318).

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WINTER ADAPTATIONS IN CASTRATED BLACK BEARS. D. L. Wellick,* R. A. Nelson, J. M. McMillin,* P. J. Palumbo,* P. Zollman.* University of Illinois and Carle Foundation Hospital, Urbana, IL 61801, University of S.D., Sioux Falls, S.D. 57105, Mayo Clinic, Rochester, MN 55901.

In two male black bears, acute castration in hibernation disrupted the state. Bears were restudied during the subsequent 2 winters, employing previously established techniques, to determine the long term effects of castration on hibernation. It was found that in both years, as in years prior to castration, bears became hyperphagic in fall and food intake declined to very low levels by Dec. When bears were put into their dens, mean value for blood urea/creatine(U/C) was 7.5 compared to 18.9 in active bears. During the 1st winter, bears were studied from mid Feb. to early Mar. Urination, which was not seen in winters prior to castration, was evident. Hematocrit,%(Hct), and blood urea,mg/dl(U) increased to 62% and 54. U/C and blood free fatty acids,mEq/L(FFA) averaged 18.5 and 594. Feb. values for U,U/C, and FFA in these bears prior to castration were 18, 4.6, and 1441. Post hibernation anorexia, seen in previous years, was not evident. In the 2nd winter, one bear studied on Feb. 28 showed evidence of defecation, FFA were low(374) and U slightly elevated (27). U/C and Hct, however, with mean of 8.2 and 47% for both bears, were comparable to values in noncastrated hibernating bears. It was concluded that although winter adaptation is not as efficient in castrated black bears, they are still able to survive 3 months without food or water and further that return to an apparently normal hibernating state is possible in time.

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MAXIMUM UPSTROKE VELOCITY IN CARDIAC PURKINJE FIBERS AS A FUNCTION OF MEMBRANE SURFACE POTENTIAL. P.M. Hogan and S.R. Besch*. Physiology Dept., State Univ.of NY, Buffalo, NY 14214.

In cardiac Purkinje fibers an increase in extracellular calcium concentration, $[Ca^{2+}]_e$, reduces the maximum upstroke velocity ($\dot{V}_{max}$) of action potentials initiated in fully polarized cells. In partially depolarized cells, however, elevated $[Ca^{2+}]_e$ increases $\dot{V}_{max}$. We have examined this phenomenon using computer simulations of both cardiac and nerve action potentials to determine if cancellation of negative surface charge alone can account for these effects. When adjusted for reduced external surface charge both models yield changes in $\dot{V}_{max}$ similar to those produced by elevated $[Ca^{2+}]_e$. Further analysis revealed that the decrease in $\dot{V}_{max}$ seen in fully polarized cells is due to a reduction in the electrochemical driving force for excitatory sodium current (I_{Na}) resulting from a reduction in $\bar{V}$, the membrane potential at the time of $\dot{V}_{max}$. The decrease in $\bar{V}$ is due to surface charge effects on the sodium activation parameter, m, exclusive of any change in the inactivation parameter h. In partially depolarized cells this effect is masked by the predominant action of reduced surface charge to restore h_∞ to its polarized level, thus permitting greater I_{Na} and $\dot{V}_{max}$ upon activation. These findings indicate that reduced external surface charge alone, without a change in maximum sodium conductance ($\bar{g}_{Na}$), can account for the diametrical effects of divalent cations on $\dot{V}_{max}$. (Supported by USPHS Grants HL-16135-CVA and PO1-HL-15194.)

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SODIUM DEPENDENCE OF CALCIUM TRANSPORT IN ISOLATED CARDIAC CELLS. Michel Desilets* and Magda Horackova. Dalhousie Univ. Halifax, Nova Scotia, Canada B3H 4H7

Myocytes from adult rat hearts were enzymatically isolated by a method similar to that of Powell and Twist, 1976(Biochem. Biophys. Res. Commun. 72, 327). Our procedure yielded 70% of rod shaped cells, 90% of which responded for several hours to field stimulation in calcium containing media. When the membrane potentials were recorded with intracellular microelectrodes, the amplitude and configuration of the action potentials were similar to those of the intact tissue. ^{45}Ca uptake by these dispersed cardiac cells suspensions was examined, using Millipore filtration technique. The ^{45}Ca uptake was increased by lowering $[Na]_o$; similar response was observed with LiCl or Tris-Cl replacing NaCl. This stimulating effect of low $[Na]_o$ on the ^{45}Ca uptake was also observed in the presence of 5mM caffeine. The Na_o-dependent ^{45}Ca uptake varied with $[Na]_i$; its level of increase at a reduced $[Na]_o$ was altered according to pre-incubation time in the low $[Na]_o$ preceding the addition of calcium. When the membrane potential was decreased by application of 50mM KCl, the Na-dependent ^{45}Ca uptake was increased, suggesting voltage-dependence of this transport. In summary, techniques to prepare viable dispersed adult cardiac cells and to measure ^{45}Ca uptake by these cells were developed. The data indicate a presence of Na-Ca exchange in these isolated myocytes and suggest voltage-dependence of this transport mechanism. (Supported by grants from MRC and Nova Scotia Heart Foundation).

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EFFECT OF ALPHA-ADRENERGIC RECEPTOR STIMULATION ON VENTRICULAR ELECTRICAL PROPERTIES IN THE NORMAL CANINE HEART. Peter R. Kowey*, Richard L. Verrier, and Bernard Lown*. Harvard Schools of Public Health and Medicine, Boston, MA 02115

While it has long been appreciated that the sympathetic nervous system contributes to the genesis of cardiac arrhythmias, the influence of alpha-adrenergic receptors on ventricular electrical properties has not been adequately explored. We therefore examined the effects of alpha-adrenergic stimulation on ventricular excitability, refractoriness and vulnerability to fibrillation. Methoxamine (M) or phenylephrine (P) was infused in ten dogs before and after aortic arch and carotid sinus baro-receptor denervation (BD), in doses which increased mean arterial blood pressure by 20-30 mmHg. M or P caused an increase in ventricular fibrillation threshold (VFT) and repetitive extrasystole threshold (RET), an effect which was abolished by BD.

	VFT (% change)		RET (% change)		
	Intact	Denervated	Intact	Denervated	
Methox	+27*	+11	+39*	-5	(*p<0.008)
Phenyl	+41*	+ 3	+28*	+8	

Neither drug had an effect on mid-diastolic threshold or on effective refractory period either before or after BD. We conclude that alpha-receptor activation exerts an indirect effect on ventricular vulnerability which is mediated by an effect on the baroreceptor. However, there appears to be no direct effect of alpha stimulation on ventricular excitability or refractoriness in the normal, intact heart.

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THE DEPENDENCE OF RIGHT ATRIAL SUBSIDIARY PACEMAKER ACTIVITY ON NOREPINEPHRINE. G. J. Rozanski, S. L. Lipsius and W. C. Randall. Department of Physiology, Loyola University Medical Center, Maywood, IL 60153.

Automaticity was studied in the isolated canine right atrium perfused through the sino-atrial node (SAN) artery. Extracellular electrodes were used to locate the site of earliest activation and record spontaneous rate (SR). The control SR of the SAN in the absence of NE (84.3±3.4 min^{-1}) increased by 25% (105.4±5.3 min^{-1}) when 10^{-8}M NE was perfused continuously. Ligation of the SAN artery was used to suppress the SAN and induce subsidiary pacemaker activity (SPA). The SR of the SPA (77.9±12.17 min^{-1}) was less ($p<.0005$) than the SAN with the same level of NE. In those preparations which developed SPA, 80% required a background level of NE in the perfusate (10^{-9}-$5x10^{-8}$M). The perfusion of propranolol ($1-3x10^{-7}$M) or NE-free Tyrode solution totally eliminated SPA. In the presence of NE, SPA was also associated with spontaneously repetitive periods of activity characteristic of triggered activity. In tissues isolated from regions which had demonstrated SPA, action potentials were recorded which showed afterdepolarizations when NE was present in the superfusate. These afterdepolarizations reached threshold and developed into triggered activity with fast pacing. We conclude that SPA is dependent upon a threshold level of NE. In the absence of SAN activity and in the presence of NE, SPA may lead to the development of dysrhythmias in the form of triggered activity. (Supported by NIH Grant HL 08682.)

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EFFECTS OF ACETYLCHOLINE ON SINUS NODE AUTOMATICITY: DEPENDENCE OF K^+ PERMEABILITY. Michael J. Mirro*, Kevin Kelly* Jeaneen Anderson*, and Dianna Johns* (SPON: M.D. Thames). Dept of Med & CV Ctr, VA Hosp & Univ of IA, Iowa City, IA 52242.

Muscarinic receptor activation depresses the slow inward current (SIC) and increases outward K^+ currents to produce slowing of sinus node automaticity (SNA). The purpose of this study was to analyze the effects of acetylcholine (ACh) on SNA under conditions of augmented SIC and restricted K^+ permeability. Conventional microelectrode techniques were utilized to determine the effects of ACh on pacemaker potentials in the presence of ↑SIC with Strontium (Sr^{2+}) and ↓K^+ permeability with 9-Aminoacridine (9-AA). Cumulative ACh concentration response curves were performed. Values = $\bar{x} \pm SE$ percent reduction in rate from control. *p < .05

	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	N
ACh alone	1±1%	9±4%	30±6%*	69±17%*	9
ACh+Sr^{2+}	0±1	4±2%	19±6 *	90±31%*	6
ACh+9-AA	0±2	1±2	8±3	9±10%	5

The effects of ACh on SNA were markedly reduced in the presence of 9-AA but not Sr^{2+}. In addition to ↓SNA, ACh ↑ maximum diastolic potential both alone and in the presence of Sr^{2+}. This effect, a reflection of ↑K^+ permeability, was not observed in tissues treated with 9-AA. We conclude that the effects of ACh on SNA are mediated primarily by alterations in K^+ permeability (supported by VA and Iowa Affiliate AHA).

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THE EFFECTS OF STROPHANTHIDIN ON DIASTOLIC DEPOLARIZATION. Mario Vassalle and Shinichi Ishikawa*. Dept. of Physiology, SUNY, Downstate Medical Center, Brooklyn, NY 11203

We studied the action of strophanthidin on the electrical and mechanical events in cardiac Purkinje fibers perfused in vitro. The fibers were driven at 60/min but the drive was interrupted for 15 sec every minute to determine how spontaneous activity was affected by strophanthidin. It was found that spontaneous discharge due to a steepening of diastolic depolarization increased rapidly during the therapeutic stage (the force progressively increased). At a later stage (the force was at its peak or was already declining), an oscillatory potential appeared which grew in size with time and resulted eventually in abrupt initiation of a fast rhythm. When the same procedures were repeated in the presence of cesium, the spontaneous activity was inhibited in the presence and in the absence of strophanthidin but the fast rhythm was not inhibited. In the presence of tetrodotoxin, spontaneous activity was inhibited although strophanthidin steepened diastolic depolarization and could induce activity. Fast rhythms (but not force development) were prevented altogether through a prevention of the oscillatory potentials. When both tetrodotoxin and cesium were present, there was an inhibition of both spontaneous activity and of fast rhythms. It is concluded that strophanthidin enhances normal diastolic depolarization during its therapeutic stage and causes fast rhythms during its toxic stage through an oscillatory potential. Supported by NIH Grant #HL-17451.

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INOTROPIC EFFECTS OF CARDIAC GLYCOSIDES ON CARDIAC MUSCLE AT DIFFERENT Na, Ca CONCENTRATIONS. Tung Li* and Mario Vassalle. Dept. Physiol., SUNY, Downstate Med. Ctr., Brooklyn, NY 11203

We studied the effect of different Na and Ca concentrations on the inotropic effect of strophanthidin in canine ventricular muscle and Purkinje fibers perfused in vitro. In Purkinje fibers, lowering $[Na]_o$ and $[Ca]_o$ at an approximately constant ratio resulted in a transient increase in force followed by a force development similar to that in Tyrode solution. The duration of the action potential decreased during the transient increase in force and lengthened again during the steady state. On returning to Tyrode solution, the force decreased transiently and then overshot the control value temporarily. In ventricular muscle fibers, similar events were noted, but at a Na/Ca ratio larger than in Purkinje fibers. When the same procedures were applied in the presence of a low concentration of strophanthidin in Purkinje fibers, force increased more initially and decreased more subsequently than in Tyrode solution. At a high concentration of strophanthidin, the force decreased initially and increased subsequently. More pronounced reactions than in Tyrode solutions were obtained also by decreasing Ca alone. In fact, in a low Na solution, the same concentration of strophanthidin increased force much more at low $[Ca]_o$ and decreased it more at high $[Ca]_o$. It is concluded that an enhancement of calcium available at the myofilaments by cardiac glycosides modifies force differently in the therapeutic and toxic stages. Supported by a New York Heart Fellowship and NIH Grant #HL-17451.

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RUTHENIUM RED: ITS EFFECT ON THE ACTION POTENTIAL AND CONTRACTION OF CARDIAC MUSCLE. K.L. Rossner, S.R. Lombardi* and H.G. Sachs*. Univ. of Hartford, W.Hartford, CT. 06117 and Univ. of Illinois at the Medical Center, Chicago, IL. 60612

Ruthenium red (R.R.) dramatically alters the configuration of the cardiac action potential monitored intracellularly from mammalian ventricular myocardium (rat and hamster) and from purkinje fibers (pig). After one hour of application the dye (50 uM) extends the duration of the action potential plateau to values between 5 and 30 seconds. Slow stimulus rates of 0.01 HZ or slower are necessary to elicit the response. Resting potentials appear unaltered by the dye, although action potential rise rates are slightly depressed. Calcium antagonists D600 (10 uM) and Mn++ (1 mM) shorten the duration of the R.R. plateau, while hypercalcemic (5 mM) and hypocalcemic (1.25 mM) conditions respectively shorten and prolong the plateau duration compared to records obtained in 2.5 mM calcium. The contraction of papillary muscle in the presence of R.R. is reduced in amplitude from control and is quickly followed by a weaker contracture which lasts the duration of the action potential plateau.

By occupying negatively charged sites on the extracellular surface of the sarcolemma, R.R. may achieve its effect by enhancing membrane conductance to calcium and/or by reducing conductance to potassium. Calcium isotope and voltage clamp experiments are in progress to investigate this phenomenon in more detail. (Supported in part by the Vincent Coffin Fund, Univ. of Hartford, and USPHS. NIH Grant HL # 18032)

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MEROCYANINE-OXAZOLONE AS A VOLTAGE-SENSITIVE OPTICAL PROBE OF PACEMAKER ACTIVITY IN SINOATRIAL TISSUE. Bruce C. Hill* and Kenneth R. Courtney* (SPON: N.B. Ingels, Jr.). Palo Alto Medical Research Foundation, Palo Alto, CA 94301

Merocyanine-oxazolone dye (NK2367) was used to optically monitor spontaneously-generated action potentials (AP) in rabbit sinoatrial (SA) tissue. SA tissue was stretched out and pinned to a Sylgard surface and bathed with oxygenated Tyrode's solution at 35°C. Incident light at 730 nm was reflected from the tissue and projected onto an image plane, where a moveable photodiode measured reflected intensity from any 0.8 mm diameter area of the tissue. Contraction from unstained tissue at normal Ca^{++} levels (1.8 mM) produced a fractional change in reflected light ($\Delta R/R$) of 1-1.5%. Reducing Ca^{++} to 25% of normal decreased this contraction signal to 0.1-0.2%. Staining the tissue by bath application of 20 mM dye for 15 min. produced signals of 0.4-0.8% having faster rise times than those associated with the contraction signals, a result consistent with the known voltage sensitivity of this dye in neural and cardiac tissue. Illumination levels sufficient to yield signal-to-noise ratios of 6:1 (for a single AP) allowed observation times of 30 min. before photobleaching necessitated restaining. These results suggest the suitability of this dye for studying propagation pathways in SA tissue with a multidetector array which monitors the AP latencies in different areas of the tissue simultaneously, thereby detecting pacemaker shifts and ectopic pacemaker phenomena. (Supported by NIH Biomedical Research Support funds.)

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Transmembrane Potentials of Paranodal Fibers Adjacent to Septal Cusp of Tricuspid Valve. Warren W. Tse, Dept. of Physiology and Biophysics, Univ. of Health Sciences/The Chicago Medical School, Chicago, Illinois 60612.

Paranodal fibers (PNFs) are groups of deep specialized cardiac muscles that cascade over the anatomical atrioventricular (AV) node (Am.H.J. 66:498, 1963). The present study of 6 experiments was to determine the transmembrane potentials (TMP) of the portion of PNFs that ran adjacent and parallel to the base of the septal cusp of the tricuspid valve of canine hearts. This deep tissue was dissected out by carefully removing the overlying atrial fibers. The exposed PNFs formed a preparation with a size about 2.0 x 4.0 mm. Histological study with Masson trichrome stain showed the PNFs had a diameter of about 6 u and were oriented in a parallel pattern. Also, these fibers were physically connected to the AV node whose fibers displayed an interwoven pattern. PNFs were excited by electrically stimulating the tissue directly or by propagating AV nodal impulses elicited by stimulating the His bundle at 70-90 beats/min. The TMP characteristics of PNFs are: the resting membrane potential was about -60 mv; the action potential (AP) has a slow rate of rise of phase 0, a slow phase 2 and a faster phase 3. The AP duration was about 200 msec. Acetylcholine (Ach) at 0.5 ug/ml suppressed the rate of rise of phase 0 and also the amplitude of the action potential. The findings suggest that these PNFs could play an important role in the AV conduction delay or block in dog hearts. Supported by NIH grant HL21574.

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HISTAMINE PROMOTES OSCILLATORY POTENTIALS AND TRIGGERED ACTIVITY IN PURKINJE FIBERS OF GUINEA PIGS. J.H. Leal-Cardoso* and J.F. Delahayes. Dept. of Physiology, Medical College of Georgia, Augusta, Georgia 30912

2 to 3 mm long bundles of purkinje tissue of Guinea Pig were superfused with Krebs solution containing 5.4 mEq of K^+ at 37°C. Action potentials (AP) were recorded from spontaneously beating preparations and from electrically driven, otherwise quiescent preparations. In spontaneously active fibers, histamine (H) (2.2×10^{-6} to 4.4×10^{-5}M) caused a progressive increase in the frequency of AP's eventually followed by a sudden change to a higher frequency sometimes associated with a shift in pace-maker site. Both increase in frequency and occurrence of sudden change were dose dependent. Quiescent fibers were stimulated with trains of 40 stimuli at various frequencies. In these preparations, H frequently induced after-depolarizations (AD) or triggered spontaneous AP's. Both AD and triggered activity were frequency and dose dependent. H did not alter the maximal rate of depolarization of AP's nor did it appreciably modify the maximal diastolic potential. AP duration was either increased, decreased or unchanged at 80% repolarization. Refractory period varied similarly to AP duration. These results suggest that triggered activity due to oscillatory potentials (late AD) occurring in well polarized Purkinje fibers is the cause of histamine induced idio-ventricular rhythms observed in whole Guinea-Pig hearts by others (Levi and Zavecs, Circ. Res., 44: 847, 1979) (Supported by Georgia Heart).

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RENAL BLOOD FLOW AUTOREGULATION: A PLAUSIBLE MYOGENIC MODEL. John C.S. Fray. University of Massachusetts Medical School, Worcester, MA 01605.

A model is developed to extend the myogenic hypothesis for the control of renal blood flow. The model predicts that by specifying the hemodynamic and other factors which alter calcium movement across smooth muscle cell membranes the mechanisms controlling renal blood flow may be accounted for. The specific and most fundamental assumption in the model is that stretch of the afferent arteriole increases the calcium permeability of the cells in the arteriole. A formula is derived which predicts that raising renal perfusion pressure increases the calcium permeability and raising intrarenal tissue pressure decreases it. The formula predicts that autoregulation of renal blood flow occurs above a certain pressure (usually 100 mm Hg). This autoregulation may be abolished by increasing intrarenal tissue pressure and by decreasing the calcium permeability. These predictions agree well with experimental evidence. The model predicts the renal blood flow patterns under a wide variety of circumstances with blood flow autoregulation being only a specific case. The myogenic model provides a framework in which to examine renal blood flow during hypertension and acute renal failure, for it suggests that hemodynamic or chemical factors which increase the influx calcium permeability of the arteriolar smooth muscle cells will induce contraction and lower renal blood flow.

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EFFECT OF RENAL NERVE STIMULATION ON RENAL BLOOD FLOW AUTOREGULATION. J.L. Osborn, L.L. Francisco* and C.F. DiBona. Dept. Int. Med., VAMC & Univ. of Iowa, Iowa City, IA 52242.

Holdaas recently reported that renal nerve stimulation (RNS) at 4 Hz decreased basal renal blood flow (RBF) and impaired autoregulation of RBF during reduction of renal perfusion pressure (RPP) below 95 mmHg (Upsala J Med Sci 26, 1979). In the present experiments, autoregulation of RBF was determined in dogs before and during RNS (10 V, 1 ms) at 0.5, 1.0, 2.0 and 4.0 Hz. Basal RPP averaged 137 ± 4 mmHg. RPP was reduced by aortic constriction in 15 mmHg decrements to 55 mmHg during control and RNS. RNS (0.5-4.0 Hz) decreased $U_{Na}V$ at all frequencies. RBF (ml/min) following reduction of RPP at each frequency of RNS are shown (mean ± SEM, C = control, n = 13):

	RPP (mmHg)					
	137	115	100	85	70	55
C	203±19	205±21	204±21	197±23	183±24	153±26
0.5 Hz	177±14	184±15	186±16	185±18	175±21	158±27
1.0 Hz	164±14	175±15	175±16	168±16	157±19	137±22
2.0 Hz	150±17	157±18	154±19	147±21	128±22	129±27
4.0 Hz	160±20	159±22	153±23	149±22	135±25	141±34

RNS reduced basal RBF but did not alter autoregulation of RBF during reduction of RPP to 85 mmHg. It is concluded that although RNS increases vascular resistance and decreases $U_{Na}V$, RBF autoregulation is not impaired.

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GLOMERULAR ADRENERGIC RECEPTORS. R. Felder*, J. Pelayo*, A. Wargo*, L. Schoelkopf*, M. Cooke*, P. Jose, and G. Eisner. Depts. Peds., Med., and Physiol. and Biophys. Georgetown Univ. Sch. Med., Washington, D.C. 20007

We have previously characterized beta and alpha adrenergic receptors(AR) in partially purified renal tubular plasma membranes(RTPM). In the adult dog RT PM, beta but not alpha AR were consistently demonstrated while in rat RTPM,both beta and alpha AR were consistently found. To determine whether there is a parallel presence of AR in vascular and tubular portions of the nephron, we characterized AR in glomeruli (G) and tubules (T) separately in adult rats and dogs. The ligands used were WB-4101 for alpha AR and dihydroalprenolol for beta AR. The results with WB-4101 binding are tabulated:

	Dog (n=4)		Rat (n=4)	
	G	T	G	T
Kd (nM)	2.35	*	2.27	1.04
Ro (fmol/mg protein)	170	*	135	222

*3 of 4 had no binding

In contrast to alpha AR, beta AR were noted in G and T in both rats and dogs. These studies demonstrate for the first time AR in G and support the thesis that neurotransmitters could directly influence glomerular dynamics.

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EFFECTS OF BARBITUATE AND OTHER ANESTHETICS ON CARDIAC INDEX (CI) AND RENAL BLOOD FLOW (RBF) IN MICROPUNCTURE PREPARED RATS. W.A. Cupples* and A.T. Veress. Dept. of Physiology, University of Toronto.

Microspheres (15 + 3 μm) were used to assess CI (ml/min-kg body wt.) and RBF (ml/min-g kidney wt.) in chronically cannulated rats which were studied before and after induction of anesthesia with different agents (Table) and following preparation for micropuncture.

Table

Inactin (I) 100 mg/kg
Nembutal (N) 50 mg/kg + supplements
α-Chloralose (αC) 100 mg/kg + supplements
Ketamine (K) 75 mg/kg (+ Atropine 0.04 mg/kg) & K 100 mg/kg-h + Atropine 0.02 mg/kg-h.

In conscious rats CI was 395 ± 46 ($\bar{x}$ ± SEM), left RBF was 5.0 ± 0.6 and right RBF was 5.1 ± 0.4. N induced rises in left RBF to 10.5 ± 2.1 ($p < 0.01$) and in right RBF to 9.6 ± 0.8 ($p < 0.05$); all other anesthetic effects were not significant (ns). With N, CI fell after surgery by 28% ($p < 0.05$); and with I by 49% ($p < 0.01$); smaller, ns decreases were seen with K (15%) and αC (19%). RBF appeared to be protected with I, but not N, as fractional flow increased from 6.6 ± 0.2% of cardiac output to 10.4 ± 1.0% (right) and from 6.4 ± 0.8% to 9.5 ± 2.5% (left) though these changes did not reach significance ($p < 0.1$).

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TUBULOGLOMERULAR FEEDBACK RESPONSES DURING RETROGRADE DISTAL TUBULAR MICROPERFUSION WITH LOW CHLORIDE ISOTONIC AND HYPOTONIC SOLUTIONS. P.D. Bell, C.B. McLean*, and L.G. Navar. Univ. of Alabama Medical Center, Birmingham, Alabama 35294.

In recent studies we found that stop flow pressure (SFP) feedback responses can be dissociated from changes in distal tubular fluid chloride concentration (Cl) during orthograde perfusion with low chloride solutions. However, previous retrograde studies have suggested that the magnitude of feedback responses depends upon perfusate Cl. The present studies were conducted to determine if Na isethionate (Naise) solutions having constant low Cl but varying osmolality were able to elicit SFP feedback responses during retrograde perfusion. For the retrograde perfusion procedure, SFP was measured after blockade of the intermediate proximal and late distal tubule with wax. Perfusion was from an early distal tubule site at 15 nl/min using isotonic and hypotonic (120 mOsm/kg) solutions of Naise and artificial tubular fluid (ATF) and a hypotonic (70 mOsm/kg) electrolyte matrix solution (Cl = 8 mEq/l). With the isotonic solutions, SFP decreased by 12 ± 1.2 mmHg with ATF (n=7) and 12 ± 1.2 mmHg using Naise (n=11). With the 120 mOsm/kg solutions SFP decreased by 13 ± 1.3 mmHg with ATF (n=9) and 11 ± 1.6 mmHg with Naise (n=9). SFP decreased by 5 ± 0.8 mmHg during perfusion with matrix solution (n=10). These results demonstrate that feedback responses elicited by retrograde perfusion do not require Cl, but may be more closely associated with changes in osmolality. (Supported by grants from NHLBI).

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EFFECTS OF ANGIOTENSIN II AND ANGIOTENSIN III ON RENAL BLOOD FLOW AND ITS CORTICAL DISTRIBUTION IN DOGS. M.J. Fiksen-Olsen*, S.L. Britton, P.C. Houck and J.C. Romero, Department of Physiology, Mayo Clinic, Rochester, Minnesota 55901.

The effects of angiotensin II and angiotensin III on total renal blood flow and its cortical distribution were compared in 10 pentobarbital anesthetized dogs. These agonists were administered as constant infusions directly into the left renal artery. Infusion rate of each peptide was adjusted to decrease total renal blood flow approximately 25% as measured with a non-cannulating electromagnetic flow probe. The distribution of renal cortical blood flow was evaluated with radioactive microspheres after 10 minutes of peptide administration. In each experiment more moles of angiotensin III than angiotensin II were required to produce a given decrease in renal blood flow. Angiotensin II was estimated to be 70-80% more potent than angiotensin III as a renal vasoconstrictor. Unilateral renal administration of these peptides produced no significant changes in arterial pressure or blood flow to the contralateral kidney. Angiotensin II and angiotensin III decreased blood flow to all renal cortical zones. The average percent decrease in blood flow to each of the four renal cortical zones produced by angiotensin II was not different from that produced by angiotensin III. Both peptides, however, caused a greater percent decrease in inner cortical blood flow relative to their effects on blood flow to the outer cortex.

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EFFECTS OF CONVERTING ENZYME INHIBITOR (CEI, SQ 14,225) ON RENAL HEMODYNAMICS AND GLOMERULAR FILTRATION RATE (GFR) AT REDUCED RENAL ARTERIAL PRESSURE. D. Jirakulsomchok*, P.D. Bell, W.C. Huang*, and L.G. Navar. Univ. of Alabama Medical Center, Birmingham, Alabama 35294.

It has been shown that inhibition of the renin-angiotensin system (RAS) causes an increase in renal blood flow (RBF), but varied GFR responses have been observed. Some studies reported that during blockade of RAS reduction in renal arterial pressure (RAP) resulted in a decreased GFR while RBF autoregulatory capability was maintained. To evaluate this problem further, the effects of CEI were determined in normal and Na depleted (1-2 wks) dogs. In the control group (n=5), both RBF and GFR showed good autoregulation when RAP was reduced to 87 ± 1 mmHg. At this RAP, CEI caused a significant increase in RBF (13%) and GFR (11%). Upon release of the renal artery clamp with continued CEI infusion, RAP increased to 119 ± 7 mmHg but RBF and GFR were not altered from values at reduced RAP with CEI. In the Na depleted group (n=8), RBF and GFR remained unchanged at reduced RAP (86 ± 1 mmHg) and when CEI was given there were significant increases in RBF (47%) and GFR (16%). With release of the clamp, RAP increased to 105 ± 6 mmHg, RBF and GFR were not significantly altered. The results of this study fail to support the suggestion that GFR and RBF autoregulation may be dissociated with CEI in Na depleted dogs. Furthermore, the increase in GFR associated with CEI induced vasodilation suggests that vascular resistances were decreased at both pre- and post-glomerular sites.

