THE PHYSIOLOGIST

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33rd Annual Fall Meeting

1982 Refresher Course—Syllabus

Society News

Cover: From Symposium on Man at High Altitude; see p. 184.

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33rd Annual Fall Meeting of the American Physiological Society

With

Division of Comparative Physiology and Biochemistry
of the American Society of Zoologists

Latin American Association of Physiological Sciences (ALACF)

IUPS Commission on Gravitational Physiology

Town & Country Hotel
San Diego, California

October 10-15, 1982

For information on Fall Meeting registration, call the APS Fall Meeting Office (301)530-7010.
For information on the meeting program, call our Membership Services Department (301)530-7171.

Refresher Course, Symposia, Tutorials, and Special Sessions

Monday, Oct 11, A.M.

Refresher Course
- Selected topics on microcirculation. B. R. Duling

Monday, Oct 11, P.M.

Methodology Tutorial Session
- Recent advances in physiological monitoring. Chaired by H. Sandler and T. F. Budinger

Tuesday, Oct 12, A.M.

Symposia
- Man at high altitude. Session 1. Man at extreme altitude. Chaired by J. B. West
- Teaching of cardiovascular physiology outside the lecture hall. Organized by W. T. Beraldo, J. A. Michael, and A. A. Rovick

Tutorials
- New concepts of nephron structure. F. W. Wright
- Physiology of gastrointestinal smooth muscle. J. H. Szurszewski
- Comparative reproductive physiology. B. Lasley

Tuesday, Oct 12, P.M.

Symposia
- Blood-brain barrier. Chaired by D. D. Heistad
- Regional vascular behavior in the gastrointestinal wall. Chaired by H. G. Bohlen

Tutorials
- The role of aldosterone sodium, chloride and potassium in metabolic alkalosis. N. A. Kurtzman
- Hormonal control of the mammalian fetus and infant C. S. Nicoll
- Neurochemical mechanisms of thermoregulatin. R. D. Myers

Bowditch Lecture (4:30)
- Electrogenic ion pumps and other determinants of membrane potential in vascular muscle. K. Hermsmeyer

Wednesday, Oct 13, A.M.

Symposia
- Man at high altitude. Session II. Sleep and respiration. Chaired by S. Lahiri
- Ionic channels in excitable membranes. Chaired by F. Benzanilla

Special Lecture
- Reflexes provoked by cytoglucopenia. C. Tiomo-Iaria, President, ALACF

Tutorials
- Endorphins. F. E. Bloom
- Cerebral cortex. R. B. Livingston

Wednesday, Oct 13, P.M.

Symposia
- Neurophysiological mechanisms controlling circadian rhythmicity. Chaired by H. Arechiga
- Differentiation of epithelial cells. Chaired by M. Cereijido

Tutorials
- Temperature regulation during exercise. C. V. Gisolfi
- Neurotoxins as tools for physiological investigation. L. Freire-Maia

Thursday, Oct 14, A.M.

Symposia
- Man at high altitude. Session III. Acclimatization in permanent residents at high altitude. Chaired by R. M. Winslow
- Anaerobic energy metabolism of invertebrates. Session I. Chaired by R. M. Winslow

Tutorials
- Neural integration at the level of autonomic ganglia. D. L. Kreulen
- Analysis of physiological systems via mathematical models. J. Hazelrig
- Pattern generators in the central nervous system of vertebrates. J. L. Feldman

Thursday, Oct 14, P.M.

Symposia
- Is efferent control of arterial baroreceptors important? Chaired by K. Sagawa
- Temperature effects on fish. Chaired by L. I. Crawshaw and J. R. Hazel

Tutorials
- Water channels in red blood cells. R. I. Macey
- New concepts in acid-base balance. P. A. Stewart
- Membrane transport processes. R. B. Gunn

APS Business Meeting (4:30)

Friday, Oct 15, A.M.

Symposia
- Exchange and compartmentation of calcium in the heart. Session I. Chaired by G. A. Langer
- Anaerobic energy metabolism of invertebrates. Session II. Chaired by W. R. Ellington
- Gravitational physiology. Session I. Chaired by H. Bjurstedt

Friday, Oct 15, P.M.

Symposia
- Exchange and compartmentation of calcium in the heart. Session II. Chaired by G. A. Langer
- Gravitational physiology. Session II. Chaired by A. S. Ushakov

Sessions of Contributed Papers by Day

1.1

THE MAGNITUDE AND "PATTERN" OF THE VENTILATORY RESPONSE TO EXERCISE IS NOT AFFECTED BY VAGOTOMY IN DOG. A. Huszczuk*, A. Oren*, L.E. Nery*, E. Shors, W. Sy*, B.J. Whipp and K. Wasserman. Division of Resp. Med., Harbor-UCLA Med. Ctr., Torrance, CA 90509

Sensory pathways mediating the exercise hyperpnea are poorly understood. To determine the role of vagal mediation, 10 anesthetized (pentobarbital Na) dogs were exercised under: a) control conditions, b) bilateral cold block (0°C) of the cervical vagi (V_{bl}), c) full recovery from V_{bl}, and d) bilateral cervical vagotomy (V_x). Gas exchange variables and minute ventilation ($\dot{V}_E$) were determined breath-by-breath and arterial PCO_2 ($PaCO_2$) was continuously displayed from an on-line PCO_2 electrode. Under the control conditions (n=48) exercise hyperpnea ($\dot{V}_E$=206±29%) did not significantly change arterial PCO_2 ($\Delta PaCO_2$=0.5±3.6 torr). There were no systematic differences between V_{bl} and V_x either at rest or during exercise. These procedures (n=37), however, did reduce resting and exercise CO_2 outputs ($\dot{V}CO_2$) by 12.1±11% and 9.3±9% respectively, although % increase of $\dot{V}CO_2$ due to exercise was unaffected. Furthermore, V_{bl} and V_x did not alter resting and exercise $\dot{V}_E$ or the proportional contribution of V_T and f to the $\dot{V}_E$ response; this unchanged "pattern" of $\dot{V}_E$ response to exercise following vagotomy being strikingly different from the response to other known hyperpneic stimuli. Under these conditions $PaCO_2$ during exercise was reduced by 1.2±4 torr. We conclude that vagal mediation is of negligible importance to the exercise hyperpnea in the dog. (Supported by ALA-CA/MG3713, AHA-GLAA/MG3760)

1.2

BREATHING PATTERN DURING EXERCISE IN TRAINED ATHLETES. Lawrence J. Folinsbee, Eric S. Wallace,* John F. Bedi,* and Steven M. Horvath. Institute of Environmental Stress, University of California, Santa Barbara, CA 93106

Breath-to-breath variation in tidal volume (V_T), inspiratory time (T_I), and expiratory time (T_E) were studied in six trained cyclists and four sedentary persons during a progressive maximum bicycle exercise test. At each workload, breath-by-breath V_T and T_I showed a strong positive relationship. Over the full range of ventilatory drive, V_T and T_I showed a weak negative relationship (r = -0.05 to -0.55). Significant negative relationships (r = -0.62 to -0.91) were observed for the min-to-min average of V_T and T_I. Within a level of ventilatory drive, larger V_T's are accompanied by longer T_I's but the increase in inspiratory flow as V_E increases is caused by an increase in V_T and a decrease in T_I except at high V_E, where only T_I changes. T_I and T_E were positively correlated in both cyclists (r = 0.56 to 0.90) and sedentary subjects (r = 0.38 to 0.73). The decrease in T_E as ventilation increased was also correlated with the increase in V_I (V_T/T_I)(r = -0.46 to -0.89). Variability of V_T, T_I, T_E decreased as ventilation increased. The transition from range 1 V_T - T_I response (i.e. increasing V_T and stable T_I) to range 2 (increasing V_T and decreasing T_I) occurred at a respiratory frequency of 25-30/min in both subject groups (T_I = 0.9-1.4 s). Tidal volume at the transition ranged from 1.9 to 2.9 liters and was correlated with individual inspiratory capacity.

1.3

THE EFFECT OF EXPIRATORY LOADING ON EXPIRATORY DURATION (Te) AND SLOWLY ADAPTING PULMONARY STRETCH RECEPTOR (PSR) DISCHARGE. P.W. Davenport and J.A. Wozniak* Department of Metabolism, University of Florida, Gainesville, Florida 32610.

PSR's have been hypothesized to be the afferents mediating the vagally dependent prolongation of Te during single breath expiratory loading. To test this hypothesis, rabbits were anesthetized, tracheostomized and the tracheal cannula connected to a loading manifold. The expiratory loads were airway occlusion at end-inspiration and two magnitudes each of resistive (R), elastic (E) and threshold (T) loads. The loads were presented in a random order 3 times each for a single expiration while recording the single unit activity of PSR's from the right vagus. Te was determined from the diaphragm EMG and volume by integration of airflow. The expired volume (Ve) and Te decreased and increased, respectively, during loading. The Te for the small R and E loads were not significantly different, however, the Ve was significantly less for the E load. This was also observed for the large R and E loads resulting in different Ve-Te curves for R and E loads. When Te was plotted against the area under the expired volume curve, a single, linear relationship was found for all types of loads. During loading, PSR activity increased as Ve decreased. A single, linear relationship was found by plotting Te against the number of PSR spikes during expiration, for all receptors recorded. These results demonstrate that the increase in Te with expiratory R, E and T loads is correlated with an increase in PSR activity.

1.4

Ability of Normal Subjects to Perceive Added Inspiratory and Expiratory Resistive Loads. S.R. Muza*, S. McDonald* and F.W. Zechman. Dept. of Physiology and Biophysics, Univ. of Kentucky Medical Center, Lexington, KY 40536.

Nine healthy male adults were studied at five levels of suprathreshold added resistance (ΔR) (range: 6.5 - 31.0 cm H_2O/l/sec) applied twice to either inspiration (I) or expiration (E) in a random sequence. Subjects squeezed an isometric handgrip dynamometer to express the perceived magnitude of the load. Peak mouth pressure (P_m), flow ($\dot{V}$), grip (G) and ΔR were analyzed to derive the exponent for Steven's Power Law. We observed: the slope and intercept for Log G vs Log ΔR was .69 ± .09 and .35 ± .10 (r = .95) for I loads and .53 ± .09 and .56 ± .14 (r = .85) for E loads respectively. These slopes and intercepts were significantly different ($p \leq .05$). However, the slopes and intercepts for Log G vs Log P_m during the same I and E loading were not significantly different. Subjects exhibiting high exponents for scaling I loads had comparably high exponents for E loads. When subjects were instructed to target I or E flow at 0.5 l/sec, we observed no difference between the slopes and intercepts for Log G vs Log ΔR during I and E loading. These results suggest: a) normal subjects performance and precision in scaling I ΔR is greater than for scaling E ΔR loads; b) the sensory information utilized in judging the magnitude of added resistance is more likely related to the force generated by the respiratory muscles (P_m) rather than ΔR per se. Supported by NIH Grant HL 24412.

1.5

LITHIUM DECREASES RESPIRATORY COMPENSATION TO RESISTIVE LOADING. Mitchell Weiner *, Alan Chausow *, Edward Wolpert *, Peter Szidon. Dept. of Medicine and Psychiatry, Michael Reese Hospital, Chicago, IL 60616

In spite of its extensive clinical use, the effects of lithium on breathing have not been studied systematically. We examined the ventilatory and occlusion pressure responses to rebreathing mixtures of 7% CO2 and 93% O2 with and without external flow resistive loading (18 cm H2O/L/sec). Eight normal males (ages 30-44) were given lithium carbonate (900 to 1500 mg/day) and placebo for 2 weeks in a double blind cross over design. Lithium blood levels ranged between .8 to 1.5 mEq/L in subjects taking lithium and were undetectable with placebo. The slope of the ventilatory response to CO2 (VI/PCO2) was unchanged with lithium either before (C) or during external resistive loading (RL). In contrast, the slope of occlusion pressure response (P100/PCO2) during resistive loading was significantly less with lithium than with placebo.

	VI/PCO2			P100/PCO2		
	PLACEBO	LITHIUM		PLACEBO	LITHIUM	
C	4.3±.6	4.7±.9	NS	1.0±.2	1.1±.2	NS
RL	2.7±.4	2.3±.4	NS	1.8±.2	1.2±.2	(p .01)

Lithium influences the respiratory compensation to resistive loading. Caution should be exercised when the drug is given to patients with increased airways resistance.

1.6

FLOW AND RESPIRATORY DRIVE RESPONSES TO INSPIRATORY FLOW RESISTIVE LOADING. Peter H. Abbrecht. Dept. of Physiology and Internal Medicine, Uniformed Services University, Bethesda, MD 20814

Mouth and esophageal pressures, inspiratory flow rates, minute ventilation, and end-tidal PCO_2 were measured continuously in normal subjects during rebreathing (Read method) without additional resistance and with inspiratory flow resistances of 12, 18, 24, or 36 cm H_2O/l/sec. Central respiratory drive was estimated from mouth occlusion pressure (P_{100}). At no load and at the lower inspiratory resistances the flow response was accurately predicted using a respiratory muscle model based on force-velocity relationship. In some subjects a more complicated model was required for the highest resistances because of changes in breathing pattern and lung volume in response to the high negative intrapulmonary pressures during inspiration. An analysis was developed to separate the CO_2-dependent and the load-dependent components of central respiratory drive during resistive-loaded breathing. The load-dependent portion of respiratory drive determined for each subject correlated well with the magnitude of esophageal and mouth pressures. (Supported by USUHS Grant RO7648).

1.7

A COMPARISON OF BREATHING PATTERNS BETWEEN STEADY STATE AND TRANSIENT HYPERCAPNIA IN CONSCIOUS DOGS. Lu-Yuan Lee and Robert F. Morton* Univ. of Kentucky, Lexington, Ky. 40536.

After a step change in F_ICO_2, the response of V_T precedes f in reaching the steady state (Loeschcke et al., Pflügers Arch. 277:671, 1963). To examine if the asynchronous responses arise from the stimulation of chemoreceptors of different responding rates, we compared the breathing patterns between steady state and transient hypercapnia (HPC) in 89 studies on 5 resting conscious dogs. Transient HPC was induced with 3-5 breaths of 15% CO_2 in air and breath-by-breath responses were analyzed continuously for 20 s after the first breath of HPC gas mixture. Steady state HPC in hyperoxia was generated by step changes in F_ICO_2 (range 3.0-5.5%) for 12-15 min at each level and steady state responses in the last 3-5 min were analyzed. Our results showed: 1) There was a linear relation between $\dot{V}_E$ and V_T in both transient and steady state HPC. However, a breakpoint emerged in the steady state $\dot{V}_E$-V_T line in all but one dog when V_T reached 843 ± 55 ml (n=4, mean ± SE). There was no apparent breakpoint in the $\dot{V}_E$-V_T line during transient HPC. 2) The slope of steady state $\dot{V}_E$-V_T line below the breakpoint was 20.5 ± 2.5 min^{-1} which was not significantly different ($p > 0.05$, n=5) from that of transient HPC (18.9 ± 2.4 min^{-1}). These results suggest that the stimulation of peripheral chemoreceptors cannot account for the asynchronous change in volume and rate following a step forcing of HPC. (NIH Grants HL-25089 and BRSG RR-05374.)

1.8

CHARACTERIZATION OF THE CO_2 RESPONSE CURVE BELOW THE OPERATING POINT BY VENOUS CO_2 UNLOADING. R. D. Tallman, Jr. Dept. of Anesthesiology, Ohio State Univ., Columbus, Ohio 43210.

Previously reported results in decerebrate ducks have demonstrated that the ventilatory / $PaCO_2$ relationship during venous CO_2 loading was the same as during CO_2 inhalation (Tallman & Grodins. J.A.P. 52:1272, 1982). A sensitivity to the increase in arterial PCO_2 was considered to be the mechanism responsible for the hyperpnea during either route of CO_2 loading. It has been suggested that the avian intrapulmonary CO_2 receptors (IPC) may affect ventilation to prevent excessive hypocapnia. The purpose of the present study was to characterize the shape of the CO_2 response curve below the operating point by unloading CO_2 from the venous blood during spontaneous breathing. Seven adult, male Pekin ducks (2.82 Kg avg. wt.) were decerebrated one week prior to the experiment. A venovenous extracorporeal blood circuit including a silicone membrane blood oxygenator was used. The animals breathed through a chronic tracheostomy. Three experimental states, venous CO_2 loading, inhaled CO_2 loading and venous CO_2 unloading were randomly administered, always bracketed by controls. Measurements were made in the steady state. Expressed as $\Delta\dot{V}_E/\Delta PaCO_2$, inhaled and venous CO_2 loading resulted in a sensitivity of 145 cc/min/Torr. Below the operating point the slope was 83.1 cc/min/Torr. The reduced CO_2 sensitivity below control levels indicates that IPC are not serving to prevent hypocapnia under these conditions. (This work was supported in part by NIH grant HL 29715).

1.9

EFFECT OF NALOXONE ON HYPERCAPNEIC VENTILATORY RESPONSE. K.R. Cooper*, B.A. Phillips* (SPON: F.L. Glauser). Medical College of Virginia, Richmond, Virginia 23298.

We have previously shown that a 24 hr. period of sleeplessness results in a significant decline in the slope (S) of hypercapneic ventilatory response (HCVR) in normal subjects. The present study was designed to determine whether the narcotic antagonist naloxone (1) had any detectable effect on HCVR or (2) abolished the decline in S induced by sleeplessness. We performed HCVR testing using the rebreathing technique on three consecutive days in 6 normal subjects. On Days 1 and 3, the subjects were well rested; however, no sleep was permitted between testing on Days 1 and 2. Each day's testing consisted of 4 trials. The first 2 trials were performed following a placebo injection and the second 2 runs were performed following intravenous injection of 50mg naloxone. The second and fourth runs were performed using an inspiratory resistive load. All runs were separated by a 10 minute rest period. Values of S (mean ± SD) under unloaded conditions were:

	DAY 1	DAY 2	DAY 3
Placebo	2.99 ± 1.29	2.90 ± 0.89	3.94 ± 0.84
Naxolone	3.48 ± 1.63	3.64 ± 1.68	4.78 ± 1.87

We conclude that 1) S was greater after naloxone than after placebo, and 2) naloxone prevented the decline in S due to sleeplessness. The addition of inspiratory resistive loading resulted in generally smaller values of S but the same relationships held as those seen during unloaded tests.

1.10

THE EFFECTS OF HYPEROXIA AND HYPOXIA ON THE VENTILATORY RESPONSE TO $IVCO_2$ LOADING. F.M. Bennett, R.D. Tallman and F.S. Grodins. Dept. of Biomedical Engineering, University of Southern California, Los Angeles, California 90089

Our previous work has demonstrated that the ventilatory response to $IVCO_2$ loading in the dog is a hypercapnic hyperpnea. Phillipson et al. (Fed. Proc. 39: 584, 1980 and Physiologist 23:87, 1980) reported that the response to $IVCO_2$ loading in awake sheep was very sensitive to small changes in Pao_2. It was suggested that the increase in Pao_2 observed in our experiments may have inhibited ventilation thus explaining our hypercapnic observation. We have reported (Fed. Proc. 40:479, 1981) that if Pao_2 was maintained at control levels during $IVCO_2$ loading the response was a hypercapnic hyperpnea that was indistinguishable from air breathing data. Also, a mild hyperoxia, Pao_2 = 150 mmHg, was found to have little effect on the results. Recently, we have studied the ventilatory response to 2 levels of $IVCO_2$ loading and CO_2 inhalation in 3 chronic awake dogs with A-V shunts during 3 treatments: 1) normoxia (Pao_2 = 103), hyperoxia (Pao_2 = 156) and hypoxia (Pao_2 = 70). Within each treatment Pao_2 was maintained constant at the control conditions. The results of 11 replicates indicate that the response was hypercapnic for both levels of loading for all treatments and resembled the response to inhaled CO_2. Also, there was no significant difference in the slope of the response, $\Delta\dot{V}E/\Delta Paco_2$, for the different treatments. (Supported by NIH Grants HL 7012, 16390 and GM 23732).

1.11

CONTROL OF BREATHING DURING HYPOXIA IN NEWBORN PIGLETS. Edward E. Lawson and Walker A. Long*. Department of Pediatrics, University of North Carolina, Chapel Hill, NC 27514.

Newborns initially respond to hypoxia with hyperventilation which is not sustained. To demonstrate that the late hypoventilation is due to diminished central respiratory drive we have used 11 anesthetized, paralyzed, and mechanically ventilated piglets-age 3.2±.4 days (±SE). The "running average" phrenic nerve activity was recorded by an on-line computer. Phrenic minute output (PMO=peak × f) was used as the index of respiratory center activity. The servocontrolled ventilator frequency (VF) was monitored as an index of CO_2 flux to the lungs. During ventilation with $P_{ET}CO_2$ held constant the inspiratory gas was changed from 100% O_2 to 15% O_2 for six minutes and then returned to 100% O_2. (*Δ from CTRL $p < .05$)

	CTRL	Hypoxia			Recovery
Time (minutes)		1.5-2	3.5-4	5.5-6	1.5-2
PMO-% CTRL 100		161±24*	144±27	104±25	57±14*
VF-cycles/m	28.0±1.0	29.9±1.2*	30.2±1.2*	30.5±1.4*	27.1±1.0

This pattern of PMO is similar to the biphasic pattern of spontaneously breathing newborns during hypoxia. Paralysis, constant $P_{ET}CO_2$, and increased ventilator rate in the piglets during hypoxia exclude changes in pulmonary mechanics or decreased metabolic rate as explanation of the paradoxical decline in respiratory output. It is unclear whether depression of excitatory input (e.g. carotid bodies) or central inhibition is the primary event leading to decreased output. (Support: USPHS HD13280 and United Cerebral Palsy).

1.12

MECHANORECEPTOR INFLUENCE ON BIOT (CLUSTER) BREATHING IN CATS. L. M. Oyer* and C. L. Webber, Jr. Dept. of Physiology, Loyola Univ. of Chicago, Stritch Sch. of Med., Maywood, IL 60153

The purpose of the study was to define the role of mechanoreceptor feedback in modifying inspiratory (Ti) and expiratory (Te) timing in Biot (clusters of breaths separated by apneic holds) breathing. Cats were anesthetized with pentobarbital (30 mg/kg, IP) and Biot breathing induced by bilateral pneumotaxic center lesions. The cat was sealed in a plexiglas box and mechanoreceptor feedback altered by increasing or decreasing the pressure within the box (Pb). Ti, Tes (duration within the cluster), Tel (duration of apneic holds) and end-tidal partial pressure of oxygen (Pet O_2) were measured in 4 cats.

Pb (cmH_2O)	Ti (sec)	Tes (sec)	Tel (sec)	Pet O_2 (torr)
-6	1.39±.20	2.47±.32	42.79±12.36*	89.1±6.0*
-3	1.33±.18	1.85±.20	32.34± 6.50	108.7±3.3
0	1.66±.09	1.89±.11	18.96± 3.39	114.0±3.2
+3	2.18±.21	1.84±.09	10.39± 2.07	120.1±3.9
+6	2.27±.37	0.92±.19*	0.00± 0.00	124.1±3.5

*significantly different ($P<.05$) from 0 cm H_2O pressure.

Increasing mechanoreceptor feedback hastens the termination of inspiration while inhibiting the onset of the next inspiration thus Ti shortens, Tes and Tel lengthen with more negative box pressures. Control of Tel duration is dominated by mechanoreceptors as Tel lengthens even as Pet O_2 falls. Mechanoreceptor input influences the timing of breaths within the cluster and the duration of the apneic holds between clusters. (Supported by the Potts Charitable Trust Fund.)

1.13

MOUTH OCCLUSION PRESSURES (P0.1) INCREASE IN PARTIALLY CURARIZED SUBJECTS DURING HYPERCAPNIC VENTILATORY RESPONSE (HCVR). R. Holle*, E. Pavlin*, R.B. Schoene* (SPON: M Hlastala) Univ. of Washington, Seattle, WA 98195

P0.1 reflects central respiratory drive (CRD), but its dependence on respiratory muscle strength is unknown. To clarify this relationship, we produced progressive levels of respiratory muscle weakness by infusion of d-tubocurarine in 8 supine, spontaneously breathing normals. HCVR was measured before curarization and at mild (mean inspiratory effort 62±3% of control), moderate (IE 42±3%) and severe (IE 23±1%) weakness. At the severe level of weakness 1) supine FRC was not significantly changed from baseline; 2) the percent of baseline slope of $\Delta P0.1/\Delta pCO_2$ (122±27%) was significantly greater ($p<0.01$) than that for $\Delta \dot{V}_E/\Delta pCO_2$ (39±10%); 3) the percent of baseline $\Delta P0.1/\Delta \dot{V}_E$ (381±46%) during HCVR was significantly increased ($p<.01$); and 4) the P0.1 response was significantly increased (by as much as 219%)from baseline at several specific levels of pCO_2 while the $\dot{V}_E$ was unchanged or significantly decreased. Similar trends were observed at mild and moderate levels of weakness, but most did not attain significance. Thus P0.1, unlike $\dot{V}_E$, did not decrease with even severe respiratory muscle weakness. Instead, P0.1 increased under circumstances when higher CRD is expected. These results imply that P0.1 may accurately reflect CRD even in subjects with severe respiratory muscle weakness. (supported by NHLBI #HL 00906 and ALA Trudeau Fellowship.)

1.14

AWAKE AND ANESTHETIZED DOGS: RELATION BETWEEN METABOLISM AND CHEMICAL DRIVE TO RESPIRATION. D.B. Jennings and W.G. Honer*. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Ventilation ($\dot{V}_E$) is depressed by barbiturate anesthesia. This is ascribed to decreased sensitivity to PCO_2 and $[H^+]$ since the slope of the $\dot{V}_E$ response to CO_2 is decreased. However, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) also decrease during anesthesia and both factors influence $\dot{V}_E$. The ventilatory drive relative to metabolism is commonly expressed as the ratios of $\dot{V}_E/\dot{V}O_2$ or $\dot{V}_E/\dot{V}CO_2$. Ventilatory sensitivity to CO_2 and $[H^+]$, independent of metabolism, can therefore be examined as the slope of the relation between $\dot{V}_E/\dot{V}O_2$ (or $\dot{V}_E/\dot{V}CO_2$) and $[H^+]$. Six dogs with tracheostomies, exteriorized carotid arteries and cisternal CSF cannulae were studied both awake and on a different day after administration of sodium pentobarbital. Measurements were obtained while the dogs breathed air and after 5%, 6.5% and 8% CO_2 were inspired for 20 min. In anesthetized dogs the threshold of the ventilation response to CO_2 was set at a higher CSF$[H^+]$ and the slope (sensitivity) averaged 20% of that in the awake state. However, when the depressant effects of anesthesia on metabolism were taken into account, sensitivity to change in CSF$[H^+]$ was 70% of that in the awake state for $\dot{V}_E/\dot{V}O_2$, but only 40% for $\dot{V}_E/\dot{V}CO_2$. O_2 uptake was more important than CO_2 output in respiratory control during anesthesia.

(Supported by the Medical Research Council of Canada).

1.15

FIRING RESPONSES OF VENTRAL RESPIRATORY GROUP (VRG) NEURONS DURING RECOVERY FOLLOWING PARTIAL INSPIRATORY INHIBITION BY VAGAL AND PNEUMOTAXIC STIMULATION. J.P. Baker, Jr. and J.E. Remmers. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

A brief train of 0.2 ms pulses, f=50 Hz, with appropriate onset time, duration, and voltage can produce partial (reversible) inspiratory inhibition followed by recovery of the inspiratory activity. Previous work has shown that phrenic activity remains depressed below control levels in the recovery period following vagal stimulation but recovers to or exceeds control levels following pneumotaxic area stimulation (Younes and Baker, in prep.). Inspiratory neurons were recorded with microelectrodes in the VRG of anesthetized, paralyzed, vagotomized cats. The firing pattern of neurons with spinal axons agreed with phrenic responses, i.e. firing remained decreased during the recovery period after vagal stimulation, but recovered to or exceeded control levels after pneumotaxic stimulation. One group of neurons which could not be antidromically activated from the spinal cord (NAA) fired in a manner similar to those with spinal axons. Other NAA neurons showed no change compared to control, while some had decreases in firing during recovery with both pneumotaxic and vagal stimuli. These results suggest: (1) different mechanisms exist for inspiratory inhibition due to vagal and pneumotaxic sources; (2) the difference is at the level of the brainstem rather than the spinal cord. (Supported by NIH grant HL-27190).

1.16

ACTIVATION OF BULBAR POST-INSPIRATORY NEURONS BY UPPER AIRWAY STIMULATION. J.E.Remmers, D.W.Richter*, D.Ballantyne*, C.R. Bainton and J.P.Klein*. Univ.of Heidelberg and Univ.of Texas Med.Br., Galveston Texas 77550

Postsynaptic potentials were recorded from bulbar post-inspiratory (postramp) neurons (Richter and Ballantyne, Neurosci. Lett. 7, 117, 1981) in chloralose anesthetized, paralyzed, vagotomized cats. Single shock (0.5-1.5 V, 0.05 ms) stimulation of the ipsilateral superior laryngeal nerve (SLN) produces compound EPSPs (latency 3-4 ms). Brief (0.1-0.2 s) tetanic stimulation (100 Hz) at the beginning of the post-inspiratory phase (PIP) enhanced spontaneous membrane depolarization throughout PIP and lengthened this phase without significantly changing the durations of ramp inspiratory (I) and neural expiratory (nE) phases. Changes in subglottic pressure, insufflation of smoke into the upper airway or application of water to the larynx produced the same effects. Sustained tetanic stimulation of both SLNs usually arrested the respiratory rhythm for a considerable time (20-30 s);sometimes a respiratory rhythm reappeared consisting of alternating I and PIP phases but without any apparent nE phase. Augmenting IPSPs in nE did not appear before stimulation ended but always preceded the next I phase. We conclude that afferent inputs from the larynx activate post-inspiratory neurons and prolong PIP. The "apnea" caused by SLN afferent activity can be characterized as a post-inspiratory apneusis.
(Supported by the DFG and NIH-Grant HL-7-1008)

1.17

RESPIRATORY OSCILLATOR MODEL BASED ON FUNCTIONAL CORRELATES DERIVED FROM PULMONARY REFLEXES. E.J. Zuperku, F.A. Hopp*, and J.P. Kampine. Med. Col. of Wisconsin and VA Medical Ctr., Milwaukee, WI 53193

A neuronal model based on recriprocal inhibition (between inspiratory, I, and expiratory, E, neurons) and postinhibitory rebound mechanisms has been developed and computer simulated. The reciprocal inhibition was assumed to result from the interaction of fast IPSPs between the two pools of neurons. The rebound mechanism has been implimented through a voltage dependent conductance with slow kinetics and is responsible for the phase timing. Previous studies suggested that central inspiratory inhibition (CII) results from slow kinetic processes similar to those which process pulmonary stretch receptor (PSR) inputs during E and I. The neuronal elements are represented by chord-conductance models in conjunction with synaptic modulated conductances. The trajectory of the transmembrane voltage, Vm, in E neurons during I is similar to the time-dependent (PSR) volume threshold and during E to the CII. Excitatory PSR inputs, to the E pool only, advance phase switching during I and retard switching during E; no gating is required. The discharge frequency which produces IPSPs is proportional to the generator potential above firing threshold and has a decrementing pattern. The augmenting patterns of bulbospinal I neurons which receive excitatory inputs (chemo and other) are assumed to result from the slow recovery of these I neurons following periodic inhibition from the E pool. (Supported by the VA)

1.18

EFFECTS OF NPBM STIMULATION ON EXPIRATORY-INSPIRATORY PHASE SWITCHING. J. Polacheck* and J.P. Baker, Jr. Univ. of Texas Medical Branch, Galveston, Texas, 77550.

A new method to determine the threshold for expiratory-inspiratory (E-I) phase switching was recently developed and then used to study temporal changes in effectiveness of an afferent vagal stimulus (Fed. Proc. 41:1691). This method uses graded withdrawal of tonic vagal stimulation. The present study was designed 1) to validate that method and 2) to determine temporal processing of another afferent stimulus. In pentobarbital anesthetized, vagotomized, paralyzed, artificially ventilated cats we monitored respiratory output from the phrenic neurogram and stimulated the pro-inspiratory region of the nucleus parabrachialis medialis (NPBM), i.e. the area which causes E-I phase switching. By varying the frequency of a brief train (fixed duration) of pulses delivered to that area during a fixed period of the expiratory phase, a threshold (T) could be determined: the frequency just sufficient to cause E-I phase switching. Next, a constant frequency conditioning stimulus was applied for variable periods preceding the time of threshold testing to either 1) the pro-expiratory region of the contralateral NPBM or 2) a vagus nerve. In both cases we found that T increased over several hundred milliseconds in response to the conditioning stimulus and then subsequently declined over several seconds (over several breaths). This behavior is similar to that found previously and indicates substantial integrative and accommodative processing. (Supported by NIH Grants HL-27671 and HL-27190.)

1.19

DIFFERENT FORMS OF RESPIRATORY MOTONEURON SYNCHRONIZATION IN HYPOXIA AND HYPERCAPNIA. A.J. Berger, J.G. Davies* and T.A. Sears*, Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG, England.

Several forms of intercostal motoneuron synchronization have been described by Kirkwood et al. (J. Physiol. 327, 1982). A light anesthetic state favors broad-peak synchronization - BPS (width wider than +/- 20 ms) while elevation of P_{ACO_2} enhances short-term synchronization - STS (width approx. +/- 3 ms). We investigated the forms of external (inspiratory) intercostal motoneuron (Ext. I.) synchronization seen with increased respiratory drive produced by peripheral chemoreceptor stimulation with hypoxia and cyanide. In paralyzed, ventilated cats under pentobarbitone anesthesia we measured arterial blood gases and recorded discharge in Ext. I. filaments from adjacent segments. The neural data were analysed by constructing spike cross-correlation histograms between these filament discharges. In moderate anesthesia, both normocapnic hypoxia (P_{aO_2} approx. 40 torr), and cyanide (i.v.) administration in normoxia, resulted in BPS. In hyperoxic hypercapnia (P_{aCO_2} approx. 45 torr) BPS was also present but at a reduced magnitude. STS was observed in both states.

Thus in moderate anesthesia, hypoxia and cyanide result in a form of motoneuron synchronization (BPS) which is the same as that seen in light anesthesia, indicating that peripheral chemoreceptor stimulation may cause arousal.
(Supported by the Brain Research Trust and a Guggenheim Fellowship to AJB).

1.20

PULMONARY RESISTANCE IS MODULATED IN STEP WITH INSPIRATORY OUTPUT IN PARALYZED CATS. C.A. Richardson, D.A. Herbert, R.A. Mitchell, Depts of Physiology and Anesthesia, and CVRI, Univ of Calif, San Francisco, CA 94143.

This lab reported (Fed Proc 41(4):986) that pulmonary stretch receptors were modulated with an inspiratory rhythm in paralyzed cats with lungs held at constant trans-pulmonary pressures. We postulated that resistance must also have an inspiratory modulation. Cats were anesthetized with chloralose urethane, intubated just above the sternum, paralyzed with gallamine, and ventilated with a volume respirator (asynchronous with neural inspiration, Vt=18ml, f=65/min). We recorded airway pressure (with pneumothorax), air flow, and phrenic nerve activity. We computed compliance and resistance by the Mead-Whittenberger technique. In 6 cats, the resistance rose from a valley of 18.0±3.7 cmW/l/s to a peak of 25.3±6.8 cmW/l/s with each neural inspiration. After atropine (0.5mg/kg), resistance was constant at 11.5±2.4 cmW/l/s. Hyperventilation to neural apnea or a bolus injection of 2mcg isoproterenol (i.v.) reduced resistance to a level close to that produced by atropine in 3 cats tested. After hyperventilation to neural apnea and a constant resistance of 14.1 cmW/l/s, hypoxia stimulated respiration and the resistance rose from a valley of 30 cmW/l/s to a peak of 42 cmW/l/s with each burst of phrenic nerve activity (results were similar in 2 cats tested). (Supported by USPH grants HL-06825, GH15575, and HL26176)

COMPARATIVE PHYSIOLOGY: CIRCULATION, MUSCLE AND LOCOMOTION I

2.1

GRAVITATIONAL BLOOD POOLING AND CONTROL OF ARTERIAL PRESSURE IN SNAKES: A COMPARATIVE STUDY. H.B. Lillywhite. Physiol. Res. Lab., Scripps Inst. Oceanography, La Jolla, CA 92093 and Univ. of Kansas, Lawrence, KS 66045.

The extent of blood pooling in the tails of snakes was measured by a plethysmographic technique during head-up tilt. In comparison with Crotalus viridis (a rattlesnake), Pituophis melanoleucus (gopher snake) maintains higher arterial pressure (means, 33 vs. 9 mm Hg at head level) and pools less blood (means, 2 vs. 10% of initial tail volume) at 60° tilt. Preliminary data indicate that C. viridis has a higher blood volume than does the other species. Thus, in comparative terms, P. melanoleucus is a higher pressure, lower volume system with superior hemodynamic control during the assumption of vertical posture. P. melanoleucus naturally engages in climbing activity, whereas C. viridis appears ecologically less subject to gravitational disturbance of the circulation. Limited data for other species of snake are consistent with this ecological correlation: e.g., blood pooling measured in both Elaphe obsoleta (a partly arboreal colubrid) and Bothrops schlegelii (an arboreal pit viper) is quantitatively more similar to that of P. melanoleucus, whereas pooling measured in aquatic sea snakes is more like that of C. viridis. It is suggested that adaptation for arboreal habits among snakes involves both effective control of arterial pressure and pooling (edema?)-preventive mechanisms. Supported by USPHS, NIH grant HL 24640 and NSF grant PCM 79-18393.

2.2

VASCULAR ADRENOCEPTORS AND ADRENERGIC INNERVATION IN THE GILL OF THE SALAMANDER, AMBYSTOMA TIGRINUM. G.M. Malvin, W.G. Dail* and S.C. Wood. Univ. of New Mexico, Sch. Med., Albuq., NM 87131

The neotenic form of the tiger salamander is a trimodal breather using skin, lungs and gills for gas exchange. Within the gills are two major circulatory pathways: 1) A respiratory path that directs blood to the respiratory lamellae, and 2) A shunt path which allows blood to flow directly from the afferent to the efferent branchial arteries. Using isolated gills, the two pathways were selectively perfused at constant flow while perfusion pressure was measured in response to adrenergic agonists and antagonists. Vascular resistance (R_v) was calculated. In the respiratory path, epinephrine (Epi) produced a marked dose-dependent increase in R_v. High doses of Epi caused a biphasic response with vasodilation following constriction. Norepinephrine (NE) produced a marked dose-dependent dilation which sometimes followed a brief constriction. Propranolol (10^{-6}M) antagonized dilations while constrictions were antagonized by phenoxybenzamine (10^{-7}M). The shunts were considerably less responsive to catecholamines. Epi generally caused only a slight rise and NE a small decrease in shunt R_v. Catecholamine histofluorescence revealed dense adrenergic innervation of the shunt path, but no adrenergic innervation of the respiratory section. These results suggest that gill flow is regulated, in part, by adrenergic innervation of the shunts and by circulating catecholamines primarily acting on α- and β-adrenoceptors in the respiratory section of the gill. (Supported by NSF Grant No. PCM 77-24264).

2.3

CARDIOVASCULAR CIRCADIAN RHYTHMS IN RHESUS MONKEYS. Bruce M. Halprvn*, Dave Murrish, Frank Sulzman, Harold Sandler. NASA-Ames Research Center, Moffett Field, Ca 94040 & S.U.N.Y. at Binghamton, Binghamton, N.Y. 13901.

Four rhesus monkeys (Mucaca mulatta) were chronically instrumented with: pressure cells in the descending aorta or left ventricle; EKG leads; and a rectal thermistor. All animals had undergone a rigorous chair restraint training regimen over a period of 2 to 3 months, prior to instrumentation, so as to allow continuous monitoring for 2 week periods. The monkeys were kept on a 12:12 light-dark cycle at all times. Circadian rhythmicity was found in all monitored parameters. Rectal temperature was higher during the day than the night with a day-night difference averaging 1.5°C. Heart rate was also higher during the day than the night with a day-night difference of 50 beats/min. Systolic and diastolic pressures also exhibited day-night differences. The acrophase (high point) however, occoured during lights off each night. This is the opposite of the pressure rhythms documented in humans. In three additionas monkeys - two instrumented with doppler flow meters on the left common carotid and one with an electromagnetic flow meter on the descending aorta, blood flow was found to be constant, or higher during the day than the night. On this basis, the increase in systemic pressure is interpreted to indicate a net increase in systemic vascular resistance during the night, presumably due to increased vasoconstriction. (Supported in part by NASA grant #NGT 33-188-800)

2.4

RESPONSE OF ISOLATED MUSKRAT AND GUINEA PIG HEARTS TO HYPOXIA. Thomas A. McKean. University of Idaho, Moscow, ID 83843

Muskrat (a diving mammal) and guinea pig hearts were removed under ether anesthesia and perfused retrograde with Krebs-Henseleit solution that was either oxygenated or unoxygenated. Left ventricular pressure was monitored with a fluid filled balloon. Heart rate was maintained at 250 beats/min by ventricular pacing. After 30-45 min of control perfusion, the heart was made hypoxic for 30 min and then reoxygenated for 30 min. In 5 animals in which insulin was omitted from the perfusion solution, glucose uptake could not be demonstrated but muskrats maintained LV pressure better than guinea pigs during hypoxia (23 vs 14 mm Hg) and reoxygenation (64 vs 28 mm Hg). In 5 muskrat and 7 guinea pig hearts perfused with insulin (12.5u/L) glucose uptake and lactate production were greater in muskrats than guinea pigs under control, hypoxia and reoxygenation conditions. Left ventricular pressure decreased faster in muskrats during hypoxia but recovered more completely during reoxygenation than LV pressure in guinea pigs. Even though muskrats utilize more glucose during hypoxia than guinea pigs, the mechanical performance of the two hearts do not differ. However, during the reoxygenation period, the muskrat heart is better able to recover. (Supported by a Grant-in-aid from American Heart Association of Idaho).

2.5

AEROBIC DIVE LIMITS OF JUVENILE WEDDELL SEALS. Gerald L. Kooyman, Michael A. Castellini,* Randall W. Davis,* and Robert A. Maue.* Physiological Research Laboratory, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

Adult Weddell seals of body mass of 400 + kg, can dive 20 min while utilizing oxygen stores to maintain metabolism through aerobic pathways. Behavioral observations and oxygen stores of juvenile animals suggest that they would have less of an aerobic capacity. We measured the postdive venous lactic acid concentrations in four juvenile animals ranging in body mass from 130 to 205 kg. All animals were diving voluntarily from an ice hole located in McMurdo Sound, Antarctica. Postdive lactic acid concentrations did not exceed resting levels until the previous dive duration exceeded 6 min in the 130 and 145 kg animals, and 13 min in the 185 and 205 kg seals. These values agree closely with the predicted aerobic dive time calculated from data on the oxygen stores and the diving oxygen consumption rate. Similar to the adults, the large majority of the juvenile dives are within their aerobic limits. (Supported by NSF DPP 79-23623 and USPHS HL17731.)

2.6

RED MUSCLE ALLOMETRY, LOCOMOTION AND TUNA ENDOTHERMY. Kathryn A. Dickson and Jeffrey B. Graham. Physiol. Res. Lab., Scripps Inst. Oceanography, UCSD, La Jolla, CA 92093.

Scaling of red muscle with body size and the distribution of red muscle in the body were compared in seven scombrid species. Red muscle amounts range from 4 to 13% of body weight. In ectothermic *Sarda chiliensis* and *Scomber japonicus* red muscle occurs along the body edge, is most concentrated posteriorly, and has weight scaling coefficients above 1. In five endothermic tunas, *Auxis thazard, Euthynnus lineatus, Katsuwonus pelamis, Thunnus albacares* and *T. alalunga*, red muscle weight coefficients are 1 or less and this tissue is set deep and anterior in the body. Positional and linkage differences account for the thunniform swimming mode of tunas which is different from the carangiform mode used by the ectothermic scombrids and other active fishes. The power required to overcome drag increases with body size and the red muscle-scaling relationships of *Sarda* and *Scomber* reflect this large power requirement. However, red muscle scaling and position may pose limitations on both the maximum attainable size and maximum power output of these species. Decreasing proportions of red muscle in larger tunas suggest these fish increase propulsion efficiency with size. This may be due to greater muscle efficiency to which both endothermy and thermoregulation could contribute. Studies of structural and biochemical characteristics related to muscle efficiency are currently underway. (Partially supported by NSF DEB 79-12235 and NIH BRSG SO7-RR07011.)

2.7

SEASONAL CYCLES IN BEHAVIORAL AND PHYSIOLOGICAL CAPACITIES IN THE LIZARD DIPSOSAURUS DORSALIS. Henry B. John-Alder. Univ. of California, Irvine, CA 92717.

Lizards (Dipsosaurus dorsalis) were collected during Winter hibernation (Group I), shortly after Spring emergence (Group II), and during the peak activity period of late Spring (Group III). Endurance was measured as walking time on a treadmill at 1.1 km/h. Standard metabolic rate (SMR), maximal rate of O_2 consumption ($\dot{V}O_2max$) during exhaustive treadmill exercise, and citrate synthase activity (CSA) of liver, heart, and hind limb muscles were measured at 40 C, preferred body temperature of this species. Plasma thyroid hormone levels were determined by radioimmunoassay. Endurance of Group I was 27% that of Group III; Group II was intermediate. Standard metabolic rates were 0.126, 0.215, and 0.164 ml $O_2/(g \cdot h)$ in Groups I, II, and III, respectively ($p<0.001$). Maximal $\dot{V}O_2$'s were 1.485, 1.826, and 1.980 ml $O_2/(g \cdot h)$ in Groups I, II, and III, respectively ($p<0.001$). Differences in endurance and organismal metabolism were reflected in citrate synthase activities. These data demonstrate very clear seasonal differences in endurance and in physiological capacities for energetic support of activity in this lizard. Activity capacity is lowest in inactive, hibernating lizards and is greatest in lizards captured during the breeding season when highest levels of activity normally occur. The results are discussed in the context of seasonal cycles in thyroid hormone levels. Supported by NSF Grants PCM 81-02331 to A.F. Bennett and DEB 81-19797 to AFB and HBJ-A.

2.8

MORPHOLOGY, STROKE FREQUENCY AND FLIGHT ENERGETICS OF HOVERING EUGLOSSINE BEES. Timothy M. Casey and Michael L. May.* Cook College, Rutgers University, New Brunswick, NJ 08903

Mass-specific oxygen consumption (VO_2) of euglossine bees during free hovering flight is inversely related to body mass (M), varying from 66 $ml O_2(g)^{-1}(h)^{-1}$ in a 1.0 g bee to 154 $ml O_2(g)^{-1}(h)^{-1}$ in a 0.1 gram bee. Individuals of the genera Eulaema and Eufriesea are morphologically very similar and differ from those of Euglossa and Exaerete. In our sample, the former had smaller wings, higher wing loading, higher wing stroke frequency (n)and higher (VO_2) relative to M than the latter. Calculated aerodynamic power requirements (induced plus profile power) represent only a small fraction of the energy expenditure and overall flight efficiency decreases with size from 7.7% in a 1.0 g bee to 3.3% in a 0.1 g bee. If mechanical efficiency is 20%, mechanical power output of hovering bees varies inversely with M from 480 to 1130 $W(Kg)^{-1}$ of muscle. These values are 1.9 to 4.5 times greater than previous estimates of maximum mechanical power output. Mass-specific energy expenditure per wing stroke (E/n) is independent of body mass and similar in both morphological types. Allometry of energy metabolism is generally similar to that of sphingid moths. Higher n of the bees compared with the moths are compensated by much lower E/n. (Supported by Nat. Geogr. Soc. #2262-80 and by NSF Grants PCM 8011158 and DEB 7912229).

2.9

ELECTRICAL AND MECHANICAL PROPERTIES OF THE "FLIGHT STARTER" MUSCLE IN THE HOUSEFLY. Moray Anderson* and Thomas A. Miller. Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, CA 92521

The tergotrochanteral muscle (TTM) in dipterans is often referred to as the "flight starter" muscle since it is believed to deliver the power necessary to push the fly from the substratum prior to flight. The muscle fibres of the TTM have input membrane resistance in the range of 175 to 350 KΩ. The electrical responses elicited in the muscle fibres by stimulation of the motor nerves or by activating a giant fibre reflex pathway through electrically shocking the eye are spike-like in appearance but are almost totally synaptic potentials, the fibres exhibiting only slight electrically-excitable electrogenesis to high depolarizing currents. The mechanical activity of the muscle in response to motor nerve stimulation indicates innervation by three motor axons. The mechanical twitches of the muscle last between 3.5 and 12.5 msec (onset to 50% relaxation). The muscle is capable of "following" stimulus frequencies up to 80 Hz but is unable to respond to higher frequencies in a synchronous manner. (Supported by NIH grant ES00814 and grants from the American Cyanamid Company. M. Anderson is a Fulbright Scholar with support from the Wellcome Trust and the British Council.)

2.10

HISTOCHEMICAL CHARACTERIZATION OF FOUR LOCOMOTOR MUSCLES IN LEPUS CAPENSIS AND SYLVILAGUS FLORIDANUS. Danny L. Schnurr* and Vernon G. Thomas* (SPON: D.W. Rodgers). Dept. of Zool., Univ. of Guelph, Guelph, Ontario. N1G 2W1.

European hare (Lepus capensis) and eastern cottontail rabbits (Sylvilagus floridanus) were collected in southern Ontario, Canada. A muscle sample from four hindlimb muscles, the biceps femoris posterior, semimembranosus, tibialis anterior and vastus lateralis, was obtained and snap-frozen. Transverse sections were histochemically examined for succinic dehydrogenase (SDH) and myosin adenosine triphosphatase (mATPase) activity, and muscle fibers were classified on the basis of high or low activity of each enzyme. Three major fiber types were identified: fast-twitch glycolytic (FG, high mATPase, low SDH), fast-twitch oxidative glycolytic (FOG, high mATPase, high SDH) and slow-twitch oxidative (SO, Low mATPase, high SDH). SO fibers constituted a minor proportion of fiber types in muscles from both species. Hare muscles stained much more intensely for SDH activity than rabbit muscles. Rabbit muscles had similar proportions of FG and FOG fibers, but hare muscles had 2-3 times more FOG than FG fibers. The transverse area of each muscle was predominantly composed of FG fibers in rabbits, but predominantly FOG fibers in hare muscles. The large number of FOG fibers and high SDH activity provide hare with the capacity for their characteristic locomotor pattern, a high speed, sustained run in open, expansive habitat. However, rabbit muscles were composed of large numbers of FG fibers which adapt them for quick darting movements in heavy, dense habitats. (Supported by NSERC and OMNR)

2.11

PROTEIN TURNOVER DURING HIBERNATION IN THE BIG BROWN BAT, EPTESICUS FUSCUS. Marshall E. Yacoe. The University of Michigan, Ann Arbor, MI 48109

Decreased turnover is central to the conservation of tissue protein during fasting in normothermic mammals. The present study examines the extent to which decreased protein turnover might account for protein sparing during hibernation in E. fuscus. Protein synthesis was measured by a technique involving IP injection of a flooding dose of ^{3}H-phenylalanine. Specific activities of the plasma and intracellular free amino acid pools equilibrate rapidly (10 min following injection) and remain constant during the measurement period. The fractional synthetic rate (FSR) of pectoralis muscle protein in hibernating bats during periodic arousals (2.2 ± 1.6 %/day) is significantly lower (P .001) than that of normothermic summer bats (8.8 ± 2.2 %/day), but hepatic FSR does not differ between these groups (53.3 ± 23.9 and 63.0 ± 27.2 %/day, respectively). Fractional breakdown rates (FBR) of pectoralis muscle and liver protein (calculated from the FSR and the rate of loss of tissue protein) during periodic arousals are equal to or greater than those in summer bats. Neither FSR nor FBR differs significantly from zero in torpid bats. These data suggest that protein turnover is rapid during periodic arousals, resembling that in short-term fasting, when protein is rapidly degraded to satisfy increased gluconeogenic demand. The lack of protein turnover during torpor bouts may be important to protein sparing in hibernation. (Supported by a Rackham Block Grant)

MYOCARDIAL METABOLISM

3.1

PYRUVATE DEHYDROGENASE FLUX IN WORKING HEART AS INFLUENCED BY ACETATE AND NOREPINEPHRINE(NE). S. Yaffe* and R. Bünger. Dept. of Physiol., Uniformed Services University, Bethesda, MD

NE can cause an activation of the pyruvate dehydrogenase complex (PDC) in working guinea pig heart utilizing pyruvate and 3-hydroxybutyrate.(Am.J.Physiol.242,H30-H36(1982)). In the present study, metabolic flux through the PDC system was examined in hearts which utilized pyruvate and glucose in physiological concentrations, both in the absence and presence of acetate and NE. PDC flux was estimated from $^{14}CO_2$ production from [1-^{14}C]pyruvate corrected for intracellular dilution of the radioactive precursor. The specific activity of coronary effluent lactate was used as a measure of the specific activity of intracellular pyruvate. Lactate was separated from pyruvate employing a Dowex-formate column and formic acid elution. PDC flux was approx. 11 μmol/min·gdrywt when hearts performed work at physiological pre-and afterloads. 1-5mM acetate diminished PDC flux by 56%-74%. However, 0.08-0.5μM NE caused a dose-dependent increase in oxygen consumption($M\dot{V}O_2$) and PDC flux. Even with 5mM acetate the NE-induced increase in PDC flux accounted for up to 83% of the increase in $M\dot{V}O_2$. Thus, PDC flux in NE-stimulated heart can become remarkably adapted to cardiac energy demand, even when physiological concentrations of pyruvate are supplied in presence of acetate as major energy-providing substrate. Evidently, adaptive changes of PDC flux can contribute importantly to the maintenance of the energy balance in acetate and ketone body perfused guinea pig hearts, particularly during NE-stimulation. (USUHS RO7638)

3.2

CYCLIC GMP-INDUCED DEPRESSION OF THE Ca^{2+}-PUMP IN CANINE CARDIAC SARCOLEMMAL VESICLES. J.G. Church* and A.K. Sen, University of Toronto, Dept. of Pharmacology, Toronto M5S 1A8.

Cyclic nucleotide modulation of the sarcoplasmic reticulum calcium (Ca^{2+})-pump has been recognized for sometime. Little is known, however, of cyclic nucleotide effects on the sarcolemmal (SL) Ca^{2+}-pump. In inside-out SL vesicles prepared from ventricular muscle by means of a recent technique (Jones et al., J.B.C. 255: 9971-9980 (1980) we have demonstrated via Millipore filtration that 10^{-8}M and 10^{-9}M cyclic GMP depressed the rate of ATP- and Mg^{2+}-dependent $^{45}Ca^{2+}$ uptake by 34% and 52% respectively ($p < 0.05$, n=6). Only at millimolar levels did cyclic AMP have any effect and the respective 5'nucleotides had no effect at all. Parallel measurement of the associated (Ca^{2+},Mg^{2+})ATPase in the presence of either cyclic or 5'nucleotides, however, revealed no concommitant depression in ATP hydrolysis. The 10^{-8}M and 10^{-9}M cyclic GMP effect on $^{45}Ca^{2+}$ uptake was associated with a 33% and 36% increase respectively in the apparent K_m for Ca^{2+} ($p < 0.01$, n=4). These results suggest that cyclic GMP may depress ventricular Ca^{2+} efflux by decreasing the enzyme affinity for Ca^{2+} and causing dissociation of the Ca^{2+} pump from ATP hydrolysis. This supports a hypothesis whereby cyclic GMP might modulate both local biochemical and electrophysiological events by an effect on a discrete regional $[Ca^{2+}]_i$. (Supported in part by OHF Grant #1-51).

3.3

ALTERATION OF CREATINE KINASE AND ITS ISOZYMES IN RESPONSE TO PRESSURE OVERLOAD LEFT VENTRICULAR HYPERTROPHY. D.E. Vatner*, S.F. Vatner, S.P. Sit*, and J.S. Ingwall. Dept. of Medicine, Harvard Medical School, Brigham & Women's Hospital, Boston, MA

To elucidate the effects of pressure overload left ventricular hypertrophy, on creatine kinase (CK), and its isozymes, four groups of animals were studied: 1 group of 7 Sprague Dawley rats with aortic banding for 3 weeks and another group of control rats; one group of 3 dogs with aortic banding for 1 year, and another group of control dogs. All changes noted for animals with left ventricular hypertrophy are in comparison to their respective control groups. Total CK was measured by the method of Rosalki, et al, and the CK isozymes were determined by cellulose acetate electrophoresis. In the rat left ventricular hypertrophy was characterized by a 31% increase in left ventricular wt/body wt, while total CK/g of tissue was unchanged. The fraction of MB-CK increased from 13.9 to 17.7% while the fraction of MM-CK decreased from 66 to 61%, and mito-CK remained unchanged. In the dog left ventricular wt/body wt doubled, while total CK/g of tissue was unchanged. In left ventricular hypertrophy, the fraction of MB-CK increased from 2.2% to 23.9%, while the fraction of MM-CK decreased from 88.7% to 66.3%, and mito-CK remained unchanged. Thus, although total CK does not change in pressure overload left ventricular hypertrophy, there is a shift towards the fetal type isozyme profile with a corresponding diminution of MM-CK.

3.4

PREVENTION OF CHRONIC ADRIAMYCIN TOXICITY WITH CARNITINE. J. Vick* and S. DeFelice* (SPON: M.A. Khan). FDA, Washington, D.C. 20204 and BioBasics International, NY 10011

Adriamycin is an anti-cancer drug widely used in the treatment of acute leukemias and lymphomas. Patients receiving this drug have shown an unusually high incidence of cardiac toxicity which has somewhat restricted its use. Preclinical studies have demonstrated that Carnitine, a naturally occurring quaternary amine, prevents Adriamycin induced cardiotoxicity while not interfering with its anti-cancer activity. This study is concerned with the ability of Carnitine to prevent cardiac damage in monkeys given a clinical course of Adriamycin. Twelve adult Rhesus monkeys were used in the study. Six monkeys served as controls and were given 5.0 Mg/Kg Adriamycin each week for six months. All six of the monkeys developed progressive cardiac myopathies (PVC's, extrasystoles, bijeminal pulse) beginning at approximately 12 weeks and becoming nearly incapacitating at six months. One of the control monkeys died at four months. At autopsy all the monkeys in this group showed both gross and microscopic cardiac damage. In contrast the six monkeys treated with 100 mg Carnitine given simultaneously with the Adriamycin exhibited no cardiovascular damage at 12 weeks or at six months. None of the six Carnitine treated monkeys showed physical deterioration, nor were any pathologies noted at autopsy. These studies indicate that Carnitine appears useful in preventing the cardiac toxicity so often observed during long term Adriamycin therapy.

3.5

EVALUATION OF CALCIUM BLOCKERS AS AGENTS TO PROTECT AGAINST MYOCARDIAL ISCHEMIC DAMAGE. S.B. Digerness, W.G. Tracy*, N.F. Andrews*, and B. Bowdoin*. University of Alabama in Birmingham, Birmingham, Al. 35294

Contracture occurs after extended exposure to ischemia, and is indicative of irreversible damage. We studied contracture and functional recovery of isolated rat hearts subjected to 37°C ischemia using a left ventricular balloon. Tissue calcium levels were measured at the end of each experiment. Untreated ischemic arrest(U) was compared with nifedipine(N), lidoflazine(L), and flunarizine(F) treatment. These drugs were given 5 min before ischemia and for the first 5 min of reperfusion. Mean ± SE time to contracture in min, % recovery of developed pressure, increase in end-diastolic pressure(EDP) slope in mm Hg/0.01 ml balloon volume, and calcium in μmol/g dry weight are:

GROUP	n	TIME TO CONTRACTURE	% RECOVERY	EDP SLOPE INCREASE	TISSUE CALCIUM
U	7	13.4±0.57	37.5±3.32	11.3±1.11	13.8±0.96
N	6	20.5±0.73**	65.2±3.29**	4.7±1.67*	14.7±0.92
L	7	15.1±1.47	59.4±1.30*	6.6±1.15*	11.9±0.50
F	6	19.2±1.60*	80.9±2.58**	5.9±1.82*	16.5±0.63

(* = P<0.05, ** = P<0.01)

Thus, N and F significantly delay onset of contracture, N,L, and F improve recovery. Calcium was significantly elevated in all groups compared to normal hearts, despite the use of blocking agents.

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3.6

EFFECT OF PROPRANOLOL ON MITOCHONDRIAL RESPIRATORY CHANGES INDUCED BY HYPOXIA IN THE ISOLATED HEART. Dennis I. Goldberg*, Joseph W. Starnes*, Linda M. Sacks*, Maria Delivoria-Papadopoulus and Ellen O. Fuller. Dept. of Physiology and Pediatrics, Univ. of Pennsylvania, Philadelphia, PA 19104.

Mitochondria from hearts exposed to transient hypoxia in an isolated perfused preparation demonstrate increased state 3 respiratory activity. To determine whether release of local stores of catecholamines are involved in the elevation of state 3 respiration, isolated hearts were treated with propranolol prior to exposure to hypoxia. 53 excised rat hearts were perfused by retrograde, single-pass perfusion through the aorta with Krebs-Henseleit buffer and 8mM glucose at PO_2=565 mmHg (normoxic) or PO_2=148 mmHg (hypoxic). All hearts were perfused at high PO_2 for 12 min. Propranolol was added to the last 2 min of this perfusion in 38 hearts. Half of the hearts were perfused at low (hypoxic) PO_2 for an additional 10 min, and half at high PO_2. Mitochondria were isolated, and state 3 respiration and RCI determined. Propranolol did not alter the hemodynamic or RCI responses to hypoxia. Hypoxia elevated state 3 respiration (natoms $O_2 \cdot min^{-1} \cdot mg^{-1}$ mito. protein) from 318±5 to 351±4 without propranolol, a 10% increase. In hearts treated with 10^{-7}M propranolol, hypoxia elevated state 3 respiration from 304±2 to 370±10, a 22% increase. Higher doses (10^{-5} or 10^{-6}M) diminished the hypoxic effect and elevated normoxic respiration. These data indicate that the hypoxic elevation of state 3 respiration involves a mechanism intrinsic to the heart tissue, independent of catecholamine action.

3.7

THE EFFECT OF GLOBAL ISCHEMIA ON FATTY ACID CONTENT AND MITOCHONDRIAL RESPIRATION OF THE RAT HEART. W.A.Dobbs,D.M.Douglas‡ L.Anisimowicz‡andR.M.Engelman*.Department of Surgery,U. of Connecticut Health Center,Farmington,CT06032.

Twenty hearts from Sprague-Dawley rats were subjected to normothermic global ischemia for 15 minutes(n=7) or served as control tissue(n=13) for measurements of mitochondrial respiration. The free fatty acid content of eight additional rat hearts was measured after a similar period of ischemia(n=4)or in the control state(n=4) using HPLC. Fifteen minutes of ischemia produced a statistically significant reduction of State III respiration,with pyruvate as the substrate,from a control value of 61.58±2.28 to 15.97±1.71nanomoleO_2/mgproteinminute and nonsignificant increases in tissue content of fatty acids C12-C18(micromole/gwetwt) given as(Mean±SEM):

CONTROL(n=4)				15MIN ISCHEMIA(n=4)			
C12	C14	C16	C18	C12	C14	C16	C18
2.46± 0.54	1.88± 0.58	5.86± 1.23	0.47± 0.40	8.87± 4.20	3.06± 1.31	8.46± 1.94	2.46± 1.38

The simultaneous rise of tissue fatty acid content and fall of mitochondrial respiration rate supports the concept of a destructive detergent effect of abnormally high tissue fatty acids in ischemic myocardium on membrane structure andfunction (Katz, A.M. and Messineo, F.C.Circ.Res.48,1-16,1981).(Supported in part by NIH Grant 22559).

3.8

THE EFFECT OF GLOBAL ISCHEMIA ON TISSUE CONCENTRATION OF H^+ AND K^+ IN THE PIG HEART. L.Anisimowicz‡D.E.Jones‡R.M.Engelman‡ and W.A.Dobbs.Department of Surgery, U. of Connecticut Health Center,Farmington,CT06032.

The hearts of eight pigs were subjected to 120 minutes of normothermic global ischemia(I) and 60 minutes of reperfusion (R). Measurements of the tissue concentration of H^+ and K^+ were obtained with ion selective electrodes placed in the anterior-lateral region of the left ventricle. During I the tissue concentration of H^+ rose at a linear rate from 0.42 to 53.36×10^{-7}M/L after 120min and fell to 6.31×10^{-7}M/L after 5 min of R and to 2.55×10^{-7}M/L after 60min of R. On the other hand tissue K^+ rose slowly during the first hour of I from 17 mEq/L to 25.5mEq/L followed by a more rapid rise to 44.07mEq/L after 75min, and 46mEq/L after 120min of I. Upon reperfusion (K^+) remained elevated but declined slowly to 37mEq/L after 60 min of R,indicating loss of integrity of the sarcolemmal membrane. Left ventricular compliance was irreversibly decreased after 60 minutes of I to approximately 0.01 times its control value of about 1.0ml/mmHg, indicating the condition of "stone heart". During R coronary resistance was approximately 4-8 times higher than the control value. These measurements indicate that marked elevation of H^+ during ischemia contributes to the failure of the sarcolemma to contain normal cellular contents and to the inability of the heart to remain compliant. (Supported in part by NIH Grant 22559)

3.9

IMPROVED MYOCARDIAL RECOVERY FROM ISCHEMIA WITH LOW DOSE ATP-$MgCl_2$. P. F. McDonagh, H. Laks*, I. H. Chaudry and A. E. Baue, Dept. of Surgery, Yale Univ. New Haven, CT 06510

To evaluate the effects of ATP-$MgCl_2$ on myocardial function following ischemia, mongrel dogs were placed on cardiopulmonary bypass with separate coronary perfusion at 80 mmHg. A ventricular balloon was used to assess cardiac function as the area under the pressure-volume curve (VP), compliance (C) and dP/dT. Control measurements were made of coronary flow (Q), myocardial oxygen consumption and VP, C and dP/dT. Myocardial ischemia was then induced for 45 minutes followed by reperfusion (R). In Group I (n=5) no ATP-$MgCl_2$ was infused into the coronary perfusion line. In Group II (n=5) low dose ATP-$MgCl_2$ (0.13 mg/min/kg) was infused from R_0-R_{30} and in Group III (n=5) high dose ATP- $MgCl_2$ (3.2 mg/min/kg) was infused from R_0-R_{30}. ATP-$MgCl_2$ produced marked coronary vasodilatation. At R_{20} coronary resistance was 81±12, 55±15, and 31±3% of control in Groups I, II and III respectively. The high dose ATP-$MgCl_2$ (Gr III) caused a marked systemic hypotension but the low dose (Gr II) did not. After 75 minutes of reperfusion (R_{75}) compliance was decreased in all groups ($p<0.05$). In Groups I and III, both function (VP) and dP/dT were significantly decreased ($p<0.05$). However, for Group II (low dose), function (VP) and dP/dT were not different from control indicating excellent recovery. Thus at low doses, ATP-$MgCl_2$ appears to be a promising adjunct to the treatment of the ischemic myocardium. (Supported by NIH HL 24328 and HL 19673)

3.10

ROLE OF ADENOSINE IN OVERDRIVE SUPPRESSION IN ISOLATED RAT HEARTS. Lois Jane Heller, Sandra Johnson*, and David E. Mohrman. Univ. Mn. Duluth, School of Med., Duluth, Mn. 55812.

Brief episodes of tachycardia often produce a transient decrease in subsequent spontaneous heart rate, a phenomenon called overdrive suppression. Although there are several mechanisms that could account for this phenomenon one possibility that has not yet been explored is that endogenous adenosine produced during the overdrive might contribute to the pacemaker slowing. This suggestion is based on previous observations that exogenous adenosine has a significant negative chronotropic effect on the heart. In order to test this hypothesis, isolated Langendorff-perfused rat hearts were subjected to brief periods of rapid stimulation and the degree of overdrive suppression was assessed by measuring the interval between the last driven beat and the next regular spontaneous beat and comparing it to the interbeat interval during the preceding control period. When adenosine was added to the Krebs-Henseleit perfusate (10^{-7} to 10^{-5} M) control spontaneous heart rate decreased and overdrive suppression increased progressively. When aminophylline (10^{-6} to 10^{-4} M) was added to the perfusate to block the effects of endogenous adenosine, control spontaneous heart rate increased and overdrive suppression decreased progressively. These data suggest that, in this model, adenosine may participate in the control of the steady state heart rate as well as in the overdrive suppression that follows rapid stimulation.

3.11

ASSESSMENT OF INTRACELLULAR ACIDOSIS AS A MECHANISM FOR NEGATIVE INOTROPIC ACTION OF HALOTHANE. P.A. Murray, T.J.J. Blanck*, Y.I. Fisher*, M.C. Rogers, M. Micelli* and W.E. Jacobus. Departments of Anesthesiology/Critical Care Medicine and Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Halothane is a highly lipophilic, volatile anesthetic with a profound negative inotropic action. Because myocardial contractility has been demonstrated to be tightly coupled to alterations in intracellular pH and because the effects of halothane on myocardial intracellular pH were unknown, we tested the hypothesis that the negative inotropic action of halothane is mediated by a reduction in intracellular pH, i.e. intracellular acidosis. Utilizing ^{31}phosphorus-nuclear magnetic resonance spectroscopy, intracellular pH was estimated from the chemical shift of the inorganic phosphate resonance measured relative to the phosphocreatine resonance. Experimental measurements were made on 8 isolated, Langendorff-perfused, isovolumically contracting rabbit hearts before and during the administration of 2.5% halothane. With heart rate and coronary perfusion pressure constant, halothane markedly decreased ($p<0.01$) left ventricular developed pressure by 48 ± 3% from 119 ± 5 mmHg. In striking contrast, intracellular pH was not significantly altered during halothane (7.22 ± 0.03) from a control of 7.21 ± 0.04. Thus, this clear dissociation between the negative inotropic action of halothane and intracellular pH indicates that intracellular acidosis does not mediate the cardiac depressant effects of halothane.

3.12

EFFECTS OF SERUM FROM AZOTEMIC PATIENTS ON CARDIAC CONTRACTILITY. K. J. Gjeltema,* J.K. Leach, P.Avasthi,* B.J. Skipper,* D. Millis.* V.A. Medical Center and University of New Mexico School of Medicine, Albuquerque, N.M. 87108.

Cardiac dysfunction in patients with renal failure may be due to fluid retention, electrolyte imbalances, anemia, hypertension, and coronary artery disease. Additionally, presence of a direct myocardial depressant factor has been suggested. This study was done to determine if sera from azotemic patients has an adverse effect on myocardial contractility. Isolated rabbit papillary muscle perfused with oxygenated Tyrode's solution was arranged for isometric force recording. Following stabilization, each muscle was perfused sequentially for 20-minute periods with Tyrode's, azotemic serum, Tyrode's, normal serum, and Tyrode's solution (sera diluted 1:4 with Tyrode's solution). The sequence of perfusion with sera was reversed in half of the experiments. Isometric contractions were recorded continuously during the entire perfusion sequence. During perfusion with sera from 6 of 8 azotemic patients, active force was less then during perfusion with Tyrode's solution or normal serum ($p<0.05$). In no instance (0 of 8) was force during normal serum perfusion significantly less than during Tyrode's perfusion. Rate of force development, time to peak force, and rate of relaxation were unchanged in all. The data suggest that azotemic sera exerted a negative inotropic effect on rabbit papillary muscle. (Supported by Veterans Administration)

MICROCIRCULATION

4.1

QUASI-PERIODIC SPONTANEOUS VASOMOTION AND THE EFFECT OF ANESTHESIA ON THE MICROCIRCULATION OF THE HAMSTER SKIN FOLD WINDOW. Antonio Colantuoni*, Silvia Bertuglia* and Marcos Intaglietta. University of California, San Diego, La Jolla, CA. 92093

The diameter of small arteries and arterioles was measured in-vivo in the dorsal skin fold window preparation implemented in the hamster which can be studied at the microvascular level without anesthesia and after the healing process mitigates the effects of surgery. Various anesthetics including nembutal, alfathesin, ethyl ether, and alfa-chloralose were given i.v., i.p. (by means of indwelling catheters) or by inhalation, according to the characteristic modality of each agent and at normal dosages. The vessel diameter was measured continuously by means of the image shearing video microscope. During control conditions all arterial vessels showed quasi-periodic dilations and constrictions with an amplitude that was directly proportional to the vessel diameter at a frequency which was inversely proportional to vessel size. Amplitudes and frequencies were determined by power spectrum analysis of the diameter records. Anesthesia always paralyzed this activity, and this effect persisted up to 30 minutes after the animal woke up. The paralyzed vessels had diameters that were as great as that of the maximum diameter achieved by the vessel during the vasomotor cycle. This diameter however, was of the order of 70% of that achieved when the vessel was made to dilate by means of pharmacologic agents.

(Supported by USPHS NIH Grant HL-12493)

4.2

THE EFFECT OF INCREASED H^+ ION CONCENTRATION ON GROWTH RATE OF CULTURED VASCULAR ENDOTHELIAL CELLS. H.W. Burton and J.K. Barclay. U. of Guelph, Guelph, Canada N1G 2W1.

Canine hindlimbs were perfused with collagenase (0.5 mg/ml) to extract vascular endothelial cells for the purpose of examining proliferation rate. Cells were cultured in Dulbecco's Modified Eagles Medium containing 10% dog serum, 50 µg/ml Gentamycin, 2.5 µg/ml Fungizone and 50 µg/ml Fibroblast Growth Factor (FGF) and maintained in an incubator at 37°C and a PCO_2 of 38.5 mmHg. After confluency was reached (2-3 weeks) cells were trypsinized and seeded at 1.25×10^3 cells/cm^2. 150 ng/ml FGF was added to each dish and the medium was changed and more FGF added at days 2 and 4. Experimental groups were exposed to either 70 mmHg CO_2 or 2.5 mM, 5 mM or 10 mM lactic acid (La). A control group was run concurrently and cell number in each group was estimated at 4 and 7 days with a hemacytometer. H^+ ion concentration ($[H^+]$) in control groups ranged from 32.4 to 39.8 n moles/l while experimental groups averaged 53.7 n moles/l at days 4 and 7 for 70 mmHg CO_2, 43.6 and 64.7 n moles/l for 10 mM La, 40.7 and 51.3 n moles/l for 5 mM La and 34.7 and 44.7 n moles/l for 2.5 mM La, at days 4 and 7 respectively. Doubling time for control groups ranged from 21-26 hr. by day 4 and 26-32 hr. by day 7. Doubling time for the experimental groups varied widely and growth was significantly depressed at day 4 and day 7 in all groups ($p < .01$). These results indicate that increased $[H^+]$ inhibits growth of vascular endothelial cells in culture.

4.3

A SIMPLE INEXPENSIVE INTRAVITAL VELOCIMETER. Jeffrey L. Borders* and Harris J. Granger. Microcirculation Research Institute and Department of Medical Physiology, Texas A&M University, College Station, Tx. 77843.

Analysis of blood flow at the microvascular level requires accurate measurements of red cell velocity. A grating type intravital velocimeter was constructed for this purpose using commonly available commercial components. The sensor head was designed around a differential grating simulated by Ronchi rulings mounted on a beam splitter. A resistive subtraction of sensor signals allowed a high transimpedance gain, 5×10^8, while noise levels were held to less than 2 mv RMS over a 5 kHz bandwidth. The sensor output, a frequency related to velocity, was converted to a dc voltage with a standard thresholding frequency-to-voltage functional block. The velocimeter has the typical characteristics of an optical doppler system - high frequency response, velocity determination - without the need for the complications of a laser doppler technique or the requirement of highly specialized microprism gratings. Since the frequency detection is a simple implementation, there are large cost and time advantages to this technique over the standard cross-correlation practices. The process requires no operator intervention or interpretation, so that experimental bias is better controlled. The velocimeter represents a cost effective approach to intravital work, and offers significant improvements in performance over standard techniques. (Supported by NIH Grant #25387)

4.4

REVASCULARIZATION OF BUNDLES OF SKELETAL MUSCLE FIBERS TRANSPLANTED INTO THE HAMSTER CHEEKPOUCH. John A. Faulkner, Stefani W. Weiss*, and John K. McGeachie*. University of Michigan Medical School, Ann Arbor, MI 48109

We tested the hypothesis that blood vessels survive the ischemia of skeletal muscle transplantation and that surviving blood vessels revascularize the transplanted muscle by establishing circulation with the host tissue. Bundles composed of approximately 20 skeletal muscle fibers were transplanted into the hamster cheekpouch. Regeneration of muscle fibers followed a course similar to that reported for autografts of whole muscles in rats. Blood vessels in the graft adjacent to vascularized host tissue survived. At 1 day, surviving blood vessels in the graft had normal structure but erythrocytes in the lumen were packed tightly rather than well-spaced. At 2 days, blood vessel sprouts had grown out of the graft into the cheekpouch. Between 2 and 2 1/2 days, anastomosis of vessels from the graft with those of the cheekpouch re-established circulation in thin-walled vessels with well-spaced erythrocytes. By 3 days, blood vessels increased in number and diameter and occupied 40-60% of the graft. Over the next 24 hours, blood vessels in the graft regressed. Few differentiated blood vessels were observed even after 5 days. We conclude that blood vessels in muscle grafts survive transplantation, and that circulation in grafts was re-established by blood vessel sprouts from the graft anastomosing with the blood vessels of the host. (Supported by Michigan Heart Association and USPHS NIH NS # 17017)

4.5

DUAL INNERVATION OF THE RAT GRACILIS MUSCLE. R.L. Prewitt and B.P. Fleming. LSU Medical Center, Shreveport, LA 71130

The rat gracilis muscle is a two-headed muscle with a thin central portion suitable for microcirculatory study. The innervation of the gracilis is thought to be the obturator nerve, but the following studies demonstrate that at least half of the sympathetic innervation enters the muscle as small branches from the femoral nerve traveling with the muscular branches from the femoral artery. Male Wistar rats were anesthetized with chloralose-urethane and the right gracilis was exposed for microcirculatory observation using quantitative stereological techniques. The obturator and femoral nerves were isolated. Capillary density was measured under innervated conditions and again following the transection of either the obturator or femoral nerve, and a third time after transection of the remaining intact nerve. Capillary density increased 14% whenever the obturator was transected, 47% when the femoral was transected first, and 27% when the femoral was transected after the obturator. Electrical stimulation of the nerves indicated that the obturator is the motor nerve, but arteriolar constriction occurred with the stimulation of either nerve after application of tubocurarine. Monoamine histofluorescence by the glyoxylic acid technique showed that catecholamines were present 3 days after the transection of either nerve, but transection of both nerves resulted in complete denervation. (Supported in part by USPHS Grants HL 28963, HL 28989 and a Grant-in-aid from the Louisiana Heart Association.)

4.6

HISTOCHEMICAL TYPING OF SPECIFIC MUSCLE FIBERS IN IN VIVO MICROVASCULAR PREPARATIONS. Evangelyn W. Kanabus, Richard D. Wetmore* and Patti J. Lute*, The Ohio State University, Columbus, OH 43210.

In order to correlate in vivo oxygen levels and capillary blood flow with specific muscle fiber types, we developed a method for marking single fibers in the cat tenuissimus microvascular preparation for subsequent histochemical identification. Alcian Blue dye is pressure injected into the in vivo preparation via micropipettes inserted into the fiber of interest. The dye spreads inside the fiber, producing a discrete spot detectable for 1500 µm along its length. Muscles are then frozen at their in situ length and stained to show oxidative capacity, twitch speed, and capillary endothelium. The blue spots remain visible throughout staining procedures for succinic dehydrogenase, myofibrillar ATPase, and alkaline phosphatase. Total fiber composition was fast glycolytic (FG): 31%, fast oxidative glycolytic (FOG): 53%, and slow oxidative (SO): 16%. Initial evaluation of fiber distributions suggest that non-oxidative fibers may be preferentially surrounded by non-oxidative fibers, while SO fibers are surrounded by fibers having demonstrable oxidative capacity.

Supported by the Central Ohio Heart Chapter.

4.7

A TEST OF THE OXYGEN SENSITIVITY OF ARTERIOLES IN THE SUFFUSED HAMSTER CHEEK POUCH. William F. Jackson and Brian R. Duling. Dept. of Physiology, Univ. of Virginia School of Med., Charlottesville, VA. 22908.

The role of O_2 in the local control of blood flow remains unclear. Oxygen could act directly on arterioles or indirectly by altering vasoactive metabolite production by the parenchyma. To separate these hypotheses we used microdissection techniques to remove the parenchyma surrounding second order arterioles. Oxygen sensitivity was assessed by changing the gas composition of the suffusate from 0% to 21% or 100% O_2. The diameters of aparenchymal arteriolar segments (APS) and control arteriolar segments (CS) were not different in 0% O_2; increased O_2 caused identical vasoconstriction in both APS and CS. Thus, O_2 induced contraction of arterioles was not solely the indirect result of an effect of O_2 on the surrounding parenchyma. However, distant parenchymal cells could have induced the vasoconstriction by causing propagation along the arteriole from CS to APS or by blood transport of a parenchyma derived mediator. To test the latter hypothesis we assessed the O_2 sensitivity of occluded APS and found normal reactivity. We infer that the O_2 sensitivity of the arterioles results from either; 1) a direct action of O_2 on some element of the arteriolar wall, 2) an effect of O_2 on some blood component or 3) an indirect action of O_2 initiated by the parenchyma and propagated by some element of the vessel wall. Supported by HL06337-01 and HL12792.

4.8

PRESSURE-FLOW RELATIONSHIPS IN THE MICROCIRCULATION DURING ACUTE ARTERIAL OCCLUSION. D.W. Haack and F.C. Johnson. Dept. Physiol., Coll. Med., Univ. Ariz., Tucson, AZ 85724

The concept of critical closure has been invoked to explain data indicating that blood flow stops 2-3s after arterial occlusion or cardiac arrest and that zero flow may occur at arterial pressures as high as 50mmHg (Bellamy, Circ. Res. 43:92, 1978). However these data were obtained from pressure-flow studies in large vessels. To determine if closure of microvessels occurs after large artery occlusion, the microcirculation of the isolated, autoperfused cat mesentery was studied by intravital microscopy. Red cell velocity and diameter were measured in arterioles (8.6-64µm, i.d.) and venules (8.6-72.8µm, i.d.) before and during a 3-7 min period in which the arterial circuit of each preparation was clamped. Arterial (Pa) and venous (Pv) pressures were measured continuously. With occlusion $\overline{Pa}$ fell from 95 to 5 mmHg while $\overline{Pv}$ dropped from 3.9 to 1.9mmHg. Mean arteriolar diameter fell from 24 to 19µm, a 20% decrease, while mean venular diameter fell from 34 to 26µm, a 22% decrease. No vessel closed completely. Dual-slit velocity measurements and visual observation reveal that flow persists for the duration of the occlusion (3-7 min). The data support the hypothesis that the capacitance of the large vessels may sustain flow for prolonged periods of time (Eng et al., Circ. Res. 50:334, 1982) and suggest that rheologic or hemodynamic factors, rather than closure, may be responsible for flow stoppage at low perfusion pressures.

4.9

EFFECT OF DEXTRAN-INDUCED HYPERVISCOSITY ON REGIONAL BLOOD FLOW. Richard Y.Z. Chen, Ronald D. Carlin, Shlomoh Simchon*, Kung-Ming Jan and Shu Chien. P & S, Columbia Univ. NY, NY, 10032

In 9 pentobarbitalized dogs, isovolumetric exchange of plasma with plasma containing 20 g w/v Dx500 (dextran m.w. 500,000) was carried out in two 200 ml steps. Plasma and blood viscosities (η_p and η_b, Couette viscometry) and regional blood flow (Q, 15 µ microspheres) were determined in control state and at 20 and 60 min after each step. Regional flow resistances (R) were calculated from pressure gradients and Q; vascular hindrances (Z) were calculated as R/η_b. Mean (± SEM) of η_p and η_b are:

		1st Dx500		2nd Dx500	
	Control	20 min	60 min	20 min	60 min
η_p (cP)	1.3±0.1	2.8±0.4	2.6±0.4	3.8±0.6	3.6±0.6
η_b (cP)	4.2±0.6	6.4±0.6	5.8±0.6	7.9±1.0	7.2±0.9

Z was negatively correlated with η_b in the total systemic and pulmonary circulations, as well as in the heart, liver (hepatic artery), brain and kidney. With increases in η_b, Q actually increased in heart and liver, while remained essentially unchanged in brain and kidney. These data indicate that dextran-induced hyperviscosity leads to a compensatory vasodilation in several vital organs, thus serves to maintain blood flow and nutrient transport. (supported in part by NHLBI Grant HL 16851 and NRSA Grant HL 07114)

4.10

COMPARISON OF ARTERIAL AND CAPILLARY FLOW VELOCITIES IN HUMAN SKIN. Daniel Richardson, Univ. of Ky., Lexington, KY 40536.

Simultaneous measurements of mean digital artery flow velocity (Vel-A) and mean capillary flow velocity (Vel-C) in the nailfold bed of the corresponding finger were obtained in 10 human subjects (age range 20 to 30 years), at a skin temp. of 32° C. Vel-A was measured by a doppler system; while Vel-C was obtained with TV microscopy and a velocity tracking correlator. Instantaneous values of Vel-A ranged from 0.5 to 22.0 cm/sec while the range of Vel-C values (excluding zero) was 0.05 to 0.60 mm/sec. The temporal pattern of Vel-A consisted of a steady state flow averaging 8.5 cm/sec randomly interrupted by brief moments during which Vel-A decreased to an average of 3.3 cm/sec. In only about 1/3 of the cases was the drop in Vel-A accompanied by a decrease in Vel-C. The correlation between Vel-A and Vel-C was first examined by selecting one capillary per subject, then obtaining from 15 to 35 paired measurements of Vel-A and Vel-C over a 5 min. period. Correlation coefficients for these 10 sets of pairings ranged from -0.414 to +0.482. A more integrated analysis of Vel-C was achieved by simultaneously video taping 4 capillaries from a given subject then periodically pairing average Vel-C values from the 4 vessels with corresponding Vel-A values. Correlation coefficients for these pairings ranged from -0.370 to +0.464. It is concluded that moment to moment variations in the arterial supply to human skin are not reflected to the level of the capillary network. Sponsored by the Ky. Heart Assoc., and KY Tobacco and Health Inst.

5.1

COMPARISON OF CARDIOVASCULAR EFFECTS OF SPACE FLIGHT AND ITS ANALOGS USING COMPUTER SIMULATIONS. R. Srinivasan* and J.I. Leonard* (SPON: C.S. Leach). Management and Technical Services Company, Houston, TX 77058

The response of the cardiovascular system to weightlessness is complex and the physiologic mechanisms are not clear. Efforts to elucidate the mechanisms underlying cardiovascular deconditioning resulting from weightless exposure have included investigations using ground-based analogs of space flight such as water immersion, supine bed rest, and head-down tilt. In the present study, the cardiovascular effects of space flight and its analogs were analyzed using computer simulations. Two different mathematical models of the cardiovascular system provided the framework to compare the results of various investigations. A short-term model was used to reproduce responses to exercise and lower body negative pressure and a long-term model simulated events lasting up to several days. The simulation results were found to be in general agreement with experimental findings, attesting to the validity of the mathematical representations. With such validation, the models were used to study the changes in cardiovascular variables that have not hitherto been measured in space flight, as well as to test hypotheses that have been suggested to explain the cardiovascular effects due to weightlessness. The results support the contention that the cardiovascular response to weightlessness cannot be explained on the basis of blood volume loss alone. (Supported by contract NAS9-16328).

5.2

FLUID SHIFTS IN VASCULAR AND EXTRAVASCULAR COMPARTMENTS OF HUMANS DURING AND AFTER SIMULATED WEIGHTLESSNESS. Alan R. Hargens, Bryan J. Tucker* and Charles M. Tipton. Depts. of Surgery and Medicine, Univ. Calif. and V.A. Medical Centers (V-151), San Diego, CA 92161 and Depts. of Phy. Ed. and Physiol. and Biophys., Univ. of Iowa, Iowa City, IA 52242

Weightlessness conditions are effectively simulated by 5° head-down tilt of humans. Eight male subjects were tilted 5° head-down for 8 hours to determine vascular and extravascular shifts of fluid. Most of the initial loss of leg volume during head-down tilt represented a passive, cephalic shift of venous blood. Facial edema, headache, nasal congestion, and pronounced diuresis were associated with this redistribution of blood volume. As measured by the wick catheter during head-down tilt, interstitial fluid pressure in lower-leg muscle and overlying subcutaneous tissue decreased 7.4 and 4.4 mm Hg, respectively. Based on previous studies of interstitial compliance, fluid is shifted from lower-leg interstitium at a rate of 12 $ml \cdot hr^{-1}$. Dehydration of lower-leg tissues probably results from decreased capillary blood pressure during tilt. During upright posture in humans, edema in the lower leg is prevented by precapillary myogenic control of blood flow and blood pressure as well as segmental variations in capillary vessel morphology. Countermeasures to maintain precapillary-muscle tone may be necessary during long space flights to prevent swelling of lower-leg tissues upon readjustment to Earth's gravity. (Supported by NASA Contract NAS 9-16039, Veterans Administration and by USPHS/NIH AM-25501, AM-26344 and RCDA AM-00602 to A.R.H.)

5.3

COMPUTER SIMULATION ANALYSIS OF THE BEHAVIOR OF RENAL-REGULATING HORMONES DURING HYPOGRAVIC STRESS. Joel I. Leonard* (SPON: C.S. Leach). Management and Technical Services Co./CE, Houston, TX 77058

Data regarding the behavior during space flight of three important hormones (angiotensin, aldosterone, and ADH) involved in renal regulation has been difficult to interpret or to reconcile with results from one-g analogs of weightlessness such as water immersion, supine bed rest, and head-down tilt. A mathematical model of renal-endocrine behavior has been used to examine these data and to assess the controlling mechanisms. These studies indicate that the behavior of these hormones may be tentatively explained by the dynamic interaction of at least two competing influencing factors: blood pressure and plasma electrolyte concentration. Initially, hormonal secretion is suppressed under the influence of volume-pressure controllers responding to headward fluid shifts. Within several days, however, hormonal levels appear to be controlled by plasma electrolytes (sodium, potassium, or osmolarity) which may be higher or lower than normal depending on such factors as diet, excretion, and tissue atrophy. Also, a reduced volume of distribution during hypogravity tends to increase plasma hormone levels. The net result is a highly dynamic biphasic or triphasic response. Confirmation of this hypothesis requires measurements of the influencing factors, including venous pressure, during acute and chronic stages of space flight. (Supported by NAS9-16328).

5.4

ATERATIONS IN GLOMERULAR AND TUBULAR DYNAMICS DURING SIMULATED WEIGHTLESSNESS. BJ Tucker* AR Hargens, CW Peterson* and RC Blantz. Depts.of Medicine and Surgery, VA Med. Ctr. Ctr. and Univ. of Calif. San Diego, CA. 92161.

There are few studies pertaining to the functional adaptation of the kidney to fluid and electrolyte shifts induced by chronic weightlessness. A 25° head-down tilt was utilized for 7 days in 6 Munich-Wistar rats (E) to simulate weightlessness. The 25° head-down posture was maintained during measurements of glomerular filtration rate (GFR), nephron filtration rate (sngfr), nephron plasma flow (rpf), afferent (AR) and efferent (ER) arteriolar resistance, proximal tubular reabsorption (APR) and compared to 6 controls. The result are as follows: (MAP= mean arterial pressure, †=$p<0.05$ to C).

	MAP [mmHg]	GFR [ml/min]	sngfr [nl/min]	rpf [nl/min]	AR [$x10^9$ dynes·sec·cm^{-5}]	ER [nl/min]	APR [nl/min]
C	116 ± 3	1.2 ± .05	40 ± 1	117 ± 7	22 ± 1	11 ± 1	18 ± 1
E	105 ± 3†	1.0 ± .06	36 ± 1†	94 ± 4†	22 ± 2	14 ± 1†	19 ± 1

Despite the reduction in sngfr due to reduced rpf, APR was maintained to facilitate retention of fluid. The major reduction in rpf was due to decreased MAP and increased ER. There was no decrease in the glomerular permeability coefficient in E which has been observed in chronic volume depletion (JCI 64:503, 1979). Conclusions: Chronic simulated weightlessness,1) did not reduce APR, although sngfr was decreased through reductions in rpf; 2) did not alter hydrostatic and colloid osmotic forces for filtration and reabsorption in the kidney. (Supported by USPHS/NIH AM-28602 and the VA).

5.5

CLONIDINE AS A COUNTER MEASURE FOR METABOLIC STUDIES DURING WEIGHTLESSNESS SIMULATION. A. Güell, Cl. Gharib, G. Gauquelin, P. Montastruc, A. Bes. Department of neurology, CHU Rangueil, 31054 Toulouse Cedex (France).

Montastruc (Europ. J. Pharmacol., 1981, 72, 373-376) show that clonidine (C) inhibites the diuretic response elected by left atrial distension in chloralose anaesthetized dogs. Furthermore the increase in the intrathoracic volume,observed in man, during space flight provokes a diuretic response.Our work involves the effects of C, in man during a simulation of weightlessness. Materials and methods : 6 young,healthy volunteers were placed in bedrest and in antiorthostatic position at-4° during 7 days ; 3 of these received 0,450 mf of C during the bedrest. Before, during and after the experiment we took blood and urine samples for the determination of the hormonal and metabolic indices involved in blood volume regulation. Results : in the subjects without C we noted increased diuresis, sodium depletion and aldosterone outflow; plasma renine and aldosterone rose after the 24 th hour. In the subjects with C the diuresis was inhibited and the sodium depletion was stopped after the 2nd day ; plasma renin and plasma aldosterone don't rise significantly : finally the ADH concentration in urine was reduced.
Conclusion : We conclude that it's interessing to use C as a counter measure for metabolic and hormonal studies during the experiments of weightlessness simulation.
Study supported by Grant from C.N.E.S.

5.6

EFFECTS OF ANTIORTHOSTATIC POSITION AT-4° ON HYDROMINERAL BALANCE. A. Güell, Cl. Gharib, G. Fanjaud, Ph. Dupui, A.Bes. Department of Neurology, CHU Toulouse Rangueil 31054 Toulouse Cedex France.

Experiments of bedrest in antiorthostatic position are conducted to simulate cardio-circulatory,metabolic and hormonal modifications observed during space flight. Materials and methods : 4 young healthy volunteers were placed in strict bedrest and in antiorthostatic position at-4° during 7 days. 1 day before, during and 2 days after the experiment, we took 2 samples daily of blood for the determination of the plasma concentration of Na^+, K^+, creatinine, urea, renin activity, aldosterone ; in the urines the same parameters were studied. Results : the haematocrit rose from 43.5 ± 1.5 to 46.8 ± 0.9% ($p<0.001$) ; the plasma sodium fell from 138.1 ± 0.5 to 136.3 ± 0.7 mmol ($p<0.01$) : plasma renin activity and plasma aldosterone rose significantly after the 24 th. hour. We alsc noted increased diuresis, sodium depletion and aldosterone out flow ; the volunteers presented with the classic clinical picture of cephalic congestion. Blood pressure was not significantly modified ; heart rate decrease by 22 % until the 4 th day. These reactions were most striking during the first 3 or 4 days, and result from a redistribution of the body's fluid volume toward the cardiac cavities and the head away from the lower part of the body.
Study supported by Grant of C.N.E.S.

5.7

CARDIOVASCULAR EFFECTS OF GRAVITATIONAL STRESSES (LBNP). THE INFLUENCE OF ANGIOTENSIN-CONVERTING ENZYME INHIBITION BY CAPTOPRIL. Flemming Bonde-Petersen, Birger Hesse,* Sten Rasmussen,* and Niels Juel Christensen.* University of Copenhagen and County Hospitals Glostrup and Herlev, Denmark.

The tolerance to Lower Body Negative Pressure (LBNP) was tested in 5 subjects during -20 and -40 Torr LBNP for 10 min periods, interspersed with a 15 min rest. Then 100 mg of Captopril was given orally, and the tests were repeated. Captopril decreased the time to pre-syncope and induced a 10 Torr lower Mean Arterial Pressure (MAP) during LBNP in face of a compensatory rise in catecholamines while resting MAP was unchanged. The findings suggest that angiotensin interacts with the sympathetic nervous system to maintain blood pressure during increased +Gz. Cardiac output (CO), stroke volume(SV), and lung tissue/blood volume were unaffected by the drug.

	CONTROL			CAPTOPRIL		
	REST	-20	-40	REST	-20	-40
CO; L/MIN	6.45	4.50	3.52	6.10	4.20	3.58
SV; ML	108	67	46	100	61	44
LUNG VOL; L	1.17	1.00	0.88	1.25	1.00	0.92
HR; BPM	60	67	75	61	69	82
MAP; TORR	90	91	84	91	80	74
TPR;MAP/CO	14	21	24	15	20	21
ARM VASC RES	3.06	2.99	4.07	3.49	3.21	3.92
EPINEPH;NG/ML	0.055	0.043	0.057	0.047	0.093	0.240
N-EPINPH;NG/ML	0.190	0.250	0.373	0.227	0.337	0.483

(Supported by the Danish Space Board 1112-33/82)

5.8

HORMONAL AND RENAL RESPONSES TO PLASMA VOLUME EXPANSION AFTER HORIZONTAL RESTRAINT IN THE RHESUS MONKEY

D.T. Dickey*, G.E. Billman, M.J. Keyl, D.A. Kem, L.C. Keil, H.Sandler and H.L. Stone: Univ. of Okla. HSC, Okla. City, OK

A 25% blood volume expansion (BVE) activates the renal compensatory mechanisms and decreases circulating levels of ADH and aldosterone in the ketamine-anesthetized rhesus monkey. 25% BVE was repeated on 5 male monkeys after 7 days of horizontal restraint in a body cast and after 14 days of restraint in 3 of the monkeys. Blood volume (BV) was previously determined using R^{125} ISA and ^{51}Cr-tagged red cells. After ketamine anesthesia, two 30 min control urine samples were taken followed by 25% BVE with iso-osmotic Dextran 75. 20 min collections of urine began 20 min after BVE and continued until 1 hr post BVE. Blood samples were withdrawn at the midpoint and end of each urine collection, this volume being replaced with Dextran.

	Control		7 Days N=5			Control		14 Days N=3	
	C	50	C	50		C	50	C	50
ADH	2.64	1.38	6.32	3.02	ADH	3.10	1.33	10.17	4.77
ALDO	12.96	3.12	6.70	2.06	ALDO	9.70	4.8	17.87	3.00

Although there was a drop in resting urine output after restraint, there appeared to be no change in the renal responses to 25% BVE. There was a clear increase in resting plasma ADH and aldosterone levels after restraint. The hormonal response to BVE was similar after restraint, but of greater magnitude. (Supported by NASA Grant NCC2-24)

5.9

HUMORAL CHANGES IN ANTIORTHOSTATIC RATS. Vojin Popovic, Pava Popovic* and Clegg Honeycutt.* Emory University, Atlanta, GA 30322.

An animal model (antiorthostatic hypokinetic rat model) was recently developed that simulates cardiovascular and some other physiological changes that occur in man during space flights. In this work sixteen Sprague-Dawley rats (200 ± 10 g), with chronically cannulated aorta (two weeks before the experiment) were exposed to seven day long head-down (antiorthostatic) hypokinesia in order to study the level of stress imposed by the placement of the harness and by the exposure to head-down position in the animals. The level of ACTH, corticosterone, and prolactin was determined in the blood (radioimmunoassays). The blood (0.3 ml) was sampled from the aortic cannula three times prior to antiorthostatic hypokinesia in resting unrestrained rats. The blood was taken three times during head-down hypokinesia in harnessed rats (on the first, third, and seventh day) and three times after release of the animals from the harness (second, fifth, and tenth day). ACTH was elevated (70 percent above the control level) on day one, and day three (30 percent) but returned to the normal range on day seven. Corticosterone was slightly increased on day one and three of the exposure while prolactin stayed in the normal limits. Catecholamine and growth hormone levels are presently measured in antiorthostatic rats. (Supported by NASA grant NAg2-87).

5.10

FLUID SHIFTS AND ERYTHROPOIESIS: RELEVANCE TO THE "ANEMIA" OF SPACEFLIGHT. C.D.R. Dunn, Northrop Services, Inc. & Baylor College of Medicine, Houston, TX, P. C. Johnson and C. S. Leach, JSC/NASA, Houston, TX 77058

To assess the hypothesis that cephalic fluid shifts and/or hypovolemia might result, through reduced erythropoietin (Ep) titers, in the anemia of spaceflight, erythroid regulation was investigated in; (1) humans subjected to horizontal or 6° head-down tilt bedrest for 7-30 days, (2) squirrel monkeys exposed to lower body positive pressure, and (3) rats subjected to antiorthostatic hypokinesia. In addition, (4) erythropoiesis was assessed in mice in which the plasma volume was reduced by water deprivation. In "model" 1, Hct was elevated, RBC production appeared reduced and the subjects became anemic. Serum Ep titers were not obviously decreased. In neither models 2 or 3 was any firm evidence obtained that the cephalic fluid shifts resulted in elevation of the Hct. Suppression of RBC production was not seen in model 2 although the rats in model 3 became anemic. Mice in model 4 showed suppression of RBC production although this occurred prior to decreases in serum Ep titers and causes other than the increased Hct are implicated. In conclusion, cephalic fluid shifts do not consistently result in Hct elevations. The mechanism by which these fluid shifts cause anemia in some models is not clear, but does not appear to be exclusively related to Hct increases or to decreases in serum Ep. (Supported by contracts NAS 9-16180, 9-15164, 9-15425, and Grant NAGW-308 with NASA.)

5.11

THE RELATIVE CONTRIBUTIONS OF GRAVITY, BUOYANCY, AND COLD TO THE CHANGES OF HUMAN PLASMA VOLUME DURING SIMULATED WEIGHTLESSNESS. Douglas R. Knight* and Steven M. Horvath. Institute of Environmental Stress, University of California, Santa Barbara, CA 93106

The effects of buoyancy, cold, and cold + buoyancy were defined by the difference between subjects' responses during 6-h exposures to gravity (27.8°C air) and their responses to 35°C water, 14.8°C air, and 29.8°C water, respectively. Weightlessness was simulated by the head-out water immersion. Plasma volume did not significantly change during prolonged exposure to gravity. Buoyancy resulted in raised plasma volumes while lowered plasma volumes were observed in the cold exposures. Biphasic changes of plasma volume occurred during 29.8°C immersion, in which buoyancy raised the plasma volume during the 1st 37 minutes and cold lowered the plasma volume thereafter. Diuresis occurred during buoyancy but not during cold. The diuretic loss of H_2O occurred from the extravascular space since there was no net loss of plasma volume. Only the diuresis of buoyancy plus cold was associated with loss of plasma volume and that loss was no greater than in response to cold alone. The additional factor of cold was required to effect the net loss of plasma volume during 29.8°C immersion. Vasoconstrictive reduction of the size of the peripheral vascular bed may explain the plasma loss because subjects' mean norepinephrine rose only in response to cold.

5.12

EFFECT OF GRAVITATION UPON THE PULMONARY CIRCULATION. Mans Arborelius, V. Lopez-Majano, P. G. Data, A. M. Andreoni and R. Martignoni. Malmo Allmanna Sjukhus, Malmo, Sweden, Cook County Hosp., Chicago, IL and Ente Ospedaliero Prov. S. Liberatore Atri, Italy.

The distribution of the pulmonary arterial blood flow was studied in nine well trained divers using 99Tc-MAA and a gamma camera, 2 control scintigrams were obtained in the horizontal and erect position. The effect of increased barometric pressure on the pulmonary circualtion was studied with Tc-99m-MAA in the divers while they were submerged in the erect position. The control and experimental lung scintigrams were obtained in a 20 second apnea. The spirometry and DLCO's were normal in all the subjects. There was a marked difference in the distribution of pulmonary arterial blood flow between the lung scintigrams obtained in the erect and supine position, i.e.; there was a marked decrease in the pulmonary arterial blood flow to the apices per unit volume when the subject was sitting up for at least 10 minutes. The lung scintigram obtained when the patient was lying down demonstrated that this gradient disappeared. The experimental lung scintigram obtained while the subject was immersed was similar to the one obtained when the patient was lying down. Thus, in apnea the pressure exerted on the pulmonary circulation by an immersion into 10 meters causes a shift of the pulmonary arterial blood from bases to apices, thus making the pulmonary circulation more homogenous.

6.1

EFFECT OF CYCLIC AMP ON THE ELECTRICAL COUPLING OF HEART CELLS. E. Estapé* and W. C. De Mello. Department of Pharmacology, School of Medicine, UPR, San Juan, P. R. 00936

Previous studies from our laboratory demonstrated that theophylline (0.4 mM) enhanced the electrical coupling of heart cells by reducing r_i. Since the effect of theophylline might be due to an increase in concentration of cAMP, experiments were made on rat trabeculae exposed to dB-cAMP (5×10^{-5} M). A small suction electrode was used to polarize the muscle and changes in membrane potential were recorded with a glass microelectrode. Measurements of conduction velocity were made with two microelectrodes. Preliminary results showed that: 1) the conduction velocity was increased by 125% (above the controls) in muscles treated with dBcAMP; 2) the time constant of the foot of the action potential did not change; 3) the space constant was increased by 45% above the controls. As the time constant of the cell membrane did not vary the conclusion is that dBcAMP enhanced the spread of electrotonic activity in heart fibers by increasing the junctional conductance. (Supported by Grant No. 5634 GM 07427-02 from NIH.)

6.2

CHANGES IN CELL-TO-CELL COUPLING DURING THE CARDIAC CYCLE. W. C. De Mello. Department of Pharmacology, School of Medicine, UPR, San Juan, P. R. 00936

Studies performed on canine Purkinje fibers beating spontaneously indicated that the space constant is gradually increased (50%) during diastolic depolarization. This increment in λ is due both to a rise in r_m (65%) and a fall in r_i (30%). In quiescent fibers epinephrine (1 µg/ml) induced spontaneous activity and triggered the changes in λ, r_m and r_i described above. In normal Purkinje fibers during the early stages of the plateau (100 ms) λ was reduced from 1.8 mm (S.E. ± .11), at rest, to .92 (S.E. ± 0.1), r_m declined from 32.4 KΩcm (S.E. ± 9.5) at rest to 12.2 KΩcm (S.E. ± 4.2) while r_i was enhanced from .94 MΩ/cm (S.E. ± 0.09) at rest to 1.38 MΩ/cm (S.E. ± 0.13) during this part of the plateau. These findings indicate that the electrical coupling changes during the cardiac cycle, a phenomenon related to variations in r_m and r_i. The possibility that cyclic AMP is involved in the effect of epinephrine in cell-to-cell coupling is discussed. (Supported from NIH, P.R. Heart Association and Angel Ramos Foundation.)

6.3

STROPHANTHIDIN, SODIUM PUMP AND CONTRACTILE FORCE. Roberto Bernabei* and Mario Vassalle. Department of Physiology, SUNY, Downstate Medical Center, 450 Clarkson Ave, Brooklyn, NY 11203.

The increase in maximum diastolic potential which presumably results from an electrogenic sodium extrusion was studied in cardiac Purkinje fibers in the absence and in the presence of strophanthidin. The changes in membrane potential were correlated with those in contractile force. The following results were obtained. 1) A zero potassium Tyrode solution exposed for 30 to 90 sec caused an initial hyperpolarization and subsequent depolarization simultaneously with an increase in contractile force. 2) During the immediate recovery from zero potassium solution there was a transient hyperpolarization with respect to control. 3) Similar exposure to a zero calcium solution caused a sharp fall in contractile force but no increase in maximum diastolic potential either during or after the exposure. 4) Simultaneous exposure to zero potassium and zero calcium solution resulted in a decrease in force and a persistance of hyperpolarization during and after the exposure. 5) Exposure to zero potassium and zero calcium solution during the administration of a dose of strophanthidin (5×10^{-8} - 10^{-7}M) which had a positive inotropic effect gave results similar to those in Tyrode solution. 6) The same procedure in the presence of a higher (5×10^{-7} - 10^{-6}M) strophanthidin concentration often precipitated toxicity and appeared to cause less hyperpolarization. It is concluded that the positive inotropic effect of strophanthidin is not necessarily associated with a depression of Na extrusion. (Supported by a NIH grant HL17451).

6.4

ISCHEMIA-INDUCED HETEROGENEITY IN REFRACTORY PERIOD SHORTENING DURING SYMPATHETIC STIMULATION IS PREVENTED BY PRIOR AUTONOMIC NERVE SECTION. Marion S. Gaide*, Robert J. Myerburg*, John S. Cameron and Arthur L. Bassett. Univ. of Miami Sch. of Med., Miami, FL 33101

Reduction of cardiac autonomic nervous system (ANS) activity before myocardial infarction (MI) decreases the incidence of ischemia-related arrhythmias. To study the interaction between the ANS and the electrophysiology of hearts with acute MI, local epicardial refractory periods (RP) from the normal (N), border (B) and MI (I) areas were measured during bilateral sympathetic nerve stimulation (BSNS) in 2 groups (Gr) of anesthetized cats with 1-hr MI created by distal coronary ligation. Gr 1: vagi and all proximal connections to the stellate ganglia (except ansa subclavia) were sectioned bilaterally just prior to MI (n=12). Gr 2: identical sectioning 1 hr after MI (n=10). Controls were sham-ligated (n=14). Control RPs, at 3 Hz drive cycles, in the N (175 ± 6; $\bar{x}$ ± SEM msec), B (174 ± 5) and I (171 ± 6) were similar to those measured 1 hr after MI in Gr 1. RPs shortened significantly ($p<0.05$) in the N (-13 ± 1; $\bar{x}$ ± SEM msec), B (-10 ± 1) and I (-9 ± 2) during BSNS, similar to controls. In contrast, RP in Gr 2 shortened in the I (-28 ± 3) 1 hr after MI ($p<0.05$), and RP shortening during BSNS was non-uniform in the 3 areas: N (-19 ± 3), B (-12 ± 4) and I (-4 ± 2). Reduction of ANS activity immediately prior to MI prevents both RP changes in I areas and heterogeneous responses to BSNS in N, B and I areas. (NIH, NIRA HL27680 and grants HL19044, HL21735, AHA of Greater Miami)

6.5

THE ACTION OF TETRODOTOXIN AND VERAPAMIL ON CARDIAC PURKINJE FIBER ACTION POTENTIALS IN A SODIUM-FREE, CALCIUM RICH MEDIUM Philip Posner and Dennis L. Kelleher Dept. of Physiology, University of Florida, Gainesville, Florida, 32610 and Defense Nuclear Agency Armed Forces Radiobiology Institute, Bethesda, MD 20014.

The ability of tetrodotoxin (TTX) and verapamil to block Ca^{++}-dependent action potentials (Ca-P) produced in isolated, superfused, canine cardiac Purkinje fibers was studied using standard intracellular microelectrode techniques. Ca^{++}-dependent action potentials were produced in fibers which were superfused with a Na^+ free (substituted by tetraethylammonium-Cl) high Ca^{++} (16.2 mM) modified Tyrode's solution (pH=7.4 or 6.0). The Ca^{++}-dependent potentials were elicited by passing 10 V, 2 msec pulses extracellularly. In 30 fibers studied two populations of resting membrane potentials (RMP) were observed. In one, the mean RMP was -43 mV ± 3 (SEM), in the second, RMP was -63 mV ± 2 (SEM). The more negative RMP was seen in solutions with pH 7.4, while the less negative RMP was observed at pH 6.0. It was possible to change the RMP by manipulating the pH of the superfusate. It was possible to block the low RMP, low pH, Ca-P with verapamil alone while the high RMP, high pH, Ca-P could be totally blocked only with a combination of verapamil + TTX. Thus, it appears that in this preparation, TTX can interfere with a portion of the current produced during a "calcium-mediated" action potential. (Supported by The American Heart Association, Florida Affiliate.)

6.6

FUNCTIONAL AND EFFECTIVE REFRACTORY PERIODS OF THE ATRIOVENTRICULAR CONDUCTION SYSTEM IN THE CHICKEN HEART. Kathleen A. Quinn*, Kevin D. Whitelaw*, Brian D. Feige*, and Jack M. Goldberg. Department of Animal Physiology, University of California, Davis, CA 95616.

The functional (FRP) and effective (ERP) periods of the atrioventricular conduction system were evaluated in the anesthetized, open chest, vagally decentralized chicken. The FRP and ERP were studied using single premature atrial depolarizations coupled to the spontaneous cycle. Atrioventricular conduction time was measured from the activation of the stimulating electrode placed over the region of the SA node to the activation of a recording electrode on the right ventricle. Additional recording electrodes were placed on the right and left atria and right ventricle. With coupling intervals ranging from 5 msec shorter than the spontaneous cycle length to a coupling interval of 120 msec atrioventricular conduction time was only increased by approximately 40 msec (60 msec to 100 msec). FRP, defined as the shortest atrial coupling interval (A_s-A_p) producing the shortest V_s-V_p interval, ranged from 130-170 msec. The ERP, defined as the shortest atrial coupling interval producing an atrial generated ventricular response, ranged from 120-160 msec. Thus, the atrioventricular conduction system of the chicken heart has a much shorter FRP and the refractory properties exhibit less time dependency than do mammalian hearts.

6.7

Na CHANNELS IN CULTURED EMBRYONIC CHICK VENTRICLE: SINGLE CHANNEL CONDUCTANCE AND OPEN TIME: EFFECT OF TTX. Robert TenEick, Norio Matsuki*, Fred Quandt*, Jay Yeh* Pharmacology, Northwestern University, Chicago, IL 60611

Current entering myocardial cells through Na channels underlies the upstroke of the cellular action potential. However, little detail is known about their properties. Using single isolated cultured ventricular muscle cells prepared from 10-11 day old chick embryos and maintained in culture for 2-5 days, we have applied patch clamping techniques to study the properties of TTX-sensitive inward current conducting Na channels. At 13-14°C when activated by 40 ms voltage clamp pulses from holding potentials of -100 to -70 mV to test potentials of -30 to -20 mV, single channel inward going rectangular current pulses were observed. Current amplitudes were normally distributed with a mean ± SDM of 1.27 ± 0.05 pA (n=6). Single channel conductance calculated from currents obtained at -40 and -20 mV was about 10 pS. A Poisson analysis of the frequency of open times gives an exponential distribution with a mean channel closing rate of 685 s^{-1}. Channel open times ranged from less than 0.8 to at least 12 ms with a mean ± SDM of 2.54 ± 0.28 ms (n=6). TTX (30 nM) reduced opening frequency about 59% (n=3), but 30-100 nM did not appear to alter single channel conductance or mean open time (n=3). This study provides direct evidence that cardiac Na channels can exist in 2 conductance states, fully conducting or completely non-conducting; that Na channels which are quite sensitive to TTX can be found in cultured chick ventricle.

6.8

NEUROCONTROL OF ATRIOVENTRICULAR CONDUCTION BY THE VAGAL EFFERENT LIMB OF BARORECEPTOR REFLEXES. M. F. O'Toole*, R. D. Wurster and W. C. Randall. Department of Physiology, Loyola University Medical Center, Maywood, IL 60153

Baroreceptor reflexes exert a profound regulatory influence on heart rate with an equally important role in modifying atrioventricular (AV) conduction. The efferent vagal component of these reflexes alter AV conduction via two oppositely directed mechanisms: 1) Direct inhibition by the release of acetylcholine. 2) Indirect enhancement by its inverse effects on heart period and AV conduction (*Ann. Rev. Physio. 43:* 443, 1981.) To delineate the vagal efferent component of baroreceptor control of AV conduction, arterial pressure was manipulated over a physiological range in chloralose-anesthesized dogs with an intra-aortic (thoracic) balloon and by hemorrhage through an intra-aortic (abdominal) catheter. The efferent sympathetic limb of the reflex was cut by spinal section at C1. AV conduction was determined with right atrial and ventricular bipolar electrodes with and without atrial pacing and after atropine or vagotomy. To define the effects of changing heart period on AV conduction, the atrial pacing rate was altered at various steady-state arterial pressures. Data show that: 1) At a constant atrial rate, increasing pressure from 60 to 160 mmHg resulted in 2:1 and 3:1 AV block in all animals with the onset of block occurring at 80±9 mmHg. 2) At a constant pressure, longer heart periods serve as a protective mechanism by improving conduction. (Supp. by NIH Grant HL 27595 & CHA C80-1)

6.9

OVERDRIVE SUPPRESSION OF ATRIAL PACEMAKER TISSUES FOLLOWING CARDIAC DENERVATION BEFORE AND AFTER SINOATRIAL NODE EXCISION. J. A. Sterba*, W. C. Randall and L. E. Rinkema. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153

Right atrial pacemakers were characterized by measuring cardiac cycle lengths following rapid atrial pacing in nine alert conscious dogs. Total intrapericardial denervation was performed to eliminate autonomic influences. Rapid atrial pacing was performed at 125%-400% of the spontaneous heart rate for 30 sec and at 200% for 30, 60, 120 and 180 sec, along with cholinergic blockade (atropine, 0.2 mg/kg IV) or adrenergic blockade (propranolol, 0.5 mg/kg IV). Corrected recovery time (CRT) was defined as the first recovery cycle length minus average control cycle length. To compare responses of sinoatrial node (SAN) and subsidiary atrial pacemakers (SAP), CRT was measured before and after SAN excision. Immediately following SAN, a junctional rhythm was demonstrated, but within min to hrs, SAP dominance was established with development of P waves and a P-R interval of 85.8±3.4 msec. CRT before SAN excision ranged from 100-300 msec. For pacing at 125%-400% of the spontaneous heart rate immediately following SAN excision, CRT ranged from 0-6000 msec. Atropine and propranolol did not influence CRT in the denervated preparation. During the 1st-9th wks following SAN excision, CRT returned toward the pre-SAN excision control values, suggesting a transition of SAP tissue characteristics toward SAN tissue characteristics. (Supported by NIH Grant HL-28205)

6.10

EFFECT OF pH ON MYOCARDIAL CELL ACTION POTENTIAL CHANGES INDUCED BY HALOTHANE. D.F. Stowe, Z.J. Bosnjak, and J.P. Kampine. Depts. of Anesthesiology and Physiology, Medical Coll. of Wis. and Wood VA Med. Ctr., Milwaukee, WI. 53193

Our aim was to determine if halothane (H) alters the effects of changes in pH on action potential duration (APD) and excitability of ventricular muscle cells. We superfused the right ventricle of 15 guinea pigs with a HCO^{-3}-free HEPES bufferred salt solution to stabilize free Ca activity during low (10% CO_2 in O_2), normal (3% CO_2 in O_2) and high pH (100% O_2). Transmembrane AP's, induced at 2.5 Hz and measured in 28 subendocardial cells impaled with 3 M KCl microelectrodes, were recorded during random step changes in the fractions of H and CO_2 gasing the suffusate. With 3% CO_2 (pH 7.43, PCO_2 35 torr) 0.7-2.1% H increased APD at 50% and 90% maximum potential from 106 up to 111 (+5ms) and 140 up to 144 (+4ms); effective refractory period (ERP) rose from 143 up to 154 (+11ms). But 2.8% H shortened APD 50 (-3ms) and prolonged ERP (+5ms). With 0% CO_2 (pH 8.08, PCO_2 8), 0.7 to 2.1% H only prolonged ERP (+9ms). With 10% CO_2 (pH 7.08, PCO_2 82) 0.7 and 1.4% H produced no effect on AP's while 2.1 and 2.8% H shortened APD 50, APD 90, and ERP (-14, -17 and -15ms). Thus alkalosis attenuates the lengthening of AP's with low levels of H whereas acidosis either enhances the shortening of AP's with high levels of H or converts an increase in APD to a decrease. Since at a given H level, acidosis increases cell excitability (decrease in ERP), acidosis may exacerbate ventricular arrhythmias.

EXERCISE I

7.1

CARDIOPULMONARY CHANGES DURING ACUTE EXERCISE-STRESS IN HEREFORD CALVES. W. D. Kuhlmann* and M. R. Fedde. Kansas State University, Manhattan, KS 66506.

Ventilatory and cardiovascular variables were measured on five Hereford steers before, during and after 5-minute acute exercise bouts on a treadmill (3° incline) running at 1.0,1.4, 1.8 and 2.2 $m \cdot sec^{-1}$. Oxygen consumption ($\dot{M}O_2$) and CO_2 production ($\dot{M}CO_2$) were measured by collecting mixed expired gas in a balloon. Blood samples were simultaneously obtained from indwelling catheters in the aorta and pulmonary artery. Minute ventilation ($\dot{V}_E$) increased in proportion to treadmill speed; respiratory frequency increased at the low treadmill speed (1.0 $m \cdot sec^{-1}$) but did not increase further at higher speeds. The remaining increases in $\dot{V}_E$ were caused by increased tidal volume. $\dot{M}O_2$ and $\dot{M}CO_2$ increased 9 times over resting values at a speed of 2.2 $m \cdot sec^{-1}$. The oxygen consumption curve suggests that these animals were at or near $\dot{M}O_2max$ at this speed. The respiratory exchange ratio, R, decreased slightly during exercise at the two lowest speeds but was unchanged at higher speeds (mean resting R = 1.034). Cardiac output and heart rate increased in proportion to speed up to 1.8 $m \cdot sec^{-1}$ but did not increase further at 2.2 $m \cdot sec^{-1}$. Packed cell volume increased from 26 at rest to a high of 36.5 at the highest exercise level. These data indicate that beef calves reach $\dot{M}O_2$ maximum at low speeds and appear to have poor tolerance to exercise, similar to that of sedentary man. (Supported in part by USDA Grant #59-2201-1-024-0).

7.2

BLOOD CHEMICAL CHANGES IN RESPONSE TO ACUTE EXERCISE-STRESS IN HEREFORD CALVES. M.R. Fedde, W.D. Kuhlmann* and W.E. Moore*. Kansas State University, Manhattan, KS 66506.

Venous blood samples were taken from indwelling jugular catheters in 5 Hereford steers, 150 to 230 kg, before, during and after a five minute exercise bout. Animals were exercised on a large animal treadmill (3° incline) at speeds of 1.0, 1.4, 1.8 and 2.2 $m \cdot sec^{-1}$. Serum was analyzed on a Technicon SMA 12/60 Microanalyzer for 12 variables. Additionally, whole blood lactic acid and plasma cortisol were measured by an enzymatic assay and a radioimmunoassay, respectively. Serum $[K^+]$ increased by 75% (4 $meq \cdot \ell^{-1}$ at rest to 7 $meq \cdot \ell^{-1}$) when samples were taken 4 minutes after the start of exercise at a medium trot (2.2 $m \cdot sec^{-1}$). Significant elevations in this variable occurred even at a slow walk (1.0 $m \cdot sec^{-1}$). Acute exercise increased serum glucose, inorganic phosphate, calcium, and sodium but caused no changes in total serum protein or chloride. Blood lactate increased at all speeds and was approximately 10 times resting values at 2.2 $m \cdot sec^{-1}$. Plasma cortisol did not increase during exercise; 10 minutes after exercise, it was 3-fold higher than at rest. Blood chemical changes induced by exercise in these young beef animals may influence a variety of regulatory or metabolic processes. (Supported in part by USDA Grant #59-2201-1-024-0).

7.3

DECREASED CELLULAR IMMUNE RESPONSES AND INCREASED VIRAL REPLICATION IN EXERCISE-STRESSED CALVES. Frank Blecha* and Harish C. Minocha* (Spon: M.R. Fedde). Kansas State University, Manhattan, KS 66506

Hereford steers, 150 to 175 kg, were acutely exercised on a large animal treadmill (3° incline) at speeds of 1.0, 1.4, 1.8 and 2.2 $m \cdot sec^{-1}$. Blood samples were obtained from indwelling jugular catheters before, during and at intervals after exercise. Serum from exercised calves was added to lymphocyte or viral cultures to evaluate its influence on lymphocyte blastogenesis and viral growth. Additionally, peripheral blood lymphocytes from exercised calves were assayed for blastogenic responses to mitogenic stimulation. Serum obtained from calves exercised at 1.8 to 2.2 $m \cdot sec^{-1}$ and incorporated into lymphocyte cultures from nonexercised calves resulted in lower lymphocyte blastogenic responses. Infectious bovine rhinotracheitis (IBR) viral growth in bovine kidney cell cultures was enhanced 4-fold when cultured with serum (10% v/v) from exercised calves. Additionally, DNA synthesis increased 2.5-fold in bovine kidney--IBR infected cultures when serum from exercised calves was added to the cultures. Lymphocytes from calves 5- and 30-min post-exercise had lower blastogenic responses when compared to pre- or 60-min post-exercise values. These data suggest that an exercise stressor causes physiological alterations in calves which may modulate cellular immunity and viral replication. (Supported in part by USDA Grant #59-2201-1-024-0)

7.4

RESPIRATORY RATE RESPONSES TO EXERCISE IN DOGS MAINTAINED AT RESTING BODY TEMPERATURE, Peter Reischl, William J. Mautz and Charles Bufalino,* Air Pollution Health Effects Laboratory, Dept. of Community and Environmental Medicine, University of California, Irvine, CA 92717

There are reports of respiratory rates near 100 breaths per minute in exercising dogs where, presumably, thermoregulation was not a significant component to this tachypnea (Szlyk et al. Respir. Physiol. 46:345-365, 1981). Because such high respiratory rates suggest panting, we exercised 4 beagle dogs in a refrigerated treadmill apparatus where body temperature was maintained near resting levels by external conduction and convection to cooled air. Dogs weighing between 8 and 13 kg breathed air through a respiratory mask described elsewhere (JAP 52:500-504, 1982) while resting or alternately exercising at 5 km/h at 0 or 15% grade for a 2 h time period. Means and standard deviations of respiratory rates were 17.6±7.8, 30.3±4.1, and 34.3±4.8 min^{-1} for rest, exercise at 0 and 15% grade respectively, with a corresponding expired minute ventilation of 2.7±0.8, 9.4±0.7, 11.5±0.7 L/min (BTPS). As rectal temperature was allowed to increase by as much as 0.5°C during exercise, respiration increased to rates above 100/min. We conclude that body temperature in exercising dogs should be maintained near resting levels when there is need to minimize effects of thermogenic tachypnea. (Supported in part by EPRI #RP1962-1 and EPA Grant R808267-01-0)

7.5

COST OF ACTIVITY IN A BIRD. W. W. Weathers, Anita M. Hayworth* and W. A. Buttemer. University of California, Davis, CA 95616

We used a specially designed 14-l metabolism chamber and an open-flow system to measure activity costs in six Budgerygahs (Melopsittacus undulatus). Activity was monitored visually as oxygen consumption was measured with an Applied Electrochemistry S-3A O_2 analyzer. Only runs in which the particular activity was maintained for at least two minutes were accepted. Subtracting basal metabolism from the measured behavior costs yielded the following activity costs: alert perching 0.75 kJ/h, grooming 0.98 kJ/h, eating 1.12 kJ/h, and shuffling 1.03 kJ/h.

(Supported by NSF grants PCM76-18314 and DEB80-22765)

7.6

CORONARY BLOOD FLOW RESPONSE TO EXERCISE FOLLOWING ACUTE AORTIC CONSTRICTION

I.Y.S. Liang and H.L. Stone, Dept. of Physiol., Univ. of Oklahoma H.S.C., Oklahoma City, OK.

Increased aortic pressure will alter the oxygen demand of the myocardium(MVO_2) at rest but no information is available to predict the changes in coronary flow (CF) or MVO_2 during submaximal exercise. Nine adult mongrel dogs were instrumented to measure CF velocity, left atrial pressure, left ventricular pressure (LVP) and to sample coronary sinus blood. A large hydraulic occluder was placed around the ascending aortic to elevate systolic LVP by 40mmHg (AO). Submaximal exercise was activated by treadmill running at various speeds and inclines. The results were: *P<0.05

COND.	SPEED/ GRADE	LVP SYST.	LVP DIAST.	CF VEL.	HEART RATE	MVO_2
Control	0/0	125±9	5±1	21±1	115±15	2.6±0.3
AO	0/0	162±9*	6±1	28±3*	121±16	3.6±0.4*
Control	6.4/16	162±10	8±2	43±5	245±15	5.9±6*
AO	6.4/16	198±6*	9±2	52±3*	253±15	7.5±0.6*

MVO_2 was increased by the elevation of systolic LVP and so was CF velocity but heart rate and diastolic LVP were unchanged. These results indicate that with the degree of aortic constriction used the increased MVO_2 during exercise was met by the increased CF velocity and no sign of myocardial ischemia was observed.

(Supported by HL-22154)

7.7

EFFECT OF ARM EXERCISE ON VENOUS BLOOD CONSTITUENTS DURING LEG EXERCISE. N. Wong*, J.E. Silver*, S. Greenawalt*, S.E. Kravik*, G. Geelen*, P.R. Barnes, and J.E. Greenleaf. Biomedical Research Division, NASA, Ames Research Center, Moffett Field, CA 94035

Blood was withdrawn through indwelling, flexible catheters from both antecubital arm veins from 5 men(22-25 yr) after 45 min of supine rest, and at 15 and 28 min of a 30-min leg exercise period (50% VO_2 max) in the supine position. Three experiments were performed during leg exercise: control (C), where both arms were maintained horizontal and passive; isotonic exercise (ITE), where the right fingers were flexed at 60 flexions/min and left arm was passive; and isometric exercise (IME), where the right hand held 10% of max contraction force and left arm was passive. At 15 min of ITE, a 19% difference in blood lactic acid measures (p 0.02) and a 4.88% difference in serum glucose (p 0.05) occured between right and left arm samples. At 15 min of IME, a 3.95% difference in glucose measures occured between right and left arm samples (p 0.02). No significant differences occur when comparing right and left arm samples for hematocrit, hemoglobin, sodium, potassium, or osmolality values. The observed differences in measures of lactic acid and glucose, when comparing right and left arm samples, indicate a possibility of errors in blood analyses due to concomitant arm exercise during leg exercise. The movement and position of the arms during leg exercise research should be carefully controlled.

7.8

ALTERATIONS IN SMALL AIRWAYS AND DIFFUSION CAPACITY AFTER A 5-MILE RUN. Daniel S. Miles and Richard J. Durbin*. Dept. of Physiol., Sch. of Med., Wright State Univ., Dayton, OH 45435.

The objective of this study was to determine the feasibility of using exercise as a model to elicit transient alterations in small airways and pulmonary diffusing capacity (DL_{CO}). Eight males ($\bar{X}$ = 28 yrs) were tested immediately before and after a 5-mile run (X run time = 40 min). Maximum expiratory flow-volume maneuvers were completed in triplicate, breathing air and 80% He/20% O_2. Residual volume (RV) was measured in duplicate using both N_2 washout and single breath He dilution. Closing volumes were measured using a single breath O_2 test and DL_{CO} was measured with the single breath technique. Forced vital capacity was reduced (P<0.05) and RV increased (P<0.05) after the run. He/O_2 flow rates were similar pre and post run; however, closing capacity (CC) was increased (P<0.05) after the run. Although DL_{CO} values were similar pre and post run, the ratio of $DL_{CO}/\dot{Q}$ (diffusion/cardiac output) was reduced. This reduction was due to an increase in the measured alveolar-capillary membrane resistance. It has been previously speculated that the increase in RV after exercise is due to an early closure of small airways. Our results support this and suggest that early airway closure may reflect the occurrence of perivascular and/or peribronchial edema. Such a change would increase lung elastic recoil and may explain the increase in CC as well as the reduction in $DL_{CO}/\dot{Q}$. (Supported by the Miami Valley Chapter of the AHA)

7.9

NOCTURNAL SLEEP, CARDIOVASCULAR FUNCTION, AND ADRENAL ACTIVITY FOLLOWING MAXIMUM CAPACITY EXERCISE. Wendy C. Bevier, David E. Bunnell* and Steven M. Horvath. Institute of Environmental Stress, Santa Barbara, CA 93106

Eight individuals (four men and four women) served as subjects to determine the influence of a maximal aerobic test on sleep. The test was performed at 1600 on day 3 (d.3) of the study with d.1 as adaptation, d.2 as baseline, and d.4 as recovery night. Half of these subjects engaged in regular daily exercise. No differences were observed between groups in $\dot{V}O_2$ max, and amount of stage 3, 4, or total slow-wave sleep (SWS) on either night 2 or 4. During night 3 after the max-exercise test both groups showed a significant elevation of heart rate in the first 2 h of sleep, a shortened first REM period, and a reduction in epinephrine excretion. The decrease in first REM period duration was greater in the sedentary group compared to the regular exercisers. Amount of SWS prior to the first REM period increased for the exercisers and decreased for non-exercisers with parallel changes in REM onset latency. Urine production and cortisol excretion tended to decrease on exercise nights. These results show that short-term maximum exercise will induce significant effects on sleep with differences in response seen between regular exercisers and non-exercisers.

7.10

REDUCED EXERCISE TOLERANCE IN MAN WITH CARBONIC ANHYDRASE INHIBITION. B. J. Brown*, L. Cordain* and J. M. Stager* (SPON: A. Tucker). Depts. of Phys. Ed. and Physiol. & Biophys. Colo. St. Univ., Ft. Collins, CO 80523

Acetazolamide (A), which inhibits carbonic anhydrase (CA), causes a metabolic acidosis by a combination of CO_2:HCO_3^- disequilibrium and increased renal excretion of HCO_3^-. Because CO_2 flux and acid base balance may be significant in defining work tolerance, it was hypothesized that clinical doses of A would impair gas exchange during exercise and decrease work capacity. Seven healthy males were studied in a double-blind crossover design using three randomly assigned treatments, (control, placebo and A) during maximal and 70% maximal treadmill tests. Expired gas was sampled continuously to determine O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and ventilation ($\dot{V}E$). Although there was no effect of A treatment on $\dot{V}O_2$ max, $\dot{V}CO_2$ max or $\dot{V}E$ max, the rate of $\dot{V}E$ increase and the $\dot{V}E/\dot{V}CO_2$, were greater ($P<.05$). Maximum work capacity, mechanical efficiency (ME) and respiratory exchange ratio were significantly decreased ($P<.05$) with A. During submaximal exercise $\dot{V}E$ was increased ($P<.05$) with A.Coupled with a lower $\dot{V}CO_2$ in A treated subjects, $\dot{V}E/\dot{V}CO_2$ and $\dot{V}E/\dot{V}O_2$ were also increased. However, there was no difference in $\dot{V}O_2$ or ME. The higher submax $\dot{V}E$, and greater slope of the $\dot{V}E$ increase indicated that ventilatory regulation during exercise is altered by CA inhibition. Furthermore, the rate at which $\dot{V}E$ increases with progressive workloads may be an important factor in limiting work capacity.

7.11

EXERCISE POTENTIATES OZONE INDUCED LUNG DAMAGE IN RATS. William J. Mautz, Thomas R. McClure* Peter Reischl, T. Timothy Crocker* and Robert F. Phalen* Dept. of Community and Environmental Medicine, Univ. of California, Irvine, Calif. 92717.

Exposure to O_3 is reported to result in a decrement of pulmonary function in exercising humans (Haak et al. Physiologist 24:80, 1981). We examined alveolar lesions in lungs of Sprague Dawley rats following exposure during treadmill exercise to 0.3 ppm O_3. Exercise exposure (3.75 h) groups consisted of 1) alternate 15 min running at 0.64 km/h with 30 min rest, 2) alternate 30 min running at 0.64 km/h with 15 min rest, 3) continuous rest, 4) clean air control with exercise protocol of group 2. Lesions, quantified as % lung section area involved, were distinguished qualitatively as Type 1) two or more cells free in alveolar lumen, Type 2) free cells in lumen and increase in numbers of nuclei in alveolar septae, and Type 3) as for Type 2 but including a marked thickening of septal wall. O_3 exposure at rest increased % incidence of Type 1 lesions over clean air control by a factor of 2 and induced Type 2 and 3 lesions. Compared to lesion areas produced by resting exposure, protocol 1 increased area incidence of Type 1 and combined Type 2 and 3 by factors of 2 and 5 respectively, and protocol 2 increased respective indicies of damage by factors of 2.4 and 14. Results indicate that exercise exacerbates O_3 induced lung damage and raises the possibility that exposure to a lower concentration of O_3 producing no apparent effect at rest might induce lung damage with exercise. (Supported by California Air Resources Board Grant 982-81).

7.12

IS HYPEROXIA ANALOGOUS TO PHYSICAL TRAINING? H.G. Welch and P.K. Pedersen*. University of Tennessee, Knoxville, TN 37996 and Odense University, Odense, Denmark.

When hyperoxic gas mixtures are substituted for air during exercise, the effects are similar to those observed following a period of endurance training. This similarity between the two stimuli raises the possibility that some of the underlying mechanisms are similar or interdependent. To test that hypothesis we conducted a series of tests on six subjects before and after a 6-wk training program. The tests were conducted under both normoxic and hyperoxic (60% O_2) conditions and included both submaximal and maximal exercise. We would interpret any change in the response to hyperoxia following training as evidence for an interdependency between the two stimuli; conversely if the two were independent, we would not expect the responses to hyperoxia to be altered. For maximal exercise we found an increase in VO_2 max of 8.4% with train-training; in submaximal exercise (75% of pretraining VO_2 max) HR after training was lower by 20 bts/min, HLa by 1.5 mM, and V_E by 8 l/min. In hyperoxia, the changes were smaller: 5-6% for VO_2 max, 6 bts/min for HR, 0.6-0.8 mM for HLa, and 4-8 l/min for V_E. Moreover, none of these effects of hyperoxia was altered with training. Because the effect of hyperoxia is relatively constant and independent of the state of training, we conclude that the effects of the two stimuli are probably related to different mechanisms. (Supported in part by grants from East Tennessee Heart Association and NIH grant 5507RR07088-11)

7.13

CARDIAC DIMENSIONAL CHANGES ASSOCIATED WITH JOGGING AND DECONDITIONING IN COLLEGE MEN. B.J. Rubal, A. Al-Muhailani, and J. Rosentswieg. Brooke Army Medical Center, Fort Sam Houston TX 78234.

The purpose of this study was to identify the changes in heart structure and function in sedentary men who participate in an aerobic conditioning program and then abruptly decondition. Ten men (ages: 19-31 yrs) jogged for 30 minutes at 70% of maximum heart rate every other day for 10 weeks, then resumed preconditioning activity for 10 weeks. Electrocardiograms, echocardiograms and maximum exercise stress tests were evaluated prior to conditioning (PC), following conditioning (C) and following deconditioning (DC). The mean (±SD) changes in resting heart rate (HR), $\dot{V}O_2$ max, left ventricular internal diastolic dimension (LVID), left ventricular posterior wall thickness (PWT) and interventricular septal wall thickness (IST) were:

	HR (bpm)	$\dot{V}O_2$ (ml/kg/min)	LVIDd (cm)	PWT (cm)	IST (cm)
PC	75±14	42±7	5.4±0.5	0.8±0.1	0.8±0.1
C	66±11	50±5*	5.7±0.4*	0.8±0.1	0.8±0.1
DC	76±13	43±4	5.2±0.6	0.8±0.1	0.7±0.1

Changes in LVID paralleled the changes (*$P < 0.05$) in $\dot{V}O_2$ max. These data suggest that jogging is associated with left ventricular enlargement. Reversion to a PC state occurs when C is terminated.

7.14

THE INTERACTION BETWEEN MAXIMAL AEROBIC CAPACITY ($\dot{V}O_{2max}$) AND HYPERCAPNIC DRIVE. Peter B. Raven, D. Rohm-Young* and D. Miller*. Department of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

In order to evaluate fitness related differences in respiratory drive eleven volunteer fit (F) subjects, with a mean $\dot{V}O_{2max}$ = 67.7 ± 2.5 $ml O_2/Kg\ min^{-1}$ were compared to nineteen unfit (UF) subjects with a mean $\dot{V}O_{2max}$ = 35.3 ± 1.4 $ml O_2/Kg\ min^{-1}$ ($P<0.01$). Camparisons included determination of hypercapnic drive (HD) and maximal apneic time (A_{max}). On a separate day after Bruce Protocol treadmill maximal testing for $\dot{V}O_{2max}$ each subject was required to determine their maximal apneic time. Following recovery from the apnea challenge each subject rebreathed [95%O_2 + 5%CO_2] into a specially adapted dry-rolling seal spriometer. Rebreathing was terminated at end-tidal CO_2 concentrations of 8.6 - 9.3% (65-70torr). Ventilation volumes were continuously recorded and used to calculate $\dot{V}E/P_{ET}CO_2$ slopes representative of HD. The time of A_{max} for F subjects averaged 57.9±6.5 sec and 48.5±4.9 sec for the UF subjects, $P<0.05$. The average $\dot{V}E/P_{ET}CO_2$ slope of the F subjects was 2.83±0.63 L/torr and 3.13±0.23 L/torr, $P<0.05$. In addition, performance time on the maximal treadmill was significantly correlated with A_{max} of the F subjects and HD (i.e. $\dot{V}E/P_{ET}CO_2$ slope) of the UF subjects. It was concluded that the hypercapnic drive for respiration is related to the level of aerobic fitness as determinted by $\dot{V}O_{2max}$ and treadmill performance time.

7.15

RESIDUAL HEART RATE AS A TESTING PARAMETER OF THE PHYSICAL FITNESS PROCESS. P.V. Carlevaro, H. Martínez Canalejo* and E. Guillén*. UAM-Xochimilco, México, DF. 04960 and Inst. Superior Ciencias Médicas, Habana, Cuba.

The solution of a linear differential equation satisfactorily describes the time course of heart rate after exercise (Carlevaro and Martínez Canalejo, 1981). For this, it is necessary to introduce a parameter V_R (residual heart rate) that divides the tachycardia that exists at the end of exercise into two complementary parts: $\Delta_1 = V(0) - V_R$ and $\Delta_2 = V_R - V_o$, where $V(0)$ is the rate at the end of exercise (t=0) and V_o is the baseline heart rate. The magnitude Δ_1 is regulated rapidly — according to a decaying exponential — that tends to an assymptote that is precisely at the level of residual rate. Δ_2, in contrast, is regulated slowly, although it also follows an exponential course. For any individual the magnitude Δ_2 of the parameter, as residual tachycardia, depends upon V_R and this parameter, as shown in experimental results, is a function of both, the effort performed (intensity and duration) and the state of physical fitness. The hardest the effort, the larger the residue. Along the process of adaptation to effort, V_R becomes progessively smaller for a given exercise test. Simultaneously, at the end of the exercise the residual tachycardia is extinguished more rapidly.

7.16

EFFECT OF 4-WEEKS OF PARTIAL EXERCISE TRAINING ON ALPHA-RECEPTOR MEDIATED CORONARY VASOCONSTRICTION IN DOGS. P.A. Gwirtz and H.L. Stone. Dalton Research Center, University of Missouri, Columbia, MO 65211 and Department of Physiology, University of Oklahoma at Oklahoma City, OK 73901

Data indicate an alteration in the neurogenic control of coronary vessels following exercise training. Studies were designed to study whether partial exercise training (PT) enhances coronary vascular adrenergic receptor sensitivity to stress. Left circumflex artery blood flow (CBF), coronary blood pressure (BP) and heart rate (HR) were measured prior to and during exercise (EX) stress. EX increased HR from 117±9 to 210±15 bpm and CBF from 54±10 to 92±10 ml/min. During EX, intracoronary injections of the autonomic receptor blockers prazosin (α_1), phentolamine ($\alpha_1+\alpha_2$), atenolol (β_1), propranolol ($\beta_1+\beta_2$) or atropine ($\bar{X}$) were given (avoiding systemic actions of the drugs on HR and BP). The vasoconstrictor response to norepinephrine (NE) and phenylephrine (PH) at rest was determined. Studies were performed before (UT) and after PT. The % change in CBF to these drugs were:

	α_1	$\alpha_1+\alpha_2$	β_1	$\beta_1+\beta_2$	$\bar{X}$	NE	PH
UT	+27±5	+10±1	-13±5	-15±6	0	-19±9	-16±3
PT	+20±7	+21±6*	-11±2	-23±5*	0	-31±6*	-35±4*

These data show (1) a potentiated increase in CBF during EX by α-blockade, and (2) vasoconstriction to α-adrenergic stimulation is enhanced (*$p<.01$) by 4-weeks of PT, prior to attaining a classical exercise trained state. (Supported in part by NIH HL18798).

7.17

EVIDENCE OF INCREASED THORACIC EXTRAVASCULAR FLUID FOLLOWING INTENSE EXERCISE IN MAN. Michael J. Buono,* Jack H. Wilmore, and Fred B. Roby, Jr.* Exercise and Sport Sciences Laboratory, University of Arizona, Tucson, AZ 85721

A series of 3 studies were conducted to help determine the physiological mechanism responsible for the previously reported (Buono et al. **Med. Sci. Sports Exercise** 13:290-293, 1981) increases in residual volume (RV) and total lung capacity (TLC) following maximal exercise. Study I showed that in 18 male volunteers, RV and TLC were significantly ($p < 0.05$) increased over the pre-exercise values through 30 and 15 min of recovery, respectively. Transthoracic electrical impedance, normalized for RV (TEI/RV), was significantly decreased below the pre-exercise value through 30 min of recovery. This suggests that there was an increase in intrathoracic fluid following exercise. By experimentally manipulating central blood volume via G-suit inflation and venous occlusion, study II (n=10) showed that post-exercise RV was relatively insensitive to intravascular fluid shifts within the thorax. This suggests that the post-exercise increase in RV was not the result of increased thoracic blood volume. Study III (n=5) showed that the post-exercise diffusion capacity (D_{LCO}/V_A) was unchanged following exercise, despite an increase in the post-exercise heart rate. This suggests that the post-exercise decrease in TEI/RV reported in study I was at least partially due to edema formation. It was concluded that a subclinical pulmonary edema is present following maximal exercise, which may be responsible for the post-exercise increase in RV and TLC. (Supported in part by BRSG S07 RR07002).

7.18

INFLUENCE OF AMBIENT TEMPERATURE ON CARDIAC OUTPUT OF RUNNING DOGS. L.W. Chapman & M.A. Baker. Div. of Biomedical Sci. & Dept. of Biology, U. Calif., Riverside, CA 92521 and Dept. of Physiol., Coll. of Osteopath. Med. of Pacific, Pomona, CA 91766.

We investigated the effect of ambient temperature (T_a) on cardiac output (CO) in dogs exercising at two work loads. Five dogs were implanted with a #7 Swan-Ganz CO catheter and trained to run on a treadmill. Arterial blood temperature (T_b) and thermodilution CO were measured at rest and during 30 min of running at 4.5 mph on the level and at a 20% grade at 25°C and 35°C T_a. Resting CO was 166 ± 7 (M ± SEM) ml/kg-min at 25°C and 203 ± 15 at 35°C. This difference was not significant (paired T-test). CO measured at the 30th min of running on the level was 340 ± 10 at 25°C and 395 ± 19 at 35°C. This difference was significant ($p < 0.05$). CO at the 30th min of running on a 20% grade was 537 ± 14 at 25°C and 660 ± 30 at 35°C. This difference was significant ($p < 0.005$). During level running, T_b rose 1.0°C at 25°C and 1.8°C at 35°C. At a 20% grade, T_b rose 3.0°C at 25°C and 4.3°C at 35°C. These results indicate that CO in running dogs is influenced by exercise intensity and by ambient temperature. (Supported by NSF Grant BNS-7901006)

7.19

STRESS ANALYSIS FOR OPERATING A MULTI-PROPULSION MODE WHEELCHAIR. E.E. Garcia*, R.M. Glaser, B.M. Fichtenbaum*, T.J. Ruchman* and J.S. Petrofsky. Wright State Univ. Sch. of Med., Dayton, OH 45435 and Rehabilitation Inst. of Ohio, Miami Valley Hosp., Dayton, OH 45409

Many individuals who are confined to manual wheelchairs have some leg function. Because of conventional wheelchair design, however, only the arms may be used effectively for propulsion. The purpose of this study was to analyze the metabolic and cardiopulmonary stresses for operating a multipropulsion mode wheelchair which could be operated by both the arms (via the handrims) and legs (via a reciprocating linear drive system). Three able-bodied volunteers operated this wheelchair over a level tiled surface at 2 $km \cdot h^{-1}$ by using the arms only, and by using a combination of the arms and legs. During the final min of 5-min exercise bouts, oxygen uptake ($\dot{V}O_2$), pulmonary ventilation ($\dot{V}E$) and heart rate (HR) were determined. For the arms only, and the arms and legs combination, respectively, $\dot{V}O_2$ = 0.382, 0.394 $l \cdot min^{-1}$; $\dot{V}E$ = 12.6, 12.3 $l \cdot min^{-1}$; HR = 81, 75 $beats \cdot min^{-1}$; whereas, maximal forward propulsion force = 40.5, 63.6 kg. These data suggest that although $\dot{V}O_2$, $\dot{V}E$ and HR were similar for both propulsion modes, the arms and legs combination was relatively less stressful because of the greater forward propulsion force capability. The practical implication is that such a wheelchair design may permit more functional muscle mass to be used for propulsion which should alleviate the stresses of locomotion. (Supported in part by the Veterans Administration.)

7.20

EFFECTS OF EXERCISE AND AMBIENT TEMPERATURE ON ANIMALS SUSCEPTIBLE TO PORCINE STRESS SYNDROME. Sylvie D'Allaire and Laszlo DeRoth. Faculté de Médecine vétérinaire, Univ. de Montréal, Québec, Canada J2S 7C6.

Porcine Stress Syndrome (PSS), a complex genetic disease, is the clinical manifestation of a triangle of conditions; the two others are Malignant Hyperthermia Syndrome and Pale, Soft, Exudative Muscle. Various factors have been suggested as triggering agents in PSS. The objective of this study was to evaluate the effects of controlled exercise (ten minutes on a treadmill at an 11° slope) in PSS-susceptible (PSS-S) animals at two ambient temperatures (14°C, 29°C), comparing the changes in heart rate (HR), plasma lactic dehydrogenase (LDH), creatine phosphokinase (CPK) and cortisol (C) with those in similarly exercised PSS-resistant (PSS-R) animals. HR reached a maximum in ten minutes at 14°C but in five minutes at 29°C. The increased HR was maintained longer in PSS-S than in PSS-R animals. Plasma C levels increased significantly following exercise ($p<0.05$) at 29°C in PSS-S animals only. LDH did not change under any of the experimental conditions. CPK increased at 14°C during and after exercise in both PSS-S and PSS-R animals. During exercise at 29°C, CPK levels were significantly higher ($p<0.01$) in PSS-S than in PSS-R subjects. PSS-S animals were more stressed by exercise and higher ambient temperature than were their normal counterparts (Grants MAPAQ and Agr. Canada).

7.21

EXERCISE EFFICIENCY: INFLUENCE OF TRAINING, WORK LOADING AND ANTHROPOMETRIC DATA. A.S.P. BARBOSA, A.K. RUSSO, T.L. BARROS NETO, A.C. SILVA, I.C.PIÇARRO, J. TARASANTCHI. Escola Paulista de Medicina, Depto. de Fisiologia, São Paulo, S.P., 04023, BRASIL.

In order to investigate the influence of training and work loading on exercise efficiency as well as its possible relationship with anthropometric parameters, three experimental groups (long distance runners, cyclists and non athletes) have been studied during cicloergometer tests at various exercise intensities under anaerobic threshold. Oxygen consumption (VO_2) has been estimated by open circuit spirometry. Net, gross, work and delta efficiency values have been calculated from work loading (100,150,200W) and VO_2 results. Anthropometric data (skinfolds, lenght and circunference of leg, sitting height) have been obtained and correlated to work efficiency values. A significant decrease in work and delta efficiency in higher loads has been observed in all groups. No significant difference between the three groups has been obtained employing different ways for estimating efficiency, and no relationship with anthropometric data could be observed. (Financial support from CAPES and FINEP).

7.22

CARDIOVASCULAR RESPONSE TO NECK MUSCLE EXERTION. Chandler A. Phillips and Jerrold S. Petrofsky. Wright State University, Dayton, OH 45435

Helicopter flight crews experience neck muscle fatigue due to eccentric loading of electronics equipment on the SPH-4 helmet. We have conducted an extensive series of experiments to evaluate the cardiovascular response which occurs during dynamic and static loading of these muscles by: the head itself (CON), the conventional SPH-4 helmet (HEL), and a combination of the SPH-4 helmet with Night Vision Goggles (NVG). Two exercise periods of 5 minutes and 35 minutes duration (5 and 35) were performed by the 6 subjects, during which a subject would rotate the head laterally (side-to-side). Immediately thereafter, the subject would position his head in an isometric head dynamometer and exert a sustained right lateral (LAT) neck contraction or forward (FOR) neck contraction at 70% of his maximum strength (MVC) until fatigue. A resting blood pressure (BP) was recorded in all subjects at rest, and as often as possible during the 70% MVC. Heart rate (HR) was continuously recorded on a strip chart-recorder. BP recordings, the time at which they occurred and HR at those times was analyzed. There was an average 40% increase in the systolic BP, an average 50% increase in the diastolic BP and an average 45% increase in the HR from resting to the end of a 70% MVC. The increase appears to be relatively independent of duration (5 and 35), head loading (CON, HEL and NVG) and contraction mode (LAT and FOR). (Supported by U.S. Army contract no. DAMD-17-C-0089).

7.23

A CT-SCAN COMPARISON OF THORACIC MORPHOLOGY IN DOGS RAISED AT LOW AND HIGH ALTITUDE. J. E. Schutte*, M. J. Landay*, R. Epstein* and R. L. Johnson, Jr. Univ of Texas Health Science Center, Dallas, Texas 75235

Our previous work found that, compared with Beagle dogs raised at sea level (SL), Beagles raised from 2 mo. to adulthood at 3100 m (HA) have larger lung volumes (V_L) measured by He dilution and larger lung tissue volumes (Vt) measured by C_2H_2 uptake. The objective of this research was to determine what morphological changes in the thorax contribute to the greater lung dimensions of the HA dogs. Nine CT-scan "cuts" were made at regular intervals on each dog with the lungs inflated to 30cmH_2O. The area and density of the lung tissue were measured at each cut, as were the AP and lateral chest diameters. Total lung air and tissue volumes were then computed:

		CT-Scan		He	C_2H_2
	WT(kg)	air(ml)	tissue(ml)	air(ml)	tissue(ml)
HA	11.94	1437	403	1414	237
SL	12.60	1256	379	1324	169

The CT-scan tissue volumes are higher, presumably because the CT-scan measures total lung tissue while the C_2H_2 method measures only the fine septal tissues. Morphometrically, most of the greater lung volume of the HA dogs can be accounted for by the longer distance between the first rib and the dome of the diaphragm in the HA dogs (15.99 vs 14.79 cm). Also, the HA dogs average 6% larger lateral and 1% smaller AP diameters than the SL dogs, giving the HA dogs an average 5.2% greater cross-sectional lung area. (Funded by NIH-HL14187)

7.24

EFFECT OF HIGH ALTITUDE AND OF PNEUMONECTOMY ON LUNG GROWTH IN BEAGLE PUPPIES. R. L. Johnson, Jr., R. F. Grover, S. S. Cassidy and A. Estrera*. Univ. of Texas HSC, Dallas, Texas 75235 and Univ. of Colorado HSC, Denver, Colorado 80262

We compared lung growth in Beagle dogs raised at sea level (SL) with that in Beagles raised from age 2 mo. to adulthood at 3100 m altitude (HA) and with that in Beagles raised at sea level after left pneumonectomy(Pn_X) performed at 2.5 mo of age. We compared lung volumes (V_L) at 30 cmH_2O transmural inflation pressure measured by helium dilution, lung tissue volume (Vt) measured by acetylene uptake and diffusing capacity of the lung (DL_{CO}) from CO uptake during rebreathing. Blood volumes (Q_B) were measured by ^{51}Cr tagging of red cells. Average results are (* = P<.05 that differences from SL are by chance alone):

	Wt(kg)	Q_B(ml)	V_L(ml)	Vt(ml)	DL_{CO}
HA	10.6	964	1685*	237*	11.9*
SL	11.9	1020	1470	169	9.6
Pn_X	11.4	1017	1232*	118*	8.4*

These data were compared to that obtained previously in Beagles in whom the HA sojourn and Pn_X were performed as adults. V_L, Vt and DL_{CO} did not change during 3 yr at HA in Beagles raised at SL; therefore, immaturity was necessary for the growth response. V_L, Vt and DL_{CO} in Beagles receiving Pn_X as adults were not different from those receiving Pn_X as pups; therefore, increased work of breathing does not cause immaturity-dependent lung growth. We conclude from these data that hypoxia, not increased work of breathing, was the stimulus for lung growth and enhanced gas exchange function at HA. (Funded by NIH-HL14187)

7.25

EXERCISE THERMOREGULATION AFTER WATER IMMERSION DECONDITIONING. W.A. Spaul*, S.E. Kravik*, N. Wong*, C.A. Elder*, and J.E. Greenleaf. Biomedical Research Division, NASA, Ames Research Center, Moffett Field, CA 94035

Rectal (T_{re}) and mean skin (T_{sk}) temperatures, heart rate (HR) and body sweat rate (SR) were measured in 6 men (20-35 yr) and 4 women (23-27 yr) during 70 min of supine leg exercise (50% $\dot{V}O_2$ max, T_{db} = 23°C) before (C) and after immersion to the neck (NI) in water (34.5°C) for 6 hr after overnight food and fluid restriction. All subjects refused drinking water during NI. Mean water balance was -1,285 ±SE 104 ml/6 hr in the men and -1,050 ±142 ml/6 hr in the women; plasma vol changes (H_b+H_{ct}) were -3.2% and +2.9%, respectively. End exercise HR during (C) and NI for men were 141 ±3 b/m and 148 ±3 b/m (P<0.05), respectively, and 138 ±4 b/m and, 142 ±5 b/m (P<0.05), respectively, in the women. Pre-exercise NI-(C) T_{re} was +0.6°C (P<0.05) in women and +0.3°C (P<0.05) in the men. In (C) and NI exercise, Δ T_{re} was +1.0 and +0.9°C in men and +0.7 and +0.6°C in women, respectively, while SR were 254 ±22 and 372 ±63 g/hr (Δ = 146%, P<0.05) in men and 188 ±19 and 304 ±33 g/hr (Δ = 162%, P<0.05) in the women, respectively. Thus, NI resulted in elevated resting T_{re} and enhanced sweating during exercise.

8.1

ANATOMY OF BRONCHIAL CIRCULATION IN SHEEP AND GOAT. Nirmal B. Charan, G. Michael Turk*, Karen A. Maier*. VA Medical Center, Boise, ID 83702

Recently it has been suggested that bronchial circulation might play a significant role in fluid reabsorption in the lung. Since the anatomy of the bronchial vein has not been established in animal models, investigators have obstructed bronchial venous blood flow by increasing pressure in the azygos vein. We studied the anatomy of bronchial circulation in four sheep and one goat. The animals were anesthetized and exsanguinated. The left chest wall was removed and the origin of the broncho-esophageal trunk from the aorta was identified. The esophageal branch of this trunk was ligated and bronchial artery was cannulated through an incision in the aorta. In one sheep bronchial artery originated directly from the aorta. A green dye was injected into the bronchial artery with an infusion pump. In both sheep and goat bronchial artery supplies pleura, part of trachea, bronchial tree (except that of right upper lobe), walls of pulmonary artery and pulmonary vein. The dye was also found in pulmonary venous blood indicating presence of intact broncho-pulmonary anastomoses. The dye flowed into the azygos vein via a single vessel joining the azygos close to the pericardium suggesting that it drains part of the bronchial arterial blood. In two sheep we infused dye directly into this vein but were unsuccessful, presumably due to the presence of valves. Thus, our study confirms that a part of the bronchial arterial blood is drained into the azygos vein. (Supported by VA Research Funds).

8.2

RESPONSE OF BRONCHIAL VENULES TO AIR EMBOLIZATION IN DOGS: AN ULTRASTRUCTURAL STUDY. A. Armer*, B.T. Peterson, R.W. Hyde, and D.P. Penney* Univ. Rochester Med. Ctr., Rochester, NY 14642.

We used colloidal carbon (35-50nm) as a tracer to determine if leakage from damaged bronchial venules contributes to the pulmonary edema caused by air emboli in dogs. Twenty-one dogs received an intravenous (iv) bolus of carbon (0.3 ml/kg) followed by an infusion of carbon (0.5 ml/min). After the iv bolus of carbon 12 dogs received iv air at 0.3 ml/kg/min and 9 dogs received no iv air. Biopsy samples were obtained either early (3-15 min) or late (90 min) in the air infusion period. We then determined the postmortem wet-to-dry weight ratio of the lungs (W/D). The results (mean ± S.D.):

	Early		Late	
	n	W/D	n	W/D
Control	3	4.7 ± 0.1	6	4.7 ± 0.1
Emboli	6	4.8 ± 0.1	6	5.6 ± 0.5

Electron microscopic examination of biopsy samples showed no carbon leakage from bronchial venules of any dogs. To determine if smaller particles would cross the endothelium 3 additional dogs received iv air and particles of colloidal gold (3-10nm). In early biopsy samples from these dogs gold was present in vesicles and in the interstitium around bronchial venules. We conclude that air emboli in dogs causes pulmonary edema but no carbon leakage from damaged bronchial venules. Preliminary studies with gold suggest that fluid transport across bronchial venules contributes to air emboli pulmonary edema. (NIH grants CA-27791, HLB-07216).

8.3

THE EFFECT OF HYPERCAPNEA ON BRONCHIAL ARTERY BLOOD FLOW ($\dot{Q}$Br) AND BRONCHIAL VASCULAR RESISTANCE (BVR). E.M. Baile* and P.D. Paré, U.B.C. Pulmonary Research Lab., St. Paul's Hospital, Vancouver, Canada.

It has been shown that hypoxia increases BVR (Amer. Rev. Resp. Dis. 125: 271,1982). In these experiments we measured the effect of graded hypercapnea (Room air; 5% CO_2; 10% CO_2) in 5 anesthetized, intact dogs. After ventilation for 1/2 hour with each of the gases, aortic (PAo), pulmonary artery and left atrial (Pla) pressures, cardiac output (CO) and arterial blood gases were measured and using a modification of the reference flow ($\dot{Q}$r) technique, ^{46}Sc, ^{153}Gd and ^{103}Ru-labelled microspheres were injected into La as a marker of bronchial blood flow. Five seconds after injection, the left main pulmonary artery (LMPa) was transiently occluded to prevent recirculation of the microspheres to the left lung (LL). After final injection dogs were killed and the lungs removed. Knowing the radioactivity in the LL and in the $\dot{Q}$r blood and the PAo and Pla, $\dot{Q}$Br (ml/min/g dry lung) and bronchial vascular resistance (cm H_2O/ml/min/100g dry lung) were calculated. Results $\bar{x}$ ± S.E.:

	Room Air	5% CO_2	10% CO_2
$\dot{Q}$Br	3.5 ± .75	5.29 ± 1.53	11.0 ± 5.28
$\dot{Q}$Br %CO	.17 ± .04	.24 ± .06	.37 ± .17
BVR	46 ± 9	43 ± 16	30 ± 17

Hypercapnea caused a significant ($P<.05$) increase in $\dot{Q}$Br and decrease in BVR. Supported by the Canadian Heart Foundation.

8.4

EFFECT OF PEEP ON BRONCHIAL BLOOD FLOW ($\dot{Q}$Br) AND BRONCHIAL VASCULAR RESISTANCE (BVR) IN DOGS. P. D. Paré, S. Lakshminarayan, E. Baile*, W. Kirk*, and R.K. Albert. Dept. of Medicine, U. of Washington, Seattle, WA., and U.B.C. Pulmonary Research Lab., St. Paul's Hospital, Vancouver, Canada.

PEEP increases pulmonary vascular resistance (PVR) but its effect on BVR is unknown. We examined this in 5 open chest anesthetized dogs using two techniques (T1, T2) to measure $\dot{Q}$Br. T1: Using a modification of the reference flow method ^{46}Sc, ^{153}Gd and ^{103}Ru-labelled microspheres were injected into the left atrium (la) as a marker of $\dot{Q}$Br to the left lung (LL) at PEEP 3, 10, 15cm H_2O. $\dot{Q}$Br was later partitioned into the left lower lobe (LLL) airway and parenchymal fractions. T2: We collected that portion of $\dot{Q}$Br that anastomosed with the pulmonary circulation and emptied into the LLL pulmonary artery (Pa) and vein. After ventilation for 10 min at each PEEP level with Pla = 0, aortic, and pa pressures and cardiac output were recorded and $\dot{Q}$Br measured by both methods. Results show that LL $\dot{Q}$Br and those fractions going to the LLL airways and parenchema (T1) and anastomosing with the pulmonary circulation (T2) all decreased, and BVR increased with increasing PEEP. Anastomotic $\dot{Q}$Br correlated best with the sum of the $\dot{Q}$Br to the LLL airway and parenchyma. We conclude that most of the LLL $\dot{Q}$Br anastomoses with the pulmonary circulation when Pla is low. PEEP increases BVR accounting for the change in $\dot{Q}$Br. Supported by the Canadian Heart Foundation.

8.5

ULTRASTRUCTURAL DAMAGE TO SMALL PULMONARY ARTERIES DURING AIR EMBOLIZATION IN SHEEP. K.H. Albertine* and N.C. Staub. Cardiovascular Research Institute and Depts. of Anatomy and Physiology, University of California, San Francisco, CA 94143.

The pathophysiology of the acute microembolism syndrome has been studied in sheep (CIRC.RES. 43:152, 1978), but without structural correlation. The role of fibrin in addition to neutrophils is controversial. In 4 anesthetized, ventilated sheep we measured hemodynamics and lung lymph flow and protein concentration. In 3 sheep, after a 2h stable baseline, we infused 1 mm air bubbles into the right atrium to double pulmonary artery pressure and lymph flow. After 1h (1 sheep) and 4h (2 sheep) we opened the chest, cross-clamped the right lung, clamped the pulmonary artery and vein to the left caudal lobe and put fixative down the lobar bronchus at 7-8 cmH_2O. We took 10 blocks each for light and transmission electron microscopy. The control lung showed no evidence of microvascular endothelial injury after 6h. During embolization neutrophils and platelets accumulated at the blood-embolus interface and neutrophils marginated in the pulmonary arteries. Even at 1h, the intima of small pulmonary arteries (<1 mm diameter) and arterioles showed foci of injured endothelial cells and disruption of the basal lamina. Gaps >1 µm were seen. No lesions were found in capillaries or bronchial vessels. One small pulmonary vein had a lesion. One obvious site of microvascular injury during air embolization is in small pulmonary arteries. Neutrophils and platelets but not fibrin are closely related to the lesions. [Supported by HL6168 (NRSA) and HL25816 (PPG)].

8.6

CARDIOPULMONARY CHANGES FOLLOWING INHALATION INJURY, D.L. Traber, D.N. Herndon*, and M.D. Stein* Univ. of TX Med. Br. and Shriners Burns Inst. Galveston, TX 77550

Inhalation injury was produced in ewes by using a bee smoker. Sheep were prepared for study by implantation of catheters for the measurement of cardiopulmonary variables, 3 had chronic lung lymph fistulas. Lung washings were done in 4 animals to obtain cell counts. Cardiovascular lesions occur 12 hrs. following injury, there is a fall in cardiac index and a rise in heart rate and pulmonary resistance. The mean arterial and mean pulmonary artery pressure show no significant changes. By 24 hrs. after injury the PaO_2 had fallen to a mean value of 72±3mm Hg from a value before inhalation injury of 109±5mm Hg. There was not a statistically significant change in $PaCO_2$. Two hrs. following injury lymph flow quadrupled and by 12 hrs. this had increased to 12X that of the control. These changes in lymph flow occurred with an increase in the lymph to plasma protein concentration ratio. Before injury the monocyte is the most numerous pulmonary cell type (74±9%), 48 hr. after injury they are reduced (36±11%) and neutrophils and lymphocytes become more numerous (43±9% and 20±7%). Inhalation injury produces a lesion of the pulmonary microvasculature causing an increased microvascular permeability to protein. This results in hypovolemia and a fall in cardiac output. The changes are related to cellular destruction of monocytes and an elevation in pulmonary lymphocytes and neutrophils in the lung.

8.7

EFFECTS OF DIMETHYLSULFOXIDE (DMSO) AND CATALASE ON THE PROTEIN PERMEABILITY OF DOG LUNG CAPILLARIES DAMAGED WITH α-NAPHTHYL THIOUREA (ANTU). D. Martin*, J.C. Parker, and A.E. Taylor, Dept. of Physiology, University of South Alabama, Mobile, AL 36688.

Mongrel dogs were anesthetized with sodium pentobarbital (30mg/kg) and a small prenodal lung lymphatic was cannulated. In addition, catheters were placed into the pulmonary artery and left atrium. Lymph flow (J_L), total protein concentration in lymph (C_L) and plasma (C_p), pulmonary arterial and left atrial pressures and cardiac output were monitored during a control (1 hr), 3 hours following ANTU challenge (5mg/kg in propylene glycol) plus either DMSO(.02gm/kg) or catalase (8mg/kg) and after elevations of left atrial pressures in the animals receiving DMSO or catalase with the ANTU challenge. Catalase did not appear to decrease the vascular damage associated with ANTU. However, DMSO appeared to partially prevent the ANTU related vascular damage in approximately one-half the animals studied. This finding is similar to the protective effects observed when superoxide dismutase was used with the ANTU experimental model. The reason that the $OH^{\cdot}$ scavenger does not totally protect the lung endothelial damage caused by ANTU is not clear at the present time, but it may be related to the number of leukocytes migrating into the lung tissue and their responses to the challenge. *D. Martin is a Parker B. Francis Pulmonary Fellow (Sponsored by HL22549).

8.8

EFFECTS OF LEUKOCYTE REDUCTION ON LUNG VASCULAR PERMEABILITY CHANGES ASSOCIATED WITH α-NAPHTHYL THIOUREA (ANTU) ENDOTHELIAL DAMAGE. A.E. Taylor, D. Martin*, and J.C. Parker, Dept. of Physiology, University of South Alabama, Mobile, AL 36688.

Leukocytes were reduced in dogs using hydroxyurea (3.75mg/kg/day). Following a 1-week hydroxyurea treatment, in which the average white cell count had decreased from 22,000 to 8,600 per mm^3, the dogs were anesthetized with sodium pentobarbital and a small prenodal lung lymphatic was cannulated in an open chested dog preparation. Both the pulmonary artery and left atrium were cannulated to measure their pressures and to elevate left atrial pressures by balloon inflation. Control lymph (C_L) and plasma (C_p) protein concentration, lymph flow (J_L), pulmonary arterial pressure, cardiac output and left atrial pressure were continuously monitored. Following a 1-hour control period, ANTU was given in the pulmonary artery (5mg/kg) and the same parameters measured for an additional 3 hours. Then, left atrial pressures were elevated and the parameters again measured at their new steady-states. The C_L/C_p was similar to previous studies in which only left atrial pressures were increased. Either the white cell depletion or hydroxyurea prevents the lung vascular permeability changes associated with ANTU challenge. The leukocytes may be responsible for the pulmonary endothelial damage associated with ANTU, but hydroxyurea may be acting as a free radical scavenger.
*D. Martin is a Parker B. Francis Pulmonary Fellow
(Sponsored by HL22549)

8.9

COLCHICINE-INDUCED NEUTROPENIA AND LUNG FLUID BALANCE IN ANESTHETIZED SHEEP. N.C. Staub, E.L. Schultz*, O. Osorio* and K.H. Albertine*. Cardiovasc. Res. Inst. and Dept. of Physiol., University of California, San Francisco, CA 94143.

In large doses colchicine causes an immediate neutropenia with some cells lodged in the lung's microcirculation (J PHYSIOL 37:50, 1908). Craddock and coworkers used colchicine to make sheep neutropenic before complement activation (NEW ENG J MED 296:769, 1977). We have studied some aspects of blood and lung neutrophil kinetics, and lung fluid balance in 5 anesthetized, ventilated, prone sheep with lung lymph fistulas. We injected ^{111}In-labeled neutrophils (MICROVASC RES 23:275, 1982). After 4h, we injected 10 mg colchicine (Lilly) into the abdominal aorta. We followed all variables for 4h more. Circulating neutrophils, both native and labeled, decreased markedly within 1h. In 2 sheep, by external gamma counting over the thorax (>90% from lung), we found that 75-85% of labeled neutrophils lost from the circulation lodged in the lung. Pulmonary artery pressure rose 3.0 ± 1.2 cmH_2O. Lung lymph and protein flow did not increase. Post-mortem lung lavage revealed no significant neutrophil migration into alveoli. Lung water content was normal. Histologically, we found clusters of neutrophils in the lung's microcirculation. Colchicine-induced sequestration of the vast majority of circulating neutrophils in the sheep lung microcirculation causes minimal obstruction and no change in lung fluid balance. [Supported by HL25816 (Prog. Proj.)].

8.10

PLATELETS AND LEUCOCYTES IN THE LUNGS AFTER ACUTE HYPOBARIC HYPOXIA. G. Coares*, C. Nahmias* and A. Thind* (SPON: E.J.M.Campbell). Dept. Radiology, McMaster University Hamilton, Ontario, Canada, L8N 3X5

Platelet aggregation and embolization to the lungs has been implicated as a mechanism in High Altitude Pulmonary Edema. In this study we asked the question does hypobaric hypoxia cause an accumulation of autologous Indium-111 labelled leucocytes or Chromium-51 labelled platelets in the lungs of rabbits. Iron-59 labelled erythrocytes were used to correct for changes in lung blood volume. In lungs of 35 control rabbits there were 4 times as many platelets than could be accounted for by lung blood alone. There was no detectable increased accumulation of platelets in the lungs after 18 hours at 350Torr (n=17). 18 hours at 440Torr (n=6) or 40 hours at 440Torr (n=6). In 6 control rabbits there were 22 times as many ^{111}In leucocytes in the lungs than could be accounted for by lung blood volume. In 6 animals at 350Torr for 18 hours this figure was reduced to 13.6. This reduction in lung leucocytes was accompanied by a peripheral granulocytosis from a mean of $(3.3\pm1.6) \times 10^9$ per litre to $(5.3\pm2.1) \times 10^9$ per litre. We conclude that hypobaric hypoxia to 350 Torr does not cause platelets to accumulate in the lungs and causes a marked reduction in the numbers of leucocytes in the lungs with a peripheral granulocytosis.
(Supported by M.R.C. of Canada)

8.11

EFFECT OF BODY TEMPERATURE ON WHITE BLOOD CELLS (WBC) DURING CARDIOPULMONARY BYPASS. M. Quiroga*, R. Miyagishima*, M. Glovsky*, B.A. Martin*, and J.C. Hogg. U.B.C. Pulmonary Research Lab., St. Paul's Hospital, Vancouver, Canada, V6Z 1Y6 and the Permenante Medical Group, Los Angeles, California.