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EFFECTS OF INDOMETHACIN AND PROSTAGLANDIN (PGE_2) IN EPINEPHRINE-INDUCED ACUTE RENAL FAILURE IN DOG. Anil K. Mandal, Francisco Llach, Jon Miller and John Nordquist (Spon: B.J. Scherlag). VA Med. Ctr. and Univ. of Okla., Okla. City, OK.

It has been reported that indomethacin (I) aggravates glycerol-induced acute renal failure (ARF) in the rabbit while PGE_2 affords partial recovery of renal function in norepinephrine-induced ARF in the dog. This study reports the effects of I and PGE_2 in epinephrine (Epi) induced ARF. Group I (12 dogs) served as control, Group II (7 dogs) received I, 100 mg daily for 4 days prior to Epi and Group III (5 dogs) was treated with I similar to Group II plus PGE_2 (10 ug/min) 1 hr. prior and during 6 hrs. of Epi. All groups received Epi infusion (4 μg/kg/min for 6 hrs). Hourly urine volume (UV), glomerular filtration rate (GFR), effective renal plasma flow (ERPF), urinary sodium excretion (UNaV) were measured. Kidneys were fixed for light and electron microscopy. There was no difference in any parameters between Group I and II. UV and UNaV at 6 hr. were significantly higher ($P < 0.001$)in Group III (.23 ± .04 ml/min; 10 ± 1 μEq/min) than in Group I (0.03 ± .01 ml/min; 1 ± .6 μEq/min) or Group II (.02 ± .01 ml/min; .6 ± .4 μEq/min). However, GFR and ERPF were not significantly different among the groups. Acute tubular lesions were present in all groups but less severe in Group II than others. Thus, this data indicates that in Epi induced ARF, indomethacin has little effect and PGE_2 induces diuresis and natriuresis but does not offer significant renal protection.

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EFFECT OF SECRETIN ON EFFECTIVE FILTRATION PRESSURE AND FILTRATION COEFFICIENT IN DOGS. G. R. Marchand, Dept. of Physiology, Sch. of Med., Univ. of Minn.-Duluth, Duluth, MN 55812.

In the dog, secretin increases renal blood flow (RBF) and single nephron glomerular filtration rate (SNGFR). To study the effect of secretin on SNGFR, the effective filtration pressure (EFP) and filtration coefficient (K_f) were determined. Recently, it has been shown in dogs that intratubule pressure is significantly reduced during collection of tubule fluid. Therefore, to calculate EFP, proximal intratubule pressure measured during collection of tubule fluid or during free flow was subtracted from stop flow pressure. Five anesthetized dogs were prepared for recollection micropuncture. Secretin infusion (100 mU/kg · min, ia) significantly increased RBF (Δ = 80 ± 39 ml/min) and whole kidney GFR (Δ = 7 ± 4 ml/min). SNGFR increased from 52 ± 6 to 65 ± 5 nl/min ($p < .05$). Although stop flow pressure was significantly increased (43 ± 1 to 49 ± 2 mmHg), an equivalent increase in intratubule pressure during collection (12 ± 2 to 18 ± 1 mmHg) or free flow (24 ± 1 to 31 ± 2 mmHg) was observed. Therefore, EFP did not change significantly, whereas calculated K_f increased from 2.74 to 3.61 nl/min · mmHg ($p < .05$). It is concluded that an increase in the filtration coefficient accounts for the effect of secretin on SNGFR. (Supported by the Minnesota Heart Association and HL 25390.)

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EFFECT OF ADENOSINE ON RENAL BLOOD FLOW AND RENIN RELEASE IN NON-FILTERING KIDNEYS. Aviad Haramati, Steven L. Britton and William S. Spielman, Department of Physiology, Mayo Clinic, Rochester, MN 55901

Intrarenal adenosine (Ado) infusion has been previously shown to increase renal blood flow (RBF) to the inner cortex and decrease renin secretion in dogs. In order to determine if an intact tubular system (i.e., Ado filtration or NaCl delivery to the macula densa) is central to the Ado-induced changes in renal hemodynamics and renin release, studies were conducted in 9 Na-depleted dogs in which one kidney was rendered non-filtering (NF) (with ureteral ligation and 2 hr. renal ischemia) four days prior to the experiment. RBF was markedly reduced in NF kidneys (63.2±5.4 ml/min) compared to the contralateral filtering kidneys (184.9±22.0 ml/min). A continuous intrarenal Ado infusion (3.3×10^{-7} mol/min) into NF kidneys caused a 27% increase in RBF (to 79.0±6.3) ($p < .01$). This increase was selective to the inner cortex since outer cortical blood flow was unchanged while inner cortical blood flow rose by 96%. Finally, renin release decreased from 309±53 to 70±26 ng angiotensin I/min ($p < .01$) without changes in arterial blood pressure or plasma Na and K. These results indicate that the Ado-mediated changes in renal hemodynamics and renin release are independent of an intact tubular system (Supported by HL14133).

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OBSTRUCTION OF SINGLE TUBULES IN THE RAT KIDNEY. George A. Tanner. Dept. Physiology, Indiana Univ. Sch. Med., Indianapolis, IN 46223

The objectives of this study were (1) to determine the nature of the signal which leads to a decrease in glomerular capillary pressure (GCP) when kidney tubules are chronically blocked, and (2) to determine the effect of chronic tubule blockade on single nephron glomerular filtration rate (SNGFR). Experiments were done on anesthetized rats using micropuncture techniques. Single tubules were blocked with solid paraffin. GCP was estimated from the stop-flow pressure. When tubules were blocked for 5-6 hr in 9 rats, GCP was decreased ($P < .01$) to 43.9 ± 1.4 (SEM) mm Hg, compared to 47.8 ± 1.3 mm Hg in nearby normal nephrons. When a hole was made in tubules upstream to the paraffin block, so as to prevent a rise in proximal tubular pressure (PTP), GCP was decreased below normal ($P < .05$) to 44.8 ± 0.9 mm Hg. In 4 rats after 24 hr of blockade, GCP in nephrons with proximal tubule blockade averaged 39.4 ± 1.1 mm Hg; with distal tubule blockade GCP averaged 40.2 ± 1.6 mm Hg. Both of these values are below ($P < .01$) GCP in normal nephrons of the same kidneys, 50.4 ± 1.9 mm Hg. Blockade of tubules for 24 hr in 5 rats reduced SNGFR from 20.4 ± 1.0 to 12.0 ± 0.8 nl/min-100 g body weight ($P < .001$). In conclusion, chronic tubule obstruction leads to a fall in GCP and SNGFR. The signal initiating this fall is not an increase in pressure upstream to or downstream from a proximal tubule block, but probably involves a decrease in fluid delivery to the distal tubule.

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PROTEINURIA AND RENAL DAMAGE IN THE SALT-SENSITIVE HYPERTENSIVE RAT. Judith B. Van Liew and Jan R. Brentjens*. SUNY at Buffalo and VA Medical Center, Buffalo, NY 14215.

We have followed the pattern of proteinuria in the salt-sensitive (S) hypertensive rat (Dahl strain) from 5-28 weeks of age. The genetically matched resistant strain (R) served as a control. Urinary protein excretion was measured by the Lowry method and fractional protein composition by polyacrylamide gradient gel electrophoresis. Systolic blood pressure (indirect tail cuff method) and creatinine clearance were measured periodically. Functional data were correlated with morphological changes at weeks 25-28. Two weeks after the administration (at 9 weeks) of a high salt diet (8% NaCl) the S rats were hypertensive (> 150 mmHg). Levels greater than 200 mmHg were achieved by week 16 and maintained. Protein excretion and histological changes in the kidney parallel the hypertension. At week 28, protein excretion was 74±15 SD mg/24 hr x 100g BW in the S group and 12±5 SD mg/24 hr x 100g BW in the normotensive (133±16 SD mmHg) R controls. This proteinuria was primarily due to an increase in the excretion of albumin. However, even in the prehypertensive low salt diet (0.3%) phase, albumin excretion is significantly ($p < 0.001$) elevated in S rats when compared to R rats (5 weeks: S = 0.90±0.45 SD mg/24 hr x 100g BW; R = 0.15±0.06 SD mg/24 hr x 100g BW). Hence an abnormality in renal albumin handling is characteristic of the S strain and is evident before the onset of hypertension and glomerular lesions. (Supported by VA Research funds and AHA grant #76-1003).

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MOST UPRIGHT SUBJECTS BREATHE AT REST WITH ABDOMINAL MUSCLE TONE. S.H. Loring and J. Mead. Harvard School of Public Health, Boston, MA, 02115.

Normal upright volunteers breathing quietly have been observed to breathe near their passive relaxation characteristic (prc). Previous papers have argued that when breathing deviates from the prc, "useless" work is being done by respiratory muscles and concluded that natural breathing is energetically efficient. However, these previous studies were done on trained respiratory subjects, and we thought it unlikely that all naive upright subjects have relaxed abdominal muscles. To examine quiet breathing in uninformed subjects, we measured abdominal and rib-cage dimensions by magnetometers in 17 healthy men and women standing at rest. Change in posture was minimized by requiring the subject to stand with the crown of the head touching a horizontal bar. Quiet breathing was observed before obtaining a prc. During quiet breathing, 14 of the 17 subjects breathed with abdominal muscle tone. Some subjects showed "hooked" characteristics suggestive of phasic end-expiratory abdominal activity. Two of the three subjects who breathed near to their prc had protuberant abdominal walls. We conclude that these subjects breathed at rest with relaxed abdominal muscles. One subject may have been unable to relax his abdomen voluntarily. We conclude that while a few standing subjects may breathe naturally with a flaccid abdomen, most uninformed subjects breathe with abdominal muscle activity. (Supported by NHLBI grants HL 22920 and 14580).

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REGIONAL VENTILATION IN SUPINE ANESTHETIZED DOGS. R.D. Hubmayr,* B.J. Walters,* J.R. Rodarte and P.A. Chevalier. Mayo Fdn., Rochester, MN 55901.

Regional lung volume (VR) during deflations from TLC to FRC was determined from the position of radiopaque parenchymal markers by a computer based video roentgenographic technique. The relationship between VR and lung volume (VL) was linear (R^2=.98) defining a ventilation index, Vi=ΔVR%/ΔVL%. In all 3 dogs there was a significant vertical gradient in Vi in upper (UL) and lower (LL) lobes. Middle lobe data were insufficient for analysis. There was substantial residual variability in Vi which did not appear to be due to experimental error since Vi data were reproducible on replicate maneuvers. A linear model adding terms related to location of lung regions in the cephalocaudal and transverse directions and their interactions reduced the residual variability in each lobe by 46 to 91%. The effect of these variables on Vi was significantly different between UL and LL. Nonvertical gradients and position interactions though reproducible within a dog, were variable between dogs. We conclude that the distribution of regional lung volume although in general vertically oriented is complex and likely reflects interactions between each lobe and surrounding structures. It is uncertain whether the variability between dogs represents real differences in respiratory system mechanics or results from variability of regions sampled or from subtle differences in position and support of the animals. (Supported in part by NIH Grants HL 21584 and HL 07222.)

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THE QUANTIFICATION OF RESPIRATORY SENSATIONS IN NORMAL SUBJECTS. David G. Stubbing*, Kieran J. Killian*, and E.J. Moran Campbell. Cardiorespiratory Dept. McMaster University. Hamilton, Ontario L8N 3Z5

The purpose of this study was to determine the sensitivity with which sensations evoked by the act of breathing are perceived, and to explore the possibility that receptors in respiratory muscles are important in generating respiratory sensations. Open magnitude scaling was used in normal subjects to define the exponent (n) of Stevens' psychophysical power law for tidal volume (V_t), inspiratory flow ($\dot{V}$), ventilation ($\dot{V}_E$), and frequency (f). The mean value of n was for V_t 1.14 ± .08 (SEM), for $\dot{V}$ 1.13 ± .15, for $\dot{V}_E$ 1.28 ± .11 and for f 1.04 ± .15. These results show that respiratory variables are judged with an expanded sensory scale. The similarity of n for V_t and $\dot{V}$ leaves open the possibility that both are sensed by the same neurophysiological mechanism. Further experiments on V_t showed that passive ventilation decreased and chest vibration increased the perceived magnitude of tidal volume suggesting that the sensation is mediated at least in part by afferent information generated by receptors in respiratory muscle. (Supported by Canadian Lung Association and Medical Research Council of Canada)

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LUNG TISSUE DISPLACEMENT FOLLOWING A CHANGE IN GRAVITATIONAL LOAD. David B. Michels and Simon C. Body.* Department of Medicine, Univ. of California, San Diego, La Jolla, CA 92093

Radiographic observations were made of lung tissue movement after a change in gravitational load. Six subjects were placed head-down on a tilt table and then rapidly rotated (1.5 sec) to the head-up position. Five PA chest X-rays were then made serially beginning 2 s after the subject had come upright (3.5 s after start of rotation) and continuing for the next 6 s. The subject breath-held at RV beginning 2s before the start of rotation. In each subject we found at least 10 distinct markings in the lung tissue which could be discerned in all 5 serial X-rays. The markings descended downward within the thorax relative to both the diaphragm and the ribs in all subjects. The maximum shifts observed exceeded 1 cm, and shifts of 4-8 mm were very frequent. We estimated the overall displacements between the head-down and head-up postures to be about 4 times the observed shifts, assuming the shifts followed exponential time courses. Time constants for the shifts varied from 0.6 to 6 s, with a mean of 2 s. Observed tissue shifts were much smaller in 2 subjects who repeated the procedure while breath-holding at RV + 1L, presumably because the tissue moves more rapidly above RV. The shape of the ribcage and diaphragm did not change between the making of the first and the last X-rays. These findings provide direct evidence that gravity causes substantial deformation of lung tissue and that it can do this without altering the shape of the chest wall. (Supported by NASA Grant J NSG 9075.)

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ADAPTATION OF THE RESPIRATORY MUSCLES TO POSTURAL CHANGE DUR - ING CO_2 REBREATHING. T.D.O'Connor,*M.Lopata* and E.Önal* (Spon: F.Al-Bazzaz). University of Illinois, Abraham Lincoln Sch. of Medicine and VA West Side Medical Center, Chicago, Il. 60680.

Changing from the sitting to supine posture, the human diaphragm gains a mechanical advantage. During CO_2 rebreathing we have evaluated electrical and mechanical properties of the diaphragm for evidence of this postural advantage and its relation to other respiratory muscles. Gastric(Pga),pleural(Ppl) and transdiaphragmatic(Pdi) pressures and thoraco-abdominal motion were monitored. Diaphragmatic EMG was measured by a bipolar esophageal electrode and quantitated as a moving time average(EMGdi). From sitting to supine, 2 of 7 subjects(Group A) showed an increased slope of the Pdi vs EMGdi relationship. The other 5 subjects(Group B) did not. At high levels of ventilation while sitting, Group B showed evidence of increased expiratory abdominal muscle activity leading to a more favorable diaphragm length and a passive descent of the abdomen-diaphragm on inspiration. In Group A while sitting, this pattern was absent. In the supine position FRC progressively increased in all subjects and the above abdominal pattern was not seen. Rib cage-Pga plots indicated no postural difference in intercostal muscle activity at any given CO_2 level. We conclude that at high levels of ventilation, the mechanical advantage afforded the supine diaphragm may be offset while sitting by the effect of the expiratory abdominal muscles placing the diaphragm in a more advantageous configuration; and by gravity the expiratory activity has true inspiratory function.

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THE MECHANICAL STRUCTURE OF THE ALVEOLAR DUCT. T. A. Wilson and H. Bachofen*. Dept. of Medicine, U. of Bern, Bern

Direct observation of transmission and scanning electron micrographs of perfusion-fixed, air and saline-filled lungs and indirect inference from the observed relation between surface area and lung recoil are consistent with the following model for the mechanical structure of the alveolar duct. There are two networks of force-bearing line elements. The first network forms the rim of the alveolar openings. It is the terminal part of the axial fiber system which surrounds bronchioli, bronchi and arteries. This network is extended by the outwardforce of surface tension and is insensitive to change in lung volume except at very high lung inflation. The length tension curve of its line elements is obtained from the surface area-recoil pressure data. The second network is an interdependent part of the peripheral connective tissue system which starts from the pleura and extends into the interlobar and interlobular fissures. At the sublobular level, however, its geometry is not yet fully clear. It is extended by changes in lung volume, not surface tension. The two dimensional alveolar wall elements are negligible mechanical components except as platforms for surface tension at the air-liquid interface. (Supported in part by NIH Grant HL-21584)

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PRESSURE AND FLOW RELATIONSHIP IN THE HUMAN BRONCHI. Marc J. Jaeger, Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610

The pressure vs flow relationship in the bronchial tree was studied in six healthy nonsmokers by measuring alveolar pressure with a body plethysmograph and intratracheal pressure with a catheter introduced through the nose and larynx after local anesthesia and pre-medication of ¼mg of atropin s.c. The catheter had lateral holes and its tip was occluded. The subjects were breathing at a frequency varying from 20 to 60 bpm with a tidal volume between .8 and .2L four gas mixtures containing either O_2 or 80% N_e, H_e, or SF_6 in O_2 (Method: Resp. Physiol. 6:113, 1968). The FRC was kept constant by having the subject monitor his/her spirogram on an oscilloscope. Bronchial resistance decreased by 24% on the average during inspiration and increased during expiration, peaking at about mid-expiration. This increase over early inspiratory baseline was inversely proportional to gas kinematic viscosity and reached 170% with SF_6. It is a phenomenon akin dynamic collapse. The ratio of bronchial resistance to total airway resistance increased linearly with kinematic viscosity and decreased with flow rate; this is presumably due to the fact that the bronchial pressure vs flow diagram is more linear than that of the upper airway.

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CALCULATION OF REGIONAL AIRWAYS RESISTANCE USING THE HORSFIELD MODEL OF THE HUMAN LUNG. T.R. Gerrity*, C.S. Garrard*, and D.B. Yeates, Section of Environmental Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60612.

To understand regional variations of ventilation and aerosol deposition in the lung it is desirable to know the resistance to airflow of individual lobes. Measurements of airway resistance in man include contributions from the terminal airways to the oropharynx. Regional variations of resistance have been measured in animals and human lung casts. We have developed an algorithm to compute peripheral resistance of the lung at any airway level from the trachea to the respiratory bronchioles assuming Poiseuille flow in the Horsfield asymmetric lung model. Total lung resistance is calculated to be 0.2 cm H_2O s/L. The right upper lobe resistance equals 0.76, right middle equals 1.49, right lower equals 0.57, left upper equals 0.76, and left lower equals 0.55. Although predicted total lung resistance is lower than measured values, the calculation does not include contributions from the oropharynx and turbulent flow. The calculated values of resistance enable the determination of flow division in the asymmetric lung. Assuming uniform pleural pressure and a 0.5 L/s inspiratory flow, the flow in the right upper lobe is 0.10 L/s, the right middle lobe 0.04 L/s, the right lower lobe 0.12 L/s, the left upper lobe 0.10 L/s and the left lower lobe 0.14 L/s. Using this model, aerosol deposition can be predicted to be greatest in the left lower lobe and lowest in the right middle lobe.

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TRACHEAL GEOMETRY AND COMPLIANCE DURING BREATHING. V. Hoffstein*, G.M. Glass, M.E. Wohl, H.L. Dorkin*, D.J. Strieder and J. J. Fredberg*. Children's Hospital Medical Center and Harvard Medical School, Boston, MA 02115, and Cambridge Collaborative, Inc., Cambridge, MA 02142.

To measure changes in tracheal geometry and tracheal compliance during breathing, we studied 7 subjects aged 27 to 47 y, whose residual volume was known. Cross-section area of the intrathoracic trachea was inferred with a high-frequency acoustic reflexion technique (JAP 48:749, 1980). Measurements were made at 0.2-0.4 s intervals during slow vital capacity maneuvers with respiratory flow averaging 1.2 $\pm$0.2 L/s. Lung volume was monitored with a spirometer and transpulmonary pressure (TPP) estimated from esophageal pressure. Tracheal area increased linearly with lung volume up to 90-100% of total lung capacity (TLC) and averaged 7.7 $\pm$ 0.5 cm^2 at TLC, with a mean TPP of 31 cm H_2O. As TPP decreased from 20 to 0 cm H_2O, tracheal area decreased by 19%, in agreement with the data of Prakash et al (JAP 45:45, 1978) for autopsy specimens. Specific compliance of the trachea averaged 1.5×10^{-2} /cm H_2O, a value similar to that of Martin and Proctor (JAP 13:337, 1958) for dog bronchi. We conclude that through a volume range comprising most of the vital capacity, tracheal cross-section area increases with lung volume but that specific compliance of the trachea is nearly constant and markedly lower than the specific compliance of the lung.

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DIMENSIONAL ANALYSIS CORRELATES EXPIRATORY FLOW OF SEVERAL GASES WITH STATIC PRESSURE DROP ACROSS A CAST OF THE HUMAN BRONCHIAL TREE. D.B. Reynolds. Mayo Clinic, Rochester, MN 55901.

Flow ($\dot{V}$) and static pressure drop (ΔP) across a latex reproduction of the human bronchial tree were measured during steady expiratory flow. The reproduction consists of the right intermediate bronchus through airways averaging about 2 mm in diameter. Flow of air, helium, argon, neon and sulfur hexafluoride was varied to obtain airway opening Reynolds number (Re) in the range 100 to 50,000. Converting pressure and flow into dimensionless parameters correlated the five gases on a single curve, described by a dimensionless equation $\Delta P/\Delta Pp = 76 + 0.0617\ Re$ ($r = 0.99$) where ΔPp is a Poiseuille pressure drop over a length equal to one diameter of the airway opening. The first dimensionless constant indicates a laminar resistance higher than computed Poiseuille resistance. Because kinetic energy per unit volume of fluid moving at the average velocity at the airway opening is $0.0156\ Re\Delta Pp$, the second constant indicates that 25% of the static pressure drop is due to convective acceleration of the flow to that point while 75% results from frictional dissipation. This is a larger frictional loss than we found in a similarly constructed cast of a canine airway (Fed. Proc. 38:1444, 1979) which had nearly equal contribution of convective and frictional pressure losses. (Supported by NIH grants HL-21584 and HL-07222).

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INFLUENCE OF GAS PROPERTIES AND FREQUENCY ON THE SHAPE OF PRESSURE-FLOW CURVES OF AN AIRWAY CAST. D. Isabey* and H.K. Chang, Biomedical Engineering Unit and Department of Physiology, McGill University, Montreal, Canada.

Recent experimental findings have contradicted theoretical predictions of the pressure-flow relationship of the lung. The pressure-flow relations obtained from human subjects breathing different gas mixtures do not form a single curve in a log-log plot of dimensionless pressure-drop vs. tracheal Reynolds number, indicating that the pressure drop is not uniquely determined by Reynolds number. To understand the reasons behind these findings, we studied the pressure-flow relationship of a central airway cast (5 generations) using air and HeO_2, SF_6O_2 mixtures. The data from steady flows in the inspiratory and expiratory directions form two single curves in a Moody plot, resembling the patterns of a straight tube. When taken from different points of the closed pressure-flow curve over the entire breath cycle, data from sinusoidal flows of 0.25, 0.5 and 1.0 liters tidal volume at 0.25, 0.5, 1.0 and 2.0 Hz exhibit similar features as the in vivo data. At frequencies above 0.5 Hz, data for different gas mixtures form distinct curves in the Moody plot; for the same gas mixture, data obtained at different frequencies also form distinct curves. By choosing a new parameter which relates local acceleration to convective acceleration in an oscillating flow, we could explain these pressure-flow relations on the basis of unsteadiness of flow.
(Supported by the Medical Research Council of Canada.)

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CORRECTION FOR THE RESPONSE TIME OF MASS SPECTROMETERS (MS). R. Arieli* and H.D. Van Liew. Physiology Dept., State Univ. of NY at Buffalo, Buffalo, NY 14214.

When we integrated data from a MS-spirometer-computer-system, we sometimes got unacceptable results; the computations showed more indicator gas exhaled than had been inhaled. When we corrected by assuming that the MS response was an exponential rise, the smaller error was still unacceptable. We changed concentration abruptly in 2 different MS: a) the responses were sigmoid shaped, not exponential, and b) time constants derived from the main part of the curves (10-90%) were 43-60 msec; single-exponent corrections using these values caused the corrected wave form to overshoot. For a better correction, we assumed that 2 exponentials are involved:

$$C^* = C + (T_1+T_2)(dc/dt) + T_1T_2(d^2C/dt^2)$$

where C is MS output as a function of time, t; C* is corrected concentration; and T_1 and T_2 are time constants. To be successful, T_1 must be smaller than a one-exponent time constant; we used 29 msec. To obtain T_2, measured data are corrected using the 29 value as a one-exponent correction, then T_2 is calculated from this partially-corrected response; use of 29 and T_2 in the formula provides the satisfactorily corrected wave form. We validated our method by integrations of amounts of gases drawn into and out of syringes and in human breaths. The method offers an advantage in assessment of the delay caused by the MS sampling tube and approximates a square output in response to a square input. (Supported by NHLBI Grant PO1-HL-14414.)

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EFFECTS OF GRAVITY AND POSITIVE PRESSURE BREATHING ON REGIONAL LUNG EXPANSION. T. Behrenbeck,* E.A. Hoffman, P.A. Chevalier, and E.H. Wood. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN

Distances measured at end expiration (FRC) between apical and basal lung parenchymal markers which serve as an index of regional lung volumes (JAP 40:118 '76) are reversibly less at the apex (average 31% ± .8) and unchanged or slightly greater at the base (average 13% ± 5) when head down (HD) as compared to the head up (HU) positions during spontaneous breathing (SB) in intact dogs. Apical intermarker distances (IMD) at FRC decreased during intermittent positive pressure breathing (IPP) when HD and increased when HU. These IPP induced changes in FRC IMD values returned gradually to the SB values following resumption of spontaneous breathing. The relative importance of gravitational and ventilatory induced strains in the lungs and associated bodily structures as causes of these changes in regional lung volumes at FRC was studied in five dogs by abolishing SB with IV succinyl choline and measuring IMD immediately after sucessive reversals between HU and HD with the airway closed and then subsequently opened in each body position. The similar body position dependent directional changes in apical IMD values observed with the airway closed and which increased further when the airway was opened confirm the primary causitive role of gravity in these effects. The mechanisms of the opposite IPP induced changes in FRC IMD values when HU, HD and paralyzed are unexplained. (Supported in part by NIH Grants HL-04664, 21584, and RR-7)

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THE EFFECT OF LUNG VOLUME ON CO_2 ELIMINATION BY HIGH FREQUENCY VENTILATION (HFV). A.S. Slutsky, J.M. Drazen, R. Kamm*, S.H. Loring, J. Lehr*, R.H. Ingram, Jr., and A. Shapiro*. Harvard Medical School, Harvard School of Public Health, and Massachusetts Institute of Technology, Boston and Cambridge, MA 02115.

In a previous paper (SCIENCE, in press) we proposed that an explanation for the effective gas exchange using HFV with tidal volumes (Vt) less than the anatomical dead space could be augmented gas transport due to (i) Taylor dispersion (laminar and turbulent) and (ii) enhanced mixing due to bifurcations via secondary motions or asymmetric velocity profiles (Haselton and Scherer, SCIENCE 208: 69, 1980). Measured CO_2 elimination ($\dot{V}CO_2$) was dependent on root mean square flow irrespective of the combination of frequency and tidal volume as predicted by theory. The model also predicted that, with isotropic lung inflation, $\dot{V}CO_2$ should be independent of lung volume. In order to test this latter prediction, we measured $\dot{V}CO_2$ using HFV (16 Hz, Vt 15-80 ml) in 3 anesthetized and paralyzed dogs at lung volumes varying from functional residual capacity to the lung volume at a mean transrespiratory pressure of 25 cm H_2O. Eucapnic ventilation was achieved with an average Vt that was about 25% of the anatomical plus equipment dead space. Consistent with the augmented gas transport theory, there was no effect of lung volume on the relationship between $\dot{V}CO_2$ and tracheal flow. (Supported in part by MRC (Canada), HL 19170, and HL 22920).

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THE MEASUREMENT OF GAS TRANSPORT BY OSCILLATING FLOW IN A TRACHEA MODEL. J.S. Lee, R.J. Sweeney*, J. Fasano*, W. Mitzner, and S. Permutt, Univ. of Virginia, Charlottesville, Va. 22908 and Johns Hopkins Med. Ctr., Baltimore, Md. 21205

To gain a better insight on the gas exchange in high frequency ventilation, we examined the gas transport in a straight circular tube. This tube is connected to a ventilator generating an oscillating flow with the condition that the bulk flow is zero. Cigarette smoke injected at the end of the tube near the ventilator is used as the tracer of gas transport. A laser-photomultiplier system is employed to detect the light scattering of the smoke particles at a specific location of the tube. By taking the intensity of scattered light as the concentration of smoke, an eddy transport coefficient is identified to describe the dispersion of the smoke bolus caused by the oscillation. A photographic system is also developed to record the light scattering of the smoke as the laser shines axially along the tube. This record provides a visual image of the progressive movement of the smoke bolus along the tube axis. It is found that the high frequepcy oscillation yields a transport coefficient which is 10^4-10^5 times the diffusion coefficient of oxygen and 10^2-10^3 times the eddy transport coefficient associated with steady state turbulence of similar Reynolds number. In summary, the oscillating flow in the simple geometry of a straight circular tube exhibits an effective turbulent mixing which may contribute significantly to the gas transport along the airway network in high frequency ventilation. (Supported by Grant HL23769, KO4-HL00004 and HL00347)

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INTERREGIONAL MIXING DURING HIGH FREQUENCY OSCILLATION (HFO). E. R. Schmid*, T. J. Knopp and K. Rehder. Mayo Clinic and Mayo Foundation, Rochester, Mn. 55901

We measured in 5 anesthetized supine dogs {body wt. 20-30 kg} regional {apical nondependent (AND), basal nondependent (BND) and basal dependent (BD)} pulmonary distribution and clearance {in duplicate} of intravenously injected 133-Xe boli {150-170 μCi dissolved in 2-3 ml saline}, once during HFO {piston pump, oscillatory frequency 27.3-31.0 cycles/sec, piston displacement 2.3-3.0 ml/kg, fresh gas (room air) 10 l/m} and again during a 45-sec period of apnea.