Chenoweth et al (1) have recently shown that complement is activated during cardiopulmonary bypass. In that study they report that the WBC count is higher at the end of bypass than at the beginning. The purpose of our study was to examine the nature of this rise in WBC during the bypass procedure and to determine if it is affected by body temperature. Ten patients were studied with hypothermia and 3 with normothermia during cardiopulmonary bypass. Complement activity was determined by measurements of C_2, C_6, C_{3a} and CH_{50} units. Blood was drawn for WBC counts every minute to 5 min while the patient was going on and off bypass and every 30 min during the bypass procedure. We found that cardiopulmonary bypass is associated with a slight fall in CH_{50}, a rise in C_{3a} and a progressive decrease in C_2 and C_6. During normothermic bypass the WBC rose gradually from $3.73 \pm .61$ to $8.79 \pm 1.05 \times 10^3$ during the bypass procedure while during hypothermia a similar rise in WBC occurred abruptly at a body temperature of ∿36°C. These studies confirm that complement is activated during cardiopulmonary bypass and that the body temperature influences the rise in WBCs that occurs during this procedure.
(1) NEJM 304:407, 1981.

8.12

THE EFFECT OF REGIONAL BLOOD FLOW ON THE SIZE OF THE MARGINATING POOL (MP) OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) IN THE LUNG. B.A. Martin*, M. Quiroga*, S. Lee* and J.C. Hogg. UBC Pulm. Res. Lab., St. Paul's Hospital, Vancouver, Canada.

Previous studies have shown retention of leukocytes by the lung is dependent on regional blood flow (1). The present experiments were designed to measure the size of the MP of PMN and determine if there were regional differences in the lung. 250 ml of blood were taken from dogs and the PMN and RBC labelled with ^{51}Cr and ^{99m}Tc respectively. Animals were anesthetized and catheters placed in the aorta (AO), pulmonary artery (PA), right ventricle (RV) and atrium (RA). A bolus of labelled PMN and RBC was injected into the RA and simultaneous samples were drawn from the RV and AO every 1/2 min for 10 min. Regional blood flow $\dot{Q}r$ was measured by injecting ^{125}I labelled macroaggregates and the animals were sacrificed followed by rapid removal and sampling of the lungs. We assumed that the labelled cells had equilibrated with the MP of the lung because there were no AV differences across the lung at the time of sacrifice. The MP of labelled cells was calculated by subtracting the ^{51}Cr-PMN in the residual blood from the ^{51}Cr-PMN in each lung sample. We found the total lung MP was $4.5 \pm .8$ x the circulating pool of PMN and that the size of the marginating pool in different lung regions varied with $\dot{Q}r$, being smallest in regions of low flow.
(1) J. Clin. Invest., June, 1982.
Supported by Medical Research Council Grant MT-4219.

8.13

HISTAMINE-INDUCED INCREASE IN PULMONARY LYMPH FLOW: HEMODYNAMICS VERSUS PERMEABILITY. F.L. Minnear*, R.J. Mullins*, D.R. Bell, and A.B. Malik. Department of Physiology, Albany Medical College, Albany, N.Y. 12208

We infused histamine (0.71-4.47 µg/kg/min) for 4h in 5 control-ventilated sheep prepared with lung lymph fistulas and tested for an increase in the pulmonary endothelial permeability to proteins by increasing left atrial pressure. The mean steady-state values ± 1 SEM are listed below: $\dot{Q}$lym = pulmonary lymph flow, L/P=lymph/plasma protein conc. ratio, $\bar{P}_{Pa}$ = mean pulmonary arterial pressure, PBF=pulmonary blood flow, PVR = pulmonary vascular resistance, *P<0.05.

	$\dot{Q}$lym	L/P	$\bar{P}_{Pa}$	PBF	PVR
Baseline	3.6±1.4	0.83±0.05	17.0±1.3	1.99±0.16	5.19±0.64
Histamine	6.8±1.8*	0.86±0.04	12.3±0.6*	1.55±0.20	3.84±0.37*

Increasing left atrial pressure by 10 mm Hg the last 2h of the infusion in 3 sheep doubled $\dot{Q}$lym and markedly decreased L/P. In 3 of the 5 sheep studied, PBF increased initially and then returned to a value below baseline. A pre-infusion of isoproterenol in 4 additional sheep elevated PBF and blocked the increase in $\dot{Q}$lym with histamine. Although these findings do not rule-out a transient effect of histamine on pulmonary endothelial permeability, they do indicate that after 2h of histamine-infusion the increase in $\dot{Q}$lym is not the result of an increase in the pulmonary endothelial permeability to proteins but probably an increase in the vascular surface area.
(HL-27016, HL-17355, HL-29273, GM-007033, HL-26807).

8.14

COMPARISON OF CRYSTALLOID AND COLLOID INFUSION ON PULMONARY FLUID BALANCE DURING INCREASED PULMONARY VASCULAR PERMEABILITY. J.C. Parker, G. Rutili*, and A.E. Taylor. Department of Physiology, University of South Alabama, Mobile, AL 36688.

A controversy exists as to the preferred solution for plasma volume replacement and cardiac output maintenance in the presence of lung injury. Crystalloid and colloid infusions were compared in dogs treated with 5mg/kg of α-naphthyl thiourea (ANTU) which produces a predictable increase in vascular permeability and extravascular water (Q_w) in the lung. A tracheobronchial lymphatic from the left lung was cannulated and left atrial (P_{La}) pressure, lymph flow, lymph/plasma protein concentration ratios and thermal dilution cardiac output (C.O.) were monitored. Test infusions were either 15 ml/kg 6% Dextran 70 (DX), or 60% ml/kg normal saline (NS) infused over 20 minutes. Solutions were infused 3 hrs after ANTU as follows: Group I (DX); Group II (DX, constant P_{La}); Group IV (NS, constant P_{La}). In groups III and IV, P_{La} was maintained constant by bleeding into a reservoir. Post-infusion Q_w increased to 9.01 and 9.14 g/g BFDW for Groups I and II, respectively, but there was no significant post-infusion increase in Q_w for Groups III and IV above the 3 hr values. However, C.O. was significantly higher at 5 hrs in Group III compared to Group IV. These studies indicate that capillary hydrostatic pressure was the major determinant of Q_w during the infusions, but that Dextran maintained C.O. significantly higher because of vascular retention of the colloids.
NIH HL22549 and 24571.

8.15

PORE-MODEL ANALYSIS OF THE EFFECTS OF CORONARY FLOW REDUCTION ON THE SHEEP LUNG MICROVASCULAR BARRIER. **J. C. Collins*, R. J. Roselli, and T. R. Harris, Departments of Medicine and Biomedical Engineering, Vanderbilt University, Nashville, TN 37232.**

Total occlusion of a coronary branch alters lung microvascular transport of fluid and protein. In order to assess the effect of reduced-flow coronary ischemia (RFCI) on lung transport, we studied 5 halothane-anesthetized sheep. Lung lymph was collected from cannulated efferent vessels from the mediastinal node. Diaphragmatic lymph was eliminated by cauterization. Left anterior descending coronary artery (LADCA) flow was supplied from the left carotid artery by an external shunt. LADCA flow, lymph flow, lymph and plasma protein concentrations (L and P) of 8 fractions, and permeability-surface area to ^{14}C-urea were measured during baseline and after reduction of coronary flow to 38% of baseline for 2-3h. The data were analyzed with a 2-pore model of the microvascular barrier. For all conditions it was assumed that the small-pore radius was 2 nm. For baseline conditions, the model estimated, by least-squares fit of L/P ratios, a large-pore radius R_L of 24 nm and a small pore/large pore frequency (F_L) of 9 X 10^{-5}. During RFCI, the model estimated: (1) a 30% decrease in the total number of large pores, suggesting derecruitment; and (2) an increase in R_L to 28 nm and a 64% increase in F_L, both of which suggest increased permeability in the remaining capillaries. (Supported in part by USPHS. NHLBI Grant Nos. HL-26811 and HL-19153.)

8.16

EFFECTS OF ALTERING INJECTATE AND PERFUSATE HEMATOCRITS ON ^{14}C-UREA EXTRACTION PS IN THE ISOLATED DOG LUNG. **R. E. Parker, R. J. Roselli, F. R. Haselton*, K. L. Brigham and T. R. Harris. Pulmonary Circulation Center, Vanderbilt University, Nashville, TN 37232.**

We conducted experiments in three isolated perfused dog lung lobes to determine if urea exchange between plasma and red blood cells significantly affects the calculation of ^{14}C-urea extraction permeability-surface area product (PSu) as determined by indicator-dilution techniques. Two injectates were utilized. The first consisted of ^{14}C-urea and ^{125}I-albumin suspended in a plasma-dextran solution. The second consisted of ^{14}C-urea and ^{125}I-albumin suspended in a plasma-dextran and ^{51}Cr labeled red blood cell solution at baseline hematocrit. Indicator-dilution curves were performed at 3-4 perfusate hematocrits with each of the two injectate solutions. Our results indicate that perfusate hematocrit has no effect on PSu provided the injectate was of normal hematocrit and pre-equilibrated with urea. The intravascular reference curve must account for urea transport in both red blood cells and plasma.

(Supported in part by USPHS. NHLBI Grant Nos. HL-19153 and HL-27169.)

8.17

BLOCKADE OF THROMBOXANE A_2 (TxA_2) SYNTHESIS PREVENTS PULMONARY HYPERTENSION BUT NOT INCREASED PERMEABILITY FOLLOWING E. COLI ENDOTOXIN INFUSION. R. Winn, J. Harlan*, J. Stothert, B. Nadir*, L. Harker* and J. Hildebrandt. Virginia Mason Research Center and Harborview Medical Center, Seattle, WA 98101.

We measured cardiac output (C.O.), lymph flow ($\dot{Q}_L$), lymph-plasma ratio (L/P), and pressures in pulmonary arteries (Ppa), pulmonary wedge (Pw), central vein (CVP) and systemic arteries (Pa) in goats. In the untreated group (UT), after a 2 hr baseline, 1 µg/kg endotoxin, was infused over 30 min, then data collected for 5½ hrs. The treated (T) group received in addition a 25 mg/kg bolus followed by a 10 mg/kg/hr infusion of dazoxiben, a specific inhibitor of TxA_2 synthesis. The increase in $\dot{Q}_L$ was greater in UT than T(2.6X vs 2.0X baseline). Peak Ppa was increased by 24 cmW in UT and by 6 cmW in T; Pw increased by 15 cmW in UT and 3 cmW in T. Plasma levels of TxB_2 (metabolite of TxA_2) were elevated in UT and had a time course similar to that of Ppa, but showed little change in T. In UT, L/P initially fell (hypertensive phase) but by 4 hrs exceeded baseline (permeability phase), whereas in T, L/P was only elevated. In UT, Pa fell by 27 torr at 2 hrs and remained depressed; in T at 2 and 6 hrs Pa was 17 and 7 torr below baseline. In UT, C.O. decreased sharply after endotoxin and remained depressed for the next 4 hrs before returning to baseline at 6 hrs; it decreased transiently in T and was at or above baseline thereafter. We conclude that dazoxiben, by blocking TxA_2, prevents pulmonary hypertension but has little effect on increased permeability caused by endotoxin. Depression in C.O. (myocardial?) was eliminated by dazoxiben. (HL25706,GM24990,GM29853).

8.18

PHENYTOIN ATTENUATES INCREASED LUNG MICROVASCULAR PERMEABILITY AFTER ENDOTOXEMIA IN SHEEP. M.R. Flick, J. Hoeffel*, M. Julien*, M. Lesser* and B. Kent. University of California, San Francisco, CA 94143 and VAMC, Bronx, NY 10468

We tested phenytoin's (DPH) effect on permeability edema caused by E. coli endotoxin (E). In 4 unanesthetized sheep, we measured lung lymph fluid ($\dot{Q}$lym) and protein ($\dot{Q}$prt) flows, lymph (L) and plasma (P) protein concentrations, and pulmonary arterial (Ppa) and left atrial (Pla) pressures. After a 2h baseline, we gave E (1µg/kg iv). E caused initial high pressure edema (Phase I) followed by increased permeability (Phase II). We repeated the experiment in the same sheep after pretreatment with DPH (10mg/kg). Mean data were:

Condition	Ppa (cmH_2O)	Pla (cmH_2O)	$\dot{Q}$lym (ml/h)	L/P	$\dot{Q}$prt (mg/h)
Endotoxin					
Baseline	15	4	5.4	0.56	175
Phase I	38	-3	22.5	0.45	600
Phase II	36	2	25.1	0.69	945
DPH/Endotoxin					
Baseline	14	1	4.7	0.53	135
Phase I	31	0	12.1	0.44	293
Phase II	24	1	12.1	0.62	397

For equivalent increases in pressures, lung lymph fluid and protein flows were 3X greater in Phase I and 1.5X greater in Phase II in untreated as compared to DPH treated sheep. DPH attenuated increased microvascular permeability after E.
(Supported by NIH Grants HL-26913 and HL-19155).

8.19

EFFECTS OF SEQUENTIAL RECRUITMENT OF PULMONARY MICROVASCULAR SURFACE AREA ON LUNG FLUID BALANCE. M.I. Townsley*, D. McClure* and W.J. WEIDNER. Dept. of Animal Physiology, UC, Davis, Davis, CA 95616.

The role of pulmonary microvascular surface area (PMSA) in pulmonary fluid balance is unclear. Using the mean transit time (M) of an intravascular marker, Evans blue dye, across the pulmonary vascular bed as an indicator of PMSA, we examined lung lymph flow (Ql) as a function of PMSA in five acutely prepared, anesthetized sheep. PMSA was recruited in each animal by elevating left atrial pressure (Pla) successively to ultimately produce a filtration-independent lymph to plasma protein concentration ratio, (L/P)min (Parker, RE, et al., Circ. Res. 49:1164-1172, 1981). Overall, a mean increase in Pla of 16.7 Torr resulted in a 50% increase in M, accompanied by a 223% increase in Ql while L/P progressively fell. These data suggest that in order to assess the effect of an experimental protocol using this model, changes in PMSA must be accounted for. In addition, we estimate the osmotic reflection coefficient, calculated as 1-(L/P)min, in this acute preparation to be 0.66, somewhat lower than the value of 0.74 found by Parker et al. in the chronic sheep preparation. This apparent discrepancy in osmotic reflection coefficient may reflect different pulmonary microvascular permeability in the acute vs chronic sheep preparation, perhaps underlying the differences in baseline Ql observed under these conditions. Aided by the Strobel Medical Research Fund American Lung Association of San Francisco.

8.20

DETERMINING ACCURATE VALUES FOR LUNG CAPILLARY PERMEABILITY-SURFACE AREAS AND LYMPH CONDUCTANCE USING THE NON STEADY STATE. A.A. Bicker*, A. Hurewitz* and E.H. Bergofsky. SUNY, Stony Brook, N.Y. 11794

A microprocessor based simulation model of transcapillary fluid exchange is used in a new method of determining the major parameters controlling lung H_2O, utilizing non steady state experimental data. The method uses a sequence of particular vascular hydrostatic & colloid osmotic pressure changes in the animal & measures consequent changes in the dependent variables, lymph flow & colloid osmotic pressure. The latter are then predicted by the model from identical vascular pressure sequences. Differences between predicted & measured values are analyzed, certain parameter adjustments are made & the procedure is repeated until the best match between experimental & predicted values is obtained. The use of this technique does not require a steady state. This method is capable of characterizing the 4 main conductive parameters governing fluid exchange: large pore permeability-surface area, upstream & downstream small pore permeability-surface area, & lymph conductance. Since the model calculates interstitial volume & protein as dependent variables, the negative feedback control feature of interstitial osmotic buffering is considered and provides more accurate values for these parameters. (VA, Wash., D.C.)

EXERCISE II

9.1

ORGAN BLOOD FLOW IN EXERCISE TRAINED (EXTN) PIGS. Brian Guth, Francis White, J.J. Wright, and Colin Bloor UCSD School of Medicine, La Jolla, California 92093

Six male Yucatan miniswine (30.3±4.6 Kg) were exercise trained (TN) for running 5 days/week on a treadmill. After 10 weeks blood flow (BF) to the left ventricle (LV), small intestine (IN), stomach (ST), spleen (SP), kidney (K), Rectus Femoris (RF), and cardiac output (CO), were measured with tracer microspheres. Blood flows (ml/min/kg) were measured at rest (R) and near maximal exercise (EX). BF values (Mean SEM) were compared to those obtained from 10 untrained pigs (C).

	LV	IN	ST	SP	K	RF	CO
RC	.84	.24	.16	2.68	1.52	.08	84
	±.05	±.04	±.02	±.47	±.15	±.01	± 5
RTN	.95	.38	.47	2.76	2.98	.10	87
	±.10	±.09	±.12	±.47	±.50	±.03	± 2
EXC	3.18	.10	.03	.18	.57	.82	253
	±.27	±.02	±.01	±.07	±.19	±.31	±15
EXTN	3.52	.23	.29	1.46	1.78	.55	416
	±.23	±.07*	±.11*	±.67*	±.80*	±.09	±25*

[* = P<0.05; unpaired t-test, EXC vs EXTN]
BF was reduced (P<0.05) to IN, ST, SP, and K during EX in untrained pigs. Reduction of BF to these organs was significantly less in TN pigs. These data suggest that increased CO from exercise training allows a relative maintenance of visceral BF during EX.

9.2

INFLUENCE OF DEMEDULLATION ON THE TRAINING ADAPTATIONS OF SYMPATHECTOMIZED SHR GROUPS. C.M. Tipton, J.M. Overton*, M.S. Sturek*, R.D. Matthes*, and J.G. Edwards*. Exercise Science Program, University of Iowa, Iowa City, IA 52242.

Previous studies have demonstrated that the acute and chronic effects of exercise are influenced by the presence of an intact sympathetic nervous system. Even though chemical sympathectomy (SYMX) will normalize resting blood pressure (RBP) in spontaneously hypertensive rats (SHR) (Physiologist 24:35, 1981), RBP was lower in the trained SYMX-SHR. To determine whether similar changes would occur in the absence of the adrenal medulla, surgical demedullation (D) was performed in SYMX-SHR (N=16) after 8 wks. They were subsequently trained (T) for 12 wks at 40-60% $\dot{V}O_2$max. Plasma and tissue catecholamine measurements at sacrifice verified the procedures were effective. RBP (mmHg) was not significantly altered by chronic exercise (NT=106±3, T=112±5). Compared to previous SHR groups, these animals had lower $\dot{V}O_2$max values. Training was associated with a 10% increase in $\dot{V}O_2$max (NT=42±2, T=47±3 ml·min^{-1}·kg^{-1}), longer run times and higher muscle cytochrome oxidase activity. No evidence (direct or predicted) was obtained for cardiac enlargement. In fact, both NT and T groups had heart weights that were lighter than predicted. Collectively, these results indicate that training adaptations can occur in the absence of peripheral sympathetic mechanisms although the intensity and duration of exercise is compromised. (Supported in part by HL 21245-05 and GM-07045-04.

9.3

BETA BLOCKADE, MAXIMAL OXYGEN CONSUMPTION AND VENTRICULAR DIMENSIONS IN EXERCISING SWINE. M.D. McKirnan, B.D. Guth*, F.C. White and C.M. Bloor. UCSD School of Medicine, La Jolla, CA 92093.

The effect of acute ß-adrenergic blockade (B) on maximal oxygen consumption (VO_2 max), left ventricular diameter (ultrasonic dimension gauges) at end-diastole (EDD) and end-systole (ESD) was studied in 6 swine (32.2±3.1kg, mean ± SD). Propranolol (1mg/kg,IV) was used to completely block the chronotropic response of isoproterenol (1ug/kg IV). B produced marked reductions in both maximal heart rate (276±8 vs 210±12) and VO_2 max (65.8±15.4 vs 42.1±14.8 cc/kg/min). The effects of B and exercise during B on ventricular dimensions were:

	Control	B		Exercise
EDD(mm)	33.6±2.8	34.7±3.3	*	34.7±3.1 NS
ESD(mm)	21.7±1.3	25.6±1.6	*	24.2±1.3‡

*=p<0.05, paired t-test Control vs B
‡=p<0.05, paired t-test B vs Exercise

These data indicate that B increases both EDD and ESD at rest. During exercise ESD decreases and EDD remains unchanged indicating an increased stroke volume in the presence of depressed cardiac function. These results emphasize the importance of sympathetic nervous system in determining cardiac function and in defining the limits of oxygen transport.

9.4

EFFECT OF REPETITIVE PACING-INDUCED TACHYCARDIA ON CORONARY REACTIVE HYPEREMIA IN CONSCIOUS DOGS. M. Hamra* and H.L. Stone. Dept. of Physiol., Univ. of Okla. HSC, Okla. City, OK 73190.

Four weeks of exercise training reduces the reactive hyperemia (RH) response to 15-second occlusions of the left circumflex coronary artery (LCCA) in the conscious dog by 30%. Exercise tachycardia might contribute to the reduced RH. The purpose of this study was to examine the effect of tachycardia alone. While resting, 4 dogs were paced daily at 260 bpm for 4 weeks according to the following schedule: week 1 for 25 minutes, week 2 for 35 minutes, week 3 for 40 minutes, and week 4 for 45 minutes. The RH response to a 15-second occlusion of the LCCA was determined before the start of the pacing protocol (control) and at weekly intervals during the protocol. All 4 dogs demonstrated a decrease in RH by week 4 of pacing. RH (expressed as % payback) decreased from a mean control value of 434 ± 35 % to 327 ± 15%. This represents an average decrease of 24 ± 4% ($P < 0.05$). The control resting heart rate (HR) (100 ± 12 bpm) was not significantly different from the week 4 resting HR (86 ± 13 bpm). Control peak RH flow velocity values (81.3 ± 4.9 cm/sec) were not significantly different from week 4 values (81.3 ± 4.3 cm/sec). These results indicate that daily pacing-induced tachycardia can reduce RH and suggest that the increased heart rate of daily exercise is probably a contributing factor to the reduced RH associated with exercise training. (Supported by HL 22154).

9.5

NONINVASIVE ECHO-DOPPLER DUPLEX MEASUREMENTS OF CARDIAC OUTPUT (CO) AND COMMON CAROTID BLOODFLOW (CCBF) DURING UPRIGHT EXERCISE (UE). E.R. Greene,* G. Cagle,* P.A. Reilly,* I.P. Miranda* (SPON: J.A. Loeppky). Lovelace Medical Foundation, Albuquerque, NM 87108

Beat-to-beat, noninvasive, and nonionizing measurements of CO and CCBF during UE have not been reported. Heart rate (HR), CO, CCBF, and common carotid stroke volume (CCSV) were calculated in the fifth min at 50, 100, and 150 watt loads of ergometer UE (50 rpm) from concurrently measured spatial average blood velocities and lumen diameters in the ascending aorta and the right common carotid with a unique, echo-Doppler duplex scanner (DS). A fast Fourier transform audio spectrum analyzer was used to calibrate the DS. The DS method was validated in vitro using hydraulic models of CO and CCBF (r=0.96) and in vivo using canine electromagnetic flow cuffs (r=0.93). Mean (±SD) results are for 10 normals at rest (R) and at the end of min 10 (Ex) of UE.

HR(bpm)		CO(l/min)		CCBF(l/min)		CCSV(ml)	
R	Ex	R	Ex	R	Ex	R	Ex
67	120*	4.7	8.5*	0.51	0.48	7.6	4.0*
±14	±12	±1.1	±2.6	±.20	±.22	±3.2	±1.9

(*p<.05 versus R) In all subjects, CCBF was cranial throughout the cardiac cycle during R but exhibited caudal direction in early diastole during UE. We conclude: 1) beat-to-beat changes in CO and CCBF can be measured reliably by DS during UE; 2) although total CCBF was unchanged during UE, CCSV decreased (p<.05) with significant waveform variations.

9.6

THE EFFECT OF HEAT STRESS AND WORK ON TOTAL PLASMA PROTEIN CONCENTRATION, ALBUMIN CONCENTRATION AND HEMATOCRIT IN ENDURANCE TRAINED AND SEDENTARY ADULT FEMALES. Vincent M. Nethery*, Peter G. Davis*, and Eugene Evonuk. Applied Physiology Lab, University of Oregon, Eugene, Oregon 97403.

Heat acclimated subjects and endurance trained males both exhibit an enhanced sudorfic response and stabilization of the cascular volume when working in a heat stress condition. Whether endurance trained females exhibit these same characteristics has not as yet been determined. The response of 8 highly trained female endurance runners and 8 sedentary females to moderate work (heart rate 140 beats·min^{-1}) on a bicycle ergometer in a moderately hot (34^{0}C db, 24^{0} wb) environment for 40 minutes was studied. Venous blood was sampled prior to and at the completion of the work period. Alterations in total plasma protein concentration, albumin concentration and hematocrit were determined from these samples. A nude weight was also taken prior to and at the completion of the work period. There were no significant differences in resting values for hematocrit, total protein or albumin concentration between the two groups. The trained females weighed significantly less than the sedentary group. At the completion of the work period, the trained females exhibited less than half of the hemoconcentration (2.6%) despite the loss of twice as much body fluids (-1.3%) of the initial body weight. This preservation of plasma volume was accompanied by a retention of plasma proteins (3.7%), primarily albumin (6.6%) within the vascular bed.

9.7

THE EFFECTS OF CHRONIC EXERCISE ON IN VITRO ADENOSINE DIPHOSPHATE (ADP) INDUCED AGGREGATION TIMES OF BLOOD PLATELETS IN RATS. J. David Symons,* James W. Agnew*and Eugene Evonuk. Applied Physiology Lab. Univ. of Oregon, Eugene, OR 97403.

The purpose of the present study was to determine the effects of chronic exercise on in vitro ADP induced aggregation times of circulating blood platelets in rats. A control and two exercise groups with 8 in each group were used. The exercise consisted of swimming with appropriate weights for one hour, five days a week for six weeks. At the end of the exercise period, in one group blood was withdrawn immediately after the final exercise bout, and in the other exercise group blood was withdrawn one hour post-exercise. In the control groups, blood was withdrawn during this same time span. After the blood was prepared for aggregate profiling, 50 µl of CLUSTER^{TM} ADP reagent (1×10^{-4} M) was added to the platelet rich plasma. The aggregation response was observed and recorded from a Bio/Data Platelet Aggregation Profiler, Model PAP-2A. The animals in the exercise group that had blood withdrawn one hour after the end of the exercise bout had a statistically significant ($P<0.05$) increased time to aggregation when compared with the exercise group with blood withdrawn immediately after exercise and the control group. The advantages for chronic exercise intervention for increasing the time to platelet aggregation are in part, obviated.

9.8

SYMPATHOADRENAL RESPONSE TO EXERCISE AFTER SLEEP DEPRIVATION. Bruce Martin and Hsiun-ing Chen.* Med. Sci. Program, Indiana University, Bloomington, IN 47405

Although sleep loss reduces long-term exercise performance, the mechanism for this effect remains unclear. Because α- and β-adrenergic receptor blockade produces many of the same symptoms as sleeplessness, some speculate that sleep loss diminishes the sympathetic nervous system response to work and thus reduces exercise tolerance. To test this hypothesis, we compared 8 subjects' sympathoadrenal responses to treadmill walking at 80% of the $\dot{V}O_2$max after normal sleep with those measured after a 50 hour sleepless period. We found an intact sympathetic response to exercise after sleeplessness: after 12 min of exercise, heart rate and plasma norepinephrine, epinephrine, and dopamine levels were similar in the two conditions. In addition, we could find no other alterations in the physiological response to exercise after sleep loss: minute ventilation, oxygen uptake, blood lactate levels, and rectal and skin temperatures were identical in the two situations. Nonetheless, despite both unaltered physiological responses to exercise, and doubled monetary incentives to work, sleep loss reduced work time to exhaustion by 20% ($P<0.01$). These results suggest that sleep deprivation leaves the sympathoadrenal response to exercise intact, and that it may reduce heavy exercise tolerance through non-physiological mechanisms. (Supported by grant DAMD 17-81-C-1023 from the U.S. Army)

9.9

OXYGEN UPTAKE AND HEART RATE DURING EXERCISE IN CHILDREN. D.M. Cooper‡ D. Weiler-Ravell‡, B.J. Whipp, & K. Wasserman. Div. of Resp. Med. and Physiol., Harbor-UCLA Med. Ctr., Torrance, CA

To understand how O_2 supply to exercising muscle changes with growth, we analyzed the relationship of HR to $\dot{V}O_2$ in 70 normal children 6-17 years old. Subjects cycled on an ergometer utilizing a continuously increasing workrate (ramp function) and gas exchange was measured breath-by-breath. We compared the children using the anaerobic threshold (AT)--a marker of O_2 delivery during exercise--and found that the O_2-pulse ($\dot{V}O_2$/HR) at AT increased with body weight to the power of ≈1. Since $(a-\bar{v})DO_2$ has been found to be either the same or greater in children than in adults during exercise, stroke volume at AT must increase in at least direct proportion to body weight. We found "m" and "b" (fig.1) enabling us to quantify dynamics of the O_2-pulse (fig.2):

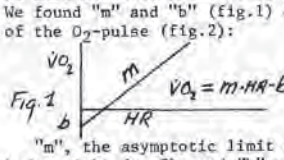
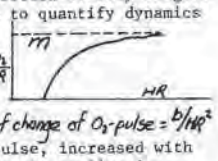


Fig. 1

Fig. 2 $\frac{\dot{V}O_2}{HR}$



rate of change of O_2-pulse = b/HR^2

"m", the asymptotic limit of the O_2-pulse, increased with body weight (r=.7); and "b" was significantly smaller in younger children demonstrating a reduced rate of increase of O_2-pulse at comparable HR. We conclude that during exercise, smaller children are closer to their limits of O_2 extraction per heartbeat, and are more dependent on increases in HR alone to augment any additional O_2 demand.

9.10

BEST WORK PERFORMANCE TESTING IN GUINEA PIGS. J.K. DUTTARER*& H.I MILLER. LSU Medical School, New Orleans, LA. 70112.

It is difficult to determine max $\dot{V}O_2$ in animals due to the inability to gain their cooperation and to motivate them. However, at times it is desireable to measure their best work performance. A blind study, using 12 male guinea pigs (25-69 wks) was initiated. Six guinea pigs were chosen from a sedentary population and 6 were from a group who ran 3 times/week at .7 mph and 10%grade. All were tested twice on each of 3 protocols. Test I used an intermittant protocol with 5 min. rest between each 6 min. run, beginning with a warm-up and then increasing progressively by .2mph or by 5% grade. Test II (a continuous protocol) used a fixed elevation (15%), began at .6mph and increased by .2mph every 2 mins. For Test III, after a warm-up, the guinea pig ran for 6 mins. at the workload which had produced the max $\dot{V}O_2$ in Test I, then after a 5 min. rest, attempted a higher workload. All tests were stopped either when the animal was unable to run or when the $\dot{V}O_2$ failed to increase with an increase in the workload. All test were done on a Rodent Treadmill modified with a metabolic cage through which air was drawn at a known rate into a Kipp Diaferometer for determination of $\dot{V}O_2$ and $\dot{V}CO_2$. The mean $\dot{V}O_2$ for Test I (39.88 ml/kg.min) was significantly greater (p .01) than the mean $\dot{V}O_2$ for either Test II (35.11 ml/kg.min) or Test III (30.07 ml/kg.min). T-test of the test-retest results showed no significant difference. We conclude that the intermittant protocol using increasing speeds and grades produced the highest $\dot{V}O_2$ and that the test protocol is reliable.

9.11

MENSTRUAL STATUS AND BODY FAT LEVELS IN ATHLETES: VALIDATION OF THE MELLITS AND CHEEK EQUATION USING HYDROSTATIC WEIGHING. Anne B. Loucks, Patty S. Freedson* and Steven M. Horvath. Inst. of Environ. Stress, UCSB, Santa Barbara, CA 93106

The purpose of the present study was to cross-validate (underwater weighing as criterion) (UWW) the Mellits and Cheek (MC) total body water prediction equation that Frisch et al. have used to estimate % fat. Eighteen runners (R) and 7 bodybuilders (BB) were studied. Cross-validation of the MC equation (n=25) resulted in a nonsignificant mean difference between the methods (UWW $\bar{x}$ %fat±SD=13.6±3.04; MC $\bar{x}$ %fat±SD= 14.4±3.36). However, a correlation analysis showed that the MC equation cannot accurately predict an individual's % fat (r=.33,p>.05). Mean (±SD) % fat was similar between the cyclic (C) (15.4%±3.08) and amenorrheic (A) (13.2%±3.36) athletes using UWW (ANOVA) whereas a significant difference was observed using the MC procedure (C:15.0%±2.59; A:12.2%±2.87) (ANOVA). The results from the present study suggest that the MC procedure can be used to adequately predict % fat for a heterogeneous sample of subjects. However, the MC equation is of limited value for an individual or for a specific homogeneous group of athletes. Although Frisch et al. have proposed that 22% fat is the minimum level required for maintenance of cyclic menstrual patterns using the MC estimate, the UWW procedure revealed that 9 R ($\bar{x}$ %fat±SD=16.2 ±2.63 and 4 BB ($\bar{x}$ %fat±SD=13.8±3.73) were cyclic in menstrual function.

9.12

PLASMA GROWTH HORMONE RESPONSE TO EXHAUSTIVE EXERCISE IN COLLEGE WOMEN FOLLOWING BASKETBALL TRAINING. C. M. Maresh*, K. L. Goetz, and S. S. Fotopoulos*. St. Luke's Hospital and Foundation, Kansas City, MO 64111

We examined the hypothesis that plasma human growth hormone (hGH) concentration following exercise can be increased by training which includes an appreciable anaerobic component. The effect of exhaustive treadmill running on plasma hGH levels was studied in nine physically-active college women (aged 18-22 yrs) before (BT) and after (AT) five months of basketball training and competition. All women were in the same phase of their menstrual cycle during both tests. Venous blood was sampled without stasis from an antecubital vein before and 5 min after exercise. Following training, treadmill time to exhaustion increased from 6.0 ± 1.8 to 7.6 ± 1.4 min ($p < 0.001$). Control levels of plasma lactate BT were 1.0 ± 0.7 and increased to 9.9 ± 2.5 mEq/L ($p < 0.001$) at 5 min after exercise. Similar values before and after exercise ($p > 0.05$) were obtained AT. HGH concentrations BT increased ($p < 0.05$) from 9.3 ± 10.2 pre-exercise to 28.8 ± 18.0 ng/ml at 5 min post-exercise. Control hGH values AT did not differ from BT values; however, a significantly greater response occurred 5 min after exercise (46.3 ± 11.2 ng/ml, $p < 0.05$). Since the $\dot{V}O_2$ max values were not significantly increased after training, these data suggest that hGH secretion during maximal exercise may be increased after prolonged training which includes a high anaerobic component.

VASCULAR SMOOTH MUSCLE I

10.1

LENGTH DEPENDENT SENSITIVITY AT LENGTHS GREATER THAN L_{max} IN VASCULAR SMOOTH MUSCLE. Joel M. Price and D.L. Davis. University of South Florida, College of Medicine, Dept. of Physiology, Tampa, FL 33612.

Previous work on vascular smooth muscle at lengths equal to and less than that for maximum active force (L_{max}) has shown that sensitivity depends on muscle length (arterial circumference). In this study dose-response curves were obtained from dog anterior tibial artery rings at lengths equal to or longer than L_{max}. The curves were compared to dose-response curves obtained at lengths less than L_{max}. The norepinephrine concentration for half maximal response (ED_{50}) was determined by graphical estimation and by calculation from a best fit curve. The results show that: 1) ED_{50} decreased when the rings were stretched from L_{max} to 1.15 L_{max} and then to 1.30 L_{max}; 2)ED_{50} decreased when the rings were stretched from 0.70 L_{max} to L_{max} and then to 1.30 L_{max}; and 3) For an NE concentration greater than the ED_{50} at L_{max}, active stress is higher at L_{max} than at 0.70 L_{max} or 1.30 L_{max}. For an NE concentration less than the ED_{50} at L_{max}, active stress was higher at L_{max} than at 0.70 L_{max} but active stress at 1.30 L_{max} was higher than active stress at L_{max}. Change in the concentration for 10% maximal response was the same as ED_{50}. We conclude that sensitivity of vascular smooth muscle continually increases with stretch and does not have a maximum at the length for maximum active force. (Supported by NIH grant #HL21103 and Am. Heart Assoc. Florida Affil.)

10.2

COMPRESSION AND DISTORTION OF PERILUMENAL WALL ELEMENTS DURING ARTERIOLAR CONTRACTION J.E. Greensmith* and E.R. Duling. Dept. of Physiology University of Virginia, Charlottesville, VA. 22908

Vasoconstriction of blood vessels with large wall thickness to lumenal diameter ratios causes encroachment of intimal and medial wall elements on the vessel lumen. The mechanisms of this lumenal encroachment have received little study. Furthermore, the relationship between lumenal reduction and distortion of perilumenal wall elements is undefined. In order to explore this problem, we isolated and cannulated primary arterioles (i.d. 50-100 microns) from the rat mesentary. Arteriolar diameters were constricted to levels between 25 and 150 % of the resting unstressed diameter using graded application of norepinephrine (luminal pressure = 50 mmHg). All vessels were glutaraldehyde fixed, stained and cross-sectional electron photomicrographs were prepared. Planimetry was performed on the photographs to assess the shape and disposition of wall elements. Constriction caused the endothelium, basal elastic lamina and inner layers of smooth muscle to be thrown into folds, the number (20-50) and amplitude (2 -5 microns) of which increased while the fold length decreased (8.5-3 microns) with greater contraction. As a consequence of this infolding, orientation of portions of the smooth muscle was made non-optimal for tangential force generation. This phenomenon will likely cause de-recruitment of contractile elements as vascular closure is approached and may amount to as much as a 15% reduction in the functional smooth muscle mass with a 80% contraction. Supported by HL 12792 and HL 13437.

10.3

CHARACTERISTICS OF SPONTANEOUS AND INDUCED RHYTHMIC CONTRACTIONS OF RABBIT CORONARY ARTERIES. Kathleen Keef*, Liu Yu-min* and Gordon Ross. UCLA School of Medicine, Los Angeles Ca. 90024.

Rabbit coronary arterial ring segments developed rhythmic contractions at an approximate frequency of 4/min in response to stretch following initial immersion in Krebs solution. This activity subsided after 15-30 minutes. Rhythmic contractions could be induced by potassium chloride (KCl) 15-25 mM and by histamine 10^{-7}-10^{-5} M. The induced rhythmic behavior was variable in pattern sometimes showing brief contractions at 12-120 second intervals, sometimes prolonged contractions every 6-24 minutes and sometimes combinations of both patterns. Phasic contractions and tone were diminished by external calcium concentrations above 5 mM or below 1 mM, by reducing oxygen tension, by verapamil (>10^{-7}M), by nitroglycerin (>10^{-5}M) and by nitroprusside (>10^{-7}M). Ouabain (>10^{-5}M) and also sodium substitution with lithium abolished phasic activity. Contractions induced by histamine were transiently abolished by low concentrations of KCl. We conclude that rabbit coronary arteries differ from those of the dog but resemble those of man. Their rhythmicity appears to be partially dependent on membrane potential, sodium-potassium ATPase activity and variations in calcium influx. (Supported by USPHS Grant #HL 26238).

10.4

Na^+-PUMP FUNCTION IN VASCULAR SMOOTH MUSCLE. J. C. Allen and R. D. Bukoski. Baylor College of Medicine. Houston, Texas 77030

Na^+, K^+-ATPase has been readily identified in vascular smooth muscle, but the specific activities of the isolated preparations have been low when compared to other tissues: ∿ 5 umoles Pi/mg pro/hr compared to ∿ 500 for cardiac muscle. The reason for this lower enzyme activity is due to a lower Na^+-pump density in vascular smooth muscle sarcolemma. ^{3}H-ouabain binding to subcellular fractions from various canine vascular smooth muscles is supported by both ATP+Mg+Na and Mg+Pi ligand conditions. The ratio of ^{3}H-ouabain binding to enzyme activity is 1-1.5 to 1 (pmoles ^{3}H-ouabain/umoles Pi/mg/hr). Recovery studies indicate that the total amount of ^{3}H-ouabain binding sites in all vascular tissues studied is significantly lower than in cardiac muscle. Calculations based on S/V ratio and extracellular space considerations confirm that vascular smooth muscle contains at least 1-2 orders of magnitude fewer sites per unit membrane area than cardiac muscle. However, both cardiac and vascular smooth muscle display similar amounts of ouabain sensitive ^{86}Rb uptake: ∿ 10-50 nmoles ^{86}Rb/mg dry wt. The calculation of fewer pump sites per μ^2 of membrane area of smooth muscle, a comparable ^{86}Rb uptake in both cardiac and smooth muscle suggests that each pump site in smooth muscle may be operating at a level closer to optimal, and that modulation of the Na^+-pump may play an important regulatory role in this tissue.
(HL 07282, HL 24585)

10.5

EVIDENCE THAT K^+-INDUCED RELAXATION MAY NOT REFLECT Na^+- PUMP ACTIVITY. R. D. Bukoski and J. C. Allen. Baylor College of Medicine. Houston, Texas 77030

Arteries contracted with norepinephrine (NE) in K^+-free PSS, are relaxed by addition of K^+ (K^+-relax). The amount of K^+-relax has been used as an index of arterial Na^+-pump activity. To clarify the role of the Na^+-pump in regulating contraction, we measured the amount and the pseudo-first order rate constant of K^+-relax of canine femoral (F) and renal (R) arteries as a function of KCl from 0.5 to 50 mM. The amount and rate were greater in R than F at $[K^+]$ from 0.5 to 3 mM. The apparent affinity ('K_D) of the Na^+-pump of R for K^+ was greater than the 'K_D of the Na^+-pump of F. These data indicate that the two Na^+-pumps are different. We also measured ^{86}Rb uptake by F and R as an independent index of Na^+-pump activity. No differences were observed in the rates of ^{86}Rb uptake or in the 'K_D's of the Na^+-pumps for Rb^+. These data indicate that the Na^+-pumps are identical. The discrepancy in the results of K^+-relax and ^{86}Rb uptake may be explained by the fact that NE stimulates ^{86}Rb uptake by R (and therefore the Na^+-pump of R), causing a shift of the rate limiting step of K^+-relax away from the Na^+-pump to a subsequent step. NE has no effect on the Na^+-pump of F. Thus, K^+-relax can be used as an index of Na^+-pump activity in F but not in R. Caution must be used when interpreting K^+-relax data in terms of Na^+-pump, because only when the pump is rate limiting can K^+-relax reflect its activity. (HL 07282, HL 24585)

10.6

MAGNESIUM BINDING OF VASCULAR CONNECTIVE TISSUE. G. Siegel* and A. Walter* (SPON: B.M. Altura). Institute of Physiology, Biophysical Research Group, The Free University of Berlin, D-1000 Berlin 33, Germany

It is known from numerous clinical observations that hypomagnesemia can lead to vasoconstrictions and spasms. Since also in vascular musculature changes of tension are voltage-dependent, the question has to be raised as to the reason of vasoconstrictor membrane depolarization in Mg^{++} deficiency. Keeping in mind the decisive role of K^+ ions for the passive potential genesis, we investigated the Mg^{++}-K^+ interactions on the cell membrane level. When a detailed compartmentation of mono- and divalent cations is computed for vascular wall, the surprising result is found that 65% of the cations are structurally bound mainly to the proteoglycans which comprise only 1% of the connective tissue, among others magnesium in a concentration of 1.5 ± 0.1 (28) mmole/kg wet wt. or 3.9 ± 0.4 (28) mmole/kg fibres. As vascular smooth muscle cells are covered by the basement membrane and a fine network of connective tissue fibres, narrow cleft spaces between cell membrane and connective tissue structures emerge. Under suitable, non-stationary conditions, even small amounts of released or adsorbed ions can lead to measurable activity changes in these tiny extracellular fluid spaces. As the K^+ concentration in the immediate neighbourhood of the cell membrane can be influenced by the microdynamic binding properties of the basement membrane and the other vascular connective tissue fibres, we studied K^+ and Mg^{++} binding and exchange in dependence on proton and cation concentration by tracer and NMR techniques. Isolated, adventitial connective tissue of the canine carotid artery has been used as test object. Intrinsic affinity constants for Mg^{++} binding to two specific sites were determined (log $K_{A,1}$ = 2.75; log $K_{A,2}$ = 3.50). The mode of interaction of an increasing Na^+ or Ca^{++} concentration on K^+ binding, or an increasing Na^+, K^+ or Ca^{++} concentration on Mg^{++} binding, is competition, while physiological concentrations of Mg^{++} ions can induce a specific change in the configuration of the polyanionic connective tissue macromolecules, which enables K^+ ions to bind cooperatively. On the other hand, an extracellular Mg^{++} deficiency effects not only a decreased binding of Mg^{++} ions to vascular connective tissue but also an increase of $[K^+]_0$ via a K^+ release or a diminished binding. K^+ accumulation close to the membrane of vascular smooth muscle cells and/or sympathetic nerve terminals leads to depolarization and vasoconstriction.

10.7

Action of arachidonic acid on uterine arteries from rabbits. Donna Moisey* and Tom Tulenko. Med Coll of PA, Phila, PA 19129.

Several investigators have suggested that prostaglandins (PG) may play a regulatory role in maintaining uteroplacental blood flow in pregnancy. The present study was undertaken to assess the response of the uterine artery from pregnant (0.92 term) and nonpregnant rabbits to the PG precursor Na-arachidonate (AA) ($C_{18:4}$). Isolated strips of uterine artery were equilibrated isometrically in physiological salt solution under their optimal resting tension. AA produced dose-dependent contractions over the dose range studied (10^{-8}-10^{-3}M) in both groups, with the responses from the pregnant group being significantly greater than those of the nonpregnant group ($P < .01$). Contractions occurred whether the strips were relaxed or precontracted with potassium chloride (30mM). The contractile responses to AA were antagonized in a competitive fashion by preincubation with the cyclooxygenase inhibitors meclofenamate (10^{-5}M) or indomethacin (10^{-5}M), evidence that the contractile response to AA was a result of its conversion by the cyclooxygenase pathway to prostanoids. The possibility that the AA response was a general fatty acid effect was ruled out since oleate ($C_{18:1}$) had no effect on the arteries. In addition, $PGF_{2\alpha}$ and PGE_2 also contracted these arteries. It is concluded from these studies that the uterine arterial wall from pregnant and nonpregnant rabbits utilizes the PG precursor, AA, for the production of prostanoid products by way of the cyclooxygenase pathway and these AA products have a net vasoconstricting effect in vitro. (Supported by NIH Grants HL07443 & HL24512).

10.8

ALTERATION IN THE ADRENERGIC INNERVATION OF THE UTERINE ARTERIES DURING PREGNANCY. Tom Tulenko and Donna M. Moisey.* The Med. Coll. of PA, Phila., PA 19129.

Many investigators have observed a decrease and/or complete loss of adrenergic innervation of the uterus and associated reproductive structures during pregnancy. The present study was designed to determine if the adrenergic innervation of the uterine artery is also altered in pregnancy. Strips of uterine artery (UA) and femoral artery (FA) from nonpregnant and full-term pregnant rabbits were isolated isometrically in physiological salt solution under their optimal resting tensions. Dose-response relations to angiotensin II (AII) ± cocaine (10^{-5}M) and tyramine were performed in all arteries and compared between the two groups. While cocaine pretreatment potentiated the AII response in UA from the nonpregnant group, it had no effect on the UA of the pregnant group. The AII response in FA was also significantly potentiated by cocaine in both groups. In addition, tyramine responses were significantly greater in UA of the nonpregnant compared to UA of the pregnant group, but were similar in FA in both groups. Pretreatment with phenoxybenzamine abolished the tyramine contractions in all arteries studied. Lastly, in separate experiments, UA from rabbits at mid-gestation (0.5 term) responded similarly to tyramine as those from the nonpregnant rabbits. Taken together, these results indicate that pregnancy in rabbits is associated with an adrenergic dysfunction which is specific to the arteries of the uterus, and furthermore, this alteration is confined to the second half of gestation. (Supported by NIH grants HL 07443, HL 24512 and RR05418).

10.9

MORPHOLOGICAL CHANGES IN VASCULATURE FOLLOWING ANGIOTENSIN II AND NOREPINEPHRINE PERFUSION. John C. Vitullo*, Ross G. Gerrity*, and Philip A. Khairallah, Cleveland Clinic, Cleveland, Ohio 44106.

Cross sections of Krebs' perfused rat mesenteric vasculature were examined histologically following the infusions of either angiotensin II (AII) or norepinephrine (NE) for 2 min at a low dose, which increased perfusion pressure 20-25 mmHg, or at a high dose, which increased pressure by 45-60 mmHg. Tissues were perfused fixed with 1% buffered glutaraldehyde at 80-100 mmHg. Microscopic examination revealed differential constrictor response among various vessel types in the mesenteric vasculature. The distributing arteries (180-200 μm) did not exhibit any morphological changes; however, the corresponding veins (300-450 μm) showed marked dose-dependent contractile responses to AII, but not to NE. Resistance vessels (60-80 μm) located mainly on the surface of the intestinal wall responded to all doses of AII and NE tested, while in the corresponding venules (90-100 μm), venoconstriction was observed in response to AII but not to NE. Electron micrographs depicting endothelial and smooth muscle contraction confirmed the constrictor responses. Reversal was seen to occur 5-10 min following termination of drug infusion.

10.10

INHIBITION OF EXOGENOUS NORADRENALINE (NA) MEDIATED CONTRACTION BY TRANSMURAL NERVE STIMULATION (TNS). M.P.J. Senaratne* and C.T. Kappagoda. Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta T6G 2G3

Although the effects of exogenous NA and TNS on vascular smooth muscle are established the interaction between the two has not been elucidated. This study was undertaken to determine the effects of TNS on the contraction brought about by exogenous NA in isolated canine saphenous veins (SV). Response to a single dose of NA was determined as a control. After washing, TNS was applied to produce a contraction of either 20-50% or 60-90% of the control value and the same dose of NA was applied against this background. Finally after washing, a control response with NA was repeated (78 observations in 16 veins). In a second series the response to transmural nerve stimulation was determined against a background of exogenous NA (71 observations in 12 veins). The response to a dose of exogenous NA was significantly less in the presence of background TNS ($P < 0.001$). However the response to TNS was not inhibited by a prior contraction caused by exogenous NA. The inhibition observed in the first series was absent when the experiment was repeated in the presence of Guanethidine (10^{-6} mol/l) but it was evident in the presence of Cocaine (10^{-5} mol/l). The inhibition was also not seen in the presence of Diltiazem hydrochloride in sufficient concentration (10^{-6}-10^{-5} mol/l) to block the effects of TNS only. These results indicate that the contraction produced by the NA released at adrenergic nerve endings inhibits the contraction induced by exogenous NA.

10.11

ISOSORBIDE DINITRATE INDUCED RELAXATION AND FORMATION OF cGMP IN CORONARY ARTERIAL SMOOTH MUSCLE. P. Galvas*, C. Westrich*, and J. DiSalvo. Dept. Physiology, U. Cincinnati, Col. of Medicine, Cincinnati, OH 45267

Although isosorbide dinitrate (ISDN) is widely used as a coronary vasodilator little is known of its mechanism of action. Because other nitrates increase tissue levels of cGMP it is thought that ISDN exerts its effects via cGMP. However temporal and dose relationships of ISDN among relaxation, and arterial cGMP are lacking. We measured isometric force, cGMP and cAMP during time and dose dependent ISDN-induced relaxation of bovine coronary arterial strips. Strips of circumflex coronary artery were contracted by addition of 30mM KCl (ED_{90}, bath conc.), stimulated with ISDN at varying doses (10^{-7}-$2x10^{-6}$M) and times (2-11 min) and frozen in liquid freon cooled by liquid N_2. The tissue was analyzed for cyclic nucleotides using an RIA acetylated protocol. cGMP increased to a maximum of 856±105% of control (KCl contracting) with no change in cAMP. The increase in cGMP was correlated to the dose of ISDN (r=.81 p<.001) and time during ISDN induced relaxation (r=.76 p<.001). Moreover, the addition of methylene blue, a purported inhibitor of guanylate cyclase, to the bathing medium significantly inhibited (p<.05) the relaxation (62±7 vs 97±2%) and increase in cGMP (.324±.058 vs .541±.045 pmol/mg protein) during ISDN stimulation. In addition, prolonged exposure (60') to ISDN resulted in a redevelopment of force (27±7 to 53±4%) with a concomitant decrease in cGMP (.695±.132 to .390±.080 pmol/mg protein). These findings suggest that the relaxation of coronary arterial strips in response to ISDN stimulation is causally linked to cGMP. Supported in part by grants from Ives Laboratories, NIH 20196 and 22619.

MECHANICS OF BREATHING: GENERAL

11.1

THE EFFECT OF MILD AIRWAYS OBSTRUCTION ON THE SITE OF ORIGIN OF LUNG SOUNDS. Steve S. Kraman. Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40511.

Normal lung sounds are thought to emanate from larger airways where turbulence is known to exist. In order to determine whether the site of lung sound generation changes in mild airways obstruction, a study was designed using a technique that yields a comparative estimate of the site of lung sound origin. 44 subjects without known lung disease (mean age 29 years) and with smoking histories of 0 to 65 pack years were studied by spirometry, He-air $\dot{V}/V$ loops and single breath N_2 washout. In addition, the degree of similarity between inspiratory lung sounds recorded by 2 microphones spaced 1 to 8 cm apart at the lung bases was determined by a flow-gated, automated sound subtraction technique. This sound subtraction intensity index (SII) was then plotted against the pulmonary function testing results and analyzed by linear regression.

The best correlations were between the SII at 2, 5 and 6 cm intermicrophone separation at the right lung base and the FEV_1/FVC, ($P < 0.01$), FEV_1 ($P < 0.01$), FEF_{25-75}, ($P < 0.01$), peak flow at 75% FVC ($P < 0.01$), and peak flow at 75% FVC ($P < 0.02$). These correlations suggest that the site of origin of the inspiratory vesicular sound moves toward the peripheral airways in mild airways obstruction. Interestingly (an unexplained), there was no significant correlation between any pulmonary function parameter and any lung sound parameter measured at the left lung base.

11.2

THREE DIMENSIONAL CONTOUR MAPPING OF LUNG SOUNDS IN NORMALS. R.A. Dosani* and S.S. Kraman. Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40511.

We have produced 3 dimensional contour maps of lung sound amplitude at the left and right posterolateral chest wall by means of a new technique (flow-corrected phonopneumography).

We studied 10 non-smokers (ages 24 to 48 years) with normal spirometry and no history of lung disease. The posterior chest wall was marked with an 18 x 18 cm grid consisting of 100 points. A microphone with a 14 mm chest piece was placed on each point and the sound of a single inspiration recorded while the subject breathed through a pneumotachograph. This was repeated for each of the 100 points on each side. The sound signals and pneumotachograph output were then processed to generate an airflow corrected lung sound amplitude index for each point. This information was then assembled into a contour map of lung sound intensity.

Analysis of these maps revealed:

1. Marked intersubject variation, some subjects having sounds 4 times as loud as others at comparable sites.
2. Frequent intrasubject variability in amplitude at adjacent locations.
3. Consistently decreased amplitude over the scapulae.

These results show in graphic form that the amplitude of the inspiratory lung sound is dependent not only on airflow (as shown in previous studies) but also on other undefined factors relating to sound production, transmission, or both.

11.3

EFFECTS OF TURBULENT AIRFLOW ON SPECTRAL CONTENT OF BREATH SOUNDS. W.J.Morgan*†, R.J.Lemen, W.Pfeiffer* and P.J.Arnott*. Dept. of Pediatrics and Physiology, University of Arizona, Tucson AZ 85724, and 3M Corporation, St. Paul MN 55101.

We determined the frequency content of tracheal and chest wall breath sounds in 5 normals and 10 subjects with cystic fibrosis ages 14-41. Airflow turbulence was varied by having each subject breathe air, 80% helium in oxygen, or 70% sulfahexafluoride in oxygen at 15,30,45, and 60 breaths/minute with a tidal volume of 25 ml/kg. Breath sounds were simultaneously recorded from the suprasternal notch and right lower posterior hemithorax using two acoustically matched microphones and stereophonic tape recorder (freq. response flat 0.05-10 kHz). The frequency spectra (FS) of 10 breaths were analyzed using a real-time spectral analyzer. From 100 to 600 Hz the FS of the tracheal sounds were flat under all conditions; however, FS of the chest wall breath sounds had a linear decrease in energy content (normals slope= -5.82±.02 DB/100 Hz, R=96, p<.001; CF slope= -5.67±.09 DB/100 Hz, R=97, p<.001. While the sound intensity increased significantly (p<.001) as turbulence increased, we failed to find any significant change in the slope of the frequency spectra. This suggests that the frequency content of breath sounds is independent of airflow turbulence. We speculate that frequency spectra of breath sounds reflect filtering characteristics of the lung and chest wall as opposed to sound production characteristics of the lung

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11.4

RESOLUTION OF ACTIVE AND PASSIVE COMPONENTS OF "EFFECTIVE" RESPIRATORY RESISTANCE" IN MAN. Arthur B. Otis and Cobern V. Peterson, Jr. University of Florida, College of Medicine, Gainesville, Florida. 32610

A subject with lung volume at FRC makes a gradually increasing inspiratory effort against a closed shutter located between the airway opening and an external resistance. The shutter opens abruptly, when a chosen pressure, P_o, has developed. Pressure at the airway opening, P_{ao}, and flow, $\dot{V}$, are continuously recorded for a second or two during the period of shutter opening. A set of six trials is made using the same P_o but a different external resistance in each trial. Values for P_{ao} are read at 0.03 sec following the shutter opening and plotted against simultaneous values for $\dot{V}$. A curve is fitted to the points. A second curve is constructed using points, (P'_{ao}, $\dot{V}'$) obtained from another set of trials using the same external resistances but with P'_o set at a value different from P_o. Respiratory muscle pressure, P_{mus}, is equal at any time to P_{ao} plus any pressure required to overcome inertance, elastance and resistance of the respiratory system. At 0.03 sec inertial and elastic forces are assumed to be negligible so $P_{mus}=P_{ao} + P_{res}$, and $P'_{mus} = P'_{ao} + P'_{res}$. Whenever $\dot{V} = \dot{V}'$, $P_{res}=P'_{res}$, and if it is assumed that $P'_{mus}/P_{mus} = P'_o/P_o$, it follows that $P_{mus} = (P_o-P'_{ao})/(1 - P'_o/P_o)$. P_o-P_{mus} = the active and $P_{mus}- P_{ao}$ = the passive components respectively of "effective respiratory resistance". (Supported by USPHS NIH Grants 23515 and 28263.

11.5

RESPIRATORY IMPEDANCE IN MAN DURING INDUCED BRONCHODILATATION AND INDUCED BRONCHOCONSTRICTION. J.C. Manço*, R.E. Hyatt and J.R. Rodarte. Mayo Clinic/Foundation, Rochester, MN 55905.

Total respiratory resistance (Rrs) and reactance (Xrs) from 3 to 30 Hz were determined by time series analysis in 12 normal subjects before and after bronchodilatation induced by aerosols of isoproterenol (I) and atropine (A) and bronchoconstriction produced by inhaled methacholine (M) and histamine (H). I resulted in a small decrease in Rrs ($cmH_2O/l/s$) at almost all frequencies: from 2.7 ± 0.8 (mean ± SD) to 2.3 ± 0.6 at 3 Hz and from 3.3 ± 0.8 to 3.0 ± 0.7 at 30 Hz ($p < .05$). Resonant frequency (fn) decreased from 7.18 to 6.51 ($p < .05$). A did not change fn and resulted in a small decrease in Rrs mainly in the higher frequencies: from 3.6 ± 0.7 to 3.2 ± 0.7 at 30 Hz ($p < .05$). M increased fn from 7.5 to 19.4 ($p < .01$) and increased Rrs mainly in the lower frequency range: from 3.0 ± 0.9 to 5.7 ± 1.9 at 3 Hz ($p < .01$) and from 3.5 ± 0.6 to 3.8 ± 0.7 at 30 Hz. H also increased fn from 7.2 to 18.6 ($p < .01$) and increased Rrs from 2.8 ± 0.9 to 5.2 ± 2.1 at 3 Hz ($p < .05$) and from 3.4 ± 0.9 to 3.6 ± 0.8 at 30 Hz. If frequency dependence of Rrs and changes in fn are considered indicators of changes in peripheral resistance, the data suggest that M and H have a pronounced peripheral effect and that I has more peripheral effect than A. (Supported by FAPESP, Brazil, Grant 80/657-4, and USPHS, NIH Grant HL-21584).

11.6

IN-VIVO MEASUREMENT OF SMALL AIRWAY DIMENSIONS ON INFLATION AND DEFLATION LIMBS OF THE P-V CURVE. W. Bennett*, W. Mitzner, P. Rosenthal*, & D. Swift*. The Johns Hopkins University, Baltimore, MD. 21205.

By measuring in dogs the recovery (RC) of a monodisperse aerosol as a function of breath holding (BH) time, we determined at a constant transpulmonary pressure the relative difference in small airway dimensions between lung volumes on inflation from FRC (V_{LI}) and on deflation from TLC (V_{LD}). The dog was ventilated with a dual pump respirator. A single tidal volume (V_T) of aerosol was introduced into the breathing cycle which was interrupted for varying lengths of time at end-inspiration. At the end of the breath hold a volume larger than V_T was withdrawn in order to collect all aerosol that had not deposited on airway walls. From continuous recordings of particle concentration with a light-scattering photometer at the mouth and flow from a pneumotachograph, fractional recovery (RC) of aerosol was calculated. We found the following: 1) at a given V_L and with increasing BH time, RC decreased at a rate inversely proportional to the mean radius of those small airways filled with aerosol; and 2) the rate of fall of RC with BH time was greater at V_{LD} than at V_{LI}. Thus, the mean radius of small airways filled with aerosol is larger at V_{LI} than V_{LD} despite the fact that V_{LD} is greater than V_{LI}. We conclude that the small airways or alveolar ducts are not expanding and contracting uniformly as the lung is inflated and deflated along the P-V curve.

11.7

UPPER AIRWAY MORPHOLOGY IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA (OSA). J.Rivlin*, WT McNicholas*, J. Kalbfleisch*, N. Zamel, V.Hoffstein*, AC Bryan. Depts of Pediatrics,Medicine and Orthodontic Surgery, University of Toronto,Toronto,Canada.

Previous reports have suggested that patients with OSA have narrowing of the upper airway during wakefulness, but have not identified a structural basis for this narrowing. In an attempt to find such a structural basis, we performed cephalometric x-rays and acoustic echography of the upper airway in 8 patients with documented OSA (Apnea index 6.7-116). Both procedures were done with the head in the neutral position. None of the patients had clinical pharyngeal abnormalities, one had a unilateral vocal cord paralysis. Cephalometric examination shows that the mandible of all patients is smaller and/or more posteriorly positioned relative to a normal population. The total posterior displacement of the mandible relative to both size and position was 6.1 mm ± 3.6. Maximum cross-sectional (X-SA) area of the pharynx by echography was less in patients with OSA (6.1 ± 3.1 cm^2) than in 6 normal controls (9.2 ± 1.8). Glottic aperture was also less in patients (0.93 ± 0.4 cm^2) than in normals (1.7 ± 0.3). Three patients had a pharyngeal X-SA of less than 50% of the normal mean values. Those patients with the most striking mandibular abnormalities had the smallest X-SA of the pharynx by echography, and also had the most severe degree of sleep apnea. The findings indicate that structural abnormalities may be found in many patients with OSA who have no obvious clinical abnormality of the upper airway. (Supp. MRC,CFF,OTS,Canada).

11.8

EFFECTS OF BRANCHING ANGLE ON OSCILLATORY RESISTANCES OF BIFURCATIONS. A.C. Jackson, M. Tabrizi* and D.L. Margolis* Primate Res. Ctr. Univ. of Calif., Davis, CA. 95616

Methods for predicting airway resistances typically compute impedances of individual tube elements, then combine these in the appropriate series - parallel network representative of the more complex airways structure. We recently reported that predicted resistances (R_p) were significantly smaller than measured resistances (R_m) in physical models of the airways (Physiol. 24:85, 1981). In the current study, we compared R_m (2-32 Hz) to R_p in single bifurcations with different branching angles (60^o, 90^o, 120^o, and 180^o) to ascertain whether or not these differences were due to the effects of bifurcations which are not included in theoretical models. In each of two networks studied (A and B), parent and daughter branches had the same diameter (D) and length (L); D = .91 cm and L = 10. cm, and D = .215 cm and L = 4.2 cm, respectively. In bifurcation A, R_m compared very closely to R_p at low flows (20 ml/sec, Re = 180) and was independent of branching angle. However at higher flows (100 ml/sec, Re = 900) R_m was significantly greater (10-100%) than R_p and was dependent upon branching angle. In bifurcation B, R_m at low flows (10 ml/sec, Re = 388) were nearly equal to R_p. At higher flows (20 ml/sec, Re = 775) R_m were again significantly larger than R_p and were branch angle dependent. These data support our earlier conclusion that the resistive properties of bifurcations contribute significantly to oscillatory resistances in the airways. Supported by NIH grants HL-26606, ES-00628 and EPA grant 807661.

11.9

FATIGABILITY OF THE CANINE DIAPHRAGM. Hylton Bark*, Rafi Levy* and Steven M. Scharf. Division of Lung Diseases, Soroka Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.

The ability of the diaphragm to maintain force over 15 minutes of supramaximal bilateral phrenic nerve stimulation was examined in 8 anesthetized dogs. Pacing parameters were: 24 trains per minute, 1 sec duration, frequencies of 20Hz, 50 Hz and 100Hz. We measured transdiaphragmatic pressure (Pdi) and the pressure-frequency curve (PFC) i.e. Pdi as a function of stimulus frequency under isovolumic conditions. At 20Hz there was no change in the Pdi or PFC. However, at 50Hz and 100Hz Pdi fell rapidly by 5 minutes to 87.8% ± 2.6% and 83.7% ± 4.2% respectively of control (p 0.025, p 0.01). By 15 minutes Pdi fell to 77.3% ± 2.7% and 78.2% ± 3.4% respectively (p 0.05, p 0.005). The PFC of the diaphragm showed significant and equal decreases at both the low (20Hz) and high (100 Hz) frequencies. When hypovolemic shock was induced by hemorrhage reducing cardiac output to 40% of the control, there was no significant change in Pdi, in the PFC or any decrease in the values produced by pacing. We conclude that the canine diaphragm can be made to fatigue by electrical stimulation of the phrenic nerves and that reduction in cardiac output does not increase the fatigability as judged by this method. (Supported by the Office of the Chief Scientist, Ministry of Health.)

11.10

EFFECT OF POSITIVE ABDOMINAL AND NEGATIVE PLEURAL PRESSURE ON DIAPHRAGMATIC BLOOD FLOW DURING INTERMITTENT CONTRACTION. B. Buchler*, S.A. Madger, and Ch. Roussos. Meakins Christie Labs., Royal Victoria Hosp., McGill Univ., Montreal, Quebec.

We investigated if diaphragmatic blood flow (Qdi) during intermittent contraction is affected by whether transdiaphragmatic pressure [Pdi = Abdominal Pressure (Pab) - Pleural Pressure (Ppl)] is generated with a high positive Pab or a large negative Ppl. Qdi was measured in mongrel dogs by the radioactive microsphere method. Intermittent contractions of equivalent Pdi's were produced at frequencies (f) between 10 and 90/min by phrenic nerve stimulation during (a) bound abdomen and open chest (+ve Pab), and (b) free abdomen, closed chest, and occluded airway (-ve Ppl). At any f, Qdi with -ve Ppl was higher than with +ve Pab ($p < 0.05$). Flows with -ve Ppl and +ve Pab were 2.18 ± 0.47 ml/min/gm (mean ± SD) and 0.797 ± 0.084 mls/min/gm respectively. Carotid blood pressure showed no consistent variation between -ve Ppl and +ve Pab, but cardiac output (measured by thermodilution) was higher with -ve Ppl ($p < 0.05$). With -ve Ppl, the diaphragm was flatter than with +ve Pab. Thus even though by Laplace's Law intramuscular diaphragmatic tension was greater with -ve Ppl than +ve Pab, Qdi was higher with -ve Ppl. We conclude that Qdi is enhanced with -ve Ppl and hindered by +ve Pab at a given Pdi; therefore diaphragmatic intramuscular tension is not the only factor affecting Qdi. (Supported by MRC of Canada.)

11.11

EFFECT OF IMMERSION ON CHEST WALL SHAPE AND INSPIRATORY MUSCLE ACTION IN SEATED SUBJECTS. Michael Reid*, Robert Banzett, and Jere Mead. Harvard School of Public Health, Boston, MA 02115

We studied 9 people (5 naive, 4 informed). Subjects were seated in a water-filled tank and instructed to maintain a constant posture while the water level was varied from hips to armpits. The subjects breathed through a mouthpiece and 440 ml dead space. We measured end-tidal P_{CO2} (P_{ETCO2}), tidal volume, dorso-ventral diameters of rib cage and abdomen, and surface electromyograms (EMG) of diaphragm and parasternal muscles. Immersion had no effect on respiratory frequency, tidal volume, or P_{ETCO2}. In each person, immersion progressively reduced abdominal diameter and increased rib cage diameter. Tidal excursions of the abdomen increased and rib cage excursions decreased in all subjects. EMGs of parasternal muscles (detectable in 9 persons) and diaphragm (detectable in 6 persons) progressively decreased with immersion. We conclude that immersion changes upright chest wall shape and increases the relative contribution of the diaphragm-abdomen pathway to ventilation. Immersion also decreases the inspiratory muscle activity required to generate a normal tidal volume. (supported by NIH grant #HL00943.)

11.12

EFFECT OF SUBMAXIMAL PARALYSIS ON CHEST WALL RELAXATION CURVES IN MAN. W. Kimball‡, S. Loring, S. Basta‡ A. deTroyer and J. Mead. Dept. of Anesthesia, Massachusetts General Hospital, Boston, MA 02114, & Dept. of Physiology, Harvard School of Public Health, Boston, MA 02115, & Meakins-Christie Laboratory, McGill University, Montreal, Canada

In order to test the hypothesis of deTroyer (JAP 47:1162) that tonic inspiratory muscle activity effects the chest wall (CW) relaxation characteristic we studied 4 highly trained relaxers using magnetometers for rib cage (RC)(AP x Lat) and abdomen (Abd)(AP x Xiphoid) and esophageal and gastric pressures, sitting 60° from horizontal. Paralysis was induced using Pavulon until RC excursion was decreased to 68% of control. Block was monitored by train-of-four twitch of Adductor Pollicis (Ali; Br. J. Anes. 47:570). FRC was measured using He dilution. During paralysis FRC points when compared to control moved in similar directions for both Konno-Mead and He dilution. (*p<.05) while Konno-Mead RC-equivalent FRC changed -66‡ -323‡ 130‡ and 42 ml. Shifts in blood volume into the thoracoabdominal cavity were determined from differences between changes in He dilution and Konno-Mead FRC as 439‡ -211, -309‡ and -434* ml. For 3 subjects both Konno-Mead and RC-Pes curves shifted in a parallel manner with changes in FRC. These changes appear to be due to blood volume shifts. We conclude that submaximal paralysis may shift blood volume which may be erroneously interpreted as changes in the chest wall relaxation characteristic. (Supported by HL-14580, 19170, and 00943.)

PHYSIOLOGICAL EFFECTS OF HYPERGRAVITY

12.1

ESTIMATION OF BODY SKELETAL MUSCLE MASS FROM BODY CREATINE CONTENT. Nello Pace and Donald F. Rahlmann. Environmental Physiology Laboratory, University of California, Berkeley CA 94720.

The body skeletal musculature is responsive to changes in loading, and numerous studies have been made of mass changes in individual muscles that are readily accessible. However, measurement of total body skeletal muscle mass by direct dissection is tedious and relatively inaccurate. It has been suggested by several investigators that since body creatine is contained largely within muscle cells, measurement of body creatine content might provide an indirect estimation of body muscle mass. The feasibility of this approach has been investigated by determination of creatine content of muscle tissue from 4 species of laboratory animals: hamster, rat, guinea pig and rabbit. Creatine content of fat-free striated muscle was found to range from 0.333% to 0.448% depending on the species, while it ranged from 0.067% to 0.075% of fat-free gastrointestinal tissue. In another series of animals, body creatine was found to be distributed about 6% in skin, 13% in viscera and 81% in skinned, eviscerated carcass. From these data it is possible to derive a factor for each species to convert body creatine content values to body skeletal muscle mass. Preliminary results indicate that grams muscle mass are approximated by multiplying grams body creatine by 243 for hamsters, 189 for rats, 198 for guinea pigs, and 181 for rabbits. Refined values will be presented. (Supported by NASA Grant NsG-7336).

12.2

ENHANCEMENT OF CHRONIC ACCELERATION TOLERANCE BY SELECTION. Arthur H. Smith. University of California, Davis, CA 95616.

Animals vary considerably in their tolerance to increased acceleration fields -- as they do to all other environmental extremes. When tolerance limits are exceeded the animals become stressed, exhibiting a frank sickness (chronic acceleration sickness). Some of the stressed animals will become physiologically adapted and tolerate the environment indefinitely and the others will die. If the survivors are reproduced, and their progeny exposed to the same treatment, a similar result obtains -- except that a greater part of the exposed group becomes physiologically adapted and survives. The mechanism is a phenotypic selection -- and the result is a change in the genetic composition of the group. In physiological terms -- the adaptates present in a group of animals become concentrated into individuals. This procedure has been applied to a strain of domestic fowl (SCWL), and the selection for acceleration tolerance (based largely on survival) has been continued for 23 generations. Changes other than acceleration tolerance are apparent in this selected strain, and these will be discussed. (Supported by grants from the Office of Naval Research and from the National Aeronautics and Space Administration.)

12.3

INCREASED GLUCONEOGENESIS IN HYPER-G STRESSED RATS. B.C. Daligcon* and J. Oyama. Ames Research Center NASA, Moffett Field, CA 94035

Male, Sprague-Dawley rats weighing 250-300 g were exposed to 3.1 G for periods ranging from 0.25-24 hr. Glucose, glucogenic substrates and hormones were determined in the blood of these rats and compared to noncentrifuged controls. Plasma glucose, insulin and glucagon were increased and glycerol was decreased at all exposure times studied. Blood lactate and plasma total catecholamines (NE+E) were increased at 0.25 and 0.50 hr; no significant differences were found with longer exposure times. In vivo incorporation rates of i.p. administered ^{14}C-(U)-labeled alanine, lactate and glycerol into plasma glucose and liver glycogen were determined in centrifuged and control rats. Rats centrifuged for 0.25, 0.50 and 1.0 hr showed higher incorporation rates (36% to 79% for plasma glucose and 186% to 513% for liver glycogen) than controls. Both the hyperglycemic and liver glycogenesis responses to hyper-G were blocked by 5-methoxyindole-2-carboxylic acid (MICA), a gluconeogenesis inhibitor. Based on these findings, it is concluded that the increase in blood glucose and liver glycogen in hyper-G stressed rats can be attributed to an increase in gluconeogenic activity.

12.4

EFFECTS OF RESTRAINT ON TWO MODES OF HEAT PRODUCTION OF COLD-EXPOSED RATS IN HYPERGRAVIC FIELDS. J. M. Horowitz, C. B. Monson* and B. A. Horwitz. Univ. of Calif., Davis CA 95616

This study was conducted to determine if restraint altered shivering and nonshivering thermogenesis (NST) in rats exposed to 3G fields and 5°C. Two groups of Long-Evans hooded male rats (425-500g) were maintained on a 12:12 light:dark cycle. One group (CA) was cold acclimated at 5°C for 6 wks at 1G so that when cold exposed at 1G, about 80% of the increased oxygen consumption would be due to NST and 20% to shivering. The second group (RT) was acclimated to 23°C so that when cold exposed at 1G, approximately 20% of the increased oxygen consumption would reflect NST and 80% shivering (Am. J. Physiol. 198: 471, 1960). Oxygen consumption was measured with closed-circuit procedures (J. Appl. Physiol. 30:50, 1971) adapted for an animal centrifuge. At 3G, restrained and unrestrained CA rats almost doubled their oxygen consumption when ambient temperature (T_a) was shifted from 24°C to 5°C. Similarly, oxygen consumption of the restrained RT rats also increased approximately two-fold. In contrast, the cold-exposed unrestrained RT rats at 3G only increased oxygen consumption by about 38%. Thus at 3G, restraint had no effect on the oxygen consumption of CA rats. However RT restrained rats consumed more oxygen than did unrestrained rats suggesting that the major mode of heat production in the RT rats, namely shivering, is suppressed in unrestrained animals. This suppression may reflect the posture assumed by the unrestrained rat in the 3G field. (Supported by NASA Grant NSG 2234).

12.5

PRIMATE THERMAL SENSITIVITY TO SHORT HYPERACCELERATION PROFILES. C.A. Fuller and B.A. Williams* Division of Biomedical Sciences, University of California, Riverside, California 92521 and Biosystems Division, NASA/AMES Research Center, Moffett Field, California 94035

We have previously shown that primates are sensitive to hyperacceleration fields. That is, when exposed to +2 G_z, the body temperature falls. This response was observed to occur within 10 minutes of the onset of acceleration. The purpose of this study was to examine the relative sensitivity of these animals to short hyperacceleration profiles which mimic the gravitational envelope seen on the space shuttle during launch (8 min, 2.9 G_z max) and reentry (19 min, 1.7 G_z max). Four mildly restrained squirrel monkeys, isolated from additional external thermal stimuli, were exposed to these profiles. The profile was initiated after a stable body temperature was observed for at least 30 minutes. During the launch profile simulation the temperatures never fell markedly below control levels. However, subsequent to return to 1 G the recovery phase showed decreases in body temperature in all four animals averaging 0.4°C over the next 10-15 minutes. The animals exposed to the reentry profile showed decreases in body temperature within three minutes of the onset of centrifugation. The maximum fall in body temperature was reached by the end of the centrifugation phase and averaged 0.7°C. It would thus appear that the temperature regulation system of this primate is sensitive even to short exposures to hyperacceleration fields. (This research has been supported in part by NASA Contract NAS2-10536 and NASA Grant NAGW-309).

12.6

ALTERED AUDITORY FUNCTION IN RATS EXPOSED TO HYPERGRAVIC FIELDS. T. A. Jones, L. Hoffman* and J. M. Horowitz. Dept. of Animal Physiology, University of California, Davis, CA 95616

Recent studies have suggested that central thermoregulatory mechanisms are impaired in rats during exposure to modest orthodynamic, hypergravic fields of +1.5G_x to +3G_x (J. Appl. Physiol.:Respirat. Environ. Exer. Physiol. 49:663-668, 1980 and 46(6):1049-1053, 1979). In the present study auditory brainstem responses were measured in rats to determine the field strengths required to alter brainstem auditory function. While thermoregulatory function is impaired at field strengths as low as 1.5G_x to 2G_x, results of the present study suggest that fields as high as +6G_x are required to produce even modest changes in auditory function. The differences in susceptibility of the two functional systems may reflect a) differences in regional tissue loading and/or the nature of the respective load-bearing structures, b) differences in the inherent susceptibility of particular neuronal assemblies to direct or indirect functional modification as might be envisioned to exist for different architectonic cellular groupings, or c) some combination fo these factors. (Supported in part by NASA grant NSG-2234 and by NASA NAGW-70.)

12.7

INFLUENCE OF ABDOMINAL RESTRICTION ON GAS EXCHANGE DURING +Gz STRESS IN DOGS. H.I. Modell, Virginia Mason Research Center, Seattle, WA 98101

It has been generally agreed that acceleration atelectasis occurs only when +Gz stress is accompanied by 100% oxygen breathing and use of a G-suit. Available data relating G-suit abdominal bladder inflation per se to pulmonary gas exchange during +Gz stress are conflicting. To reexamine this relationship, 7 spontaneously breathing, pentobarbital anesthetized, adult mongrel dogs were exposed to 60 seconds of up to +5Gz stress (onset rate=0.1 G/sec) with and without G-suit abdominal bladder inflation. Arterial and mixed venous blood were sampled for blood gas analysis during the first and last 20 sec of the exposure and at 3 min post-exposure. Little change in blood gas status was noted regardless of G-suit status at +3Gz and when the G-suit was not used at the higher Gz levels. However, with G-suit inflation, arterial Po_2 fell by a mean of 14.7 Torr during the first 20 sec at +4Gz ($P<.01$, t-test) and 20.6 Torr at +5Gz ($P<.01$). It continued to fall an additional 10 Torr during the next 40 sec at both +4 and +5Gz. Arterial Po_2 was still 5-10 Torr below control values ($P<.05$) 3 min post-exposure. G-suit inflation tended to restore cardiac output toward control values during +Gz stress, but it created increased venous admixture that persisted for as long as 3 min post G-stress. We attribute the detriment to airway closure in dependent lung regions rather than frank atelectasis. (Supported by AFOSR Contracts F49620-78-C-0058 & F49620-81-C-0055)

COMPARATIVE PHYSIOLOGY: RESPIRATION AND ACID-BASE I

13.1

RESPONSES OF ACID-BASE AND ELECTROLYTE BALANCE IN *AMBYSTOMA TIGRINUM* EXPOSED TO HYPERCAPNIA. D.F. Stiffler, B.L. Tufts* and D.P. Toews*. Acadia Univ., Wolfville, N.S., Canada BOP 1XO

Larval *Ambystoma tigrinum* were prepared by implanting a nonocclusive cannula into the truncus arteriosis of each. After 18-24 hr recovery the animals stabilized at P_{O_2}=26.6± 0.3 (SEM) torr; P_{CO_2}=7.9±0.1 torr, pH=7.833±0.023 and $[HCO_3^-]$ =15.1±0.2 mM. Exposure to 3% CO_2 (P_{CO_2}=21 torr) for 2 hr led to an increased P_{CO_2} of 25.2±0.8 torr and a decreased pH of 7.314±0.023. $[HCO_3^-]$ was 14.8±0.6 mM at this point. After 24 hr of hypercapnia blood P_{CO_2} was stable at 25.4±0.9 torr. The animals showed signs of partial compensation as pH rose to 7.485±0.027 and $[HCO_3^-]$ rose to 22.1±1.3 mM. Return to eucapnic water resulted in recovery to a normal pH of 7.841±0.033 within 12-24 hrs. Plasma electrolytes were also effected by hypercapnia. K^+ concentration increased from 2.80±0.03 to 3.50±0.14 while Cl^- concentration decreased from 89.4±1.6 to 83.2±2.6. Na^+ concentration remained constant at 105 mM. These results are consistent with the possibility of reciprocal influences between mechanisms involved in acid-base and ion transport physiology. (D.F. Stiffler was on Sabbatical leave from Calif. State Polytechnic Univ., Pomona; this work was supported by an NSERC Operating Grant to D.P. Toews).

13.2

DAY/NIGHT VARIATIONS IN BLOOD ACID-BASE BALANCE IN DESERT IGUANAS. Philip E. Bickler. Physiological Research Laboratory A-004, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

I measured P_{CO2}, pH, and P_{O2} of infraorbital sinus blood from desert iguanas (Dipsosaurus dorsalis) during mid normal photophase and scotophase (L:D 14:10). In lizards acclimated for 2 days at constant temperatures (T_b=25 and 37 °C), P_{CO2} averaged 6.8 torr greater (19.8 vs. 13.0), and pH 0.12 unit less (7.55 vs. 7.67) at night than day at 25 °C, and 3.0 torr greater (19.8 vs 22.8) and pH 0.09 unit less (7.49 vs. 7.40) at night than day at 37 °C (for each, n=7, p 0.05 t-test). P_{O2} was unchanged at about 43 torr at 25 °C and 56 torr at 37 °C, respectively. Blood pH changed by about -0.015 units/°C. Scotophase appears associated with respiratory acidosis (CO_2 retention), and changes in P_{CO2} appear to explain the changes in pH. Lizards allowed to behaviorally thermoregulate during photophase (heat lamp on 14:10 L:D cycle, T_b=38±2 °C) also showed retention of CO_2 during scotophase (T_b ca. 25 °C, P_{CO2} elevated 5 torr above expected). The CO_2 retention associated with scotophase, if reflected intracellularly, could have implications for the relative intensities of glycolysis and gluconeogenesis and night-time reductions in metabolic rate. Supported by NIH grant GM08639-01.

13.3

THE EFFECT OF TEMPERATURE ON INTRACELLULAR pH OF EEL HEPATOCYTES: AN IN VITRO TEST OF THE ALPHASTAT HYPOTHESIS. Patrick J. Walsh and Thomas W. Moon. Univ. of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

The effect of temperature on the intracellular pH (pHi) of hepatocytes isolated from the American eel (Anguilla rostrata) was studied by monitoring the distribution ratio of ^{14}C-5,5-dimethyloxazolidine-2,4-dione (DMO). In hepatocytes isolated from eels acclimated to 20°C, the pHi value measured at 20°C is 7.555 ± 0.010. At 5°C, the pHi value of cells isolated from 5°C-acclimated eels is 7.811 ± 0.042. These values, which display a $\Delta pH/\Delta T = -0.017$, are in close agreement with prior in vivo studies of eels, and are in accord with alphastat regulation. In experiments involving acute transfers of hepatocytes from 20°C to 5°C at constant medium pH (7.950), pHi increased to 7.821 ± 0.015, and this pHi adjustment was complete within 30 to 45 min post-transfer. Similarly, acute transfers from 5°C to 20°C resulted in a pHi characteristic of 20°C cells. These findings indicate that (1) liver cells can maintain appropriate pHi values independently of medium (or blood) pH, and (2) these adjustments take place more rapidly than in vivo changes in pH of the extracellular compartment which occur over a period of several hours. Such rapid, alphastat regulation would presumably lead to stabilization of protein structure and function.

13.4

INTRACELLULAR pH AND BLOOD ACID BASE STATUS AT SIMULATED HIGH ALTITUDE IN PIGEONS. Yitzhak Weinstein, Philip E. Bickler and Marvin H. Bernstein. Physiological Research Laboratory, A-004, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

We acutely exposed domestic pigeons (*Columba livia*, mean mass 0.44 kg) to simulated altitudes between sea level (SL) and 9 km above sea level (ASL) in a darkened hypobaric chamber at 25 ± 1°C. We measured body and ambient temperatures (Tb and Ta, respectively), heart rates (f_h), mixed venous blood gases ($P\bar{v}_{O_2}$ and $P\bar{v}_{CO_2}$) and $pH\bar{v}$, lactic acid concentrations (LA) and whole body intracellular pH (pHi) by the DMO method. Between SL and 9 km ASL f_h nearly doubled, Tb decreased but remained in the normothermic range; $P\bar{v}_{O_2}$ and $P\bar{v}_{CO_2}$ decreased threefold and LA increased from 1.06 to 7.16 mmol/L, $pH\bar{v}$ increased from 7.43 to 7.55 and pHi remained unchanged at 6.98. Over the same range of altitudes calculated extracellular bicarbonate concentrations (HCO_3) decreased 10.8 mmol/L, while intracellular HCO_3 decreased only 4.3 mmol/L. Close regulation of pHi may explain, in part, the ability of pigeons to tolerate extreme hypobaric hypoxia. (Supported by NIH Grant No. GM08639-01 and NSF Grant Nos. PCM 81-18956 and PCM 79-05052.

13.5

OXYGEN TRANSPORT IN ECTOTHERMIC VERTEBRATES: THEORY AND EXPERIMENTAL DATA. S.C. Wood and J.W. Hicks*. University of New Mexico, School of Medicine, Albuquerque, NM 87131.

A computer model was used to predict the effects of changes in O_2 affinity (via pH or temperature) on O_2 transport in ectothermic vertebrates. In many species, systemic arterial blood has a reduced O_2 saturation because of circulatory shunts. In theory, systemic arterial PO_2 becomes, after venous admixture, a function of O_2 content. Thus, for a given level of saturation, a right-shift of the oxygen dissociation curve (ODC) is predicted to increase arterial (and mixed venous) PO_2. When the right-shift of the ODC is temperature (or exercise) induced, the resulting increase in arterial and mixed venous PO_2 (if saturation is unchanged) could be adaptive to the increased tissue O_2 demand. The model predicts a limit to the increase in PO_2; i.e., when the ODC is right-shifted to the point where "loading" of O_2 occurs on the steep portion of the ODC. This point depends on lung PO_2 (= f (PCO_2, altitude) and on the magnitude of the temperature and Bohr coefficients. Experimental data supporting the model have been obtained in turtles (Pseudemys), snakes (Python), lizards (Varanus), salamanders (Ambystoma), and in mongrel dogs with artificial shunts. (Supported by NSF Grant No. PCM-77-24246).

13.6

SULFIDE BINDING AND TRANSPORT: A NEW ROLE FOR THE BLOOD OF THE VESTIMENTIFERAN TUBE WORM RIFTIA PACHYPTILA (JONES). Alissa J. Arp and James J. Childress. Univ. of Calif., Santa Barbara, Calif. 93106

The gas transport mechanisms of the blood of the deep-sea hydrothermal vent vestimentiferan tube worm Riftia pachyptila were examined in this study. Blood sampled immediately after recovery of the animals had high levels of sulfide (greater than 4 mM) and total carbonates (greater than 10 mM). In vitro experiments showed that the blood not only has a high capacity for sulfide (up to 10 mM) but that it binds sulfide with a high affinity and will concentrate it when dialyzed against a low sulfide medium. The abundant extracellular hemoglobin in the blood of Riftia pachyptila had a high affinity for O_2 and showed negligible effects of pH, CO_2 and temperature on the O_2 binding properties. Similarly the presence of high levels of sulfide gas (up to 5.3 mmHg) did not alter the O_2 binding characteristics. Sulfide binding by the blood suggests a transport mechanism for sulfide (as well as O_2 and CO_2) to internal chemoautotrophic bacterial symbionts. This sulfide binding may also act to protect sulfide sensitive tissues by binding of available sulfide within the animal. (Supported by NSF grant OCE80-24259 to J. J. Childress)

13.7

CO_2 SPECIFIC EFFECTS ON CRAB HEMOCYANIN OXYGEN AFFINITY. Louis E. Burnett, University of San Diego, CA 92110

CO_2 specific effects on hemocyanin oxygen affinity were assessed spectrophotometrically in buffered salines at a variety of pH's in crabs whose habitats range from subtidal to semi-terrestrial. The species chosen for the study were the relatively inactive Cancer anthonyi (15°C), the swimming crab Callinectes bellicosus (23°C), the intertidal Pachygrapsus crassipes (23°C), and the semiterrestrial ghost crab Ocypode occidentalis (20°). P_{CO_2} was varied from 1.5 to 7.5 torr in subtidal and intertidal species and from 3.5 to 14 torr in the ghost crab. The P_{CO_2}'s were chosen to represent pressures known to occur in vivo. CO_2 specific effects on oxygen affinity were not found in any of the species. Furthermore, the measured total CO_2 content of the buffered salines varied from 2 mM at low P_{CO_2} to 15 mM at high P_{CO_2}. This indicates that there is also no effect specifically attributable to bicarbonate ions. CO_2 specific effects were also absent from C. bellicosus hemocyanin incubated at 36°C, a common summer temperature in lagoons in the Gulf of California. (Supported by NSF Grant PRM-8108700.)

13.8

LACTATE PRODUCTION AND H^+ EXCRETION DURING EXERCISE IN *Callinectes sapidus*: IMPLICATIONS FOR O_2 TRANSPORT. C.E. Booth and B.R. McMahon, University of Calgary, Alberta, Canada T2N1N4

As previously reported (The Physiologist, 24:57, 1981), enforced swimming activity causes marked increases in hemolymph lactate (La^-) and PCO_2 and decreases in pH and HCO_3^- in seawater acclimated blue crabs. Although La^- increases throughout 1 hr of exercise, pH is maximally depressed within 15 min. An apparent discrepancy between the quantities of H^+ and La^- released from the tissues into the hemolymph is attributed to the excretion of large amounts of H^+ into the ambient water (or uptake of base from the water), although the mechanism is not known. Ammonia excretion increases 6 fold during exercise but the influence of this on acid-base balance is unclear. The excretion of a large portion of the hemolymph surplus H^+ load during sustained exercise has important consequences for O_2 transport: La^- increases hemocyanin O_2 affinity, and the progressive rise in La^- with no further change in pH opposes the decrease in hemocyanin O_2 affinity induced by a large Bohr shift. As a result, there is only a small rightward shift of the *in vivo* O_2 equilibrium curve, thus hemocyanin O_2 loading at the gills remains high and a venous O_2 reserve is maintained. The relationship between aerobic and anaerobic metabolism will also be discussed in terms of this species' excellent locomotor capabilities, which include both high speed "burst" swimming and long distance migrations.

13.9

BLOOD OXYGEN AFFINITY AND EQUILIBRIUM CURVE SHAPE IN EMBRYONIC AND ADULT CHICKENS. George N. Lapennas and Robert Blake Reeves. State Univ. of New York, Buffalo, NY, 14214

Oxygen equilibrium curves were recorded on blood from 4 to 18 day embryos at incubation temperature (38°C). Curves were run at the P_{CO2} measured in the air cell of each egg, which closely approximates that of arterialized blood in vivo, and therefore at in vivo arterial pH. Half saturation P_{O2}, P_{50}, increased from 36 torr at 4 days to 50 at 8 days, and then declined to 29 at 18 days. In 4-6 day blood, the Hill coefficient, n_H, increased from 1.5 at 10% saturation to a maximum of 6.5 at 85%S. After 6 days, n_H steadily increased below 30%S and decreased above that level, so that by 18 days n_H varied only from 2 at 10%S to 3.4 at 85%S. Blood of adult domestic chicken (white leghorn) and red jungle fowl (Gallus gallus, ancestor of domestic breeds) had P_{50} 47 torr (pH 7.5, 41°C, P_{CO2} 30 torr) and n_H 2 at 10%S and 4.15 at 80%S. Leghorn fixed acid and CO_2 Bohr coefficients were -0.51 and -0.54, with negligible saturation dependence between 15 and 95%S. Increasing embryonic blood oxygen affinity during the latter 2/3 of development favors oxygen loading at the falling P_{O2} that exists at the chorioallantoic surface due to increasing oxygen uptake through the fixed shell resistence. $n_H > 4$ in early embryo and adult blood presumably indicates polymerization of deoxyhemoglobin, such as occurs in sickle cell hemoglobin. Supported by NIH Grant PO1-HL-14414.

13.10

ERYTHROCYTE ORGANIC PHOSPHATE COMPOSITION AND HEMOGLOBIN FUNCTION IN MONOTREMES AND SOME MARSUPIALS. P.E. Isaacks, S. Nicol[1], J. Sallis[2] and H.D. Kim[3]. V.A. Medical Center, Miami, FL 33125; [1,2]Univ. Tasmania, Hobart, Tasmania 7001; [3]Univ. Ala. Med. Sch., Birmingham, Ala. 35294

Hematologic values, red cell organic phosphate composition, hemoglobin function, and hemoglobin composition have been determined on blood from the monotremes, the duckbill platypus and echidna, and three species of marsupials, the Tasmanian devil, wallaby, and brushtail possum. Blood from the platypus had a red cell count of 8.63 x 10^6/mm^3, a mean corpuscular volume of 49.1 μM^3, and a white cell count of 26.0 x 10^3/mm^3. The red cells from the monotremes and the three marsupials exhibited hemoglobin polymorphism, each with three hemoglobin components. Addition of ATP, 2,3-P_2-glycerate, or inositol-P_5 to phosphate-free hemoglobin from each species decreased hemoglobin oxygen affinity; the order of effect of these compounds was ATP < 2,3-P_2-glycerate < inositol-P_5. The erythrocytes of all species had concentrations of 2,3-P_2-glycerate ranging from 6.02 μmoles/ml rbc in the wallaby to 10.39 μmoles/ml rbc in the possum. The red cells from the three species of marsupials had concentrations of ATP ranging from 0.24 μmoles/ml rbc in the possum to 0.80 μmoles/ml rbc in the Tasmanian devil. The level of ATP in red cells of the platypus and echidna was 0.06 and 0.03 μmoles/ml rbc, respectively. (Supported in part by NSF Grant PCM 7815821)

REPRODUCTION

14.1

GOSSYPOL AND ENDOCRINE FUNCTION IN RATS. A. de Peyster* and H.H. Srebnik* (SPON: P.S. Timiras). University of California, Berkeley, CA 94720

Gossypol, a nonsteroidal phenolic compound extracted from cotton plants, was found by the Chinese to reversibly suppress spermatogenesis in man and several species of experimental animal. Changes in serum hormone levels were not observed in men taking gossypol for contraceptive purposes. While details of its antifertility mechanism are not yet completely understood, there is increasing evidence that gossypol does alter endocrine function in rats. Reduction of serum testosterone (T), accessory organ weight, and in some cases luteinizing hormone (LH) precede significant changes in sperm motility, count, and overall fertility, suggesting that steroidogenic processes may be initial targets of gossypol. Testosterone propionate administered concurrently with gossypol to castrate rats prevents decreases in accessory organ weight. Serum T and LH levels are restored to normal in intact rats receiving long-term gossypol treatment, indicating that steroidogenesis is not irreversibly suppressed. These observations elucidate gossypol's mode of antifertility action in rats, but they may also illustrate a difference in endocrine response to gossypol between rat and man.
(Supported in part by NIH Biomedical Research Support Grant 5-S07-RR-05441 to the School of Public Health, Berkeley.)

14.2

NATURAL AND HORMONE INDUCED CHANGES IN SKATE AND TURTLE OVIDUCTS. Ian P. Callard, John Laffan* and Thomas J. Koob*, Boston University and Harvard Med. Sch., Boston, MA.

In non-mammalian species, as in mammals, successful egg laying or parturition relies upon hormone-induced biochemical changes in the reproductive tract. Although a role for ovarian steroids is accepted, the physiological importance of the hormone relaxin in non-mammalian species is not understood. This insulin-like molecule has been demonstrated in shark ovarian tissue. We report here our recent studies of some natural changes in the mechanical and biochemical properties of the reptilian and elasmobranch oviduct, and some effects of porcine relaxin and porcine insulin on these parameters. In the turtle, "uterine", but not "cervical" extensibility increased after ovulation, and both "uterine" and "cervical" extensibility were significantly increased ($p < .001$) by injection of porcine insulin. In the skate, reproductive tract extensibility did not change between the resting and egg-laying states, but the reproductive tract showed marked increases in growth and natural circumference during egg-laying. Treatment of animals with estrogen and relaxin ($p < .001$) or insulin ($p < .05$) increased reproductive tract hydroxyproline content compared to animals treated with estrogen alone or controls. Since insulin and relaxin are postulated to have arisen from a common ancestral molecule, comparative studies of the effects of these hormones on the reproductive tract may be of significance in our understanding of hormone-hormone receptor evolution. (Supported by NSF PCM 78-08201 and NSF PCM 8104144).

14.3

IDENTIFICATION OF A COMPONENT OF RABBIT OVIDUCTAL FLUID WHICH CONTROLS HUMORAL IMMUNE CYTOTOXIC ACTIVITY. Gene Oliphant, Pat Ross*, Chris Cabot*, and Jane Svenson*. Dept. of Anatomy, Univ. of Virginia, Charlottesville, VA 22908.

Within the oviduct the maternal humoral immune system can react with the sperm on their way to fertilize the ovum and with preimplantation embryos which express paternal surface antigens at the two cell stage. The embryo or the sperm are destroyed by antibodies plus complement, thus control of humoral immune system in the oviduct is advantageous. This lab showed previously that no complement activity is present in oviductal fluid (OF). We now report an inhibitor to complement in OF. When OF was added to a standard hemolytic assay mixture containing 200 units of guinea pig complement the OF inhibited lysis by 33.7 ± 3.2%, 57.7 ± 3.1% and 75.6 ± 3.1% at concentrations of 8, 16 and 28% of OF, respectively. Likewise, OF inhibits complement-mediated sperm immobilization at as low as 10% OF. Heat treatment (100° C for 10 min.) of the OF prior to addition to the hemolytic assay resulted in the loss of an inhibition: 16.4% ± 2.9% vs. 71 ± 5.7%. Dialysis of the oviduct fluid did not remove inhibition. When OF was fractionated by Sephadex G-200 the inhibitory activity eluted at a molecular weight > 200,000. Purification by affinity chromatography suggests the inhibitor to complement mediated hemolytic activity is a sulfated glycoprotein produced in the oviductal epithelium. This may provide protection from humoral immune attack to the sperm and developing embryo while they are in the oviduct. (Supported by NIH Grant HD06573)

14.4

CHARACTERIZATION OF CONTRACTILE RESPONSES OF AMPHIBIAN WOLFFIAN DUCT TO ACETYLCHOLINE, NOREPINEPHRINE AND NEUROHYPOPHYSIAL PEPTIDES IN VITRO. R. Thomas Zoeller*and Frank L. Moore*(SPON: F.P. Conte). Oregon State Univ., Corvallis, OR 97331.

During courtship, male rough-skinned newts clasp a female for several hours before depositing a spermatophore. We undertook this study to determine if Taricha wolffian ducts contract in response to various factors known to influence mammalian vas deferens contractions. Using Gould-Statham UC2 transducing cells, we determined the minimum effective dose of norepinephrine (NE) and acetylcholine (ACh) to be 1 x 10^{-5}M and 3.2 x 10^{-8}M, respectively. The ED_{50} for NE and ACh is 1.47 x 10^{-5}M and 4.17 x 10^{-5}M, respectively. The contractile response to NE is enhanced by a beta-adrenoreceptor blocker and is attenuated by alpha-adrenoreceptor blockers. Arginine vasotocin (AVT) is significantly more effective in stimulating contractions than oxytocin. The effectiveness of lysine vasopressin is intermediate. These results demonstrate that wolffian ducts of an amphibian are capable of contracting in response to neurotransmitters and neuropeptides. In addition, because amphibian wolffian ducts are not innervated by visceral motor nerves, a humoral factor such as AVT may play a role in the temporal coordination of courtship behavior and spermatophore deposition: previous studies demonstrated that AVT can stimulate clasping behavior in male newts; the present experiments show that AVT can stimulate wolffian duct contractions.

14.5

LUTEAL LH RECEPTOR DURING DIFFERENT PHASES OF THE MARE'S ESTROUS CYCLE. **J.F. Roser* and J.W. Evans. University of California, San Francisco, San Francisco, CA 94143**

Changes in serum LH and progesterone concentrations, luteal unoccupied LH receptor numbers and affinities, luteal weights and progesterone concentrations were determined during the post-ovulatory periods in the mare. The number of unoccupied LH receptors and receptor affinity was less during the early (days 1-4) and late (day 15 through 3rd day after corpus luteum regression) luteal phases than during the mid-luteal (days 9-14) phase of the postovulatory period ($P < .01$). The number of LH receptors per corpus luteum increased 21-fold ($P < .001$) from day 1 to day 14. Receptor affinity increased 5-fold ($P < .001$) from day 1 to day 13. Receptor number was highly correlated with receptor affinity ($P < .01$) and both were highly correlated with serum and luteal progesterone ($P < .01$). During regression of the corpus luteum the number of LH receptors and receptor affinity decreased concomitantly with serum and luteal progesterone. Morphologically, luteal cell development and degeneration correlated with the changes in receptor number, affinity and luteal and serum progesterone concentrations.

14.6

LOW DOSES OF CHORIONIC GONADOTROPIN PREVENT ESTRADIOL-INDUCED LUTEOLYSIS IN CYNOMOLGUS MACAQUES. Pamela K. Westfahl* and John A. Resko. Oregon Health Sciences University, Portland, 97210 and Oregon Primate Research Center, Beaverton, 97006.

Estradiol (E_2) induces premature luteolysis in primates. This effect could be mediated via reduced gonadotropic support to the corpus luteum (CL). We studied this possibility by treating cycling monkeys with (a) E_2 alone (Group I), n=4 or (b) E_2 plus human chorionic gonadotropin (hCG, Group II), n=6. We collected blood samples once daily from day 7 of the cycle until the next onset of menses and measured E_2 and progesterone (P_4) in the serum by radioimmunoassay. Silastic capsules filled with E_2 were implanted 3-5 days after the preovulatory E_2 surge. The hCG (7.5, 10, or 15 IU) was injected daily for 10 days beginning the day capsules were implanted. The E_2 levels in Group I and Group II were 284 ± 11 pg/ml and 261 ± 22 pg/ml (mean, SE), respectively. Luteal phase length in Group I was significantly shorter (11.0 ± 0.8 days) than that in Group II (15.3 ± 0.5 days), which was not significantly different from that in untreated animals (16.6 ± 0.7 days, n=5). Serum P_4 levels in Group I were significantly below those measured in Group II. Serum P_4 in the latter group was not significantly different from that measured during untreated cycles. Therefore, we conclude that the luteolytic effect of E_2 is prevented by concurrent administration of hCG. These data suggest that E_2 induces luteolysis by reducing gonadotropic support to the CL. (Supported by NIH grants HD-06124, P50 HD-1198)

14.7

THE INFLUENCE OF NAPHTHALENE ON IN VITRO STEROIDOGENESIS BY OVARIAN TISSUE OF THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. Lester L. Rolf, Jr. and Gladys E. Pereles*. Physiological Sciences, Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078

Fish harvested in January were weighed to the nearest tenth gram and injected (i.p.) with corn oil or 100 mg/kg naphthalene (N) in corn oil. Forty-eight hrs later a 1 ml blood sample was obtained and the animals sacrificed. Ovarian tissue was excised in toto, weighed and homogenized in ice-cold Krebs-Ringer buffer, pH 7.42. Plasma cortisol levels and the production of estradiol (E2), estriol (E3) and testosterone (T) from progesterone (P) and androstenedione (A) were evaluated by radioimmunoassay. There was no significant difference between treated and control animals with respect to plasma cortisol (41.1 ± 10; 35.7 ± 10 ng/ml), ovarian protein content (3.81 ± 1.2; 3.71 ± 1.1 mg/mg ovary) and gonadosomatic index (0.002 ± .004; 0.0019 ± .004). Naphthalene treatment increased ($0.025 < P < 0.05$) the production of E2, E3 and T from P when compared to A. In control animals equal amounts of E2, E3 and T were produced from P and A. This acute exposure of fish to N apparently caused a bypass of the normal steroidogenic transformation of P→A→T→E2→E3. The data suggest that chronic exposure from polluted waters may have an important impact on reproductive cycling in fish. (Supported in part by a Presidential Challenge Grant, Oklahoma State University.)

14.8

PROGESTERONE MEDIATED INHIBITION OF NUCLEAR ESTROGEN RECEPTOR BINDING IN THE BEAGLE UTERUS. Thomas A. Gorell and Bruce A. Lessey*. Colorado State University, Fort Collins, CO 80523.

Progesterone (P_4) mediated antagonism of estradiol-17β (E_2) action within uterine cells may begin with an initial reduction of nuclear estrogen receptor (NER) binding capability. We have partially characterized the uterine NER from E_2-primed beagles utilizing a validated nuclear exchange assay with ^3H-E_2 as the ligand. Beagle uterine NER binding was specific for estrogenic compounds, was saturable at ligand concentrations of 20-30 nM, and exhibited a high affinity (K_d of 3 nM). These characteristics are similar to those we have reported for the beagle uterine cytoplasmic estrogen receptor. A portion of the ^3H-E_2 specific nuclear binding (20-30%) was resistant to extraction with increasing concentrations of KCl (0.1-0.5 M) and possessed a similarly low K_d to that obtained for E_2 binding from non-extracted nuclei. Total uterine NER binding was sharply reduced (53%) in E_2-primed beagles treated with P_4 for 12 h prior to an additional pulse of E_2 (to enhance nuclear occupation) when compared to saline treated controls. Salt-resistant uterine NER binding was correspondingly reduced (54%) in P_4 treated animals. While both total and salt-resistant NER binding were reduced similarly and significantly ($p<0.05$) with P_4 treatment, the ratio between them remained the same regardless of treatment. Nuclear occupation by estrogen receptors appears sensitive to P_4 and reduced NER binding may ultimately affect E_2 promoted growth and differentiation of the uterus. (Supported by NIH Grants HD-10613 and HD-07031).

14.9

PLASMA LEVELS OF SEX STEROID-BINDING PROTEIN INCREASE DURING SEXUAL MATURITY IN THE BAT *MYOTIS LUCIFUGUS LUCIFUGUS*. D.A. Damassa*, A.W. Gustafson and G.C. Chari*. Tufts Univ. Schools of Medicine, Boston, MA 02111

Sex steroid-binding protein (SBP) concentrations in plasma of immature male little brown bats (*Myotis lucifugus lucifugus*) were found to be low (15±2.8 nM; $\bar{x}$±SE) and stable throughout the period of hibernation which immediately precedes the onset of the first spermatogenic cycle (sexual maturity). In contrast, plasma SBP titers in spermatogenically active adults (summer) were markedly elevated (238±26 nM). When immature males were removed from hibernation and maintained on a long photoperiod at constant temperature (16L:8D; 25°C), the onset of sexual maturity was initiated in these animals and they exhibited a rapid rise in circulating SBP. A significant increase in plasma SBP occurred by 2 weeks, and by 3 weeks SBP values (234±20 nM) were indistinguishable from those measured in spermatogenically active adults. These high plasma SBP titers at 3 weeks were accompanied by increases in testicular and epididymal weights and the onset of spermatogenesis. However, no stimulation of the sex accessory glands was observed, possibly reflecting the effects of increased plasma SBP on the biological activity of circulating androgens. The ability of immature male *Myotis* to exhibit a pubertal increase in plasma SBP following simple environmental manipulation should provide a valuable model for the study of the control and action of sex steroid-binding protein. (Supported in part by the USPHS, HHS Grant No. HD14356.)

14.10

THE EFFECTS OF QUINACRINE ON FERTILIZATION. J.E. Ferguson* and S.S. Shen. Iowa State University, Ames, IA 50011

Phospholipase A2 may be necessary for the early events during fertilization. The effects of quinacrine (QA), a phospholipase inhibitor, on fertilization of the sea urchin, *Lytechinus pictus*, were examined. In a dose dependent manner, QA blocked the cortical reaction following insemination or A23187-activation. Since increasing the concentration of QA caused an increase in the degree of polyspermy, the effect of QA on the electrical properties of the egg membrane was examined. Exposure of eggs to a level of QA that would block the cortical reaction caused a transient depolarization of the membrane. This depolarization was Na-dependent and coupled with a decrease in membrane resistance. Insemination of these QA-treated eggs did not trigger a fertilization potential; however, numerous 2 to 5 mV depolarization steps were observed. In contrast, a fertilization potential was recorded upon insemination of eggs treated with a level of QA that would not inhibit the cortical reaction. Thus, QA is capable of inhibiting both the electrical and morphological blocks to polyspermy. The effect of QA on another membrane associated event, the acid release during fertilization, was examined with a pH stat. In a 1% egg suspension 100 μM QA completely blocked acid release. Decreasing concentrations of QA resulted in increasing release of acid, such that, at 10 μM QA, acid release was near normal. These preliminary experiments suggest that phospholipase A2 may regulate the cortical reaction and cause a reorganization of the membrane, which leads to the release of acid. (Supported by NSF)

14.11

EFFECT OF FOOD ON REPRODUCTIVE DEVELOPMENT IN MALE GROUND SQUIRRELS. Robert E. Liddle* and Alan R. French. Univ. of California, Riverside, CA 92521

The reproductive development of fed and unfed male Spermophilus beldingi was monitored for 30 days following termination of hibernation. In adult-sized (>400g) animals, testis weight was near maximal at the end of dormancy and changed little thereafter. Few spermatozoa were present in the epididymis after 11 days of homeothermy, but by 20 days they were abundant. Feeding had no effect on these organs. In contrast, the seminal vesicles and prostate gland were undeveloped at the end of dormancy, but showed a large (4X fed, 6.5X unfed) and progressive increase in weight during the 30 day post-hibernation period. Juvenile males (<300g), when unfed, did not stop hibernating and they maintained reproductive organs one-third to one-half the size of those in adults that just ended hibernation. However, fed juveniles terminated hibernation and their reproductive organs grew rapidly to adult size. Once an individual commits itself to becoming reproductively active, there is a constant mass-specific rate of increase in the weight of the accessory organs. This rate (9.4 mg% day $^{-1}$) is the same whether the animal is limited to internal lipid stores as a source of energy and thus undergoes a continual decrease in body mass, or has access to food resources and increases its body mass. (Supported by NSF Grant DEB 8003513)

GASTROINTESTINAL EXOCRINE AND ENDOCRINE SECRETION

15.1

THE EFFECT OF PROSTAGLANDIN SYNTHETASE INHIBITION ON GLUCAGON STIMULATED BILE FLOW IN DOGS. D.L. Kaminski, M.D. and Y.G Deshpande, M.S.*. St. Louis Univ. Med. Ctr., St. Louis, MO 63104

Both glucagon and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have previously been shown to stimulate a chloride rich choleresis in dogs. The present investigation evaluates the effects of glucagon on bile $PGF_{2\alpha}$ secretion and determines the effect of prostaglandin synthetase inhibition on glucagon choleresis. The experiments were performed on dogs with chronic biliary and gastric fistulas. Bile volume, and bile [^{14}C] erythritol clearance, bile chloride concentration and bile and systemic plasma $PGF_{2\alpha}$ concentrations and outputs were measured. Bile $PGF_{2\alpha}$ secretion increased from 80±12 pg/min during bile salt infusion alone to 1363±225 pg/min during the adminstration of 4 $\mu gkg^{-1}hr^{-1}$ glucagon. The prostaglandin synthetase inhibitor, indomethacin, significantly decreased bile flow stimulated by glucagon and inhibited the increased chloride secretion produced by glucagon. This was associated with the complete inhibition of the increased bile $PGF_{2\alpha}$ secretion produced by glucagon. Plasma $PGF_{2\alpha}$ levels were not significantly different from control values during glucagon administration or during glucagon and indomethacin administration. Indomethacin did not inhibit the [^{14}C] erythritol clearance. Inhibition of glucagon choleresis by indomethacin is compatible with the hypothesis that $PGF_{2\alpha}$ partially mediates the response of the hormone associated with increased chloride secretion and that this process is not occurring at the canalicular level. Supported by USPHS grants AM 27695 and AM 28178.

15.2

POLYAMINES PROTECT THE GASTRIC MUCOSA AGAINST ASPIRIN-INDUCED DAMAGE. J.W. Quigley*, M.R. Feller*, D.P. Moorhead*, and E.M. Brashear* (SPON: R.J. Solaro). The Procter & Gamble Company, Cincinnati, Ohio 45247

Spermine and spermidine have been reported to inhibit histamine-stimulated acid secretion in bullfrog gastric mucosa in vitro. The objectives of this study were to determine if polyamines protect the rat gastric mucosa from ulcerogenic compounds such as aspirin (ASA) and if their anti-secretory properties play a major role in this activity. Gastric damage was induced by oral administration of ASA suspensions in fasted male Sprague-Dawley rats. Oral (p.o.) administrations of spermine or spermidine given concurrently with ASA produced significant, dose-dependent gastric protection. Subcutaneous (s.c.) or intraperitoneal (i.p.) administrations of these polyamines given prior to ASA resulted in gastroprotection three times more potent than p.o. administrations of equal doses. The polyamine precurser, putrescine, did not protect the gastric mucosa when administered i.p. or p.o. Using the pyloric-ligated rat model we found that spermine but not putrescine decreased basal acid secretion. We conclude that a) spermine and spermidine have gastroprotective activity against ASA damage; b) this effect appears to be systemic, and c) inhibition of acid secretion probably mediates this gastroprotective effect.

15.3

POSSIBLE IDENTIFICATION OF THE MEDIATOR OF GI HYPERPLASIA AND HYPERGESTRINEMIA ASSOCIATED WITH TAENIA TAENIAEFORMIS (TTF) INFECTION. Lenard M. Lichtenberger and Lynne A. Graziani.* Univ. Texas Medical School, Houston, TX 77025

TTF is a parasite which encystes in the liver of rats and induces a marked 5-30 fold enhancement of GI growth and serum gastrin (SG) levels. This GI growth response is not diminished by antrectomy suggesting that gastrin is not the mediator. Two of the excretory products of TTF are volatile amines (VA) and ammonia (NH_3), compounds which are highly potent stimulants of gastrin release (Fed Proc 41:1701, 1982). We measured the concentration of NH_3 and VA in extracellular fluids of infected rats whose SG levels and stomach wts were grossly enhanced 5 and 24 fold above control levels respectively. NH_3 was determined by the glutamate dehydrogenase assay whereas VAs were determined by the difference in primary amine concentration, before and after exhaustive lyophilization in base. The NH_3 conc (μ moles/L) of control and infected rats are shown below. * = $p<0.05$ ( ) = rats/grp

	Blood	Bile	Gastric Juice
Control	25±14 (6)	68±23 (6)	77±10 (3)
Parasitized	82±12*(6)	54±13 (6)	1210±165*(4)

Similarly, VAs were elevated in the blood and gastric juice, 2.6 and 7.6 fold respectively after TTF infection. Conclusion: The dramatic alterations in GI function and structure associated with TTF may be induced by the elevations in NH_3 and VA within both the gastric juice and blood. (Supported by NIH Grants AM 20686 and AM 00842)

15.4

PANCREATIC ENZYME SECRETION AND MUSCARINIC RECEPTORS ARE MODULATED BY FASTING. L. Larose*, G.G. Poirier* and J. Morisset. Centre de recherche sur les mécanismes de sécrétion, Univ. Sherbrooke, Sherbrooke, Qué., Canada J1K 2R1.

This study examines the influence of fasting and refeeding on pancreatic enzyme secretion and on muscarinic receptors. Male Sprague-Dawley rats weighing 210-215 g at the beginning of the experiment were used. The following groups of animals were studied: chow fed control, 2 and 4-day fasted, and 4-day fasted refed for 2 days. For amylase secretion, acini were prepared and dose-response curves to carbachol 10^{-8} to 10^{-3}M were performed. The properties of the muscarinic receptors were studied on the homogenate with [^{3}H] QNB as ligand. The ED_{50} of the amylase dose-response curve to carbachol was significantly shifted from 4.8×10^{-7} (control) to 1.1×10^{-6}M after 4 days of fasting and returned to control values upon 2 days of refeeding. [^{3}H] QNB binding reveals a significant increase in Bmax from 1004 (fed) to 2248 and 1663 (2 and 4 days fasting) and 1490 (2 days refeeding) fmol/mg DNA. No significant difference was observed in K_D of [^{3}H] QNB between the 4 groups. In conclusion fasting is associated with a decreased sensitivity of the secretory response to carbachol but an increase of receptor number. Carbachol competition curves against [^{3}H] QNB will clarify which class of receptor has been modified. (Supported by MRC Grant MA-7320).

15.5

METABOLIC ENERGY SOURCES FOR ACID SECRETION IN THE HUMAN GASTRIC MUCOSA IN VITRO. J. Chacín, A. Prieto*, P.S. Cárdenas* and A. Puenmayor F.* Fac. Med., Univ. Zulia, and Dept. Gastroenterology, Hosp. General del Sur, Maracaibo, Venezuela.

The substrate-level energy dependence of acid secretion was studied in the human gastric mucosa using biopsies obtained during routine fiberoptic gastroscopies in symptomatic patients. Oxygen uptake (QO_2) and ^{14}C-aminopyrine accumulation (AP ratio) were used as indexes of secretory responsiveness. Under substrate-deplete conditions, QO_2 and the AP ratio of fundic mucosa were not significantly increased by gastric secretagogues. In the presence of 1mM glucose+1mM pyruvate+1mM butyrate QO_2 was significantly stimulated by different secretagogues: +1.67±0.15(6) µL/hr/mg.d.wt. for 0.1mM histamine, +1.53±0.07 (4) for 10mM theophylline, +1.38±0.31(3) for 0.01mM pentagastrin, and +1.36±0.40(3) for 0.1mM dimaprit. The ability of various substrates to support acid formation in the presence of 0.1mM histamine was evaluated. Respiration and AP ratio were significantly higher with glucose compared to fatty acids and no substrate. Pyruvate was less effective than glucose (glucose> pyruvate>butyrate). QO_2 values and AP ratios of antral mucosa were much lower than those of fundic mucosa and not affected by histamine. The results suggest: 1)the acid secretory response to histamine in the human gastric mucosa in vitro is absolutely dependent on a source of metabolic substrate; 2) carbohydrates seem to be the preferential substrates that support acid secretion. (Supported by CONDES of L.U.Z.).

15.6

COMBINED STIMULATION OF PEPSINOGEN SECRETION FROM ISOLATED GASTRIC GLANDS BY CHOLINERGIC AGENTS AND AGENTS THAT INCREASE CYCLIC AMP LEVELS. David E. Schafer. VA Medical Center, West Haven, CT 06516 and Physiology Department, Yale University School of Medicine, New Haven, CT 06511

We previously reported that the cholinesterase inhibitor physostigmine (PHY) and the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) synergistically stimulate pepsinogen secretion by strips of isolated gastric mucosa (Fed. Proc. 38: 885, 1979) and that pepsinogen secretion (PS) in isolated gastric glands is stimulated by acetylcholine (ACH) in the presence of 3×10^{-4} M PHY (Physiologist 23: 66, 1980) and by cholera toxin (CT), an indiscriminate activator of adenylate cyclase (Fed. Proc. 41: 1432, 1982). We have now examined the combined effects of IBMX or CT and of ACH + PHY on PS by isolated glands. In the absence of IBMX, the maximum stimulation over a 10-min interval with ACH (+ 3×10^{-4} M PHY) occurred at 10^{-5} M ACH. Stimulation by 10^{-4} M IBMX was approximately the same. Stimulation by the combination of these agents was at least twice the amount obtained with either alone. Similarly, when glands were incubated for 90 min in the presence and absence of CT, 15 µg/ml, and of ACH, 10^{-6} or 10^{-5} M (+ PHY), stimulation by the combination of CT and ACH was approximately the sum of stimulations by the two agents separately. We infer that the stimulation of PS by a combination of cholinergic agents and agents that increase cyclic AMP levels can be at least equal to the sum of the stimulations by either type of agent alone.

15.7

MECHANISM OF STIMULATION OF GASTRIC ACID SECRETION BY Ca^{++} IONOPHORE A23187. C. Jirón, M.C. Ruiz and F. Michelangeli. IVIC, Apdo. 1827, Caracas 1010A and Universidad de Carabobo, Maracay, Venezuela.

We have shown that A23187 is able to stimulate H^+ transport in toad gastric mucosa (Cell Calcium 2, 573, 1981). The mechanism of cytosolic Ca^{++} increase was investigated in isolated gastric mucosae of Bufo marinus mounted in Ussing chambers. Stimulation by A23187 was higher when added to mucosal side (with 0 Ca^{++}) and even higher in the presence of EGTA. This was due to better incorporation of A23187 as could be shown by measuring incorporation into membranes by fluorometry. Then, Ca^{++} had to enter the cell through the basolateral membrane or be released from intracellular stores or both. Mucosal A23187 did not stimulate in the absence of serosal Ca^{++}. When Ca^{++} was readded, full stimulation was achieved. Fluorescence microscopy showed that A23187 (added to lumen) incorporates into basolateral membranes and intracellular organelles, probably mitochondria. Reflectance fluorometry of mucosae incubated with chlorotetracycline, used to study intracellular Ca^{++} redistribution, indicated Ca^{++} release from cellular stores. Electron microscopy showed dissapearance of mitochondrial dense granules which probably are Ca^{++} deposits. Results indicate that A23187 incorporates into plasma membrane and organelles. Ca^{++} enters the cell through serosal membrane and is also released from stores. Ca^{++} release may require extracellular Ca^{++}.

15.8

STUDIES ON SOURCE OF SPD (SUDDEN POTENTIAL DROP) IN FROG GASTRIC MUCOSA. William Silen*, Susumu Ito, and Jeffrey Matthews. Harvard Medical School, Beth Israel Hospital, Boston, MA 02215.

Kidder has reported that an SPD and concomitant sudden drop in resistance (SR) is regularly observed in frog mucosa in the presence of anoxia, serosal pH <7.1, Cl^- bathing solutions, and a PD which changes across the region of 10mV (AJP 230:61-66, 1976). We have found in the post-SPD state that when luminal [Cl^-] is changed from 90 mM to ∿ 0 mM, or vice versa, $\Delta PD = 22.6 \pm 1.7mV$, whereas for similar Δ's in luminal [Cl^-], $\Delta PD = 8.1 \pm 0.6mV$ for control conditions and Δ PD = $12.9 \pm 1.4mV$ during anoxia with nutrient pH > 7.1. Changes in PD were negligible after Δ's in luminal [Na^+] or [K^+] in the post-SPD state, whereas there was a small but definite increased response to changes in luminal [HCO_3^-]. Nutrient Cl^- and a luminal [Cl^-] of > 40 mM are necessary for SPD and SR. Neither DIDS nor 16-16 dm PGE_2 in either the nutrient or luminal solutions prevented an SPD. An SPD and SR is observed reliably in the absence of anoxia if H^+ secretion is inhibited with metiamide or SCN^- when nutrient pH is < 7.0. Influx of Cl_{36} into the mucosa from the luminal solution is increased in the post-SPD state. No consistent morphologic changes are associated with the SPD. We conclude that the SPD is produced by a Cl^- shunt from lumen to cell through the apical membrane.

Supported by NIH Grant No. AM15681.

15.9

EFFECT OF GASTRIN RELEASING PEPTIDE ON THE RELEASE OF GASTROINTESTINAL HORMONES IN DOGS. K. Inoue*, D. Mckay, H. Yajima† and P. L. Rayford. Dept. of Physiology-Biophysics, Univ. Ark. Med. Sci., Little Rock, AR 72205, †Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

The molecular configuration of gastrin releasing peptide (GRP) is similar to bombesin, a known stimulus of cholecystokinin (CCK), pancreatic polypeptide (PP) and gastrin. The purpose of this study is to determine if GRP stimulates the release of these peptides. Methods: Six dogs were infused with 0.01 and 0.1 µg/kg-hr synthetic porcine GRP; each infusion was for 45 min. Blood samples were collected for measurement of CCK, PP and gastrin (pmol/L). Results:

Time(min)	Basal	15	30	45	I
		GRP 0.01 µg/kg-hr			
CCK	6± 2	7± 1	7± 2	5± 1	53± 35
PP	51± 9	74±26	86±28	94±32*	758±253*
Gastrin	17± 2	22± 3*	21± 4	19± 2	163± 67*
		GRP 0.1 µg/kg-hr			
CCK	6± 2	12± 3*	14± 3*	11± 2*	261± 50*
PP	64±20	112±34*	150±53*	86±19*	2266±747*
Gastrin	17± 2	29± 4*	35± 6*	25± 4*	574±150*

*=p<0.05; I=integrated response pmol - 0-45 min/L.

Conclusions: This study shows that GRP stimulates the release of CCK, PP and gastrin in a dose related manner and indicates that the biologic action of GRP is similar to that of bombesin. Supported by NIH Grant #AM-30145-01.

15.10

DISTRIBUTION AND RETENTION OF CADMIUM^{109} BY GASTROINTESTINAL TRACT AND OTHER ORGANS FOLLOWING INTRAPERITONEAL Cd EXPOSURE. P. Chowdhury*, E. Harper, and P. L. Rayford. Dept. of Physiol.-Biophys., Univ. Ark. Med. Sci., Little Rock, AR 72205

We have shown that Cd influences the BBS stimulated release of gastrin, cholecystokinin (CCK) and pancreatic polypeptide (PP). This study was undertaken to determine if cadmium is accumulated and retained in the parts of the GI tract that synthesizes GI hormones. Methods: A total of 36 rats is divided in 6 groups. Each rat received a single i.p. injection of saline or 1.22×10^{-5} M Cd/kg plus 82 µc Cd^{109}/kg. Each animal was kept in metabolic cages to collect urine. At 24, 48, and 72 hrs, they were anesthetized, blood and tissue specimens were promptly collected and radioactivity was determined for each tissue. The percent distribution of Cd per gm of tissue was calculated from the total counts for each tissue and its respective weight. A Student's paired t-test was used to determine significant differences. Results: Organs of the GI tract (fundus, antrum, duodenum, jejunum, ileum, colon) the pancreas, liver and lung showed a significant increase in Cd levels at 24, 48 and 72 hrs. Urinary and brain Cd levels were significantly elevated at 24 and 48 hrs respectively, whereas no significant Cd elevations were found in whole blood or plasma. Conclusion: Besides liver and lung all parts of the GI tract and pancreas accumulated and retained significant amounts of Cd suggesting that Cd may play a role in regulating GI hormone release after Cd exposure. Supported in part by NIH Grant AM30415-01.

15.11

SPERMINE INHIBITS GASTRIC ACID SECRETION IN PYLOROUS LIGATED RATS. Tushar K. Ray, Jyotirmoy Nandi*, Mark V. Wright* and David Fromm. Department of Surgery, SUNY-Upstate Medical Center, Syracuse, NY 13210.

Recent studies have shown that the polyamines inhibit gastric acid secretion in the chambered bullfrog gastric mucosa only when incorporated into the secretory bathing medium. However, the effects of spermine (SP) on H^+ secretion in vivo are unknown. The pylorous ligated rats were given SP (1-8 μmoles) dissolved in 2 ml of physiologic saline into the stomach cavity by oral route while the controls received saline alone. After 15 min, histamine (100 μg/kg) was injected subcutaneously. The animals were killed at 2, 4 or 6 hours. The volume and the concentration of H^+ of the gastric juice was quantitated. SP inhibits gastric acid secretion in a dose dependent manner. The SP was completely ineffective when injected by the i.v. route. Even though the SP inhibition of acid secretion decreased with time, about 35% of the H^+ transport continued to be suppressed at 6 hours following a single oral dose (5.4 mg/kg). An inverse relationship between the efficacy of SP and the age of the animal was observed. Thus, the dose of oral SP for the old (450-500 g) and young (250-300 g) animals was 4 and 8 μmoles respectively for the same extent of inhibition (about 80%) in gastric acid secretion. The data demonstrate that SP is an effective inhibitor of gastric H^+ transport from the lumen side of the stomach and the modus operandi of the polyamine is different than the H_2-receptor antagonists. (Supported in part by AM 00623)

15.12

INHIBITION OF ACID SECRETION BY ADENOSINE. M.L. Skoglund*, A.I. Vinik*, and M.R. Feller* (SPON: J. Schwartz). University of Michigan, Ann Arbor, Michigan 48109 and The Procter and Gamble Company, Cincinnati, Ohio 45247.

Adenosine has been suggested to be a purinergic neurotransmitter in the gastrointestinal tract. We have determined the effect of adenosine on gastric acid secretion and endogenous prostaglandin synthesis in isolated parietal cells. To do this, enriched fractions of parietal cells were prepared by digesting the gastric fundic mucosa with EDTA and collagenase. Separation of the cells was achieved by using ficoll density gradients. Acid secretion was measured by ^{14}-C-aminopyrine (AP) uptake at 37°C for 20 minutes. AP uptake was maximally stimulated by histamine (10^{-4}M). The effect of adenosine, adenosine N^1-oxide, 2',3'-O-isopropylidene adenosine, and cimetidine was determined on histamine-stimulated AP uptake. These compounds inhibited AP uptake in the following order: adenosine > cimetidine > 2',3'-O-isopropylidene adenosine. Adenosine N^1-oxide was completely ineffective. Endogenous prostaglandin synthesis in unstimulated or stimulated (histamine or carbachol) gastric cells was measured. Prostaglandin synthesis was not altered by adenosine.

This work shows that adenosine is a potent inhibitor of acid secretion in isolated canine parietal cells, and this effect is not mediated by endogenous prostaglandin synthesis. Thus, adenosine might be used for the treatment of gastric mucosal injury due to hypersecretory conditions.

CORONARY PHYSIOLOGY I

16.1

STEADY-STATE PRESSURE-FLOW RELATIONS IN AN UNDERPERFUSED CORONARY BED. Lillie M. Boyd* and Robert E. Goldstein, Depts. of Med. and Physiol., USUHS, Bethesda, MD 20814

To characterize coronary vessel performance during low-flow states in an animal model lacking extensive collateral circulation, we studied steady-state pressure-flow relations (PFR) in 14 open-chest pigs. The left anterior descending coronary artery was instrumented with an electromagnetic flow probe, a cuff occluder, and a distal coronary catheter. By adjusting the occluder, mean coronary blood flow (CBF) was changed in incremental descending or ascending order. CBF was maintained at 30, 20, 10, 5, and 0 ml/min until stability was achieved (1-2min); and, mean coronary perfusion pressure (CPP), systemic arterial pressure (SAP), and heart rate (HR) were measured. Pre-occlusion values ($\bar{x}$ ± SEM) were: CBF 32 ± 3ml/min; CPP 85 ± 3mmHg; SAP 95 ± 4mmHg; HR 137 ± 7/min. PFR were reproducible within the same pig; curves were not altered by the order in which CBF changes were performed. CPP plotted against CBF demonstrated a sigmoid curve, with PFR approaching linearity in the midrange (CBF 5-20ml/min). Pressure at zero flow (PZF) in 9 pigs was 9 ± 1 ($\bar{x}$ ± SEM) compared to linearly extrapolated PZF of 17 ± 3 ($P<0.05$). Thus, in our steady-state model of coronary underperfusion, resistance ($\frac{\Delta CPP}{\Delta CBF}$) increased sharply as CPP decreased toward PZF. These findings indicate that CPP must be maintained substantially above PZF to retain appreciable rates of myocardial perfusion.

16.2

PERFUSION WITH NON-OXYGENATED TYRODE SOLUTION CAUSES MAXIMAL CORONARY VASODILATION. George J. Crystal, H. Fred Downey, and Fouad A. Bashour. University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Ischemia secondary to coronary artery occlusion causes marked coronary vasodilation. Ischemia is characterized by both elevated interstitial concentrations of vasodilator metabolites and reduced myocardial PO_2, and either of these mechanisms could mediate the vasodilation. To prevent accumulation of vasodilator metabolites during hypoxia so that the coronary vasodilator capability of reduced myocardial PO_2 could be evaluated, we perfused the left anterior descending coronary artery (LAD) for 3 min with non-oxygenated Tyrode solution (Sugishita et al., Am. J. Physiol. 234: H625-H628, 1978). Coronary vasodilation was assessed from peak hyperemic flow upon restoration of arterial blood perfusion. In 5 open chest, anesthetized dogs, 3-min Tyrode perfusion increased LAD blood flow from 28.3±5.4 to 153±34 ml/min, a value not significantly different from peak reactive hyperemia following 3-min occlusion, 126±21 ml/min. These results indicate maximal coronary vasodilation when severe myocardial hypoxia is induced under conditions which favor washout of vasodilator metabolites. It appears that reduced myocardial PO_2 may be a potent dilator of coronary resistance vessels. (Supported by NIH Grant HL-21657, American Heart Association, Texas Affiliate, and the Cardiology Fund).

16.3

EPICARDIAL MAPPING OF CANINE CARDIAC SYMPATHETIC AND VAGAL AFFERENT DENERVATION FOLLOWING TRANSMURAL MYOCARDIAL INFARCTION. M.J. Barber, T.M. Mueller, B.G. Davies and D.P. Zipes, Krannert Inst Cardiol, Indiana Univ Sch Med and VA Med Ctr, Indpls, In.

We have demonstrated that transmural myocardial infarction (TMI) can interrupt transmission in both efferent and afferent (AFF) nerve fibers coursing through the infarct, producing functional denervation (DNV) apical to the TMI. To examine the extent of AFF DNV, we applied bradykinin (BK;5 mcg) and nicotine (N;50 mcg) topically on multiple epicardial sites to activate sympathetic (S) and vagal (V) afferents, respectively. Responses to BK and N were mapped in 5 open-chest dogs before and 90 min after TMI produced by embolizing a diagonal branch of the LAD coronary artery with latex. Before TMI, BK ↑ mean aortic pressure (MAP) by 16±2 mmHg (±SEM), while N ↓ MAP 8±2 mmHg at all sites tested (n=34). Ninety min after TMI, BK still ↑ MAP (16±4 mmHg) and N ↓ MAP (11±2 mmHg) when applied to sites basal and lateral to TMI, but produced no MAP change when applied to sites over the infarct zone. N (n=10) and BK (n=6) applied to sites over viable myocardium apical to the TMI also produced no MAP response. Four sites responding to BK (↑MAP 14±3 mmHg) but not N were located over diagonal branches of the LAD more apical than the diagonal which was embolized. We conclude from these data that TMI can produce regions of functional sympathetic and vagal afferent denervation in non-infarcted myocardium apical to the TMI, and that this denervation may heterogeneously interrupt sympathetic and vagal afferent innervation.

16.4

DO BEAGLES HAVE "BETTER" COLLATERALS THAN MONGREL DOGS? Leslie A. Ingram* and Konrad W. Scheel. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

Purebred beagles are an important research model. Their suitability for certain studies depends, in part, on their innate characteristics. In order to affect a comparison of coronary collateral resistances all collateral resistances to a given vessel were expressed as a ratio to the resistance of the collateral dependent vessel. The study was conducted on an isolated heart preparation during maximal vasodilation with adenosine and with constant pressure and cardiac activity.

		COL	COR	Ratio	N
Beagles	$\bar{x}$ ± SEM	14 ± 1.4	.43 ± 0.2	35 ± 3.4	20
Mongrels	$\bar{x}$ ± SEM	8 ± 1.1	.30 ± 0.2	31 ± 4.9	19
p		0.01	0.001	NS	

COL = collateral resistance to circumflex; COR = circumflex resistance (mm Hg/ml/min); Ratio = COL/COR. Similar results were obtained for the right and anterior descending coronary arteries. It appears that collateral and coronary resistances in beagles are higher compared to mongrels. However, the ratios are not significantly different. We conclude that beagles do not have "better" innate collaterals. The survival rate for beagles (20) and mongrels (200) following Ameroid application was not significantly different with a 10% mortality. This suggests that the rate of collateral development for both species also may be identical. (Supported by NIH Grant HL 28948)

16.5

EFFECTS OF RENAL HYPERTENSION ON CORONARY AND COLLATERAL VASCULATURE AND SIZE OF PERFUSION TERRITORIES IN THE DOG. Konrad W. Scheel, Allan Johnston*, Leslie A. Ingram*, Rowlyn Gordey*. Kirksville College of Osteopathic Med. and Univ. of TN Center for Health Sciences. Kirksville, MO 63501, Memphis, TN 38163

The purpose of this study was to investigate if mechanical factors (pressure) stimulate coronary collateral growth. Hypertension was produced by removal of one kidney and clamping the renal artery of the remaining kidney 1 month later. The hearts were studied at max. vasodilation on an isolated heart preparation 6 (n=6) and 16 (n=8) weeks after stenosis. Microfil was injected into circumflex, C, anterior descending, A, septal, S, and right, R, coronary vessels. Average control pressures were 127 ± 3 over 78 ± 3 mm Hg. Hypertensive pressures were 171 ± 6 over 118 ± 4. Hypertension at the end of 6 and 16 wks was not significantly (sig) different. There was no sig change in coronary collateral resistances in either group from control. Hypertrophy was sig in both groups (left ventricle to body weight ratios) but sig less in the 16 versus 6 wk groups. Perfusion territory, PT, to body weight ratios showed no increase for S-territory in either group. PT ratio for A was elevated in both groups (from control). PT ratios for C and also R were larger in 6 wk but not 16 wk groups (from control). There was no sig changes in the flow per gram ratios of any PT. It was concluded that an elevated pressure does not stimulate collateral growth. Hypertrophy was most pronounced in A territory and coronary vascularity increases with hypertrophy. (Supported by NIH Grant HL 28948)

16.6

THE EFFECT OF A REHABILITATION PROGRAM ON MYOCARDIAL INFARCTION PATIENTS IN EASTERN KENTUCKY. D.J. Saxon, R. Carpenter*, S.P. Howard*, and M. Dillon*. Morehead State University and Morehead Clinic 40351

The major objective of the study was to evaluate a current rehabilitation program at the Morehead Clinic for cardiac patients. The evaluation involved an analysis of the effect of completion or non-completion of the program on physiological risk factors and general emotional state of myocardial infarction patients. Patients were administered stress ECG's at the state of the program and again at the conclusion of the program or at an equivalent time for the patients not completing the program. Those patients completing the program exhibited a mean MET increase of 46.3%, while those not completing the program had a value of -5.05%. A MET is an energy expenditure equivalent to 3.5 mL of O_2 per kg body mass per minute. Therefore the program did greatly enhance the physical stamina of the patients. The program did reflect a significant effect in changing smokers to non-smokers. Also those patients completing the program exhibited less anxiety, as measured by their Depression Adjective Check List (DACL) score, than those not completing the program. There were no significant differences in the blood lipid profiles of the two groups. (Supported in part by MSU Faculty Research Grant)

16.7

EVIDENCE FOR A FUNCTIONAL MYOCARDIAL OPIATE SYSTEM. J.L. Caffrey and C.E. Jones, Department of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Recent studies suggest a role for endogenous opiates in the peripheral cardiovascular system. We evaluated the rat myocardium for 1) enkephalinase activity (Ease) and, 2) the sensitivity of this enzyme activity to inhibition by catecholamines, as observed in brain. The cardiac Ease was substantial (50.1 ± 2.4 nMoles/min/mg protein) although less than in brain (127.0 ± 3.4). The myocardial Ease, like the brain Ease, was inhibited by dopamine (11.2 ± 3.7) and puromycin (5.4 ± 2.4). The effect of puromycin, a known inhibitor of brain enkephalin aminopeptidase, suggests that the myocardial Ease is very similar. This is also supported by localization of both Eases in 100,000 x g supernatant fractions. The cardiac Ease is strongly suppressed by 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydorxymandelic acid but not by 3-methoxytyramine and normetanephrine. In related studies we injected pharmacologic concentrations of DOPAC or naloxone into the left coronary vasculature of dogs. Although the response was variable in degree and duration, DOPAC consistently produced transient declines in left ventricular contractile force (LCVF) and naloxone consistently increased LVCF. The rapid naloxone response suggests an action within the myocardium. We feel these indirect lines of evidence support the presence of operational endogenous opiate systems in myocardium which may be facilitated via an adrenergic inhibition of enkephalin degradation. Supported by TCOM Grant 34950 and NIH Grant HL-29371.

NEUROCHEMISTRY, NEUROPHYSIOLOGY AND SENSORY PHYSIOLOGY

17.1

ENZYMATIC DECONJUGATION OF NORMETANEPHRINE (NMN) IN HUMAN PLASMA AND RED BLOOD CELL (RBC). S. Yoneda*, N. Alexander, and N. Vlachakis* Univ. of So. Calif., Los Angeles, CA 90033

We recently applied an enzymatic approach to the deconjugation of NMN in plasma and RBC. We have compared it with a standard procedure for deconjugation by acid hydrolysis. Each plasma and RBC lysate specimen was divided into 3 parts for measuring free NMN, total NMN by acid hydrolysis plus heat (98°C, pH 1.0) and total NMN by enzymatic (sulfatase) hydrolysis. Subsequently the free and total NMN was determined by radioenzymatic assay (Vlachakis, Biochem.Med.20:107, 1978). Results are expressed in pg/ml (mean+SEM)

HUMAN (n=6)	FREE	TOTAL(Acid)	TOTAL(Enzyme)
plasma	870±74	2266±106	3709±195
RBC	7410±948	-	8392±963

The concentration of total NMN measured by enzymatic hydrolysis is significantly higher than by acid hydrolysis ($p<0.001$). Two dimension thin layer chromatography gave only one distinct peak of radioactivity at the position of NMN. These results indicate absence of cross contamination and that total NMN is present in higher concentrations than previously reported. As we reported (Alexander, Life Sci.29:471, 1981) in RBC lysate total NMN is significantly higher than in plasma ($p<.01$) and, as indicated by this study, is mostly in the free form in lysate and present in high concentrations as previously observed This indicates that RBC play an important role in metabolism of norepinephrine.

17.2

FATTY ACID AND PHOSPHOLIPID COMPOSITION OF EXCITABLE MEMBRANES AND OF A PREPARATION ENRICHED IN SODIUM CHANNEL EXTRACTED FROM THESE ORGANITES. G. Dandrifosse, S. Chapelle[X], G. Zwingelstein[X] and E. Schoffeniels. University of Liège, Belgium and University of Lyon, France.

The fatty acid (FA) and the phospholipid (PL) composition of excitable membranes arising from Maia nerves, Rattus brain and Electrophorus electric organ was determined. The predominent PL and FA of the PL in the membranes were : PC, PE, PS and 16:0, 22:6, 18:1, 18:0, 20:4 FA in Electrophorus and PE, PC, SP and 20:5, 18:1, 22:6, 16:0 FA in Maia. The must abundant PL and FA in Rattus membranes were PC, PE, PS and 16:0, 18:0, 18:1, 22:6 FA. Part of these patterns was modified after the isolation of a sodium channel enriched preparation obtained from these organites by using the same methods. The data were as follows : PC, PE, PS and 22:6, 16:0, 18:1, 18:0 FA in PL for the Electrophorus material; PC, SP and 18:1, 16:0, 20:5, 22:6 FA in PL for the Maia extract; PC, PE, SP and 18:1, 24:0, 16:0 FA for the Rattus preparation. During the purification, a lowering of the amount of PL toward this of the proteins was observed. This modification was concomitant with an enrichment of the PL containing the choline group toward the other PL. In the Maia and Rattus sodium channel preparations, the total amount of the polyunsaturated FA was lower than that measured in the membranes. These results, added to the statement of the properties of the protein phosphorylation occuring in both kinds of material, are important steps in stating precisely the molecular mechanism of the bioelectrogenesis.

17.3

REDUCTION OF MALONALDEHYDE (MA) FORMATION IN CORTICAL BRAIN SLICES BY GLUTATHIONE (GSH) AND ASCORBATE (ASC).
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Accumulation of MA by cortical brain slices during incubation indicates that oxidative degradation of fatty acids in vitro proceeds at a faster pace than detoxification of breakdown products. Development of methods for inhibition of lipid peroxidation and removal of peroxides are essential for improving existing experimental conditions. Slices were incubated in normal Krebs Ringer phosphate medium saturated with O_2. Nearly half of initial tissue ascorbate was released into the medium during incubation of control samples. Maintenance of in vivo level of cortical ascorbate during incubation by supplementation through the medium significantly reduced MA production. GSH (1-2mm) supplementation stimulated MA production. Improving cortical GSH or ASC status by i.p. injection of 2 g/kg ASC or 1 g/kg GSH 30 min prior to decapitation markedly reduced MA formation during subsequent incubation in control medium even though ≈50% of tissue ASC was released. Maintenance of ASC levels by supplementation through the medium caused further reduction in MA production. GSH supplementation was without effect. Optimal conditions were obtained with GSH pretreatment followed by in vitro ASC supplementation. In such tissue no net MA production was detected after 60 min incubation.
Supported by NIH Grant HL-08899 and ONR Contract N00014-7-C-0248.

17.4

DETECTION OF ANGIOTENSIN II FROM BRAIN TISSUE AFTER MICROWAVE IRRADIATION.
Robin A. Barraco and Howard J. Normile.* Wayne State University, Detroit, MI 48201

Although angiotensin receptors have been detected in various CNS regions and angiotensin has been measured in the brains of a variety of species by bioassay and immunoassay, the results have been equivocal. A possible explanation for this variability may be the high activity of brain angiotensinsinases. To abridge this difficulty, intact pigeons were sacrificed by microwave irradiation. Whole brain was homogenized in 0.2M NH_4HCO_3. Following ultracentrifugation the supernate was sequentially partitioned on a series of Sephadex gel filtration columns and recovery was monitored by fluorescamine assay. The eluate was assayed for angiotensin by immunoassay and bioassay. The total immunoreactive angiotensin-I (A-I) detected was 14.98 ng/g cerebrum which was eluted in a sharp peak at approximately the same Ve as synthetic A-I and prior to the Ve's of synthetic A-II and its amide. The eluate corresponding to the Ve of synthetic A-II (V_{A2}) was assayed with an in vitro muscle assay using rabbit bladder serosal strips. Approximately 2.4% of lyophilized V_{A2} produced a maximal and sustained contraction. These contractions were diminished in a dose-response fashion by saralasin, but not inhibited completely (maximal inhibition was 40%) indicating the presence of other bioactive peptides. Using RIA, much more A-II than A-I was detected. The total immunoactive A-II was calculated to be 134.73 ng/g wet wt. These studies are now being conducted on nephrectomized animals.
(Supported by a grant from the Michigan Heart Association).

17.5

THE ANTICONVULSANT EFFECTS OF OPIOIDS IN THE SEIZURE SENSITIVE MONGOLIAN GERBIL.
Randall J. Lee*, Joseph G. Bajorek*, and Peter Lomax. University of California, Los Angeles, CA 90024.

Opioid agonists were used to investigate the anticonvulsant effects mediated by μ, κ and σ opiate receptors in the seizure sensitive Mongolian gerbil. Morphine (1.0-25 mg/kg), ketocyclazocine (0.15-25 mg/kg), and N-allyl normetazocine (0.1-10 mg/kg) were used as specific prototypic agonists for μ, κ and σ opiate receptors. Morphine (n,10) reduced the seizure incidence from a control value of 80% to 40% and 10% (1.0 and 10 mg/kg), respectively. Ketocyclazocine (n,10) lowered the seizure incidence to 30% and 0% (0.15 and 0.5 mg/kg) as compared to controls (90% incidence). N-allyl normetazocine (n,9) also reduced the seizure incidence to 47% and 0% when compared to controls (78% incidence). The anticonvulsant effects of morphine (10 mg/kg) and ketocyclazocine (0.5% mg/kg) were reversed by naloxone (1.0 mg/kg) while the anticonvulsant effects of N-allyl normetazocine were not significantly changed by naloxone. Higher doses of each opioid agonist, tested for anticonvulsant effects, produced behavioral effects (specific for each receptor) which began to obscure the behavioral characteristics of the less severe seizures. The data presented are consistent with earlier studies which demonstrated the anticonvulsant effects of β-endorphin in the gerbil. Together, these studies suggests that opioids do have a modulatory role in seizure activity in the gerbil and the opioid anticonvulsant effect is not specific to one type of opiate receptor.
(Supported by ONR contract N-0014-75-C-0506).

17.6

PHYSIOLOGICAL PLASTICITY IN THE OPOSSUM AND RAT HIPPOCAMPUS: IN VITRO EXTRACELLULAR AND INTRACELLULAR RESPONSES.
John H. Ashe, Valentin K. Gribkoff* & Steven H. Spillers* Dept. of Psychology, Univ. of Calif., Riverside, CA. 92521

Neuronal processes underlying higher functions such as learning and memory, and various forms of plasticity, have been extensively investigated in the rodent and rabbit hippocampus. In view of the importance of questions concerning plasticity and its phylogenetic generality, we examined in vitro responses from transverse hippocampal slices of the North American opossum (Didelphis virginiana) and the rat. All responses were obtained from area CA_1 in the rat or its apparent equivalent in the opossum; stimuli were delivered to the Schaffer collateral system. The degree of facilitation of the intracellular response to the 2nd volley of paired pulses in opossum neurons was similar throughout the range of interpulse intervals (IPI) tested (10-100 msec); the 2nd EPSP was larger and spiking occured in response to both volleys. In contrast, in neurons of the rat the probability of spiking in response to the 2nd volley was greatly reduced at short IPI's (10-30 msec). With extracellular recording, both the population EPSP and spike in response to the 2nd pulse was enhanced at short IPI's in the opossum. In the rat, while the population EPSP could show either no change or considerable facilitation in response to the 2nd pulse at short IPI's, the population spike was greatly reduced or absent in most cases. Conditioning trains (100/sec, 5 sec) produced long-lasting potentiation of responses to subsequent test stimuli in both species.
(NIH BRSG-RR07010-17).

17.7

ELECTRICAL PROPERTIES OF ACUTELY ISOLATED HIPPOCAMPAL NEURONS.
Richard Gray*, Judianne Kellaway*, and Daniel Johnston. Prog. in Neuroscience, Dept. of Neurology, Baylor Col. of Med., Houston, TX 77030.

Considerable interest has been focused on the membrane properties of hippocampal neurons from the in vitro slice preparation. Several types of investigations, however, are difficult to pursue in the slice because of the inability to visualize individual cells. Therefore, we felt it would be advantageous to develop an isolated cell system, similar to that done with the retina (Johnston and Lam, NATURE 292:451). Slices of adult guinea pig hippocampus were incubated in proteolytic enzyme for varying times. Cells were then dispersed mechanically, resulting in small pieces of tissue with exposed cell bodies, isolated cells, and cellular debris. Exposed cell bodies with prominent dendritic processes were impaled and judged healthy (stable resting potentials greater than 55mV, time constants greater than 10 ms, overshooting action potentials, and spontaneous synaptic input). Less frequently, totally isolated cells, most often of the granule or small pyramidal cell type, were impaled and had similar electrical properties, except for spontaneous synaptic events. Successful gigohm seals, and recordings of single transmitter activated channels, have been obtained from both the partially and totally isolated cells. The further development of this preparation should make feasible studies of the biophysical properties of hippocampal neurons.
(Supported by NS15772, NS18295, and a McKnight Neuroscience Development Award).

17.8

A STUDY OF ALPHA-ALPHA' AND BETA-BETA' DICHLORODIETHYLETHER ON BIOMEMBRANE.
Wei Young, P.S. Timiras, R.W. Rosser* and John A. Parker*. Department of Physiology/Anatomy, University of California, Berkeley, California 94720, and Ames Research Center, NASA, Moffett Field, California 94035

In recent years, biomembranes, from a simple lipid bilayer to a more complicated biomembrane, such as squid giant axon, synaptic membranes, urinary bladder, gastrointestinal mucosa, and skin, have widely been utilized as a tool to probe the ionic transport and membrane potentials. Pharmacodynamic studies on such membranes are yet to be developed. This study is to utilize a simple biomembrane to probe some chemical effects on the membrane potential, $E = (RT/nF) \ln C1/C_2$, and the kinetics of the membrane. The unique protein configuration of this biomembrane permits us to assess the effects of certain radicals which interact with the active sites of the membrane. It may give some insights on the intrinsic nature of immunologic, neoplastic and aging properties of the biomembrane. We have examined the effects of some alpha-alpha and beta-beta' dichlorodiethylether and some analogs on this particular biomembrane. The kinetics of these chemicals on this biomembrane will be compared with that of the synaptic membrane in the isolated autonomic organ system.

17.9

Depression of inhibitory synaptic transmission by oxygen and pressure: Carol A. Colton and Joel S. Colton, Univ. of Nevada Medical School, Reno, NV 89557.

The effect of 100% oxygen, 100% oxygen at 10 or 60 PSIG, and helium at 10 or 60 PSIG on inhibitory synaptic transmission was studied at the lobster neuromuscular junction. Experiments were performed in a hyperbaric chamber using remote control implantation of two microelectrodes into a single surface muscle fiber. Measurement of resting membrane conductance (via current clamp) and conductance during activation of the inhibitory junction (stimulation frequency = 15 Hz) were done under control conditions and after equilibration with the appropriate gas and pressure. Inhibitory synaptic conductance (G_{syn}) was then calculated from $G_{syn} = G_{total} - G_{resting}$. In each experimental condition above, G_{syn} fell over 60 min. Air at ambient pressure controls showed no change in G_{syn} over the same time period. Since the membrane response to exogenously applied GABA was the same as control, postsynaptic receptor blockade was not the cause of the fall in G_{syn}. Complete loss of inhibitory transmission was seen in 100% O_2 only. External recording from the inhibitory axon revealed no change in action potential propagation during exposure to 100% O_2. When failure of inhibitory transmission was seen, the loss of inhibitory junction potentials was associated with a large rise in the frequency of excitatory and inhibitory miniature junction potentials. Experiments suggest that 100% oxygen and pressure decrease the presynaptic release of inhibitory transmitter.

Supported by NIH Grant NS16526.

17.10

INTRACELLULAR RECORDINGS FROM IN VITRO HIPPOCAMPUS OF THE NORTH AMERICAN OPOSSUM (DIDELPHIS VIRGINIANA): RESPONSES TO AFFERENT STIMULATION. Valentin K. Gribkoff* Steven H. Spillers* & John H. Ashe. Dept. of Psychology, Univ. of Calif., Riverside CA. 92521

The present study represents the initial part of a comparative study of hippocampal responses and plasticity in the North American opossum, and presents the characterization of responses of single neurons to afferent stimulation. Transverse slices of the hippocampus were prepared from adult and pouch-young animals. Stimuli were delivered (.2/sec) to a pathway corresponding to the Schaffer collateral system in the rat. 15 cells, located in an area similar to the CA_1 region of the rat, were examined (RP's: -65 to -75 mV). Three response types were observed: Type I: An EPSP of short duration at stimulus intensities subthreshold to spike production. Frequently, the descending phase of the EPSP is followed by a long-duration hyperpolarization (HP). Single or double spikes are seen on the ascending phase of the EPSP at high stimulus intensities. Type II: An HP at stim. intensities subthreshold to spiking. At higher stim. intensities a short-duration, small amplitude EPSP preceeds the HP. Further increase in stim. intensity result in increases in all components and spiking on the rising phase of the EPSP. Type III: A long-duration depolarization at low stim. intensities. Increases in stim. intensity above spike threshold elicit progressively larger numbers of spikes during the depolarization. (Supported by NIH grant BRSG-RR07010-17).

17.11

POSTNATAL DEVELOPMENT OF INHIBITORY CAUDATE EFFERENTS. Robin S. Fisher*, Michael S. Levine*, Chester D. Hull and Nathaniel A. Buchwald. (SPON: C.D. Clemente). Mental Retardation Research Center, UCLA, Los Angeles,CA 90024.

Extracellular unitary recordings of evoked responses to afferent stimulation in pallidal (GP-Ento) and nigral (SN) neurons of kittens (1-70 days of age) and adult cats indicate the existence of inhibitory caudate (Cd) efferents throughout postnatal development. These inhibitory inputs to the GP-Ento and SN became predominant with age. Retrograde axonal transport studies in neonates and adults show that these inputs are monosynaptic, originate exclusively from a large population of medium-sized Cd neurons, and are established by birth. Immunocytological studies demonstrate the existence of axonal terminals containing glutamate decarboxylase (GAD). Hence α-aminobutyric acid, an inhibitory neurotransmitter exists in the GP-Ento and SN of kittens as young as 5 days of age. The density of the GAD terminals increases with age. The developmental increase in these terminals which are known to originate from Cd neurons, may contribute to increasing inhibitory influences upon the output nuclei of the basal ganglia.

(Supported by USPHS Grant HD 05958).

17.12

SEROTONIN MEDIATED INHIBITION OF RENAL NERVE ACTIVITY DURING PARADOXICAL SLEEP. Henrique A. Futuro Neto and John H.Coote Departamento Ciencias Fisiologicas, CBM, UFES,29000 Vitoria, Espirito Santo, Brazil.

This study investigates the possibility that the inhibition of renal nerve activity (RN) occuring during paradoxical sleep is mediated by serotoninergic neurons. Blood pressure (BP),ECG and RN were recorded in mid-collicular decerebrated cats, previously vagotomized and with the carotid sinus denervated. Paradoxical sleep-like periods (PSI) were induced with eserine sulphate (0.1 mg/kg, i.v.). The serotonin (5HT)depleting drug p-chloro-phenylalanine (PCPA, 500 mg/kg, i.p., given 72 hrs before the experiments, n = 4) did not provoke changes in BP or heart rate. Nevertheless the inhibition of RN during PSI was significantly smaller (P 0.01) in the PCPA treated cats (10.4 ±2.1% reduction) than in the untreated controls (42.5 ±4.6% reduction,n = 11). The 5HT inhibitory pathways projecting to the preganglionic symphathetic neurons, descend through the dorsolateral funiculus of the cervical spinal cord. Therefore this region was lesioned (small cuts) in a different group of animals (n = 4). The inhibition of RN during PSI was compared before (42.8 ±6.3% reduction) and after lesions (14.3 ±2.7% reduction) and was found to be significantly smaller after the lesions (P 0.01). These re-results indicate that serotoninergic neurons participate in the inhibition of RN occuring during PSI in decerebrated cats. CAPES

17.13

ON LINE HIGH SPEED ANALOG DISCIMINATION FOR MULTIPLE NERVOUS SPIKE RECORDS. Melva A.Robinson* and Francesco Bracchi* (SPON:T.Gualtierotti) Univ.of Milan,20133 Milan,ITALY.

Analog methods are still faster than digital ones for the purpose of spike discrimination.However the need for a threshold level for detection results in the loss of a great part of the spike leading front.An apparatus has been developed which eliminates this need allowing the use of the complete signal for spike detection.All signal's maxima and minima including noise are detected.Amplitude and time difference between each minimum and following maximum and from this max. to the following minimum are displayed and measured on a memory CRT.A 4 parameter mask is then set on the apparatus i.e. the amplitude and time difference between the wanted signal's first minimum and following maximum and amplitude and time difference between this maximum to the following minimum,plus a given variability to take care of noise.Each time an event whose amplitude and time parameter correspond to the preset mask is presented at the input of the discriminator an output is obtained.Any number of discrimination channels can be used together for symultaneous analysis of any given record.From the signal's first derivative obtained from the apparatus,it is also shown that the spike leading front contains a higher degree of information with respect to the trailing front for recognition purposes.

17.14

"Limit cycle" oscillations in the power spectral band time series of squirrel monkey EEG: relation to behavioral state. C.L. Ehlers*, C.L. Franklin* and J.W. Havstad*, (SPON: F.E. Bloom). A.V. Davis Center, Salk Institute, La Jolla, CA 92037.

Rhythmic fluctuations in the distribution of power, as a function of time, in discrete frequency bands of EEG (power spectral band time series) recorded from 7 chair adapted squirrel monkeys have been uncovered. These oscillations (2-3 c/min) are the most prominent in the frequency bands between 8-16 HZ and 16-33 HZ. A fluctuation in behavioral state from "vigilance" associated with mild motor movement and visual scanning to "quiescence" associated with little visual scanning was directly associated with these oscillations in the power spectra. Administration of amphetamine (0.25 mg P.O.) produced a stable state of constant vigilance which was correlated with a decrease in amplitude and an increase in the frequency of the power spectral band time series; whereas phenobarbital (30 mg P.O.) produced an unstable state of behavior sedation, associated with a time seris which contained slower frequencies and was more chaotic. Yates (1982) has postulated that the global stability of a complex, "homeokinetic" system consists of a closed trajectory with near periodic characteristics. The observed oscillations in the power spectral band time series may represent limit cycle oscillations in information flow as described for non-linear systems. (Supported by the Klingenstein Foundation.)

17.15

DEVELOPMENT OF SOMATOSENSORY RESPONSIVENESS IN THE CAUDATE NUCLEUS IN THE CAT. Jay S. Schneider*, Michael S. Levine*, Chester D. Hull and Nathaniel A. Buchwald. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

This experiment assessed the development of caudate neuronal responses to tactile stimulation of the face in awake kittens. Observations were made of responses to "punctate or brushing" stimuli. 240 caudate units were recorded in 4 kittens. In the youngest (21-30 days), 1% of caudate cells tested responded to facial tactile stimulation. These cells had large receptive fields (i.e., entire face), responded to both touch and brushing stimuli but were not directionally sensitive. In a 60 day old kitten 5% of caudate cells responded. Fields were moderate (front muzzle and vibrissa) or large (entire face) in size. Responses occurred primarily to brushing and were mostly nondirectional. In a 90 day old kitten, 10% of the caudate cells tested responded. Receptive fields were moderate to large. 20% of these cells responded to punctate and brushing stimuli. 50% were directionally sensitive. These findings contrasted with those found in the adult cat where there are more responsive cells, more punctate responses, and more small receptive fields. The data suggest that caudate facial somatosensory responses change significantly as a function of age.

Sponsored by USPHS Grants HD 05958 and HD 07032.

17.16

EYE MOVEMENT VELOCITY AND CORNEORETINAL POTENTIAL ALTERATIONS FOLLOWING SLEEP. Eugene Aserinsky, Stephen P. Tzankoff and Joan A. Lynch*. Marshall Univ., Huntington, WV 25701.

EOG measurements were obtained on 12 young adults to ascertain whether the EOG rise in amplitude occurring in sleep is accompanied by an eye mvt. change. Following 5'-10' dark adaptation, subjects executed horizontal saccadic mvts. of 5.9° and 11.8°. This procedure was repeated in the dark upon awaking from a morning nap (ca. 4.5 hrs. sleep, REM%=26.3). Maximal eye mvt. velocity (V_M) was determined over a 6 msec period while av. velocity (V_A) was computed from estimated eye mvt. duration. Results are means ± S.E. of nasalward mvts. of rt. eye:

		V_M	V_A	EOG Pot.
Before Sleep	11.8°	338±9°/s	181±9°/s	149±14μV
After Sleep	11.8°	326±9°/s	174±5°/s	176±16μV
Before Sleep	5.9°	205±10°/s	122±8°/s	80± 8μV
After Sleep	5.9°	201± 8°/s	106±6°/s	99±12μV

Neither V_M nor V_A increased after sleep although the corneoretinal (EOG) potential did rise significantly ($p<.01$) for the larger mvts. This indicates that the EOG potential was not artifactually increased due to a velocity change affecting the recording via usage of a time constant. Since the EOG potential was 193 ± 19μV in the light before sleep, the rise in potential following the immediate drop in darkness is a membrane phenomenon not directly related to dark adaptation.

17.17

THE EFFECT OF GRAVITY ON PHYSIOLOGIC ACTION TREMOR. David J. Howard* and Robert S. Pozos* (SPON: D. E. Mohrman). Univ. of Mn.-Duluth, School of Med., Duluth, Mn. 55812.

Physiologic action tremor (PAT), an involuntary oscillation recorded from a limb being voluntarily moved, is often seen when a muscle does work against gravity. This tremor can also be demonstrated with simple flexion and extension of the foot or hand. The interaction of gravity and the muscles producing PAT were studied by altering the position of the hand. Nine subjects were seated with the forearm supported and parallel to the floor. They would then flex and extend the hand with the palm facing up, down, or perpendicular to the floor. Biopotential surface electrodes recorded electromyograms and an accelerometer taped to the hand recorded acceleration. Measurements were stored on a tape recorder and later analyzed on a MINC 11 computer. There were no significant changes in the frequency of PAT, the mean of which was 10.5 Hz, for any of the different hand positions. Six subjects showed a 4-48% increase of the extensor EMG amplitude, with a similar increase in the flexor EMG, when the hand was changed from a palm down to a perpendicular position. A further increase in amplitude was shown by 5 subjects when the hand was changed from a perpendicular to a palm up position. In all subjects, PAT was predominately associated with the phasic contractions of the extensors as the flexors initiated motion of the hand. These data suggest that gravity has an influence on the amplitude but not the frequency of PAT.

17.18

THE EFFECTS OF PRENATAL UNILATERAL ENUCLEATION UPON THE FUNCTIONAL ORGANIZATION OF THE CAT'S LATERAL GENICULATE NUCLEUS. Robert W. Williams and Leo M. Chalupa* Department of Psychology and Physiology Graduate Group, University of California, Davis CA 95616.

In the mature cat crossed and uncrossed retinal projections to the lateral geniculate nucleus (LGN) are segregated within distinct laminae. Removal of one eye more than two weeks before birth prevents the formation of these laminae, and the retinal input from the remaining eye innervates virtually the entire ipsilateral and contralateral LGN. Given this diffuse pattern of projection to the LGN of prenatally unilaterally enucleated cats, in the present study we asked two fundamental and related questions: (1) Is the extensive retinal projection functional, and (2) does it have a normal visuotopic organization? For this purpose, extracellular single cell recordings were taken from the LGN contralateral and ipsilateral to the remaining eye of adult animals which had one eye removed more than two weeks before birth. We have found that apparently all regions of the LGN are functionally innervated by the retinal input from the remaining eye. Furthermore, the retinotopic organization is similar to that of the normal cat. However, portions of the LGN which are presumably innervated by an aberrant retinal input show a somewhat greater degree of scatter in receptive field position, and in these regions the size of receptive fields are also significantly larger than normal. Supported by GM 07416 from NIH and a University of California Research Grant.

17.19

CURRENT SOURCE DENSITY ANALYSIS OF OLFACTORY BULB EVOKED POTENTIALS. Diane P. Martinez* and Walter J. Freeman. Dept. of Physiol.-Anat., Univ. of Calif., Berkeley, CA 94720

The sign of action of periglomeruluar (PG) cells on the apical dendrites of mitral cells in olfactory bulb glomeruli was investigated by measurement of current source density (CSD) maps constructed from potentials evoked by primary olfactory nerve (PON) and lateral olfactory tract (LOT) stimulation. Evoked potentials were recorded and averaged from anesthetized rabbits simultaneously with a 1x16 array of electrodes placed normal to the bulb surface. A one-dimensional CSD analysis with depth was made in the center of PON- and LOT-evoked activity. Maps were examined at specific times during the oscillatory AEP: at the peaks of the first surface-negative wave (N1), the first surface-positive wave (P1), and the second surface-negative wave (N2). N1, P1, and N2 correspond to excitation, dis-excitation, and dis-inhibition of the granule (GR) cells through mitral basal dendrites. GR cells were the major generator of both PON and LOT AEPs. When N1 and P1 or P1 and N2 maps were combined, the GR source/sink pair was eliminated and other secondary source/sink pairs revealed. PON-evoked N1+P1 or P1+N2 maps showed a source/sink pair not present with LOT stimulation. These results indicated that a long-lasting excitation of the mitral cell was taking place at the glomerular layer (GL) and the GL/external plexiform layer border. This excitation was due to PG cells or possibly to prolonged monosynaptic PON excitation of the apical dendrite. Supported by MH06686.

17.20

GUSTATORY NERVE RESPONSE AND TASTE PREFERENCE FOR NACL AND KCL IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS. Fay Ferrell* and Sarah D. Gray. Depts. of Nutrition and Human Physiology, Univ. of California, Davis, CA 95616

The relationship between neural gustatory sensitivity and the expression of behavioral taste preferences is poorly understood. We compared neural sensitivity in weanling SHR and WKY by recording integrated responses of the chorda tympani nerve to 0.1M NaCl and KCl flowed over the tongue. Taste preferences for NaCl and KCl at concentrations from 0.001M to 1.0M were examined using 24-hr two-bottle preference tests of each salt vs distilled water. Compared to WKY, SHR had higher preferences for NaCl concentrations at and above 0.01M and for KCl concentrations at and above 0.032M. Additionally, the "most preferred" concentration of NaCl and of KCl was higher for SHR than for WKY. Age-related changes occurred in acceptance of high concentrations of each salt, with each genotype showing stronger preferences as weanlings than on retests conducted two months later. SHR and WKY exhibit no readily observable differences in neural sensitivity to NaCl, but show marked differences in sensitivity to KCl relative to NaCl. KCl elicits less integrated neural activity relative to NaCl in SHR than in WKY. Potassium may somewhat alleviate the hypertensive condition through its role in sodium excretion; thus the diminished afferent activity observed might provide information to the CNS which signals SHR to increase KCl ingestion.

17.21

BULLFROG VESTIBULAR PRIMARY AFFERENT DEPOLARIZATION BY ELECTRICAL STIMULATION OF THE VESTIBULAR NERVE.
Francesco Bracchi* (SPON:T.Gualtierotti) Univ.of Milan, 20133 Milan,ITALY.

By electrical stimulation of the Bullfrog vestibular nerve two potentials can be extracellularly recorded in the same nerve,a direct one with a .2-.3 ms.delay and a second one with a longer latency (3-5 ms.) so far thought to be expression of afferent fibers activity (1,2).Intra fibral recordings from identified primary afferents show that a single shock stimulus of low intensity,even lower than the threshold for direct stimulation,elicits a depolarizing potential of about 3 mV amplitude,30 ms.duration and 2 ms.delay.Increasing the strength of the stimulus,action potentials are generated over this wave.At stimulation frequencies over 20 stim./s.single responses fuse into a steady depolarization of up to 40 mV. Upon interruption of the stimulation the membrane potential recovers in a few seconds.Since the above phenomena are present also in most fibers of the central stump of a severed VIII nerve,but in none of the peripheral portion,it is suggested that it raflects a depolarization in the central terminal of primary afferents.(1) F.Bracchi:Boll.Soc.Ital. Biol.Sper.45:159,1969. (2) R.Llinas et al.:Exp Brain Res. 9:16-29,1969

17.22

EFFECT OF INTRAVITREOUSLY INJECTED SCORPION VENOM ON THE ELECTRORETINOGRAM.
Adnan A. Alawi and Amal F. Jeryes.* Faculty of Science University of Jordan, Amman, Jordan.

The electroretinogram (ERG) of the frog Rana ridibunda is studied after the intravitreous injection of different concentrations of the yellow scorpion Leiurus quinquestriatus crude venom (experimental), or the same volume of Conway's solution (control). The ERG is recorded from the perfused isolated eye-cup preparation by cottonwick electrodes. The crude venom is collected by electrical milking of the scorpions collected from the hilly areas of Jordan.