During both apnea and HFO, 133-Xe was similarly distributed, namely, preferentially to BD, indicating a vertical gradient in regional perfusion. When 133-Xe was injected during HFO, relative regional 133-Xe concentrations, corrected for chest wall contribution {C_rXe}, reached in BD a peak concentration within 5-8 sec from beginning of injection and thereafter decreased continuously. In contrast, C_rXe increased for 20-38 sec in AND and BND before it began to decrease. After 36-58 sec, C_rXe became similar in all regions and thereafter regional clearance rates were similar. During apnea, increase in C_rXe in AND and BND was less, suggesting that cardiogenic mixing and redistribution of 133-Xe via pulmonary perfusion accounted for a small proportion of the initial increase in AND and BND. We conclude that interregional mixing occurs during HFO; it may affect pulmonary gas exchange. {Supported in part by USPHS, NIH grant HL 21584, and a grant from Ohio Chemical, Madison, Wi.}

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SUBSTRATE UTILIZATION IN THE DEVELOPING RAT LUNG. M. V. Konicki, D. A. Filler, and R. A. Rhoades, Dept. of Physiology, Indiana University School of Medicine, Indianapolis, IN 46223.

Fetal lung maturation is critically dependent upon substrate utilization. The present investigation examined [U-^{14}C]glucose, [U-^{14}C]glycerol, [1-^{14}C]palmitate, and [Me-^{14}C]choline incorporation into 19 and 21 day fetal (term 22 days), and 1 day neonatal rat lung slices. Glucose oxidation to CO_2 showed a linear decrease from the 19 day fetal to the 1 day neonate while palmitate oxidation showed a linear increase. Despite this inverse relationship, glucose served as a major energy fuel. Glucose, glycerol, and palmitate were preferentially incorporated into lung phospholipids (PL), with maximum incorporation occurring at 21 days. Although glycerol incorporation into disaturated phosphatidylcholine (DSPC) increased from 19 to 21 days, the percent contribution into DSPC decreased (22-12%, respectively). DSPC showed a four-fold increase in both content and choline incorporation from 19 to 21 days while palmitate esterification increased to a much lesser degree (less than 1 fold increase. These data show with increased lung maturation: 1) glucose is preferentially incorporated over glycerol into the "backbone" of DSPC, 2) a source of fatty acids, other than an exogenous source, is used in the synthesis of DSPC, and 3) substrate utilization is greatly enhanced with the onset of DSPC production prior to birth. (Supported by NIH grant HD 10670 and HL KO4-317).

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ALPHA- AND BETA-ADRENERGIC BLOCKADE: EFFECT ON LUNG LIPIDS AND STATIC LUNG COMPLIANCE (C_{STAT}) IN STELLATE-STIMULATED CATS. Daniel J. Crittenden* and David L. Beckman, Department of Physiology, East Carolina Univ., Greenville, NC 27834

Previous work has shown that stellate ganglion stimulation (SGS) can reduce C_{stat}. Elucidation of causative mechanisms may be complicated by the possibility that alpha- and beta-adrenergic pathways mediate separate effects of SGS on peripheral lung function. In order to distinguish between alpha and beta effects, phentolamine (PA) or propranolol (PR) were given to anesthetized cats before SGS. Lung lobes removed during continuous SGS were washed (Oyarzun and Clements, 1977) and the lavage fluid analyzed for lipids. Beta-blockade with SGS resulted in less cholesterol (chol) than unstimulated, non-drug treated controls (0.25±.02 (SE) vs. 0.16±.01 mg/g lung; P<0.05). Alpha-blockade with SGS resulted in increased chol (0.33±.08 mg/g lung). Preliminary data indicate that alpha-blockade did not prevent the increase in disaturated phosphatidylcholine (DSPC) or total phospholipids normally produced by SGS. In order to assess possible surface tension effects of altered alveolar lipids, C_{stat} was measured in lungs in situ in cats given PA or PR. Lungs were inflated twice to 100, 93.75, 87.5, 81.25, 75, 50, and 25% TLC, alternating SGS with no SGS. Loss of C_{stat} caused by SGS was blocked by alpha- and beta-blockade. The data are consistent with the view that beta receptors mediate release of DSPC and chol into the subphase, and suggest that alveolar surface tension and lung compliance are under adrenergic influence. (Supported in part by N.C. United Way.)

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EFFECTS OF CORONARY OCCLUSION AND ALLOPURINOL ADMINISTRATION ON ERYTHROCYTE 2:3 DIPHOSPHOGLYCERATE LEVELS IN DOGS. Chandra M. Banerjee and John K. Walsh, Jr*, Southern Illinois University School of Medicine, Carbondale, IL 62901.

We investigated the effects of acute induced coronary occlusion on erythrocyte 2:3 diphosphoglycerate (2:3 DPG) concentration up to a period of six hours following occlusion. Since Allopurinol has an effect on infarcted myocardium, we also investigated the effects of allopurinol administration on 2:3 DPG with or without coronary occlusion. Twenty-two male mongrel dogs were used for this study. Coronary occlusion were induced by a ligature occluding the circumflex branch of left coronary artery for three and one-half minutes. Arterial blood gas tensions, hemoglobin, hematocrit, CPK and end-tidal P_{CO2} were analyzed before and after treatment. Analysis of 2:3 DPG concentration was carried out by Sigma Assay before and after coronary occlusion at intervals up to a period of six hours. The dogs were divided into four groups--first group with open chest acute coronary occlusion. The second group is a sham group identical to the first group but without ever occluding the coronary artery. The third group consisted of pretreatment of Allopurinol before coronary occlusion. And the fourth group were sham occlusion with Allopurinol administration. We observed no significant change in 2:3 DPG molar ratio following occlusion. We observed a significant drop of 2:3 DPG molar ratios in dogs which were treated with Allopurinol and then given myocardial infarction. There was significant rise ofarterial P_{O_2} in the coronary occlusion dogs treated with Allopurinol.

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RELATIONSHIP OF PULMONARY FUNCTION PARAMETERS TO HISTAMINE SENSITIVITY OF SPONTANEOUSLY BREATHING GUINEA PIGS. S. A. Silbaugh, J. L. Mauderly, and C. A. Macken*. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87115.

The relationship of pulmonary function parameters to airway histamine sensitivity was examined in 54, three mo. old Hartley guinea pigs. Animals were anesthetized with halothane and a pleural catheter was inserted to measure transpulmonary pressure. When recovered from anesthesia, animals were placed in a pressure plethysmograph and tidal volume, respiratory frequency, minute volume, peak inspiratory and expiratory flows, transpulmonary pressure, total pulmonary resistance and dynamic compliance (C_{dyn}) were measured. Each animal was then individually exposed to a series of increasing histamine dihydrochloride concentrations (mass median aerodynamic diameter = 0.6 μm). Concentrations were doubled every 2 minutes until C_{dyn} decreased to 50% of its pre-challenge value. Histamine response dose (HRD) was calculated as the sum of concentration x time products required for the C_{dyn} decrease. Correlations of pre-challenge function values and subsequent HRD values were examined. Only minute volume and C_{dyn} were significantly related to HRD. Both were negatively correlated ($p < 0.001$) and together explained approximately 33% of the between animal variability in HRD. These results suggest certain pulmonary function measurements may be predictive of the airway sensitivity of spontaneously breathing guinea pigs. (Research supported by EPA and the U.S. Department of Energy under DOE Contract DE-AC04-76EV01013.)

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RESPIRATION OF UNSEDATED RATS AND HAMSTERS CONFINED IN INHALATION EXPOSURE TUBES. J. L. Mauderly. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87115.

The effects of exposure tube confinement on respiration of rats and hamsters were studied. Ten young adult F-344 rats and Syrian hamsters were trained to sit quietly and breathe through a valve allowing collection of expirate (*Lab. An. Sci.* 29: 323, 1979). Baseline "unconfined" respiratory frequency ($\bar{f}$), tidal volume (V_T), minute volume ($\dot{V}_E$), O_2 uptake and CO_2 output were measured. Tests were then repeated at 15-min intervals for 1 hr with animals confined in cylindrical plastic exposure tubes (ID = 4.1 cm for hamster, 4.5 cm for female rat and 5.1 cm for male rat). Body surface temperatures were obtained from thermistors on the rubber stoppers behind the animals. Hamster $\dot{V}_E$ rose progressively in the tubes from 170 to 200% of baseline, due to an increased f. Rat $\dot{V}_E$ in the tubes was unchanged from baseline, but f was increased and V_T decreased. Assuming deadspace = 1/3 of baseline V_T, alveolar ventilation of hamsters increased from 163 to 209% of baseline, and that of rats was constant at 85 to 95% of baseline during tube confinement. A progressive increase in hamster temperature paralleled the increase in $\dot{V}_E$, but rat temperature fell slightly during confinement. The increased temperature, due to the tighter confinement necessary for hamsters, probably caused the increased $\dot{V}_E$. (Research performed under U.S. Department of Energy Contract No. DE-AC04-76EV01013.)

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EFFECTS OF INTRATRACHEALLY-INSTILLED ELASTASE OR HISTAMINE ON SINGLE-BREATH N_2 WASHOUTS IN THE RAT. S. A. Likens* and J. L. Mauderly. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87115.

The single-breath N2 washout (SBNW) was compared to other lung function tests in detecting experimentally-induced disorders in rat lungs. Pre- and post-instillation tests were performed on groups of 15, 3 mo old male F-344 rats treated with histamine, elastase or saline. Histamine-treated rats were tested at 5 min, and other rats at 4 wks post-instillation. Rats were anesthetized with halothane, intubated with oral tracheal and esophageal catheters and tested by plethysmography. SBNWs were done by measuring N_2 during slow exhalation of a vital capacity of O_2 inhaled from residual volume. The slope of phase III (slope III), closing volume (CV) and closing capacity (CC) were calculated. Other tests included breathing patterns, dynamic compliance (C_{dyn}), pulmonary resistance (R_L), lung volumes, and quasistatic and forced exhalation. Histamine increased slope III but did not change CV or CC. Tidal volume and C_{dyn} were reduced, and respiratory rate and R_L were increased. Elastase increased CV and CC, but slope III was unchanged. Forced expiratory time and functional residual capacity were increased and forced flowrates were reduced. The SBNW thus discriminated between the two disorders and had equal or greater sensitivity than other tests. (Research performed under U.S. Department of Energy Contract Number DE-AC04-76EV01013.)

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PARTICLE SIZE DEPENDENCE OF SULFURIC ACID MIST HEALTH EFFECTS. R. K. Wolff, S. A. Silbaugh*, B. A. Muggenburg, R. L. Carpenter* and A. R. Dahl*, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87115

Studies have been carried out with sulfuric acid aerosols of two distinct sizes both of which fall within the typical size range of urban particulates: 0.3-0.4 μm and 0.8-0.9 μm mass median aerodynamic diameter (MMAD), with geometric standard deviations of 1.2 to 1.4. Acute mortality studies were carried out in guinea pigs involving 8-hour exposures to the two aerosols at a relative humidity of 80%. The concentration required to produce 50% mortality (LC_{50}) was 30 mg/m^3 for the 0.8-0.9 μm aerosol. For the 0.4 μm aerosol, the LC_{50} was above 109 mg/m^3, the highest concentration obtainable at that particle size. Tracheal mucous clearance studies were carried out in Beagle dogs. Depressions in mucous velocities were observed at 1.0 and 0.5 mg/m^3 with the 0.8-0.9 μm MMAD aerosols following 1 hour exposures. However, no changes were observed following similar exposures to 0.3-0.4 μm MMAD aerosols at 1 mg/m^3 and even 5 mg/m^3 levels. Higher aerosol deposition has been observed in the upper airways for the 0.8-0.9 μm aerosols which may lead to an increased response from the irritant receptors in this area. The observations in these studies may help explain the observed adverse health effects during high pollution episodes when conditions usually tend to increase particle sizes. (Research supported in part by the Environmental Protection Agency and the U.S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.)

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SODIUM DEPENDENCY OF SULFATED MUCIN SECRETION IN DOG TRACHEA, IN VITRO. J.P. Zorn*, J.A. Estep*, M.G. Marin. University of Rochester, Rochester, NY 14642

Previously, we developed an in vitro method to determine the secretion rate, pool size, and turnover time of the pool of sulfated mucins of dog trachea. Because the Na gradient present across the submucosal cell membranes may mediate the uptake of precursors of sulfated mucins into tracheal cells, we examined the effect of Na concentration and ouabain on the kinetics of sulfated mucin secretion. In 5 tracheas, we compared the secretion rate (pmol $SO_4/cm^2/hr$), pool size (pmol SO_4/cm^2), and turnover time (hr) in modified Krebs-Henseleit solution containing various Na concentrations:

	Na Concentration (mM) 0	10	30	100
Secretion Rate	179±25*	319±66*	286±64*	457±76
Pool Size	197±75*	527±141*	476±104*	973±122
Turnover Time	1.06±0.32	1.55±0.16	1.67±0.08*	2.18±0.13

Variability expressed as ± SEM, * = $p < 0.05$ vs. 100 mM Na

Secretion rate, pool size, and turnover time correlated significantly with the media Na concentration indicating that Na plays an important role in the process of sulfated mucin secretion. In 8 tracheas, 10^{-4} ouabain added submucosally reduced significantly both secretion rate and pool size, further supporting the hypothesis that the Na gradient has an important effect on mucin secretion. (Supported in part by NIH Grant #HL-21314 and a CF Foundation Grant).

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THE EFFECTS OF ISOPROTERENOL ON THE GOBLET CELLS OF THE RABBIT TRACHEA. Louis A. Gatto and Anita Maysonet*. Department of Biological Sciences. SUNY/Cortland, N. Y. 13045

The respiratory tract of the rabbit is lined with mucous secretions originated in the lungs and in the serous (neutral) and mucous (acidic) goblet cells of the upper airways. The trachea of the albino rabbit (*COBS*® New Zealand White, Charles River) was histochemically studied for morphological changes resulting from the subcutaneous administration of a β-adrenergic drug, Isoproterenol, for 20 consecutive days. Alcian blue at pH 2.6 was used to stain goblet cells with an acidic secretion consisting of sulfated and sialylated mucus. PAS counterstain identified neutral mucus. Only the cells which reach the surface of the epithelium were counted, and their histochemical types were therefore expressed as percentages of all cells reaching the lumen: neutral, acidic, and non-staining cells. Acidic goblet cells increased 60% with drug treatment, while neutral goblet cells decreased 41%, and non-staining decreased 25%. It is believed that the latter group represents ciliated, brush, sensory epithelial, and other surface epithelial cells which do not contain mucus in their cytoplasm. The findings in this study suggest a direct relationship between systemic stimulation with a β-adrenergic drug and an increase in the number of mucous goblet cells with a concomitant decrease in the serous and the non-staining cells of this epithelium. This relationship is indicative of adrenergic influence over the mucus-secretory functions of the rabbit trachea.

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RESPONSIVENESS AND SENSITIVITY (pD_2) OF ISOLATED TRACHEAE (T), BRONCHI (B) AND PARENCHYMAL STRIPS (P) TO HISTAMINE (H), ISOPROTERENOL (I) AND CIMETIDINE (C) IN YOUNG (Y), MIDDLE AGED (MA) AND OLD (O) FEMALE AND MALE GUINEA PIGS. C. Brink*, P. Ridgway* and J.S. Douglas* (Spon: Arthur B. DuBois). J.B. Pierce Fdn., New Haven, CT. 06519.

Maximal force (g) increased during aging in all tissues while force/dry weight (gmg^{-1}) and tension (gmm^{-2}) decreased in T and B but not in P. Induced force in preparations (T,B,P) from MA male and female animals was the same whereas tension to H in female T vs male T were significantly different ($1.06 gmm^{-2}$ ± 0.06 vs $0.59 gmm^{-2}$ ± 0.10 respectively). pD_2 of T to H decreased during development (Y,5.55 ± 0.07;MA,5.19 ± 0.04;O,4.91 ± 0.07); this change was not seen in B or P. During aging pD_2 to I of T was unchanged but decreased in B (Y,8.31 ± 0.09;MA, 8.12 ± 0.04;O,7.94 ± 0.06). Female T preparations were significantly more sensitive to H than male T (MA group:5.19 ± 0.04; 4.92 ± 0.04, respectively). Barium was 10x more potent (pD_2) in MA male T than in MA female T. pD_2 to H for B preparations from Y females were not altered by C(5×10^{-5}M), B preparations from O females were significantly increased (pD_2 to H before:5.22 ± 0.22 after:6.06 ± 0.04). C(2×10^{-7} and 2×10^{-6}) reduced responsiveness to H in female T but had no effect on responses in male T. pD_2 to H was significantly increased by C(2×10^{-6}M) in male T but not altered in female T.

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EFFECTS OF PARAQUAT ON ANGIOTENSIN CONVERTING ENZYME IN MICE LUNGS. Sang Joo Kim*, John F. Roberts*, and Joo Ok Koo* (SPON: M. J. Galvin, Jr.). North Carolina State University, Raleigh, N.C. 27650, and NIEHS, Research Triangle Park, N.C. 27709

Thirty-one adult ICR Mus musculus, weighing 30-34 g, 6-8 weeks old, were treated intraperitoneally with paraquat (PQ: 1,1'-dimethyl-4,4'-bipyridylium dichloride), 30 mg/kg body weight. Activities of lung angiotensin converting enzyme(ACE) were measured in the PQ-treated group and in 13 vehicle-treated controls after 1,2,3,4, and 5 days. The ACE in the PQ-group increased linearly($r=0.92$, $P<0.01$), and the levels were significantly elevated over the controls(Mean±SE;37.5 ± 5.4 vs 5.9±0.6, $P<0.01$ at day 1, and 60.9±7.3 vs 7.1±1.5 Units/mg protein, $P<0.001$ at day 5). The lung weight(Lwt) in the PQ-group increased progressively ($r=0.98$, $P<0.01$; 0.24 ± 0.01 vs 0.17±0.01, $P<0.01$ at day 1, and 0.45±0.02 vs 0.21 ± 0.01 g, $P<0.005$ at day 5). Protein concentrations in the PQ-group decreased ($r=0.81$, $P<0.05$; 49.4±3.2 vs 69.0±1.2, $P < 0.05$ at day 1, and 32.4±1.2 vs 61.1±6.8 mg/g tissue, $P < 0.001$ at day 5). The Lwt/body weight ratio exhibited an increasing profile($r=0.97$, $P<0.01$; 0.005 vs 0.009, $P<0.01$ at day 1, and 0.007 vs 0.021, $P<0.001$ at day 5). SQ 14225 inhibited the ACE activity completely. These data indicate the enhanced level of ACE and the presence of a regulatory mechanism for its sustained activity despite a diminished concentration of protein in PQ-induced pulmonary pathology.

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EFFECTS OF GANGLIONIC BLOCKADE AND STIMULATION ON NONADRENERGICALLY-MEDIATED BRONCHODILATION IN THE CAT. Mark N. Gillespie*, John P. Sabo* and Louis Diamond. University of Kentucky, College of Pharmacy, Lexington, KY 40506.

In cats pretreated with atropine and propranolol, vagal stimulation reverses the increase in lung resistance produced by an infusion of serotonin (Science 208, 185, 1980). Because this response, termed vagally-mediated nonadrenergic bronchodilation (VMNB), may involve a novel component of the autonomic nervous system, we investigated the ability of ganglionic blockade to inhibit and ganglionic stimulation to mimic VMNB. Intravenous administration of the ganglionic blocking drugs hexamethonium (2 mg/kg) and the structurally unrelated compound mecamylamine (4 mg/kg) consistently abolished VMNB. Surprisingly, intravenous administration of the ganglionic stimulants acetylcholine (.05 mg/kg), nicotine (.05-.1 mg/kg) and DMPP (1,1-dimethyl-4-phenylpiperazinium iodide, 0.1 mg/kg) in doses sufficient to cause a marked increase in arterial blood pressure, failed to mimic VMNB. Thus, although inhibition of VMNB by ganglionic blockade is in accord with classical concepts of autonomic pharmacology, the failure of ganglionic stimulation to initiate the response is an interesting point of divergence. These results suggest that there may be fundamental differences between the nonadrenergic inhibitory pathways to airways smooth muscle and the neural pathways to other autonomic effector organs in the cat.

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TRAPPED GAS IN LUNGS OF INTACT, ANESTHETIZED RATS. J.J. Morgan* and D.G. Frazer. ALOSH, NIOSH, CDC, DHHS, and Dept. of Physiology, WVU, Morgantown, WV 26506.

The purpose of this study was to evaluate the effects of end expiratory pressure (P_{min}) and inflation rate (R_t) upon gas trapping in lungs in vivo. Anesthetized and paralized Long Evans hooded male rats were ventilated in a whole body plethysmograph following O_2 degassing. The effects of P_{min} on gas trapping were determined by recording 5 different P-V curves for each animal at an inflation rate of 15.5 cc/min and a deflation rate of 26.5 cc/min. Each curve consisted of 6 cycles in which P_{min} for the first 5 cycles was either +7, +1, -2, -5, or -8 cm H_2O. On the sixth cycle the lung was deflated to -8 cm H_2O where the volume of gas trapped (V_m) was determined and normalized with respect to maximum lung volume (V_{max}). The ratio V_m/V_{max} was then plotted verses P_{min}. This relationship showed that airway closure for lungs of live rats follows a normal distribution centered about a mean value of P_{min} equal to -1.84 and a SD of 2.22 cm H_2O. In a second series of P-V curves the effects of inflation rate on gas trapping was determined by recording 3 curves, each consisting of 5 cycles recorded between P_{min} = -8 cm H_2O and a P_{max} = 30 cm H_2O with an inflation rate of either 69.6, 26.5, or 15.5 cc/min and a deflation rate of 45.9 cc/min. The ratio V_m/V_{max} was plotted verses R_t. It was found that a decrease in R_t caused an increase in V_m/V_{max} similiar to that seen by Frazer and Weber (J.A.P. 40:915, 1976) for excised lungs. (Supported in part by ERDA contract EY-77-21-8087)

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FORCED EXPIRATORY TIME CONSTANT HISTOGRAM. R.L. Pimmel*, T.M. Miller*, J.C. Eyles*, J.M. Fouke*. (SPON: P.A. Bromberg) Dept. of Medicine, Univ. of North Carolina, Chapel Hill, N.C. 27514

We have developed an algorithm for computing a time constant histogram from a maximum forced expired volume signal. The algorithm is based on a model containing 20 exponentially emptying parallel compartments with fixed time constants ranging from 0.1 to 10 s. The histogram defines the fraction of vital capacity from each compartment. The algorithm is based on a least squares solution of a discrete version of this model's equation with both smoothness and nonnegativity constraints applied. A simulation study using unimodal and bimodal histograms indicated that accurate histograms can be computed even in the presence of random volume fluctuations. Histograms computed from independent forced expirations were reproducible in normal children and adults and in patients with lung disease. Normal children generally had unimodal distributions with the mean time constant (T) ranging from 0.12 to 0.64 s. Normal adults had bimodal histograms with more than 75% of the vital capacity in a fast compartment (T= 0.12 to 0.55 s) and the remainder in a slow compartment (T=1.3 to 2.7 s). In patients with obstructive lung disease a larger fraction was found in the slow compartment. This technique may provide an alternate way of interpreting forced expirations and a set of parameters that is more dependent on the configuration of the waveform than are conventional parameters. (Supported in part by HL-00207, HL-19118, and HL-23822).

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STATE-SPACE ANALYSIS OF FORCED OSCILLATION MEASUREMENTS. W. K. Johnson* (SPON: B. A. Muggenburg). Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87115

Respiratory system impedance is a function of frequency, flow, and volume. Sensitivity analysis of mathematical models indicated that the variation in respiratory system impedance during normal breathing was sensitive to a variety of disease processes, including diseases affecting the small airways. A modified forced oscillation technique was developed which measures the effects of frequency, flow, and volume on respiratory system impedance during spontaneous breathing. This technique involved the application of a pressure function composed of octaves from 1 to 64 Hz. Evaluation of respiratory system impedance as a function of forcing frequency, flow, and volume is accomplished by computer analysis of the flow response signal. This analysis used digital filtering and pattern recognition techniques. Acute NO_2 exposure was used to induce a small airway disease in 10 Beagle dogs. Two additional dogs served as controls. Pulmonary function tests including spontaneous breathing pattern, dynamic mechanics, alveolar gas exchange, forced expiratory maneuvers, imposed deadspace, and the state-space forced oscillation technique were performed prior to NO_2 exposure and at 0, 1, 2, 3, 7 and 14 days post exposure. The state-space forced oscillation technique was as sensitive to the small airway disease resulting from NO_2 exposure as any of the other function tests used in this study. (Research performed under U. S. Department of Energy Contract Number DE-AC04-76EV01013.)

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RESONANT FREQUENCIES OF RESPIRATORY FLOW TRANSDUCERS. R. Gelfand* and J. DeLong* (Spon: C.J. Lambertsen). Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Laminar flow transducers and spirometers were tested for dynamic responses to step function changes in flow rate with three gases (helium, oxygen, sulfur hexafluoride). For laminar flow transducers, a differential pressure manometer with resonant frequency 170 Hz in air at 1 ATA (not degraded by increased gas density) was used to convert flow to an electrical signal (Francis, Gelfand, and Peterson. J. Appl. Physiol: Resplrat. Environ. Exercise Physiol. 47(3): 631-637, 1979). All three laminar flow transducers and a dry spirometer tested showed underdamped responses to step changes in flow. For laminar flow transducers, the oscillatory response frequency is directly proportional to the velocity of sound in the test gas and inversely proportional to the connecting tubing length and can be computed as f = C/4L; C = velocity, L = tube length (acoustic model). For spirometer, the oscillatory response is due primarily to inertia of the moving system and compression of the enclosed gas volume (mass-spring model). These findings have particular relevance to measurements of pulmonary function with sulfur hexafluoride, to wide bandwidth measurements of pulmonary system impedance, and to measurements at high ambient pressures encountered in deep diving. Supported in part by NIH Grant HL 08899 and jointly by the Office of Naval Research and the Naval Medical Research and Development Command through ONR Contract N00014-7-C-0248.

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VOLUME DEPENDENCY OF TOTAL RESPIRATORY RESISTANCE. C.V. Peterson, Jr.* and A.B. Otis, Department of Physiology, University of Florida, Gainesville, Fla. 32610

Resistive properties of the human breathing system at 5 different lung volumes were studied using an apparatus consisting of a pneumotachograph, a variable resistance, a shutter, and a mouthpiece with a connection to a pressure transducer. The subject made a constant inspiratory effort against the closed shutter until a predetermined pressure (P_o) was reached which activated a solenoid to open the shutter. Peak airflow, $\dot{V}$, and the simultaneous mouth pressure (P_m) from repeated trials at the same lung volume were varied by adding external resistances to the apparatus. A plot of P_m vs. $\dot{V}$ for each lung volume fits the straight line $P_m = K\dot{V} + P_o$ where K has the units $cmH_2O\ L^{-1}$ sec. K increased in a curvilinear manner as lung volume was decreased. Esophageal pressure in the subject was measured to estimate changes in alveolar pressure (P_a) from the time the shutter opened to the time at which $\dot{V}$ was measured. The greatest change in P_a was found at the highest $\dot{V}$, however, even this pressure difference was small (1-2 cmH_2O). The relationship between P_a and $\dot{V}$ was not affected by changes in lung volume. A model using a bottle to replace the subject demonstrates that the shortening of inspiratory muscles minimizes any effect of lung volume per se on the value of K. The increase in K with decreasing lung volume reflects the associated increase in airways resistance. (Supported by NIH Grant 1 R01 HL2351502)

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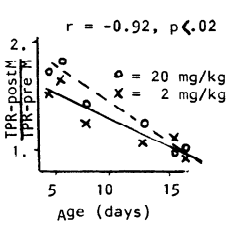
EFFECTS OF MECLOFENAMATE ON PULMONARY HEMODYNAMICS DECREASE WITH POSTNATAL AGE IN ANESTHETIZED PIGLETS. Gregory J. Redding*, Ivan F. McMurtry, and John T. Reeves. CVP Research Lab, Univ. of Colo. Health Sciences Ctr., Denver, Colo. 80262.

The pulmonary vasodilation required for a successful transition to extrauterine life at birth may be mediated in part by prostaglandin (PG) vasodilators. We measured total pulmonary resistance (TPR) in 6 pigs, aged 5-17 days, during normoxia and hypoxia before and after treatment with 2 and 20 mg/kg sodium meclofenamate (M), a PG synthetase inhibitor, to assess the influence of PGs on pulmonary hemodynamics soon after birth. During normoxia, M treatment produced an increase in TPR in the younger pigs and little change in animals older than 2 weeks. This increase in TPR was greater after treatment with the higher dose. M did not alter the piglets' pulmonary hypoxic pressor response. We conclude that endogenous PG vasodilators help to maintain low pulmonary vascular tone during normoxia around the time of birth, but that these compounds modify pulmonary hemodynamics less with increasing postnatal age.