It is observed that the crude scorpion venom affects the amplitude of the ERG waves, and these effects are dependent on the venom dose and the intensity of the light stimulus.With low doses of the venom, the amplitude of the ERG waves increases as the intensity of the light stimulus is increased. But, with high doses of the crude venom, the amplitude of the ERG waves starts to decrease as the intensity of the light stimulus is increased. The mechanism of the venom action on the retina is discussed. (Supported by the University of Jordan.

17.23

EFFECTS OF L-GLUTAMIC AND ASCORBIC ACID IN THE SLEEP OF NORMAL AND INSOMNIAC SUBJECTS. Santibáñez, I. Depto. de Fisiología y Biofísica. Fac. de Medicina. Universidad de Chile. Casilla 137-D. Santiago, Chile.

Ascorbic Ac. (Ascorb) has shown an agonistic effect on the synthesis and release of brain catecholamines, and a stimulating effect on performance during wakefulness. L. Glutamic A. (L-Glut) in a single oral physiological dose, alone or plus L-Tryptaphan (L-Tryp), shows a facilitatory effect on REM-S in normal humans and cats (Santibáñez et al). A single dose of L-Glut (50 mg/Kg) and/or Ascorb (1 gr total dose) and/or L-Tryp (30 mg/Kg) was administered at night to every subject of a selected sample of 18 normal good-long sleepers (G-L-S) and insomniacs for 10 days. The EEG, EOG and EM were recorded in the laboratory, the 1st, 5th and 10th nights.

L-Glut plus L-Tryp plus Ascorb, produced a notorious increase in the REM-S percentage and in the amount of Total Sleep in normal and also in insomniac subjects. An increase of X 54% in REM-S was observed in G-L-S (mainly during the first night) and an increase of X 25% in REM-S in insomniacs (higher in the 5th and 10th nights). Comparing our normal values with those of other authors, we found that the proportion of Superficial versus Profound SWS was reversed in every subject (G-L-S and Insomniacs) during the first night of sleep in the laboratory, also presenting an increase of Profound SWS.

18.1

NONADRENERGIC NONCHOLINERGIC NERVE MODULATION OF GUINEA PIG TRACHEALIS MUSCLE RESPONSES TO 5-HYDROXYTRYPTAMINE IN VIVO.
S.E. Chesrown* and C.A. Cockrell, Jr.* (SPON: J.A. Mangos). Dept. of Pediatrics, Univ. of Florida, Gainesville, FL 32610

Nonadrenergic noncholinergic (NANC) nerves relax trachealis muscle during electrical stimulation (ES) of vagus nerves in anesthetized guinea pigs treated with atropine, 6-hydroxydopamine and propranolol. This response is decreased 62-78% after cutting the recurrent laryngeal nerves (RLN) (Chesrown et al., JCI 65:314-320, 1980). We inferred changes in trachealis muscle tone from changes in intratracheal pressure (P_T) measured within a saline-filled segment of cervical trachea. We studied NANC nerve modulation of trachealis contractions to 5-hydroxytryptamine (5-HT) injected into the left ventricle. In 10 animals, injections of 2 and 4 µg 5-HT increased P_T by 3.8 ± 0.2 (mean ± SEM) and 5.5 ± 1.1 cmH_2O respectively. When 5-HT was injected 10 s after beginning ES, responses were attenuated to 1.2 ± 0.5 and 3.0 ± 0.3 cmH_2O ($p < .05$). Sectioning of RLN prior to vagal ES resulted in increased P_T responses to 5-HT of 2.4 ± 0.3 and 4.4 ± 0.4 cmH_2O ($p < .05$). In 12 other animals, interruption of the NANC nerve pathway with bilateral vagotomy had no effect on P_T responses to 5-HT ($p > .05$). We conclude that NANC nerves attenuate trachealis contraction responses to 5-HT via a RLN pathway during ES of the vagus nerves, but vagal NANC nerves did not reflexly attenuate trachealis contraction responses. Supported by NIHLBI grant HL-26831 and the Cystic Fibrosis Foundation.

18.2

EPITHELIAL WATER FLUXES IN SHEEP TRACHEA.
R.J. Phipps*, S.M. Denas*, (SPON: A. Wanner). Div. of Pulmonary Disease, Mt. Sinai Medical Center, Miami Beach, FL 33140

In studies of airway mucus secretion it has been argued that water movement is determined by osmotic gradients set up by net transport of ions, notably Cl^- and Na^+, across the airway. In this study we attempted to show that changes in airway transepithelial water movement, as determined by the epithelial fluxes of tritiated water (THO), do parallel changes in transepithelial ion fluxes. Pieces of sheep trachea were mounted in perspex chambers. Fluxes of THO (in $ul/cm^2/h$) were measured simultaneously with, and in the same tissues as, fluxes of Cl^- and Na^+ (in $uEq/cm^2/h$), under short-circuit conditions. Net fluxes were calculated from paired tissues. Tissues were challenged with Ascaris suum antigen (25ug protein/ml) which has previously been shown to produce significant changes in Cl^- and Na^+ fluxes in airways of allergic sheep. Changes in net ion fluxes towards the tracheal lumen (JNet SM→L) produced by antigen were paralleled by significant changes in net THO movement. Values are mean (±SD).

JNet SM→L	Control	Antigen	Post Antigen
Cl^-(n=5)	+0.60(0.37)	-0.99(0.50)	+0.89(0.69)
Na^+(n=7)	-0.33(0.71)	-1.17(0.62)	+0.70(1.26)
THO(n=12)	+25(115)	-133(139)	+108(131)

Thus, THO fluxes may be a reliable indicator of water movement across the airway. (Supported by NIH HL 20989).

18.3

RELATIONSHIP BETWEEN AIRWAY REACTIVITY AND AIRWAY PERMEABILITY IN NORMAL AND ALLERGIC SHEEP. W.M. Abraham, J.C. Delehunt*, L. Yerger* and B. Marchette*. Div. of Pulmonary Disease, Mt. Sinai Medical Center, Miami Beach, FL 33140

Allergic (sensitized) sheep have greater airway responsiveness to inhaled histamine as compared to normal (non-sensitized) sheep. The purpose of this study was to determine if this difference in airway responsiveness (AR) to inhaled histamine is related to a difference in the permeability of histamine across the airway wall (AP) between the two groups. AR was assessed by measuring the change from baseline mean pulmonary flow resistance (R_L) following a controlled 2 min inhalation challenge with 1% histamine, containing 200uCi/ml ^{3}H-histamine. The rate of appearance of ^{3}H in plasma obtained from serial samples of arterial blood collected during the inhalation challenge was used to estimate AP. Inhalation of histamine (0.7ml) produced a greater ($p=0.02$) change in R_L in 11 allergic sheep (median 140%, range 51-362%) than it did in 15 normal sheep (median 68%, range 0-332%). AP was also significantly greater in allergic sheep (median 109dpm/min, range 62-225 dpm/min) than in normal sheep (median 54 dpm/min, range 23-165 dpm/min). Considering all values for AR and AP there was a weak but significant correlation between the two variables. We conclude that the increased AR to inhaled histamine in allergic sheep is associated with an increase in AP. Supported by NIEHS-02668.

18.4

CHOLINERGIC NEUROTRANSMISSION IN AIRWAY GANGLIA: INHIBITION BY NOREPINEPHRINE. D.G. Baker, D.A. Herbert* and R.A. Mitchell. Cardiovascular Research Inst., Univ. Calif., San Francisco, CA 94143.

In airways, adrenergic fibers terminate in parasympathetic ganglia but the action of norepinephrine on ganglionic transmission is not known. To determine this action, we recorded intracellularly from tracheal ganglion cells that were identified in sections of ferret trachea, stained with neutral red and perfused with 37° Krebs-Henseleit solution. Input resistances ranged from 20 to 60 mohms, time constants from 2 to 8 msec and membrane potentials from -45 and -70 mv. In the presence of atropine ($5x10^{-6}$M), synaptic potentials were evoked by stimulating preganglionic fiber tracts and abolished by adding hexamethonium ($5x10^{-4}$M) to the recording bath. With the addition of acetylcholine ($5x10^{-4}$M), both membrane potential and input resistance were reduced. When norepinephrine (10^{-5}M) was added, the amplitude of the evoked potential was diminished and sometimes abolished, the effect being blocked by phentolamine ($5x10^{-5}$M) but not by propranolol ($5x10^{-5}$M). In addition, neither membrane potential nor input resistance was appreciably changed. Our results support the hypothesis that norepinephrine inhibits cholinergic transmission acting via presynaptic alpha receptors and suggest that catecholamines dilate the airways, in part, by blocking transmission in airway ganglia. (Supported by Parker Francis Fellowship and NIH Grant # HL27319).

18.5

Alpha-adrenergic contractions induced by stimulation of sympathetic nerves in the tracheas of anesthetized dogs. James K. Brown* and Cary Jones* (Spon: W.M.Gold). Department of Medicine, VA Medical Center, San Francisco CA 94121 and CVRI, UCSF.

In 13 dogs receiving a beta-adrenergic inhibitor, we tested the effect on tracheal tension of electrical stimulation of sympathetic fibers in the superior and recurrent laryngeal nerves. In 7 dogs treated with atropine (0.5 mg/kg iv, to inhibit contractions induced by stimulation of parasympathetic fibers in the same nerves), supramaximal stimulation (30V,20Hz) did not alter tension. After tracheal injections of histamine, repeat stimulation produced contractions (14±3 g/cm), which were inhibited by injections of bretylium (to block release of transmitter from adrenergic nerves) or yohimbine ($6x10^{-8}$mol/kg, to inhibit alpha-2 responses) but not prazosin ($6x10^{-8}$mol/kg, to inhibit alpha-1 responses). In 6 other dogs, frequency dependence of cholinergic contractions, produced by stimulation (30V) before atropine, was compared to that of alpha-adrenergic contractions, produced by stimulation after atropine and histamine. Alpha-adrenergic contractions were 56-90% of cholinergic contractions at low frequencies (2-6Hz) and 37-47% at higher frequencies (10-20Hz). We concluded that tracheal contractions induced by stimulation of sympathetic nerves were present only after histamine, were mediated by alpha-2 receptors, and were of significant magnitude, compared to cholinergic contractions, particularly at low frequencies of stimulation. (Supported by New Investigators Research Award HL-27669, Research Advisory Group of the VA, and Academic Senate, UCSF)

18.6

ATROPINE PREVENTS THE REFLEX TRACHEAL RELAXATION ARISING FROM THE STIMULATION OF INTESTINAL AND GRACILIS MUSCLE AFFERENT ENDINGS. K.J. Rybicki* and M.P. Kaufman* (SPON: J.H. Mitchell). Univ. of Texas Health Science Ctr., Dallas, TX 75235

In previous studies, we have shown that stimulation of afferent endings in both the intestine and gracilis muscle reflexly relaxed tracheal smooth muscle in dogs. In the present study we examined the efferent limb of these reflex arcs. In 5 chloralose anesthetized dogs we reflexly evoked decreases in tracheal tension both by topical application of capsaicin (200 µg/ml) to the small intestine and by the injection of capsaicin (20 µg) into the gracilis artery, maneuvers which decreased tracheal tension by 8±1 and 7±1 grams, respectively. Following I.V. administration of 1.5 mg/kg atropine methyl nitrate, a cholinergic blocking agent, the decreases in tension evoked by both of these maneuvers were abolished. In addition, in 3 dogs we attempted to block these reflex tracheal relaxations with butoxamine (4 mg/kg), a β_2-adrenergic blocking agent. However, butoxamine had little or no effect on these reflex decreases in tracheal tension. We conclude, therefore, that the efferent pathway for tracheal smooth muscle relaxation evoked by the stimulation of intestinal and gracilis muscle afferents is cholinergic. (Supported by the Lawson and Rogers Lacy Research Fund and Program Project HL06296)

18.7

POST-SYNAPTIC POTENTIALS (PSPs) IN FERRET PARATRACHEAL GANGLION CELLS. R.F. Coburn and A.R. Cameron.* Dept. of Physiology, Univ of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have previously described a preparation suitable for study of the electrical properties of individual cells in the ferret paratracheal ganglion. The preparation consists of a network of neurons on the posterior surface of trachealis muscle including: input nerves arising in the recurrent and inferior laryngeal nerves (LN), interganglionic nerve trunks (IGN), and ganglia. Two types of ganglion cells have been identified on the basis of electrical properties:"A" which exhibit action potentials with intracellular cathodal current injection, and "B" which do not. We report now on the pattern of PSPs resulting from electrical stimulation of LN and IGN. Sixteen "A" and 36 "B" cells were studied in preparations from 18 animals.

Cell Type	LN	IGN
"A"	fast EPSP (9)	fast EPSP (3)
	no response (0)	antidromic response (2)
		no response (2)
"B"	fast IPSP (1)	slow EPSP (15)
	no response (7)	fast IPSP (6)
		no response (7)

These findings indicate "A" cells receive presynaptic input from LN. Axons from some of these cells run in the IGN and may allow communication between adjacent ganglia. Type "B" cells which did not receive input from the LN but which appear to have multiple presynaptic inputs may be involved in integration and modulation of LN input.(Supported by HL 19737 from NHLI).

18.8

Comparative Distribution of Smooth Muscle Contractile Responses in Canine Trachea and Bronchus in situ. A.R.Leff,N.M.Munoz* and S.Hendrix*. Dept. of Medicine,Univ. of Chicago,Chicago,IL 60637

We compared the responses of canine tracheal and bronchial smooth muscle to intravenously (iv) and intra-arterially (ia) administered constrictor agonists in 28 mongrel dogs. Tracheal and bronchial responses were measured isometrically *in situ* (Am Rev Respir Dis.125:223,1982) in the same dogs. In 9 dogs, dose-response curves were generated with ia acetylcholine (ACh) and histamine (10^{-10} to 10^{-6} mol) in a 4 cm tracheal segment and a 1 cm segment of third order canine bronchus. The tracheal response to ia histamine was 31.0 ± 7.5% of the response to equimole doses of ACh ($p < 0.001$); in bronchus, the contraction caused by ACh and histamine was nearly identical. In 5 dogs with β-adrenergic blockade, contraction to 10^{-8} to 10^{-6} mol ia norepinephrine (NE) was 33.3 ± 2.5% of the response to ACh in trachea. However, contraction to NE was 219 ± 26% of the response to equimole doses of ACh in bronchus ($p < 0.001$). Similar ratios for α-adrenergic/cholinergic contraction were obtained with iv methacholine and NE after β-blockade. Phentolamine (PA) 200 - 400 µg/kg ia caused complete blockade of the tracheal and bronchial response to ia NE in β-blocked dogs. We demonstrate substantial heterogeneity in the contractile responses of trachea and bronchus in situ. Relative to cholinergic contraction, both histamine and α-adrenergic stimulation cause substantially greater smooth muscle contraction in bronchus than in trachea. (Supported by NHLBI Grant HL-28806).

18.9

AIRWAY SENSITIVITY TO INHALED OUABAIN AND HISTAMINE IN CONSCIOUS GUINEA PIGS. Krishna P. Agrawal. Mayo Clinic/ Foundation, Rochester, MN 55905

The tracheal tissue of guinea pigs with high in vivo sensitivity to histamine shows high levels of lysophosphatidylcholine (LPC) and increased (Na^+-K^+)-ATPase activity. (Na^+-K^+)-ATPase, which may be activated by Na^+ loading of the cell through ion pores formed by LPC, would repolarize the cell membrane. Its inhibition by ouabain would produce membrane depolarization and smooth muscle contraction, as shown by Souhrada and Souhrada (Res.Physiol.47:69,1982) in isolated guinea pig tracheal smooth muscle. We wanted to know how closely this excitatory effect of (Na^+-K^+)-ATPase inhibition was related to airway sensitivity to histamine in vivo. The doses of ouabain and histamine producing a 30% fall in specific airway conductance were determined in intact conscious guinea pigs, giving 30 seconds exposure to aerosols of increasing concentrations of ouabain or histamine (.03 - 1%), at an interval of two days. A highly significant direct correlation ($r = .76$, $p < .002$) was observed between them. The results show that inhibition of (Na^+-K^+)-ATPase produces a bronchoconstrictive effect proportional to the airway sensitivity to histamine. Determination of airway sensitivity to ouabain may provide the same information about airway reactivity as do exercise or isocapnic hyperventilation breathing cold dry air, which also seem to act through inhibition of (Na^+-K^+)-ATPase by cooling of the airways. (Supported by grants from the Parker B. Francis Foundation and USPHS HL-21584).

18.10

RELATIONSHIP BETWEEN COUGH AND BRONCHOCONSTRICTION INDUCED BY DISTILLED WATER AEROSOL. D. Sheppard,* H.A. Boushey, R.A. Bethel,* San Francisco General Hospital and Cardiovascular Research Institute, San Francisco, CA 94110.

We studied the relationship between the cough and bronchoconstriction caused by inhaled distilled water aerosol in 8 subjects with asthma by measuring specific airway resistance (SRaw) and recording cough while subjects breathed serially increasing volumes of distilled water or normal saline aerosol produced by an ultrasonic nebulizer. We performed the distilled water dose-response curves after no treatment and after treatment with cromolyn (40 mg), lidocaine aerosol, or atropine aerosol in doses of 0.2 mg and 2.0 mg on separate days. Without prior treatment, distilled water aerosol caused cough in 7 of 8 subjects and a marked increase in SRaw in every subject, whereas only one subject coughed and no subject had a greater than 50% increase in SRaw after inhalation of saline. The two doses of atropine caused an equivalent reduction in baseline SRaw, but 2.0 mg caused greater inhibition of water-induced bronchoconstriction than did 0.2 mg. Neither dose of atropine inhibited cough. These data suggest that water-induced bronchoconstriction involves cholinergic nerves, and that water-induced cough is not dependent on bronchoconstriction. Lidocaine inhibited cough, but not bronchoconstriction, whereas cromolyn inhibited bronchoconstriction but not cough, suggesting that cromolyn does not inhibit bronchoconstriction by an inhibitory effect on airway afferent nerves. (Supported in part by Cal. Air Res. Contract #AO-13232)

18.11

EFFECT OF DL-PROPRANOLOL (PR) ON AIRWAY AND ON CARDIOVASCULAR RESPONSES TO INHALED HISTAMINE IN AWAKE GUINEA PIGS. C.J. Setzer*, E.H. Boykin*, J.A. Graham* and M.J. Wiester. U.S.EPA, Research Triangle Park, NC 27711 and Northrop Services Inc., Research Triangle Park, NC 27709.

Threshold doses of histamine aerosol were determined by using changes in dynamic compliance (C_{dyn}) as the measurement of response in male Hartley guinea pigs (682 ± 20 g) pretreated with 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/kg Pr (i.p.). Arterial pressure and blood gas responses to histamine were observed in animals that had received 5 mg/kg Pr. Intrapleural and carotid artery catheters were implanted one day prior to the experiment. Pulmonary measurements, ECG, blood pressure and blood gases were obtained during histamine challenges with the g. pig sealed in a body plethysmograph. Administration of Pr alone did not influence C_{dyn}, tidal volume, breathing frequency, minute volume, inspiratory flow or airway resistance. Thirty minutes after Pr injection, heart rate and blood pressure were significantly depressed. Heart rate decreased exponentially with Pr dose, 377 ± 75 beats/min without Pr to 249 ± 21 beats/min with 10 mg/kg. Mean blood pressure dropped from control values of 74 ± 4 to 65 ± 5 mm Hg with 5 mg/kg. Propranolol had no effect on the initial threshold response to histamine aerosol over the entire dose range used. It did, however, have a very profound effect on the animals' ability to recover from the histamine challenge. Whereas control g. pigs were essentially back to normal 6 min after the histamine challenge, most of the g. pigs that had received Pr died within 3 minutes; 33% at 1 mg/kg to about 80% at 10 mg/kg Pr.

18.12

MECHANISM OF AIRWAY CONSTRICTION AND SECRETION EVOKED BY LARYNGEAL ADMINISTRATION OF SO_2 IN DOGS. H.D. Schultz, A.M. Roberts*, H.L. Hahn*, J.A. Nadel, H.M. Coleridge, and J.C.G. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Low concentrations of SO_2 cause bronchoconstriction in asthmatic patients. Since low concentrations of SO_2 may be totally absorbed in the upper airways and since the upper airways appear to be very sensitive to SO_2, we have explored the possibility that SO_2 evokes reflex effects by engaging afferent nerves in the upper airways. We recorded smooth muscle tension in an innervated segment of the upper trachea in anesthetized dogs. To examine tracheal submucosal gland secretion, we coated the mucosa of the segment with tantalum powder and counted the rate of appearance of secretion "hillocks" in the tantalum layer. SO_2 (200-500 ppm) delivered to the larynx through the cranial stump of the trachea for 2 min caused tracheal smooth muscle contraction and increased submucosal gland secretion. Effects were reduced or abolished by section of the superior laryngeal nerve (SLN). To investigate the afferent arm of this reflex, we recorded impulses from SLN. Many fibers (conduction velocity, 20-40 m/sec) were stimulated by SO_2 (50-200 ppm); some responded to concentrations as low as 3 ppm. Afferent endings stimulated by SO_2 usually had a sparse and irregular control discharge and were located above the vocal cords. We suggest that the reflex effects of SO_2 delivered to the upper airways are mediated by these afferent endings. (Supported by HL-24136 and HL-07192 from NHLBI.)

18.13

AFFERENT VAGAL C-FIBERS ARE RESPONSIBLE FOR THE REFLEX AIRWAY CONSTRICTION AND SECRETION EVOKED BY PULMONARY ADMINISTRATION OF SO_2 IN DOGS. A.M. Roberts*, H.L. Hahn*, H.D. Schultz, J.A. Nadel, H.M. Coleridge, and J.C.G. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Low concentrations of SO_2 induce bronchoconstriction in asthmatic patients, an effect prevented by atropine. SO_2 also stimulates airway secretion. We have examined the mechanism of these effects in anesthetized dogs. We recorded smooth muscle tension in an innervated segment of the upper trachea. To examine tracheal submucosal gland secretion, we coated the mucosa of the segment with tantalum powder and counted the rate of appearance of secretion "hillocks" in the tantalum layer. Administration of 200-500 ppm SO_2 for 2 min to the lower airways caused tracheal smooth muscle contraction, which occurred in two distinct peaks, and increased submucosal gland secretion. Both effects were abolished by cooling the vagus nerves to 0°C. To identify the afferent arm of this reflex we recorded afferent impulses from vagal fibers with endings in the lower respiratory tract. Of 34 C-fibers arising from the lower trachea, bronchi or lungs, 15 were stimulated. Firing sometimes occurred in 2 or more distinct peaks whose timing corresponded with that of the peaks in tracheal tension. Effects on slowly adapting stretch receptors and irritant receptors were small. We conclude that SO_2 stimulates lung and airway vagal C-fibers to evoke airway constriction and submucosal gland secretion. (Supported by HL-24136 and HL-07192 from NHLBI.)

18.14

SULFUR DIOXIDE INDUCED BRONCHOCONSTRICTION IN FREELY BREATHING, EXERCISING, ASTHMATIC SUBJECTS. R.A. Bethel,* J. Epstein,* D. Sheppard,* J.A. Nadel, and H.A. Boushey. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143

We sought to determine if low concentrations of ambient sulfur dioxide (SO_2) caused bronchoconstriction in 10 subjects with mild asthma as they exercised in an exposure chamber, breathing freely through the nose and mouth. We determined specific airway resistance (SRaw) before and after each subject exercised for 5 min on a cycle ergometer at a moderately heavy workload (750 kilopond meters/min). In the first phase of the study, the subject breathed filtered air containing 0.5 ppm SO_2 on one day and SO_2-free air on another day. In the second phase, the subject breathed 0.25 ppm SO_2 on one day and SO_2-free air on another. The order of administration of SO_2 or SO_2-free air was randomized and was double blind. The chamber temperature and relative humidity and the subject's SRaw before exercise did not differ significantly between SO_2 and SO_2-free trials. The mean change in SRaw ± SD (L x cm H_2O/L/s) from before to after exercise was:

First phase	no SO_2	2.2 ± 2.3	0.5 ppm SO_2	13.6 ± 9.2
Second phase	no SO_2	3.6 ± 4.8	0.25 ppm SO_2	7.1 ± 6.1

Thus, 0.5 ppm SO_2 caused significant bronchoconstriction in asthmatic subjects performing moderately heavy exercise and breathing freely. 0.25 ppm SO_2 caused a lesser degree of bronchoconstriction. (Supported in part by USPHS Grants HL-24136, HL-07185, and Cal. Air Resources Contract #AO-13232)

18.15

RELATIONSHIP BETWEEN NONSPECIFIC BRONCHIAL REACTIVITY AND PERIPHERAL AIRWAYS OBSTRUCTION IN SMOKERS. K. Clark*, S. Khaladkar*, K. Bergstrom* and J.A. Dosman, Section of Respiratory Medicine, Department of Medicine, University of Saskatchewan College of Medicine, Saskatoon, Canada. S7N OXO.

We measured the concentration of inhaled aerosolized histamine acid phosphate required to reduce forced expired volume in one second (FEV_1) by 10% (PC_{10}) in 12 heavy smokers shown to respond to inhaled histamine (mean age ± 1 SD: 38.4 ± 9.4 yr; mean pack years 23.0 ± 10.5). We also measured maximum expiratory flow volume curves with air and also a mixture of 80% helium and 20% oxygen (HeO_2) to determine the percent increase in maximal flow at 50% vital capacity breathing HeO_2 as compared to air ($\Delta\dot{V}max50$), and the slope of phase III of the single breath oxygen test ($\Delta N_2/\ell$). In the smokers, PC_{10} ranged from 1.4 mg/mℓ to 10.2 mg/mℓ (mean 5.5 ± 3.3 mg/mℓ) and $\Delta\dot{V}max50$ ranged from 7.1% to 68.4% (mean 39.6 ± 18.3%). There was a significant positive correlation between $\ell n\ PC_{10}$ and $\Delta\dot{V}max50$ ($r = .78$, $p < .01$). Similarly, there was a significant correlation between $\ell n\ PC_{10}$ and $\Delta N_2/\ell$ ($r = -.57$, $p < .05$) and between $\ell n\ PC_{10}$ and pack years ($r^2 = -.41$, $p < .05$). These results suggest that smokers with evidence of peripheral airways obstruction have greater nonspecific airways reactivity than smokers without evidence of peripheral airways obstruction (Supported by the Medical Research Council of Canada).

18.16

EFFECT OF INSPIRATORY GAS TEMPERATURE ON NONSPECIFIC BRONCHIAL REACTIVITY IN ASTHMATIC PATIENTS. W.C. Hodgson*, D.J. Cotton, D.W. Cockcroft* and J.A. Dosman, Section of Respiratory Medicine, Department of Medicine, University Hospital, University of Saskatchewan College of Medicine, Saskatoon, Canada. S7N OXO.

We measured the extrapolated concentration of inhaled histamine acid phosphate required to reduce the forced expired volume in one second (FEV_1) by 20% (PC_{20}) in 8 asthmatic patients (mean age ± 1SD: 26.9 ± 11.6 yr) both while inhaling warm dry air (temperature: 25.0 ± 1.5°C, water content: 2.37 ± 0.02 mg H_2O/ℓ) and while inhaling cold air (-18.8 ± 1.9°C, 1.19 ± 0.18 mgH_2O/ℓ). Patients alternatively breathed warm or cold air [minute ventilation ($\dot{V}_E$) warm air: 8.11 ± 1.31 ℓ/min; $\dot{V}_E$ cold air: 8.54 ± 2.81 ℓ/min; respiratory heat exchange (RHE) warm air: 0.23 ± 0.04 Kcal/min; RHE cold air: 0.37 ± 0.14 Kcal/min] for 10 minutes prior to and also during 1 minute challenges with doubling concentrations of aerosolized histamine ($\dot{V}_E$ warm challenge: 11.00 ± 2.73 ℓ/min; $\dot{V}_E$ cold challenge: 12.54 ± 3.60 ℓ/min). Cold air PC_{20} (0.63 ± 4.26 mg/ml) was significantly lower than warm air PC_{20} (2.54 ± 2.41 mg/ml), ($p < .01$, paired t test). Five of 8 patients reduced PC_{20} by two or more doubling concentrations during cold as compared to warm air breathing. These results suggest that during cold air breathing, nonspecific bronchial reactivity is increased in asthmatic patients. (Supported by Medical Research Council of Canada).

COMPARATIVE PHYSIOLOGY: OSMOTIC AND IONIC REGULATION I

19.1

AN EPHEMERAL URINARY BLADDER IN NEONATAL LIZARDS: A WATER STORE FOR SURVIVAL? C.A. Beuchat, E.J. Braun and D. Vleck, Depts. of Physiology, and Ecology and Evolutionary Biology. University of Arizona, Tucson, AZ 85724.

The iguanid lizard species Sceloporus jarrovi and Urosaurus ornatus do not have a urinary bladder as adults, but neonates of both species possess a bladder that is fluid-filled at birth (S. jarrovi) or hatching (U. ornatus). In both species, the bladder has degenerated in individuals only four weeks old. In neonates of the viviparous species S. jarrovi, the osmolality of the bladder fluid, which comprised about 10% of the mass at birth, averaged 50 mosm/kg H_2O, while plasma averaged 300 mosm/kg. In contrast, bladder fluid in hatchlings of the oviparous species U. ornatus is isosmotic with plasma (~325 mosm/kg). Four days after initial urine collection at birth, neonatal S. jarrovi given access to water had refilled their bladders. The osmolality of this fluid averaged 76 mosm/kg. The low solute concentration in bladder fluid of neonatal S. jarrovi may result because the embryos can exchange salts and water with their mother across the placenta. The large amount of hypoosmotic (S. jarrovi) to isosmotic (U. ornatus) fluid in bladders of neonatal lizards could represent a significant reserve of water to prevent desiccation in the first few days of life. NSF DEB 8202880; NIH AM 16294.

19.2

EPIDERMAL LIPIDS AND INTEGUMENTARY WATER LOSS IN REPTILES. J.B. Roberts* and H.B. Lillywhite, Univ. of Kansas, Lawrence KS 66045.

Rates of cutaneous evaporative water loss (CWL) were measured from shed epidermis of 12 species of squamate reptiles from widely ranging habitats. Rates of CWL were inversely related to habitat aridity and increased 2.6 to 32.3-fold when lipids were extracted prior to measurements. Lipid extraction from epidermis of one xeric-adapted species, Crotalus vegrandis, increased CWL to rates comparable to that from a free water surface. Measurements from epidermis of albino and scaleless mutants indicate that cutaneous pigments and scale structure per se do not significantly influence CWL. The data indicate that epidermal lipids play a major role in limiting CWL of sequamates. Lipids were demonstrated histochemically to be located primarily in the mesos layer of the epidermis, with some lipid also located in the alpha and beta keratin layers. This corroborates recent ultrastructural findings published by L. Landmann. The lipid barrier appears to function bidirectionally and is present in both dorsal and ventral integument of at least some species. Neither quantity of lipid removed nor biological transition points of epidermal lipids were related to rates of CWL.

19.3

SERINE METABOLISM IN THE GILL OF THE RIBBED MUSSEL (Modiolus demissus). Lehman L. Ellis, James M. Burcham and Stephen H. Bishop. Iowa State University, Ames, IA 50011

During isosmotic adaptation to increasing environmental salinities, the tissue concentrations of serine, and glycine increase. The small amounts of serine and glycine synthesized from glucose [(^{14}C) tracer experiments] do not account for the actual glycine or serine accumulation. Serine is catabolized to pyruvate by serine dehydrase and to glycine by mitochondrial and cytosolic serine hydroxymethyltransferase (SHMT). Both pyruvate and glycine are catabolized in the mitochondria. Chloroalanine (CA) and aminooxyacetic acid (AOA) are strong inhibitors of SHMT and weak inhibitors of SD. [^{14}C]-CO_2 production from tissues incubated with [^{14}C]-serine is low at high salinity and high at low salinity. Addition of AOA and CA to tissues incubated at high and low salinity with [^{14}C]-serine blocked serine catabolism. The principle catabolic route for serine metabolism is probably through the SHMT and mitochondrial glycine catabolism. This pathway is inhibited in tissues incubated at high salinity. Serine accumulation in the tissues during hyperosmotic stress is apparently due to reduced catabolism of serine derived from preformed sources (proteins, peptides, phospholipids). (Supported in part by NSF Grant PCM-80-22606.)

19.4

CHARACTERIZATION OF ASPARATE OF AMIONTRANSFERASE (AAT) ISOZYMES FROM BIVALVE (Modiolus demissus) GILL TISSUE. K.T. Paynter,* L.L. Ellis, J.M. Burcham and S.H. Bishop. Iowa State University, Ames, Iowa 50011

Asparate levels and turnover change during cellular osmoregulation and anaerobiosis. Some regulation of aspartate metabolism may be due to specific differences in the cytosolic (cAAT) and mitochondrial (mAAT) isozyme forms. Both were purified from tissue homogenates by salt fractionation, heat treatment and hydroxylapatite chromatography. With starch gel-electrophoresis (Tris-borate, pH 6.5), the cAAT migrated cathodally and the mAAT migrated anodally. All tissues had the same electrophoric forms and the cAAT showed some polymorphism among individual animals (3 allele system). The mAAT was less heat stable than the cAAT. Although both isozymes were inhibited by aminooxyacetic acid (I_{50} = 2-3 μM), neither were inhibited by up to 10 mM vinylyglycine or 3-chloroalanine. The apparent K_m's for aspartate and 2-oxyglutarate were 5.2 mM and 0.044 mM, respectively, for the cAAT and 0.59 mM and 0.58 mM, respectively, for the mAAT. Differences in properties of the AAT's may account for the low aspartate levels in the mitochondia and the accumulation of high aspartate levels in the cytosol. (Supported in part by NSF Grant PCM-80-226606)

19.5

SIMULTANEOUS DETERMINATION OF NET FLUX FOR 14 AMINO ACIDS IN TETRAHYMENA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. James P. Davis* and Grover C. Stephens.
University of California, Irvine, Irvine, CA. 92717

Kinetics of entry of ^{14}C-labelled aspartate, glycine and leucine were studied by following the decrease of radioactivity in an inorganic medium containing an axenic suspension of Tetrahymena. Influx was apparently mediated in part by carrier systems which showed half-maximum rates at 2.2 - 9.5 µM. However, data suggest that entry also occurred by way of a nonsaturable or unsaturated pathway. Simultaneous fluorometric determination of changes in primary amines in the medium showed a steady increase with time at substrate concentrations less than 25 µM; observations at 70 µM showed a net decrease in primary amines with time. Changes in the ambient level of 14 amino acids supplied at initial levels of 0.5 µM and 5.0 µM were studied using high performance liquid chromatography (HPLC). There was a net increase in total amino acids in the medium at the lower substrate level (7.0 µM total) and a net decrease at the higher level (70 µM). Observance of a net increase or decrease in the medium of specific amino acids depended on the ambient level supplied initially and the particular substrate. We conclude that carrier-mediated influx of labelled substrate may be accompanied by net entry or net loss of that substrate. This work was supported in part by NSF Grant PCM 78-09576.

19.6

SALT AND WATER BALANCE IN METAMORPHOSING LARVAE OF THE BONEFISH (ALBULA). Edward Pfeiler. Instituto Tecnológico y de Estudios Superiores de Monterrey, Guaymas, Sonora, México 85400

The changes in water and Na^+, K^+ and Cl^- content of leptocephalous larvae of the bonefish were followed during metamorphosis. Metamorphosis is characterized by a period of pronounced shrinkage during which larvae may lose about half of their original body length in as few as 6 days. In addition to the resorption of the extracellular, gelatinous matrix, shrinkage is accompanied by a loss of approximately 382 mg of water and about 70 µEq of both Na^+ and Cl^-. This corresponds to a 78% loss of total body water, an 83% loss of Na^+ and a 91% loss of Cl^-. No significant change in K^+ content was noted. However, when the values are converted to a per kg wet weight basis, a 3-fold increase in K^+ concentration is seen during shrinkage, accompanied by a loss of about half of the Cl^- concentration. These results are consistent with the view that extracellular fluid volume decreases, and intracellular fluid volume increases, during metamorphosis. It is proposed that water loss is the primary cause of shrinkage and that resorption of the gelatinous matrix provides a source of energy for tissue formation and possibly osmotic and ionic regulation.

19.7

BRANCHIAL CHLORIDE CELLS IN GILLICHTHYS: ULTRASTRUCTURAL EVIDENCE FOR MEMBRANE RECYCLING IN THE APICAL REGION. Byron A. Doneen. Univ. of Michigan, Ann Arbor MI 48109

Branchial chloride cells (CC) secrete Na^+ and Cl^- in sea water-(SW) adapted fish, but absorb these ions after adaptation to fresh water (FW). Few models proposed to account for this directionality have considered that ion (and water) transport might be mediated by movement of apical vesicles between mucosal membrane and tubular system. T. Bradley (J. Exp. Zool. 217: 185, 1981) used osmium fixation to reveal interactions of tubular system and vesicles with the apical surface of CC in SW-Poecilia and Fundulus. The same fixation scheme has been used to examine Gillichthys adapted to 200% SW and to FW. Frequency of apical fusion events was increased in the hypersaline environment. Apical blebbing of the tubular system into extracellular spaces was confined to spaces also occupied by processes of adjacent CC. Some apical vesicles appeared to be derived from (or to fuse with) tubular system. FW-adapted CC displayed no interactions with adjacent CC, and showed reduced development of tubular and vesicular membranes. However, tubular system did penetrate to the apical surface. This region also contained dense and multivesicular bodies. Golgi and rough ER were prominent in the apices of FW-CC. These results support the existence of extensive membrane recycling in the apex of SW-CC. Membrane biogenesis appeared to be accelerated in FW-CC, but ultrastructural evidence for recycling was not obtained. Supported by NSF PCM-7922985.

19.8

OSMOREGULATION AND SALT GLAND Na,K-ATPase ACTIVITY FOLLOWING EXPOSURE TO THE ANTICHOLINESTERASE FENTHION. B. A. Rattner, W. J. Fleming* and H. C. Murray*. Patuxent Wildlife Research Center, Laurel, MD 20708.

Salt gland function and osmoregulation in aquatic birds drinking hyperosmotic water has been suggested to be impaired by organophosphorus insecticides. To test this hypothesis, adult ducks (Anas rubripes) were provided various regimens of fresh or salt (1.5% NaCl) water (FW,SW) and mash containing vehicle or 21 ppm fenthion (Fn) on days 1-7 and 7-12 of this study. The 8 treatments (day 1-7:day 7-12) included FW:FW, FW:FW+Fn, FW:SW, FW+Fn:SW, FW:SW+Fn, FW+Fn:SW+Fn, SW:SW and SW:SW+Fn. Ducks were bled by jugular venipuncture on days 1, 7 and 12, and then sacrificed. Brain and salt gland acetylcholinesterase activities were substantially inhibited (44-52% and 14-26%) by Fn. However, plasma Na, Cl and osmolality, as indirect but cumulative indices of salt gland function, were uniformly elevated in all SW groups including those receiving Fn. In a second experiment, salt gland Na,K-ATPase activity was reduced after in vitro incubation with DDE (40 and 400 uM; positive control), but was unaffected by Fn and its oxygen analog (0.04-400 uM). The present findings suggest that environmentally realistic concentrations of organophosphorus insecticides do not affect osmoregulatory function in adult ducks.

19.9

EFFECTS OF SOLUTES ACCUMULATED DURING DESICCATION ON THE PHYSICAL PROPERTIES OF MODEL INTRACELLULAR FLUID. Mary E. Clark. San Diego State Univ., San Diego, CA 92182-0057

The three classes of organic solutes that are accumulated in large quantities by partially desiccated organisms (polyols, selected amino acids, and methylated amines, especially TMAO [trimethylamine-N-oxide]) have been studied with respect to their effects on several important physical properties of model intracellular solutions. Increasing concentrations of glycerol, TMAO, and amino acids markedly decrease the self-diffusion coefficient of water in a linear and additive manner. At 1.0 M, TMAO reduces the relative self-diffusion coefficient by 30%; the effects of glycerol and amino acid mixtures are slightly less. These solutes, particularly TMAO, significantly alter the activities of monovalent cations, measured by specific ion electrodes. At 0.5 M, TMAO decreases the activity of potassium ion between 30 and 40% and increases the activity of chloride ion between 5 and 10% over the physiological salt concentration range. Finally, these solutes have parallel effects on the dielectric properties of dilute salt solutions. Thus, neither water molecules nor simple ions retain their conventional properties in the presence of these organic solutes. The results may help explain the stability of native macromolecular structure at high ionic strengths, such as occur in hyperosmotic environments, during dehydration, or during freezing.

20.1

CAPILLARY DISTRIBUTION IN CAT PAPILLARY MUSCLE. Barbara M. Doerr* and Evangelyn W. Kanabus. The Ohio State University, Columbus, Ohio 43210.

To determine diffusional parameters in order to relate O_2 transport to heart muscle performance, we measured capillary density (CD), intercapillary distance (ICD), and distance-to-nearest-capillary (DtC) in six right ventricular papillary muscles. Muscles were excised from perfused hearts, frozen at their in situ diastolic lengths, and 12 µm crosssections stained for capillary endothelium. Counts from 2-4 microscopic fields (0.192 ± 0.001 mm^2) in each muscle gave an average CD of 2098 ± 398 capillaries/mm^2. Mean ICD, estimated using a hexagonal model (1), was 22.3 ± 7.8 µm. The frequency distribution of distances from random points in the tissue to the nearest capillary, measured by the closest individual method (2), was right-skewed with a mode of 6.5 µm (mean 9.5 ± 4.3 µm). Our findings agree with literature values of diffusional distances in the myocardium. These values, along with the parallel fiber orientation found in the papillary muscle, allow realistic predictive modeling of O_2 levels in the heart muscle. (Supported by the Central Ohio Heart Chapter)

(1) Vetterlein, et al. Am. J. Physiol. 242:H133, 1982.

(2) Kayar, et al. Fed. Proc. 41:1759, 1982.

20.2

MYOCARDIAL PERFUSION AND CAPILLARY DENSITY IN EXERCISE TRAINED PIGS WITH CARDIAC HYPERTROPHY

F.C. White, E.A. Breisch, B. Guth and C.M. Bloor, UCSD School of Medicine, La Jolla, California, 92093.

Experimentally induced cardiac hypertrophy (CH) results in decreased capillary density (CD) and increased cross-sectional area (CA) of myocardial cells. Superimposed physiologic stress (PS) induces subendocardial underperfusion. It is not known how these parameters are affected when CH is induced by exercise training. Four Yucatan pigs instrumented with catheters in the aorta and left atrium were exercise trained (EXT) for 11 weeks. Myocardial perfusion was obtained by microspheres at 10 weeks while the pigs were exercising at maximal heart rate. Arterial pO_2 remained normal. Myocardial blood flow per unit mass of myocardium was similar in both EXT pigs and C. Compared to 15 instrumented sedentary pigs (C), EXT pigs during PS had decreased subendocardial flow resulting in decreased endo-epi flow ratios (.92±0.05 for EXT vs. 1.04±0.04 for C, $p<0.05$). At autopsy EXT pigs had increased left and right ventricular weights, 31% and 38%, respectively, compared to C ($p\leq0.05$). CD was decreased in EXT pigs (1379±70/mm^2 vs 1650±75/mm^2, $p<0.01$) while CA increased in EXT pigs 561µm^2 vs 406 µm^2, $p<0.001$). Although EXT may induce CH, the stress did not induce comparable hyperplasia of the myocardial microvasculature. This may cause greater subendocardial underperfusion when PS is superimposed.

20.3

CAPILLARY TRANSPORT RESERVE IN THE CORONARY CIRCULATION OF NORMAL DOGS. M. Harold Laughlin, K. Rouk* and C. Mitchell*. Dept. of Physiol., Oral Roberts Univ. Med. School, Tulsa, OK 74171

The purpose of these studies was to determine normal coronary capillary transport reserve (CTR). Myocardial extractions and permeability surface area products (PS) of ^{51}Cr EDTA were determined with the single injection indicator diffusion method in intact working hearts of 11 anesthetized dogs. The left anterior descending branch of the left coronary artery was cannulated and pump-perfused while mean aortic, central venous and coronary perfusion (CAP) pressures, heart rate, ECG, and coronary flow were continuously monitored. All parameters were measured in the control state at 4-6 pts along the pressure-flow autoregulation curve. Maximal vasodilation was then produced with ia adenosine (A) or A+ α receptor blockade with prazosin. All parameters were then measured at constant flow (F) and at 3 different pressures (80-120 Torr). Results are: (PS-ml/min/100g)

	Control		Max Vasodilation Constant F		Constant P		α Block	
	CAP	PS	CAP	PS	CAP	PS	CAP	PS
X	101	17	46	12	99	24	97	28
SE	3	2	3	1	5	3	12	5

Maximum vasodilation with constant coronary perfusion pressure resulted in a 200% increase in plasma flow. The CTR of the coronary bed, defined as the difference between resting PS and PS obtained during maximal vasodilation under constant pressure conditions, is 7 ml x min^{-1} x $100g^{-1}$ or 44%. Supported by NIH #HL26963.

20.4

THE EFFECT OF MYOCARDIAL ISCHEMIA ON CORONARY PERFUSED CAPILLARY DENSITY. D.M. Cohen,* P.F. McDonagh, J. Suaudeau,* and H. Laks*. Depts. of Surgery, Yale Univ. New Haven, CT 06510 and Univ. of Calif. Los Angeles, CA 90024

To determine the effects of ischemia and reperfusion on myocardial perfused capillary density, rat hearts were excised, kept ischemic for thirty minutes then reperfused with blood from a support animal. Control hearts were isolated and perfused for either 10 or 60 minutes before injection of a vascular marker (Monastral Blue-blood mixture, 30 sec at 100 mmHg). The ischemic hearts were perfused with Blue after 10 minutes of reperfusion. The perfused capillary density (e) and capillary/fiber ratio (C/F) were measured from frozen transverse sections of the left ventricle. For 10 and 60 minute control hearts, the epicardial e values were 2324±476 caps/mm^2 and 2378±330 SD. The endo/epi e ratio was not significantly different from unity for either control group. For the ischemic group, e was significantly decreased both in the epicardium (707±515 caps/mm^2) and endocardium (130±30 caps/mm^2) ($P<0.05$). The endo/epi e ratio was significantly less than unity for the ischemic group ($P<0.05$). The C/F ratio analysis yielded the same results. The decrease in both e and CF ratio indicate that 30 minutes of ischemia followed by 10 minutes of reperfusion caused a marked "no-reflow" phenomena in isolated, supported hearts. The no-reflow was most pronounced in the endocardium. (Supported by NIH HL 24328)

20.5

ADENOSINE CONCENTRATIONS IN CARDIAC LYMPH DURING ADMINISTRATION OF ISOPROTERENOL. Jack E. McKenzie, Booker T. Swindall*, Jodi Johnston*, and Francis J. Haddy. Uniformed Services University, Bethesda, Md. 20814

To evaluate the release of adenosine by the heart, adenosine concentrations in cardiac lymph were studied in five open chest anesthetized dogs. A main cardiac lymph vessel was isolated and cannulated (PE 90 or 190) within the pericardium, proximal to the first lymph node. Verification of cardiac lymph was made after the study by subepicardial injection of Patent Blue dye. All animals were primed with 1L of Krebs solutions and lymph flow was sustained with a 5.3 ml/min infusion of the same solution. Cardiac performance was increased by intravenous infusion of isoproterenol(I) at 3.9 mg/min. This infusion of I increased dP/dt 2.2 times with little change in mean arterial blood pressure. The lymph was collected in chilled tubes of perchloric acid (1N) and samples were processed for adenosine determination. Transit time for lymph from heart to collection was 2±2 seconds. The adenosine concentration was 19.2±2.1 pmoles/ml at control and increased to 83.0±2.1 pmoles/ml with I infusion. Values did not return to control within the 30-min recovery period, but did significantly decrease (39.6±1.7 pmoles/ml). Cardiac lymph flow (0.13±0.03, 0.13±0.03,and 0.18±0.05 ml/min) was used to calculate adenosine release: 2.3±0.7, 13.1±1.1, and 4.6±0.7 pmoles/min for control, I, and recovery samples, respectively. These data support the concept that adenosine is released from the heart during increased cardiac performance produced by I. (Support USUHS CO7635 & NIH G57601)

20.6

TISSUE ADENOSINE CONTENT REQUIRED TO MAINTAIN MAXIMAL CORONARY VASODILATION. Emma L. Bockman, H. Fred Downey, George J. Crystal and Fouad A. Bashour. Dept. Physiol.; Uniformed Serv. Univ., Bethesda, MD 20814 and Univ. Texas Health Sci. Ctr., Dallas, TX 75235.

To determine the tissue adenosine content (ADO) just sufficient to maintain maximal coronary vasodilation, the left anterior descending coronary artery (LAD) was occluded for 3'. Tissue samples were obtained by drill biopsy and freeze clamping (<2 sec) at the end of 3' of occlusion (3'-OC; N=5), at 28±7" after release of occlusion when blood flow had just reached a plateau (3'-Peak; N=6), and at 109±14" after release of occlusion when blood flow had just started to decrease (3'-DCR; N=6). Control blood flow was 31±5 ml/min and increased to 421±20% of the control value at 3'-Peak. At 3'-DCR, blood flow was 88±1% of the 3'-Peak. Control ADO (N=4) was 1.0±0.1 nmoles/g. ADO was 33±7 nmoles/g at 3'-OC and had decreased significantly to 18±5 nmoles/g at 3'-Peak. At 3'-DCR, ADO was 4.7±1.6 nmoles/g. ADO at 3'-DCR was similar to that after 90" occlusion (3.1±0.4 nmoles/g; N=7) which also causes maximal vasodilation but of a shorter duration. Intracoronary infusion of adenosine to achieve an LAD flow of 283±61% of control resulted in ADO=2.8±0.2 nmoles/g (N=3). Thus, ADO was similar when maximal coronary vasodilation was elicited in three different ways, including infusion of adenosine. These data indicate that changes in coronary blood flow from control to maximal values are associated with a narrow range of ADO of <10 nmoles/g. (Supported by USPHS Grants HL-26345 & HL-21657)

20.7

EFFECTS OF REGIONAL ISCHEMIA ON THE FUNCTIONAL AND METABOLIC STATE OF THE NON-ISCHEMIC SEGMENT. J. Vinten-Johansen,* H. Edwards,* G.D. Buckberg,* R.J. Barnard, E.R. Rosenkranz,* F. Okamoto,* and H. Bugyi*. UCLA Med. Ctr., Los Angeles, Ca. 90024.

Acute myocardial ischemia abolishes contraction and severely depletes energy levels (ATP) in the affected area. This places an increased functional burden on the normally perfused region in order to maintain adequate systemic circulation. This study documents changes in contractile function and ATP levels in the ischemic and non-ischemic compensating zones after reperfusion. In thoracotomized dogs, ultrasonic dimension transducers were placed in the equatorial plane of the anterior (ischemic) and posterior (non-ischemic) left ventricle to measure segmental shortening. The proximal left anterior descending coronary artery (LAD) was ligated for 40 minutes and subsequently reperfused for two hours. Upon occlusion, the ischemic segment ceased contracting and showed continuous paradoxical motion throughout the study, while subendocardial ATP levels fell from 4.0±0.14 to 1.01±0.23 μMol/gm*. Conversely, the non-ischemic zone increased shortening by 41±10% to maintain cardiac output and pressure within normal limits, while a depletion of subendocardial ATP from 4.0±0.14 to 2.20±0.32 μMol/gm occurred. We conclude that compensatory increases in function of the non-ischemic zone results in depletion of subendocardial ATP stores. This depletion may be exacerbated when the normal zone is supplied by a stenotic vessel, resulting in compromise in compensatory effort and possible failure. (Supported in part by USPHS Grant HL16292).

20.8

ALPHA-SYMPATHETIC VASOCONSTRICTION IN SEVERELY STENOTIC CANINE CORONARY ARTERIES. G. Heusch*, A. Deussen*, G. Arnold. University Düsseldorf, FRG

Electrical stimulation of postganglionic cardiac sympathetic nerves was performed in 6 anesthetized, vagotomized dogs. Critical stenosis of circumflex coronary artery was defined by absence of any reactive hyperemia to a 15 s occlusion. Without stenosis, sympathetic stimulation decreased circumflex coronary resistance from 1.20 ± 0.13 to 0.90 ± 0.09* resistance units (RU) and increased O_2-consumption of circumflex-perfused myocardium from 7.5 ± 1.3 to 10.1 ± 1.4* ml/min · 100 g. With critical stenosis, sympathetic stimulation increased circumflex resistance from 2.30 ± 0.59 to 2.99 ± 0.89* RU; O_2-consumption was unchanged, but O_2-content of a cannulated circumflex vein fell from 3.9 ± 0.5 to 3.5 ± 0.5* ml/100 ml. After administration of phentolamine (2 mg/kg i.v.) sympathetic stimulation decreased stenotic circumflex resistance from 1.38 ± 0.24 to 1.16 ± 0.23* RU and increased O_2-consumption from 5.1 ± 1.9 to 6.8 ± 2.3* ml/min · 100 g. These data suggest that alpha-sympathetic vasoconstriction is unmasked in severe coronary stenosis and can be prevented by phentolamine.

* $p < 0.05$

20.9

SIMILARITY OF BLOOD FLOW-SYSTOLIC WALL THICKENING RELATIONS AT REST AND DURING EXERCISE IN DOGS. Kim P. Gallagher, Masunori Matsuzaki*, W.Scott Kemper*, John Ross,Jr.,Univ.of California, San Diego, La Jolla, CA 92093

To determine how steady-state exercise affects the relationship between regional myocardial blood flow (MBF)and regional myocardial function (measured as systolic wall thickening,%WT), ten dogs were instrumented to measure MBF (microspheres) and %WT (sonomicrometry). Variable degrees of coronary stenosis (CS)were produced with a hydraulic occluder on the circumflex artery at rest and during treadmill exercise.The slope of the relation between mean transmural MBF (ml/min/g)and %WT during exercise (y=11.6x-1.9,r=0.90)was significantly different from the resting relation (y=25.3x-2.1,r=0.80). When mean MBF per beat was plotted versus changes in %WT (fraction of control %WT at rest), however, no significant difference was detected in the slopes or intercepts of the two relations (exercise:y=102.34x-0.13,r=0.81; at rest:y=88.34x+0.02,r=0.61). Likewise, subendocardial MBF per beat versus changes in %WT at rest and during exercise were not significantly different.Thus, the level of contractile performance and flow per beat in both conditions were the same. Our findings suggest that "relative" ischemia with exercise plus CS exists only transiently, since progressive dysfunction follows which achieves a steady-state with reduced myocardial oxygen requirements. The steady-state is characterized by a level of regional contractile performance appropriate to the available blood flow which is the same, per beat, whether at rest or during exercise.

20.10

THE EFFECT OF REPERFUSION AFTER PROLONGED SUBENDOCARDIAL ISCHEMIA IN CONSCIOUS DOGS. Masunori Matsuzaki*, Kim P Gallagher, John Ross,Jr., Univ. of California, San Diego, La Jolla, California 92093

In 8 chronically instrumented dogs,we measured left ventricular(LV) pressure and systolic wall thickening(%WT,sonomicrometry) during 5 hours(Hr) of partial circumflex coronary stenosis(PCS,hydraulic occluder)and after reperfusion. Myocardial blood flow(MBF,microspheres) was measured before and after PCS,as well as after reperfusion. During PCS, %WT was continuously monitored, maintaining a reduction of %WT from -25% to -50% of control, an average reduction from control of -35.3%(%WT: 31.2±9.5 to 20.2±7.5%,p<0.01). MBF in the ischemic area at 4 Hr of PCS was reduced only in the subendocardial third(ENDO)(0.72±0.14 to 0.36±0.10 ml/min/g,p<0.01),accompanied by significant ST segment elevation on ENDO electrogram(ST, 0.8 to 4.2 mV,p<0.01), and decreased LV dP/dt(3565±461 to 2943±353 mmHg/sec,p<0.01). Within minutes after complete release of PCS, ENDO ST returned to normal,and ENDO MBF markedly increased(1.30±0.33,p<0.01), but %WT and dP/dt remained depressed at 1,2,24 and 48 Hr after reperfusion. %WT was depressed ,but not significantly,at 78 Hr, and MBF was normal. By 7 days, %WT and dP/dt had returned to control. At autopsy, only the posterior papillary muscle exhibited gross evidence of scar and/or hemorrhage. Thus, reperfusion after 5 Hr of PCS is followed by full recovery of regional and global contractile function within a period of one week without gross infarction of the LV free wall.

20.11

SUPERIORITY OF THE PRESSURE-VOLUME AREA DERIVED FROM PRESSURE-DIAMETER MEASUREMENTS IN PREDICTING MYOCARDIAL O_2 DEMAND IN IN VIVO CANINE HEARTS. M.C. Hume,* J. Vinten-Johansen,* J.C. Finkenberg,* R.J. Barnard, G.D. Buckberg*. UCLA Med Ctr.,Los Angeles, California.90024.

The pressure-volume area (PVA) has been shown to be an accurate predictor of left ventricular O_2 consumption (MVO_2) in excised hearts, but has not been tested in vivo. In the present study, the feasibility and accuracy of PVA, derived from pressure-diameter data, in predicting MVO_2 in comparison to other indices was determined in in vivo canine hearts. A right heart bypass preparation was established to independently control cardiac output and pressure at constant heart rate (150). Left ventricular pressure and minor axis diameter (sonomicrometry) were measured continuously; PVA was derived from transformation of diameter to volume. Indices based on pressure (tension-time index, pressure-rate product) failed to provide a consistent correlation with MVO_2 (r=0.43, 0.56, resp.). In contrast, better correlations with MVO_2 were obtained for wall tension (r=0.71). Integrated pressure-diameter curves correlated highly (r=0.90±0.02) with MVO_2 in each dog, but failed to provide accurate predictions in composite data (r=0.59, n=60). The PVA provided the most superior correlation with MVO_2 in individual hearts (r=0.92±0.01) as well as in composite data (r=0.86). We conclude that derivation of PVA from pressure-diameter data provides accurate prediction of MVO_2 during pressure-loading and volume-loading in the in vivo setting. (Supported in part by USPHS NIH Grant HL16292.)

20.12

MYOCARDIAL BLOOD FLOW CHANGES WITH ATRIAL FIBRILLATION. Howard S. Friedman, John Scorza*, Stephen Kottmeier*,Regina McGuinn*. The Downstate Medical Center, (SUNY), Brooklyn, N.Y.

The changes produced by atrial fibrillation on myocardial blood flow and energetics is unclear. Accordingly, the effect of atrial fibrillation on myocardial blood flow and oxygen consumption were studied in 18 anesthetized dogs. With atrial fibrillation cardiac output(from 2.8±0.2 to 2.0±0.2 L/min, P<0.001, L/min)and mean aortic pressure(from 101±5 to 87±6 mmHg,P<0.001)declined. Although average myocardial blood flow (from 108±10 to 99±0.7 ml/min/100g)and oxygen consumption (7.7±0.7 to 8.1±0.7 ml/min/100g)did not change, the alterations in these variables correlated strongly(r=0.95,P<0.01). Myocardial blood flow changes correlated with those of tension time index and peak dp/dt(r=0.64,P<0.05). Distribution of left ventricular regional flow remained uniform irrespective of the directional changes in blood flow. However,average left ventricular inner to outer wall declined by 0.10±0.04,P<0.05,and myocardial oxygen extraction increased by 0.4±0.17 ml/dl, P<0.05. Thus, with atrial fibrillation,myocardial blood flow does not change in a consistent fashion although myocardial blood flow still reflects myocardial oxygen demand. Relative hypoperfusion of subendocardium and an increase in myocardial oxygen extraction suggests that with atrial fibrillation delivery of myocardial blood flow is inadequate.

21.1

A FRESH LOOK AT THE VASCULAR ARCHITECTURE OF THE RAT STOMACH. K.W. Ballard, P.H. Guth and S.S. Sobin. VA Wadsworth/UCLA School of Medicine and the Cardiovascular Research Laboratory, University of Southern California, Los Angeles, CA.

For study of the gastric circulation and function, knowledge of the branching order and other dimensions of the microvessels is essential. Rat stomach vessels were perfused with latex at arterial pressure, cleared in glycerin and flat-mounted between glass slides. Visualization by stereomicroscope and photomontages from sequential photos were used to a) trace arterial and venous pathways, b) measure vessel diameters and lengths and c) diagram generations of branching order. The left gastric artery (LGA) divides into several branches which penetrate external muscle, lie in the submucosa (SM) and send smaller branches back to the muscle, where they branch into capillaries. Some offshoots supply muscle before entering the SM. The branches of the LGA radiate to the greater curvature and are bridged by arcades of smaller interconnecting arteries which branch extensively as they continue inward toward the mucosa, finally terminating into capillaries there. Based on ongoing histological studies, distal SM arteriolar plexuses may actually lie in the base of the mucosa, beyond the muscularis mucosa. Along the greater curvature, branches from the gastroepiploic artery penetrate into the SM and interconnect with branches from the LGA. On average, there were 4-6 orders of branching from supplying artery to capillaries. Venous patterns were similar to the arterial. No A-V anastomoses were seen.

21.2

LYMPHATIC MICROVESSELS IN THE RAT STOMACH. H. Nagata* and P.H. Guth. CURE, VA Wadsworth Medical Center and UCLA School of Medicine, Los Angeles, CA 90073.

A new approach using in vivo fluorescence microscopy technique has been successfully used to study the gastric lymphatic system. A rat was anesthetized, laparotomized and the stomach exteriorized. A fluorescein-albumin conjugate was injected into the interstitial space of different layers of the stomach via a micropipet. Fluorescence was stimulated by epiillumination and the movement of conjugate was visualized and recorded by a closed circuit TV microscopy system. Results. Layer injected: I. Mucosa — The conjugate filled a honeycomb-like network of interglandular sinuses in the mucosa, entered the submucosal lymphatic network and later the large lymphatic vessels on the lesser and greater curvatures. II. Submucosa — Conjugate flowed from the injection pool into a network of lymphatic vessels (20 to 60 μm in diameter) that was clearly separate from the submucosal arterioles and venules. III. External muscle — The muscle layer fluoresced diffusely, suggesting rapid spread of injected conjugate, without demonstrating muscle lymphatics. Later conjugate appeared in large lymphatics (80 to 200 μm in diameter) on the lesser and greater curvatures, paralleling branches of the left gastric and right gastroepiploic arteries and veins respectively. Valve leaflets were seen in large lymphatics. Studies are continuing to ascertain the connections among lymphatics of the three gastric layers and the large draining lymph channels on the lesser and greater curvatures.

21.3

CAPILLARY FILTRATION COEFFICIENTS IN THE STOMACH. Michael A. Perry*, Peter R. Kvietys, and D. Neil Granger, Dept. of Physiology, University of South Alabama, Mobile, AL 36688.

Capillary filtration coefficients (K_f) were measured in the isolated perfused canine stomach under control conditions and during infusions of norepinephrine, isoproterenol and pentagastrin. Under control conditions K_f was 0.18 ml $min^{-1}mmHg^{-1}$ $100g^{-1}$. Infusions of norepinephrine (5μg/min) reduced blood flow to 46% of control values, and reduced K_f to 60.7% of control. Mechanical reduction in blood flow to the same level achieved by norepinephrine infusion reduced K_f to 44% of control values. Isoproterenol infusions (0.4μg/min) increased blood flow to 212% of control and increased K_f to 167% of control. When blood flow was increased with a pump to the same level achieved by isoproterenol infusions, K_f increased to 166% of control. Pentagastrin (0.2μg/min) caused no change in blood flow but increased K_f to 210% of control values. (Supported by a grant from the Alabama Affiliate of the American Heart Association)

21.4

POST MORTEM COLLECTION OF SUBCUTANEOUS INTERSTITIAL FLUID FROM RATS, G C Kramer, K Aukland*, L Sibley*, and E M Renkin. Dept. Human Physiology, University of California, Davis, CA 95616

To eliminate effects of inflammation on protein concentration of wick fluid, we have explored the possibility of collecting wick fluid after stopping the circulation. Male Wistar rats 200-320g were rapidly killed by injection of KCl solution. Three to six nylon wicks (~1mm thick) were implanted into the subcutis of the back for 1 hr. Extracted wick fluid was analyzed for total protein concentration (TP) by Lowry assay. We preloaded wicks with serial dilutions of rat serum or saline. Fluids sampled from the more dilute wicks were concentrated, while the more concentrated wicks were diluted. Plotting TP of loading solutions versus that of collected wick fluid allowed a cross-over TP to be calculated where neither dilution nor concentration would occur. Mean cross-over TP (12 rats) was 44.5 ± 4.7 (SD) mg/ml of wick fluid; while the TP of plasma was 64.5 ± 5.7 mg/ml. Measured oncotic pressures were 14.5 and 18.9 mmHg, respectively. In 8 of the rats, saline loaded wicks implanted and removed before sacrifice were compared with serum loaded wicks implanted after death. The cross-over TP for the post mortem wicks was always greater than TP for saline wicks (33.6 ± 3.7 mg/ml). Our results suggest the presence of regions with high filtration rates in the interstitium of living animals. (Supported by NIH HL 18010)

21.5

MODIFICATION OF LYMPH DURING PASSAGE THROUGH THE LYMPH NODE: EFFECT OF HISTAMINE. M. B. Maron, Physiology Program, Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272

The possibility that the fluid and protein composition of lymph may be significantly altered during passage through the lymph node was evaluated using the canine perfused popliteal lymph node preparation. With this preparation it is possible to perfuse the node via an afferent lymphatic with artificial lymph of known composition and to collect the total efferent effluent for analysis of potential changes in volume and composition. In 7 dogs, the node was perfused at an average flow rate of 0.26 ml/min with artificial lymph containing 3.85 g% albumin. Under steady state conditions, 97 and 98% of the infused volume and protein, respectively, were recovered in the efferent lymph. The addition of histamine to the infusate (2-3 ug base/ml) caused the efferent lymph flow to increase to 0.30 ml/min (19.4%↑, $p<0.01$), the efferent protein concentration to increase to 4.22 g% (8.5%↑, $p<0.01$), and the efferent protein flux to increase from 9.91 to 12.87 mg/min (29.9%↑, $p<0.01$). Mass balance calculations indicated that the addition of 0.05 ml/min of fluid with a protein concentration of 6.2 g% (plasma protein concentration was 6.6 g%) to the lymph could account for the observed increases in efferent lymph flow and protein concentration. These data suggest that histamine increased the permeability of the lymph nodal blood-lymph barrier allowing protein-rich fluid to pass into the nodal lymph. (Supported by the American Lung Association and the American Heart Association, Akron District Chapter).

21.6

FORELIMB WEIGHT DURING THE INFUSION OF HISTAMINE AND ARTERIAL HEMORRHAGE. Connie Y. Soika*, Andre J. Premen, David E. Dobbins, and Joe M. Dabney. Department of Physiology, Uniformed Services University, Bethesda, MD 20814

The prior administration of norepinephrine or isoproterenol prevents histamine-mediated increases in lymph flow, protein concentration and protein transport. We have previously reported that bilateral carotid occlusion or hemorrhage to 40 to 55 mmHg (Physiologist 23:89, 1980; Fed. Proc. 40:599, 1981) 30 min after beginning an infusion of histamine significantly reduced lymph flow. However, bilateral carotid occlusion 1 min after beginning an infusion of histamine did not affect forelimb total weight gain or weight gain per 100g forelimb weight (Fed. Proc. 41:1271, 1982). In the current study, forelimb weight was measured in the constantly perfused canine forelimb during an intraarterial infusion of histamine (4 μg base/min) for 30 min. One minute after beginning the histamine infusion, the animals were hemorrhaged from a cannulated femoral artery until a mean systemic arterial pressure of 55 mmHg was obtained. In a control group, forelimb weight was measured for 30 min in the absence of hemorrhage. In the hemorrhaged animals from 10 minutes onward, forelimb total weight gain and total weight gain per 100g of forelimb weight were significantly less than that seen during the infusion of histamine alone. These data indicate that the hemorrhage-induced physiological release of endogenous catecholamines is capable of significantly attenuating the edematogenic action of histamine in the canine forelimb.

21.7

THE ACUTE EFFECTS OF ANGIOTENSIN II (AII) ON CANINE INTESTINAL VASCULAR PERMEABILITY. Richard A. Nyhof* and Harris J. Granger
Microcirculation Research Institute and Dept. of Medical Physiology, Texas A&M University, College Station, TX 77843

The acute effects of AII on intestinal vascular permeability were examined by analyzing lymph (L) and plasma (P) protein concentration ratios (L/P) as venous pressure (Pv) was elevated from 5 to 35 mmHg in 5 mmHg steps. Pre-nodal L was collected from an isolated autoperfused jejunal segment in each animal, and P was obtained from the segmental vein. In 5 of 11 animals AII was infused (50ng/100ml blood) locally via arterial side-branch for 30 min prior to initial readings and continued for the remainder of the experiment. The results are as follows ($\bar{X} \pm$ SEM, * = p less than 0.05 compared to control):

	Pv (mmHg)	Vascular Resistance (mmHg/ml/min/100gm)	Lymph flow (ml/min/100gm)	Protein flux (mg/min/100gm)
Cont.	5	2.87 ± 0.09	0.018 ± 0.003	0.76 ± 0.33
AII	5	3.79 ± 0.48*	0.007 ± 0.002*	0.28 ± 0.05*
Cont.	35	8.25 ± 0.88	0.173 ± 0.048	1.75 ± 0.51
AII	35	6.22 ± 1.11	0.306 ± 0.028	1.30 ± 0.28

The estimated osmotic reflection coefficients (1-L/P when L/P plateaued at highest L flows) were 0.85 ± 0.02 for controls, and 0.93 ± 0.01* for AII animals. Therefore, at low Pv, acute infusion of AII did not alter L/P but did reduce L flow and protein flux. At high Pv, AII appears to reduce vascular permeability, but does not change lymph flow or protein flux. (Supported by HL-25387 and a Biomedical Rexearch Support Grant)

21.8

INTESTINAL CAPILLARY EXCHANGE CAPACITY AT DIFFERENT METABOLIC RATES. P.R. Kvietys, M.A. Perry*, and D.N. Granger, Department of Physiology, University of South Alabama, Mobile, Alabama 36688.

Blood flow ($\dot{Q}_B$), arterial oxygen content (A_{O2}), arteriovenous oxygen difference ($A\text{-}V_{O2}$) and the capillary filtration coefficient (K_f) were measured in the autoperfused canine ileum. Ileal metabolism, or oxygen uptake ($\dot{Q}_V \times A\text{-}V_{O2}$), was either increased (intraluminal glucose) or decreased (graded reductions in intraluminal temperature). The K_f was directly related to intestinal $A\text{-}V_{O2}$ and indirectly related to the oxygen delivery-to-demand ratio ($\dot{Q}_B \times A_{O2} / \dot{Q}_B \times (A\text{-}V_{O2})$). These findings support the hypothesis that intrinsic modulation of oxygen extraction is mediated by vascular elements which govern capillary exchange capacity, and that capillary exchange capacity is inversely related to the oxygen delivery-to-demand ratio.

(Supported by NHLBI 27659 and 26429.)

21.9

EFFECT OF VASODILATOR AGENTS ON CAPILLARY SURFACE AREA AND TISSUE OXYGENATION IN THE HAMSTER CHEEK POUCH. R.N. Pittman, Dept. of Physiology, Medical College of VA, Richmond, VA 23298.

Six different vasodilator agents (acetylcholine, adenosine, histamine, isoproterenol, papaverine and sodium nitrite) were administered in the bicarbonate-buffered Ringer's solution superfusing the hamster cheek pouch. The cheek pouch was viewed on a closed circuit TV system and scenes of the microcirculation were taped for later analysis. The surface areas of perfused capillaries were estimated by measuring capillary lengths. Surface area was calculated as $\pi \cdot$capillary diameter$\cdot$capillary length. PO_2 was recorded at locations selected to represent the least oxygenated tissue sites. Graded, but not statistically significant, increases in perfused capillary surface area resulted with increasing concentrations of vasodilator, except that 0.1 mM isoproterenol caused a decrease in surface area. Graded increases in vasodilator concentration caused increases in tissue PO_2 for papaverine and sodium nitrite, biphasic changes in PO_2 for adenosine and histamine, and decreases in PO_2 for acetylcholine and isoproterenol. Changes in perfused capillary surface area and tissue PO_2 were positively correlated for adenosine, histamine, isoproterenol and papaverine; negatively correlated for acetylcholine; and not correlated for sodium nitrite. These results suggest that vasodilator agents do not necessarily improve tissue oxygenation even though they relax arteriolar smooth muscle and increase perfused capillary surface area. (Supported by PHS grant HL 18292.)

21.10

ADENOSINE FAILS TO INCREASE CAPILLARY PERMEABILITY-SURFACE AREA IN THE ISCHEMIC CANINE MYOCARDIUM. K. A. Overholser and T. R. Harris, Vanderbilt University, Nashville, TN 37235

In 11 anesthetized dogs we studied the effect of adenosine (A) on sucrose permeability-surface area (PS) and on resistance (R) to myocardial blood flow before and during flow-reduction ischemia. We used the multiple-tracer method in which mixtures of isotopes were injected into a shunt between the carotid and left anterior descending coronary (LADCA) arteries. Measurements were made during baseline conditions, after a 4-min LADCA infusion of 8 ± 2 (s.e.m.) µg A/ml plasma, after 45-min of reduced LADCA flow, and after a second infusion of A (300 ± 90 µg A/ml plasma) during which the arterial shunt remained partly occluded. The results are shown in the Table, in which F indicates plasma water flow to the LADCA:

Condition	F(ml/min)	R(mm Hg·min/ml)	PS(ml/min)
Baseline	30.2 ± 3.2	2.18 ± 0.20	18.5 ± 1.9
Baseline + A	55.9 ± 4.0*	0.83 ± 0.08*	21.3 ± 1.6*
Ischemia	13.0 ± 1.3*	2.44 ± 0.36	8.4 ± 1.1*
Ischemia + A	28.4 ± 3.4	0.95 ± 0.11*	10.6 ± 1.9*

*greater or less than baseline, p less than 0.0175

Although A enhanced blood flow during flow-reduction ischemia, the effect was not associated with a significant increase in PS. Thus A probably did not lead to a recovery of capillary surface area for exchange, but simply raised the flow through capillaries which were already functioning. (Supported by HL 19370.)

PLANT GRAVITY RECEPTORS: STRUCTURES AND BIOCHEMICAL TRANSDUCERS

22.1

CHARACTERISTICS OF STATOLITHS FROM ROOTCAPS AND COLEOPTILES. F.D. Sack* & A. C. Leopold* (SPON: T. Halstead). Boyce Thompson Institute for Plant Res., Cornell Univ., Ithaca, NY 14853

Intact amyloplasts have been successfully isolated from corn coleoptiles and fixed for electron microscopy. Their envelopes are double and continuous. The surface charge on freshly isolated amyloplasts has been further characterized using several refinements in the measurement of zeta potential including: (1) a vertically oriented cataphoresis cell with the microscope on its side; this allows reliable measurements of mobilities within the plane of focus of the stationary layer. (2) the use of Nomarski optics and green (light-grown) amyloplasts to aid in the identification of intact plastids. Values for the zeta potential vary from 10-28 mV for amyloplasts regardless of coleoptile age (5-9 days) or days in light (0-9). Starch grains from the same preparations have potentials 1-2 mV lower. The measured potential of spinach chloroplasts (57 mV) closely agrees with values in the literature. Rootcap statoliths have been shown by our lab (Science 216:1221-2) to be rich in calcium. Chlorotetracycline, which fluoresces in the presence of membrane-bound calcium was applied to 40µm sections of living rootcap tissue of Zea mays and Vicia faba. The absence of fluorescence from the amyloplasts in statocytes (although nuclei fluoresced) suggests that the calcium in these organelles is not membrane-bound. (Supported by NASA Grant # NAG-W3)

22.2

ISOLATED STATOCYTES FROM ETIOLATED PEA EPICOTYLS: A MODEL SYSTEM FOR THE STUDY OF GRAVIPERCEPTION AT A CELLULAR LEVEL. John J. Gaynor and Arthur W. Galston, Dept of Biol, Yale Univ, New Haven, CT 06511.

The cells responsible for the perception of gravity in dicotyledonous stems surround the central vascular bundle and are referred to collectively as the "starch sheath" or perivascular parenchyma. These starch sheath cells, or statocytes, contain large multigranular amyloplasts which sediment in response to a 1xg force; likewise, when these cells are de-starched by cold treatment, the plants become agravitropic. By using standard protoplasting techniques we have produced a suspension of statocytes from the etiolated epicotyls of 7-day old Pisum sativum (cv. Alaska). These columnar-shaped cells are between 50-150 µm in length, and are highly vacuolated with numerous cytoplasmic strands. Rapid cytoplasmic streaming is quite common in these cells but amyloplasts, seen as bright refractile bodies, are not transported by this streaming. However, when one gravistimulates such an isolated statocyte by changing its orientation with respect to gravity, amyloplasts are seen to sediment to the lower end of the cell (average time = 5 min to fall 50 µm). We would like to suggest that these isolated starch sheath cells have potential as a model "statocyte" system which may allow one the opportunity to directly examine physiological and biochemical changes associated with gravistimulation or graviperception at a cellular level, instead of the traditional organ or whole plant level.

22.3

THE MODE OF GRAVITY SENSING IN PLANT CELLS.
G. Perbal*(SPON : P. E. Pilet). Université Pierre et Marie Curie, Paris.

In gravireacting plant organs, amyloplasts containing voluminous starch grains are found in the cells which are responsible for the perception of gravity. The density of starch is greater than that of the surrounding cytoplasm and the statoliths can therefore sediment under the influence of gravity.

In a vertical root, the amyloplasts are sedimented on large aggregations of endoplasmic reticulum lining the distal wall of the cells. When the root is stimulated by a change in orientation, the amyloplasts move along the longitudinal wall. The intensity of the response is dependent upon :
- 1 the sine of the angle of the root's inclination relative to the direction of gravity ;
- 2 the number of contacts between the amyloplasts and the parietal cytoplasm ;
- 3 the displacement of these organelles along the longitudinal wall.

It is proposed that the action of the statoliths is transmitted to the plasmalemma by the more viscous parietal cytoplasm.

22.4

REDISTRIBUTION OF AMYLOPLASTS IN INVERTED CAULERPA PROLIFERA RHIZOME TIPS. Michael B. Matilsky. Department of Biology, Princeton University, Princeton, N. J. 08544

The sedimentation of gravity sensor organelles (e.g. amyloplasts) against the cell membrane or other organelles directly adjacent to the membrane is a current hypothesis for the triggering of gravity perception in plants. Caulerpa prolifera, a multinucleated, single-celled, marine, green alga can be used to test this hypothesis because although a giant single cell, Caulerpa differentiates into 3 "organs" each with a specific orientation with respect to gravity. A horizontally growing, tubular rhizome gives rise to root-like rhizoids from the bottom surface and erect blades from the top surface. Inverted whole plants immediately switch the site of new organ differentiation to correspond with the new gravity stimulus (Jacobs and Olson, 1980). Light microscopy of PAS-stained serial sections of rhizome tips fixed 24 hours after inversion of whole plants has revealed that the switch in the site of organ differentiation is accompanied by the sedimentation of starch-containing organelles to the bottom of the rhizome. Sedimentation occurs in a region which extends from 200-800 µm behind the tip of the rhizome. The presence of starch in these sedimenting organelles is confirmed by the appearance of a characteristic birefringent pattern when viewed through crossed polarizers. The quantitative distribution of the amyloplasts in the rhizome tip as well as the time course of redistribution is under investigation. (Supported by NASA Grant NSG-7280 to W. P. Jacobs and NASA Fellowship NAGW-70 to M. B. Matilsky).

22.5

LINKAGE BETWEEN GRAVITY PERCEPTION AND RESPONSE IN THE GRASS LEAF-SHEATH PULVINUS. Peter B. Kaufman & P. Dayanandan. Div. of Biol. Sciences, University of Michigan, Ann Arbor, Mi 48109

Plant responses to gravity stimulation are a reflection of several biochemical changes that occur immediately prior to elongation response. However, the only observed cellular change is the prominent sedimentation of statoliths. Besides observing the fine structure of the statolith interaction with cellular components, we made use of several inhibitors to disrupt the early response to observe cellular changes. Inhibitors known to disrupt tubulin such as colchicine, griseofulvin, rotenone, podophyllotoxin and isopropyl N-chlorophenyl carbamate were supplied along with gravistimulation. A cellulose synthesis inhibitor, dichlorobenzonitrile (DCBN) was also used. All these agents suppress gravitropic curvature. Colchicine and DCBN cause characteristic swelling that reflects the inability of responding cells to elongate normally. Instead, the pulvinus cells increase in diameter, the amount of increase being proportional to the response at that locus as expressed previously (The Physiologist 24: No.6 S-113). DCBN induces lens-shaped swellings on the radial cell walls of barley pulvinus. These swellings are associated with pit connections and seem to be related to disruption of cellulose synthesis machinery. It may also indicate regions of accumulation or transport paths for growth-promoting hormones. In control treatments, sedimentation of statoliths leads to an interaction of these amyloplasts with tonoplast membranes. (Supported by NASA Grant NAGW-34).

22.6

GRAVITATIONALLY INDUCED ASYMMETRY IN THE DISTRIBUTION OF INDOLE-3-ACETIC ACID. Robert S. Bandurski and A. Schulze*. Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824-1312.

Bio-assays of a plant growth hormone (auxin) have demonstrated that more auxin diffuses from the lower side of a geostimulated plant shoot than from the upper side. This laboratory has shown by gas chromatography-selected ion monitoring-mass spectrometry that more free indole-3-acetic acid (IAA) is present in the lower side of a geostimulated shoot of Zea mays. It has also been shown that the vascular stele of the mesocotyl portion of the shoot contains 80% free IAA-20% ester IAA, whereas the mesocotyl cortex contains 20% free IAA, 80% ester. Thus, it becomes important to determine whether the IAA asymmetry occurs in both vascular and cortical tissues so that the point of transduction of the geo-stimulus may be located. Now, using an isotope dilution assay of the pentafluorobenzyl ester of IAA, we have demonstrated that the percentage of free IAA in the lower half of the mesocotyl cortex of Zea mays is 57 ± 3, 56 ± 2 and 50 ± 0% following 90, 30 and 0 minutes of geostimulation. Thus, the asymmetric distribution occurs in the cells of the mesocotyl cortex and it remains to be determined whether the hormone asymmetry is also found in the vascular stele.
(Report of work supported by the Life Science Section of the Space Biology Program (NASA-NAGW-97, ORD 25796) and the Metabolic Biology Program of the National Science Foundation (PCM 79-04637).

22.7

ROLE OF AUXIN AND PROTONS IN PLANT SHOOT GRAVITROPISM. David L. Rayle and Fernando Migliaccio.* San Diego State University, San Diego, CA 92182

The role of auxin and protons in the gravitropic response of sunflower hypocotyls has been investigated. A lateral proton gradient along gravistimulated hypocotyls was visualized using a brom cresol purple agar technique. Vanadate (an inhibitor of auxin-enhanced H^+ secretion), PCIB (an anti-auxin), and TIBA (an auxin-transport inhibitor) not only prevented observable proton excretion but also inhibited gravicurvature. Vanadate, PCIB, and TIBA inhibition of gravicurvature could be reversed with acid treatment of the lower surface of a gravistimulated hupocotyl. Auxin treatment to the lower surface of a gravistimulated hypocotyl did not reverse vanadate-induced inhibition, but it partially reversed PCIB- and TIBA-induced inhibition. These results indicate a close relationship between auxin and proton excretion in the differential growth responses of the sunflower hypocotyl during gravitropism. This link is supported by data indicating a close spatial correspondence between those regions of a hypocotyl which maximally respond to protons and auxin, and those zones which exhibit differential growth during gravistimulation. Further preliminary data indicate there is a rapid (within 20-30 min) redistribution of ^{14}C-IAA to the lower epidermal layer of gravistimulated shoots. This redistribution precedes or is coincident with the accelerated growth of this tissue, which in turn initiates gravicurvature.

22.8

PROTEIN SYNTHESIS IN GEOSTIMULATED ROOT CAPS OF CORN. Lewis J. Feldman. Dept. of Botany, Univ. of California, Berkeley, CA 94720.

The root cap is the site of perception of gravity. When geostimulated the cap is thought to produce an inhibitory growth substance leading to downward bending of the root. In some roots light is also required for the gravity response. Using light as a trigger we have examined biochemical events required for inhibitor production. Protein synthesis is necessary. By culturing caps in light or dark and employing a double label (^3H and ^{14}C) and a combination of fluorography/autoradiography, we have shown that light stimulates the formation of many but not all pre-existing proteins. Moreover, it appears that several new proteins are formed as a result of the light treatment. The growth substance auxin, at a concentration of $10^{-9}M$, must also be included in the culture medium for inhibitor production. Deleting auxin reduces total protein synthesis to approximately half of that observed in caps cultured in light plus auxin. Specific proteins form when auxin is included. The relationship between protein synthesis and the translation of the perception of gravity into a growth response will be discussed. (Supported in part by the National Aeronautics and Space Administration (NASA) Grant NAGW-234)

22.9

QUANTITATION OF CHLORPROMAZINE-BOUND CALMODULIN DURING CHLORPORMAZINE INHIBITION OF GRAVITROPISM. Stanley J. Roux and Ronald L. Biro. Botany Dept., U. of Texas, Austin, TX 78712

The regulatory protein, calmodulin (CaM), controls the activity of a plasma membrane-localized ATPase in plants which serves to pump calcium out of cells. Recent data are consistent with the hypothesis that activation of this pump is one of the early transduction steps necessary for gravitropism. Chlorpromazine (CPZ), a CaM antagonist, reversibly inhibits both the calcium pump and gravitropism in oat coleoptiles at concentrations which permit normal growth rates. We used ^{14}C-labeled CPZ to photoaffinity label endogenous CaM in vivo to learn whether the drug is actually binding to some portion of endogenous CaM when it inhibits gravitropism. During 12 hr perfusions with 10^{-7}M CPZ, as much as 20% of the endogenous CaM in gravistimulated oat coleoptiles is bound to CPZ and gravitropism is totally inhibited for over 1 hr following the treatment. Shorter perfusion times yield less prolonged inhibition and a lower % of CaM bound to CPZ. By radioimmunoassay, about 1% of the acetone-precipitable protein in etiolated oat coleoptiles is CaM, some of it tightly associated with mitochondria and nuclei. (Supported by NASA Life Sciences Program Grant NSG 7480)

22.10

ASYMMETRIC EFFLUX OF PROTONS AS A MEDIATOR OF GRAVITROPISM IN ROOTS. MICHAEL L. EVANS and TIMOTHY J. MULKEY. Ohio State Univ., Columbus, OH 43210

Using a computerized measuring device, we find that the growth of seedling roots of maize (Zea mays) is stimulated by low (e.g. 4 to 5) pH. The stimulation is as large as 10 fold and occurs within 3 min. When seedlings are placed on agar containing a pH indicator dye, acid efflux is observed along the elongation zone of the root but not in non-growing regions. When the root is oriented horizontally, acid efflux from the elongation zone increases on the top and decreases on the bottom and this asymmetry develops before gravitropic curvature. It has been suggested that root gravitropism results from accumulation of one of the two hormones, indole-3-acetic acid (IAA) or abscisic acid (ABA) to growth-inhibiting levels on the lower side of the root. We have obtained the following data indicating that redistribution of IAA is more likely to be important: 1) high concentrations of IAA inhibit growth and acid efflux in root cells, 2) low concentrations of IAA stimulate growth and acid efflux in root cells, 3) inhibitors of IAA redistribution prevent gravitropism and 4) the initial effect of ABA is to stimulate root cell elongation. Our data are consistent with the model that gravity induces asymmetric redistribution of IAA in roots and that this causes asymmetric acid efflux leading to the differential growth causing gravitropism. (supported by NASA Grant NAGW-297)

22.11

THE MECHANICS OF GRAVITROPIC BENDING IN LEAFY DICOT STEMS. Frank B. Salisbury, Wesley Mueller*, Julianne Sliwinski*, Raymond Wheeler*, and Chauncey Harris*. Plant Science Dept., Utah State University, Logan, Utah 84322

We confirm that when a stem is laid on its side, the top of the stem almost immediately stops growing and may even shrink by a significant amount; the bottom continues to grow at the same or an accelerated rate. We have measured this with conventional photography and with stereophotogrammetry. The later method has been refined for use with a digitizer and a computer. We have gained insight by studying plants that have been restrained in a horizontal position for 48 hours or more and then released. In such plants, cells on the bottom of the stem continue to grow, stretching cells on the top, so that, upon release, cells on top become shorter and thicker, while cells on the bottom become longer and thinner, causing bending in one to a few seconds. Cell volume is apparently conserved during the rapid bending; the dimensional changes must depend upon the properties of the cell wall, and gravitropic bending must occur as cell-wall properties begin to change in response to reorientation with respect to the gravitational field. Ethylene is known to reorient cell-wall microfibrils, and we have shown that ethylene plays a positive role in gravitropic bending of dicot stems. Auxin is also required for the gravitropic response. We are also measuring the forces developed under stem restraint and comparing our results with current theories of cell growth in plants.

FETAL AND NEONATAL BIOLOGY

23.1

RENAL ARTERY CLIP AND CONTRALATERAL NEPHRECTOMY IN FETAL SHEEP. J.Job Faber and Debra F. Anderson, Dept Physiol. School of Medicine, OHSU, Portland OR 97201

Blood pressures and daily plasma renin activities (PRA) were recorded in 10 clipped fetuses and 9 controls who met the following criteria for acceptance: survival $\geq$ 1 week and pH $\geq$ 7.35 and evidence of renal function (measurable PRA or urine production). There were no significant differences between the 2 groups in weight (3.0 Kg), duration (mean 17 days), pH, PO2, heart rate, or PRA, 12 ng/(ml.hr). Life time mean arterial blood pressures were 50.1 and 41.3 mm Hg in clipped fetuses and controls ($P<0.01$) but this difference was due to a transient hypertension in the first week; blood pressures in the second week and in the period thereafter were the same. There was no significant correlation between lifetime mean PRA and arterial blood pressure. Although clips were too tight in several fetuses whose kidneys became nonfunctional (rejected from this series), we were unable to produce lasting hypertensions or increases in PRA with renal artery stenoses. We conlude that the fetal kidney does not determine long term fetal arterial blood pressure.
(Supported by a grant from the National Heart Lung and Blood Institute, HL27194).

23.2

MATCHING OF MATERNAL AND FETAL PERFUSION IN THE PLACENTA OF THE SHEEP. J.H.G. Rankin, M.K. Stock* and T.M. Phernetton*. Dept. Physiol., Univ. Wisconsin Med. Sch., Madison, WI 53706.

Local interaction of maternal and fetal placental blood flows was studied in 5 near-term sheep. Radioactive microspheres (15u) were administered simultaneously to mother and fetus before (control) and during (test) infusion of norepinephrine to the fetus (6 ug/kg/min for 11 min). Maternal (M) and fetal (F) relative activities (RA) defined as (% total placental radioactivity)/(% total placental weight) were calculated for each isotope. Pieces of cotyledons (1 g) were defined as matched if the direction of change in RA from control to test was the same for both M and F. Pieces in which M and F RA did not change, or changed in opposite directions, were defined as not matched. In the absence of an interaction between M and F placental blood flows the probability of a piece of cotyledon being matched is 0.5. We tested the alternate hypothesis that $P>0.5$ using the Z-test.

SHEEP	MATCHED	NOT MATCHED	TOTAL	% MATCHED	P
1	224	143	367	.610	<.001
2	153	127	280	.546	.07
3	199	156	355	.561	.01
4	137	111	248	.552	.06
5	203	137	340	.597	<.001

These data suggest that the majority of the placental mass reacts in such a way that changes in relative perfusion are qualitatively matched in the adjacent M and F placental circulations. (Supported by Univ. Wis. Sea Grant, NOAA)

23.3

FETAL-MATERNAL CORTISOL KINETICS IN CONSCIOUS, CHRONICALLY CATHETERIZED SHEEP. **D.D. Kitts*, G.B. Anderson* and G.H. Stabenfeldt. Departments of Animal Science and Reproduction, University of California, Davis, CA 95616.**

Pregnant ewes and their fetuses were injected simultaneously with [4-^{14}C] and [2-^{3}H] cortisol (F) respectively, to study the changes in steriod clearance, placental transfer and metabolic interconversion during the immediate prepartal (PRE-P) period. Fetal plasma F blood production rates (PR_F) and secretion rates (SR_F) increased from 0.97 + 0.09 mg/24 h and 0.69 + 0.11 mg/24 h (Mean + SEM) at D-8 to 5.605 + 0.28 mg/h and 5.510 + 0.32 mg/h at D-1.5 PRE-P (n=5). There was a positive correlation between fetal plasma F concentration and PR_F (r=0.966; $p < 0.01$; n=11) and SR_F (r=0.965; $p < 0.01$; n=11). A similar correlation was also observed in the ewe (r=0.672; $p < 0.05$; n=12). A bidirectional transfer of F between maternal (M) and fetal (Fe) compartments was noted, however, no activity was detected in the amniotic fluid from either source. The proportion of fetal F turnover involving corresponding M→Fe and Fe→M F transfer was 11.25 + 2.25 µg/h and 0.232 + 0.06 µg/h at D-8 and 3.977 + 2.79 µg/h and 4.713 + 0.52 µg/h at D-1.5 PRE-P. Dual labelled experiments (n=2) performed in twin pregnancies showed transfer of F between fetal siblings was less than 1%. A substantial proportion of fetal F was converted to cortisone(E) (46.11 + 12.87%), however there was no evidence of a maternal contribution to fetal E activity. The results demonstrate that maternal contribution of fetal F metabolism decreases in the PRE-P and that increased fetal plasma F levels are directly related to fetal adrenal autonomy.

23.4

LONG-TERM PARTIAL OCCLUSION OF THE DISTAL AORTA IN FETAL LAMBS. Debra F. Anderson, Kent L. Thornburg and J. Job Faber. Dept. of Physiology, OHSU, Portland, OR 97201.

Bilateral nephrectomy or renal artery stenosis do not appear to affect long term arterial pressure in fetal lambs. To test the hypothesis that the placenta determines arterial blood pressure, a flow sensor and an inflatable occluder were placed around the aorta (below the level of the renal arteries) in 6 fetal lambs. After 7 + 1 days (mean ± sem) blood flow was reduced to 65 + 6% of control. This degree of occlusion was maintained for 16 days (range, 6 to 34). Daily measurements show (pre- vs post-occlusion): plasma renin activity changed from 10.8 and 10.0 (N.S.), brachial artery blood pressure during free blood flow from 42 to 43 mm Hg (N.S.) and heart rate from 175 to 163 bpm (N.S.). Weekly measurements showed PO2 changed from 19.6 to 16.5 mm Hg ($p<.01$), pH from 7.39 to 7.37 (N.S.) and PCO2 from 45.8 to 50.3 mm Hg ($p<.01$). We conclude that a reduction of placental blood flow does not lead to a compensatory increase in arterial blood pressure. (Supported by a grant from the National Heart, Lung and Blood Institute, HL27194).

23.5

AIRWAY COLLAPSIBILITY IN NEONATAL LAMBS: ROLE OF TRACHEAL SMOOTH MUSCLE AND MECHANICS. Vinod K. Bhutani*, Randall J. Koslo*, and Thomas H. Shaffer, Dept. Newborn Pediatrics, Pennsylvania Hospital and Dept. Physiology, Temple Univ. Sch. of Medicine, Philadelphia, PA 19140.

The interrelationships between tracheal mechanics and its smooth muscle tone were investigated during airway collapsibility in 4 neonatal lambs (1-5 days). Proximal extrathoracic tracheal segment (mean + SE length = 3.2 ± 0.1 cm) was bypassed in the anesthetized (Pentobarbital®, 30 mg/kg/i.p.) lamb, while spontaneous normoxic and normocapneic ventilation was maintained through the distal segment. Airway volume and radius were measured; pressure-volume relationships by plethysmography and pressure-flow relationships were determined at transmural pressures (P_{tm}) of 0, 10, 20, 30, and 40 cm H_2O. Tracheal resistances (R_T) were calculated at flows ($\dot{V}$) of 0 to 0.5 L/sec. In addition, tracheal compliance (C_T) and R_T were measured during maximal tracheal active tension (AT) induced by Bethanecol® infusion (0.16 mg/kg/i.v. bolus). At P_{tm} = 0, increased AT resulted in 47% decrease in C_T and over 350% increase in R_T; at P_{tm} = 40, R_T increased both before (675%, $p < 0.01$) and after (550%, $p < 0.01$) Bethanecol. Further, at P_{tm} = 40 the airway collapsibility was significantly reduced ($p < 0.01$) with increased AT and directly correlated ($r = 0.7$; $p < 0.05$) to the decreased C_T. These data suggest that neonatal tracheal smooth muscle tone directly alters C_T and R_T and plays a significant role in controlling airway collapsibility. (Supported in part by HL22843).

23.6

A ROLE FOR ANGIOTENSIN II IN THE DEVELOPMENT OF THE HEART. Maurice S. Holder (SPON: K. F. A. Soliman). Florida A&M University, School of Pharmacy, Tallahassee, Florida 32307.

Developing Sprague-Dawley pups from birth through 10 weeks show a continuous decrease in ventricular weight/body weight (VW/BW) ratio, suggesting a different growth rate for the heart than for the body. Kidney weight to body weight ratio nor liver weight to body weight ratio do not demonstrate the same pattern during development. Angiotensin II inhibits or reverses the decreasing pattern and also induces the development of larger hearts in offsprings of mothers treated during pregnancy. I^{125} labelled A-II, injected into pregnant females resulted in high levels of radioactivity in hearts of offsprings. Saralasin administration to the females caused a lower VW/BW in the pups at birth while verapamil reduced an A-II induced hypertrophy. Changes in overall growth rates of the offspring did not adversely affect the decreasing pattern in VW/BW, nor did Indomethacin treatment or adrenergic blockade. A-II content in myocardial tissue was highest in offsprings of mothers treated during pregnancy. The results support the contention that Angiotensin II levels have a positive influence on the development of the myocardium and suggests that postnatal myocardial tissue growth may be proportional to A-II levels. (Supported by Grant #RR 08111-12 from MBRS and NHBLI of NIH)

23.7

CARDIAC OUTPUT AND UTEROPLACENTAL BLOOD FLOW IN MALNOURISHED, DIET-REPLETED PREGNANT RATS. R.A. Ahokas,* G.D. Anderson, * and J. Lipshitz* (SPON: C.M. Blatteis). Univ. Tenn College of Medicine, Memphis, TN 38163.

Diet-restricted pregnant rats fed ad libitum during the last week of gestation develop normal-sized fetuses (J.Nutr. 110:883, 1980). The objective of this study was to determine the effect of dietary repletion on the uteroplacental circulation of malnourished rats during the last week of gestation. Cardiac output (CO), total uterine (UBF) and placental(PBF) blood flow, and fetal weight were measured on days 14, 20 and 21 of gestation using radioactive-labeled 15 µ microspheres in anesthetized pregnant rats fed: 1) ad libitum throughout gestation; 2) 50% of the average daily amount of food consumed by the ad libitum fed dams throughout gestation; and 3) the 50% restricted diet until day 14 of gestation and ad libitum until day 21 of gestation. Diet restriction reduced CO 30%, UBF and PBF 65%, and mean fetal weight 20% by day 21 relative to the ad libitum fed controls. Dietary repletion significantly increased mean fetal weight, UBF and PBF, but not CO, to near ad libitum fed control values by day 21. Therefore, the increased UBF and PBF were primarily a result of an increase in the fraction of CO distributed to the uterus (decreased uterine resistance). The results suggest that when adequate dietary nutrients are available again the placental circulation expands to meet the nutritional demands of the fetus and normal fetal growth resumes.

23.8

EFFECTS OF CITALOPRAM ON TEMPERATURE PREFERENCE IN MATURING MICE. Cecilie A. Goodrich. Cleveland State Univ., Cleveland, OH 44115.

Citalopram (Lu 10-171) is a specific inhibitor of the neuronal uptake mechanism for 5-hydroxytryptamine (5-HT; serotonin), causing decreases in brain 5-HT and its metabolite 5-hydroxyindole acetic acid and decreased rate of 5-HT turnover in both adult and maturing mice. The effects of a single IP dose (20 mg/kg) of citalopram have been tested in maturing mice in a thermal gradient 18-37° C and 20 cm long. Treated and littermates injected with saline vehicle only (controls) were tested "double blind" at ages from 1 to 10 days postpartum. At all ages tested, treated mice showed significant increases in mean preferred temperature (T_{pref}) when tested 24 hr after injection. Mean minimum times required to move to within 1 cm of the final preferred location in the gradient (min-t) were significantly reduced by this treatment. Minimum body temperature (T_b) at constant ambient temperature (23° C) within 20 min was not affected at 3 days, but was significantly decreased at 7 days. At ages 1, 3 and 5 days, citalopram treatment had no effect on T_{pref} 2 hr after injection, although min-t was again reduced compared with controls. Animals aged 6, 7 or 10 days showed similar effects to those seen after 24 hr: increased T_{pref} and decreased min-t. Since citalopram increases synaptic 5-HT, these results suggest that a 5-HT dependent component of the thermoregulatory mechanism matures between 3 and 7 days postpartum, with some events occuring between 6 and 7 days.

23.9

NEONATAL PLASMA GLUCOSE AND INSULIN FOLLOWING MATERNAL ETHANOL INGESTION. Linda Witek-Janusek. Dept. of Physiology, Loyola Univ. of Chicago, Stritch Sch. of Med., Maywood, IL 60153

Since pups of rats ingesting ethanol (E) during pregnancy have decreased hepatic glycogen (Fed.Proc. 41:923, 1982), the effect of maternal E ingestion on neonatal plasma glucose (PG) and immunoreactive insulin (IRI) was studied. Female rats were placed on either a liquid E (6%v/v; 30% daily cal.), isocaloric liquid pair fed (PF), or ad lib rat chow (RC) diet 3 wk prior to mating and throughout gestation. E intake was 1.85±.05 g/100g/day. Trunk blood was collected from rat pups at 6, 12, and 24 hr of age. PG (mg/dl) and IRI (μU/ml) results are shown below (*P<.05 compared to PF):

Group	PG-6hr	IRI-6hr	PG-12hr	PG-24hr	IRI-24hr
E	49±8*	28±7	77±2	71±4	36±4
PF	67±2	48±8	75±2	75±2	31±5
RC	60±2	32±4	67±5	66±1*	31±4

No differences were seen in IRI's of E pups but at 6 hr IRI's were greater (P>.05) in PF pups. This may be due to maltose-dextrins substituted for E cal. in the PF diet. PG's were less (P<.05) in E pups at 6 hr and increased 57% by 12 hr but only 12% in PF and RC pups. After 6 hr PG's in E and PF pups were similar, but PF pups had greater (P<.05) PG's than RC pups at 24 hr. The lower 6 hr PG in E pups suggests a more severe or prolonged "transient hypoglycemia" seen in normal neonates at 2 hr postnatal, while the greater increase over time suggests compensation by homeostatic control systems. (Supp. by USPH 5 S07 RR05368 BRSG).

NEURAL CONTROL OF CIRCULATION I

24.1

NUCLEUS RAPHE MAGNUS INHIBITION OF SPINORETICULAR NEURONS ACTIVATED BY INJECTION OF BRADYKININ INTO THE LEFT ATRIUM. C. Dale Chapman*, W. Steve Ammons* and Robert D. Foreman, Dept. of Physiol., University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190

The object of this study was to determine the effect of nucleus Raphe Magnus (RM) stimulation on spinoreticular (SR) neurons in the thoracic spinal cord activated by injection of bradykinin (BK) into the left atrium. Our laboratory has demonstrated that injection of BK in the left atrium activates SR neurons which receive viscerosomatic convergent input. In cats anesthetized with α-chloralose the RM was electrically stimulated at current intensities of 50-700 μA. Neurons of the SR tract were antidromically activated by stimulation of the medullary reticular formation. All of the cells studied could be activated by stimulation of the left stellate ganglion and by mechanical manipulation of their somatic fields. For 6 SR neurons, injection of BK produced an increase in cell activity. During the BK response stimulation of the RM inhibited the response in all 6 SR neurons for the duration of the stimulation. It can be concluded that activation of SR neurons by a noxious cardiac input can be inhibited by stimulation of the RM. Supported by National Heart, Lung and Blood Institute, Grants HL22732, HL07430, HL00557.

24.2

THORACIC VAGAL INHIBITION OF PRIMATE SPINOTHALAMIC NEURONAL RESPONSES TO INTRACARDIAC INJECTIONS OF BRADYKININ. W. Steve Ammons* Robert W. Blair*and Robert D. Foreman. Dept. Physiol. Univ. of Okla. Hlth. Sci. Ctr., Okla. City, OK 73190.

The goal of this study was to determine if thoracic vagus nerve stimulation alters the response of spinothalamic neurons of the thoracic spinal cord to intracardiac injections of bradykinin (BK). All experiments were performed on monkeys (Macaca fascicularis) tranquilized with Ketamine and anesthetized with α-chloralose. The T_1 to T_5 segments of the spinal cord were searched for antidromic responses to electrical stimulation of the contralateral ventral posterior lateral nucleus of the thalamus. All cells studied could be activated by electrical stimulation of the caudal ansa subclavia and by manipulation of their somatic fields. For 5 spinothalamic neurons injection of BK into the left atrium increased cell activity from 15±9 to 29±13 spikes/sec. At the peak of the response the left thoracic vagus was stimulated (10-20V, 2 msec, 20 Hz) for 3 to 5 sec resulting in decreased cell activity to 17±10 impulses/sec. Turning off the stimulator caused the activity to return toward the BK stimulated value. For 2 out of 2 cells left cervical vagotomy prevented the inhibitory effect of vagal stimulation. These data suggest that vagal afferent fibers modulate spinothalamic neuronal responses to noxious cardiac events, presumably via descending inhibitory pathways originating in the brain stem. Supported by National Heart, Lung and Blood Institute Grants HL22732, HL07430 and HL00557.

24.3

RECEPTOR MECHANICS OF CARDIOVASCULAR AFFERENTS. H. Rodney Holmes*, J. Andrew Armour, C. Dale Chapman*, Robert D. Foreman and Robert W. Blair*. University of Oklahoma, HSC, Oklahoma City, OK 73190.

Receptors in the heart and great vessels respond to mechanical stimuli which have been described in terms of blood pressure or deformation of receptive fields. To determine the mechanical properties stimulating receptors, we recorded single afferent fiber activity from cardiac nerves and sympathetic rami of 11 dogs, determined conduction velocity, mapped receptive fields, measured blood pressure in the chambers under the fields and measured the three dimensional movements of the fields with sonomicrometers sutured just outside the fields. We recorded afferents from the ventricles (4), atria (4), aorta (3). During arrhythmias, volume injections, great vessel occlusions, and administrations of phenylephrine, dobutamine, and methylcholine, the changes in afferent activity were consistently correlated with changes in receptive field dimensions but not with the magnitude of blood pressure changes. Stretch receptors were oriented within their receptive fields such that deformation due to field-specific length changes determined activation. The sonar length measurements gave the more accurate description of those discrete conformational changes of a receptive field which stimulated primary afferent activity. (Supported by NHLBI Grants, HL027260, HL00557, HL07430).

24.4

INTRACELLULAR RECORDINGS FROM THE MIDDLE CERVICAL GANGLION (MCG) OF THE CAT. Zeljko J. Bosnjak, and John P. Kampine. Medical College of Wisconsin and Wood VA Medical Center, Milwaukee, Wisconsin 53193

Earlier studies from our laboratory demonstrated that the neurons of the stellate ganglion receive synaptic information from peripheral nerves (Am. J. Physiol. 242:R237, 1982 and J. Physiol. London 324:273, 1982). The purpose of this study was to examine the possibility of a peripheral synaptic input to the MCG. The influence of peripheral and central inputs to the MCG of the cat was studied in vitro in six preparations by means of intracellular recording. Preganglionic electrical stimulation, via the ventral or dorsal ansa subclavia, and postganglionic electrical stimulation, via the ventrolateral cardiac nerve, evoked graded synaptic responses which led to the discharge of one or more action potentials. The conduction velocity of these pathways ranged from 0.4 to 0.9 m/sec. 10% of the cells impaled were inexcitable, even with direct intracellular depolarizing current, while 80% of the neurons tested received a synaptic input from fibers of both central and peripheral origin. In addition, subthreshold synaptic inputs from peripheral and central origin sum to discharge the cell, suggesting an integration of neural inputs in the MCG. This evidence indicates that sympathetic efferent nerve activity can be modified by peripheral excitatory inputs and that these inputs may function as pathways for a peripheral reflex at the level of the MCG. (Supported by NIH Grant GM 29641 and the VA).

24.5

INFUSION OF PGE_2 ROSTRAL TO THE CAROTID SINUS OF CONSCIOUS SHEEP RAISES ARTERIAL PRESSURE. J.E. Chimoskey and B.A. Breuhaus*. MI State University, East Lansing, MI 48824.

Hull and Chimoskey have shown that infusion (I) of PGE_2, 10 ng/kg/min, into the common carotid arteries (CCA) of conscious animals raises arterial pressure (AP), and does not do so by stimulating a carotid chemoreflex (Fed. Proc. 40:527, 1981). Hull and Chimoskey have also shown that I of PGE_2 into CCA resets the arterial baroreflex (Physiologist 24:5, 1981), and raises AP by increased sympathetic alpha-adrenergic activity (Fed. Proc. 41:1344, 1982). Because I of PGE_2 into the cerebral ventricles of conscious animals raises AP (Hypertension 3:426, 1981), I of PGE_2 into CCA probably acts in the cerebral circulation to raise AP. PGE_2 does not stimulate the carotid sinus (CS) in anesthetized animals (Quart. J. Exp. Physiol. 59: 63, 1974), but I of PGE_2 into CCA also does not raise AP in anesthetized animals. We now have evidence that I of PGE_2 rostral to CS raises AP. In sheep the external carotid artery (ECA) is the major cerebral supply; the internal carotid origin is absent, the CS region is near the occipital artery origin, and the vertebral arteries supply only the medulla caudal to the obex (Quart. J. Exp. Physiol. 45:243, 1960 and J. Anat., Lond. 97:203, 1963). The ECA of sheep were chronically cannulated non-occlusively rostral to the lingual artery origin and CS region. PGE_2, 10 ng/kg/min, I into ECA of conscious sheep raises AP. Thus I of PGE_2 into CCA acts rostral to CS to raise AP. Supported by MI Heart Association.

24.6

BARORECEPTOR FUNCTION IN SENESCENT RATS. Michael C. Andresen. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

In previous studies of development of baroreceptor (BR) characteristics (Andresen et al., 1980) we found that BR threshold distortion levels increased with age up to 20 wks to match the increase in vessel distensibility and maintain a constant pressure threshold (P_{th}). Data from 30 wk old rats suggested that vessel wall and BR characteristics had stabilized. Here BR and aortic wall properties were examined in 15 WKY rats 2 yrs old (mean=115 wks) and compared to the earlier data. An in vitro aortic arch-aortic nerve preparation was used. Static vessel wall properties were derived from diameter. Threshold and suprathreshold sensitivity of 41 BR were expressed both in terms of pressure (P_{th}, S_{th}) and wall strain ($\Sigma=r-r_o/r_o$, r=midwall radius, r_o = unstressed r).

Age (wks)	5	8	14	20	30	115
P_{th} (n)	88.2(14)	90.7(15)	86.5(28)	92.0(11)	93.9(19)	85.7(41)
S_{th}	1.02	1.50	1.65	1.80	2.01	1.13
Σ_{th}	.275	.309	.395	.480	.438	.377
S_Σ	261	222	217	247	348	250

Normotensive aged WKYs had increased vessel wall thickness and decreased distensibility from the 30 wk. BR Σ_{th} decreased to maintain a constant P_{th} despite drops in both S_{th} and S_Σ. Rapid resetting was not different from younger rats. Thus BR sensitivity appears to be more closely linked to vessel mechanics while the operating point may be importantly regulated by rapid resetting keeping P_{th} near MAP. Amer. Heart. Assoc., TX

24.7

TOTAL SUBFORNICAL ORGAN (SFO) DE-EFFERENTATION FAILS TO INHIBIT THE PRESSOR RESPONSE TO CIRCULATING ANGIOTENSIN II. R.W. Lind*, L.E. Ohman*, M.B. Lansing*, and A.K. Johnson. Univ. of Iowa, Iowa City, IA 52242

Much evidence suggests that circulating angiotensin II (AII) acts in the SFO to initiate drinking and pressor responses. We recently demonstrated with knife-cut lesions that neural efferents from the SFO to the median preoptic nucleus are important for the AII-induced drinking response but not for the pressor response. In order to test the hypothesis that SFO efferents to other neural structures carry information relevant to the pressor response, cuts were made of the ventral stalk of the SFO which would interrupt all known SFO efferents. Rats were tested for drinking responses to a large (3 mg/kg) dose of subcutaneously injected AII and cuts of the ventral stalk blocked the response. Animals with cuts of the dorsal stalk (which is not known to carry any SFO efferents) drank an amount similar to that of rats with sham knife-cuts and animals with cuts that separated the caudal half of the SFO from the rostral half had a 50% reduction in drinking. Pressor responses were measured following intravenous injections of 75, 150, and 300 ng/kg of AII. Dose-dependent pressor responses were elicited but no group differences were found. Thus, knife-cuts of SFO efferents interrupted AII-induced thirst while failing to inhibit the pressor response. These findings may indicate that information related to blood pressure from angiotensin receptors in the SFO is carried by a humoral messenger rather than by ventrally directed nerve fibers.

Supported by PHS Grants HLP-14388 and 1 RO1 HL24102

24.8

HUMAN CAROTID BAROREFLEX CONTROL OF HEART PERIOD AND ARTERIAL PRESSURE IS MAINTAINED DURING ISOMETRIC EXERCISE. TJ Ebert*, GM Janik*, KJ Kotrly*, JA Barney*, A Stadnicka* and JJ Smith. Dept. of Physiol., Med. Col. of Wisconsin, Milwaukee, 53226, and VA Med. Ctr., Wood, WI 53193

The simultaneous rise in heart rate and arterial pressure during isometric handgrip exercise (IHE) suggests that arterial baroreflex function may be deranged. We used 6 intensities of neck suction (+20 to 0,-10,-20,-30,-40,-50 mmHg) to examine human carotid baroreflex function at rest and during 5 separate 3 min sequences of IHE at 20% of subject's maximum grip strength. Arterial pressure was monitored through a radial artery catheter. Random 5 sec neck suctions (NS) were applied during end-expiratory apnea. Each NS was initiated 0.8 sec prior to the ECG p-wave. Seven healthy young men were studied. IHE elevated mean arterial pressure (MAP, 10 ± 1 mm Hg) and decreased R-R interval (R-RI, 124 ± 19 msec). R-RI and MAP responses to similar carotid sinus distending pressures (systolic pressure - NS intensity) were diminished during IHE compared to control. We derived stimulus-response curves relating carotid sinus distending pressure to R-RI and MAP. These curves were displaced to the right during IHE indicating a change in threshold (set point) had occurred. However, calculated gains (slopes) were not different from those determined during control. Thus our data indicate that during mild isometric exercise carotid baroreflex modulation of R-RI and arterial pressure is maintained. (Supported by AHA/Wisconsin Affiliate and VA Medical Center).

24.9

THE EFFECTS OF LEFT AND RIGHT STELLECTOMY ON BAROREFLEX CONTROL OF HEART RATE. G. E. Billman, P. J. Schwartz*, H. L. Stone. Dept. of Physiol., Univ. of Okla. HSC., Okla. City, OK 73190.

Ten mongrel dogs (14.5 to 25 kg) were chronically instrumented to measure arterial blood pressure and ECG. After a three-four week surgical recovery period, the baroreceptor reflex control of heart rate was assessed before and after beta blockade (BB 1.0 mg/kg propranolol HCl). The animals were given i.v. bolus injections of nitroprusside (100 µg/kg) and phenylephrine (10 µg/kg) to raise or lower systolic arterial pressure 30 to 50 mm Hg. R-R interval and heart rate were plotted as a function of systolic pressure. The slope (an index baroreflex sensitivity) was determined by linear regression ($r > .80$). The left (LSGX n = 6) or right (RSGX n = 4) stellate ganglion was removed and after a recovery period the experimental protocol above repeated. The control (pre-drug) heart rate was significantly ($p < .01$) elevated by LSGX (control 98.7 ± 10.6 vs LSGX 120.2 ± 12.2 bpm). These differences were eliminated by BB. Neither BB, LSGX nor RSGX significantly altered the baroreflex slope (HR slope control 1.6 ± 0.2 BB 1.4 ± 0.3 LSGX 1.4 ± 0.6 RSGX 1.2 ± 0.3 beats/mm Hg). These data suggest that a) reflex adjustments of heart rate in response to arterial pressure changes are mediated primarily through the vagus nerves in the dog and b) unilateral stellectomy does not alter baroreflex control of heart rate. (Supported by NIH Grants HL07430 and HL22154).

24.10

SUSTAINED INTERRUPTION OF AORTIC BAROREFLEX BY LEFT CERVICAL VAGOTOMY. S.C. Walgenbach, Department of Physiology and Biophysics, Mayo Medical School, Rochester, Minnesota 55905.

During periods of elevated aortic pressure in dogs, the major inhibition from aortic baroreceptors to the vasomotor center is relayed by the left aortic nerve. The present study examined whether the minor inhibition exerted by the right aortic nerve would be augmented with time. In 7 conscious dogs, the rise in arterial blood pressure (ABP) and heart rate (HR) in response to bilateral carotid occlusion (BCO) was measured. Before left cervical vagotomy (LCV), the BCO induced rise in ABP was 28±12 mmHg, and HR did not change. LCV increased resting ABP by 13±5 mmHg and HR by 20±6 beats/min. Immediately after LCV, BCO induced greater rises in ABP (63±10 mmHg) and in HR (38±7 beats/min). In the next 3 weeks, resting ABP and HR declined, while the increase in ABP and HR in response to BCO were unchanged. Right cervical vagotomy (RCV), 3 weeks after LCV, resulted in an increase in resting ABP of 36±9 mmHg and in HR of 95±11 beats/min. The BCO induced increase in ABP before and after RCV were similar, 59±8 and 66±8 mmHg, respectively. Thus LCV interrupts, for at least 3 weeks, the majority of aortic arch inhibition of a BCO induced rise in ABP. (Supported by NIH grant HL 05883.)

24.11

ATTENUATION OF BAROREFLEXES DUE TO ISOFLURANE. E.O. Elegbe*, J.L. Seagard, Z.J. Bosnjak, F.A. Hopp*, and J.P. Kampine. College of Medicine, Univ. of Ibadan, Nigeria; Med. College of Wisconsin and Wood VA Center, Milwaukee, Wisconsin 53193

The effects of isoflurane (I) on the baroreceptor reflex have not been thoroughly investigated. This study examined the relative importance of effects of I on the carotid sinus baroceptors and their afferent input; the central nervous system, through sympathetic preganglionic nerve activity (NA); ganglionic transmission, through postganglionic NA; and the direct end-organ response of the heart, through direct stimulation of sympathetic and parasympathetic efferent nerves 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 six conscious and anesthetized (1.3% and 2.6% I) dogs. Both levels of I significantly attenuated the reflex changes in heart rate, in spite of an accompanying sensitization of the baroreceptors seen as an increase in carotid sinus afferent NA for a given pressure. Dose-dependent decreases in baseline and reflex changes in postganglionic sympathetic NA were significantly greater than those in preganglionic NA, indicating an effect of I on ganglionic transmission. Finally, both levels of I significantly blunted heart rate changes produced by direct stimulation of cardiac efferent nerves. Therefore I attenuation of the baroreflex appears to have multiple sites of action. (Supported by NIH Grant GM 29641 and the VA).

24.12

RELATIVE INFLUENCE OF THE SINOAORTIC AND CARDIOPULMONARY BARORECEPTORS ON THE RENAL AND LUMBAR SYMPATHETIC OUTFLOW David R. Brown* and Marc D. Thames. Cardiovascular Center, University of Iowa College of Medicine, Iowa City, IA

Reflex control of sympathetic outflow to different regional vascular beds is nonuniform. This nonuniformity may be due in part to differential influences of sinoaortic(SA) and vagal cardiopulmonary(CP) baroreflexes on specific regional outflows. The purpose of this study was to determine the relative influence of SA and vagal CP baroreflexes simultaneously recorded on renal(RNA) and lumbar(LSNA) sympathetic outflow during phenylephrine induced increases in arterial pressure (AP). Experiments were done in anesthetized rabbits with baroreflexes intact, following SA or CP denervation(D), and after SAD and CPD. The mean (±SE) slopes of the regression relationships relating changes in AP and changes in nerve activity are summarized in the table (*p<0.05 RNA vs LSNA).

	C(n=15)	SAD(n=8)	CPD(n=7)	SAD+CPD(n=3)
RNA	-3.11±.4	-1.46±.4	-3.02±.4	-.09±.051
LSNA	-2.45±.5*	-.80±.3*	-2.52±.5	-.09±.09

There is greater inhibition of RNA than of LSNA during increases in AP. These responses are mediated by the SA and CP baroreceptors (abolished after SAD and VD). The results also suggest the CP baroreceptors may contribute to the differential responses of RNA and LSNA (responses after SAD). Thus, nonuniformity of baroreflex control of sympathetic outflow to different regional beds is due to differential influences of SA and CP baroreflexes.

CARDIAC DYNAMICS I

25.1

ASSESSMENT OF IMPEDANCE CARDIOGRAPHY FOR MEASURING CARDIAC OUTPUT DURING HYPOXIA. William R. Sexson*, Daniel S. Miles, Robert W. Gotshall and Bruce S. Bradley*. Coll. Sci. & Egr., Sch. of Medicine, Wright State University, Dayton, OH 45435

Hypoxia is a consequence of most conditions which necessitates monitoring of cardiac output ($\dot{Q}$), therefore, a reliable, non-invasive method of assessment of $\dot{Q}$ is desirable. The purpose of this study was to correlate $\dot{Q}$ and stroke volume (SV) measurements obtained by thermal dilution (TD) with those obtained by impedance cardiography (IC). Eight spontaneously breathing dogs were anesthetized with pentobarbital and monitored during ambient and hypoxic conditions. Progressive hypoxia (F_{IO_2}=.12 and .08%) was maintained for 30 minutes at each level, with 30 minutes of ambient air recovery after each period of hypoxia. Central hemodynamic variables were measured simultaneously by TD and IC. Both techniques demonstrated a similar increase in $\dot{Q}$ ($p<0.05$) during hypoxia. This increase in $\dot{Q}$ was due to an increase in heart rate with no significant change in SV. SVs obtained by both methods were significantly correlated (r=.734). The highest correlation for SV occurred during the ambient control period (r=.877). The lowest correlation (r=.566) was found during the recovery from breathing 8% oxygen. In conclusion, IC does appear promising as a technique with which to assess central hemodynamics for both normoxic and hypoxic conditions. (Supported by the Miami Valley Chapter of the AHA)

25.2

MODULATION OF SYNCHRONY OF MYOCARDIAL CONTRACTION AND EJECTION DURATION BY REGIONAL ADRENERGIC STIMULATION IN CONSCIOUS DOGS. Dean Franklin, Patricia A. Gwirtz and Daniel P. McKown,* Dalton Research Center, Univ. of Missouri, Columbia, MO 65211

The sympathetic innervation of the left ventricle provides a potential mechanism for modulation of synchrony of myocardial contraction by higher integrative centers. We tested the potency of this mechanism. Two dogs were instrumented for measurement of left ventricular pressure (LVP), segment length in the region perfused by the circumflex artery (CSL) and in the region perfused by the anterior descending artery (ASL). A catheter was implanted within the circumflex artery. After recovery (>14 days) norepinephrine (NE) was injected into the circumflex artery in bolus doses sufficiently small to produce no direct systemic effects.

		Control	0.3 µg NE	0.5 µg NE
Segment Shortening, %	ASL	18.3	22.2	22.0
	CSL	13.9	20.5	20.7
Mean Shortening Velocity (end diastolic length/sec)				
	ASL	1.1	1.3	1.3
	CSL	.81	1.9	2.1
Shortening Duration (msec)	ASL	165	175	175
	CSL	170	110	100
LV Ejection Duration (msec)		155	90	85

There was no change in LV systolic pressure. These results were confirmed in 2 acute preparations. Thus, profound changes in the synchrony of contraction and the duration of ejection may be produced by regional adrenergic stimulation.

25.3

CARDIOVASCULAR CHANGES FROM EXPIRATION TO INSPIRATION DURING MECHANICAL VENTILATION. William P. Santamore, James L. Heckman & Alfred A. Bove. Bockus Research Institute, Philadelphia, PA 19146.

In vitro studies indicate that the volume of one ventricle influences the diastolic distensibility of the opposite ventricle. In addition, right ventricular systolic function was depended upon left ventricular volume and structural integrity. Based on these in vitro observations, ventricular interdependence was examined in 12 anesthetized dogs. Right and left ventricular volume and pressure and esophageal pressure were simultaneously measured during mechanical ventilation at zero-end expiratory pressure. The volumes were calculated from the cinefluorographic positions of endocardial, radiopaque markers. Comparing expiration to inspiration, esophageal pressure decreased (4.6 ± 0.3 to -2.1 ± 0.2 mmHg, $p < 0.05$); a right ventricular diastolic volume increased (31.0 ± 1.3 to 36.3 ± 1.6 ml, $p < 0.05$) and ejection fraction increased (0.48 ± .03 to 0.55 ± 0.3, $p < 0.05$); while, left ventricular diastolic pressure increased (6.8 ± 1.6 to 8.3 ± 1.5 mmHg, $p < 0.05$), diastolic volume decreased (33.6 ± 1.3 to 32.9 ± 1.3 ml) and ejection fraction decreased (0.54 ± 0.3 to 0.52 ± .03, $p < 0.05$). These data indicated a decreased left ventricular distensibility as right ventricular diastolic volume increased. The decreased left ventricular ejection fraction suggest a possible systolic interaction between the ventricles. (Supported by USPHS NIH Grant HL28459).

25.4

CARDIAC FUNCTION AND STRUCTURE AFTER HEMODILUTION WITH A FLUOROCARBON BLOOD SUBSTITUTE. G.P. Biro*, F.C. White, B. Guth*, E.A. Breisch, C.M. Bloor. Univ. of California at San Diego, La Jolla, California 92093 and Univ. of Ottawa, Ont. K1N 9A9, Canada.

Fluosol-DA, a putative "blood substitute" which allows the transport of significant amounts of dissolved oxygen in the plasma-phase during ventilation with oxygen, was used to replace 60% of the estimated blood volume in anesthetized pigs, reducing the hematocrit to 13±2%. Regional myocardial blood flow (using 15 µm microspheres) and left ventricular wall thickness (using ultrasonic dimension gauges) were measured before (PRE-EXCH.) and after (POST-EXCH.) the exchange. The findings in the territory of the left anterior descending coronary artery were:

	PRE-EXCH.	POST-EXCH.
End-diast. wall thickness (mm)	8.34±.69	8.14±.75 (N.S.)
End-syst. wall thickness (mm)	10.30±.86	10.16±.81 (N.S.)
Systolic thickening (%)	23.4 ±3.4	24.2 ±3.6 (N.S.)
Endocardial flow (ml/g/min)	0.93 ±.29	1.42 ±.26 ($p<.005$)
Epicardial flow (ml/g/min)	0.97 ±.27	1.34 ±.21 ($p<.02$)

One hour after the exchange the LV myocardium was perfusion fixed and examined electron microscopically. The subendocardial, mid-wall, and subepicardial layers revealed normal mitochondrial, myofibrillar, and sarcoplasmic ultrastructure. Thus, ventricular function and structure are not significantly affected by the short-term replacement of a large fraction of blood volume with a fluorocarbon "blood substitute".

26.1

IN VIVO MEMBRANE POTENTIALS FROM THE CAUDAL ARTERY OF THE RAT. Howard J. Bryant, David R. Harder, Stephen J. Huot, Motilal B. Pamnani, Francis A. Kutyna*, and Francis J. Haddy. Department of Physiology, Uniformed Services University, Bethesda, MD 20814 and Department of Physiology & Biophysics, University of Vermont, Burlington, VT 05405

Membrane potentials measured in vivo may differ significantly from those measured in vitro in part due to circulating plasma factors, innervation, and vessel wall tension. These studies were initiated to determine whether it is feasible to measure membrane potentials in vascular smooth muscle cells in rat caudal artery in vivo. Anesthetized normotensive (systolic BP<130 mmHg) male Wistar rats (250-325g) were intubated and mechanically ventilated. The caudal artery was exposed from the ventral side and the entire tail was submerged in a temperature-controlled chamber at 37°C. After a stabilization period of 30 minutes, fixed (non-floating) microelectrodes were inserted into the smooth muscle cells. Membrane potentials ranged from 68 to 29 mV and generally contained oscillations correlated with the blood pressure. Spontaneous activity was observed in some of the in vivo cells. In contrast, however, spontaneous activity was never observed in the in vitro preparation. When action potentials occurred, they fired at a rate of about 7 per minute. Action potentials did not overshoot nor did they have a plateau phase on repolarization. Both single and multiple spikes were observed. (Supported by NIH Grants HL21525-05, HL27862 and USUHS Grants CO7605, CO7607).

26.2

INCREASE IN ALPHA-ADRENOCEPTOR AFFINITY IN DOG SAPHENOUS VEIN CAUSED BY PROFOUND COOLING. N.J. Rusch,* J.T. Shepherd and P.M. Vanhoutte. Department of Physiology and Biophysics, Mayo Foundation, Rochester, MN 55905.

Moderate cooling of the dog saphenous vein from 37° to 24°C increases the affinity of postjunctional alpha-adrenoceptors, causing an augmentation of the contraction to norepinephrine (Janssens and Vanhoutte, Am. J. Physiol. 234: H330, 1978). This augmentation is still present at temperatures as low as 10°C (Rusch et al., J. Physiol. 311:57, 1981). The present study investigated whether the enhanced response to norepinephrine at 10°C could be explained by an increase in adrenoceptor affinity. Rings of dog saphenous vein were mounted in organ baths for isometric tension recording. Dose-response curves to norepinephrine were performed in paired rings at 10° and 37°C in control solution and in solutions with increasing concentrations of phentolamine. The results show that the dose-response curve to norepinephrine at 10°C is shifted in a parallel manner to the left of the curve at 37°C. The pA_2 for phentolamine is significantly higher at 10° (7.84) than at 37°C (7.21). These findings indicate that the affinity of the alpha-adrenoceptors is increased at 10°C. This increase in affinity could contribute to the maintenance of vasoconstriction in cutaneous veins at very cold temperatures. (Supported by NIH grant HL 05883.)

26.3

CALCIUM AND α-ADRENOCEPTOR ACTIVATION IN ISOLATED CANINE BLOOD VESSELS. T.J. Rimele* and P.M. Vanhoutte. Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905.

The different subtypes (α_1 or α_2) of postjunctional α-adrenoceptors may be linked preferentially to either the entry of extracellular or the mobilization of cellular calcium. The present study was designed to compare the effects of calcium entry blockers on the responses of isolated canine blood vessels to norepinephrine (NE), sympathetic nerve stimulation (ES) and synthetic α-adrenoceptor agonists with varying affinities for α_1 (phenylephrine; PE) and α_2 (xylazine; XYL) adrenoceptors. The preparations were mounted for isometric tension recording in organ chambers filled with physiological salt solution. In the portal vein NE and PE were full agonists while XYL produced only a minimal response; the K_B's for prazosin and thymoxamine were comparable to those obtained in other tissues containing only α_1-adrenoceptors. Diltiazem and verapamil markedly inhibited the responses to NE, PE and ES. Similar results were obtained in the coronary artery. In the saphenous vein, which contains both α_1 and α_2-like adrenoceptors, diltiazem and verapamil had only moderate effects on the responses to NE, PE and ES but markedly reduced that to XYL. These experiments suggest that the dependency of α-adrenoceptor activation upon calcium entry is determined by the functional differentiation of the smooth muscle cells rather than by the apparent pharmacological characteristics of their α-adrenoceptors.

26.4

DOSE-TIME RELATIONSHIPS OF NE-INDUCED CALCIUM INFLUX IN VASCULAR SMOOTH MUSCLE. D. L. Davis, J. M. Price and C. H. Baker, University of South Florida, Tampa, Florida 33612.

Calcium influxes were obtained from dog anterior tibial and ulnar arteries. The adventitia was removed, the vessels were cut into lengths weighing 10-15 mg, and mounted on stainless steel holders. After 60 min equilibration in normal PSS the vessels were exposed to 10^{-7}, 10^{-6}, $3\cdot10^{-6}$, 10^{-5}, and 10^{-4} M NE for 1-5 min. Calcium influxes, obtained by ^{45}Ca uptake procedures using the cold lanthanum method, were calculated from consecutive 60 sec uptake periods during exposure to NE. Resting calcium influx rates averaged 0.17 μ moles/Kg tissue/ sec. Calcium influx rates were not significantly increased during exposure to 10^{-7} M NE. Dose-dependent increases in calcium influx occurred at concentrations greater than 10^{-7} M NE. Peak rates of calcium influx occurred earlier during the exposure period as the concentration of NE was increased. At all concentrations of NE decreases in influx rate occurred during the latter portion of the exposure period. Although a clear dose-dependent effect of NE on calcium influx was evident, there was no indication of a delayed increase in calcium influx at the low concentrations of NE, or of a biphasic increase in calcium influx at NE concentrations which have been reported to produce biphasic mechanical responses (Supported by American Heart Association, Florida Affiliate and HL-24151).

26.5

VERAPAMIL ATTENUATES THE RENAL AND CARDIOVASCULAR RESPONSES TO VANADATE IN THE CONSCIOUS DOG. W. D. Sundet*, B. C. Wang, M. O. K. Hakumaki*, J. S. Mapes*, and K. L. Goetz. St. Luke's Hospital and Foundation, Kansas City, MO 64111

We investigated the hemodynamic and renal responses of intravenous vanadate in 5 conscious dogs. Infusion of sodium orthovanadate (0.32 μM/kg/min for 30 min) significantly increased mean aortic pressure (27 mm Hg), total peripheral resistance (0.50 PRU), mean pulmonary arterial pressure (4.2 mm Hg), left atrial pressure (3.6 mm Hg), and cardiac output (158 ml/min). Plasma renin activity and urine flow decreased significantly, but plasma vasopressin was unchanged throughout the experiment. Since in vitro studies have indicated that vanadate inhibits Na-K-ATPase and Ca-ATPase, both of which affect the intracellular calcium concentration, we evaluated the effects of vanadate after administration of the calcium channel blocker, verapamil. No changes in cardiac output, plasma renin activity, urine flow, or plasma vasopressin occurred when vanadate was given in the presence of verapamil. In addition, mean aortic and pulmonary arterial pressure responses to vanadate were significantly reduced in the presence of verapamil, and the effects on total peripheral resistance and left atrial pressure were attenuated, but not significantly. These results are consistent with the hypothesis that renal and hemodynamic changes produced by vanadate in the conscious dog are mediated in part by changes in intracellular calcium concentration.

26.6

Functional alterations of vascular smooth muscle in hypercholesterolemia. R.Broderick* and T.Tulenko. Med Coll of PA, Phila., PA 19129.

Atherosclerosis is classically described as a disease of the large arteries. This study was designed to determine if the smaller arteries were functionally altered in this disease state. Dutch Belt rabbits were fed a high cholesterol/fat (HC) diet for 6 months resulting in plasma cholesterol levels of 1270 ± 181 mg/dl in the HC group and 40 ± 10 mg/dl in the control group. Histology of the HC group revealed marked atherosclerosis in the aortas and mild lesions in the renal arteries while the smaller femoral arteries were free of lesions and appeared normal; all arteries of the control group appeared normal. Femoral arteries of both groups were perfused with a physiological salt solution under constant flow (2ml/min) while perfusion pressure was monitored to determine vascular resistance. Dose-response analyses demonstrated a 10-fold increase in sensitivity to norepinephrine in the femoral arteries of the HC group compared to those of the control group ($p < .01$), and similarly, a 3-fold increase in sensitivity to histamine ($p < .05$). Additional studies measuring Rb^{86} uptake as well as K^+ relaxation of arterial strips demonstrated enhanced Na^+-pump activity in the arteries of the HC group compared to those of the control group. We can conclude that femoral arteries with no atherosclerotic lesions obtained from the hypercholesterolemic atherosclerotic rabbit were functionally altered in that they a) were supersensitive to norepinephrine and histamine, and b) demonstrated enhanced Na^+-pump activity when compared to arteries from normal rabbits. (Supported by NIH Grants HL07443 and HL 24512).

26.7

EFFECT OF GUANETHIDINE ON HISTAMINE-INDUCED INCREASE IN CORONARY RESISTANCE IN RABBIT HEARTS. Jack T. Saari, University of North Dakota, Grand Forks, ND 58202.

Histamine (HA) increases flow resistance in the isolated, perfused rabbit heart, but the effect is transient. The transient nature implies either a desensitization of HA receptor sites or release of a secondary vasoactive substance which is gradually depleted. Literature evidence supports the latter concept in that HA may modify catecholamine (CA) release in various tissues. A prior test of this hypothesis in our preparation showed that phentolamine, an α-adrenergic blocker, inhibits the response to histamine. In current work, we tested whether guanethidine (GE, Ismelin SO_4, CIBA), a CA release blocker, inhibits the HA-mediated vasoconstriction. The protocol consisted of perfusing a given heart with HA (as 2HCl, 20 mg/L) for four successive intervals of 3.5 min each, separated by recovery intervals of 12 min. In the average control heart we observed an initial 114% resistance increase and an exponential decline in the resistance increase with each successive perfusion with HA. In a typical heart with GE (50 mg/L) in the perfusion solution before (6 min) and during the second perfusion with HA, the resistance increase was reduced 86% as indicated by the difference between the measured resistance change during GE and that predicted by interpolating between the first and third HA perfusions. Inhibition of the HA response by GE further supports the concept that HA acts by causing secondary release of CA. (Support: AHA-Dak. Aff. Grant DA-G-05/605 and NIH Grant HL 28217.)

26.8

AGGREGATING PLATELETS AND 5-HYDROXYTRYPTAMINE INHIBIT CANINE CORONARY ARTERY SMOOTH MUSCLE TONE BY AN ENDOTHELIUM-MEDIATED PROCESS. R.A. Cohen,* J.T. Shepherd and P.M. Vanhoutte. Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905.

Vasoactive substances, including 5-hydroxytryptamine, released from aggregating platelets could mediate localized spasm of coronary arteries. Isometric tension was measured in isolated ring segments of canine coronary arteries suspended in organ chambers. These rings contracted when exposed to 5-hydroxytryptamine (10^{-8}-10^{-5}M); the contractions were potentiated in rings physically denuded of endothelium. In rings precontracted with prostaglandin $F_{2\alpha}$, exposure to platelets (10^5/ml) and 5-hydroxytryptamine caused relaxation. This relaxation did not occur in rings denuded of endothelium. The relaxation on exposure to platelets was blocked, and that to 5-hydroxytryptamine competitively antagonized by the serotonergic $5HT_2$-antagonist, ketanserin ($K_B = 2 \times 10^{-8}$M). These studies indicate that 5-hydroxytryptamine released from aggregating platelets can inhibit the contractile responses of coronary vascular smooth muscle, presumably by an endothelium-mediated process. Thus, the coronary endothelium protects the vascular smooth muscle from the constrictor action of vasoactive substances released from aggregating platelets. (Supported in part by grant HL 05883.)

26.9

THE EFFECT OF PREGNANCY ON PERIPHERAL VASCULAR RESPONSIVENESS STUDIED IN VITRO. R.J. Porcelli, E.H. Bronstein*, J.F. Monckton* and S. McGillicuddy*. VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

The present study investigated the role of pregnancy (CR>45mm) in altering tension development of isolated femoral arterial segments to the biogenic amines (NorEpi, NE; Histamine, Hist; 5HT)and KCl. Vessel segments from control (C) and pregnant (P) cats were isometrically suspended in tissue baths and cumulative dose response curves (DR) developed. All agents showed linear increased in tension with increasing doses (/ml bath) and the ED 50, slope (m) and maximum responses (Max Resp) analyzed:

Agent:	(n)	ED50	m	MaxResp	(n)	ED50	m	MaxResp
NE(0.06-6000)	(34)	8.0±.1	112±18	409±52	(19)	8.0±.1	79±17	265±56
Hist(.09-9000)	(31)	8.1±.1	114±24	508±72	(17)	8.0±.1	58±10*	216±35*
5HT(.06-6000)	(27)	8.7±.1	86±12	336±63	(17)	8.5±.1	56±19	167±30*
KCl(10-640mM)	(29)	132±14	2.3±.6	486±78	(17)	169±24	.5±.1*	162±28*

P significantly reduced Max Resp to Hist, 5HT & KCl. The DR curves were unchanged except for m, suggesting ↓ efficacy of these agents. This ↓ Max Resp is not due to overall ↓ contractility since NE was not significantly reduced. Since KCl ↑ tension by depolarization, P seems to interfere with this mechanism. ↓ Max Resp to Hist & 5HT may have some relation to this possibility or P results in a more receptor specific alteration in this case. Furthermore, NE seems to develop tension by mechanisms that are less influenced by pregnancy. *p<0.05 (Supp. by Vets. Admin. and by NHLBI # 23210.)

26.10

INHIBITION BY METHACHOLINE OF DRUG-INDUCED RELAXATION AND cAMP-DEPENDENT PROTEIN KINASE (PK) ACTIVATION IN CANINE TRACHEAL SMOOTH MUSCLE. T.J. Torphy*, G.A. Rinard, S.M. Peterson*, Z. Cong* and S.E. Mayer. UCSD, La Jolla, CA 92093.

Cumulative concentration-response curves to d,l-isoproterenol (ISO), prostaglandin E_2 and forskolin (agents thought to relax smooth muscle via the cAMP-PK cascade) were conducted after precontracting trachealis strips with 0.3 or 3.0μM methacholine (MCH). ISO produced an 80±6% reversal of the contraction induced by 0.3μM MCH with a pD_2=6.32±0.10. ISO concentration-response curves were shifted to the right (pD_2=5.42±0.17) and the maximum response reduced (29±8% reversal) by 3.0μM MCH. Pretreatment with MCH also decreased the ability of ISO to elevate PK activity ratios (active enzyme/total enzyme). Mean values (±S.E.) from 8 paired experiments are shown below:

[ISO]μM	[METHACHOLINE]μM 0	0.3	3.0
0	0.28±0.01	0.25±0.01	0.23±0.03
30	0.50±0.03	0.39±0.02	0.31±0.03

Mechanical and PK responses to prostaglandin E_2 and forskolin were also greatly depressed by 3.0μM MCH. In contrast, relaxation produced by sodium nitroprusside, an agent which acts by a cAMP-independent mechanism, was only depressed slightly by increasing the MCH concentration from 0.3 to 3.0μM. These data indicate that muscarinic stimulation of canine trachealis antagonizes the mechanical responses to several smooth muscle relaxants and that a portion of this antagonism may be due to a suppression of PK activity. (HL24621 and HL06264)

26.11

EFFECT OF SKELETAL MUSCLE RELAXANTS ON BLADDER SMOOTH MUSCLE. F.M. Ayyat*, K.V. Kuhlemeier and L.K. Lloyd* Urology Rehabilitation and Research Center, University of Alabama in Birmingham, Birmingham, AL 35294

Baclofen, dantrolene sodium and diazepam are widely used to allievate skeletal muscle spasms of various neuromuscular disorders. Bladder smooth muscle hyper- or hypo-tonus is a common complication of these disorders. This study was conducted to determine the direct effect of skeletal muscle relaxants on bladder smooth muscle contractions. Rat bladder muscle strips were incubated in a 50-ml organ bath filled with physiological salt solution, aerated with 95% O_2, 5% CO_2 and maintained at 37°C. Contractions were induced both electrically and with acetylcholine (Ach). Contractile strength was measured before and after 30-70 minutes incubation with baclofen (4 mcg/ml), dantrolene sodium (4 mcg/ml) or diazepam (0.25 to 1.0 mcg/ml). Neither baclofen nor dantrolene sodium had any effect (p>0.3) on electrically- or Ach-induced contractions but diazepam potentiated both electrically-induced (115% of pre-drug contractile strength, p<0.05) and Ach-induced (122% of pre-drug contractile strength, p<0.01) contractions. While diazepam is thought to exert its effects on skeletal muscle through CNS inhibition, our results suggest that diazepam may effect smooth muscle directly. (Supported in part from Grant No. 16-P-55680 7/4 by National Institute for Handicapped Research).

27.1

HEAVY METAL-INDUCED INHIBITION OF INTESTINAL TRANSPORT IN THE RAT. ANTAGONISM WITH SELENIUM. S.J.Iturri*, A.Peña*, P.Yavar*, J.L.Torres* and C.Pino*. (SPON: R.Gallardo). Fac.Cs.Básicas y Farm. Univ. of Chile. Santiago, Chile.

Cadmium and lead are capable of inducing a wide variety of toxic manifestations after parenteral or oral administration. Also, there is evidence that selenium may interact with these heavy metals, rendering these substances less toxic. The present studies were carried out to determine the effect of cadmium and lead on sugar and aminoacid active transport through rat intestinal epithelium. Everted intestinal sacs from Sprague Dawley rats were incubated at 37°C in Krebs-Henseleit solution containing D-glucose (10mM) and L-tyrosine (2mM). The active transport of both compounds was evaluated by determining their increase in concentration inside the sac at 60 minutes. Cadmium and lead (10^{-4} and 10^{-5}M) inhibit the active transport of glucose and tyrosine, this effect being due to a direct action of these ions on the active transport mechanism as is shown by a decrease of the Na^+/K^+ ATPase activity from rat intestinal mucose membrane fractions. When selenium (10^{-6}M) was added to the medium of these everted sacs, the active transport of glucose and tyrosine was inhibited only 4-10%. These results suggest that the glucose and tyrosine active transport is inhibited by cadmium and lead affecting the Na pump, but in the presence of selenium this inhibition is almost eliminated.
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27.2

EFFECT OF DIETARY CARBOHYDRATE ON INTESTINAL GLUCOSE TRANSPORT IN MICE. William H. Karasov and Jared M. Diamond. Physiology Department, UCLA Medical Center, Los Angeles, Calif. 90024.

We measured active glucose absorption by the small intestine in vitro in laboratory mice fed high-carbohydrate (HC) and carbohydrate-free (NC) diets. Measured tracer fluxes were corrected for adherent fluid and passive uptake. At every position along the intestine except the ileum, HC mice exceeded NC mice in active transport per cm or mg of intestine, and summed transport over the whole small intestine was 40% higher in HC mice. In the jejunum, the region where transport is greatest, HC mice exceeded NC mice both in maximal transport rate V_{max} (300 ± 16 [S.E.M.] vs. 180 ± 27 n-moles/cm,min) and in apparent Michaelis constant K_t (3.6 ± 0.4 vs. 1.7 ± 0.1 mM). The differences in apparent K_t may be an artifact of unstirred layers, given the difference in V_{max}. Villus surface area did not change with diet. Hence the simplest explanation of the results is that dietary carbohydrate stimulates glucose transport by induction of glucose carriers, as expressed in the increased V_{max}. This effect is rapid: V_{max} changes within 24 - 48 hrs of a change in dietary carbohydrate. (Supported by NIH grants GM 14772 and Center for Ulcer Research and Education AM 17328).

27.3

ENHANCED ABSORPTION OF THE CARCINOGEN BENZO(A)PYRENE DURING LIPID HYDROLYSIS AND ABSORPTION. Russell D. Vetter* and John S. Patton* (SPON: J. Crim). Univ. of Georgia, Athens, Ga. 30602

Benzo(a)pyrene has a water solubility of 3.8 ng/g but is soluble in triolein to 19.8 mg/g. In experiments designed to measure the effect of various diets on the absorbability of B(a)P, natural triglyceride enhanced the absorption of a single dose of B(a)P as much as 10 times over that absorbed from a strictly protein diet. In vitro fluorescence measurements indicate that the effect is not due primarily to the increased solubility of B(a)P in lipid but to the quantitative transfer of B(a)P from the initial triglyceride droplet to the product phase (fatty acid - monoglyceride) during hydrolysis by pancreatic lipase. Subsequent dispersion by bile salt mixed micelles allows B(a)P to follow a continuous hydrophobic domain to the absorptive epithelia resulting in enhanced uptake. The coabsorption of lipid does not appear to effect the postabsorptive fate of B(a)P. The results suggest a mechanism to explain the unexpectedly high accumulation potential of other extremely hydrophobic toxicants such as polychlorinated biphenyls and DDT when consumed in conjunction with natural lipid. (Supported by USPHS. NIH Grant AM #27304)

27.4

FACTORS REGULATING PANCREATIC DUCT PRESSURES. David A. Dreiling, Ehud Klein* and Hugo Grateron*. The Mount Sinai Medical Center, New York, NY 10029.

Increased pancreatic duct pressure has been implicated in several hypotheses elucidating the pathogenesis of pancreatic inflammation. Pancreatic ductal pressure is known to depend directly on the rate of secretion and indirectly upon pancreatic blood flow. This study investigates the effect on PDP of vagal tone, sympathetic activity, intravenous and peroral alcohol administration, as well as anesthesia and stimulation of the periampullary duodenal mucosa. Studies of pressures in canines were performed before and after vagisection, celiac ganglionectomy, intravenous, intragastric and intraduodenal alcohol, as well as lidocaine applications to and electrical stimulation of the papilla of Vater. The results indicate that pancreatic ductal pressures are regulated in part and sensitive to local and remote duodenopancreatic reflexes which exert their effects upon the rate of blood flow, the rate of secretion, as well as directly on the ducts themselves and the Odditic sphincteric mechanism.

27.5

HEPATIC LYMPH FLOW AND LYMPH PROTEIN CONCENTRATION IN THE RAT. Jui S. Lee, Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Holtzman rats (300-450 gm B.W.) were anesthetized by pentobarbital. After the abdomen was opened by crisscross incisions, the hepatic lymph duct was cannulated by inserting a 22-gauge hypodermic needle tip into it, which was attached to a 20-cm length of polyethylene tubing. In about 40% of rats there are two hepatic lymph ducts, and the rest have one duct adjacent to the celiac artery. Both hepatic and intestinal lymph ducts were cannulated in some experiments. In the fasted or unfasted rats, hepatic lymph flow (J_L) was 0.2 ± 0.1 ml/kg·hr, lymph protein concentration (C_L) was 4.3 ± 0.5 gm%, plasma protein concentration (C_p) was 6.2 ± 0.3 gm%, and C_L/C_p was 0.7 ± 0.1 (mean ± S.D., N = 12). Hepatic J_L was at most about one-eighth of intestinal J_L. Hepatic J_L increased and C_L decreased during biliary obstruction or during saline infusion into the liver via bile duct (= 0.7 ml/min) at an infusion pressure of 20 mmHg, indicating close relationship between biliary and lymphatic systems. Similar to other organs, hepatic J_L increased by 2 to 8 x and C_L decreased to about 1 gm% during intravenous infusion of saline (2.5 ml/kg.min) due to increased sinusoidal filtration. Furthermore, during intestinal digestion and water absorption, both hepatic J_L and and C_L did not change. (Supported by NIH grant AM 18085).

27.6

A QUANTITATIVE ANALYSIS OF DIGESTIVE ENZYMES IN PENAEID SHRIMP, INFLUENCE OF DIET, AGE AND SPECIES. Phillip G. Lee and Addison L. Lawrence. Texas Agricultural Experiment Station and Department of Wildlife and Fisheries Sciences, Texas A&M University, P.O. Drawer Q, Port Aransas, TX 78373

The effect of protein level and source on the digestive potentials of penaeid shrimp was evaluated using digestive enzyme assays. Several sizes (2.5-15g) and species of penaeid shrimp, Penaeus aztecus, P. setiferus, P. occidentalis, P. stylirostris and P. vannamei were utilized in a series of thirty day growth trials. The shrimp were fed once daily (4% body weight) with pelleted diets having standardized protein levels (22, 29 and 36%) and animal protein to plant protein ratios (2:1 and 1:1). Upon termination of the growth trials an extract of the digestive gland was analyzed for the specific activities of seven digestive enzymes. The following activities were detected using specific synthetic substrates: trypsin, carboxypeptidase A, carboxypeptidase B, amylase, non-specific esterase and lipase. The extracts lacked chymotrypsin activity. Individual shrimp within each experimental treatment exhibited large differences in enzyme activities. Although no quantitative differences in the levels of activity were related to species or sex, there were quantitative differences in relation to age and diet. In conclusion, digestive enzyme analysis provides a useful technique for assessing the digestive potentials of penaeid shrimp. (Research supported in part by a Texas A&M University Sea Grant Marine Fellowship).

27.7

Ca^{++}-cAMP INTERACTIONS IN THE ACTIVATION OF GASTRIC ACID SECRETION. F. Michelangeli, M.C. Ruiz and C. Jirón. IVIC, Apartado 1827, Caracas 1010A, Venezuela.

Cyclic-AMP has been proposed as the intracellular messenger in the activation of the gastric secretory process by histamine. Based on studies in isolated membranes we have proposed (J.M.B. 42, 301, 1978) that Ca^{++} may be the final activator. We have studied the possibility of participation of the two messengers in isolated toad gastric mucosa. Ca^{++} ionophore A23187 stimulated H^+ secretion. At high doses, it also stimulated histamine release. H_2-blocker metiamide inhibited 70% of response to A23187 whereas completely blocked histamine. Amount of histamine released was insufficient to explain secretory rates attained with A23187 as indicated by parallel experiments with other histamine-releasing conditions like K^+-depolarization. These results indicated that histamine was required but not sufficient to induce secretion, suggesting the joint participation of Ca^{++} and histamine-induced cAMP. Histamine also affected distribution of intracellular Ca^{++} as seen from chlorotetracycline fluorescence. In metiamide inhibited mucosae subliminal doses of db-cAMP or IMX potentiated response to A23187. In mucosae maximally stimulated by histamine or db-cAMP, A23187 induced a further increase in secretion. The results suggest the joint participation of Ca^{++} and cAMP as co-messengers in the activation of H^+ secretion by secretagogues.

27.8

DIRECT ACTION OF ACh ON THE GASTRIC OXYNTIC CELL. M.C.Ruiz and F. Michelangeli. IVIC, Apartado 1827, Caracas, Venezuela.

The possibility of a direct action of acetylcholine (ACh) on the oxyntic cell, not mediated by histamine release, was studied in isolated frog gastric mucosae. H^+ secretion and histamine release, were measured. ACh released histamine and stimulated H^+ secretion. Dose-response curve showed a roughly parallel increase in H^+ secretion and histamine release by ACh. Dose-response curves to exogenous and endogenous histamine (released by ACh) were compared. ACh decreased apparent K_m and increased V_{max} and slope for endogenous histamine. Comparison of the effects of ACh to other experimental conditions such as K^+ depolarization or Na^+-free nutrient solution, showed that the amount of histamine released by ACh is insufficient to explain the observed rates of secretion. Stimulation by ACh was faster, larger and more transitory as compared to that by histamine. In mucosae maximally stimulated by histamine, ACh induced a further increase in H^+ secretion. The results are consistent with ACh having a dual action on oxyntic and histamine-releasing cells. It is suggested that ACh and released histamine act on the oxyntic cell through different second messengers (Ca^{++} and cyclic AMP, respectively) resulting in the potentiated response.

27.9

ADENOSINE PREVENTS THE FORMATION OF ASPIRIN-INDUCED GASTRIC DAMAGE. M.R. Feller* and D.P. Moorhead* (SPON: R.R. Warner). The Procter & Gamble Company, Cincinnati, Ohio 45247

A natural cell component, adenosine, inhibits histamine-stimulated acid secretion in isolated canine parietal cells (Skoglund, et al., this issue). In the present study, we evaluated the effect of adenosine on the rat gastric mucosa exposed to aspirin (ASA). In the control group, gastric damage was induced in fasted male Sprague-Dawley rats by oral administration of ASA suspensions (125 mg/kg). Concurrent administrations of adenosine (125 mg/kg) with ASA completely prevented the formation of gastric damage. This effect was found to be dose-dependent (ED_{50} = 10 mg/kg). Acute or subchronic oral administrations of ASA/Adenosine mixtures caused a similar effect. Prior oral administrations of indomethacin (10 mg/kg) did not prevent the gastroprotective effects of adenosine suggesting that prostaglandins do not mediate this activity. We conclude that adenosine is a highly potent, orally active gastroprotective compound. This compound might be beneficial in the treatment of gastric mucosal injury induced by aspirin and hypersecretory conditions.

27.10

EFFECT OF BOMBESIN ON THE RELEASE OF CHOLECYSTOKININ AND PANCREATIC POLYPEPTIDE IN RATS. P. L. Rayford*, K. Inoue, and D. W. McKay. Dept. of Physiology-Biophysics, Univ. Ark. Med. Sci., Little Rock, AR 72205.

Bombesin (BBS) releases pancreatic polypeptide (PP) and cholecystokinin (CCK) in man and dog and there is an interrelationship between the release of the two peptides. This study was conducted in rats to determine the effect of BBS on the release of CCK and PP. Methods: After an overnight fast, groups of 6 rats were anesthetized & infused via jugular vein with saline or BBS (5 and 25 µg). Blood samples were collected from the portal vein -15, 0, 15 and 30 min for measurement of CCK and PP by specific radioimmunoassays. Results: Plasma levels of CCK and PP did not change significantly with saline or with 5 µg of BBS. With 25 µg of BBS, CCK levels increased from a control value of 59±16 pmol/l to 89±17 and 94±16 pmol/L ($p<0.05$) and PP increased from 7±2 to 14±2 and 16±2 pmol/L ($p<0.05$). Integrated values were 729±191 for CCK and for PP 160±47 pmol/L-30 min. Correlation coefficients between release of CCK and PP was -0.14, prob. 0.67. Conclusions: This study demonstrates that in rats both CCK & PP are released by 25 µg, but not by 5 µg, of BBS suggesting that the response to BBS may be dose related. The nonsignificant correlation between the release of CCK and PP suggests that the PP response to BBS in rats, in contrast to man dog, may not be due to the release of endogenous CCK. Supported by NIH Grant #AM-30145-01.

27.11

HYPOTHALAMIC KNIFE CUTS DISRUPT ELEVATION OF RAT SERUM GASTRIN LEVELS BY INTRACISTERNAL BOMBESIN. Mark W. Gunion*, Carlos V. Grijalva*, Yvette Tache, John H. Walsh, and Donald Novin*. Center for Ulcer Research and Education and Department of Psychology, U.C.L.A., Los Angeles, Ca. 90024.

Intracisternal injection of bombesin into rats reliably increases serum gastrin levels. We recently found that lesions of the lateral hypothalamus block this effect. To help clarify the route of the neural systems involved in the gastrin response to bombesin discrete bilateral transections were made on the anterior, posterior, medial, or lateral borders of the lateral hypothalamus in 24 hour food deprived adult male hooded rats under methohexital anesthesia. Bombesin (500 nanograms, 10 microliters) or saline was immediately injected intracisternally. Two hours later the animals, all conscious, were decapitated and blood and gastric contents taken. The 400% increase in serum gastrin caused by bombesin in sham operated rats was diminished equally by lateral and posterior cuts to about 125%. Anterior and medial cuts both suppressed the bombesin increase to about 250%. None of the cuts altered gastrin levels in saline injected rats. Further, none of the cuts altered the effect of bombesin on pH, titratable acidity, or gastric secretion volume. Lateral hypothalamic lesions may block gastrin release by disrupting fibers passing through the lateral hypothalamus. (Supported by AM30110 to Y.T., 17328 to C.U.R.E., and NS 7687 to D.N.)

27.12

CONTAMINATION OF NATURAL VIP AND GLUCAGON BY CHOLECYSTOKININ-LIKE SUBSTANCE. Travis E. Solomon and Iris Tanaka*. Dept. of Physiology, Univ. of MO, and Research Service, Truman VA Hospital, Columbia, MO. 65201

We measured amylase secretion (% of total) from enzymatically dispersed guinea pig pancreatic acini in response to a range of concentrations of natural bovine glucagon, natural and synthetic porcine VIP (nVIP, sVIP), and synthetic secretin. A maximally effective concentration (0.3 nM) of synthetic porcine CCK8 (C-terminal octapeptide of cholecystokinin) was included in each preparation for comparison. Natural VIP (highly purified, supplied by V.Mutt) at 3 µM stimulated amylase secretion to 118% of the CCK8 value; values for natural glucagon were 134% at 10 µM. Synthetic VIP (Peninsula) and secretin (Squibb) caused maximal observed amylase secretion of 19 and 20% of the CCK8 value at 3 nM and 1 µM, respectively. Atropine, 1 µM, had no effect on amylase secretion in response to any stimulant except bethanechol (87% inhibition of response to 0.1 mM). Dibutyryl cGMP, 1 mM, decreased the maximal responses to natural VIP and glucagon to 22% of CCK8 value but had no effect on synthetic VIP or secretin. The response to CCK8 was decreased 83% by dbcGMP. We conclude that natural VIP and glucagon both contain a CCK-like substance. (Supported by NIH Grant AM 30705 and Research Service, Veterans Administration).

27.13

EFFECTS OF SUBSTANCE P ON ELECTRICAL AND MECHANICAL ACTIVITY OF CANINE GASTRIC SMOOTH MUSCLE. Shu Huai-de* and J.H. Szurszewski. Mayo Medical School, Rochester, MN 55905

The effects of substance P (SP) on mechanical and intracellular electrical activity of canine gastric circular muscle were studied in vitro. In the antrum, SP (5×10^{-8} M - 1×10^{-7} M) increased the frequency of spontaneous phasic contraction. This effect was biphasic. The force of contraction at first decreased and then gradually increased. The initial effect was associated with a decrease in the amplitude and the duration of the action potential whereas the increase was associated with an increase in the size of the plateau potential. SP had no effect on basal tone and resting membrane potential. In corporal circular muscle, SP not only increased the force of phasic contraction, but also the level of basal tone. In the fundus, SP significantly increased basal tone. All changes in mechanical activity in the corpus and fundus were not companied by definite changes of electrical activity. There were no significant changes in the resting membrane potential nor in the plateau potential. The calcium antagonist (D600, 10^{-5} M) antagonized the excitatory effect of SP on phasic contraction of antrum and corpus. However, D600 did not antagonize the effect of SP on the basal tone of the corpus and fundus. These data suggest that the mechanism of action of SP on phasic and tonic contractions are different. In the former case, SP may increase Ca^{2+} influx, while in the latter, SP may release intracellular calcium. (Supported by NIH Grant AM 17238.)

27.14

EFFECT OF NUTRIENT DENSITY AND COMPOSITION OF LIQUID MEALS ON GASTRIC EMPTYING IN THE FEEDING RAT. Theodore J. Kalogeris*, Roger D. Reidelberger and Verne E. Mendel. Depts. Animal Physiology, Animal Science and Food Intake Laboratory, U.C. Davis, Davis, CA 95616.

Rats were fitted with gastric cannulas to determine gastric emptying rates of liquid test meals after a 16-hour fast. They drank ^{14}C-polyethylene glycol (PEG)-labelled Vivonex High Nitrogen (VHN) or Intralipid Fat Emulsion (IL) at 0.25, 0.50 and 1.00 kcal/ml under two conditions: drinking to satiety (VHN) or drinking to a 15 ml constant volume (VHN, IL). Gastric emptying rate (ml/min) during a meal varied inversely with nutrient density of the test meal. However, caloric emptying rate (kcal/min), as well as total caloric load delivered to the intestine by meal's end, remained constant over the range of nutrient densities tested. The constancy of caloric emptying rate as diet nutrient density increased was independent of nutrient composition, supporting the hypothesis that gastric emptying is determined by diet caloric density. When rats were allowed to drink to satiety, caloric intake was not regulated, but increased with increasing nutrient density, suggesting that control of meal size is independent of regulation of gastric emtpying. (Supported in part by NIH Training Grant AM 07355).

27.15

EFFECT OF INTRALUMINAL PRESSURE ON REBOUND EXCITATION OF GUINEA-PIG SMALL INTESTINE. G. R. Athey* (SPON: D.L. Beckman). East Carolina University, School of Medicine, Greenville, NC 27834

Rebound excitation of smooth muscle is believed to be a myogenic event resulting from termination of electrical activation of intrinsic inhibitory neurons. These inhibitory neurons are tonically active and involved in control of the contractile tone of the smooth muscle. Reflexes, such as the peristaltic reflex, involve mechanoreceptors which are likely to influence the smooth muscle through action on these inhibitory neurons. To examine the effect activation of mechanoreceptors might have on inhibitory neurons, postinhibitory rebound excitation was performed while perfusing the lumen of the intestine with a constant pressure. Increased intraluminal pressure abolished rebound excitation and initiated spontaneous, rhythmic contractions (λ=6-9/min) of the small intestinal smooth muscle. These contractions continue for as long as the intraluminal pressure remains above a threshold level in the range of 4-6cm H_2O for guinea pig small intestine. Following contractions the muscle remains refractory for several minutes. It is suggested that increased intraluminal pressure activates mechanical receptors which are affecting tonic inhibitory neuronal control of the small intestinal musculature.

27.16

UPTAKE OF VITAMIN B-12 INTO ISOLATED ILEAL ENTEROCYTES. R. C. Beesley Dept. of Physiology and Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73008

Ileal cells incubated with intrinsic factor-^{57}Co-vitamin B-12 complex (IF-B-12) at 37°C took up 25 times more B-12 than jejunal cells. Uptake of B-12 by ileal cells was dependent on Ca^{++} and IF. B-12 taken up by ileal enterocytes could be divided into two components: 1) B-12 which was removed by chelation of Ca^{++} (bound) and 2) B-12 which was not removed by chelation of Ca^{++} ("internalized"). Incubation of ileal cells at 4°C or in the presence of 2,4 dinitrophenol (DNP) reduced incorporation of B-12 into the internalized but not the bound component. Ileal enterocytes were incubated with IF-^{57}CoB-12, washed and reincubated with unlabeled IF-B-12. Reincubation resulted in a decrease in the amount of ^{57}CoB-12 in the bound component with a concomitant increase in that in the internalized component suggesting transfer of ^{57}CoB-12 from bound to internalized components. Addition of DNP reduced transfer from bound to internalized components by 50%. When ileal cells were incubated with IF-B-12, washed with EDTA to remove bound B-12, homogenized and fractionated, more than 85% of the B-12 was recoverd in the 100,000xg supernatant. These results indicate that uptake of B-12 into isolated ileal enterocytes involves initial energy independent binding of IF-b-12 to its receptor followed by energy dependent transfer of B-12 from the receptor into the cell. (Supported by NIH grant AM30594).

ENVIRONMENTAL PHYSIOLOGY I

28.1

EFFECTS OF NO_2 EXPOSURE ON THE LUNG. James J. McGrath and Robert M. Bidwell*. Department of Physiology, Texas Tech University School of Medicine, Lubbock, TX 79430.

Experiments were conducted to assess the effects of nitrogen dioxide (NO_2) exposure on the lung. Mice were exposed in inhalation chambers to filtered air or 0.5, 1.0, 1.5 ppm NO_2 for 3 months or 5 ppm NO_2 for 7 days. Chamber NO_2 concentrations were monitored continuously by a Thermoelectron Chemiluminescent Analyzer verified by the Saltzman procedure. Lung weights increased 28% over control ($P<.05$) in 7 week old mice exposed to 5 ppm NO_2 for 7 days. Glucose-6-phosphate dehydrogenase (G6P-DH) activity was unchanged by exposure to 0.5, 1.0 or 1.5 ppm NO_2 for 3 months. 6-phosphogluconate dehydrogenase (6PG-DH) and G6P-DH activities increased, respectively, 13 and 12% above control ($P<.05$) in 7 week old mice and 61 and 38% above control ($P<.05$) in 16 week old mice exposed to 5 ppm NO_2 for 1 week. These results suggest that increased activity of the two pentose shunt enzymes may be related to the added demands for the formation of NADPH under oxidative stress. The lack of a significant increase in the activity of G6PDH in the chronic study may indicate the development of enzymatic adaptation to oxidant stress. (Supported in part by Center for Energy Research - Texas Tech University).

28.2

CHARACTERIZATION OF THE GROWTH RESPONSE OF THE MONTANE VOLE (MICROTUS MONTANUS) IN DIFFERENT PHOTOPERIODS. Teresa H. Horton* (SPON: S.J. Fidone). Univ. of Utah, Salt Lake City, UT 84112.

Juvenile Microtus montanus exhibiting slowed growth and delayed sexual maturity begin to appear in field populations during late June and early July. Exposure of juveniles to short photoperiods in the laboratory has been shown to reduce growth rates of males and female M. montanus. The laboratory results to be presented are in response to several questions about the interplay between photoperiod and the physiological responses which may determine seasonal changes in growth rate of this species. The following hypotheses have been tested:
A) Growth rate shows a threshold rather than graded response to the number of hours of light per day.
B) Exposure to a particular photoperiod prior to weaning influences a vole's growth response when exposed to a new photoperiod following weaning.
An ancillary question to hypothesis B has been to determine the age at which voles become conditioned to their pre-weaning photoperiod. These data will provide an estimate of the age at which the internal system of photoperiod measurement matures. (Supported by NSF grant # DEB-79-21059 to N.C. Negus and P.J. Berger)

28.3

DEVELOPMENT OF THE BIOLOGICAL CLOCK IN THE MATURING RAT. Lynne A. Farr*, College of Nursing, Judith A. Ramaley, Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska.

There is evidence that development of biological clocks plays an important role in puberty onset. This study attempts to confirm and examine the development of estrous-like cycles in two overt, measurable manifestations of the clock. We measured locomotor activity and oxygen consumption in 12 individual animals isolated in metabolic cages equipped with an infrared activity monitoring system, for 20-30 day periods beginning at weaning age. Data were analysed for evidence of ultradian, circadian and infrared rhythms. Closely parallel locomotor activity and oxygen consumption ultradian and circadian rhythms were observed. Both rhythm peaks tended to shift into the light period every 6 days. The activity peak shifts preceeded those of oxygen consumption in most cases. Two infradian rhythms with periods slightly longer than 5 days were observed. Activity rhythms peaked the day before the oxygen consumption rhythm in most animals. When oxygen consumption was adjusted for activity (oxygen consumed/activity event), a third 4 to 5 day rhythm was detected which was not explained by the activity. We conclude that the pre and peripubertal animal has a stable clock which requires periodic resetting with photoperiodic cues and which is loosely coupled to overt phenomena. Supported in part by NIH grant HD8703 and NSF grant PCM-7903252 (JAR)

28.4

THE EFFECT OF PHOTOPERIOD AND TEMPERATURE ON BROWN ADIPOSE TISSUE. K. S. Kott*, S. J. Wickler and B. A. Horwitz. Univ. of Calif., Davis, CA 95616

Two separate experiments were conducted to determine the effects of photoperiod and temperature on brown adipose tissue (BAT) weight and oxidative capacity in male Syrian hamsters. In the first experiment, 9 hamsters were subjected to a photoperiod of L:D 9:15 (short day) and another 9 hamsters were exposed to L:D 15:9 (long day). Both groups were at 8°C. After 81 days, the animals were sacrificed. Cervical (CBAT) and interscapular brown adipose tissue (IBAT) were excised, weighed, and analyzed for citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (HOAD) activity. The short day animals had significantly more CBAT and IBAT as % body weight than did long day animals. Maximal specific activities, μmoles product/gram tissue·min as well as total activity (μmoles product/brown fat pads·min) of CS and of HOAD were higher in short day animals. There were no differences in protein concentration (mg/g tissue). In the second experiment, 18 hamsters were maintained on L:D 15:9. Nine of these were at 24°C and 9 at 8°C. After 79 days, there were no significant differences between warm and cold animals in the relative amounts (% body wt) of IBAT or CBAT; but the BAT from the cold animals had significantly higher CS and HOAD specific as well as total activities. The BAT from the cold animals also had higher protein concentrations. These data indicate that photoperiod and temperature affect thermoregulatory capacity via different mechanisms. (This study was supported by NSF grant PCM 81-09875.)

28.5

INCREASED DENSITY OF β-ADRENERGIC RECEPTORS IN BROWN FAT OF WINTER ACCLIMATIZED ALASKAN VOLES. Dale D. Feist. Institute of Arctic Biology, University of Alaska, Fairbanks, Ak. 99701

To assess a possible mechanism for the enhanced thermogenesis of cold acclimated and winter acclimatized red-backed voles (Clethrionomys rutilus), β-adrenergic receptors of brown fat (BF) were characterized by specific binding of (-)(^{3}H) dihydroalprenolol (^{3}H-DHA) to isolated BF membranes from warm control (23°C), cold acclimated (5 wk or 5 mo at 5°C), wild summer and winter acclimatized voles. Scatchard analysis to determine the equilibrium dissociation constant (K_d) and the maximum number of binding sites (B_{max}) for control BF membranes gave a K_d=4.45nM ^{3}H-DHA and B_{max}=249 fmol ^{3}H-DHA bound/mg protein. β-adrenergic agonists competed for specific binding sites with an order of potency typical of the β_1 subtype of adrenergic receptors: (-) isoproterenol>(-) norepinephrine> (-) epinephrine. After cold acclimation for 5 wk or 5 mo, the K_d and B_{max} for adrenergic binding sites were similar to controls. BF mass was 1.5 times greater than controls after 5 wk cold but similar to controls after 5 mo cold. Winter voles had a 1.7 times higher B_{max} and 1.6 times more BF than summer voles. Thus seasonal acclimatization to winter in red-backed voles appears to involve an increase in β-adrenergic receptors in BF but cold acclimation does not. (Supported in part by USPHS. NIH Grant AM 26864.)