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INDEPENDENCE OF THE CENTRAL SYMPATHETIC RESPONSE TO CO_2 OF THE CENTRAL INSPIRATORY ACTIVITY (CIA). Andrzej Trzebski and Leszek Kubin. Dept. Physiol., Institute Physiol. Scienc., Medical Academy, Warsaw 00927, Poland.

In vagotomized, debuffered and artificially ventilated cats anaestnetised with chloralose-uretan mixture phrenic nerve activity, cervical, splanchnic and renal sympathetic nerve as well as single cervical preganglionic units activity was recorded. CIA was abolished by hyperventilation alone or combined with bilateral stimulation of the pontine pneumotaxic center or laryngeal superior nerve. CO_2 threshold for the increase of the sympathetic activity was lower than CO_2 threshold for the reappearance of the phrenic rhythmic activity. CO_2 threshold for the pressor and splanchnic nerve excitatory responses was lower than CO_2 threshold for the renal and cervical nerves sympathetic responses. In some experiments CO_2 produced slow component oscillations of the sympathetic discharge prior to phrenic nerve rhythmic response. Excitability of the phrenic motoneurones tested as the amplitude of the evoked potential increased along with the sympathetic excitatory response to CO_2 thus anticipated reappearance of the rhythmic phrenic activity. It is concluded that central chemoreceptors may augment sympathetic discharge in the absence of CIA. However, during the transient state from hypocapnia to normocapnia some subtreshold excitatory input to phrenic motoneurones occurs.

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RELATION BETWEEN BICARBONATE SECRETION AND CHLORIDE ABSORPTION IN AMPHIUMA SMALL INTESTINE. Michael A. Imon* and John F. White. Dept. Physiol. Emory University, Atlanta, Ga.

Amphiuma Small Intestine absorbs Cl^- by a process requiring HCO_3^- (White, J.F., J. Membrane Biol 53: 95-107, 1980) Bicarbonate secretion ($J^{HCO_3^-}$) determined by the pH stat technique was 1.10±0.06 μeq/hr cm^2 in normal media. The difference between $J^{HCO_3^-}$ and the short circuit current ($J^{HCO_3^-}-I_{sc}$) μeq/hr cm^2, (0.94±0.09 μeq/hr cm^2), was highly significant and consistent with Cl^- absorption. Replacement of Cl^- in the media with $SO_4^=$ significantly reduced $J^{HCO_3^-}$ (0.48±0.03 μeq/hr cm^2) and $J^{HCO_3^-}-I_{sc}$. Replacement of the serosal HCO_3^-/CO_2 buffer with a phosphate buffer reduced $J^{HCO_3^-}$ to 0.11±0.02 μeq/hr cm^2 and $J^{HCO_3^-}-I_{sc}$ was not significantly different than zero (P>0.10). $J^{HCO_3^-}$ and $J^{HCO_3^-}-I_{sc}$ were dependent on serosal HCO_3^- in a manner resembling saturation kinetics, the change in $J^{HCO_3^-}$ correlating significantly (r=0.94) with changes in $J^{HCO_3^-}-I_{sc}$. $J^{HCO_3^-}$ was measured with the mucosal pH statted at 8.0, 7.4, and 6.0 while the serosal pH was buffered at 7.4 in normal media. $J^{HCO_3^-}$ was maximal at pH 7.4 and $J^{HCO_3^-}$ again correlated (r=0.92) with $J^{HCO_3^-}-I_{sc}$. The stilbene SITS significantly reduced both $J^{HCO_3^-}$ and $J^{HCO_3^-}-I_{sc}$ in this study. These results strongly indicate a link between chloride absorption and bicarbonate secretion.

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INTESTINAL Cl^- TRANSPORT AND INTRACELLULAR ACTIVITY OF Cl^- IN THE PRESENCE OF THEOPHYLLINE. John F. White. Dept. Physiol. Emory University, Atlanta, Ga.

Simultaneous bidirectional tracer fluxes of ^{22}Na and ^{36}Cl were measured under short-circuited conditions in isolated stripped segments of Amphiuma small intestine incubated in media containing Cl^- and HCO_3^- (25 mM). When exposed to 10 mM theophylline the short-circuit current (I_{sc}) was increased significantly. Simultaneously net Cl^- absorption was eliminated while the residual flux was increased. Net Na^+ absorption was negligible in controls and unchanged by theophylline. Acetazolamide (10^{-4}M) reduced the theophylline-stimulated I_{sc} but did not re-establish Cl^- transport. Using microelectrodes it was observed that theophylline depolarized the mucosal membrane of villus epithelial cells by an average of 8.6 mV. Using double-barreled Cl^--specific microelectrodes the intracellular activity of Cl^- averaged 27.1 mM in the presence of theophylline, consistent with active accumulation of Cl^-. These findings indicate that the inhibition of Cl^- absorption by theophylline is not due to inhibition of mucosal Cl^- uptake and provide evidence that HCO_3^- secretion is enhanced by theophylline.

(Supported by NIH grants AM 17361 and AM 26870)

904

K ABSORPTION BY A NA-DEPENDENT, OUABAIN-SENSITIVE PUMP AT THE LUMINAL MEMBRANE OF TURTLE BLADDER. R.F. Husted* and P.R. Steinmetz. University of Iowa, Iowa City, IA 52242.

In the absence of transepithelial electrochemical gradients, the turtle urinary bladder transports K from the mucosal (M) to the serosal (S) solution. To characterize the K absorptive pump we examined the influence on K absorption of an imposed voltage, of sodium removal and of ouabain addition. Hemibladders were mounted in lucite chambers, short-circuited and bathed on both surfaces with identical Ringer's. In the first group of experiments, active K absorption at OmV was estimated as the M→S K flux minus the passive S→M flux. The passive flux was corrected for potential using the Nernst-Planck relationship. The imposition of a 58 mV (S positive) potential, which would be expected to decrease M→S K flux, caused an increase in the active portion of K absorption. A S negative potential of 25 mV caused a decrease in active K absorption. Thus the active K flux appeared to be coupled to a flow of positive charge in the opposite direction, possibly representing Na secretion in excess of K absorption. In separate experiments in Na-free media (Na replaced by Cs) active K absorption was abolished (Control K fluxes: M→S: 101±5; S→M: 59±4; Na-free: M→S: 66±8 and S→M: 70±18 n moles/hr/8 cm^2). In the third series of experiments active K absorption was abolished by addition of ouabain to M, but not by addition to S. The reverse electrogenicity, Na-dependence and ouabain-sensitivity of K absorption indicate that the K pump of the mucosal membrane has the characteristics of a Na-K ATPase. (Supported by NIH grant AM 24022)

905

EXCRETION OF K^+ BY SKIN OF *RANA PIPIENS* WITH RATE VARYING WITH K^+ LOAD. FRAZIER, L.W. AND VANATTA, J.C. Baylor Coll. of Dentistry and U. of TX Southwestern Med. Sch. Dallas, TX 75246; 75235.

Frogs of either sex of both northern and southern subspecies were used in these experiments. The anus was closed with a purse string suture after injecting a small volume of methylene blue solution into the cloaca. The frogs were then placed in 60 ml of 2 mM NaCl for 5 to 6 hr. The outside solution was collected, concentrated to 0.1 or 0.05 times its original volume and K^+ determined by flame photometery. Group I consisted of 11 control animals. Group II consisted of 11 toads injected with 20 mM KCl, 0.1 ml/G two times the day before the experiment, and one time just before the immersion period. Urine was collected from some of the animals in each series following the immersion, and $[K^+]$ determined on it. K^+ excretion was normalized to μM (50 G frog)$^{-1}$(hr)$^{-1}$(Units). The skin excretion was 0.77 + 0.18 units for Group I, and 1.87 + 0.43 units for Group II. (± SEM, p<0.025) In 7 frogs of Group II, skin excretion averaged 41% of total excretion. In the control group, K^+ was not detectable in the urine of 3 frogs, with skin excretion estimated to be at least 90% of total in these animals. Na^+ was absorbed from the bathing media, however there was not increased absorption by the K^+ loaded animals. We conclude that the frog skin can excrete K^+ and that the skin is an important organ for K^+ balance. (Supported in part by NIH Grant AM 18689 and by NIH General Research Grant 5 SO 1-RR05426-14).

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IONIC ACTIVITIES IN NECTURUS GALLBLADDER-EFFECT OF ADENOSINE-3'-5'-CYCLIC MONOPHOSPHATE (cAMP). A.Diez de los Rios* and W. McD. Armstrong, Dept. Physiology, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

The effects of cAMP (6 mM, added to the serosal medium) on apical membrane potential (E_m), transepithelial P.D., and intracellular Na+, K=, and Cl^- activities (a^1_{Na}, a^1_K, a^1_{Cl}) were studied in Necturus gallbladder. Isolated gallbladders were mounted, at 23°C, between identical oxygenated HCO_3^--free Ringer solutions (Na^+=Cl^-=100 mM; K^+=5.4 mM; pH 7.2) in divided chambers. In 13 experiments the average (± SEM) control E_m (-52 ± 2) did not differ from E_m in the presence of cAMP (-50 ± 2 mV). cAMP changed P.D. from virtually zero (-0.1 ± 0.2 mV) to a small, significant (P < 0.05) positive value (0.8 ± 0.2 mV). In 4 experiments cAMP reduced (P < 0.05) a^1_{Na} from 8.5 ± 1.1 to 5.5 ± 0.7 mM and a^1_{Cl} from 14 ± 2 to 9 ± 1 mM. The latter a^1_{Cl} value corresponded to transmembrane Cl^- electrochemical equilibrium. In 5 experiments, cAMP increased a^1_K from 84 ± 5 to 113 ± 6 mM. (P < 0.05). In 3 experiments with Cl^--free (gluconate) media, cAMP did not affect E_m (48 ± 2 mV), P.D. (1.0 ± 0.1 mV), or a^1_K (80 ± 5 mM). These results indicate that, (i) cAMP inhibits coupled electroneutral transapical Na-Cl entry into the cells, (ii) the cAMP induced increase in a^1_K seen in media containing Cl may reflect inhibition, when the outwardly directed transmembrane electrochemical driving force for Cl^- is abolished, of coupled K-Cl baso-lateral efflux. Supported by USPHS AM 12715, HL 23332. A.D.R. was a Fellow of the Ministerio de Univ. e Invest., Spain.

907

ROLE OF K^+ IN MAINTENANCE OF THE TRANSMURAL POTENTIAL AND SHORT CIRCUIT CURRENT ACROSS THE PHARATE PUPAL INTEGUMENT DURING RESORPTION OF MOULTING FLUID. Paul D. Cooper*, Lewis E. Deaton* and Arthur M. Jungreis. Zool. Dept., U of Tenn, Knoxville, TN 37916

The production and subsequent resorption of moulting fluid across the integument in lepidopteran larvae are important processes in the transformation from larva to pupa. During the period of resorption, integumentary P.D.'s and I_{sc}'s of the tobacco hornworm, Manduca sexta, were studied in vitro in solutions of differing ionic composition. After 2-3 hrs in 32 mM $KHCO_3$-180 mM Mannitol (K-M-HCO_3), the P.D. was ca. +10 mV, exuvial side positive with respect to hemolymph side, and the I_{sc} ca. 15 uAmps-cm^{-2}. Substitution of 32 mM Na-M-HCO_3 red reduced the I_{sc} by ca. 10 uAmps-cm^{-2}, while 32 mM NH_4-M-HCO_3 or 32 mM Ch-M-HCO_3 eliminated the I_{sc}. Replacement of HCO_3^- with Cl^-. $SO_4^=$ or OAc^- had no effect on either P.D. or I_{sc}. If K^+ is added to the exuvial side while the preparation was bathed in NH_4-M-Cl^-, the I_{sc} immediately increased. Addition of K^+ to the hemolymph side had no effect. We propose that the observed current across the integument is generated by the electrogenic exchange of K^+ and H^+ on the exuvial side of the epidermal cells. (Supported in part by NIH Grant AI #12779)

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STIMULATION OF SODIUM TRANSPORT BY 18-HYDROXYDEOXYCORTICOSTERONE, 18-HYDROXYCORTICOSTERONE AND ALDOSTERONE IN THE TOAD SKIN. R. Beauwens*, J. Crabbé* and M. K. Birmingham. Université Catholique de Louvain, Département de Physiologie, B-1200 Brussels, Belgium and McGill University, Department of Psychiatry, Montreal, Quebec, H3A 1A1.

Ventral skin of Bufo marinus was divided into 4 or 6 pieces, mounted in Ussing chambers and exposed on the serosal side to 50 nM steroid or to vehicle only. After 16 h incubation went on with fresh, steroid-free medium and the short-circuit current (SCC) was monitored. Aldosterone (ALDO), 18-hydroxydeoxycorticosterone (18OHDOC) and 18-hydroxycorticosterone (18OHB) significantly increased sodium transport:

n	Vehicle	ALDO	18OHDOC	18OHB
40	14 ± 2	27 ± 3	23 ± 3	
		(Δ 13 ± 2)	(Δ 9 ± 2)	
16	16 ± 4	31 ± 4		25 ± 4
		(Δ 15 ± 4)		(Δ 8 ± 2)

Corticosterone and deoxycorticosterone on the other hand had no significant effect on SCC. The 18-oxygenated steroids thus stimulated sodium transport at a concentration which is physiological for ALDO and may be for 18OHB, both major ACTH-responsive, amphibian adrenocortical hormones. These findings caution against the definition of a mineralocorticoid solely in terms of effects on urinary Na^+/K^+ ratios. A pathophysiological role for 18-oxygenated steroids not limited to ALDO is conceivable. (Supported in part by the Medical Research Council of Canada.)

909

Lectin-affinity Separation of ATPase activities in Membrane Fractions of Turtle Bladder Epithelial Cells. Youmans*SJ, Durham*,JH, Heinz,E, Masur,SK* and WA Brodsky. Mt Sinai and Cornell Schools of Medicine, New York, NY, 10029

Preliminary results indicate that ConA, visualized by electron microscopy after peroxidase staining, binds predominantly to apical membranes of suspended granular cells from urinary bladders of P. scripta turtles. This suggests that ConA (bound co-valently to Sepharose beads, Sigma) might bind predominantly to right-side out apical plasma membrane vesicles in a suspension of randomly-oriented vesicles of apical and basolateral membranes. Such a suspension, isolated by a previously used technique (BBA 556:490,1979), was incubated for 1 hr. at 0 C with ConA beads, after which the supernatant of unbound membranes was decanted. Membranes bound to the beads were then eluted by resuspending the bead pellets in 0.5M methyl-a-D-glucopyranoside. The ouabain inhibitable ATPase activity of the original mixed membranes was recovered exclusively in the fraction which did not bind. No ouabain sensitive ATPase was found in the fraction which was bound to ConA. It is tentatively concluded that lectin binding surfaces or membranes of epithelial cells can be selectively isolated by this technique. (Supported by NSF grant PCM-79235 and NIH grant, 16928).

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METHYL GLUCOSE TRANSPORT BY LOBSTER HEPATOPANCREAS. Gregory A. Ahearn, Elizabeth A. Monckton* and Eugene Montes* Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822

Isolated hepatopancreatic lobes of the Atlantic lobster, Homarus americanus, accumulate exogenous 3H-3-O-methyl-D-glucose at 15°C from a saline approximating hemolymph in composition. The time course of 1 mM sugar uptake was significantly reduced in the presence of 0.2, 0.5, or 1.0 mM phoridzin or 1 and 10 mM D-glucose, suggesting the occurrence of a carrier mechanism for the sugar analog. Methyl glucose influx (10 min exposure intervals) was a hyperbolic function of analog concentration after correcting for radioactivity associated with the tissue extracellular space (3H-mannitol used as the ECS marker). Approximate kinetic constants for the sugar carrier mechanism determined using a Lineweaver-Burk plot were: K_t^{MG} = 4.2 mM and J_{max}^{MG} = 2.5 μmoles g^{-1} hr^{-1}. Addition of 1 mM ouabain or 1 mM sodium azide to the incubation medium failed to reduce the rate of methyl glucose uptake by this tissue over a 60 min accumulation period. These preliminary results suggest that sugar uptake by lobster hepatopancreas occurs through an energy-independent carrier system that may not exhibit sodium-cotransport and is most likely localized to the baso-lateral pole of the tubular epithelium. (Supported by NSF grant number PCM 76-84105)

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THE ACTIVE TRANSPORT OF THROMBOXANE-B_2 (TxB_2), PROSTACYCLIN (PGI_2) AND 6-KETO-$PGF_{1\alpha}$ BY SOME MAMMALIAN TISSUES. F. E. DiRenedetto* and L. Z. Bito. Dept. Ophthal., College of P & S, Columbia Univ., N.Y. 10032

The concentrative accumulation or exclusion of some arachidonic acid derivatives (AADs) were studied by incubation of suspended rabbit erythrocytes and tissue pieces or slices in solutions containing ^{3}H-TxB_2, ^{3}H-PGI_2 or ^{3}H-6-keto-$PGF_{1\alpha}$ or by bolus intra-arterial injection of these tracers into isolated perfused kidneys. Following a 30-min incubation with ^{3}H-TxB_2 the choroid plexus (CP) exhibited the highest tissue to medium (T/M) ^{3}H accumulation ratio (7.4 ± 0.7) followed by kidney cortex (KC)>anterior uvea>vagina>lung>1.0. Following 5 min of incubation with ^{3}H-PGI_2, KC showed the highest ^{3}H T/M (2.7 ± 0.1) followed by CP>anterior uvea>2.0. T/M ratios were less than unity for PGI_2 in the lung and for all three tracers in erythrocytes, spleen, kidney medulla or aorta. The intracellular compartments of spleen and erythrocytes were clearly not accessible to these AADs. KC, but not lung slices accumulated ^{3}H-PGI_2 more effectively than ^{14}C-$PGF_{2\alpha}$. Probenecid (10^{-4}M) inhibited the concentrative uptake of all three tracers. These results show that the basic cell membrane is essentially impermeable to these AADs but some tissues which were previously shown to have a PG transport capacity can actively transport them. The only exception is the lung, which does not appear to have an effective PGI_2 transport capacity. (Supported by EY 00402; NIH).

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GLUCOSE AND ALANINE TRANSPORT IN UPPER AND LOWER INTESTINES OF CARNIVOROUS AND HERBIVOROUS MARINE FISHES. Ronaldo P. Ferraris* and Gregory A. Ahearn. Department of Zoology, University of Hawaii, Honolulu, HI 96822

The initial uptake rates of glucose (glu) and alanine (ala) (0.01-25 mM) in isolated upper (UI) and lower (LI) intestines of a carnivorous moray eel (Gymnothorax undulatus) and a surgeonfish (Acanthurus mata) were compared. First, a linear, non-saturable component of influx was found in all four tissues for both solutes and may represent diffusion. Second, a saturable influx component was also found in the eel UI and LI and surgeonfish UI. Affinity constants (K_t; mM), maximal influx rates (J_{max}; nmoles $cm^{-2}min^{-1}$) and diffusion constants (K_D; nmoles $cm^{-2}min^{-1}mM^{-1}$) are shown below:

		glucose		alanine	
		UI	LI	UI	LI
surgeonfish	K_D	3.76	4.35	7.94	10.82
	K_t	0.70	-	0.10	-
	J_{max}	13.00	-	1.64	-
eel	K_D	3.31	0.86	5.69	1.50
	K_t	0.31	0.38	1.44	0.31
	J_{max}	4.35	3.07	14.10	4.78

Results suggest that (1) K_t's and J_{max}'s are linked to the probable organic solute concentrations in the diet of these organisms and (2) nutrient transport differences exist between fish upper and lower intestines.

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THE EFFECTS OF HARMALINE ON NONELECTROLYTE INFLUX ACROSS THE MUCOSAL MEMBRANE OF RABBIT INTESTINE. John F. Schaeffer* and Jerel W. Yokel* (SPON: W. McD. Armstrong). Indiana University School of Medicine, Evansville, IN 47732.

Treatment of the mucosal membrane with the hallucinogenic alkaloid, harmaline (HARM) at concentrations > 0.5mM (1mM, 2.5mM, and 5mM) inhibits the influx of phenylalanine (Phe), cycloleucine and 3-O-methyl-α-D-glucopyranoside across the brush border. The time course of Phe influx inhibition (5mM HARM) is 50% at 1 min, 70% at 15 min, and 80% at 30 min. This inhibitory effect (5mM HARM, 30 min) is not readily reversed by a 30 min wash in low sodium medium containing 140mM choline chloride, 10mM $KHCO_3$, 0.2mM KH_2PO_4, 1.2mM $K2HPO_4$, 1.2mM $CaCl_2$, and 1.2mM $MgCl_2$. The treatment (5mM HARM, 30 min) blocks both Na^+-dependent and Na^+-independent Phe influx to the same extent. It is suggested that when the mucosal membrane is exposed to harmaline in a low sodium medium, the effects of harmaline are irreversible and non-Na^+-specific; whereas, the modification of the tissue by harmaline in a "normal" sodium medium, results in a largely Na^+-dependent, reversible blockage such as reported by Aronson and Bounds, Amer. J. Physiol. 238: F210-F217, 1980. (Supported in part by USPHS, NIH Grant AM17414).

914

PREPARATION OF PROXIMAL TUBULE CELLS FROM DOG KIDNEY.
W. Pinos* and M. Silverman. Univ. of Toronto, Toronto, Ont.

A homogeneous population of proximal tubule cells would be a useful tool in the investigation of transport and metabolism phenomena. The following method is a preliminary attempt at isolating a viable and relatively homogeneous population of proximal tubule cells. Cells are prepared using a two step perfusion method. First, a Ca^{2+}- and Mg^{2+}-free modified Collins solution is perfused through the renal artery to remove blood contaminants. Second, a high Na^{+}, low K^{+} digestion media containing 0.1% collagenase and 0.05% DNase recirculates through the kidneys at 37°C to promote tissue dissaggregation. Following digestion, the kidney cortex is dispersed with gentle shaking. After filtering, the cells are separated on a 30% Percoll density gradient. Yields of cells vary between 10 and 15 mls with an average cell concentration of 7×10^{7} cells/ml. Approximately 84 to 88% of the cells exclude trypan blue. Using phase microscopy, two morphologically distinct cell populations can be identified, the major difference being size. From the total preparation, 75% of the cells contain histochemically demonstrable alkaline phosphatase. Greater than 90% of the cells bind rabbit anti-dog antibody specific for the proximal tubule brush border membrane as shown by indirect fluorescent staining (Kidney Int. 11:348-356, 1977). It is hoped that this cell preparation can be used to investigate sugar transport and other transport mechanisms.

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THE EFFECTS OF HEMORRHAGIC SHOCK ON THE PRODUCTION AND/OR RELEASE BY THE RAT LIVER OF CLOTTING FACTORS VII, IX AND X. P. Kokas* and P.G. Iatridis. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408.

There is no available data regarding changes in clotting factors' activity in isolated perfused rat liver during different pathological conditions. In the present experiments the clotting factors' activity were studied in pre and post-hemorrhagic shock (HS) of SPD male rats in (a) platelet poor plasma (PRP), (b) liver perfusate (Prf.), and (c) ascites (Asc). The HS was according to A.G.B. Kovach and P. Sandor (1972) as modified by P. Kokas. In the pre-HS control (intact) animals the liver was removed immediately after the blood sampling, whereas in the post-HS animals the liver was removed 60 min. after the induction of the HS. In both cases the liver was placed in the perfusion chamber as described by Miller (1951) and modified by Kupcsulik and Kokas (1979). The liver was then perfused for 60 min. with Krebs-Henseleit solution plus 4% bovine albumin. Samples of the Prf. were collected at 0', 30', and 60'. The results indicate that there is a significant decrease in the production and/or release of factors VII, IX , and X in the post-HS rat liver at 0' and 30' post-perfusion, whereas, at 60', post-perfusion, only factor VII was significantly decreased. Factors VII and IX were significantly decreased in Asc., of the post-HS rats, but not factor X. (Supported in part by a grant from the LCMCDA).

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COMPARISON OF THE ABILITY OF VARIOUS COLLAGEN PREPARATIONS TO PROMOTE PLATELET AGGREGATION AND PLASMA CLOT FORMATION. P. A. Gentry, M. D. Schneider* and J. K. Miller.* Univ. of Guelph, Guelph, Ont. and Comparative Animal Research Laboratory, Oak Ridge, TN 37830.

Pepsin treated acid soluble collagen preparations isolated from burro and horse aortic tissue and acid soluble collagen from human umbilical cord will all promote platelet aggregation. At a final concentration of 2 µg per ml plasma, each preparation produced a similar degree of aggregation, but did not activate the coagulation mechanism even when incubated with plasma at 37C for up to 30 min at concentrations up to 100 µg per ml. By contrast, fibrillar form collagen from burro aortic tissue was able to stimulate both platelet aggregation and plasma clot formation. A maximal aggregation response was observed at concentrations as low as 1 µg per ml. A dose related activation response, as judged by the degree of shortening of plasma clotting times, was seen after incubation of plasma with collagen at concentrations from 20 to 100 µg per ml. Heat denaturation at 56C for 15 min produced substantial losses in both platelet aggregation ability and plasma activating ability. The results suggest that the coagulation mechanism will only respond to collagen preparations which closely resemble native collagen. (Supported by NSERC Grant #A6462, DOE Contract #DE-AC05-760R00242 and USDA Grant #901-15-168.)

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RELATIONSHIP OF PLASMA FIBRONECTIN TO THROMBOTIC RESISTANCE. J.E.Kaplan, S.H.Baum*, P.W.Snedeker*. Department of Physiology, Albany Medical College, Albany, NY 12208.

Studies suggest that the reticuloendothelial system (RES) and plasma fibronectin (Fn), an opsonic protein, are components of physiologic anti-thrombotic mechanisms. This study addresses the hypothesis that Fn deficiency results in enhanced sensitivity to thrombosis whereas increased Fn results in enhanced resistance. Rats were injected with antiserum to Fn, purified Fn, or vehicle prior to intra-aortic infusion of saline, ADP (1mg/100g) or thrombin (15U/100g). Infusion of ADP or thrombin following vehicle or Fn was non-lethal as was the administration of anti-Fn and saline. Following anti-Fn, thrombin infusion resulted in 87% mortality and ADP infusion 54% mortality. Infusion of thrombin into controls led to 50% depletion of fibrinogen, low levels of FDP and little effect on platelet levels. Infusion of ADP resulted in a 50% reduction in platelets with a delayed consumption of fibrinogen and appearance of FDP. Fn alone resulted in reduced circulating fibrinogen while anti-Fn resulted in modest reduction in fibrinogen and platelets. When ADP or thrombin were infused after antiserum, fibrinogen and platelets fell to 10-30% of control values with high FDP. When ADP or thrombin were infused following Fn there was no additional change in fibrinogen, FDP or platelet levels. The current study produces evidence that reduction of Fn levels increases sensitivity to thrombosis and supplementation of Fn increases resistance to thrombosis. (GM-25946, HL-07194)

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PLASMA COMPONENT THAT AMPLIFIES FIBRONECTIN MEDIATED KUPFFER CELL PHAGOCYTOSIS. F.A.Blumenstock*, T.M.Saba, E.Cho* (SPON: G.Kanter). Dept. of Physiology, Alb. Med.Col., Albany, NY 12208

Kupffer cell phagocytic uptake of gelatinized colloid is dependent upon opsonic α_2SB glycoprotein which is identical to plasma fibronectin. This protein can be purified by gelatin affinity chromatography with retention of opsonic activity and the specific activity of the purified protein (opsonic activity/mg fibronectin) is less ($p<0.05$) than in plasma (15.8± 5.8 vs 66.1±6.4). Plasma deficient in fibronectin minimally supports non-bacterial particulate phagocytosis (0.9±0.1%ID/100mg). Reconstitution of affinity absorbed opsonin deficient plasma with fibronectin resulted in the restoration of opsonic activity. Dialysis of affinity absorbed plasma, at 4°C, did not remove the co-factor activity indicating that the moiety mediating amplification of opsonic activity has a molecular weight greater than 10,000 daltons. The co-factor precipitated at 35% saturated $(NH_4)_2SO_4$ at 4°C and migrated with the gamma globulin fraction during isolation by preparative free flow electrophoresis. There was a stoichiometric relationship between fibronectin and its co-factor with respect to maximal opsonic activity. Therefore, reticuloendothelial depression demonstrated in the presence of elevated immunoreactive plasma fibronectin during some pathologic states may be due to deficiency of this co-factor. (CA-16011 and GM-21447)

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COMPARISON OF PLASMA PREKALLIKREIN ASSAYS BY ESTEROLYTIC, AMIDOLYTIC, AND IMMUNOLOGIC TECHNIQUES. Carol A. Fisher,* Robert W. Colman, L. Henry Edmunds, Jr., and V. Paul Addonizio. U. of PA and Temple U., Phila., PA 19104.

To date there is no consensus for assaying prekallikrein, an essential component of the earliest phases of coagulation. We therefore compared the best procedures currently available. Two enzymatic assays using L-tosyl-arginine methyl ester (TAMe) and H-D-Prolyl-L-phenylalanyl-L-arginine-p-nitroanilide dihydrochloride (S-2302) as substrates, which provide susceptible ester and amide bonds respectively, were both compared with an immunologic assay in two groups - normal random donors (N=19) and women receiving oral contraceptives (N=21). The amount of enzyme generated from its respective substrate by kaolin was slightly higher in women taking oral contraceptives which probably reflects estrogenic stimulation of hepatic protein synthesis. Correlation coefficients (r) between groups are summarized as follows:

	Normals		Oral Contraceptives	
TAMe vs S=2302	r=0.89	p<0.001	r=0.66	p<0.01
TAMe vs Immunologic	r=0.68	p<0.01	r=0.90	p<0.001
S-2302 vs Immunologic	r=0.72	p<0.001	r=0.76	p<0.001

Although the immunoreactive procedure is useful, it does not distinguish prekallikrein from kallikrein bound to its inhibitor. Although the amidolytic procedure is colorometric and may thus suffer interference from certain pigments (e.g. bilirubin), this technique is favored due to its technical ease, lower Km of its substrate, and ability to perform precise time course studies in purified systems. (Support - HL-22346)

920

PLATELET PHAGOCYTOSIS OF BACTERIA. D.R. Absolom,* W. Zingg, P.L. Chang,* C.J. van Oss,* and A.W. Neumann.* Hospital for Sick Children, Toronto, Ontario M5G 1X8

We have previously investigated the adhesion of platelets to polymers as a function of surface tension and the free energy of adhesion. The results indicated that the process of cell adhesion could be described entirely by thermodynamic considerations. To test the supposition that the two processes of platelet adhesion and platelet phagocytosis of bacteria might be governed by the same thermodynamic principles we have investigated platelet phagocytosis on this basis. For this purpose we have determined the surface tension of washed pig platelets and four bacterial species. Our results may be summarized as follows: (a) There was no detectable bactericidal activity of the platelets upon any of the test micro-organisms. Bacteria were ingested but not digested by the platelets. (b) Engulfment increased with increasing hydrophobicity of the bacteria in contradiction of the thermodynamic model. (c) The more virulent, pathogenic bacteria were found to be the most hydrophilic and were the least engulfed. (d) The degree of engulfment could be considerably modified by opsonizing the bacteria. When opsonized significantly fewer hydrophobic bacteria and significantly more hydropholic bacteria are engulfed. These results seem to preclude specific antibody receptors playing a role in non-opsonized platelet phagocytosis. (Supported by Medical Research Grant No. MA 5462)

921

RESPONSES OF CIRCULATING HEMOCYTES OF THE CRAYFISH, ORCONECTES VIRILIS, TO INJECTIONS OF LATEX AND BACTERIA. Douglas P. Merrill and Susan E. Fisher.* Department of Zoology, University of Vermont, Burlington, VT 05405.

It is well established that the principal line of defense of invertebrates against nonself materials is cellular. Although the terminology is often confusing and contradictory, crustaceans are generally recognized as having two populations of circulating blood cells: agranular or hyaline hemocytes and granulocytes or granular hemocytes. Transition forms may exist and various developmental sequences have been proposed. We have described two classes of agranular hemocytes and four classes of granulocytes in the crayfish, Orconectes virilis. By means of a differential blood stain, we examined the responses of each class to the injection of either 3 x 10^7 latex microspheres or Staphylococcus epidermidis. The responses varied considerably. Following the injection of latex microspheres, the percentage of mature granulocytes in circulation increased dramatically while the percentage of all other cell types decreased. The total cell count decreased only slightly. The injection of S. epidermidis caused an 85% reduction in the number of circulating hemocytes; all cell types declined in proportion to their preinjection concentrations. Following either injection, the numbers and relative proportions of blood cells returned to preinjection levels after 48 hours. (Supported by a Biomedical Research Support Grant, PHS 07125-08, from the University of Vermont)

922

NODULE FORMATION IN THE GILLS OF THE CRAYFISH, ORCONECTES VIRILIS. Stephen A. Mongeon and Douglas P. Merrill, Department of Zoology, University of Vermont, Burlington, VT 05405.

The course of clearance of Staphylococcus epidermidis from hemolymph of the gills of the freshwater crayfish, Orconectes virilis, was followed for times ranging from 1 min. to 30 days after the injection of 3x10^7 bacteria. The gills were studied histologically by means of a staining procedure which differentiated bacteria, blood cells and gill epithelium. S. epidermidis were found free in the hemolymph of the gills for only up to 5 min., after which they were observed in association with hemocytes. Large numbers of bacteria were phagocytized by agranular hemocytes while the remainder were found adhered to granulocytes or sequestered in an extracellular amorphous material in the proximity of ruptured granulocytes. Within 3 hrs. of injection, distinct aggregations of blood cells were observed in the gill lumens. A progression in the appearance of the aggregates, from loose masses of 10-15 cells, in a ratio of 2:1 granulocytes to agranular cells, to tight nodules of 2-5 cell layers in thickness was observed. After 24 hrs., nodules began to accumulate a pigmented amorphous core which darkened with time, taking on the appearance of melanin. The retention of such nodules for long periods of time is apparent, suggesting that they are the end-point in the cellular reaction to S. epidermidis. (Supported by Grant #PHS 07125-08 from the University of Vermont.)

923

FASCIOLIASIS: EVIDENCE THAT THE ANEMIA IS DUE TO A SUBSTANCE RELEASED FROM THE WORM. Robert N. Spengler* and Hadar Isseroff. SUNY College, Buffalo, New York 14222.

Anemia is a characteristic symptom of chronic fascioliasis. The cause of that anemia is controversial: Some investigators have proposed that a toxic substance emanating from the fluke is the cause while others have argued for hematophagia. In the present study the anemia was investigated in rates. Hematological parameters measured over the course of infection showed significant decreases in erythrocyte counts and hemoglobin. Significant increases were observed in reticulocyte counts and mean corpuscular volume. Urobilinogen levels increased in early stages of infection but returned to near normal levels after the worms reached the bile duct.

To determine whether the anemia could be due to toxic substances emanating from the fluke, worms were inserted into fine mesh sacks and implanted into the peritoneal cavity of uninfected rats. The sacks prevented access to erythrocytes. Hematological parameters indicate that the anemia produced in rats with implanted worms was very similar to that produced by mature per os infections. This study indicates that the anemia is due to a substance released by the worms and supports previous work suggesting that proline released from the worms may be inducing the anemia.
(Supported in part by grant AI-09911 from the NIH-NIAID.)

924

PROSPECTIVE LYMPHOCYTE MEDIATED CYTOTOXICITY ON B-CELL TARGETS AND THE SELECTION OF HUMAN KIDNEY ALLOTRANSPLANT RECIPIENTS. P. Nathan, R.T. Plessinger*, J.W. Alexander* and R.M. First*. Shriners Burns Institute and Univ. of Cinti., College of Medicine, Cincinnati, Ohio 45219.

Lymphocyte mediated cytotoxicity (LMC) was performed using mixtures of recipient effector cells and donor target cells labeled with a fluorescent dye (Tissue Antigens, 14:205,1979). Frozen aliquots of specific cells were rapidly thawed and used as effectors. Purified donor B-cells labeled with fluorescein diacetate served as target cells (Proc. Clin. Dialysis and Transpl. Forum 8:62, 1978). Loss of fluorescence was equated with cell death. During 1979, transplants were performed in 54 LMC negative patients in whom renal function was followed for 3-12 months. Kidney survival in this group was 70%. Eight of the 16 non-functioning kidneys were lost due to rejection. A similar group of 30 recipients, not evaluated for LMC, received kidney allotransplants during the previous year, 1978. Only 46% of these patients retained their kidney functions when evaluated for a 3-12 month interval after renal allotransplantation ($p < 0.05$). Fourteen of the patients in this group rejected their allotransplanted kidney. The results indicate that prospective LMC tests using specific B-cell targets to selectively eliminate donor-recipient matches with positive cell mediated reactions improve kidney allotransplant survival.

925

AMMONIA EXCRETION IN THE BLUE CRAB: DIFFUSION AND NOT NA^+/NH_4^+ EXCHANGE. Gregg A. Kormanik and James N. Cameron. Univ. Texas, Marine Sci. Inst., Port Aransas, TX. 78373.

Controversy exists as to the means by which ammonia is excreted across the gills of water breathing organisms. Callinectes sapidus, the blue crab, was examined to determine if ammonia excretion occurred via Na^+/NH_4^+ exchange, or by passive NH_3 diffusion across the body surface. Net ammonia efflux was determined during a sea water control period. Transfer of the crabs to 2.5mM Na, K-free ASW increased net ammonia efflux to 108±16% (n=9) of the control. Transfer of the crab to 2.5mM Na, K-free ASW containing amiloride (10^{-4}M.) slightly reduced ammonia efflux to 90±40% (n=3) of the control; in neither case was the difference significant ($p>0.1$). We concluded that ammonia efflux is not via Na^+/NH_4^+ exchange in the seawater blue crab. To see if NH_3 diffusion can account for net ammonia excretion, the crab was transferred to sea water at pH 9.5, to elevate external pNH_3. Ammonia efflux was reduced to 31±5% (n=10) of the control, which is explained by the change in NH_3 gradient. Thus ammonia excretion across the gill of the seawater crab appears to be via NH_3 diffusion. (Supported by NSF grant PCM77-24358 to JNC).

926

ISOLATION AND CHARACTERIZATION OF NA,K-ATPase INDUCED IN CRABS, *Callinectes sapidus*, EXPOSED TO OSMOTIC STRESS. Anthony F. Almeida* and John B. Pritchard. NIEHS, c/o CV Whitney Lab., Univ. of Fl., RFD 1, Box 121, St. Augustine, Fl. 32084 USA

The Na,K-ATPase increases in the gills of blue crabs exposed to dilute sea-water. It is not certain if this increase is a consequence of *de novo* synthesis or of activation of existing sites. Differential contrifugation was employed to prepare enzyme enriched membranes from crabs exposed to 20% sea-water and to normal sea-water. An increase in total gill Na,K-ATPase and a small increase in Na,K-ATPase specific activity could be shown in gill homogenates from adapted animals. These increases were much more marked in the 9500 G pellet. However, the 48000 G pellet, which showed the highest specific activity, did not show a change in either total content or specific activity in adapted animals. Thus, the increased gill Na,K-ATPase activity could be isolated in the 9500 G fraction. This increase was apparent as early as three days after exposure to 20% sea-water and increased with time thereafter. Therefore, adaptation to dilute sea-water results in an increase in Na,K-ATPase activity and an alteration in its sedimentation characteristics. However, other enzyme characteristics, for example, affinity for the cardiac glycoside ouabain, were unchanged in all fractions from control and adapted animals. These results appear to reflect synthesis of new enzyme probably in newly formed "chloride" cells (Neufeld *et al.*, *J. Expt. Zool.*, 1980) and suggest that differences in membrane properties may lead to the differential sedimentation of otherwise identical enzyme.

927

TRANSPORT ATPASES IN THE OSMOREGULATING HERMIT CRAB CLIBANARIUS VITTATUS. T. D. Sabourin and D. G. Saintsing. Louisiana State University, Baton Rouge, LA 70803.

Changes in specific activity (SA) of the gill Na^++K^+-ATPase are associated with the salinity acclimation regime and level of hyperosmotic regulation in the crab C. vittatus. Crabs acclimated to 30 o/oo salinity for two weeks had a gill SA of 1.09±0.31(n=10) umoles Pi/min/mg protein compared to a significantly greater level of 5.67±1.17(6) for crabs acclimated to 10 o/oo. When crabs were abruptly transferred from 30 to 10 o/oo, the SA increased significantly by 1 hour after transfer and continued to increase to a maximum of 14.76±3.01(5) at hour 3. Following this peak activity, a decrease in SA occurred, stabilizing by hour 12 to levels comparable to those for the 10 o/oo steady state animals. When crabs were subjected to a simulated diurnal tidal salinity fluctuation (30-10-30 o/oo) the Na^++K-ATPase SA increased to 10 o/oo steady state levels by hour 6 and remained at this level throughout the 24 hour experiment. Gill microsomal HCO_3^--ATPase SA was low (0.34-1.66 umoles P_i/min/mg protein) and exhibited no salinity related differences under steady state conditions. A small but significant increase in SA was seen by hour 1 with abrupt transfer (ANOVA;$P<0.01$). Na^++K^+-ATPase appears to be rapidly modulated in hermit crabs under all of the above conditions. (Supported by a Grant-in-Aid from Sigma Xi)

928

OXYGEN UPTAKE AND TRANSPORT IN THREE AIR BREATHING CRABS. Brian R. McMahon and Warren W. Burggren. Dept. of Biology, University of Calgary, Calgary, Alberta, Canada. and Zoology Dept., U. Mass., Amherst, Mass. 01003.

Hemolymph oxygenation has been studied *in vivo* and *in vitro* in three terrestrial crabs, the anomurans *Birgus latro*, the coconut crab, and *Coenobita brevimanus* a land hermit crab and in the brachyuran *Cardisoma carnifex*. These animals differ both in the degree of reduction of gill area and in the degree of development of accessory air breathing structures, and in their specific habitat within the supralittoral zone. Prebranchial oxygen tensions are similar (Pv_{O2} = 11-13 torr) in all species. Postbranchial oxygen tensions are low (Pa_{O2} = 22-32 torr) compared with marine crabs, but adequate tissue oxygen supply (a-v O_2 content = 0.4 m mol.l^{-1}) nonetheless occurs. Oxygen equilibrium curves constructed *in vitro* from pooled hemolymph for each species indicate high oxygen capacity and sufficiently high oxygen affinity to ensure 65-85% oxygenation of postbranchial hemolymph. *Coenobita*, a species with reduced gills and the least development of accessory air breathing structures, exhibits the lowest postbranchial oxygen tensions, but also has the highest oxygen capacity and thus is able to deliver equivalent amounts of oxygen. Cooperativity is high (n_{50} = 3.6-3.9) in all 3 species but P_{50}, ΔH and the magnitude of the Bohr effect (ϕ) vary between species. Possible relationships between hemocyanin oxygen affinity and the degree of terrestriality are discussed. Work supported by NSERC grant A5762 and conducted aboard R.V. Alpha Helix.

929

THE VASCULATURE OF THE GILL FILAMENT OF THE BOWFIN AMIA CALVA. K.R. Olson. Indiana University School of Medicine, South Bend Center, Notre Dame, IN. 46556.

The gill microcirculation in Amia calva was examined by scanning electron microscopic analysis of methyl methacrylate corrosion replicas. The gill vessels of Amia are organized into three pathways, respiratory (R), nutritive (N) and interlamellar vascular sinusoids (ILV). The respiratory pathway consists of an afferent filamental artery (AFA), afferent lamellar arteriole (ALA), lamellae (L) efferent lamellar arteriole (ELA) and efferent filamental artery (EFA). The L contain an outer marginal channel (OMC) and a less organized inner marginal channel (IMC). The lamellar sinusoides are organized into distinct channels which impart longitudinal directionality to blood flow. The nutritive circulation is formed from vessels that arise from the medial border of the EFA along its length and large vessels that are formed from the confluence of up to 100 small vessels (SV) which originate from the base of the EFA. The SV follow a tourtous course before anastomosing into the large nutrient vessel. The ILV originates from branches of the AFA, near the ampulla. The ILV network surrounds the AFA and forms a network of vessels that traverse the filament body between the IMC. The ILV also receive flow from N and both N and ILV are drained by several filamental veins. The similarities between gill vascular replicas of Amia and teleosts suggests that both infraclasses may regulate gill physiological activity through similar vasomotor mechanisms. Supported in part by NSF Grant # 79-23073.

930

INTRINSIC HYPOXIC VASOCONSTRICTION IN SALINE PERFUSED TROUT GILLS (SALMO GAIRDNERI). William F. Jackson and Paul O. Fromm. Dept. of Physiology, Michigan State University, East Lansing, MI 48824

The first pair of gill arches in rainbow trout are known to contain receptors which mediate the hypoxic bradycardia response. We were interested in determining (a) if there is any intrinsic vasomotor response to environmental hypoxia in isolated gills perfused at constant flow with air saturated, albuminated Cortland solution and (b) if there is any difference in the response of first and second gill arches to environmental hypoxia. When environmental oxygen tension was decreased from 20 to less than 1 kPa, gill vascular impedance increased transiently and then returned to, or slightly above, control levels. At the peak of the response, impedance was elevated by 51% in first arches, 14% in second arches and 10% in first arches perfused with epinephrine. Venous outflow from gills remained unchanged during the period of vasomotion, an indication that the site of vasoconstriction was proximal to efferent lamellar arterioles. The data demonstrate that isolated gills display an intrinsic hypoxic vasoconstriction and that first and second arches differ in the magnitude of their response to environmental hypoxia. (Supported by HL 07404-02)

931

CHLORIDE TRANSPORT IN THE ISOLATED PERFUSED TELEOST GILL
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Net Cl extrusion is demonstrated in the isolated perfused gill of the pinfish, Lagodon rhombiodes, bathed bilaterally with Ringers solution. The magnitude of the unidirectional Cl efflux in both Ringers (789 ± 71 μM 100 g $fish^{-1}$ h^{-1}, n = 41) and sea water (1985 ± 337 μM 100 g $fish^{-1}$ h^{-1}, n = 2) is 45% that of the intact animal. The transepithelial electrical potential (TEP) across the isolated gill in sea water, +9.61 ± .83 mV (n = 4), is equal to that of the intact animal. The TEP in Ringers, +.73 ± .075 mV (n = 41), is slightly lower than that of the intact animal. Afferent and efferent flow rates were carefully monitored and the difference between the two or "leak" value was subtracted from the observed unidirectional efflux. The unidirectional efflux varies linearly with the afferent flow rate (slope = 5.74, r = .86). When bathed bilaterally by Ringers, furosemide inhibits 57% of the unidirectional efflux. Removal of Na from the perfusate inhibits 30% of the unidirectional efflux; a significant portion of this inhibition is not potential mediated. Bilateral Na removal produces an inhibition of net Cl extrusion that is not potential mediated. Microfil injection of the arch shows that afferent and efferent filamental arteries and presumed basal lamellar channels are well perfused in this preparation.

932

MECHANISMS OF TRANSIENT ACID-BASE REGULATION AFTER STRENUOUS ACTIVITY IN RAINBOW TROUT.
George F. Holeton, Peter Neumann* and Norbert Heisler*. Dept. Zoology, Univ. of Toronto, Canada and Dept. Physiol., Max Planck Institute Exp. Medicine, D-3400 Göttingen, FRG.

Rainbow trout held in a closed system were induced to exercise to exhaustion over a four minute period. Blood was sampled via a catheter in the dorsal aorta. Immediately following the exercise there was a 15 mM increase in plasma [Na^+] which probably reflected an osmotic uptake of extracellular water into the muscle due to the formation of lactic acid. The equimolar disappearance of Cl^- from, and appearance of lactate in the blood suggested that efflux of lactate from the muscle was accompanied by Cl^- uptake. The formation of large amounts of lactic acid within the muscle was reflected by an abrupt acidaemia and a delayed rise in blood lactate levels which peaked after 2 hours. There were no measurable amounts of lactate excreted to the water and net amounts of electrolytes transferred to and from the water were small compared to the changes in the extracellular fluids. The H^+ formed in the muscle with the lactate was excreted into water and, to a minor extent, stored in the extracellular fluid. Unlike the elasmobranch *Scyliorhinus stellaris*, the trout took up only small amounts of bicarbonate from water. Instead H^+ was transferred to the ambient water in the form of a transient increase in NH_4^+ excretion. Later, as lactate was metabolized, H^+ was taken up from the extracellular and environmental compartments.

933

TEMPERATURE RELATED CHANGES IN RESPIRATION AND ACID-BASE BALANCE IN AN AIR BREATHING FISH.
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In Lepisosteous oculatus, a bimodal breather, the extracellular pH changes with temperature as it does in water breathing fish. Temperature related acid-base balance, however, is regulated by different mechanisms in Lepisosteous than it is in water breathing fish. The extracellular change in pH with temperature is -0.015. Blood HCO_3^- did not decrease significantly from 20° to 30°C, as it does in water breathing fish. There were also no significant changes in branchial or renal acid (or base) efflux rates as temperature rose. The acid-base adjustment was due to an elevation in blood P_{CO_2} from 5.6±0.6 (s.e.) torr at 20 to 10.5±0.5 torr at 30°C. Pulmonary ventilation increased from less than 1 breath per hour to 7.6 breaths per hour, and pulmonary $\dot{M}O_2$ more than doubled at 30°C. Branchial ventilation, $\dot{M}O_2$, and $\dot{M}CO_2$ did not change significantly as temperature rose. Pulmonary $\dot{M}CO_2$ increased slightly, but because of the low lung R value of 0.09, CO_2 excretion from the lung was limited. Thus, by increased dependence on pulmonary gas exchange the gar met its elevated oxygen demands and simultaneously regulated acid-base balance at elevated temperatures. This response to temperature change is similar to that shown by air breathing ectotherms, but involves an indirect regulation of P_{CO_2}. (Supported by NSF Grant PCM77-24358 to J.N.C.)

934

ACID-BASE STATUS IN TWO SALAMANDER SPECIES, *Siren lacertina* AND *Amphiuma* , IN RESPONSE TO ENVIRONMENTAL HYPERCAPNIA.
Norbert Heisler*, Gordon R. Ultsch and John F. Anderson. Dept. Physiol., Max Planck Institute Exp. Medicine, D-3400 Göttingen, FRG.

Partial pressure of CO_2 (PCO_2) in part of the natural aquatic habitat of *Siren* and *Amphiuma* frequently rises to values up to 70 Torr due to hindrance of gas exchange between water and air by dense water hyacinth mats. Even when both species are able to satisfy their oxygen demand by gas exchange via their lungs, considerable amounts of CO_2 can be expected to be taken up from the water via the skin. Specimens of both species exposed to water PCO_2 of 47 Torr showed increases in arterial PCO_2 from 12 to 35 for *Siren* and from 17 to 36 Torr for *Amphiuma*. The resultant drops in plasma pH of more than 0.4 or 0.3 units for *Siren* and *Amphiuma*, respectively, remained uncompensated, whereas the intracellular pH of white muscle and heart of *Siren* did not change in hypercapnia due to greatly elevated intracellular bicarbonate concentrations. This pattern of highly compensated intracellular pH with constant extracellular bicarbonate was also maintained during a considerable increase of water bicarbonate. Even bicarbonate infused into *Siren* was quantitatively excreted and produced no increase in extracellular bicarbonate. These results suggest that the type of regulation described, which is in contrast with that in all other investigated vertebrates except *Synbranchus* (a tropical freshwater teleost), is characteristic for these animals and is not dependent on the availability of bicarbonate ions.

935

THE EFFECTS OF HYPERCAPNIA ON THE INTRACELLULAR AND EXTRACELLULAR ACID-BASE STATUS IN THE TOAD *Bufo marinus*.
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Twenty-eight adult tropical toads were used to determine changes of extracellular and intracellular pH (pHe, pHi) effected by a step increase of 36 Torr in ambient PCO_2. Animals were chronically cannulated in the femoral artery for blood sampling and allowed 24 hrs. recovery prior to experimentation. Determinations of $PaCO_2$, pHa and pHi (by the DMO distribution technique) were made while animals were breathing air, 5% CO_2 for 1 hr., or 5% CO_2 for 24 hrs., respectively. Mean normal pHa fell from 7.81 to 7.40 due to 1 h CO_2 exposure, and only slightly recovered to 7.44 after 24 hrs. exposure. Mean plasma [HCO_3^-] rose from 21.7 to 26.3 (1 hr.) and stabilized at 32.7 (24 hrs) while $PaCO_2$ rose from 11 to 35 and 41 Torr over the same time course. The large pHe changes were not mirrored intracellularly as heart muscle pHi changed only from 7.24 to 7.09 and then to 7.12 in the above mentioned regime and skeletal muscle pHi from 7.15 to 6.93 (1 hr.) and 6.96 (24 hrs.). The results show that a large hypercapnia-induced pHe depression does not drastically alter pHi because of a 2 1/2 fold increase in intracellular [HCO_3^-] and therefore shows that pHi is regulated much more effectively than pHe.

936

ENERGETICS OF OSMOREGULATION IN A FRESHWATER TELEOST, THE BROWN BULLHEAD CATFISH, ICTALURUS NEBULOSUS.
Philip B. Furspan* and Henry D. Prange. Indiana Univ., Bloomington, IN 47405.

Groups of brown bullhead catfish were acclimated to different concentrations (0, 120, 200 mOsm/Kg) of either NaCl (artificial seawater) or $MgSO_4$ solutions. Oxygen consumption was reduced when the gradient between the external solution and internal body fluids was decreased (average plasma concentration in freshwater was 260 mOsm/Kg). In the NaCl solutions the reductions were 16% and 31% whereas in the $MgSO_4$ solutions reductions of 13% and 27% were measured. The consistent difference between the two sets of values suggests that although a large portion of the fishes' metabolism is devoted to osmoregulation only a small part of this is required for ionic transport. The concommitant decrease in urine flow rate (40% in 120 and 75% in 200 mOsm/Kg in both media) suggests that the kidneys are the main sites of reduced metabolic activity due to the reduced necessity for sodium retention. Measurements of glomerular filtration rate should corroborate this. Consideration of the values found in this study and the calculated minimum thermodynamic work (Potts, 1954) for osmoregulation suggests that it is a relatively inefficient process. The results of this study suggest that this inefficiency involves kidney function.

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EFFECT OF INSPIRATORY FLOW RATE ON INTRAPULMONARY GAS DISTRIBUTION. T. J. Knopp and K. Rehder. Mayo Clinic and Mayo Foundation, Rochester, Mn. 55901

The decrease in efficiency of pulmonary gas exchange associated with general anesthesia and mechanical ventilation in normal subjects appears to be due to an altered distribution of inspired gas. We examined whether changing the inspiratory flow rate ($\dot{V}_I$) alters the intrapulmonary distribution of inspired gas and thus might improve the efficiency of gas exchange.

Total (mouth) and regional (apical and basal for each lung) 133-Xe clearances following equilibration of the lung with 133-Xe were measured in 5 awake subjects and another 5 anesthetized-paralyzed subjects while they were in the right lateral decubitus position. Each awake subject was studied at 3 $\dot{V}_I$'s (0.4 to 1.0 l/s); each anesthetized-paralyzed subject at 4 $\dot{V}_I$'s (0.2 to 1.6 l/s). Tidal volume and breathing frequency were maintained constant for each subject.

The clearance from each lung region in each state was well described by a monoexponential function after correction for chest wall contribution. Regional 133-Xe clearances were significantly more uniform ($P < 0.001$) in the anesthetized-paralyzed state than the awake. No consistent differences in total or regional 133-Xe clearances were observed between the various $\dot{V}_I$'s in either state. We conclude that altering $\dot{V}_I$ during anesthesia-paralysis is probably not a useful means of improving the matching of ventilation to perfusion and thus intrapulmonary gas exchange in subjects with normal lungs.
(Supported in part by NIH grant HL 21584.)

938

PLEURAL PRESSURES BY WICK CATHETERS. E.A. Hoffman, S.J. Lai-Fook, and E.H. Wood. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN

We previously reported a technique (Fed Proc 39:575 '80) whereby distances between percutaneously inserted x-ray opaque apical and basal lung parenchymal markers (LPM) (JAP 40:118 '76) were calibrated to known regional transpulmonary pressures in intact dogs. Apical intermarker distances in head up dogs indicated an expansile force at FRC about 10 cm H_2O greater than pleural pressures (Ppl) measured simultaneously at the same vertical height in the thorax by liquid filled catheters. (Catheter values agreed with Banchero et al. JAP 23:228 '67). This discovery prompted comparisons of Ppl measured simultaneously by apical and basal pairs of identical percutaneously inserted liquid filled catheters, one open ended (OE) and the other wick ended (WE) containing a 3 cm long bundle of 25 μm fibers from #1 dexon suture unbraided and tethered by an intraluminal thread so that about 1 cm of the wick extended beyond the catheter tip. WE and OE values were equal in dependent regions of the thorax. However, apical non-dependent WE values were about 10 cm H_2O more negative than OE values in 4 dogs and equal in 3 others. "Improper, i.e., retrograde relative to the catheter tip, positions" of the external segment of wick was observed at autopsy in the latter 3 dogs. Greater negativity of "properly positioned" WE values along with prior estimates by LPM suggest apical expansile forces in head up dogs greater than indicated by Ppl measured by liquid filled OE catheters. (Grants HL 4654, 21584, and RR-7)

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THE REGIONAL DISTRIBUTION OF GAS DURING HIGH FREQUENCY OSCILLATIONS. L. Forkert* and J.E. Burks* (SPON: J. Remmers). Univ. of Texas Medical Branch, Galveston, Texas 77550.

Recent investigations have shown that paralyzed animals may be adequately ventilated with high frequency oscillations. The purpose of this study was to determine how gas is distributed to apical-basal lung regions during such oscillations. A speaker, generating oscillations at 5 Hz, was attached to the mouthpiece of healthy adults seated in a plethysmograph. The distribution of gas to horizontal lung regions from apex to base was determined with scintillation detectors positioned against the subject's back. A 1 mCi ^{133}Xe bolus was injected into the subject's mouth while he breathheld at FRC + 1 liter, FRC, FRC - 1 liter or RV. Oscillations were maintained at the mouth until regional count rates were constant. Oscillations were then terminated and the subject inspired to TLC for count rate measurements. The control distribution was determined with boluses inspired at .2 l/sec from the same lung volumes to TLC. At FRC + 1 l and FRC, boluses distributed during oscillations (D_{OS}) had a more uniform apex-base gradient than the basal predominance of control boluses (D_{con}). At FRC - 1 l these differences diminished and at RV both distributions had an apical predominance and were similar. These results indicate that D_{OS} changes with lung volume and may be determined by a gradient of regional resistances at FRC + 1 l and FRC in contrast to the regional compliances of the D_{con}. At the lower volumes the effects of airway closure may predominate and determine both D_{OS} and D_{con}. Supported by NIH Grant HL-25745.