28.6

HIBERNATION: A NATURAL ANALOG TO EXERCISE INACTIVITY? Steven Wickler, Kayleen Kott* and Barbara Horwitz. Dept. of Animal Physiology, University of California, Davis 95616

Exercise training may result in a number of profound physiological and morphological changes such as muscle hypertrophy, increase in $\dot{V}O_{2max}$, and increase in oxidative capacity of skeletal muscle. Conversely, inactivity may result in muscle atrophy and reduced oxidative capacity. During hibernation, mammals may spend prolonged periods of time in an inactive state. Skeletal muscle masses and enzymatic indicators of oxidative capacity were measured in skeletal muscle of hibernating and nonhibernating hamsters (Mesocricetus) to assess the degree to which hibernation modifies muscle function. Animals were sampled for mass of gastrocnemius (GAST) and semitendinosus (ST). Citrate synthase (CS, an indicator of oxidative capacity) and HOAD (an indicator of β-oxidation) activities (μmoles/g·min) were also measured. Hibernators (n=7) weighed less (112±6 vs 141±6g, n=9) and showed a pronounced muscle atrophy (177±14 vs 252±13mg for GAST; 110±16 vs 295±23mg for ST). However, unlike exercise studies, inactivity during hibernation was accompanied by substantial increases in the maximal activities of oxidative enzymes in hibernators. CS increased significantly in all muscles: 47±2 vs 41±1 in GAST and 29±2 vs 19±1 in ST. Similarly HOAD increased: 16.2±1.2 vs 12.6±1.1 in the GAST and 10.8±1 vs 5.5±.3 in the GAST. (Supported in part by a UCD grant to SW.)

28.7

HIBERNATION, LYMPHOID ORGANS AND IMMUNE RESPONSES IN THE LEOPARD FROG. Richard K. Wright and Edwin L. Cooper*. Univ. of California, Los Angeles, CA 90024

Hibernation is an intrinsic function and a normal phase in the annual life cycle of the leopard frog, *Rana pipiens*. During winter hibernation, there is a progressive loss of splenic lymphocyte populations and loss of bone marrow hemopoietic populations. Lymphocyte loss suggests that bone marrow hemopoiesis ceases and that spleen lymphocyte populations become depleted due to non-renewal of lymphocytes from bone marrow. Immune responses are temperature dependent and in general, as environmental temperatures decrease, immunological reactivity decreases. Primary and secondary (memory) immune responses to antigenic challenges cannot be elicited during hibernation. Splenic lymphocyte aplasia may account for the absence of primary immune responses during hibernation. Memory lymphocytes generated during primary responses prior to hibernation however, appear to survive the hibernating period. responding rapidly to antigen challenges when hibernation is terminated. Non-expression of memory during hibernation may be attributed to the thermal sensitivity of some phase(s) involved in generating memory responses. Survival of memory lymphocytes during hibernation confers obvious adaptive survival advantages to frogs until lymphocyte populations can be renewed after hibernation. These observations raise the possibility of other regulatory controls on the activity of the immune system influenced by the environment. (Supported by NIH Grant HD 09333-07)

28.8

CORRELATION BETWEEN METABOLIC RATE AND THE DURATION OF AROUSAL EPISODES DURING MAMMALIAN HIBERNATION. Alan R. French. Univ. of California, Riverside, CA 92521

The length of time mammals remained at high body temperatures following periodic arousals from hibernation was inversely related to their mass-specific rates of euthermic metabolism. At any one environmental temperature, large individuals generally remained euthermic in midwinter longer than small animals. This was true among individuals of different species that spanned three orders of magnitude in size [$Duration_{(hr)} = 1.44\ Mass_{(g)}^{0.32}$], as well as within species that exhibit large size polymorphism at the time of hibernation. Likewise, the duration of mid-winter arousal episodes in similar-sized individuals increased as ambient temperature increased over the range of 5-20°C. This trend was evident in comparisons among different animals that hibernated at different temperatures, and in individuals that hibernated at different temperatures in successive years. In addition, less time was spent at high body temperatures following bouts of torpor that were interrupted prematurely by environmental disturbances. These results are consistent with the theory that arousals are initiated by, and euthermic metabolism necessary for the elimination of, some chemical imbalance that develops while hibernators remain at low body temperatures. (Supported by NSF Grant DEB 8003513 and by grants from the University of California Research Committee)

28.9

HYPERTHERMIC RESPONSES IN GASTROINTESTINAL MUCOSA OF THE RAT. Thomas J. Sernka, Daryl M. Lechner* and Mark E. Collins*. Physiology Dept., Wright State Univ., Dayton, OH 45435

During fever the gastrointestinal mucosa may respond to the greater need for nutrients and fluid by increasing absorption of sugar and water. To test this hypothesis, gastrointestinal mucosal tissue was isolated from the anesthetized rat and incubated at normothermia (37°C) and/or hyperthermia (42°C). Uptake of ^{14}C-labeled 3-O-methyl glucose into everted jejunal sacs was used to measure active absorption of sugar. As determined by the serosal/mucosal ratio, the uptake was not significantly different in sacs incubated at 37°C or 42°C. Flux of ^{14}C-urea from submucosa to mucosa of gastric mucosa was used to measure passive permeability to fluid. Unidirectional flux of urea was increased significantly from 30.8 ± 1.4nEq/cm^{2}h at 37°C to 57.6 + 8.4 nEq/cm^{2}h after 15 min exposure to hyperthermia. Since hyperthermia may release prostaglandins, the effect of 10^{-8}M 16,16 dimethyl prostaglandin E_2 was tested in the same preparation after a second control period at 37°C. Mucosal addition of dmPGE$_2$ at 37°C significantly increased urea flux by 8.4% during the subsequent 15 min. We conclude that hyperthermia such as would be encountered in fever brings about nonspecific increases in gastrointestinal mucosal absorption of nutrients and fluid and that prostaglandins may mediate this change in permeability.

28.10

INFLUENCE OF HYPERTHERMIA ON BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEP). Gilles Géraud and Joseph Coll (SPON : A. Guell)
Departments of Neurology and ORL, CHU RANGUEIL, TOULOUSE, France

Six healthy subjects ranging from 30 to 48 years of age were studied. The BAEPs were obtained by monoaural stimulation. The clicks had a duration of 100 µsec, a frequency of 20 Hz and an intensity of 90 dbHL. Each measurement consisted of 2,048 clicks. The BAEPs were recorded before and after the raising of body temperature. Warming was achieved by immersion in a hot bath at 40°C for 30 to 40 minutes. The mean increase of body temperature was 1.4°C (ranging for 1.2°C to 1.6°C). On emerging from the bath, the subject was wrapped in a survival blanket and the second BAEP measurement was immediatly taken.
Wave V latency decreased 10 times, twice remained invariable and slightly increased in one case ; the mean value changed from 6.03 ± 0,17 msec in the basal state to 5.88 ± 0,10 msec (p 0,001) in hyperthermia. The results are similar for the other measurable parameters of the BAEPs.

Our study shows that, in normal subjects, induced hyperthermia results in a slight decrease of BAEP latencies. This latency shortening could be due to an acceleration of axonal conduction, or, more probably, to a change in synaptic processes. This acceleration of conduction velocity in normal subjects stands in opposition to the results obtained in demyelinating diseases and this discrepancy can be explained by the blocking temperature phenomenon.

28.11

A POTENTIALLY HAZARDOUS EFFECT OF HIGH POWER, 2.8 GHZ, PULSED MICROWAVE RADIATION. R.N. Friedman*, J.R. Jauchem*, M.R. Frei*, G.D. Hubbard*, and F. Heinmets* (SPON: T.C. Smith). Life Sciences Div., Technology Inc., San Antonio, TX 78216, Trinity Univ., San Antonio, TX 78284, and USAF Sch. of Aerospace Med., Brooks AFB, TX 78235.

Studies comparing bioeffects of continuous wave (CW) and pulsed microwave radiofrequency radiation (RFR) on ketamine-anesthetized rats with a cannulated carotid artery(a.) exposed to pulsed RFR at 2.8 GHz, 2 µsec pulse width, at two pulse rates and average power densities showed a mortality rate (500 pps: 50%, N=10 ♀ @ 60 mW/cm^2; 50%, N=4 ♀ @ 30 mW/cm^2; 60%, N=5 ♂ @ 60 mW/cm^2; 250 pps: 67%, N=3 ♀ @ 60 mW/cm^2) significantly greater than that of carotid-cannulated rats exposed to CW (no mortality) and pulsed RFR at 5.6 and 9.3 GHz with various power and pulse characteristics and control rats exposed to identical 2.8 GHz fields. Controls (5 each): tied carotid a., RFR; cut vagus, RFR; carotid cannula, no RFR; ketamine; ketamine, RFR; femoral a. cannula, RFR. We applied far-field RFR intermittently, cycling colonic temperature between 38.5 and 39.5°C 8-10 times in ~6 hrs. Gross and microscopic study of all organ systems and 5 brain regions (N=25 rats) revealed no lesions. A dye fixitive showed unilateral deprived brain circulation due to carotid ligation or cannulation. Results suggest that specific RFR fields may interact with implanted objects to produce effects that can be more hazardous than RFR exposure alone. Controls show that mortality due to deprived brain circulation is unlikely. (USAF Contract No. F33615-80-C-0614)

28.12

HEART RATE CHANGES DURING INTERMITTENT EXPOSURE TO PULSED AND CONTINUOUS WAVE MICROWAVE RADIATION. J.R. Jauchem*, R.N. Friedman*, M.R. Frei*, and F. Heinmets* (SPON: T.C. Smith). Life Sciences Division, Technology Incorporated, San Antonio, TX 78216 and Trinity Univ., San Antonio, TX 78284.

35 female rats, anesthetized with ketamine, were exposed to pulsed or continuous wave (CW) microwave radiation at frequencies of 2.8, 5.6, or 9.3 GHz, at an average power density of 60 mW/cm^2. Pulses were of 1 or 2 µsec in duration and were applied at a rate of 250, 500, or 1000 pulses/sec. Irradiation was performed intermittently to maintain colonic temperature (Tc) between 38.5 and 39.5°C for 2.8 and 5.6 GHz exposures, and between 37.5 and 38.5°C for 9.3 GHz exposures. 10 periods of exposure were applied in each animal. Results of exposure to 2.8 GHz (1 µsec, 500 pulses/sec) on heart rate (HR) are:

Power	On - - - - - -		Off - - - - - -	
Tc	39.0	39.5	39.0	38.5
Δ HR (mean±SE)	+11±2	+22±1	-15±2	-25±2

(Δ HR is expressed as Δ from value at 38.5° (avg HR 285±7 beats/min) during "on" period and as Δ from value at 39.5° (avg HR 307±7) during "off" period; N=30 exposure periods in 3 animals). Similar results were obtained using other pulse characteristics. HR consistently increased during irradiation and returned to baseline levels when exposure was discontinued. Pulsed radiation resulted in greater increases in HR than CW radiation. (Performed at School of Aerospace Medicine, Brooks AFB, TX; supported by USAF Contract No. F33615-80-C-0614).

28.13

THERMOREGULATORY EFFICIENCY OF RATS INTERMITTENTLY EXPOSED TO CONTINUOUS WAVE RADIOFREQUENCY RADIATION. M.R. Frei,* F. Heinmets,* J.R. Jauchem* and R.N. Friedman* (SPON: T.C.Smith). Trinity University, San Antonio, TX 78284 and Technology Incorporated, San Antonio, TX 78216.

Measurements of thermoregulatory efficiency (TE) were obtained by intermittently exposing rats to radiofrequency radiation (RFR). Intermittent exposure which results in the production of 1°C temperature cycles permits the introduction of electromagnetic energy into a biological system without exceeding the physiologically tolerable temperature range. TE was measured by T_r/T_c, where T_r=1°C rise time, T_c=T_r+Td (Td=time of recovery to initial temperature). While continuously monitoring and recording the colonic temperature, the respiratory rate, the ECG, and arterial blood pressure, individual rats were exposed to continuous wave (CW) RFR at average power densities of 10,20,30 or 60mW/cm^2 with a carrier frequency of 2.06 GHz. Exposure to CW-RFR at power densities of 20-60mW/cm^2 resulted in the production of 8-10 1°C temperature cycles within 6-8 hrs. of experimentation. Exposure at 10mW/cm^2 did not result in the desired temperature cycling. Repeated cycling during a single day's exposure caused no observable changes in the animals' TE. However, an inverse relationship between average power density and TE was noted. The efficiency of thermoregulation will be presented relative to the physiological parameters monitored. (Work performed at the Sch. of Aerospace Med., Brooks AFB,TX and supported by USAF Contract No.F33615-80-C-0614).

28.14

AN EVALUATION OF LACTATE AND RESPIRATORY RESPONSES TO INCREMENTAL AND CONSTANT LOAD WORK BOUTS. S. Constable, M. Joyner,* Y. Tsao,* J. Bunt,* T. Rotkis,* and J. Wilmore. ESSR Lab, University of Arizona, Tucson, AZ 85721

The applicability of an energy metabolism model incorporating two distinct lactate thresholds identified as the aerobic threshold (AerT) and the anaerobic threshold (AT), was evaluated during level treadmill work at various running velocities. Three unbiased observers found the identification of both AerT and AT, using independent lactate and respiratory gas exchange "breakpoints," very subjective in nature. The test-retest reliability was generally poor and varied considerably among both the observers and the individual predictor variables. The validity of the respiratory gas exchange predictors was then evaluated against the criterion lactate thresholds using the estimates of a fourth, more experienced observer. Resultant correlation coefficients were generally not high, while t-test evaluation demonstrated significant differences in 50% of the cases. When these ventilatory predictors were pooled for one best estimate, they consistently underpredicted the criterion lactate thresholds. This finding suggests that increases in running speeds may confound the normal association between plasma lactate and ventilation as observed during cycle ergometry. Arbitrary lactate concentrations of 2mmol/L and 4mmol/L consistently overpredicted the criterion lactate thresholds in terms of $\dot{V}O_2$. The lactate responses for constant work at the estimated AerT and AT intensities were also varied. These observations suggest the reliability of detection and true physiological significance of both the AerT and AT may be questioned during treadmill running.

28.15

THERMOREGULATION IN ERYTHROCEBUS PATAS: A THERMAL BALANCE STUDY. Margaret A. Kolka and Reynaldo S. Elizondo. Indiana Univ. School of Med., Physiol. Sect., Bloomington, In. 47405

A thermal balance study over an ambient (T_a) range of 15-35°C was performed on four non-acclimated patas monkeys (Erythrocebus patas) weighing between 3.9 and 5.8 kg. Total body surface area and the relative contributions of each area to the total were determined. Oxygen consumption, CO_2 production, mean weighted skin temperature ($\bar{T}_{sk}$), rectal temperature (T_{re}), respiratory evaporative water loss (E_{resp}), and total evaporative water loss (E_{tot}) were measured continuously after equilibration at each T_a. $\bar{T}_{sk}$ increased as T_a increased, whereas T_{re} was maintained between 37.7 and 38.1°C at T_a's from 15 to 35°C. Total evaporative water losses increased with increasing T_a to a mean value of 116 W/m^2 at 35°C. Panting was not observed, and E_{resp} was relatively constant and increased from 1.1 to 8.5 W/m^2 at 15 and 35°C, respectively. Whole body conductance was similar to that previously reported for Macaca mulatta over the same temperature range. The data demonstrates that the patas monkey can maintain its core temperature within a narrow range over a wide range of T_a's. Additionally, E_{tot} in the patas monkey is significantly higher than what has been reported in other non-human primates and approaches that reported for man. It is concluded that the patas monkey can serve as an appropriate animal model for temperature regulation studies which for technical or ethical reasons cannot be performed in man. (Supported by Grants PHS AM 16703 and NASA OR335)

28.16

CIRCADIAN RHYTHM IN THERMOREGULATION Lou A. Stephenson, Ethan R. Nadel, B. Helen O'Donovan* and C. Bruce Wenger. J.B. Pierce Fndn. Lab. and Yale Univ. Sch. of Med., New Haven, CT 06519

We examined whether there is an active variation in regulated temperature and/or a change in the senstivity of the heat loss mechanisms which corresponds to the circadian variation in body temperature. Five male subjects exercised at 60% $\dot{V}O_{2max}$ at 25° C on six different days at times equally spaced over the 24 hour day. Esophageal temperature (T_{es}) and chest sweating rate ($\dot{m}_{sw}$) were measured continuously, and forearm blood flow (FBF) was measured 1-2 times per min. The thresholds for forearm vasodilation were significantly higher at 1600 and 2000h, than at 2400 and 0400h, with the threshold 0.65°C higher at 1600 than at 0400h. The gain of the FBF:T_{es} relation was also different over the course of the day, and was lowest at 0400h. The threshold for sweating was also significantly higher at 1600 and 2000h than at 2400 and 0400h, averaging 0.57°C higher at 2000 than at 0400h. The $\dot{m}_{sw}$:T_{es} relation was not significantly different at the various times. Blood volume, estimated from Hct and Hb, did not vary in any consistent pattern. Regulated core temperature, as determined from thresholds for $\dot{m}_{sw}$ and for vasodilation, varies over the 24 hour day with the zenith occurring around 1600 and the nadir at 0400h. However, while the sensitivity of $\dot{m}_{sw}$ response to increasing T_{es} was constant over the 24 hour day, the sensitivity of the FBF response tended to increase between 0400 and 2400h.

AGING, CALCIUM AND CALCEMIC HORMONES, ADRENAL CORTEX AND SEX HORMONES

29.1

CORTICAL BLOOD FLOW AND FRONTAL DISTRIBUTION IN AGING STUDIED BY 133 XE INHALATION TECHNIQUE. A. Bès, A. Guëll, N. Fabre, G. Fanjaud, G. Géraud, Department of Neurology, CHU Rangueil, 31054 TOULOUSE CEDEX (France)

Our work dealt with locoregional Cerebral Blood Flow (l.r CBF) in normal subjects of different ages. Particular attention was paid to the CBF in prefrontal regions. l.r CBF was measured by the 133 XE inhalation technique using a 32 detectors system, 16 in each hemisphere. Fl represents the grey matter flow in ml/100 g/mn. l.r CBF was studied in 105 subjects divided in 7 groups in relation to age. We arbitrarily isolated the values obtained from the most frontal detectors.

Group	1	2	3	4	5	6	7
n	15	15	15	15	15	15	15
Mean age (years)	13,9 ±1,9	25,3 ±2,7	35,3 ±2,7	45 ±2,6	54,7 ±2,5	65,1 ±2,9	74,6 ±2,7
Fl ml/100 g/mn	87,4 ±12,3	80,2 ±6,4	78 ±4,4	73,6 ±5,1	77,2 ±5,9	67,2 ±7,2	65,2 ±7
Frontal pattern (%)	+12,2 ± 4,1	+11,6 ± 1,6	+10,3 ± 2	+9,5 ±1,3	+8,7 ±1,4	+6,3 ±1,9	+6,8 ±2,4

We observed a hyperfrontal distribution of CBF in normal subjects : the CBF measured by the detectors in the most frontal positions was greater than the CBF of the rest of the hemisphere ; this frontal predominance decrease with the age.

29.2

CHOLESTEROL AND Na^+ -K -ATPase IN YOUNG AND OLD RAT KIDNEY PLASMA MEMBRANES. R. Marín*, T. Proverbio* and F. Proverbio. Centro de Biofísica y Bioquímica, IVIC, Apartado 1827, Caracas 1010 A, Venezuela.

Several authors (1,2) have found that there exists an inverse relationship between the Na^+ -K^+-ATPase activity of several membranes and their cholesterol/phospholipids molar ratio. In the present work we have found that Basolateral plasma membranes from old rat (24 months) kidney proximal tubular cells, show a lower Na^+ -K^+-ATPase activity, a higher cholesterol content and similar phospholipids content than young rat (3 months) membranes. Accordingly the cholesterol/phospholipids molar ratio increased from a value of 0.47±0.02 (for young rats) to a value of 0,64±0.03 (for old rats). The cholesterol content of the membranes can be lowered by treatment with cholesterol-oxidase. Thus, old rat membranes treated with this enzyme, lowered their cholesterol/phospholipids molar ratio to 0.50. The Na^+ -K^+-ATPase activity of these membranes showed a 35% increment. From these results it may be concluded that the increased cholesterol/phospholipids molar ratio present in the old rat membranes, could be the responsible of the lowered Na^+ -K^+ -ATPase activity. 1) Fiehn, W. & Seiler, D. (1975) Experientia, 31:773. 2) Hegner, D. & Platt, D. (1975) Mech. Ageing Dev., 4:191. (Supported in part by CONICIT, Grant DF S1-1245).

29.3

ARTERIAL DEGENERATION IN URBAN CHINESE SUBJECTS. Michael F. O'Rourke, Chen Shang-gong*, Wang Ruo-ping*, Zhang Chun-lai*, Li Mei-feng*, Albert P. Avolio*. St. Vincent's Hospital, Sydney and Fu Wai Hospital, Peking.

Arterial stiffening with age in Occidental subjects is apparent as increase in aortic pulse wave velocity (PWV) and pulse pressure (PP). This has been attributed to medial degeneration (an ageing change) and concomitant atherosclerosis (a disease with high prevalence in Western society but low prevalence in the East). The contribution of each has not been established.

Aortic PWV was measured from simultaneously recorded brachiocephalic and femoral transcutaneous Doppler flow recordings in 480 Chinese subjects aged 3-89 years. None had clinical evidence of atherosclerosis; serum cholesterol was low (4.5mM/L; SD 1.0). Aortic PWV increased with age, (PWV(cm/sec) = 9.2 age + 615, r=.69, $p<0.001$.) No sex difference was apparent. Mean arterial pressure (BP) and brachial PP also increased with age ($\overline{BP}$(mmHg) = .55age + 74.5; r = .62, $p<0.001$; PP(mmHg) = .37age + 40, r = 0.47, $p<0.001$). When compared at different ages there was no significant relationship between PWV and $\overline{BP}$.

Chinese subjects show similar PWV, PP and $\overline{BP}$, and similar changes with age as Western subjects, despite lower prevalence of atherosclerosis and lower serum cholesterol. Findings suggest that atherosclerosis is a minor factor and medial degeneration the major factor in causing increased arterial stiffening with age in Oriental and Occidental populations.

29.4

OXYGEN CONSUMPTION OF MAMMALIAN FAST AND SLOW SKELETAL MUSCLES: EFFECTS OF AGE AND DIET. Roger McCarter, E.J. Masoro and B.P. Yu. Dept. of Physiology, Univ. of Texas Health Science Center San Antonio, Texas, 78284.

Oxygen consumption of lateral omohyoideus (LOMO) and soleus muscles of Fisher 344 rats was measured in vitro at 35C using polarographic methods. Muscles were maintained at the optimal length at rest and rates of oxygen consumption were measured at 6, 12, 18, 24, 27 and 30 months of age. Group 1 rats were fed ad libitum a diet containing 21% protein; Group 2 rats were fed 60% of the food consumed by Group 1 rats from 6 weeks of age; Group 3 rats were fed ad libitum a diet containing 12.6% protein from 6 weeks of age. Muscles of Groups 1 and 3 rats exhibited an age-related decline in oxygen consumption, with the rate of decline being greater for the fast LOMO muscles than for the slow soleus muscles. Muscles of Group 2 rats exhibited little change in rate of consumption with age and the rates of LOMO and soleus muscles were not significantly different at any age. The results demonstrate that the well known decline of muscle oxygen consumption with advanced age is modulated by the life-prolonging action of food restriction and that this modulation is not influenced by restriction of protein intake only. Also, age-related decline in muscle oxygen consumption is more marked in the case of fast-twitch type 2B muscle fibers than for slow-twitch type 1 muscle fibers. (Supported by NIH Program Project AG 01188).

29.5

THE EFFECT OF EXERCISE ON MYOCARDIAL CAPILLARY BED AND CONNECTIVE TISSUE IN SENESCENT RATS. M.B. Finch*, C.L. Stebbins*, T.I. Musch*, W.G. Reddan, and E.L. Smith* (SPON: F.J. Nagle). Biogerontology Lab., Univ. of Wisconsin, Madison, WI 53706

The combined effect of exercise-training and aging on the morphology of the heart was studied in three groups of female Fischer-344 rats: two control groups, C1, aged 21 months, and C2, aged 26 months; and an exercise group, E, treadmill-trained from 21 to 26 months of age. Alkaline phosphatase stained ventricular sections were used for the determination of fiber diameter, capillary density, and capillary-to-fiber ratio. Ventricular hydroxyproline content was determined spectrophotometrically. Exercise training resulted in a relative ventricular hypertrophy, milligrams ventricular weight/100 grams body weight (E=253±9.2), beyond that seen with age (C1=186±11.3, C2=214±8.4, $p<.001$), an indication of a training effect. Fiber diameter was larger ($p<.001$) in both C2 (17.26u±0.13) and E (17.07u±0.12) than in C1 (15.80u±0.16), indicating an age related fiber hypertrophy. C2 capillary density (2218/mm2±32) was not different from C1 (2161/mm2±40). C2 capillary-to-fiber ratio (1.242±0.016) was larger ($p<.001$) than C1 (1.156±0.020). E capillary density (2086/mm2±31) and capillary-to-fiber ratio (1.71±0.016) were similar to C1, but lower ($p<.02$ and $p<.001$ respectively) than the age-matched C2. There was no apparent effect of age or exercise-training on hydroxyproline content. Exercise-training may attenuate age related changes in the morphology of the heart. (NIH R127)

29.6

THE EFFECT OF EXERCISE ON ENDPLATE ELABORATION IN AGED RAT SKELETAL MUSCLE. C.L. Stebbins*, E. Schultz*, R.T. Smith*, and E.L. Smith* (SPON: J.A. Dempsey), Biogerentology Lab, University of Wisconsin, Madison, WI 53706

Age-related endplate elaboration and myofiber atrophy were studied in the distal (GAS)trocnemius (∿90% Type II fibers) and (SOL)eus (∿85% Type I fibers) muscles of female Fischer-344 rats 21 and 26 mos. of age. These parameters were also studied in rats who performed treadmill running from 21 to 26 mos. of age. Endplate and muscle fiber morphology were demonstrated by staining for acetylcholinesterase and myosin ATPase activity. Endplate elaboration was assessed by determining the incidence of terminal sprouting, expressed as the % of growth configurations (%GF). Fiber atrophy was evaluated as the % of small diameter fibers (SDF) seen in cross section. In all three groups, the incidence of SDF was significantly smaller and the %GF significantly larger in the SOL when compared to the GAS ($\bar{x} \pm$ S.E.):

	GAS		SOL	
	SDF	%GF	SDF	%GF
21 mo Norm	9.8± .89	21.3±1.90	6.3±.98	30.4±1.06
26 mo Norm	16.0±1.12	28.4±2.78	9.8±.66	37.5±1.55
26 mo Ex	13.4± .60	27.6±1.65	8.8±.38	47.3±2.40

These data suggest a differential aging and exercise response in the SOL with respect to the GAS. The combination of a high % of GF and a low incidence of SDF in the Ex. SOL may relate to an enhanced neuromuscular contact during aging, resulting in a reduced rate of myofiber atrophy. (NIH R127)

29.7

FLUCTUATIONS IN BLOOD CALCIUM AND PHOSPHORUS FOLLOWING INTENSE ACTIVITY IN THE SALMONID FISH ONCORHYNCHUS AND SALMO. Brett A. Adams* (SPON: A.F. Bennett). Oregon State University, Corvallis, Oregon 97331

Serum concentrations of calcium and phosphorus increase by 28% (2.50 to 3.20 mM) and 60% (2.75 to 4.42 mM), respectively, following intense activity and blood pH depression in Oncorhynchus and Salmo. In addition, serum levels of these elements remain significantly different from resting levels for as long as eight hours post-activity. Whole blood calcium and phosphorus concentrations rise 24% (1.68 to 2.08 mM) and 13% (41.8 to 47.4 mM), respectively, while no detectable change is observed in the calcium or the phosphorus content of epaxial muscle. Significantly, whole blood millimolar increments in calcium and phosphorus appear in the ratio of 1 Ca:14 P immediately following the activity period(10 min.). This ratio is unlike that found in bone (10 Ca:6 P), and seems to indicate an additional, non-skeletal origin for phosphorus released into the blood during and following activity. The absolute, post-active increments in blood calcium and phosphorus suggest that calcium is much more tightly regulated than phosphorus during periods of acute metabolic acidosis, at least in the genera examined.

29.8

ELECTROPHYSIOLOGY OF NORMAL HUMAN PARATHYROID CELLS: RELATION SHIP TO HORMONE SECRETION. James T. Posillico* and Nels Anderson, Jr. Depts of Surgery and Physiology, Duke University Med. Ctr., Durham, NC 27710

The transmembrane potential (TMP) of normal human parathyroid cells (NHPC) was measured by continuous intracellular microelectrode recordings. The mean resting potential of the cells in 2.5 mM Ca Krebs-Henseleit (KHB) was -22.6 mV± 0.3 mV. Exposure to low Ca (0.5 mM) initiated a rapid hyperpolarization (HYP) to -63.8 mV± 2.7 mV. The HYP response was progressive and demonstrated concentraton dependent changes as the Ca was lowered from 2.5 mM to 0.5 mM. In synchrony with the HYP response, parathyroid hormone (PTH) secretion increased: basal secretion in 2.5 mM Ca KHB was 0.05 pg/ml/mg ± 0.01 pg/ml/mg which increased to 106.5 pg/ml/mg ± 15.9 pg/ml/mg in 0.5 mM Ca. The HYP state in low Ca was rapidly reversed by the addition of 0.5 mM LaCl or 10^{-5}M methoxy-verapamil (D-600) (in 0.5 mM Ca). Both the D-600 and LaCl blockage of the HYP response was reversible upon re-exposure of the tissue to 0.5 mM Ca alone. Similarly, increased extracellular potassium concentration (25 to 75 mM in 0.5 mM Ca) initiated a depolarization of the HYP state. Preliminary data suggest that D-600 and LaCl inhibited PTH secretion at the doses used, however, increased K^+ stimulated hormone secretion in a progressively additive fashion over that of 0.5 mM Ca alone. These data suggest that the low CA induced HYP in NHPC: 1) is correlated with PTH secretion, and 2) arises from a D-600 sensitive, calcium activated potassium conductance.

29.9

EFFECT OF SHORT AND LONG TERM CORTICOSTEROID ADMINISTRATION ON MINERAL AND PROTEIN METABOLISM IN MAN. **Herta Spencer, Daniel F. DeBartolo*, Dace Osis*, and Clemontain Norris*. Metabolic Section, Veterans Administration Hospital, Hines, IL 60141.**

Corticosteroids cause bone loss and osteoporosis. Decreased intestinal absorption of calcium and increased PTH secretion have been implicated in causing bone loss. Controlled metabolic balance studies of calcium, phosphorus, and nitrogen were carried out in adult males during short and long term therapy with corticosteroids. The absorption of calcium was determined, using oral tracer doses of $^{47}CaCl_2$. During short term administration, urinary calcium increased, while fecal calcium was unchanged, indicating a lack of change in intestinal calcium absorption, confirmed by ^{47}Ca absorption studies. Urinary nitrogen was markedly increased. After long term use of corticosteroids, urinary calcium was high, while stool calcium was low, indicating high absorption of calcium, confirmed by ^{47}Ca absorption (81%-92%). The initial effect of corticosteroids is a marked increase in urinary nitrogen, a moderate increase in urinary calcium, while calcium absorption is unchanged. In the late phase of corticosteroid administration, calcium absorption is high, urinary calcium is somewhat increased, and nitrogen metabolism is normal, indicating a compensatory mechanism to counteract the continued protein loss during corticosteroid administration. **(Supported by a grant from USDA, CSRS, S&E.)**

29.10

THE EFFECTS OF CONFINEMENT STRESS ON CIRCULATING PERIPHERAL LEUCOCYTES IN RATS. Mary E. McNaughton* and Robert W. Reynolds. Univ. of Calif., Santa Barbara, 93106.

The effects of confinement stress on circulating leucocyte populations were studied in intact and adrenalectomized (ADX) rats. Rising levels of plasma glucocorticoids have been linked to decreasing levels of leucocytes over 3 hours. We looked for a more rapid effect over 1 hour. Tail vein samples were taken every 5 minutes from animals confined to restraint boxes. Both groups showed a slight increase in leucocyte levels at 5 and 10 minutes compared to baseline. In the intact animals leucocytes dropped to one half their initial number by 40 minutes. The ADX animals stabilized at a level slightly above baseline. We attempted to separate the effects of the confinement stress from the handling accompanying the sample. Intact animals were confined for 5 minute increments, then returned to their home cages and resampled at 40 minutes. Leucocyte counts did not drop, and in some cases showed a rise. Animals which were simply confined and sampled at the end of each 5 minute increment gave similar results. While stress alters leucocyte level over a short time it may be that handling is more critical than the confinement itself.

Supported by UCSB Faculty Grant #144.

29.11

THE INHIBITORY EFFECT OF STRESS ON ORNITHINE DECARBOXYLASE ACTIVITY OF NORMAL AND REGENERATING ADRENAL GLAND. Karam F. A. Soliman and Matty O. Udoye*. School of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Adult male Sprague-Dawley rats kept under controlled environmental conditions were adrenomedullectomized or sham-operated and 11 days after surgery they were sacrificed at 0600 h and 1800 h. In this experiment, one group was exposed to immobilization stress for one hour, another group was exposed to immobilization and was treated with dexamethasone (dex, 1 mg/kg) 2 hr. prior to stress, a third group was treated only with dex and a fourth group was injected by the drug vehicle. After sacrificing, adrenal ornithine decarboxylase (ODC), adrenal corticosterone, plasma corticosterone and plasma ACTH were assayed. The results show that immobilization stress resulted in more than 50% decline in ODC activity at 1800 h in both sham-operated or adrenomedullectomized groups. Dex pretreatment did not abolish the stress effect on ODC in both groups. However, treatment with dex alone inhibited ODC activity significantly in the groups studied. The results of this experiment indicate the dissociation of adrenal ODC activity from its dependency on ACTH, confirming our previous results in the hyperthyrodism rat where ODC activity was also inhibited. (Supported by a grant from NASA, NSG 2183)

29.12

SUPPRESSION OF CORTICOSTERONE SERUM LEVELS AND LACTATIONAL PHYSIOLOGY IN POSTPARTUM RATS TREATED WITH DIMETHYLBENZANTHRACENE (DMBA) DURING GESTATION. Howard S. Pitkow, William Urbas*, Michael Goldman*, and Linda Hill*. Penna. Col. of Podiatric Med., Phila., Pa. 19107

Our laboratory has reported that DMBA administered to pregnant rats caused a significant decrease in mammary gland RNA and DNA contents on day 1 of lactation. No significant differences were found in these parameters on days 6 and 11 postpartum when compared to controls. The retarded litter weight observed in early lactation was normal by day 11. These observations suggest a mammary gland rebound phenomenon. In order to determine the effects of DMBA on hormones essential for lactation, female adult virgin Long Evans rats (12 animals/group) were intraperitoneally injected daily with 1 mg DMBA in 0.2 cc sesame oil on days 8 through 12 of pregnancy. On days 1 and 8 postpartum prolactin and corticosterone serum levels were determined by radioimmunoassay. On day 1 of lactation we observed a significant decrease in corticosterone levels (32.8+2.7ug/100ml) in DMBA rats when compared to controls (55.9+5.2ug/100ml). No significant differences were found in corticosterone levels on days 8 nor prolactin levels on days 1 and 8. Our data suggests that DMBA in pregnancy suppresses the normal increase in corticosterone levels in early lactation thereby depressing mammary gland growth (i.e., DNA) and secretion (i.e., RNA). We believe the normalization of corticosterone levels by day 8 between control and DMBA groups account for the mammary gland rebound phenomenon.

29.13

ESTROGEN METABOLISM IN NEW WORLD MONKEYS: URINARY EXCRETION OF ESTRADIOL METABOLITES BY SQUIRREL MONKEY. P.I. Musey, D.C. Collins, K.G. Gould*, and J.R.K. Preedy*. Emory University, Atlanta, GA 30303.

Previous reports from this laboratory have shown that steroid secretion and metabolism in the chimpanzee, rhesus and baboon are quite similar to the human. In this report, we extend these studies to the metabolism of 17β-estradiol (E_2) in the squirrel monkey. ^{3}H-E_2 was injected into 2 female squirrel monkeys and total urine collected daily for 4 days. The urine samples were analyzed by standard procedures for total excreted radioactivity as well as for free and conjugated metabolites. Approximately 15% of the ^{3}H was excreted in the first 24 hr. Less than 1% was collected in the next 3 days. Thus, the excretion of estrogens in urine is much lower in the squirrel monkey than in the human or Old World Monkeys. Estradiol disulfate was quantitatively the major metabolite of E_2 and accounted for 54% of the urinary radioactivity in the first 24 hr. 17β-Estradiol-17-sulfate (11%) and estrone sulfate (E_1S) (5%) were also identified. The estrogen glucosiduronates accounted for 27% of the ^{3}H in the first 24 hr urine collection. Estrone glucosiduronate accounted for more than 50% of the label in this fraction. These results indicate that the metabolism of E_2 in the squirrel monkey is unlike the human and nonhuman primates thus far studied. The excretion of estrogen metabolites in this species is much reduced, and the primary route of metabolism is via the sulfate pathway. (Supported by NIH grants CA-24616 and HD-13083).

29.14

THE SQUIRREL MONKEY: PLASMA METABOLITES OF ^{3}H-ESTRADIOL-17β (^{3}H-E_2). D.C. Collins, P.I. Musey, V.C. Musey*, and K.G. Gould*. Emory University, Atlanta, GA 30322.

As part of our ongoing studies of estrogen metabolism, we have determined the metabolism of ^{3}H-E_2 in the squirrel monkey, a species of New World Monkeys. ^{3}H-E_2 was injected into the leg vein of 2 adult female squirrel monkeys and blood was collected at 30, 60, 120, and 240 min post-injection. Plasma estrogen metabolites were separated and identified by standard procedures. The unconjugated fraction accounted for only 3-6% of ^{3}H present in each plasma sample. Estrone (E_1) and E_2 were the major metabolites of E_2. 17β-Estradiol-3,17-disulfate (E_2diS) (78%) and 17β-estradiol-17-sulfate ($E_2$17S) (20%) were the major plasma metabolites at 30 min in 1 animal. Thereafter the quantitative importance of the estradiol disulfate fraction diminished to 60% at 240 min with a simultaneous increase in $E_2$17S to 35%. At the same time, but not prior to 120 min, estrone sulfate (E_1S) appeared. Results from the second animal showed E_2diS increased from 71% at 30 min to 86% at 240 min with concomitant reduction of $E_2$17S from 18 to 12%. E_1S appeared earlier in this animal and accounted for 8% at 60 min. These results suggest that the metabolism of E_2 in the squirrel monkey is different from that seen in any other primate species thus far studied. E_2diS and $E_2$17S are the preponderant estrogen metabolites in plasma. This is the first time that E_2diS has been reported as a plasma metabolite in any human or nonhuman primate. (Supported by NIH grants CA-24616 and HD-13083).

29.15

BIOSYNTHESIS OF ESTROGEN SULFATES BY NEONATAL GOAT LUNG AND LIVER. Musey, V.C.*, and P.I. Musey. Department of Medicine, Emory University School of Medicine, Atlanta, GA 30303.

Previous reports from this laboratory suggest that the canine lungs are an active site at which hydrolysis of estrogen glucosiduronates occurs in vivo. In this report, we extend these studies to in vitro sulfation of estrogens by goat liver and lung. The 110,000 g cytosol fractions of neonatal goat liver and lung were incubated with ^{3}H-estrone (E_1), ^{3}H-estradiol (E_2) and ^{3}H-estriol (E_3) in the presence of ATP and $MgSO_4$ at 37C for 1 hr. Both lung and liver preparations were very active in the sulfation of estrogens. When E_1 was incubated with lung, more than 70% of the conjugate fraction was identified as E_1S. Similarly, 77% of the conjugate fraction was E_1S and 7% was E_2S when E_2 was used as substrate. Corresponding results from the liver studies were 69 and 19% as E_1S and E_2S, respectively, from E_1 incubation, and 13 and 73%, respectively, when E_2 was the substrate. However, the lung was particularly poor in sulfating E_3, only 20% of the water soluble metabolites was present at E_3S as compared to 79% from the liver. The oxidation of E_2 to E_1 was heavily favored by both tissues, but the lung cytosol could not reduce E_1 to E_2. These results clearly show that the lung has enzymes capable of C-17 oxidation of E_2 to the less active E_1. Furthermore, the neonatal lung and liver contain sulfotransferases which readily conjugate estrogens as sulfates. Whether these enzymes, particularly in the lung, persist into adulthood remains to be determined. (Funds CA24616, 5K08AM).

29.16

BINDING OF DANAZOL TO CYTOSOL PROTEIN RECEPTORS IN ENDOCRINE TISSUES OF THE RAT. D.T. Magrane*, D. Spencer* and G. Russell* (SPON: D.M. Miller). Morehead State University, Morehead, Kentucky 40351

Danazol, (Danocrine), an oral synthetic steroid derivative of ethinyl testosterone is clinically used for the treatment of endometriosis. Danazol has been shown to displace ^{3}H-dihydrotestosterone (DHT) and ^{3}H-corticosterone (B) from their cytoplasmic receptors which accounts for its mild androgenic and lowered glucocorticoid levels respectively. Estrogen (E_2) receptors appear to be weakly bound by Danazol. This study measured the in vitro binding of Danazol to cytosol receptors in uterus, mammary tissue, adrenals and hypothalamus. The evaluation of ^{3}H-progesterone, ^{3}H-E_2, ^{3}H-DHT, and ^{3}H-B displacement of cytosol receptors confirms the stronger binding of Danazol to progesterone and DHT receptors and weaker binding of B and E_2 receptors. These data were supported by in vivo studies following 10 daily injections of 4mg/kg Danazol.

29.17

EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE ON PLASMA PROGESTERONE AND ESTRADIOL IN THE RABBIT. Christopher M. Fredericks, Rajesh S. Mathur*, Sara C. Landgrebe* and Sarah H. Ashton* Med. Univ. of S.C., Charleston, S.C. 29425

The purpose of this study was to determine the effect of exogenous vasoactive intestinal polypeptide (VIP) upon the plasma levels of progesterone (P) and estradiol (E_2) in the rabbit. Virgin adult New Zealand does were infused for 60 min with VIP (75pmol/kg/min) and the plasma levels of P and E_2 determined. VIP was administered to both estrous and ovulatory (120 min post coitum & 75 I.U. hCG) animals. In estrous does, P increased during the VIP infusion, reaching a peak at 60 min. The P levels at 60 min (313±93ng/dl;X̄±SEM), 90 min (255±70), and 120 min (235±71) were all greater ($p<.05$) than at time 0 (99±9). In both VIP-treated and vehicle-only ovulatory animals, P rapidly increased following the ovulatory stimuli, peaked at 180 min (1975±272, 1350±240: treated and control, respectively), and returned to baseline within 24 hrs (150±10; 178±29). Although the VIP-treated levels were not significantly different from those of the vehicle controls, they were 20-30% greater during and at 30 and 60 min after the VIP infusion. No changes were observed in E_2 levels in association with VIP treatment in either estrous or ovulatory animals. In conclusion, VIP infused at 75pmol/kg/min induces an increase in plasma P but not E_2 in the rabbit. Endogenous VIP may play a role in the regulation of plasma P levels. (Supported in part by S.C. Appropriation for Biomedical Research).

29.18

MIDPREGNANCY ELEVATION OF SERUM ANDROSTENEDIONE LEVELS IN THE C3H/HeN MOUSE: PLACENTAL ORIGIN. Michael J. Soares* and Frank Talamantes. Thimann Laboratories, University of California, Santa Cruz, CA 95064

Recent studies suggest that androgens are involved in the maintenance of pregnancy in the mouse and rat (Biol. Reprod. 20:733 and 21:53, 1979). The primary source of androgen production during the second half of pregnancy appears to be the placenta (Biol. Reprod. 24:249, 1981). Although testosterone's role in pregnancy has been investigated extensively, the mouse placenta synthesizes significantly more androstenedione (A) than testosterone (T) (J. Steroid Biochem. 2:111, 1971). The purpose of this study was to characterize the serum profile of A during pregnancy. Serum A and T levels were measured by radioimmunoassay on days 5-18 of gestation. Both profiles were characterized by a prominent increase on days 9 and 10 of pregnancy. Serum A levels were higher than T levels throughout pregnancy. At midpregnancy serum A levels were about five times greater than serum T levels (A: 2.9 ± 0.2 ng/ml vs T: 0.5 ± 0.1 ng/ml). On day 10 of pregnancy *in vitro* A production by the yolk sac placenta (17.9 ± 4.8 ng/4 hr) was greater than by the chorioallantoic placenta (3.5 ± 0.6 ng/4 hr), ovary (0.2 ± 0.1 ng/4 hr) or adrenal gland (not detectable). These results suggest that A may be the principle androgen of pregnancy in the mouse and that the placenta is the site of its production. (Supported by NIH grants RR08132 and HD14966 to FT).

29.19

FOLLICLE-STIMULATING HORMONE AND PROLACTIN SECRETION IN HEMI-DECORTICATE FEMALE RATS. J.C.Bedran de Castro; K.O. Nonaka,A. M.Reis, A.L.V. Favaretto and J.Antunes-Rodrigues, Dept. of Physiology. School of Dentistry of Araçatuba (UNESP) and School of Medicine of Ribeirão Preto, USP. Ribeirão Preto, SP, BRAZIL.

Previous studies utilizing hemidecorticate (HD) as an experimental model for the investigation of extra-hypothalamic structures involved in reproductive physiology we have shown changes in endocrine gland weights, in the accessory sex organs, in the estrous cycle and in the synthesis and release of LH. Now we are showing that HD rats differs from the control (C) by releasing greater quantities of follicle-stimulating hormone (FSH) in the afternoon of the proestrous (HD = 1372,1 + 145,1 and C = 907,7 + 190,2 ng/ml) and lower ones of prolactin (PRL) (HD = 409,3 ± 64,1 and C = 536,2 ± 34,3 ng/ml). After 14 days of bilateral castration (CB), a similar data to FSH was obtained (HD = 2431,6 + 94,5 and C = 2008,8 ± 260,4 ng/ml) and 21 days of CB to PRL (HD = 20,2 ± 2,3 and C = 32,8 ± 2,7 ng/ml). These data suggest that HD would release the hypothalamus-hypophysis axis from extra-hypothalamic modulations.

(Supported by FAPESP-FINEP).

30.1

CHRONIC HYPOXIA PRODUCES DIFFERENT HEMATOLOGICAL AND RESPIRATORY MORPHOMETRIC EFFECTS IN LARVAL VS. ADULT BULLFROGS (RANA CATESBEIANA). Warren Burggren and Alan W. Pinder. Univ. of Massachusetts, Amherst, MA 01003.

Larval and adult bullfrogs were exposed to 4 weeks normoxia, hypoxia (P_{O_2}=70mmHg) or hyperoxia (P_{O_2} > 300 mmHg), and changes in blood hematology and respiratory properties and on morphometrics of skin, lungs and gills (if present) were determined. Larvae responded to chronic hypoxia with extreme gill hypertrophy, doubling of lung volume and 1° septation and doubling of skin capillarization and halving of skin gas diffusion distance. Respiratory properties of the high O_2 affinity blood were unchanged. Adults responded to chronic hypoxia with large increases in [Hb], O_2 capacity, RBC count and Hb-O_2 affinity, the last change probably being mediated by a fall in [NTP] and [2,3-DPG] in RBCs. There were no changes in the morphometrics of the lungs and skin of adults. Chronic hyperoxia produced no effect in either larvae or adults. The profound morphological changes in larvae, but not adults, reflects a greater morphological 'plasticity' of larvae, which undergo radical morphological change during metamorphosis. Hematological changes, rather than changes in gas exchanger morphology, are either the only possible changes in hypoxic adults, or are metabolically less costly. In any event, much larger absolute increases in O_2 -Hb affinity and attendant increases in O_2 loading can occur in adults compared with larvae.

30.2

TRANSITION TO FISH AIR BREATHING: *ANCISTRUS* AND *SYNBRANCHUS*. Jeffrey B. Graham. Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, CA 92093

Ancistrus chagresi, a facultative air breather, uses its stomach as an air-breathing organ (ABO). Comparisons of control and fish acclimated to hypoxia assessed the effects of this treatment on bimodal gas exchange capacity. Acclimation elicits physiological and biochemical changes that enable *A. chagresi* to increase O_2 utilization by gills and ABO. Hypoxia-acclimated *Ancistrus* have a higher Hb-O_2 affinity, more Hb and can maintain a higher $\dot{V}O_2$ in hypoxic water. They have a 25% larger ABO volume, hold each air breath longer, and can reduce ABO-$P_{\bar{E}}O_2$. CO_2-release occurs primarily through gills. An air breath instantly causes reduced gill ventilation rate and tachycardia. Lower cardiac and gill ventilation rates in hypoxic water during air breathing suggest acclimated fish are more adapted for hypoxia. While an air breath is held, hypoxia acclimated fish exhibit more coordinated phase shifts in gill ventilation and cardiac rates. These may favor an initial phase of efficient aerial O_2 uptake from the ABO and transport through the body followed by a period of aquatic CO_2 release from the gills. *Synbranchus marmoratus* uses gills and branchial epithelium while air breathing in water. During amphibious sojourns or exposure to air in burrows skin also functions for air breathing. Hypoxic water has no effect on the cardiorespiratory adaptations of this species. However, since the gills and mouth are utilized in air breathing, the avenues of CO_2 release in this species are different. (NSF DEB 79-12235)

30.3

CONTROL OF VENTILATION BY PERIPHERAL CHEMORECEPTORS IN TWO LIZARDS, Tupinambis nigropunctatus and Varanus exanthematicus. Ronald M. Jones, Dartmouth Medical School, Hanover, N.H. 03755

The role of chemoreceptors responding to changes of pO_2 in the peripheral circulation in the control of ventilation has been examined in Tupinambis nigropunctatus (T) and Varanus exanthematicus (V). Ventilation of unanaesthetized lizards was measured with a pneumotachograph and air tight masks at 35° C. Ventilatory response to a reduction of inspired pO_2 was measured during a 1 min. experimental period of N_2 breathing. Total ventilation ($\dot{V}_I$) during the period was 140% of control in T and 280% of control in V. $\dot{V}_I$ significantly increased during the first 10 seconds of N_2 in V and after 30 seconds in T. During O_2 breathing ("continuous O_2 test") $\dot{V}_I$ fell to 65% of control within the first 2 breaths in V. Total time for the 2 breaths was always >1 minute. T averaged 90% of control $\dot{V}_I$ during the 1 minute of O_2, with a maximum reduction to 65% of control between 40-50 seconds. Control $\dot{V}_I$ averaged 78 ml/kg min in T and 50 ml/kg min in V. Because the response was measured before the stimulus to central chemosensitive areas was likely to have been substantially altered (<1 min) these responses have the characteristics of peripheral chemoreceptors analogous to mammalian carotid bodies. Functionally Varanus has a more mammalian-like response to transient peripheral chemoreceptor stimulation than does Tupinambis. (Supported by NIH HL07449-02 and HL02888-25.)

30.4

CENTRAL VASCULAR SHUNTING DURING THE BREATHING CYCLE IN VARANUS EXANTHEMATICUS. N. Heisler*, P. Neumann* and G.M.O. Maloiy* (SPON: P. Scheid). Max-Planck-Institut für experimentelle Medizin, Physiology Dept., D-3400 Göttingen, FRG

In order to study the extent of blood shunting in the heart of Varanus exanthematicus during breathing episodes and apnea, microspheres (15μ) with different radioactive label were injected simultaneously through indwelling catheters into the right and the left atrium of unrestrained specimens mounted with tracheal pneumotachograph sensors. The relative activity of the microsphere label injected into the left atrium (right atrium) trapped in the lungs (systemic tissues) yielded the magnitude of the intracardiac L-R shunt (R-L shunt, respectively) (see: Berger and Heisler, J. Exp. Biol. 71, 111-121, 1977). In nine specimens, shunting, which was corrected for recirculation of microspheres (1.5-4.0 %), was not found to be significantly different between breathing periods and apnea: The R-L shunt averaged 29 and 31%, respectively, the L-R shunt was 11% in both conditions. The observed shunting was rather constant with time and during the breathing cycle, but was extremely variable between different specimens (R-L: 10-63 %, L-R: 3-23 %). It is concluded that the extent of central vascular shunting in Varanus is not modulated according to different periods of the breathing cycle, but is the result of the peculiar anatomical arrangement and the extremely variable relative size of the heart chambers.

Supported by Deutsche Forschungsgemeinschaft

30.5

ADAPTIVE SIGNIFICANCE OF ARRHYTHMIC BREATHING PATTERNS IN LIZARDS. William K. Milsom and Timothy Z. Vitalis*. Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C., Canada.

The normal breathing pattern of the Tokay lizard, Gekko gecko, is an arrhythmic pattern consisting of a series of one to several breaths separated by a highly variable respiratory pause commencing at end inspiration. When respiratory drive is increased (3.7x) by warming (10°C), increases in pulmonary ventilation are caused solely by increases in respiratory frequency due to shortening of the periods of breath-holding. There is no significant change in tidal volume, breath length (and ∴ instantaneous breathing frequency), or the work performed in producing each breath. The breathing frequencies associated with minimum mechanical work loads for the levels of minute ventilation recorded in awake animals were determined from pressure-volume loops derived from forced ventilations in anaesthetized animals. These optimum breathing frequencies were found to be almost identical to the instantaneous breathing frequencies obtained in the awake animals. These data suggest that at low metabolic rates where continuous breathing is not required to meet metabolic demands, an arrhythmic breathing pattern in which the breath-hold length is the major controlled variable while instantaneous breathing frequency and tidal volume are fixed at optimum values, represents an adaptive strategy to reduce the energetic cost of breathing.

30.6

STRUCTURE AND FUNCTION OF THE LUNG OF THE TEGU LIZARD, TUPINAMBIS NIGROPUNCTATUS. Hlastala MP, TA Standaert*, DL Luchtel* and DJ Pierson*. Univ. Wash. Seattle, WA 98195.

The walls of the unicameral Tegu lung enclose a large axial air chamber. Microscopic examination revealed approximately three generations of septa which subdivided the wall into tubular-shaped gas-exchange chambers. At the anterior end of the lung, wall thickness averages 3-4 mm, is 1.5-2.5 mm in the middle portion of the lung and decreases to 1.0 mm or less at the posterior end. The axial chamber comprises approximately 70% of the lung volume. The average thickness of the blood air barrier (epithelial - basal lamina - endothelial cell layer) ranged from 1.0 μm to less than 0.50 μm.

Ventilation-perfusion (V_A/Q) distribution was evaluated using the multiple inert gas elimination method. The lizards (.5-.9 kg) were anesthetized with halothane and ventilated with a 6 ml tidal volume and frequency sufficient to maintain arterial P_{CO_2} at 27 torr. Body temperature was maintained at 35°C. Arterial and mixed venous P_{O_2} averaged 75±5 torr and 36±3 torr while air breathing and 221±21 torr and 44±5 torr while O_2 breathing. V_A/Q distributions were broad and shifted to the high V_A/Q region. Inert gas elimination showed a right to left shunt varying from 0% to 74%.

Despite the lack of a typical alveolar structure, gas exchange does not appear to be diffusion limited. The lungs exhibit a very high effective mean V_A/Q with a somewhat broadened distribution.

30.7

INFLUENCE OF ISCHEMIA AND HYPOXIA ON BREATHING IN DUCKS. Richard S. Lillo* and David R. Jones. Dept. of Zoology, Univ. of British Columbia, Vancouver, Canada.

The influence of ischemia and hypoxia on breathing in White Pekin ducks, Anas platyrhynchos, excluding pathways involving the carotid bodies, was examined. Occlusion of the abdominal aorta in unanesthetized ducks produced immediate development of hypertension. Ventilation was unaffected for the first minute; a tachypnea then developed rapidly and persisted for the duration of the occlusion resulting in a 25% increase in minute ventilation ($\dot{V}_E$). Following thoracic spinal section, all ventilatory responses to occlusion were eliminated. Carotid body denervated birds breathing 10% or 5% O_2 developed a tachypnea after a latency of 30-100 s. The tachypnea was more pronounced with the more severe hypoxia resulting in almost a doubling of $\dot{V}_E$. Experimental perfusion of the brain and single intact carotid body in unanesthetized ducks with hyperoxic blood during low O_2 breathing (6-9% O_2) resulted in tachypnea also after a considerable latency. Cerebral hypoxia accentuated by ischemia likewise produced tachypnea. These results suggest that severe hypoxia can affect breathing in birds via pathways other than those involving the carotid bodies.

30.8

CARDIOPULMONARY CONTROL DURING EXERCISE IN THE DUCK. J.P. Kiley and M.R. Fedde. Dept. of Anatomy and Physiology, Kansas State Univ., Manhattan, KS 66506.

To determine the importance of nonhumoral drives to exercise hyperpnea in birds, we exercised 12 adult Pekin ducks on a treadmill (3° incline) at 1.44 $km \cdot hr^{-1}$ for 15 min during artificial, unidirectional ventilation. This ventilation procedure allowed the regulation of intrapulmonary gas concentrations and arterial blood gases, while allowing ventilatory effort to be measured during both rest and exercise. At a flow rate of 12 $\ell \cdot min^{-1}$, P_{aCO_2} could be maintained within ± 2 Torr of resting values throughout exercise. Arterial P_{O_2} did not change significantly with exercise. Heart rate, mean arterial blood pressure, and mean right ventricular pressure significantly increased during exercise. Ventilatory effort increased approximately 400% over resting levels because of increases in both tidal volume and respiratory frequency. CO_2-sensitivity curves, obtained for each bird during rest, indicated that a 1.5 Torr increase in P_{aCO_2} during exercise would account for only about 5% of the increase in ventilation over resting levels. During exercise, arterial $[H^+]$ increased approximately 4 nM/ℓ; this increase could account for about 18% of the rise in ventilation. We conclude that only a minor component of the exercise hyperpnea in the duck can be accounted for by a humoral mechanism; other factors appear responsible for most of the hyperpnea observed during running. (Supported, in part, by a grant from the American Heart Assn., Kansas Affiliate, Inc.)

30.9

RESPIRATION IN HEAT-STRESSED RAVENS. Michael A. Taylor and Marvin H. Bernstein. New Mexico State University, Las Cruces, NM 88003.

Body temperature (T_b), respiratory frequency (f), and tidal volumes (V_T) were determined in resting White-necked Ravens, Corvus cryptoleucus (mean mass, 0.45 kg). At air temperatures (T_a) from 22 (thermal neutrality) to 44.5°C, T_b rose from 41.0 to 45.6°C, f increased 10-fold, and V_T nearly doubled. Minute volume ($\dot{V}$) also increased, as did respiratory evaporation ($\dot{m}_e$), calculated from the difference between inspired and expired V_T. Exhaled air temperature (T_E), calculated from V_T and ($\dot{m}_e$), was 1°C less than T_b in thermal neutrality. The difference between T_E and T_b decreased with increasing T_a; the two were equal at a T_a of 44°C. Breath-by-breath analysis of inspiratory and expiratory V_T's demonstrated simple panting with no evidence of the compound ventilation found in flying ravens and in other species during panting. Further, panting V_T was always greater than tracheal volume. The data show that heat-stressed ravens thermoregulate by use of typical avian mechanisms. The apparent flushing of tracheal dead space during panting raises questions about maintenance of blood gas and acid-base status. Supported by an NSF Graduate Fellowship (MAT) and by NSF grant PCM79-21856.

30.10

DISTRIBUTION OF AIR SAC VENTILATION DURING HIGH FREQUENCY VENTILATION IN DUCKS. Randolph H. Hastings* and Frank L. Powell. Dept. of Med. M-023, Univ. Calif. San Diego, La Jolla, CA 92093

We investigated the f and V_T dependence of the distribution of ventilation to air sacs of the Pekin duck. We measured helium washouts of the interclavicular (ICS) and posterior thoracic (PTS) air sacs of an anesthetized, tracheostomized duck during low frequency control ventilation (CV), f=10.4 min^{-1}, V_T=213 ml, and several levels of high frequency ventilation (HFV) with a piston pump, f=3-12 sec^{-1}, V_T=9-18 ml. A bias flow of 10 l/min provided fresh air for HFV and V_T was obtained by integrating the signal from a frequency compensated pneumotachograph placed between the bias flow and the trachea. Two catheters were sealed into each sac for simultaneous injection of helium and sampling of air sac gas at 15 ml/min by mass spectrometer. The ratio of effective air sac ventilation ($\dot{V}_{eff}$) to $\dot{V}_E$ was .151 for ICS and .243 for PTS during CV. With HFV, $\dot{V}_{eff}/\dot{V}_E$ decreased in both sacs but more in PTS (e.g. .092 for ICS and .056 for PTS at f=12 sec^{-1}, V_T=9.1 ml.) This may be due to a) rebreathing of dead space gas containing helium into the sacs and b) a change in the distribution of ventilation to air sacs on HFV. On HFV, $\dot{V}_{eff}/\dot{V}_E$ in ICS increased with V_T but showed no relation to f while $\dot{V}_{eff}/\dot{V}_E$ in PTS decreased with increasing f and was weakly correlated with V_T. The V_T dependence may be due to convective transport between dead space and gas exchange regions while f dependence may reflect changes in aerodynamic valving. Supported by NIH grants HL 26050 and GM 07198.

30.11

MORPHOMETRY OF RAPIDLY FROZEN AVIAN LUNG. F.L. Powell and R.W. Mazzone. Dept. of Med., Univ. of Calif., San Diego, La Jolla, CA 92093

Recent measurements of compliance of the avian lung indicate that it may not be as rigid as was previously thought. To better understand the structural basis of such compliance we rapidly froze domestic and Canadian goose lungs in situ at end-expiration. The pleura was exposed in the 1st, 2nd and 3rd intercostal spaces and flooded with liquid Freon 22 (-155°C). Frozen samples were obtained from the dorsal lung surface and processed by freeze substitution fixation. Random sections were photographed on a Zeiss EM9 transmission electron microscope and analyzed stereologically. Folds, pleats, and narrowed capillaries were observed, indicating surface areas and volumes may change with physiologic variables. In domestic and Canadian geese, respectively, air capillaries occupied 47% and 56% and blood capillaries occupied 34% and 26% of exchange tissue in the parabronchial mantle; surface:volume ratios were 8019 and 7712 cm^{-1} for air capillaries and 12,976 and 13,357 cm^{-1} for blood capillaries. Our air:blood capillary volume ratio for Canadian geese is larger than Duncker's previous estimates on perfusion fixed lungs (2.13 vs. 1.34) but the effective exchange tissue area:volume ratio is similar (2515 vs. 2450 cm^{-1}). Such differences may be due to regional differences within the lung or fixation technique. Analysis of rapidly frozen avian lung will allow further investigation of the effect of physiologic variables on air-blood capillary morphology. (NIH grants HL 26050, HL 28305)

EXERCISE III

31.1

REPRODUCIBILITY AND DISTRIBUTION OF PULMONARY RESPONSES RESULTING FROM OZONE EXPOSURE. W. McDonnell*, D. Horstman, S. Salaam*, and D. House*. US EPA, Chapel Hill, NC 27514

The magnitude of pulmonary function changes resulting from exposure to low levels of ozone (O_3) varies among individuals. The purposes of this study were to determine the reproducibility of responses to O_3 exposure and to characterize the distribution of response resulting from exposure to 0.18 ppm O_3 (level at which we have observed small, but statistically significant, mean decrements). To determine reproducibility we twice exposed 32 subjects to either 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3. To determine distribution of response, we exposed 67 subjects to 0.18 ppm O_3 and 26 subjects to air. Subjects were healthy, young males. Exposures consisted of 2 hours of alternating 15 minute periods of rest and heavy exercise (V_E 35 l/min. m^2BSA). In the reproducibility study, a 14 ± 3% decrement in FEV_1 was observed after both exposures. The correlation coefficient between individual changes in exposures 1 and 2 was 0.89. Following exposure to 0.18 ppm O_3, the mean decrease in FEV_1 was 10 ± 1%, and 67% of subjects had FEV_1 decrements less than 10% while 33%, 15%, and 9% of the subjects had decrements in FEV_1 greater than 10%, 20%, and 30%, respectively. Following air exposure, FEV_1 was decreased by 2 ± 1%; all but one subject had decrements less than 8%. We conclude that some individuals are more sensitive to O_3 than others, and exhibit marked decrements in pulmonary function even at low levels of O_3.

31.2

NONLINEARITY OF CO_2 OUTPUT DYNAMICS DURING MODERATE EXERCISE. N. Lamarra*, B.J. Whipp and K. Wasserman. Div. Resp. Med., Harbor-UCLA Medical Center, Torrance, CA 90509.

O_2-uptake ($\dot{V}O_2$) dynamics have commonly been described as linear for moderate-intensity exercise, both at the muscle and at the lung. CO_2-output ($\dot{V}CO_2$) dynamics, on the other hand, are likely to be more complex, owing to the influence of the large tissue CO_2 stores (capacitance); however, they have been assumed to be dynamically linear without formal verification of this assumption. To characterize these dynamics, 6 healthy males performed 4 step-transitions each of 6-min duration on a cycle ergometer, for each of 3 moderate-intensity protocols: A) 0-75W; B) 50-125W; and C) 0-125W. Breath-by-breath calculations of ventilation, $\dot{V}CO_2$ and $\dot{V}O_2$ were interpolated, time-aligned, and averaged to obtain their characteristic responses to each protocol. Model-order estimation showed that, surprisingly, $\dot{V}CO_2$ dynamics were first-order, following the initial "cardiodynamic" region of gas exchange. However, the time constant changed with work rate, being faster from a baseline of moderate exercise. Since $\dot{V}CO_2$ dynamics at the lung are related to those at the tissue via a transfer function which depends on the capacitance of the intervening stores, this finding would be consistent with reduced capacitance at moderate exercise levels, compared to light exercise. Consequently, the more-loaded the CO_2 stores, the tighter will be the coupling between $\dot{V}CO_2$ kinetics at the lung and those of the contracting muscles.

31.3

EFFECT OF INCREMENT DURATION ON THE REGION OF ISOCAPNIC BUFFERING DURING CYCLE ERGOMETER EXERCISE. J. A. Davis, B.J. Whipp, and K. Wasserman. Division of Respiratory Physiology and Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509

During incremental exercise in which the work rate is increased rapidly, a region of $\dot{V}O_2$ can be identified between the beginning of the systematic increase in $\dot{V}_E/\dot{V}O_2$ (anaerobic threshold, θan) and that of $\dot{V}_E/\dot{V}CO_2$, i.e., the onset of respiratory compensation. As $P_{ET}CO_2$ is maintained constant in this region, it has been termed "isocapnic buffering". To investigate this region further, we examined how isocapnic buffering was influenced by the duration of the work-rate increment. Five males, aged 26.6 ± 4.0 yrs, performed four incremental cycle ergometer tests. The size of the increment was always 25 W while the durations were 0.5, 1, 2, and 4 min. Ventilation and gas exchange were measured breath-by-breath. For the 4 min incremental test, θan and the onset of respiratory compensation, as evidenced by the systematic reduction in $P_{ET}CO_2$, occurred at the same $\dot{V}O_2$; thus there was no region of isocapnic buffering. For the 2 min test, a small or nonexistent isocapnic buffering region was found. However, as the increment duration became shorter, the isocapnic buffering region became progressively longer and extended to over 1 $l \cdot min^{-1}$ of $\dot{V}O_2$ above θan for the 0.5 min incremental tests. Because isocapnic buffering is a requirement for rigorous detection of θan from gas exchange measurements, these results indicate a necessity for work-rate durations of 1 min or less for incremental exercise testing designed to determine θan.

31.4

VENTILATION AND GAS EXCHANGE EFFECT OF HYDRALAZINE IN CHRONIC AIRWAY OBSTRUCTION. C.Keller, G.F.Dolan, D.S.Chun, P.Vasquez and V.D.Minh. St. Louis VAH(GRECC), St. Louis Univ. and Univ. Ca., Irvine, 92668.

We studied the effect of Hydralazine(total dose 200 mg over 24 hours)in 8 patients with chronic airway obstruction (VC 2.5±.81, $FEV_{1.0}$ 1.1±.41, and DLCO 44±15% pred. val.), at rest and during maximum treadmill exercise. Improvement in cardiac output, pulmonary pressures, work capacity, systemic blood pressures etc. was consistent with previous data from others (Rubin, NEJM 302:69, 1980). New data are given below, comparing ventilation and gas exchange before (BEF) and after (AFT) administration of Hydralazine.

REST	PaO2	PaCO2	VD/VT	Q̇va/Q̇t	C̄vO2	P̄vO2	V̇E	VT
BEF.	61±6	45±9	60±8	22±6	12±2	28±2	15±2	.6±.3
AFT.	64±8	35±7	53±8	22±12	14±2	32±4	18±8	.9±.3
p<	.01	.001	.05	NS	.01	.05	NS	.05
EXER.								
BEF.	48±5	48±10	51±10	25±5	7±1	21±2	27±4	.8±.4
AFT.	60±9	39±6	47±6	18±6	9±1	24±2	37±9	1.2±.4
p<	.01	.001	NS	.01	.01	.01	.01	.001

The data indicate that: 1) Hydralazine improves ventilation and gas exchange particularly during exercise. 2) The improvement of arterial O2 stems from an increase in mixed venous oxygenation (PV̄O2 in mmHg, and CV̄O2 in vol%), and a reduction in venous admixture (Q̇va/Q̇t). In conclusion, Hydralazine is a potent drug that has a beneficial effect in patients beyond the hemodynamic improvement.

31.5

SYSTEMIC OXYGEN TRANSPORT AT NORMAL AND DECREASED P_{50} DURING NORMOXIC AND HYPOXIC TREADMILL EXERCISE IN THE DOG. P.T. Schumacker*, A.J. Suggett*, P.D. Wagner and J.B. West. Dept. of Med., U. of Calif., San Diego, La Jolla, CA 92093

Previous studies have suggested that a left-shifted oxyhemoglobin dissociation curve (decreased P_{50}) may improve peripheral O_2 transport during exercise at high altitude. To clarify this further, we have studied male, tracheostomized dogs at rest and at several levels of steady-state exercise (9 km/hr; 0,5,10 and 15% incline). Inspired gas was room air, 12% O_2 or 10% O_2 in N_2. Measurements were made at normal P_{50} (27-29 torr) and after the Hb-O_2 curve was chronically left-shifted (P_{50}=17-19 torr) by administration of Na cyanate. The increase in cardiac output with $\dot{V}O_2$ at all levels of F_{IO_2} was not affected by left-shift of the curve, nor was heart rate. Arterial O_2 saturation was increased by left-shift at all F_{IO_2} levels. Mixed venous PO_2 was significantly lower at rest and at all levels of $\dot{V}O_2$, at all levels of F_{IO_2}, after left-shift of the curve. Base defecit was small at rest (2-7 mEq/L) at all F_{IO_2} and all levels of exercise and was not affected by the change in P_{50}. The data from this study suggest that left-shift did not improve systemic O_2 transport in the dog during normoxic or hypoxic exercise, nor did it significantly impair O_2 delivery. However, since left-shifted hemoglobin may lower the gradient for O_2 diffusion in the peripheral capillaries, it could conceivably limit peripheral unloading of oxygen at higher levels of work. (Supported by Grants HL 27410, HL 17731 and MRC, London)

31.6

DOES EXERCISE-INDUCED REDUCTION OF MUSCLE GLYCOGEN ALTER THE ANAEROBIC THRESHOLD? V.J. Caiozzo*, J.A. Davis, C.A. Wills*, A.W. Hawksworth*, R.B. Vandagriff*, C.A. Prietto*, W.C. McMaster*, and R.A. Baird*. Dept. of Surgery, Univ. of Calif., Irvine, CA 92717.

While it is known that muscle glycogen reduction induced by prior exercise lowers blood lactate concentration [HLa] at heavy exercise intensities, it is not known if this intervention alters the onset of a lactic acidosis, i.e., anaerobic threshold. To resolve this question, 11 male subjects performed a 20 $W \cdot min^{-1}$ incremental cycle ergometer exercise test, and 1 wk later the test was repeated 2 hrs after the subjects completed an exercise protocol designed to lower muscle glycogen by ≈70%. During both tests, measurements of [HLa], ventilation, and gas exchange were made every 30 s. The anaerobic threshold (θan) corresponded to the $\dot{V}O2$ where there was 1) a systematic increase in [HLa], and 2) a systematic increase in $\dot{V}E/\dot{V}O2$. θan discerned from [HLa] was 1.66 ± 0.09 $l \cdot min^{-1}$ before and 1.70 ± 0.08 $l \cdot min^{-1}$ after muscle glycogen reduction. Discerned from gas exchange, θan was 1.68 ± 0.10 and 1.71 ± 0.11 $l \cdot min^{-1}$, respectively. None of the four values for θan were significantly different from one another. During both tests, the concentrations of HLa were virtually identical up to and at θan, but beyond θan there was a progressive divergence with peak [HLa] of the second test being significantly lower. Thus, the onset of a lactic acidosis, as discerned from alterations in either [HLa] or gas exchange, is not affected by exercise-induced reduction of muscle glycogen.

31.7

MUSCLE GLUCOSE UTILIZATION FOLLOWING EXERCISE: RELATION TO GLYCOGEN DEPLETION. L.P. Garetto*, E.A. Richter*, M.N. Goodman and N.B. Ruderman. Boston Univ. Med. Ctr., Boston, MA 02118

Glucose utilization by skeletal muscle is enhanced following exercise presumably to replete diminished glycogen stores. To investigate the mechanism for this, untrained, fed, male rats were run on a motor-driven treadmill, immediately after which their hindquarters were isolated and perfused. Hindquarters from rats run at 18 m/min for 45 min (E) had the same rate of glucose uptake as non-exercised controls (C) in the absence of insulin. When insulin was added to the perfusate at concentrations as low as 30 μU/ml, glucose uptake was significantly higher in E. This effect persisted for at least 4 h and was mainly due to an increase in insulin sensitivity (K_m insulin: C-480 μU/ml, E-150 μU/ml). Biopsies taken at the start of the perfusion indicate that E had already substantially repleted their muscle glycogen. To study rats that were glycogen depleted, a second group was exercised at 36 m/min for 24 min. In these rats glucose uptake was enhanced even in the absence of insulin. The greatest increases occurred in rats with the most marked glycogen depletion. Based on these results, a two phase model of glycogen resynthesis following exercise is proposed. Immediately after exercise when glycogen stores are diminished there is an insulin independent increase in muscle glucose uptake. Then, as glycogen stores are repleted the increase in glucose uptake is dependent on insulin. The increase in insulin sensitivity during the latter phase may, in part, explain the phenomenon of supercompensation.

31.8

THE ROLE OF WORK RATE AND PHYSICAL FITNESS ON THE KINETICS OF O_2 UPTAKE DURING MODERATE EXERCISE. M.A. Gausche*, T. Harmon*, N. Lamarra*, K. Wasserman and B.J. Whipp. Div. Resp. Physiol. and Med., Harbor-UCLA Med. Center, Torrance, CA 90509.

O_2 uptake ($\dot{V}O_2$) is commonly described as responding exponentially to moderate-intensity exercise with a time constant (τ) which does not vary with work rate. This has been based upon estimating the best-fit exponential to a single work-rate transition and without separating the early "cardiodynamic" phase from the response. In an attempt to characterize the effect of a sufficiently wide range of work rates upon τ, we utilized highly-fit subjects (i.e. anaerobic thresholds, θan, up to 50 ml/kg/min). The subjects performed four repetitions of cycle ergometer exercise, each for six minutes, from a baseline of unloaded cycling to each of 50, 100, 150, 200 watts etc; but constrained to be < θan. $\tau\dot{V}O_2$ was computed breath-by-breath and the best-fit exponential extracted for each work rate from the mean of the four repetitions; the cardiodynamic phase having been deleted. The results demonstrate that τ was systematically related to fitness; being smallest in the subjects with the highest θan. However, τ was not constant among tests in a given subject, becoming shorter as the imposed work rate increased but then increasing again as the rate increased further. We therefore conclude that $\dot{V}O_2$ during moderate exercise does not evidence linear, first-order kinetics and that there appears to be a work rate at which the O_2-flow to the contracting muscles is "optimized" thereby minimizing the O_2 deficit.

31.9

GLUCOSE AND PALMITATE METABOLISM DURING REST AND EXERCISE IN THE GRAY SEAL. Michael A. Castellini* and Peter W. Hochachka, University of British Columbia, Vancouver. V6T 2A9.

The metabolic fuel requirements of gray seals were determined while the animals were resting, swimming and diving in a swimming-mill chamber at the University of Guelph. A bolus of 14C tagged glucose or 14C palmitate was injected arterially during the experimental procedures and blood samples taken while the seal was resting or working for up to 8 hours after the injection. The resting apparent turnover rate of glucose was determined to be about 9 μmoles/min-kg, in close agreement to values from harbor seals, but doubled when the animals were swimming at 1.5m/sec for up to 2 hours. During a dive, the turnover rates became very low, most likely due to a redistribution of blood flow to different body compartments. The free fatty acid results followed a similar pattern with half life times significantly increasing for the dive experiments, then returning to normal during the post dive period. Combined with values of oxygen uptake, carbon dioxide production, blood oxygen levels and heart rate, we can estimate the metabolic costs for the gray seal while swimming and gauge the preference for glucose or free fatty acid metabolism while diving.

31.10

ENDURANCE TRAINING ENHANCES CORI CYCLE ACTIVITY DURING EXERCISE. Casey M. Donovan* and George A. Brooks. Exercise Physiology Laboratory, University of California, Berkeley, CA 94720.

The effect of endurance training upon Cori cycle activity was assessed by continuous infusion of [U-^{14}C]-lactate (1.0 μCi/min) in rats. Measurements were made under three metabolic conditions: rest (Re), easy exercise (EE, 13.4 m/min, 1% gradient), and hard exercise (HE, 26.8 m/min, 1% gradient). Lactate turnover rates (R_t) were not different between groups at any metabolic condition and demonstrated a linear increase from Re to HE. Following infusion of [U-^{14}C]-lactate, incorporation of ^{14}C into glucose decreased from 306 ± 43 dpm/μg in Re to 220 ± 27 dpm/μg in EE. No significant differences in ^{14}C-GSA were observed between controls and trained animals at Re and EE. During HE controls demonstrated a further decrease in ^{14}C-GSA to 130 ± 26 dpm/μg, while trained animals maintained ^{14}C-GSA at 244 ± 36 dpm/μg. Mean values for rates of gluconeogenesis from lactate (L → G) were calculated (R_{glu} was obtained from a parallel study employing [6-^{3}H]-glucose). In trained animals L → G increased from 20.8 μMol/kg/min in Re to 50.2 μMol/kg/min in EE and 70.2 μMol/kg/min in HE. Control animals had similar values for L → G in Re and EE, but demonstrated a significantly lower rate in HE (34.2 μMol/kg/min). Trained animals demonstrate an improved conservation of carbohydrate carbon during hard exercise as a result of enhanced Cori cycle activity. (Supported by NIH Grant AM19577).

31.11

ISOMETRIC STRENGTH AND ENDURANCE OF ELECTRICALLY STIMULATED LEG MUSCLES OF QUADRIPLEGICS. R.M. Glaser, J.S. Petrofsky, J.A. Gruner* and B.A. Green*. Wright State Univ. School of Med., Dayton, OH 45435 and Univ. of Miami School of Med., Miami, FL 33136

The purpose of this study was to determine isometric strength and endurance of paralyzed leg muscles. For this, the quadriceps muscles of four male quadriplegic volunteers (C4-6, $\bar{x}$ age = 21.5 yr, $\bar{x}$ time paralyzed = 3.8 yr) were electrically stimulated via a computer controlled feedback stimulator. During testing, each subject sat in a specially constructed exercise chair, and his right ankle was coupled to a strain-gauge transducer for force determinations. Maximal voluntary contraction (MVC) was 10.9 kg. Endurance time for 40% MVC was 55 sec. During the fatiguing contractions, systolic blood pressure increased by 46% (81 to 118 mm Hg), whereas diastolic blood pressure increased 52% (60 to 91 mm Hg). Heart rate responses were variable (0 to 38% increase). These data indicate that substantial isometric strength and endurance can be obtained by electrically stimulating paralyzed muscles in cervical spinal cord patients. Blood pressure responses appeared to be similar, whereas heart rate responses were not as marked as for able-bodied individuals. (Supported in part by the Spinal Cord Society)

31.12

THE EFFECT OF ELECTRICALLY INDUCED BICYCLE ERGOMETER EXERCISE ON BLOOD PRESSURE AND HEART RATE. J.S. Petrofsky, R.M. Glaser, C.A. Phillips and J.A. Gruner*. Wright State Univ., Dayton, OH 45435

Blood pressure and heart rate were monitored during exercise on a Monark bicycle ergometer induced by electrical stimulation of iliopsoas, quadriceps and gastrocnemius muscle groups in four male quadriplegics. Stimulation was applied by sequentially discharging square wave impulses through three electrodes placed above the active muscle groups with voltages which were varied between 0 and 150 volts at a pulse width of 100 usec. Feedback of the position of the joints of the legs and the position of the bicycle peddles was detected and transduced for the microprocessor based stimulator. The appropriate muscles were then stimulated at the contraction level and proper sequence of time to allow the Monark bicycle ergometer to be peddled at a speed of 50 rpm. For max exercise, heart rate was quite variable (remained at rest level; increased or decreased), and depended to a great extent on the level of the injury. In contrast blood pressure (both systolic and diastolic) increased markedly (59.7%) in all four subjects: $\bar{x}$ rest = 84/59 mmHg; $\bar{x}$ max exercise = 147/88 mmHg. It appears that electrically induced exercise in paralyzed muscle causes a severe afterload on the heart. This increased work load on the heart may prove hazardous for individuals who have a deteriorated cardiovascular system due to a number of years of paralysis in a wheelchair. (This work was supported in part by the Spinal Cord Society)

CARDIAC DYNAMICS II

32.1

CONTRIBUTION OF THE AORTA TO LEFT VENTRICULAR MIXING IN DOGS. J.Mickelson*, D.Abendschein*, C.J.Carlson, E.Rapaport. Medical Service, San Francisco General Hospital, Dept. of Medicine & CVRI, University of California, San Francisco 94110

Thermal mixing within the left ventricle (LV) and aorta (Ao) was assessed by simultaneous temperature measurements using thermocouples in 7 LV subregions and 2 Ao subregions. End diastolic temperature (T) after the LV injection of iced saline at a paced heart rate of 100 bpm was measured. Residual fraction (K) is the ratio of temperature change for consecutive beats ($\Delta T_n/\Delta T_{n-1}$). The following variables were calculated from at least three LV injections: mean beat to beat K at each site, standard deviation of K at each site for each beat, and the difference in K's between each LV site and the Ao sites. The mean K did not stabilize and the standard deviation did not reach a minimum for at least two beats after injection at any site. The difference in K's between any LV site and the distal Ao site after two beats was essentially zero. This suggests that there was no contribution of the proximal aorta to mixing after an LV injection of iced saline.

32.2

INTRAOPERATOR AND INTEROPERATOR VARIABILITY IN SERIAL NONINVASIVE PULSED DOPPLER MEASUREMENTS OF STROKE VOLUME DURING REST AND UPRIGHT EXERCISE. G. Shaw*, C. Johnson*, K. Wimer*, M. Sutton*, G. Cagle*, E.R. Greene*, (SPON: J.A. Loeppky) Lovelace Medical Foundation and University of New Mexico, Albuquerque, NM 87108

We determined the intraoperator (ITR) and interoperator (ITE) variability in the noninvasive measurement of stroke volume (SV) using suprasternal pulsed Doppler echocardiography (PDE). Serial measurements during 3 trials (T_1, T_2, T_3) were performed independently by 5 novice operators on 5 normal subjects under steady-state conditions. Measurements of ascending aortic blood velocity (v) were made during supine rest (SR), upright rest (UR), and upright ergometer exercise (UE) at 300 kpm/min. SV (range 44.1-143.7 ml) was calculated using resting M-mode aortic root diameters (D). The ITR given by the mean ($\bar{x}$) and range (R) of the coefficients of variation (CV) by single operators in repeated trials were as follows: SR, $\bar{x}$=12%, R=5-11%; UR, $\bar{x}$=14%, R=11-22%; UE, $\bar{x}$=12%, R=5-19%. The ITE (values of CV in %) during each trial were as follows:

	SR			UR			UE		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
$\bar{x}$	22	16	14	19	14	17	20	14	14
R	9-32	5-33	9-20	14-28	11-23	9-28	13-28	8-20	9-18

Based on the statistical results of repeated measures ANOVA, we conclude that 1) ITR is less than ITE in SR, UR, and UE, and 2) ITE improves with repeated trials in all conditions but only significantly ($p<.05$) in SR.

32.3

CARDIAC OUTPUT CHANGES WITH POSTURE DURING PREGNANCY. K. Wimer* and M. Eldridge* (SPON: J.A. Loeppky). Lovelace Medical Foundation, Albuquerque, NM 87108

Techniques for measuring maternal cardiac output (CO) and stroke volume (SV) include radiography and catheterization which are not easily repeated and require restrictive postures. We used a 5 MHz pulsed Doppler velocimeter to noninvasively measure ascending aortic blood velocity (v) and estimated vessel diameter (d) with M-mode echocardiography to derive CO, SV and heart rate (HR). Subjects were measured in supine (Su), sitting (S), standing (St), hands and knees (K), and right and left lateral (RL and LL) positions. Subjects included 8 nonpregnant (Grp-1) and 15 pregnant women (Grp-2 = 12-20 week, n=4; Grp-3 = 20-30 weeks, n=7; Grp-4 = 30-term, n=4). CO and SV peaked after 25 weeks (Grp-3) in all positions with highest values in Su and K. CO and SV showed a decreasing trend in late gestation (Grp-4) while HR rose in early pregnancy (Grp-2 vs Grp-1) and remained elevated to term. LL and K remove the uterine compression on the inferior vena cava but CO and SV decreased in Grp-4 in all postures, suggesting that factors other than mechanical compression reduce CO and SV. CO was greater in K than Su in Grp-3 and Grp-4 but less than SU in Grp-1 and Grp-2. We conclude that CO and SV decrease in all positions in later pregnancy which cannot be solely explained by a reduction in venous return.

32.4

CARDIAC OUTPUT IN HEALTHY PRETERM AND TERM NEWBORN INFANTS. M. Eldridge*, D. Alverson*, J. Johnson*, R. Burstein*, L. Papile*, T. Dillon*, S. Yabek*, W. Berman, Jr.* (SPON: J.A. Loeppky). University of New Mexico, Department of Pediatrics and Lovelace Medical Foundation, Albuquerque, NM 87131

A 5MHz pulsed Doppler velocity meter was used to measure mean ascending aortic blood flow velocity (v) in 8 preterm (mean birth wt. 1718gms; mean estimated gestational age 33.3 wks) and 14 term (mean birth wt. 3127gms; mean estimated gestational age 39.8 wks) healthy infants under one week of age. Measurements were made from a suprasternal approach. The internal ascending aortic diameter was determined echocardiographically and aortic cross-sectional area (A) was calculated. Ascending aortic blood flow (Q) was then computed as $Q(ml/min)=v(cm/sec) \times A(cm^2) \times 60(sec/min)$. No infant showed clinical evidence of patent ductus arteriosus or intracardiac defects. Flow determinations were normalized to body weight. The 8 preterm infants had a mean $\dot{Q}$ of 221±56 ml/kg/min. The 14 term infants had a similar mean Q of 236±47 ml/kg/min. The mean Q of all 22 infants was 230±50 ml/kg/min. This study establishes normal values for cardiac output in the first week of life. These values are similar to previously reported systemic blood flows determined by cardiac catheterization and thermodilution methods in newborn infants.