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Pulmonary Gas Exchange During High Frequency Ventilation
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Pulmonary gas exchange in 16 normal anesthetized dogs during high frequency ventilation with a piston pump and bias flow (HFV) (frequency 10HZ, TV 4 ml/Kg) was compared to standard mechanical ventilation (MV) using either room air (RA) or 20% O_2 in Helium (Heliox). The inert gas infusion technique was used to describe distributions. With the same exhaled V_E the (A-a) D_{O_2} was unchanged comparing HFV and MV on room air (23.6 torr vs 24.1 torr), but the Bohr V_D/V_T was higher on HFV (60.1% vs 50.6%, $p<0.01$). The mean (A-a) D_{O_2} during HFV was higher on RA than with Heliox (24.1±8.6 torr vs 13.3±7.8 torr $p<.001$), and the Bohr V_D/V_T on HFV also increased with gas density (Heliox 48.9%, RA 60.1%). V_A/Q distributions on HFV demonstrated a bimodal distribution of ventilation with one mode near the mean V_A/Q and the other between V_A/Q of 10 and 100. The inert gas or "anatomic" dead space fraction was reduced on HFV in comparison to MV, (MV .40±.07, HFV .14±.10; $p<.001$). Although the Bohr V_D/V_T predicted from the inert gas data agreed well with measured values during MV, a discrepancy was apparent during HFV (mean V_D/V_T 50.7% predicted vs 60.1% measured, $p<.01$). The bimodal distribution of ventilation may represent different phases of the ventilatory cycle rather than distinct anatomic regions. Comparison of gas exchange with RA and Heliox suggests that convective mixing is more significant in HFV than MV. (Supported by the U. of Washington Graduate School Research Fund and NIH HL05777)

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VENTILATION-PERFUSION RATIOS DURING HIGH FREQUENCY OSCILLATORY VENTILATION. R.D. McEvoy*, F.L. Mannino*, P.T. Schumacker*, F.C. White*, P.D. Wagner and J.B. West. Department of Medicine University of California, San Diego, La Jolla, CA 92093

We studied gas exchange during high frequency oscillatory ventilation (HFOV) using the inert gas method of Wagner et al. Results were compared during constant volume mechanical ventilation (MV) and HFOV in 6 anesthetized, paralyzed dogs. Frequencies during HFOV ranged from 20 to 25 Hz and pump stroke volumes from 2.5 to 4.5 mls/Kg body weight. For MV, arterial P_{O_2} was 87.5 ± 1.8 torr (mean ± S.E.), and arterial P_{CO_2} was 35.5 ± 0.9 torr. Corresponding values for HFOV were 89.2 ± 1.3 torr and 33.9 ± 0.9 torr. Mean airway pressure was 1.66 ± .14 torr during MV and 3.79 ± .53 torr during HFOV. Ventilation-perfusion ($\dot{V}A/\dot{Q}$) distributions obtained during MV exhibited single modes of ventilation and perfusion centered on a $\dot{V}A/\dot{Q}$ ratio = .8. By contrast, HFOV distributions were bimodal, with 49.4 ± 1.7 % of alveolar ventilation being distributed to a high mode, $\dot{V}A/\dot{Q}$ = 50. To determine whether this high mode was due to a redistribution of pulmonary blood flow similar to that seen with PEEP ventilation (JAP 47(5):938-946, 1979), further studies of regional pulmonary perfusion were performed using radioactively labelled microspheres. The distributions of pulmonary blood flow were similar for MV and HFOV. We conclude that a high $\dot{V}A/\dot{Q}$ mode is recovered by the inert gas elimination method during HFOV and that it is not explained by a topographical redistribution of pulmonary blood flow.
(Supported by the Australian NH and MRC grant HL 17731.)

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HIGH FREQUENCY OSCILLATION COMPARED TO CONVENTIONAL POSITIVE PRESSURE VENTILATION IN OLEIC ACID INDUCED PULMONARY INJURY: WK Thompson*, BE Marchak*, AC Bryan and AB Froese. Research Inst. Hospital for Sick Children, Toronto, Ontario, Canada.

Gas exchange and cardiac output were compared at identical mean airway and pulmonary artery occlusion pressures once hemorrhagic pulmonary edema had been induced by infusing 0.04-0.08 ml/kg of oleic acid intra atrially into anesthetized dogs. In a randomised order animals were ventilated either using a volume cycled ventilator with PEEP (CPPV; V_t 16-27 ml/kg, f 16-20/min) or a variable volume piston pump operating at 15 Hz (HFO). Ventilators were adjusted so as to match mean intratracheal airway pressure (MAP). Arterial gas tensions, thermodilution cardiac outputs, pulmonary arterial and occlusion pressures (P_{AOP}) and acid base status were measured for 12 data sets matched for MAP over a range of 7.5 to 25 cmH_2O, with F_{IO_2} 0.5 throughout. Mean values are shown below:

	A-aDO_2 torr	$PaCO_2$ torr	pH	CO l/min	P_{AOP} mmHg
HFO	207.6	45.2	7.34	1.65	2.9
CPPV	223.5	43.8	7.35	1.69	2.9

With both forms of ventilation PaO_2 progressively improved as MAP increased. We conclude that in a shunt model of acute lung injury there are no significant differences in oxygenation or cardiac output when high frequency oscillation is compared to conventional positive pressure ventilation with PEEP at equivalent mean airway pressures.
(Supported by MRC of Canada MA6342).

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ANATOMICAL EQUIVALENTS OF LOW VENTILATION/PERFUSION (V/Q) UNITS IN THE LUNG. R.L. Conhaim and N.C. Staub. Cardiovasc. Res. Inst and Physiol. Dept., Univ. of Calif., San Francisco, CA 94143

Multiple inert gas analysis reveals that there appears to be a population of units having V/Q < 0.1 in some normal subjects and many patients. No anatomical structures accounting for such low V/Q units have been shown. We have recently described the flux of oxygen across the walls of small pulmonary arteries (JAP:43(5),1980). Theoretically, small arteries in gas-filled cavities, or regions of microatelectasis could account for low V/Q units. We modeled the V/Q of a 190 μm diameter pulmonary artery and 100 μm half-thickness atelectatic plate, using Roughton's advancing front equation for 02 uptake or outward diffusion of inert gas (SF6). In our calculations we used blood transit times of 1 and 10 seconds.

	Calculated V/Q Ratio			
Transit Time	Pulmonary Artery		Atelectasis	
(sec)	02	SF6	02	SF6
1	.01	.002	.03	.002
10	.1	.04	.08	.01

The V/Q is sensitive to the time available for gas exchange. The reason for the discrepancy between 02 and SF6 is not clear, but both 02 and SF6 models confirm the concept that very low V/Q may be due to blood flowing through regions of microatelectasis, or through small arteries in emphysematous cavaties. When breathing 100% 02, such units appear as shunts, but the amount of shunt will be less than that calculated during air breathing. (Supported in part by HL6285)

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INCOMPLETE BLOOD-GAS EQUILIBRATION OF CARBON DIOXIDE IN LUNGS: MECHANISMS AND EVIDENCE. J. Piiper, M. Meyer* and P. Scheid. Dept. Physiol., Max Planck Inst. Exp. Med., D-3400 Göttingen, W. Germany.

The positive arterial-alveolar PCO_2 difference ($aAD(CO_2)$), frequently observed in man and in the dog, is usually attributed to alveolar dead space ventilation. However, additional mechanisms may be involved. (1) Recent studies have produced evidence for stratification of CO_2 (and O_2) in lungs due to incomplete mixing of inspired gas with resident gas in the alveolar space. For the dog, estimations of $aAD(CO_2)$ attributable to stratification have yielded values ranging from 1 to 3 Torr. (2) The results of our studies on blood-gas equilibration kinetics of CO_2 in lungs (J. Piiper et al.: Physiologist 22,101,1979) suggest that in resting man the blood-gas equilibrium is close to complete, but for heavy exercise an $aAD(CO_2)$ of several Torr may occur. On the other hand, a negative $aAD(CO_2)$ or reduction of an existing $aAD(CO_2)$ may arise from: (1) continuing gas exchange during expiration; (2) negative blood/gas PCO_2 difference in (rebreathing) equilibrium, which has been claimed, but could not be reproduced in later studies (Scheid, P. and J. Piiper: Respir. Physiol. 39,1-31, 1980). The quantitative role of these individual mechanisms of $aAD(CO_2)$ cannot yet be assessed with sufficient accuracy.

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REGIONAL DIFFUSING CAPACITY/ALVEOLAR VOLUME IN APICAL AND BASAL SEGMENTS OF NORMAL LUNGS. N.R. MacIntyre* and J.A. Nadel Cardiovasc. Res. Inst., UCSF, San Francisco, CA 94143.

To study CO diffusion separately in apical and basal regions of the lung, we gave 5 seated normal subjects 20-30cc boluses of 50% CO and 50% CH_4 either near residual volume (RV) or near total lung capacity (TLC) during a slow inhalation of room air. Diffusing capacity/alveolar volume (D_L/V_A) was determined throughout the subsequent slow exhalation by repeated calculation of the CO disappearance slope with respect to the inert gas, CH_4. Scans after similar bolus inhalation of xenon^{133} confirmed that RV boluses went preferentially to apical regions and that TLC boluses went preferentially to basal regions. Boluses delivered near RV (i.e., to apical regions) resulted in a D_L/V_A at high lung volumes of 5.2 ± 2.1 (mean ± S.D.) that increased during exhalation to a maximum of 9.4 ± 1.6 near RV. Boluses delivered near TLC (i.e., to basal regions) resulted in a D_L/V_A at high lung volumes of 8.2 ± 0.9 that increased during exhalation to 11.1 ± 1.7 near functional residual capacity (FRC) and then decreased to 9.6 ± 1.6 near RV. We conclude that: D_L/V_A increases from lung apex to lung base; that the determinants of D_L in both apical and basal regions are relatively unaffected by V_A from TLC to FRC; and that below FRC, the decrease in basal D_L/V_A could reflect either a true decrease in determinants of D_L or be the result of airway closure in the lung bases. (Supported in part by USPHS NIH Grant HL-24136.)

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REGIONAL DIFFERENCES IN THE CONDUCTANCE-PERFUSION RELATIONSHIP OF AVIAN EGGS. Mary Anne Rokitka, Amos Ar* and Hermann Rahn. Dept. of Physiology, State Univ. of New York at Buffalo, Buffalo, N. Y. 14214

The gas spaces created by the fibers of the membranes that lie between the avian egg shell and the chorioallantoic capillaries are analagous to the alveolar gas spaces in the mammalian lung except that convective alveolar ventilation ($\dot{V}_A$) is replaced by the diffusive egg shell conductance (G). Thus any regional differences in the O_2 and CO_2 concentrations in the gas spaces of the shell membranes should reflect regional differences in the conductance-perfusion ratio ($G/\dot{Q}$). A technique was devised for sampling regional gas concentration which shows that the blunt end of the egg has lower CO_2 and higher O_2 tensions than the pointed end. Since it had previously been shown that regional conductance decreases from the blunt to the pointed end, one can assess regional differences in chorioallantoic perfusion which increases from the blunt to the pointed end.

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CONVECTIVE GAS FLOW CAUSED BY DIFFUSION EXPLAINS POSITIVE HYDROSTATIC PRESSURE IN HEN'S EGGS. A. Ar*, C.V. Paganelli, L.E. Farhi and H. Rahn. State University of NY at Buffalo, Buffalo, NY 14214.

Gas exchange in incubating hen's eggs occurs primarily by diffusion through pores in the shell. Since there is a net loss of mass (as water vapor), diffusion is non-equimolar in character, and results in a small hydrostatic pressure difference (ΔP) across the shell, as well as partial pressure differences for O_2, CO_2 and H_2O. We measured an average ΔP in 17 incubating hen's eggs of 24 (± 2.4 SE) Pa (2.4 mm H_2O), inside positive, while ΔP calculated from Poiseuille's Law using known values of mean pore number, diameter, and length was 4 Pa. The 6-fold difference between measured and calculated ΔP probably results from pore size distribution: in relatively few large pores, convection predominates, while in many small pores, diffusion prevails. A simple model was developed to partition gas conductances of the hen's eggshell into purely diffusive and convective components. Convection facilitates CO_2 and water transport by 3 and 5%, respectively, and hinders O_2 transport by as much as 16%. (Supported in part by NIH Grants # RO1-HL-18022 and PO1-HL-14414 and PO1-HL-23190.)

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RELATIVE 02 AND CO AFFINITY OF SHEEP WHOLE BLOOD. J.-Rene Haag*, Martin Tschopp*, Aldo Tempini* and Pierre Haab* (SPON: Mario Wiesendanger). Dept. of Physiology, Univ. of Fribourg, CH-1700 Fribourg, Switzerland

In order to investigate whether hemoglobin saturation has an influence on the magnitude of Haldane's constant M (M = [HbCO/HbO2]·[PO2/PCO]) blood was equilibrated in long duty tonometers with O2-CO-N2 gas mixtures in which PO2 and PCO were insufficient to saturate hemoglobin. The experiments were performed on the blood of four sheep for which the dissociation curves for O2 and CO alone were also determined. Gas mixture composition was controlled by Scholander and infrared analyses. CO and O2 blood concentrations were measured by Van Slyke and gas chromatography. The results show that when Hbred is present, M is an increasing function of HbO2 and a complex function of HbCO. Comparison of O2 and CO dissociation curves shows that the ratio of the PO2 and PCO corresponding to the same Hb saturation also varies but to a much smaller extent than M. The data suggest that the presence of one ligand on the Hb molecule may modify the heme-heme interaction for the other ligand. In practice, they point to the fact that calculation of blood PCO by the Haldane's equation cannot be performed with an unique value of M. (Supported by SNSF, grant no 3665-0.75)

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THE EFFECT OF URINARY pH ON INTRARENAL FORMATION OF KININS
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Although urinary kallikrein (UKK) has been measured and assumed to be a good indicator of the intrarenal formation of kinins, other factors such as pH, amount of substrate, kininases, inhibitors, etc. could affect kinins release from kininogen. We studied whether U pH affects the intrarenal release of kinins. Rats were anesthetized and catheters placed in the jugular vein, femoral artery, and in both urethers. Only rats in which blood was not detected in urine (Multistix) were studied. After control clearance of 1 hr (iv 2.5% dextrose infusion), the rats were infused with 4% Na_2SO_4 to acidify the urine. U kinins and KK (RIAs), pH, osmolarity (Osm), volume (V), inulin and PAH clearance were measured. The Na_2SO_4 infusion decreased U pH from 6.05±0.08 to 5.12± 0.11 ($p<0.001$), and kinins from 33±9.5 to 10±2.3 pg/min ($p<0.05$). U Osm increased from 217±21 to 418±65 mOsm/Kg ($p<0.05$), and KK from 0.7±0.1 to 1.2±0.19 µg/min ($p<0.05$). U kinins correlated with pH ($r=0.6$, $p<0.001$) and Osm ($r=-0.40$, $p<0.001$). A strong correlation between U kinin in the left and right urethers was observed ($r=0.91$, $p<0.001$). UKK correlated with both pH and Osm ($r=-0.59$ and 0.48, respectively). No correlation was found between U kinins and KK, V, GFR or renal plasma flow. It was concluded that changes in pH and/or Osm influenced the intrarenal formation of kinins and that UKK is not necessarily a good indicator of the intrarenal formation of kinins.

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Ca-DEPENDENT INHIBITORY EFFECT OF ANTIDIURETIC HORMONE (ADH) ON RENIN SECRETION (RS). Paul C. Churchill and Monique C. Churchill.* Wayne State University, Detroit, Mi. 48201

Previous studies have shown that depolarization of the JG cell inhibits RS, presumably by increasing Ca influx through voltage-sensitive channels as D-600, a specific Ca-antagonist, blocks the inhibitory effect. As shown below, ADH inhibited RS during two consecutive periods of incubation of rat kidney slices.

ADH, U/L	RS Rate, ng hr^{-1} mg^{-1}/30 min period	
0	287 ± 15	309 ± 21
5	233 ± 22	275 ± 22
50	153 ± 16	158 ± 14
500	114 ± 21	104 ± 17

Buffering extracellular Ca to approximately 10^{-8} M stimulated RS and abolished the inhibitory effect of ADH. However as can be seen below, $5x10^{-7}$/M D-600 completely blocked the inhibitory effect of K-depolarization, but 100 U/L of ADH still inhibited RS by approximately 50%. Thus inhibition of RS by ADH requires Ca influx and / or mobilization but not Ca influx via voltage-sensitive channels. (Supported in part by NIH # HL 24880-01)

initial period:added between initial and final periods	RS Rate, ng $hr^{-1}mg^{-1}$/20 min initial	final
60 mM K:diluent	5 ± 2	2 ± 2
60 mM K:D-600	8 ± 2	184 ± 25
60 mM K + ADH:D-600	5 ± 2	90 ± 23

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RENIN SECRETION (RS) BY INTACT (IK) AND DENERVATED (DK) KIDNEYS IN RESPONSE TO CAPTOPRIL IN THE DOG. J.P. Girolami*, J.L. Ader*, T.C. Lee, T. Tran Van*, M.P. Cavalier*, M.H. Maxwell and J.M. Suc*. Faculte de Medecine de Rangueil, Toulouse, France, and Cedars-Sinai Medical Center, Los Angeles, Ca 90048

Chronic renal denervation suppresses RS, but the effect of acute denervation on RS and the responsiveness of renin secreting cells have not been studied. Thus, we assessed the latter by comparing the renin secretory responses of the DK and the contralateral IK of dogs (n=12) to inhibition of angiotensin II formation produced by the converting enzyme inhibitor Captopril (1 mg/kg, iv). Renal functional parameters were monitored 30 min after surgery for 4 consecutive periods. After denervation, Na excretion ($U_{Na}V$), fractional Na excretion (FE_{Na}) and renal blood flow (RBF) were greater by 134, 86 and 22%, resp., while glomerular filtration rate (GFR) did not differ and RS rate was less by 40% when compared to the IK. Captopril produced similar percentage increases in $U_{Na}V$, FE_{Na} and RBF in both kidneys above their resp. controls and GFR remained constant. In contrast, whereas RS rate in the IK rose progressively after Captopril, the RS rate of the DK did not rise beyond an initial increase and remained significantly below those of the IK at all time periods. Our results thus indicate that relative refractoriness of the renin secreting mechanism occurs soon after denervation, reflecting possibly the net effect of an increase in the transmural pressure at the baroreceptor site, an increase in Na delivery to the macula densa and/or a reduction in direct neural input to the renin secreting cells.

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ACUTE EFFECTS OF LEAD ON PLASMA ANGIOTENSIN II AND HEPATIC REMOVAL OF RENIN. Jay M. Goldman*, Arthur J. Vander, Joan Keiser*, and M. Gary Nicholls*. Univ. of Michigan, Ann Arbor, Mich. 48109.

Our earlier studies established that intravenous Pb acetate (3mg Pb/kg) acutely raises plasma renin activity (PRA) without an associated increase in renin secretion. The present experiments evaluated acute effects of Pb on hepatic removal of renin and on arterial angiotensin II (AII) levels in pentobarbital-anesthetized dogs. Hepatic removal of renin was completely inhibited in all 3 Pb-treated dogs; extraction fell from 38% (baseline) to -5% by 3h after Pb. In controls, PRA was raised by tightening an aortic clamp placed proximal to the renal arteries; renin extraction fell slightly as PRA rose, but total removal of renin increased. Despite the striking rises in PRA, arterial AII did not increase after Pb. Plots of AII as a function of PRA were established for control dogs (n=9) at 1,2, and 3h after either no intervention or after acutely raising PRA by aortic clamping or furosemide administration; the linear relationship of AII to PRA was unchanged with acute rises in PRA. After Pb, however, the correlation was lost; by 2h after Pb, AII levels in 6 of 9 dogs and, by 3h, AII levels in 8 of 9 Pb dogs, were below the 98% confidence limits for AII levels as a function of PRA in controls. We conclude that the major mechanism by which Pb acutely raises PRA is by completely inhibiting hepatic removal of renin; further, Pb prevents AII from rising proportionately with PRA, probably by inhibiting angiotensin-converting enzyme.

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PLASMA PRORENIN-RENIN IN BARTTER'S SYNDROME, CYSTIC FIBROSIS, AND OTHER HIGH RENIN STATES, AND THE EFFECT OF PROSTAGLANDIN INHIBITORS. L.L.Chan*, D.H. Osmond, J.W. Balfe*, M.L. Halperin*, and M. E.Goldstein*. Depts. of Pediatrics, Physiology, and Medicine, University of Toronto, Toronto, Canada M5G 1X8.

High renin states imply a strong stimulus for renal renin secretion and/or increased conversion of "prorenin" to renin in the blood. Little is known about such conversion, so we looked for evidence of a reciprocal relationship between plasma levels of prorenin and renin. 15 normal subjects, aged 19-42, and 8 patients with extracellular fluid contraction were studied; of whom 5 had Bartter's Syndrome, 1 had Cystic Fibrosis and 2 were chloride deficient,(1 with hypokalemia). All patients had elevated plasma renin activity (PRA, range 24-475 ng angiotensin I/ml/hr vs 2.3 ± 0.3 in the control group). After activation with trypsin, 0.5 mg/ml plasma, prorenin as % of active renin averaged 103 ± 24 in the controls but was essentially zero in 5/8 of the patients, and sub-normal in the other 3. These data suggest that endogenous prorenin conversion in vivo was so active to preclude further activation by trypsin and that part of the PRA elevation was due to prorenin conversion, implying reciprocity of prorenin/renin. Prostaglandin inhibition for 1 week in 3 Bartter's patients reduced PRA by 50-75% and increased prorenin as % of active renin 2-5 times. These effects may be due to inhibition of both renal renin release and prorenin conversion to renin.
Supported by Ontario Heart Foundation and MRC.

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EFFECTS OF INDOMETHACIN ON RENIN (RS) STIMULATED BY AORTIC CONSTRICTION (AC), ISOPROTERENOL (I) AND FUROSEMIDE (F). Edward H. Blaine, Rose Marie Evans* and Terry Schorn.* Merck Institute for Therapeutic Research, West Point, PA 19486

Mongrel dogs were anesthetized with 60 mg/kg of vinbarbital and a right nephrectomy performed. An electromagnetic flow probe was placed around the left renal artery and an indwelling 22 g needle was inserted distal to the probe. An 18 g needle was inserted into the renal vein and catheters were placed into the femoral artery and vein. After a 40-min control period, renin secretion was measured during aortic constriction (▲BP-40 mm Hg), intrarenal artery isoproterenol infusion (0.1 µg/kg min) and intravenous furosemide (1 mg/kg) in 3 dogs. The three stimuli were applied sequentially for 40-min periods with RS measured at 10-min intervals. A second group of 5 dogs received 5 mg/kg indomethacin 30 min prior to the control period and were tested in an identical manner. The data suggest that blockade of prostaglandin synthesis partially inhibits the renin stimulatory effects of decreased renal perfusion and furosemide. RS stimulated by isoproterenol infusion was not blunted and may have been augmented.

Control, ng AI/min		AC, ng AI/min	
s Indo	c Indo	s Indo	c Indo
185 ± 165	161 ± 74	533 ± 203	304 ± 56
I, ng AI/min		F, ng AI/min	
s Indo	c Indo	s Indo	c Indo
442 ± 244	933 ± 345	299 ± 151	147 ± 56

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COMPARISON OF ISOLATED GLOMERULI FROM RAT AND DOG AND THEIR RESPONSE TO ARACHIDONIC ACID. S. Schryver*, W.H. Beierwaltes, E. Arne* and J.C. Romero, Dept. of Physiology, Mayo Clinic, Rochester, Minnesota 55901.

Isolated rat glomeruli superfused with arachidonic acid have been shown to synthetize prostaglandins and release renin. A technique for isolating glomeruli from the dog has been developed because of the restricted yield of glomeruli imposed when using the smaller rat. Dog kidneys were removed and flushed with Krebs ringers. Glomeruli were harvested from cortical tissue using a passive sieving technique as used previously in the rat. Dog kidneys yielded 686±56 (SE) mg wet wt of glomeruli/animal (protein=4.3±0.4 g%) compared to 43±5 mg glomeruli/animal obtained from rats, p<0.001, (protein=4.1±0.4 g%). For comparison, 50 mg glomeruli from dogs or rats were suspended in glass chambers and superfused with Krebs (0.3 ml/min), and the effluent was collected in 10 min fractions. Arachidonic acid was added to the superfusate (1.6×10^{-4}M) and the effluent was measured for PGE_2, PGI_2 and renin concentration. PGE_2 increased significantly in dogs (29±7 to 908±150 pg/ml) and in rats (20±2 to 610±81 pg/ml). PGI_2 also increased significantly in dogs (71±20 to 1536±462 pg/ml) and in rats (0 to 42±6 pg/ml). Renin concentration rose in dogs (4.6±2.5 to 8.4±2.0 ng A-I/ml/hr) and in rats (4.4±1.5 to 14.1±6.0 ng A-I/ml/hr). These results suggest that isolated dog glomeruli are similar to isolated rat glomeruli in that they respond to arachidonic acid, while offering the advantage of simplified recovery and a significantly greater yield of useable tissue. (NIH HL 16496)

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EFFECT OF SAR^1-ILE^8-ANG II AND SQ 14225 ON RABBIT URINARY PGE_2 EXCRETION AND RENAL TISSUE PGE_2 PRODUCTION. A.A. Attallah*, R.A. Stahl*, D.L. Bloch*, J.L. Ambrus* and J.B. Lee, SUNY at Buffalo, N.Y. 14215

To test the hypothesis that renal prostaglandin (PG) biosynthesis is regulated by angiotensin II (A II), rabbits were chronically treated with the A II antagonist Sar^1-Ile^8-A II (Sar^1) (250 mg/kg/3 h, s.c.) or with the converting enzyme inhibitor SQ 14225 (20 mg/kg/24 h, p.o.). Following sacrifice, slices of renal cortex, outer medulla and papilla were either extracted for initial PGE_2 concentration or incubated in Krebs-Ringer HCO_3^-, 95% O_2-5% CO_2, 37° C, 30 min for PGE_2 production in vitro. Sar^1 at a dose which blocked the rise in blood pressure, induced by 0.2 mg A II, produced a marked reduction in daily $U_{PGE_2}V$. Sar^1, furthermore, significantly decreased cortical, medullary and papillary PGE_2 biosynthesis. SQ 14225 at a dose which blunted the vasopressor action of A I also inhibited renal PGE_2 biosynthesis. During SQ 14225 administration, $U_{PGE_2}V$ was not significantly changed possibly due to the concurrent increase in urine flow rate. We conclude that A II blockade with Sar^1 or converting enzyme inhibition with SQ 14225 results in a marked reduction in renal PGE_2 biosynthesis suggesting that A II may normally modulate basal renal PG biosynthesis in vivo.

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FAILURE OF INDOMETHACIN TO BLOCK RENAL VASCULAR RECEPTOR CONTROL OF RENIN RELEASE IN THE CONSCIOUS DOG WITH A NONFILTERING KIDNEY. R.H. Freeman, J.O. Davis, S.F. Echtenkamp* and J.R. Dietz* Dept. Physiol., Univ. Mo. School Med., Columbia, Missouri 65212

Plasma renin activity (PRA) was measured in conscious dogs (n=5) with a single, denervated nonfiltering kidney during step reductions in renal perfusion pressure (RPP) before and after the administration of indomethacin (8mg/kg BW, IV). Prior to indomethacin administration, a reduction in RPP from 112±4 to 50±3 and 57±2 mmHg (p<.01) resulted in an increase in PRA from control levels of 0.32±0.13 and 0.32±0.14 ng/ml/hour to 1.50±0.49 and 1.81±0.71 ng/ml/hour (p<.05) after 30 and 40 minutes, respectively. Following release of the constriction, RPP returned to 116±6, 113±4, and 116±4 mmHg and PRA returned to 0.70±0.25, 0.50±0.23, and 0.14±0.05 ng/ml/hour (p>.05) after 30, 60 and 90 minutes. Indomethacin was then given IV as a bolus. After 30 and 60 minutes, RPP was 114±3 and 113±3 mmHg and PRA averaged 0.19±0.12 and 0.24±0.14 ng/ml/hour (p>.05) respectively. RPP was reduced to 52±4 and 58±2 mmHg (p<.01) and again PRA increased to 0.97±0.47 and 1.38±0.77 ng/ml/hour (p<.05) after 30 and 40 minutes. Following release of the constriction, PRA returned to 0.35±0.17 and 0.18±0.07 ng/ml/hour (p>.05) after 30 and 60 minutes. These data suggest that prostaglandins do not mediate renin release elicited by decreased RPP in conscious dogs with a denervated nonfiltering kidney and a nonfunctional macula densa mechanism.

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TEMPERATURE INDUCED CONVERSION OF RENAL ALPHA AND BETA ADRENERGIC RESPONSES. Elizabeth J. Corwin*, and Richard L. Malvin, University of Michigan, Ann Arbor, Michigan 48109.

Adrenergic stimulation of renal beta receptors increases renin release. Alpha adrenergic effects on renin release appear to be inhibitory. We suggest that altered temperature may induce an interconversion of α and β responses. Renal cortical slices from dogs were incubated in a physiological salt solution. Pre-incubations were carried out at 20°C, during which time the α antagonist phenoxybenzamine (POB) was added to 1/2 of the slices. After 1 hour, the slices were placed in new media kept at 37°. Isoproterenol (ISP) was added to slices which had received the POB and to control slices. Slices which received only ISP showed a 48% stimulation of renin release. Slices which were exposed to POB at 20° were unresponsive to ISP. These experiments were repeated keeping the temperature at 37° throughout. In these experiments pretreatment with POB did not prevent the slices from responding to ISP. One may conclude from these results that at 20°, but not at 37°, POB can bind β receptors irreversibly. In summary, with a change in temperature there is a change in the renal response to adrenergic stimulation. This phenomenon may be due to an interconversion of the renal α and β receptors. (Supported in part by NIH Grant HL18575).

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RENAL Na-K-ATPase IN OKAMOTO (SHR) AND DAHL HYPERTENSIVE (SS) RATS. C. Rodriguez-Sargent*, J.L. Cangiano, S. Opava-Stitzer, M. Martínez-Maldonado, Research and Medical Service, Veterans Administration Center, San Juan, PR. 00936

Plasma renin activity (PRA) (ng/ml/hr) plasma aldosterone (PA) (ng%) and renal Na-K-ATPase activity (μmPO_4/protein/hr) were measured in 8 SHR and 8 SS and their controls (NR & SR) after 2 weeks on a normal and free sodium diet, respectively (see table). After a normal sodium diet PRA and PA were lower in both groups of hypertensive rats. Na-K-ATPase was lower in SS. In SHR it was not different from control. On a sodium free diet PRA and PA remained low in SHR but ATPase increased. In contrast, PRA, PA and ATPase were lower in SS. These results suggest that renal Na-K-ATPase regulation in SHR is independent of PA. SS is a model of volume expansion hypertension.

	Normal sodium diet			Sodium free diet		
	PRA	PA	ATPase	PRA	PA	ATPase
SHR	6.1	20	72.6	5.6	27.6	163.2
NR	12.9	37	83.5	29.2	68.8	150.7
SS	3.7	18	42.1	8.1	43.4	86.7
SR	9.8	31	78.8	16.2	59.4	174.2

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RESPIRATORY PATTERN CHANGES ACCOMPANYING ACUTE FALL IN CARDIAC OUTPUT. J. Fisher, R. Wise, M. Pinsky, M. Weisfeldt, S. Permutt and W. Ehrlich. Johns Hopkins Hospital, Baltimore, Maryland 21205

Recent studies have shown that cyclic elevation in intrathoracic pressure has beneficial circulatory effects during cardiac arrest and experimentally produced cardiac depression. We investigated immediate respiratory changes accompanying a precipitous fall in cardiac output (CO) for pleural pressure (PP) changes, as increased PP swings might represent a homeostatic response. Using chronically instrumented dogs, CO was acutely increased or decreased by rapid ventricular pacing (mean 213 vs 347 bpm, respectively) for 30 seconds. Decreased CO (3.2±.1 to 2.0±.1 L/min, p <.001) was accompanied by increases in tidal volume (498 to 763 ml, p <.001), and minute ventilation (14.5 to 20.6 L/min, p <.001) within 2 to 4 breaths without changes in respiratory rate (43.4 to 46.8,ns). Furthermore, end-expiratory pressure increased (-7.5 to -3.1 mmHg, p <.001) and end-inspiratory pressure decreased (-17.6 to -26.8 mmHg, p< .001) resulting in a marked increase in the PP "swing" (10.1 to 23.5 mmHg, p <.001). Accompanying these changes were an increased arterial pH and pO_2 with a decreased pCO_2. These PP and ventilatory changes did not occur when rapid ventricular pacing increased CO (3.4±1.2 vs 4.1±1.3 L/min, p <.001). Thus, in the unanesthetized dogs there are immediate increases in pleural pressure swings, tidal volume and minute ventilation accompanying a tachycardia-induced acute fall in CO. Neither arterial hypoxemia nor rapid ventricular pacing is responsible for these respiratory changes.

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THE EFFECTS OF MECHANICAL VENTILATION ON RESPIRATORY DRIVE AND OFF-SWITCH. A. Jasper*, C.S.H. Tan*, and D.H. Simmons. Dept. of Med., Univ. of Calif., Los Angeles, CA.

We studied 8 dogs anesthetized with barbiturates during 16 pairs of 30-minute periods of spontaneous breathing (SB) and mechanical ventilation (IPPB) maintaining $PaCO_2$ and pH constant (± 1 torr, ± 0.01) in each pair. We randomly selected tidal volume (V_t) during IPPB to equal (n=7) or exceed (n=9; mean increase 56%, range 7-120%) V_t during SB. Wires were placed in the diaphragm to obtain the electromyogram (EMG) and its moving average. During controlled IPPB breaths (i.e., triggered by the examiner) there was no phasic diaphragmatic activity and no muscular work of breathing. During assisted IPPB (i.e., triggered by the dog's inspiratory effort) the diaphragm was active during part or all of inspiration, and muscular work of breathing persisted but was less than during SB, especially with higher V_t. The inspiratory volume at which diaphragm activity ceased (off-switch volume) was equal to that during the paired SB in 10 of 16 (within 10% in 13 of 16) and was independent of V_t during IPPB. When V_t during assisted IPPB and SB were equal, the initial slope of the moving average EMG, an index of respiratory drive, was unchanged in 6 of 7 periods. When V_t during assisted IPPB exceeded that during SB, the slope fell in 7 of 9 (mean decrease 28% ± 24 (SD), range 0-54%; one increase +20%). Summary: 1) During controlled IPPB breaths there was no diaphragm electrical activity; 2) during assisted IPPB, respiratory drive fell with increasing V_t and decreasing work of breathing; 3) off-switch volume during assisted IPPB was the same as during SB.

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INCREASED VENTILATORY DRIVE DURING POSITIVE PRESSURE BREATHING AT CONSTANT ARTERIAL P_{CO_2} IN MAN. R. Banzett, J. Mead, K. Strohl* and B. Geffroy*. Harvard School of Public Health, Boston, MA 02115

Inspiratory muscle activity increases when lung volume is increased by continuous positive pressure in conscious human subjects (Green, et al, Resp. Physiol. 35: 283-300; Banzett et al, Fed. Proc. 38: 1300). Because end-tidal PCO_2 did not change, these increases have not been attributed to chemoreflexes. However, continuous positive pressure breathing at 20 cmH 0 influences the end-tidal arterial PCO_2 differences (Folkow and Pappenheimer, JAP 8: 102-110). We have compared end-tidal with arterial P_{CO_2} in healthy human subjects exposed to continuous positive airway pressure (10 cmH 0) or continuous negative pressure around the thorax (-25 cmH 0) sufficient to increase mean lung volume by about 650 ml. The relationship between end-tidal and arterial PCO_2 was not altered, and we conclude that end-tidal P_{CO_2} is a valid measure of chemical drive to ventilation in such circumstances. We, too, observed substantial increases in respiratory muscle EMG during pressure breathing which we conclude must originate by proprioception. On average, the compensation thus afforded was complete in terms of arterial partial pressures but the wide variability of individual responses suggests that there was a large cerebral cortical component in the responses seen here. (Supported by NIH Grants HL 4580-09 and GM 07560)

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IMPORTANCE OF NEURAL DYNAMICS IN CAT LUNG VOLUME FEEDBACK REGULATION. Stan Yamashiro and Alfred Cheung* USC, Los Angeles, California 90007

The hypothesis that the neuronal pool responsible for central inspiratory activity (CIA) has intrinsic dynamics important to lung volume regulation has been explored. If these dynamics are present, the volume-time threshold of inhibition should be a function of stimulus duration as well as magnitude. To test this possibility, experiments were conducted in pentobarbital anesthetized (30mg/Kg i.p.) and gallamine paralyzed cats using the servo-respirator method. CIA responses as measured by rectified and moving average filtered (25msec) phrenic nerve activity (C5) were compared for step tracheal pressure inflations initiated at 0 and 400 msec following the start of phrenic activity. The threshold of inhibition was estimated by the volume-time point where inflation responses just began to decrease below 0 inflation responses (respirator grounded). Four spontaneous breaths were allowed between each test inflation for recovery. All responses were ensemble averaged over 5 trials. In 4 cats, threshold inhibition for delayed inflations occurred 120-260 msec after the corresponding point with 0 delay inflations. Stimulus duration does appear to affect the volume-time threshold of inhibition. (Supported in part by Am. Heart Assoc. Grant #642, L. A. Chapter and NIH Grant #HL07012.)

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EFFECT OF BACKGROUND ELASTIC LOADING ON MAGNITUDE ESTIMATION OF INSPIRATORY ELASTIC LOADS. W.R. Revelette*, R.L. Wiley and F.W. Zechman. Miami Univ., Oxford, OH 45056 and Univ. of Kentucky Med. Cntr., Lexington, KY 40506

The purpose of this study was to determine whether a background elastic (E) load affected subjects' ability to estimate magnitudes of added E loads. 7 control E loads from 3.9 - 21.9 cmH_2O/L were randomly presented 4 times each. Magnitude estimation of loads was expressed by squeezing a handgrip dynamometer. Above a 3.9 cmH_2O/L background load on every breath, 6 loads from 3.5 - 18 cmH_2O/L were similarly scaled. Assuming Steven's power law relationship, a linear regression was calculated on the averaged responses and the E load stimuli. The slopes, or exponents of the power law were 0.68 for control and 0.62 with background load and were significantly different ($p > .20$). These data indicate that this level of background E loading does not alter the perceptual performance for estimating added E loads. This is consistent with magnitude estimation with background stimuli of other sensory modalities when the added stimuli are of greater intensity than the background stimulus. (Supported in part by NIH Grant #HL24412)

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THE ROLE OF END-INSPIRED POSITION IN THE PERCEPTION OF INSPIRED VOLUME. L.J. Folinsbee, J.A. Gliner, and S.M. Horvath. Inst. Environmental Stress, U. Calif. Santa Barbara, CA 93106

Man is capable of reproducing inspired lung volume (IV) with relative precision (Gliner et al FED PROC 39:831). In order to determine the importance of cues which may aid the subject in determining IV, we performed two experiments. In each experiment, the subject began by inspiring a breath corresponding to 25, 50, or 75% of IC and then expired to near RV. On the ensuing breath, the subject either tried to reproduce the IV (VOL-RV reproduction) or tried to reproduce the previous end-inspired position (EIP reproduction). These data were compared with previous data where the subject attempted to reproduce IV after expiring to the normal FRC (VOL-FRC reproduction). Seven subjects performed 30 reproductions of each volume (25, 50, 75% IC) for each type of reproduction. The Just Noticeable Difference (JND) at small IV (25% IC) for EIP (139 ml) was larger than VOL-FRC (109 ml). For large IV (75% IC), EIP (138 ml) and VOL-FRC (139 ml) were identical and about 30% less than VOL-RV reproduction (202 ml). For VOL-RV reproductions JND increased with increasing volume but JND remained constant for EIP reproduction. These data suggest that cues other than EIP are important in perceiving IV at small tidal volumes (VT). At large VT, EIP is an important cue. However, the overall lower JND for VOL-FRC reproductions indicates that both cues related to EIP and volume change are used to perceive inspired volume.

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CAROTID SINUS AND MEDULLARY CHEMORECEPTOR CONTRIBUTIONS TO PHRENIC AND HYPOGLOSSAL NERVE RESPONSES DURING REBREATHING. E.N. Bruce*, N.S. Cherniack, J. Mitra*, D. Weiner*, and J. Salamone*. CWRU, Cleveland, Ohio 44106.

Peak phrenic (Phr) and hypoglossal (Hyp) nerve activities do not increase in parallel as chemical drive (hypercapnia or hypoxia) increases. At low drive peak Phr increases more with drive than does peak Hyp; at high drive the reverse is true. We studied the relative contributions of carotid sinus and medullary chemoreceptors to Phr and Hyp responses to hyperoxic hypercapnia by comparing Hyp to Phr nerve activity during rebreathing runs done: 1) before and after bilateral carotid sinus nerve section (CSNX) (7 animals), or; 2) before and during cooling of the intermediate (I) area on the ventral surface of the medulla (4 animals). The studies were performed in chloralose-anesthetized, vagotomized, paralyzed and artificially ventilated cats. CSNX caused small or no decrease in peak Phr or average rate of rise of Phr as a function of PCO_2 during rebreathing, but Hyp was diminished significantly.[2] Consequently CSNX resulted in less Hyp activity at a given level of Phr activity. On the contrary, cooling of the I area depressed Phr response to hypercapnia much more than Hyp response, resulting in more Hyp at a given Phr. We conclude that during hyperoxic hypercapnia phrenic activity is dependent more on central (medullary) than on carotid sinus chemoreceptor input, while in contrast hypoglossal nerve activity is more dependent on peripheral (carotid sinus) chemoreceptor input. (Supported by NIH Grant HL 20847).

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HUMAN EXTERNAL INTERCOSTAL EMGs. T. Lynne-Davies*, J. Bowden*, R. Yamane*, B. Muller*, and P. Lynne-Davies. Dept. of Medicine Wayne State University, Detroit, Michigan 48201.

We recorded parasternal surface EMGs in the second and third right intercostal spaces during 19 series of breaths in five normals. Averaged power spectra were computed from DC-1 KHz for each series, and centroids (fc) calculated for the bandwidth 25-1,000 Hz. Three types of spectra were observed: A. those with a single high-frequency peak; B. those with two discrete concentrations of power, between 25-125 and 251-501 Hz; and C. those with a single low-frequency peak:

		% POWER				
TYPE	N	25-125	126-250	251-500	501-1,000 Hz	fc (Hz)
A.	10	21.2	28.7	36.7	13.5	284
B.	6	36.4	24.1	28.4	10.7	242 $p<0.02$
C.	3	63.5	17.5	14.3	4.7	157 $p<0.001$

These differences reflected the magnitude of pectoralis major EMG, assessed by another electrode sited over the anterior axillary fold. This was greatest in type C. High-frequency signals resembling type A were recorded in one subject over the triangle of ausculatation, at the posterior angle of the scapula. We conclude: a) external intercostal EMGs contain significant high-frequency components; b) interference from other muscles occurs often; c) this renders intercostal EMGs unsuitable for studies of respiratory muscle fatigue. (Supported by California Lung Association and NHLBI Grant #HL 26327)

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VAGAL MECHANISMS IN RECRUITMENT OF ABDOMINAL EXPIRATORY MUSCLES. J.P. Farber. Dept. of Physiology and Biophysics, University of Oklahoma HSC, Oklahoma City, OK 73190

Abdominal expiratory muscles can be recruited when breathing against continuous positive airway pressure (CPAP), and in several mammals vagotomy abolishes this response. To more fully evaluate the role of vagal input in expiratory motor activation, studies were performed in Inactin-anesthetized adult opossums using graded vagal cooling and unit recording of afferent vagal discharge. Diaphragm and abdominal muscle electromyograms (EMG) were measured along with end-tidal CO_2 levels. With 6-8 cmH_2O CPAP, cooling the vagi to 4-10°C among animals completely supressed the abdominal EMG. Variable increases were observed in diaphragm EMG amplitude and duration but these effects did not typically lower the end-tidal CO_2. Presumably, the loss of expiratory motor activity impaired ventilatory efficiency. With further cooling of the vagi, additional increases in diaphragm discharge and lowering of end-tidal CO_2 were observed. In separate studies, one vagus nerve was cut and recordings were made from thin filaments of its central stump. The discharge rate of vagal units with low threshold to lung inflation was greatly reduced by cooling the nerve to levels compatible with blockade of abdominal expiratory activity. Peak activity of vagal units during inspiration was most readily affected by cooling. A reflex involving low threshold pulmonary stretch receptors may contribute to abdominal expiratory activity during CPAP. (Supported by HL-24865 from NIH)

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FREQUENCY SPECTRUM OF HUMAN STERNOCLEIDOMASTOID EMGs. R. S. Fine*, J.A. Bowden*, and P. Lynne-Davies. Department of Medicine, Wayne State University School of Medicine, Detroit, MI 48201.

Sternocleidomastoid (SCM) is the only accessory muscle of respiration from which it should be possible to record surface electromyograms (EMGs) with little risk of interference from other muscle groups. We performed spectral analysis on such signals recorded in 3 normal subjects via skin electrodes applied over the superior aspect of the body of the muscle, lateral to platysma. Significant EMG activity was detectable at 5-10 Hz and extended to approximately 250 Hz, while motion artefact (when present) was concentrated in the range DC-5 Hz. We therefore calculated the centroid (fc) for the bandwidth 10-250 Hz. When subjects voluntarily increased their ventilation, SCM activity was first reliably detected at an average tidal volume ($\pm$ SD) of 2.76 $\pm$ 0.38 L. In these circumstances fc averaged 72 $\pm$ 4 Hz. During sustained fatiguing contractions (lateral rotation of the head against a fixed resistance, and maximum inspiratory efforts) fc fell to 52 $\pm$ 6 and 50 $\pm$ 7 Hz, respectively ($p < 0.01$ in both cases). We conclude that monitoring EMGs recorded from SCM may be helpful in the detection of ventilatory muscle fatigue. Supported by NHLBI, Grant No. HL26327

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PNEUMOTAXIC CENTER NEURON RESPONSES TO INSPIRATORY INHIBITORY REFLEXES FROM INTERCOSTAL AND ABDOMINAL MUSCLES. R. Shannon, D.L. Freeman* and B.G. Lindsey*. Dept. of Physiology, Col. of Med., Univ. of So. Florida, Tampa, Florida 33612.

Intercostal and abdominal muscle proprioceptor afferents (Group I) have been shown to inhibit medullary inspiratory (I) neuron activity. Experiments were conducted to determine if these reflexes influence respiratory units (RU) in the rostral pons (pneumotaxic center, PC). Mid-collicular decerebrated, vagotomized, paralyzed, artificially ventilated cats were used. Phrenic (PA) and pontine RU extracellular activities were recorded during electrical stimulation of intercostal nerve afferents (INS). INS prematurely terminated the activity of phasic I-neurons (5), and inhibited the I modulated portion of tonic firing I-neurons (10) without altering the tonic firing rate. INS during I shortened T_I and the subsequent T_E; there was a concomitant decrease in the duration of phasic E-neuron (3) activity and the E modulated portion of tonic E-neurons (5). INS during E prolonged the duration of activity of phasic E-neurons and the E portion of tonic E-neurons, without altering the slope of E-neuron activity. It is concluded that intercostal and abdominal muscle proprioceptor afferents do not directly affect pneumotaxic center neurons. The inhibition of PC I-neuron activity by these afferents is most likely secondary to the inhibition of medullary I-neurons that drive PC I-neurons. The change in PC E-neuron activity appears to be secondary to alterations in PC I-neuron activity. (Supported by USPHS, NIH Grant HL-17715).

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SPINAL INHIBITION OF PHRENIC MOTONEURONS BY STIMULATION OF AFFERENTS FROM PERIPHERAL MUSCLES. F. L. Eldridge, P. Gill-Kumar*, D.E. Millhorn and T.G. Waldrop*. Univ. of North Carolina, Chapel Hill, North Carolina 27514

Phrenic nerve responses to stimulation of calf muscle receptors or their afferents were studied in 36 cats. End-tidal P_{CO_2} was kept constant by a servo-controller. One group involved 17 paralyzed, vagotomized and glomectomized animals with intact central nervous systems. Stim. of calf muscles immediately increased frequency by shortening both T_I and T_E. It also caused magnitude and rate of rise of phrenic activity to increase, but only after an initial inhibition lasting about 5 sec. In a second group of 19 high (C_1) spinal animals, stim. of calf muscle or tibial n. uniformly caused inhibition of spontaneous tonic phrenic activity and that evoked by the intercostal-to-phrenic reflex. As stimulation continued, the inhibition lessened and poststimulus augmentation of phrenic activity also occurred. We conclude that muscle afferent input has predominantly facilitatory respiratory effects when acting through brainstem controller mechanisms, but a purely inhibitory effect on phrenic motoneurons when acting via spinal mechanisms. We suggest that the early inhibition of phrenic activity found in intact animals reflects the direct spinal effect. Since rate of rise of phrenic activity also decreases, the possibility of spinal modulation of this measurement must be kept in mind when it is used as an index of central respiratory drive. (Supported by USPHS grants HL-17689, NS-11132, & Pulmonary Training Grant HL-07106)

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THE EFFECT OF HYPERTONIC DEXTRAN ON RENAL BLOOD FLOW (RBF) IN THE NONFILTERING KIDNEY. Robert W. Gotshall. Wright State University School of Medicine, Dayton, Ohio 45435

Increases in plasma oncotic pressure have been shown to increase RBF. The mechanism by which this occurs is unknown, but may involve macula densa feedback regulation of GFR; the increased oncotic pressure reduces GFR which results in an alteration of the tubular signal to the macula densa resulting in vasodilation of the afferent arteriole to return GFR toward normal. To evaluate this possibility, 20% dextran (m.w. 79,000) was infused into the renal artery of normal and nonfiltering (NFK) kidneys. RBF in normal kidneys (n = 7) increased from 357 ± 47 to 468 ± 61 ml/min/100 gm ($P < 0.01$) in response to dextran; while RBF in NFK's decreased from 281 ± 38 to 247 ± 36 ml/min/100 gm ($P < 0.05$). Both dextran induced vasodilation in normal kidneys and vasoconstriction in NFK's was a consistent finding. Thus, the dextran vasodilation may be dependent on an intact tubular system for macula densa feedback. Dextran may also result in both vasodilation and vasoconstriction, with vasodilation dominating in normal kidneys and vasoconstriction dominating in NFK's when the vasodilatory response has been eliminated.
(Supported by USPHS. NIH Grant #HL21382)

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IN VIVO TISSUE THERMAL CONDUCTIVITY AND LOCAL BLOOD PERFUSION MEASURED WITH A HEAT PULSE-DECAY METHOD. K.R. Holmes and M.M. Chen*. Departments of Veterinary Biosciences and Mechanical Engineering, and Bioeng. Faculty, Univ. of IL, Urbana, IL 61801.

A method is described for measuring local tissue thermal conductivity k (W/mK) and volumetric perfusion rate p (ml/ml s). The method consists of inserting a thermistor microprobe (0.3 mm nom.) into the tissue and, after reaching thermal steady state, sending a short voltage pulse into the probe. The resulting small temperature increment T_0 subsequently decays due to thermal diffusive spreading and local blood flow. The relationship of T_0 at long post-pulse measurement times (typically 5-15 s) shows that with tissue perfusion, the apparent conductivity behaves as an exponential curve, where the exponential parameter is equal to p. Simultaneous calculations give $k/(\rho c)^{1/3}$. Preliminary experiments in dog myocardium and kidney cortex gave p's agreeing with published values obtained by other indicators. Important advantages over previous constant temperature methods are: This is an absolute measurement of p and no calibrations or stop-flow measurements are required; the sampling volume is shown to be considerably greater than the volume of microprobe traumatized tissue; results are independent of probe shape, size and properties; the electronics and calculations are extremely simple. The opportunity is offered to measure local blood flow in tissues previously inaccessible with trapped-indicator methods. (Supported in part by: NIH Radiation Oncol. Training Grant USPHS CA 09067, the Bioeng Program, and a Basic Research Support Grant from the Sch. of Life Sci. NIH RR07030, at the Univ. of Ill.-U.

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ACUTE HEMODYNAMIC EFFECTS OF THE CALCIUM ANTAGONIST DILTIAZEM IN CONSCIOUS WISTAR RATS. B.L. Pegram, S. Kulik, D. Duckworth and E.D. Frohlich. Alton Ochsner Medical Foundation, New Orleans, LA 70121.

Systemic and regional hemodynamics were determined in 8 conscious male rats before and during intravenous infusion of diltiazem (D). Catheters were placed in the left ventricle and femoral artery and vein under ether anesthesia. Three hours after recovery control measurements were obtained. D was then infused at an initial rate of 300 µg/kg/min for 20 minutes and then allowed to stabilize at 150 µg/kg/min for 10 min. Under these conditions mean arterial pressure decreased significantly (131±6 to 104±4 mm Hg; $p<0.001$). Cardiac index (reference sample method), total peripheral resistance index and heart rate all tended to decrease during D infusion, but were not significantly reduced. Significant regional hemodynamic changes, as determined with radioactive labeled microspheres, were noted only in skin and heart. Fractional flow decreased from 0.05 ±0.01 to 0.03±0.00 % CO/g tissue in skin but increased from 2.43±0.27 to 5.94±0.69 % CO/g tissue in heart. Blood flow was likewise decreased from 0.07±0.01 to 0.04±0.01 ml/min/g tissue ($p<0.005$; skin) and increased from 3.19±0.38 to 7.55±1.00 ml/min/g tissue ($p<0.005$; heart). While blood flow tended to increase to brain and to decrease to kidneys and splanchnic organs, it remained constant to skeletal muscle during D infusion. These data clearly indicate that while the antianginal agent D markedly increased cardiac flow in the conscious rat, blood flow to other organs was not compromised.

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FREQUENCY ANALYSIS OF DOPPLER SIGNALS FROM PERIPHERAL VESSELS. R.R. Gonzalez, Jr. and L.A. Aamodt*. Loma Linda University, Loma Linda, CA 92350

The Doppler ultrasonic flowmeter has been used for a number of years to estimate blood flow in intact vasculature. Typically the Doppler signal is amplitude modulated to produce a voltage which is proportional to the instantaneous average velocity of blood in a given vessel. We have chosen to evaluate the Doppler signal itself. The signal is a spectrum whose frequencies represent velocities of moving particles within the sound beam of the sensor. We obtained spectra from peripheral arteries with the use of a continuous-wave, direction-resolving Doppler in conjunction with a transcutaneous sensor. The spectra were digitized and transformed by a fast Fourier technique. Systolic blood flow into a normal resistive vascular bed (i.e. brachial) was characterized by a narrow frequency distribution around 2kHz, and diastolic flow by a low-frequency reversal or a low amplitude, low frequency forward transient. Systolic blood flow into a low resistance bed (i.e. cerebral) showed a broad frequency distribution around 2.5 kHz. The diastolic component was always forward and broadly distributed around 1.5 kHz. Post fist-clench hyperemic brachial flow resembled flow in the internal carotid artery, whereas common carotid flow after hyperventilation resembled resting brachial blood flow. These data suggest that changes in vascular resistance are reflected by alterations of the Doppler spectrum. Supported in part by the Department of Surgery and the Walter Macpherson Society, Loma Linda University.

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EFFECT OF VARIATIONS OF LOAD ON SKELETAL MUSCLE FUNCIONAL HYPEREMIA. T.W.B. Moore, H. W. Burton and P. A. Jackson Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W2.

This study investigated the role of load changes in determining functional hyperemia of gastrocnemius-plantaris in situ preparations of dogs (N=18) and cats (N=10). Stimulation frequencies (dog: 3Hz and 20 150 msec trains/min (7impulses/train); cat: 1Hz) caused minimal fatigue. Muscles contracted isometrically and against loads of 10, 40 and 70% of the isometric load in dogs and 10 and 50% in cats. Fifteen minute control periods separated contraction periods of 3.5 minutes duration. We determined arterial and venous concentrations of potassium and inorganic phosphate (Pi). Isometric twitch and tetanic contractions resulted in a consistent net release of potassium while the Pi response varied greatly. Twitches in cat and tetanii in dog caused a net release of Pi but twitches in dog resulted in a consistent net Pi uptake. Flow was highest in dog twitch experiments followed by dog tetanic and cat twitch experiments. Within individual experiments, there was no significant effect of load on blood flow or the release of either vasodilator. In cat muscle, the conductance change was related to Pi flux ($r = -.779$) and to potassium flux ($r = -.883$). Functional hyperemia and vasodilator release in both species was independent of load but dependent upon the metabolic rate as determined by stimulation parameters.
Supported by a grant from Natural Science and Engineering Research council of Canada.

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HEMODYNAMIC FORCES AND THE ARTERIALIZATION OF SEPHENOUS VEIN GRAFTS IN DOGS, J.F. Cornhill, J. Teijeira*and J.S. Vasko* Columbus, Ohio and Sherbrooke, Quebec.

Hemodynamic forces (e.g. wall shear stress) may be important in both atherogenesis and failure of aorto-coronary saphenous vein by-pass grafts. The effect of these forces on vein grafts were studied in 10 English Foxhounds in which saphenous vein homografts were interposed between the right and the left iliac arteries with ligation of the proximal left iliac. Velocity profiles were measured at 7 locations in the graft and arteries using an 8 channel pulsed ultrasonic velocity meter and wall shear stresses were calculated. The animals were allowed to survive for 4 months and the velocity measurements were repeated. The vessels were fixed in situ at pressure and wall morphology and biochemical composition were studied. The hemodynamic data has shown that in the graft, two major hemodynamic phenomena occured. Firstly, the velocity profile was severely skewed caudally especially in the proximal region. The degree of skewness decreased distally but remained asymetric. Secondly, the cephalic proximal region was a low flow region with possible boundary layer separation. In general, the cephalic region of the graft experiences relatively low wall shear stress particularily in the proximal section; whereas, the caudal region experiences high wall shear stress. In this model, different portion of the graft experience very different hemodynamic forces and these are currently being correlated with morphological and biochemical parameters to determine their role in arterialization.
(Supported by the National Heart, Lung and Blood Institute.)

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EXERCISE PROGRAM IN INTERMITTENT CLAUDICATION. Stefan A. Carter, Eugene R. Hamel,* Judy M. Paterson,* and Christopher J. Snow.* Vascular Laboratory, St. Boniface Hospital & Depts. of Physiology & Medicine, U. of Manitoba, Winnipeg, Canada.

To assess effects of an exercise program 34 patients with stable claudication were studied including 10 with arterial obstruction proximal and 20 distal to inguinal ligament; and 4 with combined disease. Disease was bilateral in 23. Brachial (BP) and ankle (AP) systolic pressures were measured at rest and after a standard 5-minute walk at 3.2 km/h on 7% grade, and maximal walking distance (MW) tested on the treadmill on an upgrade and on the level. Training consisted of supervised walking 1 hour 3/week for 3-6 months. At entry AP at rest in the leg with more severe disease averaged 87 mm Hg or 64 ± 3 (± SEM) % of BP. After training AP increased significantly by 2.7 ± 1.2% of BP at rest, and 5.3 ± 2.5 mm Hg after standard walk; and brachial-minus ankle difference after standard walk decreased by 8.0 ± 3.4 mm Hg ($P < 0.05$). MW increased after training by 125 ± 24 m (27%) on an upgrade and 428 ± 72 m (66%) on the level ($P < 0.01$). During training patients walked an average of 3.3 ± 0.3 km without stopping at average speed of 4.0 ± 0.2 km/h. Remarkable walking ability can be achieved by patients with proximal or distal arterial obstruction and measurements suggest that improved collateral circulation plays a part. (Supported by Manitoba Medical Service Foundation)

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THE FUNCTIONAL IMPORTANCE OF DIFFERENCES BETWEEN INSTANTANEOUS PRESSURE FLOW RELATIONS IN DIFFERENT ARTERIAL BEDS. W. Ehrlich. Johns Hopkins Medical Institutions, Baltimore, Md. 21205

COMPARISON OF INSTANTANEOUS P/Q VALUES FOUND IN DIFFERENT BEDS

EXPERIMENTAL ANIMAL	INVESTIGATED BED	METHOD USED	Ped in mmHg	Ra in mmHg ml^{-1}/min	PUBLICATION
Awake Dogs	Total arterial bed transducer on ascending aorta	Paced heart rate increase	49	0.0167	Ehrlich et al, AJP, 229:1261-1267, 1975
Awake Dogs	Circumflex branch of left coronary artery	Long diastole	45	1.55	Bellamy, Circ. Res., 43:92-101, 1978
Anesthetized Dogs	Femoral arteries	Without occlusion	~ 100	----	Ehrlich et al, Circ. Res. 47: 1980
		Vagal arrest after 30 second occlusion	35	1.2	
Anesthetized Dogs	Renal artery	Vagal arrest after 30 second arterial occlusion	20	0.6	This study

If the arteries supplying coronary or skeletal muscles have relatively high effective downstream pressures (Ped) and arterial resistances (Ra), these parameters can fall precipitously in the case of need, many more arterioles recruit and the flow can increase greatly without change in inflow pressure. High P/Q parameters are not necessary in the renal arterial bed. There is no emergency which needs an enhanced kidney flow. Quite the opposite in an emergency Ped and Ra can be elevated so that the renal artery flow can be restricted.

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EFFECTS OF ABSORPTION AND SECRETION ON COLONIC LYMPH FLOW: A PHYSIOLOGICAL AND ANATOMICAL ANALYSIS. W. Wilborn*, P. R. Kvietys*, and D. N. Granger (Spon: L.Eddy) Depts. of Physiology and Anatomy, University of South Alabama, Mobile.

In the small intestine lymph flow increases dramatically during absorption and decreases during active secretion. The effect of net transmucosal volume movement (absorption or secretion) on colonic lymph flow has not been assessed. In an isolated autoperfused canine colon preparation we studied the effects of net volume absorption rate (Tyrode solution) and net volume secretion rate (40 mm theophylline in the lumen) on colonic lymph flow. Over a wide range of absorption rates (0.15-0.87 ml/min/100gm) and secretion rate (0.16-2.28 ml/min/100gm) lymph flow was not significantly altered. Ultrastructural analyses of colonic tissue were undertaken to determine the location and caliber of the terminal lymphatics. The terminal lymphatics of the mucosa were sparse and the caliber of the vessels was orders of magnitude less than the terminal lymphatics of the small intestine. The characteristic central lacteal found in mucosa of the small intestine was lacking in the colon. Although the lymphatics of the small intestine contribute significantly to the removal of absorbed volume it appears that the removal of the absorbed volume in the colon is accomplished solely by blood capillaries. (Supported by NHLBI 15680).

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RESPONSE OF TONGUE CIRCULATION TO LOCAL CHANGES IN PERFUSION PRESSURE AND BLOOD IONIC COMPOSITION. Robert A. Weiss*, Rudy A. Bernard and Jerry B. Scott. Dept. of Physiology, Michigan State Univ., E. Lansing, MI 48824

We studied the blood circulation to the tongue in 16 dogs anesthetized with Na pentobarbital (33 mg/kg) i.v. and maintained with artificial respiration. The lingual arteries were isolated at the level of the angle of the mandible, tied proximally and cannulated distally. Isolation was verified by the absence of back flow and a perfusion pressure (PP) less than 20 mm Hg when blood flow was interrupted. Heparinized (5mg/kg) blood from the femoral artery was perfused through the tongue by a constant-flow pump. Changes in PP were used as an index of the variations in vascular resistance. After interruption of blood flow for 30, 60 and 120 sec the vasculature responded with increasing amounts of reactive dilation of 29.0, 37.2 ($p < .01$) and 47.4 ($p < .05$) mm^2. Autoregulation was indicated by a relatively constant resistance over a PP range of 100-270 mm Hg. Isotonic solutions of KCl, $MgCl_2$, $CaCl_2$ and hyperosmotic NaCl were infused at progressively increasing rates. K^+ initially dilated, but tended to constrict at the highest infusion rate. The other ions caused progressive changes: Mg^{++} dilated ($.05 < p < .1$), Ca^{++} constricted ($p < .01$), whereas hyperosmotic Na^+ decreased ($p < .01$) vascular resistance. This study suggests that the vasculature of the tongue resembles that of skeletal muscle in its response to alterations in pressure, flow and ionic composition. (Supported in part by an NIH BRS Grant to Mich. State Univ. Coll. of Vet. Med.)

982

RELATIONSHIP BETWEEN EEG STATE AND CEREBRAL OXYGEN CONSUMPTION ($CMRO_2$). William J. Pearce, Eduardo H. Rubinstein, Oscar U. Scremin, and Ralph R. Sonnenschein. UCLA Dept. of Physiology, Los Angeles, CA. 90024.

The relationship between EEG state and $CMRO_2$ was studied in paralyzed rabbits anesthetized with .5 to 1% halothane in 70% N_2O - 30% O_2. Halothane concentration was adjusted in each animal so that EEG oscillated spontaneously between synchronized (S) and desynchronized (D) states. A shift from a D to an S state was defined as a 3-fold increase in the peak-to-peak amplitude of the EEG voltage. EEG was measured with two homolateral electrodes placed 5 mm from the midline, one 5 mm anterior and one 5 mm posterior to the parietal suture. Cerebral blood flow (CBF) was determined by timed collection of the venous effluent from a catheter placed in the superior sagittal sinus. O_2 content of anaerobically collected arterial and cerebral venous samples was determined by two independent methods. In all animals, a shift from an S to a D state was associated with an increase in CBF of 12.6 ±5.8% (SEM) and an increase in $CMRO_2$ of 21.1+ 6.9% (SEM). These results suggest that cortical arousal is associated with an increase in the cerebral metabolic demand for oxygen.

(Grant support: AHA-GLAA 4371G and NIH-HL 17903)

983

MESENTERY AND CREMASTER MUSCLE ARTERIOLAR TO VENULAR BLOOD FLOW DISTRIBUTION. C.H. Baker, F.R. Wilmoth*, D.L. Davis, Dept of Physiol., Coll. of Med., Univ. of So. Fla., Tampa, FL 33612.

Techniques for obtaining by videomicroscopy time-concentration curves in parallel and series coupled microvessels have been previously reported. Intra-arterial bolus injections (0.05ml) of blood containing sulphemoglobin-RBC (SH-RBC) or FITC-dextran (FID) are made into exposed cat mesentery and rat cremaster muscle. The mean arterial pressures averaged 89±3 mm Hg and 85±4 mm Hg respectively. Since the arteriolar curve can be considered to be the input function to the capillary circuits and the venular curve the output function from them, differences in the curves would be due to blood flow distribution through the capillaries. The arteriolar curves from both the mesentery and cremaster muscle are not significantly different in regard to mean transit time ($\bar{t}$), 2.2 sec, appearance time (t_a), 0.9 sec, curve width (t_E), 3.9 sec, dispersion ratio ($t_a/\bar{t}$), 0.45. However, the venular curves from the two tissues were markedly different from each other. The venular curves from mesentery had $\bar{t}$ of 3.5 sec, t_a of 1.3 sec, t_E of 5.1 sec, and dispersion ratio of 0.32. The cremaster muscle values in contrast to the mesentery were extended markedly with $\bar{t}$ of 23.3 sec, t_a of 11 secs, t_E of 38 secs and dispersion ratio of 0.48. The parallel circuits would seem to be physically longer and/or have lower flow velocities or greater capacity in the cremaster muscle as indicated by the greater $\bar{t}$, t_a and t_E. (Supported by NIH grant HL-18866 and the American Heart Assn., Suncoast Chapter).

984

EFFECT OF HYPERCAPNIA ON BLOOD-BRAIN PERMEABILITY AND BRAIN BLOOD VOLUME DETERMINED BY AUTORADIOGRAPHY. A. M. Thompson, R. E. Crandall*, R. G. Blasberg*, C. S. Patlak, and J. D. Fenstermacher. NIH, Bethesda, Md. 20014

The purpose was to use a new method to determine whether brain capillary permeability increases in hypercapnia and, if so, in what regions and at what blood pressures. Since blood volume increase might lead to an apparent increase in permeability, blood volume was also determined. By an autoradiographic method involving α-aminoisobutyric acid (^{14}C-AIB), the brains of rats were analyzed in 11 areas. This method permits detail analysis and visualization of permeability in localized areas by densitometry and image analysis. Rate constants inward (K_1) of 1.2 to 3.2 x 10^{-5}/sec were obtained. After two hr exposure to 20% CO_2-80% O_2, only small and statistically insignificant increases could be observed in the brain. In the caudate nucleus and thalamus, 2 to 3-fold increases from the very small initial value may have occurred. Blood pressure measurements showed no correlation with degree of increased permeability. Microvascular blood volume was determined by autoradiography using ^{125}I-albumin. By avoiding larger vessels in densitometry the plasma volume was found to be 0.4-0.5% in 11 areas and to remain quite constant in hypercapnia. This suggests that the blood volume increases found in hypercapnia by other methods are due mainly to increased volume of large vessels and there is little, if any, increased microvascular permeability with this large degree of hypercapnia.

985

COMPUTER SIMULATION OF THE VASCULAR RESPONSES OF CANINE INTESTINE TO CHANGES IN VENOUS PRESSURE. Carl F. Rothe, Bruce L. Johns* and Tom D. Bennett*, Department of Physiology, Indiana University, Indianapolis, IN 46223.

Canine intestinal volume changes in response to changes in venous pressure were measured by the integral of inflow minus outflow. The pattern of the volume change was well described by an equation with two exponential terms and a ramp. We hypothesized that the fast component represented the elastic distension of the vasculature, the slow (ca. 30-sec time constant) exponential component represented a visco-elastic distension of the vasculature, and the ramp represented transcapillary filtration. An alternative hypothesis was that the slow component represented distension of the interstitial space. We developed a four-compartment model (arterial, capillary, venous and interstitial space), fitted parameter values to the model by judicious manipulation of values, and found that an excellent fit could be made without assuming a visco-elastic vasculature component, pressure dependent changes in arteriolar or venous resistance, or changes in interstitial oncotic pressure. Supplemental information, such as A-V differences in hematocrit or other indicator, is necessary to separate vascular from interstitial distension. Using the model, the magnitude of probable error was estimated when determining capillary pressure or vascular compliance using autoperfusion, weighing and/or plethysmographic techniques. (Supported by USPHS Grant HL-07723.)

986

EFFECTS OF Ca^{++} and Ca^{++}-ANTAGONISTS ON THE INTESTINAL VASCULATURE. K.M. Walus*, J.D. Fondacaro and E.D. Jacobson. Dept. Physiology, Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

Reports on the influence of Ca^{++} and Ca^{++}-antagonists on intestinal circulation have been scarce. The present study was conducted to elucidate these effects. The action of Ca^{++} and Ca^{++}-antagonists, nifedipine (N) and dilitiazem (D), on small intestinal blood flow (BF), flow distribution, oxygen extraction (A-V_{O_2}) and uptake (V_{O_2}) and intestinal motility were studied in anesthetized dogs. Both N and D (0.1, 1.0 and 10.0 µg/kg-min) dose-dependently increased BF, decreased A-V_{O_2} and intestinal motility, redistributed BF to the mucosa-submucosa but did not change V_{O_2}. $CaCl_2$ (0.001-0.5 mg/kg-min) had a mild constrictor effect, but at a dose of 1.0 mg/kg-min caused vasodilation. The latter was reversed by digoxin suggesting that high [Ca^{++}] stimulates Na^+,K^+-ATPase. $CaCl_2$ also decreased A-V_{O_2}, stimulated intestinal motility but did not change V_{O_2} except at the highest dose, when it was increased. N completely blocked Ca^{++}-induced intestinal vasoconstriction and partially attenuated constrictor effect of norepinephrine (NE). N also relaxed isolated mesenteric arterial strips *in vitro* contracted by KCl or NE in the presence of external Ca^{++} and NE-contracted strips in the absence of external Ca^{++}. Ca^{++}-antagonists *in vivo* seem to act mainly on resistance vessels without increasing the nutrient circulation. *In vitro* studies suggest that these agents relax smooth muscle by interfering with both Ca^{++} influx and release of Ca^{++} from intracellular stores. (Supported by Am. Heart Assoc. grant 79-968.)

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DEPENDENCE OF MICROSPHERE SHUNTING UPON THE DISTRIBUTION OF MICROSPHERES WITHIN THE INTESTINAL WALL. L.C.Maxwell and A.P.Shepherd, U. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

We recently quantitated the effect of microsphere size upon the distribution of 7-30 µm spheres within canine intestine. The mucosal/submucosal (M/S) ratio of trapped spheres increased markedly as sphere diameter decreased. Regional delivery of small spheres may more accurately represent regional blood flow than large spheres. However, a fraction of spheres small enough to distribute in proportion to blood flow "shunt" through the tissue to venous blood. If each layer shunted small spheres in proportion to its blood flow, determinations of regional blood flow could be corrected for shunting. Therefore, we tested the hypothesis that the percentage of shunted spheres is independent of the intramural distribution of trapped spheres. We injected radioactive 9 and 15 µm spheres into 12 isolated canine gut loops. M/S ratio for 9 µm spheres exceeded but was highly correlated (r=0.94) with the M/S ratio for 15 µm spheres. The percentage shunting varied fourfold and was unrelated to the percent of 15 µm spheres trapped by muscularis. However, percentage shunting was inversely related to the M/S ratio for 9 µm (r=0.68) or 15 µm (r=0.74) spheres. We, therefore, reject the hypothesis that the shunting of spheres is independent of their intramural distribution. The inverse relationship between shunting of 9 µm spheres and the mucosal/submucosal ratio of trapped spheres suggests that mucosa contributes fewer 9 µm spheres per ml of venous blood than the remainder of the intestinal wall. (Supported by HL-23435)

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A COMPARISON OF MEASURED VERSUS ESTIMATED TRANSCAPILLARY FLUID MOVEMENT DURING HISTAMINE INFUSION. J.J. Szwed, B.L. Johns*, P.A. Kesler*, and J.J. Friedman. Depts of Med. and Physiol, Indiana Univ., Indianapolis, IN 46202

The measurement of transcapillary fluid movement (FM) is considered to be the sum of changes in tissue weights (ΔFM_G), luminal secretions (ΔF_S), and lymph flows (ΔF_L). To these measured extravascular components, we compared FM derived from intravascular measurements. These intravascular measurements were changes in venous blood colloidal osmotic pressure (ΔFM_O) which is considered to exclusively represent FM due to ΔP_C, together with FM induced by the change in the oncotic gradient ($\Delta\pi$) resulting from the increase in protein leakage (ΔPL) which occurs with histamine infusion (ΔFM_{PL}). In 7 experiments, intraarterial infusion of histamine at 100 µg/kg/min produced the following changes in FM expressed as mean ml/min · 100 gm ± S.E.

$$\Delta FM_G + \Delta F_S + \Delta F_L = \Delta FM_O + \Delta FM_{PL}$$

$$.21 \pm .08 + .022 \pm .03 + .03 \pm .01 = .13 \pm .02 + .11 \pm .02$$

$$.26 \pm .04 = .24 \pm .02$$

Therefore even during histamine infusion, a circumstance which alters capillary permeability substantially, measured and estimated transcapillary fluid movements are statistically identical.

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GROWTH AND SKELETAL MUSCLE CAPILLARITY. Lynn Aquin*, Andrew J. Lechner and Natalio Banchero. University of Colorado Medical School, Denver, CO 80262 USA

The medial head of the gastrocnemius muscle (GN) from young growing rats, guinea pigs and the entire GN of growing dogs was disected out from the anesthetized animal, weighed and bisected transversely. A 1 cm thick cross section of the muscle was quick frozen in isopentane cooled to -130°C with liquid nitrogen. Ten µm thick sections of the cross section at the midpoint of the muscle were cut in a cryostat and stained with the myosin ATPase technique after preincubations of pH 4.0 and 4.5 to visualize capillaries and typify fibers. In the rat and guinea pig only the center area of the cross section was analyzed whereas in the dog values from all parts of the cross section were averaged. In all three species, muscle weight (MW) was linearly related to body weight (BW), fiber cross sectional area (FCSA) was linearly related to both BW and MW and capillary to fiber ratio (C/F) was linearly related to FCSA. The fibers of the GN of the newborn rat and dog were more immature than the GN of the guinea pig. The initial differences in the GN muscle of the three species disappeared however, and in the adult animal, the muscles were similar in respect to FCSA, C/F and fiber composition. The BW's of the adult animals were 666 g, 1274g and 23.0 Kg, whereas the mean FCSA's were 4900, 4200 and 4700 µm^2 in the rat, guinea pig and dog, respectively. The C/F's at these FCSA's were 2.4, 2.8 and 2.3, respectively. (Supported by USPHS, NIH Grant HL-18145).

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COMPARISON OF TOTAL PROTEIN IN GASTRIC AND HEPATIC LYMPH. M. Jack Keyl and Alvin CK Chang*. Dept. of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

We have reported that gastric lymph collected from the non-secreting stomach of dogs had a lymph/plasma ratio (L/P) for protein of .80-.90 (Fed. Proc. 38:1137, 1979). This was much higher than had been reported by Bruggeman (Gastroenterology 68:1204, 1975) who collected lymph by micropuncture from the stomach of animals with the duodenum ligated at the pylorus and the stomach distended with air. This study was designed to determine if stomach distention and/or pyloric ligation would alter gastric lymph protein concentration and to compare the concentration of total protein in gastric and hepatic lymph. Gastric L/P for protein was .83 ± .04 before and .81 ± .04 after ligating the pylorus with no change in gastric venous pressure or lymph flow. Pyloric ligation and air distention caused a decrease in L/P from .85 ± .04 to .69 ± .04 with no significant difference in maintained venous pressure or lymph flow. The gastric L/P and the hepatic L/P were .80 ± .04 and .83 ± .02 respectively. It was hypothesized that the drop in L/P after air distention was due to a shift in mucosal blood flow to the muscularis layer.

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CARDIOVASCULAR AND RENAL EFFECTS OF ISOPROTERENOL INFUSION IN YOUNG SWINE. N.M. Buckley, A.N. Charney, P.Brazeau*, G. Feldman*, S.Cabili* and I.D. Frasier*. Albert Einstein Coll. of Med., and N.Y.V.A. Hospital, New York, N.Y. 10461 and 10010.

Isoproterenol (ISP) infusions (0.1 and 0.2 μg/kg/min) were studied in 2-week old swine anesthetized with 20 mg pentobarbital/kg. Aortic pressure, heart rate, and femoral and renal blood flows were recorded. Left and right ureters were catheterized. ISP was given i.v. in 8 animals thoracotomized to record right ventricular pressure and its first derivative; and i.a. in 5 others while perfusing the left kidney in situ at constant flow and recording perfusion pressure. Renal vascular resistance (R) was calculated. Glomerular filtration rate (GFR) was determined by ^{14}C-inulin clearance and fractional sodium excretion (FE_{Na}) was calculated. After a 10-20 minute control clearance period, ISP was infused for 10-20 minutes before and then during another clearance period. ISP given i.v. had positive cardiac inotropic and chronotropic effects, decreased renal R and FE_{Na}, and increased renal blood flow and GFR. When ISP was given i.a at constant renal blood flow, renal R and FE_{Na} decreased while GFR was unchanged or decreased. Stepwise increases in renal blood flow increased GFR, and pressure elevation increased FE_{Na}. We conclude that the renal vasodilator action of ISP in 2-week old swine is accompanied by blood flow-dependent changes in GFR and perfusion pressure-dependent changes in sodium excretion. (Supported in part by USPHS. NIH Grant HL-21865).

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ROLE OF ADENOSINE IN THE CEREBRAL HYPOXIC HYPEREMIC RESPONSE. Thomas E. Emerson, Jr. and Richard M. Raymond*.Dept. of Physiology, Mich. State Univ., E. Lansing, MI 48824

We previously reported that the increased cerebral blood flow (CBF) during moderate hypoxia (PaO_2=47 mmHg) could be completely reversed by the competitive adenosine antagonist theophylline(Physiologist 20:78, 1977). However, a valid criticism of this experiment was that the hypoxia and hyperemia were insufficient to test the hypothesis. The present study is an extension of the earlier work. The experiments were completed in 5 mongrel dogs anesthetized with nembutal and ventilated artificially. CBF was measured using the Rapela-Green technique. Severe hypoxia was induced by ventilating the dogs with a gas mixture of 5%O_2, 2%CO_2, and 93%N_2. Theophylline was infused at 0.01 ug/min into the brachiocephalic artery with the subclavian arteries ligated just distal to the origin of the vertebral arteries. During severe hypoxia (PaO_2=20 mmHg; cerebral PvO_2=11 mmHg), CBF increased by 98%, cerebral vascular resistance (CVR) decreased by 40% and arterial blood pressure (ABP) was unchanged on the average. When theophylline was infused during hypoxia, CBF decreased by 27% and CVR increased by 17%; ABP was unaltered. These variables returned to hypoxic,non-theophylline infusion levels when theophylline infusion was stopped and hypoxia maintained. CBF and CVR returned to control levels when the animals were subsequently returned to the normoxic gas mixture. Theophylline infusion during normoxia had no effect on any variable. These data support the hypothesis that adenosine is involved in cerebral hypoxic hyperemia.

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CONTINUOUS MEASUREMENT OF CEREBRAL BLOOD FLOW IN CATS & DOGS. D.W. Busija*, D.D. Heistad and M.L. Marcus. CV Center & Dept of Med, Univ of Iowa & VA Hosp, Iowa City, IA 52242.

We have developed a method for continuous measurement of changes in cerebral blood flow (CBF) in experimental animals. Flow is determined from the product of blood velocity and cross-sectional area (CSA) in the same cerebral artery. Velocity was measured using a pulsed Doppler crystal positioned under a cerebral artery. CSA was determined by measuring pial artery diameter with an electronic micrometer through a pial window. Using this method, vessel caliber could be measured every 2-3 seconds. CBF was determined in 12 anesthetized cats during: 1) control 2) hypocapnia 3) hypercapnia and 4) hypercapnia plus hypertension. Microspheres were injected under steady-state conditions to compare the 2 methods. During control, diameter of cerebral vessels observed was 388±28 μM (mean±SE) and CBF measured with microspheres was 40±4 ml/min per 100 gm. During hypocapnia, CBF (velocity x CSA) decreased 24±3%; during hypercapnia CBF increased 122±28% and during hypercapnia and hypertension, flow increased 351±47%. The correlation coefficient between changes in flow (velocity x CSA) and microspheres during steady-state conditions was 0.94 and the slope of the regression line was 0.84±0.06. In similar studies in 7 dogs, the correlation coefficient between velocity x CSA and microspheres was 0.98 and the slope of the regression line was 0.89±0.04. We conclude that the product of blood velocity and CSA of a pial artery provides accurate, on-line measurement of changes in CBF.

ADDITIONAL ABSTRACTS*

MATERNAL ALPHA-METHYLDOPA AND POST-NATAL DEVELOPMENT OF NEURAL CONTROL OF HEART RATE IN THE CONSCIOUS, NEWBORN RAT. Edward J. Hoskins*, Edward K. Holly* and Gary W. Gulizia*. (SPON: D.V. Priola). Point Loma College, San Diego, CA 92106

During the last one-third of gestation (term=21 days), 8 time-mated pregnant Sprague-Dawley derived albino rats were injected (s.c., 1/day) with either sterile saline (1.0cc/kg, Control Group) or alpha-methyldopa (1.0cc/kg=50mg/kg, Drug Group), an antihypertensive routinely encountered in obstetrical practice. The rats were allowed to deliver normally. At 1,2,4, and 6 weeks after birth, resting autonomic control of HR was evaluated in 48 conscious, newborn rats by monitoring HR (from the external tail pulse) before and after sequential autonomic blockade with propranolol (10mg/kg, i.p.) followed 30 minutes later by atropine (5mg/kg, i.p.). In the control group, propranolol decreased HR by 36±4%, n=6 and 31 ±7%, n=6 at 1 and 2 weeks respectively. In the drug group, propranolol decreased HR by only 6±10%, n=6 ($p<0.02$) and 5±8%, n=6 ($p<0.05$) at 1 and 2 weeks respectively. These results demonstrate that early in the neonatal period, newborn rats exposed pre-natally to maternal alpha-methyldopa have a significantly diminished resting sympathetic neural control of HR.

UNILATERALITY OF SYMPATHETIC INNERVATION IN THE HEMIPADS OF RAT INTERSCAPULAR BROWN ADIPOSE TISSUE (IBAT). D.O. Foster*, F. Depocas and G. Zaror-Behrens* (SPON: O. Héroux), Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6.

In 6°C-acclimated, male rats derived from the S.-D. strain unilateral sympathectomy of IBAT by cutting the 5 intercostal nerve bundles entering one hemipad 24h before measurement of each hemipad's noradrenaline (NA) content, total dopamine β-hydroxylase (DBH) activity, and in vivo blood flow (with microspheres) had precisely the same effects in the denervated hemipad as bilateral sympathectomy: i.e., denervated hemipads of either treatment group had (i) 3% of the NA and 42% of the DBH found in intact hemipads of sham-operated or unilaterally sympathectomized rats, and (ii) 0% of the 10 fold increase in blood flow that occurred in intact hemipads when the rats were exposed unanesthetized to -6°C. This increase in IBAT blood flow is relative to flow measured in rats exposed to 22°C and is an index of sympathetically mediated calorigenesis in the tissue. IBAT bilaterally denervated for 24h was as responsive in terms of its increase in blood flow during infusion of rats with NA as intact IBAT. These results negate the existence of a significant degree of sympathetic cross-innervation of the hemipads of rat IBAT as postulated (Seydoux et al. 1977. J. Physiol. Paris 73, 985) from an histofluorescence-based assessment of the effects of surgical sympathectomy on adrenergic fiber density in the tissue.

*Abstracts in this section are not scheduled for formal presentation.

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XXIX MEETING OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES, SYDNEY, 1983

THE VENUE

The 29th International Congress of Physiological Sciences will be held at the University of New South Wales from August 28-September 3, 1983.

The modern campus of this University is in the suburb of Kensington, about 7 km south east of the centre of the City of Sydney and only 1-2 km from several Pacific Ocean beaches.

Sydney was established in 1788. It is the oldest city in Australia and is situated on a spectacular harbour. The population is approximately three million. It offers a rich variety of activities to resident and visitor — there are numerous ocean and harbour beaches, sailing on the harbour and nearby bays is extremely popular; the opera, concert and theatre seasons are in full swing in August; there are numerous national parks close to Sydney, notably the famous Blue Mountains.

The Conference is timed for the end of Winter, beginning of Spring. Average August temperatures in Sydney are 17.4°C (68.3°F) maximum, and 8.8°C (47.8°F) minimum. Average August rainfall is 81 mm.

ACCOMMODATION

Accommodation has been reserved in a variety of hotels and University Colleges (student halls of residence). There is abundant accommodation available in the adjacent beach suburbs. As the organizers need information on the likely demand for various grades and costs of accommodation, an information card is being distributed with this brochure.

TRAVEL

Qantas Airways, the Australian airline, is the official international carrier to the Congress. Qantas is well-known throughout the world as an efficient airline with a reputation for excellent service. Its offices around the world will be responsible for co-ordinating group travel programmes for Congress members who require this less expensive form of travel.

Group travel or excursion fares exist from most parts of the world to Australia, representing a considerable saving on normal fares. Qantas will assist in co-ordinating delegations, so allowing delegates to take advantage of these fares.

Ansett Airlines has been appointed the official domestic airline and will arrange a number of pre and post Congress tours to enable delegates to see as much of Australia as possible. The range of tours includes such areas as:

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2. Central Australia — known as the Red Centre — witness the grandeur of Ayers Rock — visit the Macdonnell Ranges — Alice Springs, almost exactly in the geographical centre of Australia.
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PROGRAMME

The programme will cover the full range of disciplines embraced by IUPS. There will be both free communications and symposia, although the details of the format will be influenced by the review currently being undertaken by IUPS Council of conference structure.

The opening session will be held in the Concert Hall of the Sydney Opera House and the closing ceremony in the Sydney Town Hall. The scientific sessions will all be at the University of New South Wales.

A special concert by the Sydney Symphony Orchestra, and a performance by the Australian Opera are being arranged, both in the Sydney Opera House. Other social activities, taking particular advantage of Sydney Harbour, are being organized.

Sporting activities that are readily available in Australia include swimming and surfing, water and snow ski-ing, sailing, bush walking.

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The Congress is being sponsored by the Australian Academy of Science through its National Committee for Physiology.

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