32.5

THE EFFECT OF THE PATENT DUCTUS ARTERIOSUS ON LEFT VENTRICULAR OUTPUT IN PREMATURE INFANTS. D. Alverson*, M. Eldridge*, J. Johnson*, R. Burstein*, L. Papile*, T. Dillon*, S. Yabek*, W. Berman, Jr.* (SPON: J.A. Loeppky). University of New Mexico, Department of Pediatrics, and Lovelace Medical Foundation, Albuquerque, NM 87131

We used a 5 MHz pulsed Doppler velocity meter to measure mean ascending aortic blood flow velocity (v) noninvasively. Studies were performed from a suprasternal approach in 18 preterm infants with patent ductus arteriosus (PDA). Measurements were made serially in each patient before and after medical or surgical closure of the PDA. The internal ascending aortic diameter was determined echocardiographically and aortic cross-sectional area (A) calculated. Ascending aortic blood flow (Q) was computed as $Q(ml/min) = v(cm/sec) \times A(cm^2) \times 60(sec/min)$. Flow determinations were normalized to body weight. Prior to PDA closure, Q averaged 343 ml/kg/min, well above predicted normal values. After PDA closure, Q fell to 252 ml/kg/min, significantly lower than the preclosure level ($p<.001$), but slightly higher than the mean cardiac output of healthy term infants (236 ml/kg/min). This study demonstrates the effect of left to right ductal shunting on left ventricular output and emphasizes the demands placed on the neonatal left ventricle by a PDA.

32.6

FILLING AND ARTERIAL PRESSURES AS DETERMINANTS OF FETAL LEFT VENTRICULAR STROKE VOLUME. K.L. Thornburg, M.J. Morton*, D.F. Anderson, and J.J. Faber, Oregon Health Sci Univ, Portland, Oregon 97201.

Neonatal left ventricular (LV) stroke volume (SV) increases dramatically at birth despite increased arterial pressure. We investigated chronically instrumented near-term fetal lambs in order to determine the separate effects of filling and arterial pressure on LVSV. Nine lambs received ascending aortic electromagnetic flow sensors and vascular catheters. Function curves relating LVSV to mean left atrial pressure (LAP) were generated during rapid withdrawal and reinfusion of blood at normal and pharmacologically elevated and reduced arterial pressure (AP). Control blood values were pH 7.38 ±.02 (mean ± SD), P_{CO_2} 45.4 ± 2.2 mmHg, P_{O_2} 19.2 ± 0.9 mmHg, Hct 34 ± 2.8%. Hemodynamic values were: AP 46 ± 7 mmHg, HR 172 ± 11 min^{-1}, LAP 3 ± 1 mmHg, QLV 260 ± 131 ml min^{-1} kg^{-1}, SV 1.5 ± 0.8 ml·kg^{-1} and were unchanged by cardiac autonomic blockade. LVSV fell sharply with reductions of LAP below control, but rose to 1.7 ± 0.8 ml·kg^{-1} ($P < .01$) at elevated LAP (8.3 ± 1.9 mmHg). Increased arterial pressure (76 ± 8 mmHg) did not reduce maximal LVSV. It appears that 1) fetal LV preload reserve is small but may be important in the transition period, 2) acute increases in fetal arterial pressure similar to those at birth do not decrease maximal LVSV, 3) increased LV contractility at birth is necessary to raise LVSV to postnatal levels. Supported by NICHD Grant # 10034.

32.7

TWO-DIMENSIONAL ECHOCARDIOGRAPHIC MEASUREMENT OF LEFT VENTRICULAR MASS IN THE CONSCIOUS CANINE: ACCURACY AND REPRODUCIBILITY L. E. Ginzton*, P. Delaney*, G. Adomian*, D. Garner*, M. Laks Harbor-UCLA Medical Center, Torrance, Calif. 90509

Two-dimensional echocardiography (2D-echo) is reported to measure left ventricular (LV) weight in dogs, but the accuracy and reproducibility in conscious dogs has not been elucidated. Fifteen conscious random source dogs (weight 18-36 kg) had 2D-echo on 2 days using a rotating head mechanical scanner. Long and short axis views were obtained from the right and left sides. Measurements of LV mass were made at end-diastole (ED) and end-systole (ES) according to previously reported formulae: area-length prolate ellipsoid (AL), truncated ellipsoid (TE), and single-plane Simpson's rule. Comparison was made with autopsy LV weight (range 41-183 gms). The best correlation was obtained using Simpson's rule algorithm of ED from the left side ($r=0.89$, $p<0.001$). The AL and the TE formulae correlated poorly with autopsy LV weight ($r= -.43$ to $+.50$, $p>.1$). The ED Simpson's rule measurement of LV mass on different days were reproducible ($r=0.99$, $p<0.001$, SEE= 7.0 gms).

Conclusions: 1) Measurement of LV mass by 2D-echo correlates significantly with autopsy LV weight. 2) LV mass by the single-plane Simpson's rule algorithm at ED from the left side correlated most highly with autopsy LV weight. 3) The geometry of the dog LV muscle mass is not represented by an ellipsoid model. 4) Measurement of LV mass by 2D-echo is highly reproducible, and therefore is a valuable technique for noninvasively assessing the development of changes in LV mass.

32.8

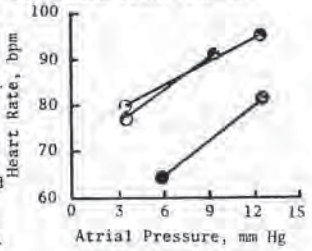
THE RELATIONSHIP OF MYOCARDIAL FIBER ANGLE TO LV SEGMENT SHORTENING. G. FREEMAN, J.W. COVELL, M. LeWINTER, AND R. ENGLER. VAMC AND UC, SAN DIEGO, CA.

Since it is commonly felt that shortening of a segment of the ventricular wall is maximal in the direction of the local fibers, ultrasonic dimension gauges (UDG's) are generally implanted in that direction. To examine the relationship between myocardial fiber angle (MFA) and segment shortening, we placed 3 UDG's in 8 open chest, anesthetized mongrel dogs, one in line with predicted MFA (in line UDG) and the others out of line with MFA. An external long axis (ELA) from the bifurcation of the L coronary artery to the apex was used as a reference line. UDG position and the relationship between the ELA and internal Z axis (IZA) was determined by post mortem X-rays. The MFA was defined by histology and the relative angle between the intragauge axis and MFA determined. The ELA was a good predictor of the IZA being within 6.5° of it (avg 3.3°) in the frontal plane. The in line UDG's were from .5° to 41.5° (avg 14.3°) off the MFA. If the relative angle between the intragauge axis and the MFA was > 30°, the measured shortening was significantly reduced: 7.2±1.6% compared to 11.8±3.0%, $p < .05$. We conclude 1) the ELA provides a good reference for prediction of MFA, 2) UDG's can be placed within 20° of MFA using this external axis, and 3) measured midwall shortening will be reduced in UDG's which are off the MFA by > 30°.

32.9

NON-REFLEX EFFECTS OF RIGHT ATRIAL PRESSURE CHANGES ON HEART RATE. A.M. Scher and T.D. Bennett. Dept. of Physiology and Biophysics/SJ-40, University of Washington, Seattle, WA 98195.

In 1915, Bainbridge described the tachycardia which follows intravenous infusion of saline. Bainbridge considered the response neural since it was abolished by vagotomy. Many studies have verified Bainbridge's observation, but the afferent and efferent nerves necessary for the effect have not been clearly delineated. A similar tachycardia has been recorded in isolated atria and isolated hearts. This has led to the hypothesis that the tachycardia might be due to stretch of pacemaker cells. To test this, we studied anesthetized animals with both vagi sectioned and carotid sinuses distended to a constant size with balloons. These animals have no functional arterial or cardiopulmonary baroreceptors. The distal right vagi were stimulated to lower heart rate to 50-90 bpm. Right atrial pressure was servo-controlled by a pump and was changed in steps. Changes in atrial pressure produced significant changes in heart rate (1-2.5 bpm/mmHg venous pressure). We agree with others that the response to changes in venous and atrial pressure may well be partly non-reflex. (Supported by NIH/NHLBI Grant HL16910-08).



32.10

EFFECTS OF CHRONIC CARDIAC ENLARGEMENT ON THE PERICARDIUM, M. LeWINTER and G. FREEMAN, VAMC AND UC, SAN DIEGO, CA.

In order to examine the response of the pericardium to chronic cardiac enlargement, we studied 7 normal dogs (nls) and 9 dogs with chronic volume overload (CVO) resulting from a systemic AV fistula. The pressure-volume relation (PVR) of the pericardium was determined after KCL arrest and with the cardiac chambers empty. The pericardial surface area (SA) and mass were then determined, as was total heart (TH), LV and RV mass; these values were all normalized to pre-operative body weight (bw). CVO dogs had larger TH/bw ratios (8.8±1.2 vs 6.4±.6 g/kg bw, $p<.0001$), with biventricular enlargement. The pericardial SA was larger (7.2±.9 vs 5.7±.5 cm^2/kg bw, $p=.003$), as was pericardial mass (.23±.05 vs .17±.05 gm/kg bw, $p=.054$). The PVR in CVO dogs was shifted to the right: at a pressure of 2 mmHg the volume was 12.3±.5 ml/kg bw compared to 8.2±1.6 ml/kg bw in nls, $p=.006$. Analysis of the slope of the PVR by linear fit was done in the pressure ranges of 1-6 mmHg and > 8 mmHg (r values > .91 in all cases). The slope was less in CVO dogs in both ranges: 0.23±.004 vs .041±.006 mmHg/ml for 1-6 mmHg, $p=.007$, and .117±.060 vs .238±.007 mmHg/ml for > 8 mmHg, $p=.03$. We conclude that specific pericardial adaptations to CVO occur, including pericardial enlargement as evidenced by increased surface area and mass, as well as increased pericardial compliance in the pressure ranges from 1-6 and > 8 mmHg.

32.11

EFFECTS OF VERAPAMIL ON STRESS VENTRICULAR FUNCTION IN CONSCIOUS DOGS. W. French*, D. Garner*, W. Averill* and Michael Laks. Harbor-UCLA Medical Center, Torrance, California 90509

Left ventricular ejection fraction (LVEF) is the standard measurement of LV function (LVF). However, resting LVEF may not be sensitive in detecting early LV failure or in assessing reserve LVF. The stress ventricular function test (SVF) relating changes in LV end diastolic pressure (LVEDP) to changes in aortic pressure (AP) is a valid index of LV contractility. The purpose of this study is to determine the amount of verapamil (V) needed to depress LVF using the SVF test. Five conscious dogs with right atrial, LV and aortic catheters were studied. SVF involves an infusion of methoxamine (M) at .33 µg/ml and .67 µg/ml for 10 minute periods while recording pressures every 3 minutes on 3 separate days. A slope (S) relating AP to LVEDP was derived using linear regression analysis. On different days a loading dose of .1 mg/kg of V was given for 2 minutes followed by an infusion of .005 mg/kg/min for 10 minutes and SVF repeated. The dose of V was doubled if the SVF slope was not depressed during the initial infusion. During M the maximum systolic AP was 204±17 mmHg* and with V was 164±18 mm Hg (P=NS). The max LVEDP with M was 26±3 mmHg and during V was 23±3 mmHg (P=NS). The mean serum level of V chronically was 380 µg/ml. Summary: 1) SVF is a sensitive, reproducible method to determine depressed LVF as well as to assess reserve myocardial contractility in conscious dogs. 2) V produced depressed SVF in consious dogs at similar therapeutic levels as used in humans. *$\bar{x}$ ± SD (Supported by AHA Grant MG-3765)

32.12

A COMPARISON OF VARIOUS TREATMENTS OF CYANIDE POISONING USING THE ISOLATED PERFUSED DOG HEART. H.L. Froehlich*, J.A. Vick*, E. Herman*, and S. Sarnoff*, (SPON: E.G. Cummings). F.D.A. Labs, Washington, D.C. 20012

Acute cyanide poisoning continues to be of interest to both the industrial and military community. Previous studies have shown that in addition to the disruption of the cytochrome oxidase system, cyanide appears to have a direct effect on the myocardium. A series of isolated perfused Langendorff heart preparations were used to study the effect of Amyl nitrite, Sodium nitrite, Dimethylaminophenol HCl (DMAP) and Hydroxylamine HCl in reversing the cardiac effects of cyanide. Heart rate, EKG, force of contraction and coronary perfusion pressure were continuously recorded. Within 3-5 minutes after cyanide, 10mg, there was a dramatic decrease in force and rate with but a slight decrease in pressure. All 10 cyanide control hearts failed at from 5-15 minutes. Treatment, when given, was administered when force decreased to approximately 50% of control. Amyl nitrite restored force and rate to control levels within 10 minutes. Sodium nitrite and DMAP were less effective. Hydroxylamine HCl, 10mg, produced an almost immediate return of force and rate to control levels following cyanide. High doses of Hydroxylamine (20-40mg) reversed the toxic effects of cyanide however the hearts failed within 45 minutes. It appeared that the failure was due to methemoglobinemia.

PERIPHERAL CIRCULATION I

33.1

FUNCTIONAL EVALUATION OF THE PALMAR ARCH CIRCULATION. A. Fronek and D. King*. Dept. of Surgery and Bioengineering, University of California, San Diego, La Jolla, CA. 92093.

A functional evaluation of the patency of the palmar arch is essential in many cases of impaired digital blood supply. We developed a quantitative examination which is based on the well known Allen test in which disappearance of blanching of the palm is compared after compression of the radial and ulnar artery respectively. This test, however, identifies only the respective participation of these two arteries and in addition is highly subjective. In our study based on examination of 15 normal male and female subjects, we recorded the digital pulse volume from the 1st,3rd and 5th finger during alternative compression of the radial and ulnar artery. The pulse volume was sensed with a photoplethysmographic and strain gauge sensor and its output amplified by an A-C amplifier with a low frequency cut-off point of 1 Hz. The results are as follows: compression of the ulnar artery leads to reduction of pulse volume oscillations by 27%± in the 1 digit while the reduction in oscillations in the 3 and 5 digits are: 25 and 25% respectively. The compression of the radial artery yielded the following reductions: 1st = 24%, 3rd = 29% and 5th = 21%. Preliminary tests, especially when combined with finger pressure and radial and ulnar artery flow velocity determination, indicate that this test can help to differentiate flow obstructions of the main supply arteries from those in the palmar arch. This may significantly influence the therapeutic decision making.
(Supported by NIH Grant HL-18977)

33.2

HYSTERESIS AND ACUTE RESETTING IN ATRIAL RECEPTORS IN THE DOG. John R. Ledsome and Gerald McFetridge*. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Stimulus-response curves for arterial baroreceptors demonstrate hysteresis which probably depends upon rapid resetting (Coleridge et al., Circ. Res. 48: 676). Hysteresis has also been reported for stimulus-response curves for atrial receptors but the time course of any resetting is not clear. In chloralose anesthetized dogs, type B or intermediate type atrial receptor activity was related to mean atrial pressure and peak V-wave pressure. Atrial pressure was changed by rapid infusion or withdrawal of a dextran-blood mixture. Continuous infusion and withdrawal at a rate of 20% of blood volume/min was associated with clear hysteresis of the stimulus response curves. Stepwise infusion and withdrawal with recording at 3 min after each step was not associated with hysteresis. Rapidly increasing or decreasing atrial pressure by 10 cm H_2O caused respectively, an overshoot or undershoot of activity with adaptation to a new steady value in 60-120 sec. This behaviour is similar to that described for aortic baroreceptors even though the atrial receptors lie in the wall of an actively contracting chamber. (Supported by B.C. Heart Foundation and MRC.)

33.3

THE EFFECT OF AORTIC CHEMORECEPTOR DENERVATION DURING ANEMIA. P.C. Szlyk, C. King, D.B. Jennings, S.M. Cain and C.K. Chapler. Department of Physiology, Queen's University, Kingston, Ontario K7L 3N6 and University of Alabama in Birmingham, 35294.

The role of aortic chemoreceptors in the circulatory and metabolic responses occurring during acute anemia were studied in anesthetized dogs. Data were obtained from 6 dogs in which the aortic chemoreceptors were surgically denervated and from 7 sham operated dogs. Values for cardiac output (Q_T), limb blood flow (Q_L), limb and whole body oxygen uptake ($\dot{V}_{O_2}$) were obtained at a normal hematocrit (Hct = 48%) and at 30 min of anemia (Hct = 13%) induced by an isovolemic dextran-for-blood exchange. During anemia, Q_T increased in both the sham operated (109%, $p \leq 0.01$) and the aortic denervated dogs (42%, $p \leq 0.05$). Stroke volume (SV) also rose in both the sham (94%, $p \leq 0.01$) and denervated groups (32%, $p \leq 0.05$). The changes in Q_T and SV were significantly less in the aortic denervated animals compared to the sham operated dogs. Arterial blood pressure fell to comparable levels in both groups during anemia. Similar increases in Q_L and reductions in limb resistance were observed during anemia in the two groups while limb $\dot{V}_{O_2}$ was unchanged. These results show that the aortic chemoreceptors contribute to the cardiac output response during acute anemia but are not involved in the regulation of hindlimb blood flow.

(Supported by the OHF and MRC of Canada).

33.4

THE ROLE OF AORTIC CHEMORECEPTORS DURING CO HYPOXIA. C.E. King*, S.M. Cain and C.K. Chapler. Depts of Physiology, Queen's University, Kingston, Ont K7L 3N6 and Univ of Alabama in Birmingham, 35294.

The importance of aortic chemoreceptors in the circulatory and metabolic responses occurring during carbon monoxide (CO) hypoxia were studied in the whole body and hindlimb in spontaneously breathing anesthetized dogs. Control data were obtained and then CO hypoxia was induced using in situ dialysis which reduced arterial O_2 content about 65%. Measurements were obtained at 30 and 60 min of CO hypoxia. Cardiac output (Q_T), hindlimb blood flow (Q_L), total body and hindlimb O_2 uptake ($\dot{V}_{O_2}$) were determined. In one group of animals (n=9), the aortic chemoreceptors remained intact while in a second group (n=6) the chemoreceptors were denervated. The data for the whole body at 30 and 60 min of CO hypoxia were similar in the two groups and included (1) a rise in $\dot{Q}_T$ of about 70% ($p \leq 0.01$) (2) decreases in both mean arterial pressure ($p \leq 0.01$) and total peripheral resistance ($p \leq 0.01$) and (3) $\dot{V}_{O_2}$ was maintained at the control values. Hindlimb $\dot{V}_{O_2}$ decreased ($p \leq 0.05$) during CO hypoxia in the denervated group and was less ($p \leq 0.05$) than that in the intact group at 30 min. Hindlimb resistance was decreased at 30 and 60 min in both groups and was significantly less in the denervated group at 60 min. The data indicate that aortic chemoreceptors are necessary for maintenance of $\dot{V}_{O_2}$ by the hindlimb during severe CO hypoxia. (Supported by MRC of Canada).

33.5

EFFECTS OF SYMPATHETIC NERVES ON CEREBRAL BLOOD FLOW DURING HYPOTENSION AND HYPERCAPNIA IN AWAKE RABBITS. D.W. Busija. Dept. of Anesthesiology/Critical Care Medicine, The Johns Hopkins University, Baltimore, MD 21205

Although sympathetic nerves play an important role in attenuating increases in cerebral blood flow (CBF) during hypertension, it is not clear whether sympathetic nerves have important effects during hypotension. The purpose of this study was to examine neural effects on CBF during hypotension and hypercapnia in awake rabbits. Hypercapnia results in profound dilatation of cerebral resistance vessels and may unmask responses to sympathetic nerves. Sympathetic nerves supplying cerebral vessels were interrupted on one side in 7 rabbits. CBF was measured with 15 μ microspheres. During hypercapnia (PCO_2 > 60 mmHg) CBF was measured during normotension (87 ± 3 mmHg), moderate hemorrhagic hypotension (64 ± 2 mmHg) and severe hemorrhagic hypotension (42 ± 3 mmHg). During normotension, CBF was not different between the denervated and intact sides (220 ± 33 and 208 ± 27 ml/min per 100 g, respectively). However, when arterial pressure was decreased by hemorrhage, CBF was lower on the intact side. During moderate hypotension, CBF was 166 ± 21 and 148 ± 18 ml/min per 100 g on the denervated side and intact sides, respectively ($p < 0.05$), and during severe hypotension CBF was 121 ± 13 on the denervated side and 104 ± 11 ml/min per 100 g on the stimulated side ($p < 0.05$). Thus, in awake rabbits during hypercapnia, reflex activation of sympathetic nerves during hemorrhagic hypotension reduces CBF.

33.6

POSSIBLE COEXISTENCE OF ACETYLCHOLINE AND VASOACTIVE INTESTINAL POLYPEPTIDE IN VASODILATOR NERVES TO CAT CRANIAL ARTERIES. Georgette M. Buga*, Ian L. Gibbins*, Sami I. Said and John A. Bevan. Dept of Pharmacology, Univ. of California School of Medicine, Los Angeles, CA 90024 and Dept of Medicine, Univ. of Oklahoma, Oklahoma City, OK 73190.

Previous studies (Bevan et al. (1982) Circ. Res. 50:470-476) have provided evidence that the atropine-sensitive component of neurodilation observed in cat cerebral and certain extracerebral cranial arteries is due to the release of acetylcholine. The following observations suggest a role for vasoactive intestinal polypeptide (VIP) - a potent vasodilator in the same group of vessels, in causing the atropine insensitive component of vasodilation. (1) VIP containing neurones are found at the adventitio-medial junction of these vessels. (2) VIP is present only in the walls of arteries that show atropine insensitive neurogenic vasodilation. (3) VIP is released from nerves within the artery wall by electrical field stimulation. (4) An antibody to VIP preferentially reduces the dilation to nerve stimulation and VIP in comparison to that to acetylcholine and papaverine. All neurodilator effects in these vessels are abolished by tunica intima destruction. Our findings suggest that acetylcholine and VIP are released from neurones in the wall of certain cranial arteries to cause vasodilation via the tunica intima. (Supported by USPHS grants HL 15805 and 20581).

33.7

DIFFERENCES IN THE CEREBROVASCULAR RESPONSE TO K^+ AND OUABAIN IN DOGS ANESTHETIZED WITH CHLORALOSE AND URETHANE OR PENTOBARBITAL. J.T. O'Neill, L.M. Schwartz*, and F.J. Haddy. Uniformed Services University, Dept. of Physiology, Bethesda, MD 20814

In previous studies on chloralose and urethane (CU) anesthetized vagotomized dogs (Fed.Proc. 40:455,1981) we showed that intracarotid injections of potassium (K^+) raised systemic arterial pressure (SAP) but did not change cerebral vascular resistance (CVR) either before or during ouabain (OU) infusions. Our current studies attempt to examine the roles of anesthesia and the vagus in the cerebral vascular response to K^+ and OU. Cerebral venous outflow (CF) was measured with the Rapela-Green technique in 5 pentobarbital (PB) anesthetized dogs with vagi intact. Common carotid blood flow was measured with a perivascular electromagnetic flow probe. SAP was also measured. K^+ and OU were given via catheters placed into the carotids by retrograde cannulation of the thyroid arteries. Isosmotic KCl was injected (0.5, 1, 2, and 3 ml) into each carotid before and during OU infusion (7.6μg/min/carotid). In contrast to the K^+ response in CU dogs, K^+ did not increase SAP in PB dogs. However, K^+ did cause a small increase in CF (5, 7, 10 and 13%) due to a small but consistant decrease in CVR. CVR was elevated during OU infusion but was not altered by K^+ injections. These data indicate that the canine cerebral vasculature may be more sensitive to K^+ under PB than CU anesthesia or that the vagus is necessary to elicit K^+ cerebral vasodilation. In either case, the K^+ cerebral vasodilation may involve the membrane Na-K pump.

33.8

EFFECTS OF THEOPHYLLINE (T) ON CEREBRAL BLOOD FLOW (CBF). H. R. Winn, S. Morii*, A. C. Ngai*, and R. M. Berne. Depts. of Neurosurg. & Physiol., Univ. of Va., Charlottesville, Va.

We treated rats with theophylline (0.2 μmol/g, IP) to determine if this adenosine receptor blocker would alter CBF during hypoxia. Adult rats (300-420 gms) were initially anesthetized with halothane, tracheostomized, and mechanically ventilated to maintain their PaO_2 > 100 mmHg and their $PaCO_2$ between 30 and 40 mmHg. Blood pressure and rectal temperature were continuously monitored. Transient hypoxia (30 sec) was induced by switching the animals' tracheostomy tube to a second respirator containing almost 100% nitrogen. CBF was measured by the retroglenoid venous outflow technique. CBF increased 160% in the untreated animals during hypoxia, but only 75% ($p < 0.01$) in the T-treated animals. A similar response in CBF was noted in animals treated with 0.05 μmol/g (IP) of T. T levels in CSF and blood were between 10^{-5} and 10^{-4}M. No changes in brain C-AMP were observed with T treatment prior to hypoxia. Moreover, no attenuation in response of CBF to hypercarbia was observed with T. The significant attenuation of the increase in cerebral blood flow during transient hypoxia in the theophylline treated animals supports the hypothesis that adenosine is a critical factor in regulation of cerebral blood flow.

33.9

DIPYRIDAMOLE POTENTIATION OF EXTRACELLULAR ADENOSINE IN ISCHEMIC DOG GRACILIS MUSCLE. Richard E. Klabunde, West Virginia Univ., Med. Ctr., Morgantown, WV 26506.

Dipyridamole (DIPY), an inhibitor of cellular transport of adenosine (ADO), has been shown to potentiate the duration of postischemic vasodilation (PIVD) following 5 min, but not shorter periods of ischemia in dog gracilis muscles (Microvasc. Res. 20:115, 1980). The purpose of this study was to determine if the hemodynamic effects of DIPY are associated with an elevation of whole tissue and extracellular ADO concentrations. Constant-flow, dog gracilis muscles were treated with intra-arterial infusions of saline, DIPY (3 nmol/ml blood), or DIPY plus adenosine deaminase (ADA, 3 U/ml blood). In each muscle, a non-ischemic tissue sample was obtained, followed by additional samples at 1, 3, and 5 min of ischemia. Samples were assayed for ADO content by high pressure liquid chromatography. Saline-treated muscles showed a 2-fold increase in whole tissue ADO content after 5 min of ischemia. DIPY potentiated ADO accumulation 2 to 3-fold at 1, 3, and 5 min of ischemia compared to the saline-treated group. Infusion of ADA + DIPY completely abolished the elevation of tissue ADO brought about by DIPY. Since the infused ADA is confined to the extracellular space, the results indicate that DIPY potentiates extracellular ADO. Furthermore, this increase in extracellular ADO is not necessarily associated with increased PIVD duration. This suggests that ADO is not an important factor regulating PIVD. (Supported by NHLBI grant #HL25412; and Grant-in-Aid from West Virginia Affiliate, AHA.)

33.10

FAILURE OF THEOPHYLLINE TO BLOCK THE EFFECTS OF ADENOSINE ON ADIPOSE TISSUE. Sharon E. Martin* and Emma L. Bockman. Dept. of Physiol., Uniformed Services Univ., 4301 Jones Bridge Rd., Bethesda, MD 20814

To study the role of adenosine (ADO) in the local control of adipose tissue blood flow, an ADO antagonist, theophylline (TH), was used. Inguinal fat pads of female dogs were isolated, denervated, and perfused under free flow conditions. TH (10mg/kg; N=5) or saline (control; N=6) was given intravenously. Before TH or saline was given, blood flow (basal Q) was 6.4±0.6 and 8.3±1.5 ml/min/100g in TH and saline groups, respectively. Close i.a. boluses of 10^{-6} moles of ADO increased blood flow by 144±14% in TH and 143±17% in saline groups. Thirty min after TH or saline, ADO increased blood flow by 102±18% and 44±6%, respectively, when compared with basal Q. However, when compared with the blood flow immediately preceding the bolus, ADO increased blood flow by 161±50% and 149±34% in TH and saline-treated groups, respectively. Sixty min after drug administration, blood flow had decreased by about 35% in both TH and saline-treated groups. TH appeared to increase basal lipolysis since arterial free fatty acid concentrations were increased significantly by TH (38±14%) but not by saline. However, the presence of TH appeared to have no effect on the vascular or metabolic response of adipose tissue to norepinephrine (1µg/kg/min, i.v.). These data indicate that TH has no effect on basal blood flow nor on the vascular effect of exogenous ADO in adipose tissue. Supported by USUHS Grant R07639.

33.11

EFFECT OF THEOPHYLLINE (THEO) ON RENAL VASOACTIVITY OF ACETATE (AC) AND ADENOSINE (ADO). Robert P. Steffen, John T. O'Neill, and Francis J. Haddy. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814.

We have proposed that AC participates in skeletal muscle exercise hyperemia (Pflugers Arch 392:315, 1982) and that the vasoactivity of AC may be due, in part, to ADO (Fed. Proc. 41:1681, 1982). The purpose of this study was to further examine the role of ADO in AC's vasoactivity. Since ADO causes renal vasoconstriction, we used the kidney to characterize the vasoactivity of AC and ADO before and during THEO treatment. In 6 anesthetized dogs, the left renal artery was exposed and an electromagnetic flow probe placed around the renal artery near the aorta. Arterial pressure, blood gases, and pH were monitored. Isosmotic solutions of AC (9 mg/ml), ADO (1 µg/ml) and THEO (1.8 µg/ml) were given via a 22g Angiocath into the renal artery (natural flow) distal to the flow probe. AC and ADO were administered as bolus doses and THEO was infused at a rate that did not affect renal vascular resistance. During THEO, ADO's threshold vasoconstrictor dose was increased from 0.25 to 1.5 ml ($P<0.05$). The renal vascular response to AC was biphasic, first constriction and then dilation. THEO increased the threshold AC vasoconstrictor dose from 3 to 6 ml ($P<0.05$). During THEO, ADO and AC constrictor response curves were shifted to the right. The AC vasodilator response (threshold 1 ml) was unaffected by THEO. These data support the suggestion that the vasoactivity of AC is, in part, due to ADO but a second mechanism of AC vasodilation exists.

PERCEPTION OF GRAVITATIONAL STIMULI IN ANIMALS

34.1

STRIATED ORGANELLES IN HAIR CELLS OF RAT INNER EAR MACULAS: DESCRIPTION AND IMPLICATIONS FOR TRANSDUCTION. Muriel D. Ross, Ph.D., Univ. of Michigan, Ann Arbor, Mi. 48109

Current theories of transduction in hair cells are based largely upon concepts that include stereociliary deformation with current flow proceeding from the stereociliary tips into the cell and out its basolateral membranes. In recent research we used low concentrations of glutaraldehyde as a primary fixative for inner ear tissue in preparation for possible collection of such tissue during space flight. This revealed that the cuticular plates of both types of hair cells were striated, with much of the apparently contractile organelle oriented vertically; and that an elaborate striated neck apparatus existed in Type I hair cells but was virtually lacking in Type II hair cells. Additionally, the cuticular plate of Type I hair cells appeared to function as a prominent microtubular assembly region. These results suggest that the transductory process in hair cells is more complicated than expected and also more intricate in Type I hair cells. Both kinds of striated substances appeared to be linked to the plasma membrane, and the striated neck apparatus lay directly beneath the upper end of the calyx nerve ending. Contraction or tension in these organelles may regulate cell permeability while concurrent streaming of substances distally along microtubules could affect activities of more distal parts of Type I hair cells. Ca^{++} transients and calmodulin are implicated in regulation of the actin assemblies discussed here. (Supported in part by NASA NSG 9047 and by NASA NAS2-10535)

34.2

TIMING OF NEURON DEVELOPMENT IN THE RODENT VESTIBULAR SYSTEM. J.Richard Keefe .Case Western Reserve Univ.,Cleveland,OH 44106

The development of the vestibular and proprioceptive systems involves cell birth, cellular migration and differentiation of both primary and central integrative pathways. Since any of these processes may be adversely impacted by exposure to spaceflight, we are determining the timing of cell birth (H^3-thymidine), embryonic cell death and onset of differentiated function (H^3-2-deoxyglucose) in both systems in Wistar-SPF rats, C57Bl/6j and Pallid mutant mice under quiescent, rotated and centrifuged states. The present report will present data on cell birth in the peripheral vestibular apparatus and vestibular nuclei in normal Wistar rats. Thymidine was administered to pregnant rats on days E8 thru birth and sacrificed after 3 hours or at 30D postnatal. Serial sections of the head were dipped in Kodak NTB-2, processed and stained with either modified Protargol or H+E. Sections were quantified utilizing computer-aided analyses of section tracings and labelled neurons. The first neurons to undergo terminal mitosis in the peripheral vestibular system are in the utricular macula on day E10, followed closely by cells in the macula of the saccule. First cells in the cristae of the semicircular canals were in the superior canal on day E12, with all cristae showing first cells by day E13. Cells of the lateral vestibular nucleus (Dieters) were also first established on day E10, with inferior (day E11), superior (day E12) and medial (day E12) nuclei demonstrating similarity to the pattern of peripheral afferent development. (Supported by NASA Grant NAGW-83.)

34.3

OCULOMOTORIC RESPONSE TO VOLUNTARY HEAD ROTATIONS DURING PARABOLIC FLIGHTS. Søren Vesterhauge, Arne Månsson†, Torben Stæhr Johansen† and Kaj Zilstorff†. Rigshospitalet and the Royal Danish Air Force, Denmark.

8 voluntary subjects were exposed to zero-G environment during parabolic flights and to two-G conditions during the same flights. The oculomotoric response to voluntary horizontal head rotations either at 0.4 Hz or with a random frequency pattern between 0.2 and 0.8 Hz was measured and computed as the transfer function between the head rotations and the eye movements. The responses varied proportionally to the G-forces, though the variations were small. The gain of the responses and the phase lag decreased during zero-G and increased during two-G periods. Furthermore, the quality of the responses was considerably poorer at the two abnormal G-levels compared to the ground experiments. Both factors might be of importance for the development of space sickness, giving rise to unsteady and unprecise eye movement responses to head rotations during unusual G-environments.

34.4

RELATION BETWEEN PHYSIOLOGICAL EFFECTS OF GRAVITATIONAL FORCES AND THAT OF MAGNETIC FORCES—III. H. Saiki, Y. Saiki*, M. Sudoh*, M. Nakaya*, M. Abe*, M. Kohno* and K. Shioda*. Space Medicine Lab., The Jikei Univ. Tokyo, 105, Japan, and The Saiki Institute of Nutrition, Tokyo, 143, Japan.

We had reported about experimental results in comparison of responses of many physiological functions of mice and rats during and after low magnetic field (LM) exposure, under simulated hypogravic conditions (LG), to those of geomagnetic field exposure(NM), under normodynamic terrestrial life (NG). And, the results of the same experiment by high magnetic fields (HM) which is several hundreds times as much as the geomagnetic field (NM), in its intensity level, under the same LG, was also reported. In this report, using mice and rats as the subjects, high and low magnetic field (HM and LM) exposure experiments under normodynamic terrestrial life (NG) and active or passive hyperdynamic life (HG) will be reported. For the experimental parameters, wound healing index, swimming capacity, and tolerability of erythrocyte-membrane to bacterial toxin, for mice, urinary excretion rate of such minerals as K^+, Na^+ and Ca^{2+}, resting metabolic rate, and blood pressure at v. coccygea and v. cava anteriol-posteriol for rats were examined. Summarizing the findings, we were able to note the following characteristics. LM effects to attenuate the biological reactivity of the animal subjects induced by orthostatic hypodynamics. And HM is opposite in general functions. The findings related to the basic functions as aging etc. will be also discussed.

34.5

REVERSAL OF EARLY PATTERN FORMATION IN INVERTED AMPHIBIAN EGGS. George M. Malacinski. Dept. Biology, Indiana University, Bloomington, IN 47405

Newly fertilized anuran (Xenopus) and urodele (Ambystoma) eggs were inverted prior to the first cleavage division. The animal/vegetal cleavage pattern was completely reversed, as was the site of involution at gastrulation. Histological analyses were performed to determine the extent to which the distribution of cytoplasmic components such as yolk platelets was altered in inverted eggs. In Xenopus eggs the yolk platelet distribution pattern was substantially altered. The larger yolk platelets moved to the hemisphere which faced gravity. In inverted Ambystoma eggs the yolk platelet pattern was completely reversed. Prior to the formation of the first cleavage furrow the location of the egg nucleus was also monitored. It was discovered that the position of the zygote nucleus shifted to correspond to the egg's novel orientation to gravity.

By employing embryological grafting experiments it was possible to determine the extent to which developmental programs were altered in inverted eggs. All inverted eggs displayed a reversal of the early developmental pattern (cleavage division and gastrulation). Yet none developed beyond the involution stage. Tissue grafts demonstrated, however, that the pattern of developmental competence was reversed in inverted eggs. (Supported in part by NASA NAGW-60.)

34.6

AXIS SPECIFICATION AND TWINNING BY GRAVITATIONAL AND CENTRIFUGAL FORCE IN AMPHIBIAN EMBRYOS. Steven D. Black. Dept. of Molecular Biology, U. C. Berkeley 94720.

The position of the dorsal-ventral axis in embryos of the frog Xenopus laevis can be predicted 20 minutes after fertilization when a visible sperm entrance point (SEP) forms. The SEP side of the egg becomes the ventral side of the tadpole. I embedded eggs in gelatin so that they were held in place with their animal poles up and their SEP sides oriented in one direction. Eggs turned SEP side up soon after fertilization with their animal-vegetal axes perpendicular to gravity for 20 minutes develop normally, but their dorsal sides form on the SEP-predicted ventral sides--dorsal-ventral polarity is completely reversed. The dorsal structures may be placed on any side of the egg by tipping in the appropriate orientation. Centrifugal force also affects the position of the axis. When centrifuged very soon after fertilization at 10-50 g for 4 minutes, eggs form their dorsal sides centrifugally; at somewhat later times, centrifuged eggs form their dorsal sides centripetally (equivalent to the 1 g result above). There is an abrupt transition between the two sensitivities. When centrifuged at the time of the transition, an average of 60% of the embryos form two conjoined dorsal axes. Twins form at high frequency, and always in the same orientation with respect to centrifugal force, regardless of the orientation of the SEP side of the egg to centrifugal force.

34.7

GRAVITY AND POSITIONAL HOMEOSTASIS OF THE CELL. George W. Nace. Univ. of Michigan, Ann Arbor, MI 48109

Normally bilateralization takes place in the presence of Earth's gravity which produces torque, tension and compression acting upon the naked aggregates of cytoplasm in the zygote which is only stabilized by a weak cytoskeleton. Buoyancy and torque, L=Fℓ sin θ (F=ma, a=g), the only quantity considered here, acting upon a dumbbell shaped cell with heavy, H (storage granules), and light, L (lipid vacuoles), masses ($m=V\rho$, $V_B=V_H>V_L$) at either end and "floating" in a medium, m, yields a net torque expressed as

$$L_n = g\ell \sin\theta \frac{V_H\rho_m}{(V_H\rho_H+V_L\rho_L)}(V_B\rho_H - V_L\rho_L).$$

Using crude values for the variables, torque of 2.5×10^{-13} to 3.2×10^{-3} dyne-cm is found to act upon cells ranging from 6.4 to 1700 μm (frog egg). (Six microtubules can yield 4.9×10^{-9} dyne-cm.) (1) Gravity imparts torque to cells, (2) torque is reduced to zero as gravity approaches zero, and (3) torque is sensitive to cell size and particulate distribution. Cells must expend energy to maintain positional homeostasis against gravity. Although not previously recognized, Skylab 3 results confirmed this hypothesis: tissue cultures used 24% more glucose on Earth than in space. The implications for developmental biology, physiology, genetics, and evolution are considered. At the cellular and tissue level "gravity receptors" are irrelevant. (Supported in part by NSA Grant NAGW-29 and Contract NAS 2-10945)

34.8

GRAVITO-INERTIAL SENSITIVITY OF THE SPIDER;ARANEUS SERICATUS. Alfred Finck *(SPON.T.W.Halstead). Temple Univ. Phila. Pa. 19122.

Spiders employ gravity cues in web construction and body orientation. Reflexes of the neurogenic heart of the spider are employed as the dependent variable for the evaluation of gravity effects on the unrestrained animal. This is accomplished by laser plethysmograph. The absolute threshold of 'g' is on the order of 1.005. The functional relationship between heartrate and 'g' is logarithmic. A presentation time of 10 minutes angular acceleration is sufficient to elicit a stable cardiac reflex. Post-rotary recovery is on the order of presentation time. Tilt stimuli evoke substantial increases in heartrate and amplitude. Recovery during tilt is extended. Spiders do not present a receptor morphologically dedicated for linear or angular acceleration. We have hypothesized that the patellar lyriform organ transduces the gravity stimulus. This structure is exquisitely sensitive to cuticular deformation produced by compression of the exoskeleton. In a "working" model;the lyriform organs communicate changes in the magnitude and direction of 'g' to the CNS pacemaker. The hydraulic system is modulated by the heartpump, thus the legs are adjusted to maintain equilibrium of the spider. (Supported by NASA Grant NAGW-242).

34.9

GRAVITY RECEPTORS IN A MICROCRUSTACEAN WATER FLEA: FUNCTION OF ANTENNAL-SOCKET SETAE IN DAPHNIA MAGNA. Dewey G. Meyers. Biology Dept., Univ. Pennsylvania, Philadelphia, PA 19104

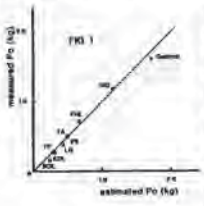
Equilibrium and spatial orientation in the continuously swimming zooplanktors, Daphnia magna, are essential for coordinated locomotion and precise diurnal vertical migration, and are believed to be directed by light cues during the day and gravity at night. Quantitative analysis of video recordings of the swimming paths and antennal beat frequencies of neutrally buoyant D. magna in infra-red illumination (to eliminate visual cues) reveals disorientation and indicates a lack of internal gravity sensing organs, e.g. statocysts, common in higher order crustaceans. These findings suggest that daphnids sense gravity indirectly by the flow of water past their bodies as they passively sink between upward swimming strokes. Gravity receptors were, therefore, postulated as being mechanoreceptive setae on the exoskeleton. Selective removal of paired setae on the basal socket of the swimming antennae resulted in daphnids that swam normally in water with overhead illumination and exhibited disorientation in the dark. These observations suggest that the antennal-socket setae as essential for the indirect detection of gravity through changes in the direction and velocity of water currents. Continuing investigations of this orientation mechanism have revealed details of its structure and sensitivity. (Supported by NASA Grant NAGW-70).

SKELETAL MUSCLE PHYSIOLOGY

35.1

PREDICTABILITY OF SKELETAL MUSCLE TENSION OUTPUT FROM ARCHITECTURAL DETERMINATIONS. P.L. Powell*, R.R. Roy*, P. Kanim*, M. Bello*, V.R. Edgerton. Kinesiology, UCLA,L.A., CA 90024

Recently, Spector et al. (J. Neurophysiol., 1980) reported similar specific tensions (P_{sp}=2.3 kg/cm^2) for cat hindlimb slow and fast muscles. These results suggest that, assuming a constant P_{sp}, the maximum tetanic tension (P_o) of a muscle can be predicted from its architecture (Sacks and Roy, J.Morphol.,1982). Our purpose was to examine if this relationship also exists in a smaller mammalian species, the guinea pig(GP). Muscle weight (MW), fiber length (FL) and mean angle of pinnation (Θ) were determined for 8 GP hindlimb muscles. Cross-sectional area was calculated as follows: CSA=(MW)(COSΘ)/(FL) (specific density). P_o was estimated as follows:$P_o$est=(CSA)(P_{sp}). In situ measurements of P_o ($P_o$meas) for individual muscles were made by maximal stimulation via the nerve. $P_o$meas was compared to $P_o$est (Fig.1). The results indicate that a variety of GP fast/mixed muscles have similar P_{sp} generating capabilities and that, similar to cat muscle, 2.3 kg/cm^2 is a reasonable estimate of this potential. However, the GP soleus (100% slow-twitch) appears to have a P_{sp}=1.5 kg/cm^2. The reason for this interspecies difference is unclear at this time. (NIH grant NS16333)



35.2

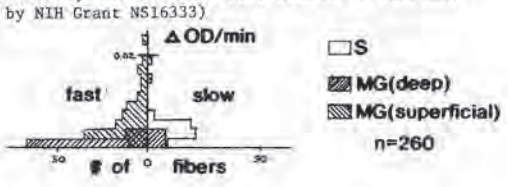
ULTRASTRUCTURE AND CONTRACTION KINETICS OF STRIATED MUSCLE. Robert K. Josephson. University of California, Irvine CA 92717.

Quantitative correlations between ultrastructure and isometric kinetics were determined for cicada tymbal muscles, chosen because of their homogeneous fiber composition. Muscles from 10 species were examined; of these 9 were synchronous muscles and one an asynchronous (oscillatory) muscle. In the synchronous muscles twitch rise time, decay time and total duration were directly correlated with myofibril dimensions; inversely correlated with relative fiber volume as sarcoplasmic reticulum (SR) and T-tubules; and not significantly correlated with fiber size or sarcomere length. The best predictor of twitch duration was fibril cross-sectional area (r=0.95). The asynchronous muscle differed from the synchronous muscles in having very little SR and in producing long isometric twitches. The twitch rise time in the asynchronous muscle was as would be predicted from the fibril dimensions and much faster than would be predicted from the SR volume. Conversely, twitch decay time was much slower than would be predicted from fibril dimensions and about what might be expected on the basis of SR volume. It is proposed that fibril size is a principal determinant of twitch rise time and SR abundance a major determinant of twitch decay time. (Supported in part by USPHS. NIH Grant NS14564).

35.3

APPARENT REGIONAL DIFFERENCES IN THE OXIDATIVE CAPACITY OF SINGLE MUSCLE FIBERS IN THE CAT HINDLIMB. C.E. Blanco*, V.R. Edgerton and K. Castleman*, UCLA, L.A., CA 90024 and Jet Propulsion Laboratory, Pasadena, CA 91103

The variability in enzyme characteristics of individual fibers within skeletal muscle having a variety of motor unit types is visually apparent from standard staining procedures of fresh frozen sections. To quantify this fiber-to-fiber variability, the superficial and deep sections of the cat medial gastrocnemius (MG) and the soleus (S) muscles were stained for succinic dehydrogenase (SDH) by incubating 10 μm thick sections for 8 min, a time at which the enzymatic reaction is linear for all fibers. A serial section was then stained for myosin ATPase with an alkaline preincubation to classify fibers as high and low ATPase ("fast- and slow-twitch"). In neither region of the MG was a bimodal distribution observed (see graph). These data illustrate the wide range in activities among fibers with the MG and a smaller range for S fibers, most of which fall between MG extremes. (Supported by NIH Grant NS16333)



35.4

REGULATION OF NERVE-MUSCLE CONNECTIVITY. R.D Sacks*, R.R. Roy*, C. Blanco*, J. McDonagh* and V.R. Edgerton. Kinesiology Dept. and BRI, UCLA, L.A. CA 90024

The spatial arrangement of fibers belonging to single motor units (SMU's) in the cat tibialis posterior (TP) was determined. SMU's were glycogen depleted (McDonagh et al., J. Neurophysiol., 44:696, 1980) and the depleted fibers were identified from PAS sections using an image analysis system. The section containing the highest density of fibers (n=162) belonging to the SMU was analyzed. The fibers of the SMU were localized in about 25% of the total muscle area. Because the TP has 60 SMU's (see above reference), the number of SMU's occupying a given area was assumed to be 15. Counts were made of muscle fibers which either had no adjacent depleted fibers or were found in groups of two, three, or four. This distribution was compared to that of the probability of adjacency for a truly random mix assuming the number of SMU's occupying a given area to be 15 (Willison, Muscle & Nerve, 3:360, 1980).

Group Size	One	Two	Three	Four
Random Probability (%)	65	24	9	1
TP SMU (%)	89	10	1	0

These results demonstrate a minimization of adjacent fibers belonging to SMU and that the arrangement of the muscle unit is not random but ordered. Supported by NIH Grant NS16333.

35.5

MOTOR UNIT PROFILE OF AUTOGRAFTED RAT EXTENSOR DIGITORUM LONGUS. Claude Côté* and John A. Faulkner. University of Michigan Medical School, Ann Arbor, MI 48109

Our purpose was to determine in the rat the characteristics of motor units in extensor digitorum longus (EDL) muscles transplanted with nerves intact. We tested the hypothesis that the decrease in maximal tetanic tension (P_o) observed in mature autografts was due to a decreased average motor unit P_o rather than a decrease in the total number of motor units. Nerve-intact grafts were transplanted orthotopically in 15 female rats. Between 40 and 50 days after surgery, contractile properties of both whole muscle and single motor units were measured *in situ*. In 10 rats, single motor units were mapped histochemically using the glycogen depletion technique. Mean P_o for grafts was 81% of the control EDL value. This decreased muscle P_o was accounted for by a concomitant decrease in average motor unit P_o from 3.8 to 3.3 g. Total number of motor units was estimated to be 47 in grafts compared to 48 in control muscles. In control EDL, motor units contained fibers broadly dispersed throughout approximately one half of the muscle cross section. In nerve-intact grafts, 60% of the motor units mapped had a distribution similar to the control muscles while the rest showed a tight clustering of fibers within a smaller portion of the muscle cross section. These data support our hypothesis that compared to control muscles the decrease in P_o in nerve-intact grafts resulted from a decreased average motor unit P_o. (Supported by MDA, Canada and USPHS NIH # 17017)

35.6

REGENERATION IN NERVE-INTACT AND FREE AUTOGRAFTS OF SKELETAL MUSCLE. **Leo C. Maxwell. University of Texas Health Science Center, San Antonio, TX 78284.**

Extensor digitorum longus (EDL) muscles of adult cats were transplanted as free autografts (FRA) with the nerve severed, or as nerve-intact autografts (NIA) with the nerve retained. Histochemical and contractile properties of NIA and FRA were analyzed at selected times from 1 to 14 weeks following surgery. Regeneration was qualitatively similar in NIA and FRA. Regenerating fibers were observed in both NIA and FRA within 2 weeks. After 14 weeks there were fewer Type I fibers in both NIA and FRA than in control EDL muscles. NIA had a lower percentage of Type IIB fibers and a greater percentage of Type IIA fibers than FRA, but NIA percentages of Type IIA and IIB fibers approached control values by 14 weeks. Mean fiber area, muscle mass, and absolute tension development were greater in NIA than FRA, but did not reach values for control muscles. Muscle mass, mean fiber area, contractile properties and the proportion of Type IIA and IIB fibers, but not the proportion of Type I fibers, develop toward control values more quickly in autografts with the nerve left intact. **(Supported by American Heart Association)**

35.7

EXERCISE-INDUCED GROWTH OF REGENERATING MUSCLE IN RATS. T.P. White, J.F. Villanacci*, P.C. Morales and D.J. Grossman*. Dept. of Kinesiology, Univ. of Michigan, Ann Arbor, MI 48109

We tested the hypothesis that growth of autografted soleus muscle (SOL) resulting from endurance (E) running (30 m/min; 60 min/day) would be greater when initiated at the nadir in the non-running (NR) graft growth curve (28 days post-transplantation (PT)) than at other times. Transplantation of SOL with nerve implant was performed in female Wistar rats, and the contralateral SOL was sham-operated for control (N=40). Differences in the time between transplantation and start of running and the duration of running were studied. At 42 days PT, there were no differences in mass of grafts between NR and E from rats which started running at 14 or 28 days PT. At 84 days PT, graft mass of E was 40% greater than NR ($P<0.05$) and was not different if running started 28 or 56 days PT. We then tested the hypothesis that growth of grafts would be greater in rats (N=18) required to sprint (S) run (50 m/min; 10 min/day) than E. Running started 28 days PT and after 14 days, S grafts were 40% larger than E (67 $\pm$ 6 mg; $\bar{X}$ $\pm$ SEM) ($P<0.05$). After 28 days of running there were no differences between S and E, and mass was 30% greater than NR (82 $\pm$ 11 mg) ($P<0.05$). Changes in total protein paralleled those in mass. Histochemistry showed no difference in intercellular area of E, S and NR grafts. We conclude that running which started: 1) before 28 days PT did not affect graft growth; 2) at 28 or 56 days PT induced comparable growth if duration was at least 28 days. There were no persistent differences between E and S. (USPHS AM 29732 and NS 17017)

35.8

POLYSOME CELL-FREE PROTEIN SYNTHESIS OF SOLEUS MUSCLE AUTOGRAFTS. David A. Essig*, Timothy P. White, George H. Jones*, and Pedro G. Morales. Departments of Kinesiology and Cellular and Molecular Biology, Univ. of Michigan, Ann Arbor, MI 48109

We hypothesized that polysome cell-free protein synthesis would be increased in regenerating soleus (SOL) skeletal muscle compared to control. Following unilateral orthotopic transplantation with SOL nerve implant, 12 female Wistar rats were killed and bilateral SOL were removed at 23, 42 and 56 days post-transplantation (PT) (N=36). The contralateral SOL was sham-operated for controls. SOL mass (mg) : body mass (g) ratios for controls remained constant (0.50 $\pm$ 0.02) at all times PT. Autograft ratios were lower than controls at all times PT (0.20 $\pm$ 0.01, 0.23 $\pm$ 0.02, 0.32 $\pm$ 0.02), and increased 60% from 28 to 56 days. The concentration of polysomes for control SOL remained constant PT (23.3 $\pm$ 2.3 $A_{260}U$ / g muscle wet weight). In contrast, the polysome concentration of autografts was 130% of control at 28 days PT, equivalent to control at 42 days PT, and 46% of control at 56 days PT. Polysome concentration curves were performed and the concentration eliciting peak incorporation was used for comparisons between groups. Polysome translational activities of autografts were 206, 138 and 123% of control at 28, 42 and 56 days PT, respectively. Protein synthetic capacity up to 42 days PT, as evidenced by polysome concentration and translational activity, was greater than control. As the autograft stabilized in mass, the protein synthetic capacity was less than control. (Supported by USPHS AM 29732 and NS 17017).

35.9

ADAPTATION OF RAT SKELETAL MUSCLE TO HYPOKINESIA. Chris E. Kasper*, Timothy P. White, and Leo C. Maxwell. Department of Kinesiology and School of Nursing, Univ. Michigan, Ann Arbor, MI 48109 and Dept. Physiology, Univ. Texas, San Antonio, 78284

Our aim was to characterize the chronology of change in selected morphological variables of skeletal muscle due to hypokinesia. Adult female Wistar rats (N=25) were placed in hypokinetic slings for 4,7,14 and 28 days. Following hypokinesia, the rats were anesthetized and the soleus muscle was removed, weighed, and frozen in isopentane and dry ice. Non-hypokinetic controls (N=6) were analyzed at the same times. Transverse sections were cut at 10 µm thickness for histochemistry. Serial sections were incubated for myofibrillar ATPase and succinate dehydrogenase activities. Soleus mass (mg) : body mass (g) ratios decreased to 70 and 55% of control value (0.5 $\pm$ 0.02) at 14 and 28 days, respectively ($p < 0.05$). Mean fiber area was 81% of control value (3700 $\pm$ 400 μm^2) at day 7 and 54% at day 28 ($p<0.05$). At day 28, the percent number of type I fibers was 84% of control value (94 $\pm$ 4%) and the percent number of type IIA fibers was 380% of control (5 $\pm$ 2%) ($p<0.05$). At day 28 the percentages of total muscle cross-sectional area comprised of type I and IIA fibers were 90 and 290% of control values (96 $\pm$ 3%; 4 $\pm$ 2%), respectively ($p<0.05$). We conclude that the hypokinetic sling model induced a significant loss of muscle mass due to atrophy. These data indicate a conversion between fiber types due to 28 days of hypokinesia. (Supported by USPHS AM 29732 and NS 17017, American Nurses Foundation and American Heart Association.)

35.10

CONTRACTILE AND FATIGUE PROPERTIES OF THE COMPENSATORY OVERLOADED CAT PLANTARIS. I.D. Meadows*, R.R. Roy*, P.L.Powell*, V.R. Edgerton. Kinesiology Dept. and BRI, UCLA, L.A., CA 90024

Compensatory overload (O) of the rat plantaris (P) results in a conversion of the contractile properties of the fast P towards that of a slow muscle (Roy et al. JAP, 1982). Our concern was whether similar changes would occur in a species which is innately less active. The soleus and gastroc of cats were removed bilaterally and the cats were housed in sedentary cages for 12 weeks. Two O cats were exercised (E) on a treadmill for ~5 min, 3 times/week. Control (C) and O muscles were tested *in situ* at ~ 36°C. Muscle weight (MW,g), contraction time (CT,ms), maximum tetanic tension (P_o,N), tension/cross-sectional area (P_o/CSA,N/cm^2), maximum shortening velocity (Vmax,mm/s/1000 sarc) and a fatigue index (FI) were measured.

GROUP(n)	MW	CT	P_o	P_o/CSA	Vmax	FI
C (3)	7+2	40+2	82+15	22+2	31+6	38+5
O (3)	8+1	46+4	93+4	22+2	31+6	35+4
OE(2)	12+2	45+2	143+20	23+2	30+3	38+5

Muscles in O cats were relatively unaffected. OE cats showed increases of 65% in MW and 75% in P_o which probably reflect the enhanced activity induced by the forced E. Vmax, P_o/CSA, FI, and CT were unchanged. These data indicate that marked hypertrophy can occur without affecting the fatigability or intrinsic speed and force properties in cat OP. Possibly, a greater "overload stimulus" (increased activity) is necessary to produce changes comparable to that in rat. (NIH NS16333)

35.11

SOURCES OF CALCIUM INFLUENCING MOUSE SLOW TWITCH SKELETAL MUSCLE FUNCTION IN VITRO. J.K. Barclay and M.M. Murdoch.* University of Guelph, Guelph, Canada. N1G 2W1

A soleus was immersed in Krebs-Henseleit solution bubbled with 95% oxygen and 5% CO_2 at 27°C for 105 min. (control). After a 60 min. immersion under control conditions, the contralateral muscle either remained in control solution (n=4) or was exposed to Krebs-Henseleit solution containing either 35 μM sodium dantrolene (NaD) (n=5), no calcium (loCa) (n=6), 100 μM verapamil (n=5), NaD plus loCa(n=5), or NaD plus verapamil (n=6) for the remaining 45 min. Both muscles were stimulated for 45 min at 0.6 tetanic contractions per min (500 msec, 50 Hz). Stimulation began within 90 sec of immersion in an experimental solution. Tension development was expressed as a % of the first contraction's tension(%P). The %P of paired muscles under control conditions did not differ significantly. Exposure to NaD for 45 min decreased %P by 17.8±1.6% more than control with 81% of this occurring in the first 10 min. Verapamil resulted in a 22.6±6.8% lower %P while loCa decreased %P by 17.5±4.4%. In both cases 29% of the decrease had occurred by 10 min. The NaD plus loCa solution decreased %P by 58.7±8.0 from the paired control while NaD plus verapamil resulted in a 70.8±2.3% drop. In both solutions, 56% of the change had occurred by 10 min. Thus slow twitch skeletal muscle function was decreased to a similar degree by a SR calcium release blocker and a calcium influx blocker and low extracellular calcium indicating an involvement of both intracellular and extracellular calcium stores.

35.12

FITTING AND COMPARING FORCE-VELOCITY CURVES OF SKELETAL MUSCLE: ALTERNATIVES TO THE HILL EQUATION. Richard L. Marsh and Albert F. Bennett. Univ. of California, Irvine, CA 92717.

Force-velocity data on skeletal muscle have most often been described with the Hill equation: $V=[b(P_0-P)]/(P+a)$ where V= velocity, P_0=maximum isometric force, P=force, and a and b are constants with dimensions of force and velocity, respectively. This equation has no theoretical justification, and in many cases does not adequately describe the experimental data. We measured isotonic contractile properties at 7 different temperatures on the iliofibularis muscle of a lizard Dipsosaurus dorsalis. An iterative non-linear curve-fitting procedure was used to compare the Hill equation with an alternative exponential equation: $V = \alpha EXP-\beta P/P_0-\gamma P/P_0 + \delta$ where α, γ, δ are constants with dimensions of velocity and β is a dimensionless constant. Fitting this alternative equation resulted in lower residual sums of squares than did fitting the Hill equation to the same data. The improved statistical fit was due largely to a better description of the data at high levels of force. Adequate description of the data over the entire range of forces used has important consequences for the predicted power output. As an overall descriptor of the force-velocity curve, a/P_0 derived from the Hill equation can be replaced by the dimensionless power ratio: $\dot{W}_{max}/(V_0P_0)$ where, $\dot{W}_{max}$ is the maximum power output and V_0 is the maximum shortening velocity. This ratio reflects the curvature of data regardless of the equation used. Supported by NSF grant PCM 02331 and NIH K04 AM00351.

PITUITARY

36.1

DOPAMINERGIC MECHANISMS INHIBIT THE RELEASE OF IMMUNOREACTIVE BETA-ENDORPHIN FROM BOTH THE ANTERIOR AND INTERMEDIATE LOBES OF THE RAT PITUITARY. J.M. Farah, Jr.*, D. Sapun-Malcolm* and G.P. Mueller. Dept. of Physiology, USUHS, Bethesda, MD 20814

The secretion of beta-lipotropin (β-LPH) in vitro distinguishes the anterior lobe (AL) from the intermediate lobe (IL) of the rat pituitary. The two lobes also differ in that glucocorticoids selectively inhibit AL release whereas dopamine selectively inhibits IL release of β-LPH and β-END respectively. Using a radioimmunoassay that recognizes both β-END and β-LPH equally we have examined the different molecular forms of β-END immunoreactivity (β-END-I) released in vivo after dopamine receptor blockade by haloperidol (HAL, 2.5 mg/kg, 60 min). HAL increased total levels of plasma β-END-I 3-to 4-fold over control values. Sephadex G-50 gel filtration revealed that this rise was due to two major forms of immunoreactivity, one coeluting with β-LPH standard and the other with β-END. Since both forms of β-END-I were increased approximately equally, the ratios of β-END to β-LPH in control and HAL-treated rats were the same (55-65% β-END; 35-45% β-LPH). Pretreatment with the glucocorticoid, dexamethasone (50 μg/kg, 3h), selectively reduced the form resembling β-LPH whereas pretreatment with the dopamine agonist, bromocriptine (5 mg/kg, 2h), preferentially blocked the release of β-END-I corresponding to β-END in size. Together these findings suggest that HAL in vivo evokes the release of β-END-I from both the AL (β-LPH-sized and sensitive to glucocorticoid inhibition) as well as the IL (β-END-sized and sensitive to bromocriptine inhibition).

36.2

EVIDENCE FOR SEROTONERGIC REGULATION OF PITUITARY β-ENDORPHIN RELEASE FROM THE ANTERIOR LOBE. D. Sapun-Malcolm*, J. Farah,Jr.*, G.P. Mueller. Dept. of Physiology, USUHS, Bethesda, Md. 20814

Using gel filtration chromatography and radioimmunoassay for β-endorphin (β-END) and β-lipotropin (β-LPH) we investigated the site [anterior lobe (AL) vs. intermediate lobe (IL)] for serotonergic control of pituitary β-END immunoreactivity (β-END-IR) in the rat. Since the in vitro secretion of β-LPH by the AL clearly distinguishes β-END-IR release from the IL, we interpreted changes in plasma levels of IR resembling β-LPH to reflect β-END-IR release from the AL. Chromatographic profiles of plasma from control animals reveal that β-LPH and β-END contribute 20% and 80% respectively to total β-END-IR. Following tryptophan administration, nearly 100% of the rise in total plasma β-END-IR (0.49±.04 vs 1.27±.07 ng/ml) was due to the IR form resembling β-LPH in molecular size. Similarly, 5-hydroxytryptophan treatment elevated IR corresponding to β-LPH and to a lesser extent increased the form resembling β-END standard. Quipazine, a serotonin receptor agonist, dramatically elevated plasma IR coeluting with β-LPH 9-fold whereas IR corresponding to β-END was only increased 1-fold over control values. Following blockade of serotonin reuptake by fluoxetine, 86% of of the rise in β-END-IR was due to β-LPH-sized IR. In summary, serotonergic drugs which stimulate pituitary β-END-IR secretion predominantly influence the release of β-END-IR resembling β-LPH in molecular size and therefore suggest that serotonin neurons preferentially regulate the release of β-END-IR from the AL in vivo.

36.3

CORTICOTROPHIN-RELEASING FACTOR STIMULATES RELEASE OF PRO-OPIOMELANOCORTIN-DERIVED PEPTIDES FROM THE INTERMEDIATE LOBE OF THE RAT ADENOHYPOPHYSIS. Jacob Kraicer, Timothy C. Gajewski*, John C. R. Randle* and Bruce C. Moor*. Queen's Univ., Kingston, Ont. K7L 3N6.

A 41-residue corticotrophin-releasing factor (CRF) stimulates the release of ACTH and related peptides from the pars distalis of the adenohypophysis (Vale et al., Science 213, 1394, 1981). Since the cells of the pars intermedia (PI) also synthesize and release peptides derived from pro-opiomelanocortin, we tested the effect of synthetic CRF on the release of peptides from rat nervosa-intermedia (NI). Ten to 15 NI's, perifused with Krebs-Ringer bicarbonate containing 0.1% BSA, were subjected to 30 min pulses of synthetic CRF. Radioimmunoassays (RIA) and bioassays (BA) were then carried out on the acidified perfusate: ACTH-RIA (West antibody), ACTH-BA (Sayers), αMSH-RIA (Vaudry antibody), MSH-BA (Anolis skin assay), LPH-RIA (Lis and Chrétien antibody) and receptor assay for morphine-like activity (MLA). CRF stimulated the release of all assayable peptides. The response was immediate, sustained, dose-related (1-50 ng/ml), reversible and repeatable. Thus CRF may be a physiological secretagogue for the control of release of the pro-opiomelanocortin-derived family of peptides from the PI. It remains to be seen whether CRF acts via a humoral route, or by direct innervation of the cells of the PI. (Supported by MRC.)

36.4

IDENTIFICATION OF AROMATASE ACTIVITY IN RODENT PITUITARY CELL STRAINS. Gloria V. Callard, Zoltan Petro*, and Armen H. Tashjian, Jr., Harvard Med. Sch. & Sch. of Public Hlth., Boston, MA 02115

Aromatization of androgen to estrogen occurs in the brain of many vertebrates where it mediates certain central androgen actions. Based on mammalian studies, the hypophysis was thought to be aromatase-negative; however, we found recently that the teleost gland is capable of synthesizing substantial amounts of estrogen. To reinvestigate this paradox, established clonal strains of rodent pituitary cells were cultured for 6-8 h in serumless medium with [7-^{3}H]-androstenedione. Metabolites were isolated by solvent extraction, thin-layer chromatography, and phenolic partition and authenticity verified by derivative formation and crystallization to constant specific activity. Estrone and estradiol-17β were identified in cultures of two prolactin/growth hormone-secreting clones (GH_3> GH_4C_1) but not in an ACTH-secreting line (AtT20/D16) or in a line of presumptive gonadotropes (courtesy, Dr. E. Leiter). Large amounts of 5α-reduced and conjugated androgens were also produced. We conclude that the expression of aromatase activity in hypophysial cells is not a property of all transformed lines but may be dictated by secretory cell type. Although low, estrogen yields in GH cultures resemble those in brain and other peripheral estrogen targets. (Supported by PCM-78-23214 and AM 1101. Present address: GVC, Boston University)

36.5

EFFECTS OF NEONATAL TREATMENT WITH DIETHYLSTILBESTROL (DES) & 17α-HYDROXYPROGESTERONE CAPROATE (HPC) ON IN VITRO PITUITARY PROLACTIN (PRL) SECRETION. J. Lopez*, L. Ogren* and F. Talamantes. Univ. of Calif., Santa Cruz, CA 95064

This study examined whether neonatal exposure of mice to exogenous estrogens & progestins alters PRL synthesis. Female C3H/MTV+ mice were treated with 0.001 µg/day DES, 2.5 µg/day DES, 15 µg/day HPC, 150 µg/day HPC or sesame oil on days 1-5. Mice were killed when 2,4,6,8 & 10 wks old. PRL synthesis was measured by ^{3}H-leucine incorporation. Neonatal treatment with 2.5 µg/day DES increased PRL synthesis at 2 wks of age but at 10 wks levels were suppressed. Neonatal treatment with 150 ug/day HPC suppressed PRL synthesis at 10 wks. PRL synthesis was increased at 2 wks by treatment with 0.001 µg/day DES and at 2 & 4 wks with 15 µg/day HPC. Neonatal treatment with 2.5 µg/day DES suppressed serum PRL levels at 6 wks. In addition, PRL secretion by pituitaries from mice treated neonatally with 0.001 µg/day DES, 2.5 µg/day DES or sesame oil was examined at 8 mo. PRL secretion during 12 days of culture and pituitary PRL content was lower in mice treated with 2.5 µg/day DES than in controls. Treatment with 0.001 µg/day DES did not affect PRL content, but PRL secretion was lower on day 2 and higher on day 4 of culture in DES-treated than in control mice. In summary, PRL synthesis by pituitaries from young mice was altered by neonatal treatment with DES & HPC. Similarly PRL secretion by pituitaries from adults in long-term organ culture was also altered by neonatal treatment with DES. (Supported by N.I.H. Grant CA 28393 to F.T.)

36.6

DOES PROLACTIN (PRL) PLAY A ROLE IN THE ACQUISITION OF A CONDITIONED AVOIDANCE RESPONSE (CAR)? D. Yelvington*, D. Beach*, G. Weiss and A. Ratner. Univ. New Mexico., Albuq., NM 87131.

Hypophysectomy (HYPOX) has been claimed to attenuate the acquisition of a CAR in rats. It has also been claimed that ACTH, ADH, GH, MSH and endorphin will abolish this deficit. We previously reported that psychological factors play a major role in control of PRL secretion and that PRL increases during acquisition testing. Others have reported recently that hyperprolactinemia facilitates the acquisition of active avoidance behavior. It was our intention to determine if blocking the PRL increase during acquisition testing would decrease acquisition and to determine if the reported HYPOX-induced deficit in acquisition could be corrected by PRL replacement.

In one series of experiments we found that lergotrile mesylate (LM) completely blocked the PRL response to testing, but did not alter acquisition of a CAR. In a second series of experiments, HYPOXed rats and sham-operated rats were subjected to acquisition testing. Contrary to previous reports there was no difference in acquisition between the shams and fully recovered HYPOX rats.

These data are inconsistent with the idea that pituitary hormones, including PRL, play a role in the acquisition of a CAR. They also point out the need for consideration of recovery time as a factor in assessing the acquisition of behavior in HYPOXed rats. (Supported in part by MSB Grant No. RR-08139-08 and NIH Grant No. HD-13780.

36.7

DIFFERENTIAL SECRETION OF BIOASSAYABLE GROWTH HORMONE BY TWO TYPES OF RAT SOMATOTROPHS. R.E. Grindeland, W.C. Hymer, P. Lundgren*, and C. Edwards*. NASA-Ames Research Center, Moffett Field, California 94035 and Pennsylvania State University, University Park, Pennsylvania

Secretion of bioassayable GH (BA-GH), an immunochemically modified form of GH, was investigated in two types of rat somatotrophs. The cell types were isolated by dispersion of anterior pituitary cells and separation on a density gradient. Unfractionated cells ("Starts"), Type 1 and Type 2 cells were cultured for 12 days with media changed and collected for assay every 4 days. BA-GH and immunoreactive GH (IR-GH) were measured in cell extracts and media by tibial assay and RIA. Start, Type 1 and Type 2 cells secreted essentially constant amounts (mug/1000 cells) of IR-GH over the 12 days incubation period. In contrast BA-GH secretion was constant for Type 1 cells, but fell by 60% for Start and Type 2 cells. Nevertheless, Type 2 cells secreted 3 - 4 times as much BA-GH as Type 1. BA-GH secretion by Start and Type 2 cells from cold exposed rats (4 C, 1 hr) fell 40 and 75%, respectively, from room temperature control values. Cold exposure did not affect BA-GH secretion by Type 1 cells or IR-GH secretion by any cell type. All cell types showed net synthesis of IR-GH and BA-GH, with IR-GH synthesis similar for control and cold exposed cells; in contrast BA-GH synthesis fell 50% for Type 1 and 60% for Type 2 cells. Clearly the cell types differ in their secretion of BA-GH and their responses to cold exposure.

36.8

ARGININE VASOPRESSIN RECEPTORS ON HUMAN PLATELETS. Lydia S. Ho* and M. Husain Jawadi* (SPON: G.S. Greenwald). Univ. of Kansas Sch Med-Wichita and VA Med Ctr, Wichita, KS 67211

The investigation of interaction between arginine vasopressin (AVP) and its receptors in the human has been hampered by lack of accessible tissues containing AVP receptors. We have developed a procedure to study AVP binding to platelets using a relatively small amount of blood. Platelets were isolated by differential centrifugation at room temperature from anticoagulated blood obtained from fasting human volunteers. After incubation of cell suspension in the presence of ^{125}I-AVP with and without cold AVP, bound and free AVP were separated by microcentrifugation. At platelet concentration of $5x10^{5}$µl, specific binding ranged from 4-17% of the total radioactivity added, while nonspecific binding was approximately 0.2%. Specific binding was saturable with time and was linear with cell number. Scatchard analysis showed an apparent Kd of $2.9x10^{-10}$M and 70 binding sites/cell. The relative binding affinity for AVP, related peptides and analogs were: AVP=LVP (Lysine Vasopressin)>oxytocin>dDAVP (Deamino 8-D Arginine Vasopressin). Binding was readily reversible by adding excess amount of cold AVP after binding has reached max. We confirm the presence of AVP receptors in human platelet and conclude that our method provides an easy and reproducible technique using the readily accessible tissue (platelets) for human AVP receptor studies. This technique allows us to perform receptor assays in various states of altered water and electrolyte metabolism.

NEURAL CONTROL OF CIRCULATION II

37.1

REFLEX EFFECTS OF HINDLIMB AND FORELIMB EXERCISE ON ARTERIAL BLOOD PRESSURE IN THE DOG. Orren Beaty III. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

Rhythmic contraction of skeletal muscle will increase or decrease arterial blood pressure (ABP). Dogs were anesthetized with chloralose. The contribution of hind and forelimb contraction to the reflex changes in ABP, non-exercising hindlimb perfusion pressure (HLPP), and heart rate (HR) were examined. Contractions were evoked electrically 4, 8, 16, 32, and 48 Hz for 90 sec. The left hindlimb was perfused at constant flow. All blood pressure regulating mechanisms were intact. Ventilation was 12 breaths/min. Right hindlimb contrations evoked a frequency dependent reflex change in ABP and HLPP. 48 Hz increased ABP, 11 ± 2 and HLPP 19 ± 6 mm Hg. 16 Hz only transiently affected ABP and HLPP. 4 Hz reduced ABP 5 ± 4 and HLPP 8 ± 4 mm Hg. HR increased by 14.6 ± 9 beats/min. Right forelimb contractions evoked a different reflex response pattern. 48 Hz increased ABP 12 ± 3 mm Hg. 16 and 4 Hz evoked no sustained change. HLPP was augmented by all frequencies of contraction (average 12 ± 3 mm Hg). Heart rate was increased an average of 18 ± 4 beats/min. Skeletal muscle paralysis abolished these reflex responses. Thus, hind and forelimb contractions appear to evoke different reflex changes in the cardiovascular system. Specifically, hindlimb contractions appear to evoke reflex vasoconstriction and dilation while forelimb contractions appear to evoke only reflex vasoconstriction. (Supported by AOA 80-04-313, 84-04-012)

37.2

RESPONSES OF GROUPS III AND IV MUSCLE AFFERENTS TO DIFFERENT LEVELS OF TENSION DEVELOPMENT BY THE TRICEPS SURAE. M.P. Kaufman*, J.C. Longhurst , K.J. Rybicki* and J.H. Mitchell Univ. of Texas Health Science Ctr., Dallas, Texas 75235

Muscular contraction, induced by electrical stimulation of the L_7-S_1 ventral roots has been shown to reflexly increase arterial pressure, an effect whose magnitude is directly related to the level of tension development by the contracting skeletal muscle. Although the activation of groups III and IV muscle afferents is believed to be responsible for this reflex pressor response, the responses of these afferents to different levels of tension development by the contracting muscle has not yet been determined. Therefore, in anesthetized cats, afferent impulse activity arising from the triceps surae was recorded from the dorsal roots, while this muscle group was contracted by stimulation of the cut peripheral end of the L_7 ventral root. We found that 6 of 7 group III muscle afferents increased their responses to contraction as the level of tension developed by the triceps surae increased from 1 to 6 kg. By contrast, 4 group IV muscle afferents did not display tension related responses to muscular contraction. We conclude, therefore, that group III afferents appear to be at least partly responsible for the tension-related reflex pressor responses evoked by muscular contraction, although a contribution from group IV afferents cannot, at the present time, be excluded. (Supported by the Lawson and Rogers Lacy Research Fund and Program Project HL06296)

37.3

HEMODYNAMIC AND CATECHOLAMINE RESPONSES TO CRYPTENAMINE DURING PROGRESSIVE HEMORRHAGE IN THE CONSCIOUS DOG. E. Hasser*, C. Heesch* and V.S. Bishop. Dept. Pharmacology, Univ. TX Hlth. Sci. Ctr., San Antonio, TX 78284.

This study examined the hemodynamic and plasma epinephrine (E) and norepinephrine (NE) effects of activation of vagal afferent fibers during progressive hemorrhage (HEM) in the conscious dog. Dogs were instrumented with catheters in the left atrium and aorta. Animals were subjected to HEM of 20 and 30 ml/kg. The response to 6-8 µg/kg cryptenamine (CRP) was examined under control conditions and at each level of HEM. HEM of 20 and 30 ml/kg significantly reduced left atrial pressure (LAP) and significantly elevated heart rate (HR) and plasma E (+540 and 1962%) and NE (+207 and +323%). Arterial pressure (AP) was significantly reduced (75 vs 97 mmHg) at HEM of 30 ml/kg. Prior to HEM, CRP significantly reduced AP (-25 and -15 mmHg) and HR (-23 and -22 bpm) at 2 and 5 min. Plasma E and NE concentrations were insignificantly elevated. Following 20 and 30 ml/kg HEM, CRP elicited significantly greater falls in AP at 2 min (-31 and -33 mmHg) and 5 min (-27 and -27 mmHg). During HEM, HR also fell to a greater extent in response to CRP at 2 min (-58 and -79 bpm) and 5 min (-54 and -81 bpm). Plasma E and NE concentrations were increased in response to CRP, but these changes were not significant at any level of HEM. Data suggest that activation of vagal afferents causes decreased AP, the magnitude of which is increased during HEM. No significant effects of vagal afferent activation on adrenal E and NE secretion could be demonstrated. (Supported by NIH Grant HL-12415 and AFOSR 78-3657).

37.4

EFFECT OF PENTOBARBITAL ON OPIATE MEDIATED SYSTEMIC AND CORONARY VASOCONSTRICTION. S. Pasyk, W. Pluta*, J. Walton*, R. Grekin*, and B. Pitt, University of Michigan Medical Center, Ann Arbor, MI.

Previous studies have shown that intracerebral (I.C.) administration of the opiate fentanyl (F) results in incr. systemic (SVR) and coronary vascular resistance (CVR) accompanied by release of vasopressin (AVP). Since pentobarbital anesthesia (P) has previously been found to be useful in treating opioid mediated cerebral ischemia, 6 dogs with a chronically implanted cannula in a lateral cerebral ventricle; electromagnetic flow transducers around the left circumflex coronary artery and aorta; as well as catheters in the left atrium, central aorta, and superior vena cava, were studied. F 1.3 µg/kg was injected I.C. in the conscious state (C) and on a separate occasion after P 30 mg/kg. Heart rate beats/min (HR); SVR units; CVR units; mean left atrial pressure mmHg (LAP); and pg/ml AVP were determined before and after F.

	HR	SVR	CVR	LAP	AVP
C	85	.028	2.9	4.3	2.02
C-F	52*	.072*	6.1**	11.3**	133.50*
P	114	.029	2.1(n=4)	5.3	4.63
P-F	111	.029	1.6*(n=4)	3.7	6.70*

C compared to C-F, P compared to P-F, *p<.05, **p<.001.

Thus, P blocks the increase in SVR, CVR, and limits AVP release following I.C. F. The effectiveness of P in reducing vascular resistance in various pathophysiologic conditions may be due to blockade of opiate mediated AVP release.

37.5

SYMPATHETICALLY MEDIATED INCREASES IN THE MAXIMAL RATE OF FALL OF VENTRICULAR PRESSURE IN AWAKE DOG. D.C. Randall, G.E. Billman & T.L. Skinner*. Dept. Physiol. & Biophys., Univ. Kentucky College Medicine, Lexington, KY 40536.

The role of the sympathetic nervous system in controlling the maximal rate of fall of left ventricular pressure (-P'max) in the intact animal is unclear. We have used classical appetitive (n=6) and aversive (n=8) conditioning to increase cardiac sympathetic nervous activity and observed concomitant increases in -P'max. Conditioning was accomplished by following a 30 sec conditional stimulus (CS+) tone by food or shock, respectively; food delivery lasted 30 sec. while shock duration was 1 sec. Data were averaged during 30 sec. pre-CS+, the tone itself, and a 30 sec. post-CS+ period (ie, following shock or during food). Along with the usual increase in blood pressure (BP) and heart rate (HR), -P'max increased during conditioning:

	pre-CS+	CS+	post-CS+	(Average -mmHg/sec; * = p<.01 relative
appetitive	2040	2227*	2346	to previous period)
aversive	2598	4007*	3646	

No significant changes occurred during a different tone (CS-) never followed by food or shock. β-blockade eliminated the increase in -P'max during CS+ for both paradigms. The conditional increase in -P'max was not significantly altered when HR was paced (tested for aversive conditioning only) at a constant 182 bpm; also, the conditional increases in mean BP were similar during control, paced and β-blocked trials. The data indicate that activation of the β-adrenergic system in intact dogs increases the rate of fall of ventricular pressure. (HL 19343)

37.6

EFFECTS OF PENTOBARBITAL ANESTHESIA ON SYMPATHO-ADRENAL AND RENIN-ANGIOTENSIN SYSTEMS. M. Zimpfer*, W.T. Manders*, A.C. Barger, and S.F. Vatner. From the Depts. of Med. and Physiol., Harvard Med. School, Brigham & Women's Hospital, Boston, MA, and the NERPRC, Southboro, MA

It is generally held that general anesthesia with pentobarbital stimulates the sympatho-adrenal system, while its effects on the renin-angiotensin system remain controversial. To study this 7 dogs were instrumented with arterial pressure catheters, aortic electromagnetic flow probes, and left ventricular pressure gauges. Three-six weeks after recovery from operation, pentobarbital, Na, 30 mg/kg, was administered i.v. to the conscious dogs, while resting quietly. With ventilation controlled by a respirator, pentobarbital did not alter mean arterial pressure (85 ± 2.6 mmHg) or total peripheral resistance (0.029 ± 0.002 mmHg/ml/min), but increased plasma renin activity from 0.7 ± 0.1 to 2.5 ± 0.5 ng Angiotensin1/ml/hr, and decreased left ventricular dP/dt from 3350 ± 90 to 2020 ± 150 mmHg/sec, and plasma norepinephrine from 254 ± 30 to 119 ± 28 pg/ml, and epinephrine from 188 ± 18 to 125 ± 24 pg/ml. Thus, in the healthy, trained dog general anesthesia with pentobarbital, but in the absence of surgical trauma, stimulates the renin-angiotensin system, but does not appear to augment sympathetic tone sufficiently to increase arterial pressure, myocardial contractility, or peripheral resistance, and actually depresses levels of circulating catecholamines.

37.7

PENTOBARBITAL DEPRESSES SPLENIC CONTRACTION IN RESPONSE TO HEMORRHAGE. T.A. Patrick *, W.T. Manders*, and S.F. Vatner. Dept. of Medicine, Harvard Medical School, Brigham & Women's Hospital, Boston, MA., and the New England Regional Primate Research Center, Southboro, MA.

Hemorrhage reduces arterial pressure significantly more in barbiturate anesthetized dogs than in conscious dogs. Since the spleen plays an important role in the response to hemorrhage in the conscious dog, it is conceivable that some of the difference in response to hemorrhage in conscious and anesthetized dogs could be due to differences in responses of the spleen. To study this, we utilized 3 pairs of ultrasonic crystals and an ultrasonic dimension gauge to define a cubic volume index of the spleen (length x width x thickness). Experiments were conducted 1-4 weeks after implantation of the transducers, and an arterial pressure catheter. In conscious dogs mean arterial pressure fell by 28 ± 7% (from 85 mmHg) after 30 ml/kg of hemorrhage. The splenic volume index fell by 40 ± 5% from 3.3 cc. Pentobarbital, Na, 30 mg/kg, did not alter arterial pressure but increased splenic volume index, by 21 ± 5%. In anesthetized dogs hemorrhage, 30 ml/kg, reduced mean arterial pressure more (by 55 ± 5%), and splenic volume index less (by 27 ± 3%) than was observed in conscious dogs. Thus, barbiturate anesthesia, while dilating the spleen, interferes with its contraction capacity in response to hemorrhage. This could explain, in part, the inability of the anesthetized dog to maintain arterial pressure in response to hemorrhage.

37.8

ROLE OF THE SPLEEN IN THE RESPONSE TO HEMORRHAGE IN THE CONSCIOUS DOG. W.T. Manders*, M. Zimpfer*, and S.F. Vatner. Dept. of Medicine, Harvard Medical School, Brigham & Women's Hosp., Boston, MA., and the NERPRC, Southboro, MA.

Experiments in anesthetized dogs indicate that the spleen contributes significantly in the response to hemorrhage. However, the extent of its contribution to the maintenance of arterial pressure and to the rise in peripheral resistance in the conscious animal is not known. To study this, effects of hemorrhage were examined in 7 conscious dogs before and after recovery from splenectomy. These dogs were previously instrumented with electromagnetic flow probes on the aorta and arterial pressure catheters. In intact, conscious dogs mean arterial pressure was well maintained to 15 ml/kg of blood loss and fell by 18 ± 6% (from 85 mmHg) at 25 ml/kg of hemorrhage. In contrast, after splenectomy mean arterial pressure fell significantly, by 16 ± 6%, at 15 ml/kg of hemorrhage (from 89 mmHg), and by 42 ± 5% at 25 ml/kg of hemorrhage. In intact dogs total peripheral resistance rose by 78 ± 12% (from 0.033 mmHg/ml/min) at 25 ml/kg of hemorrhage, but only by 39 ± 8% (from 0.033 mmHg/ml/min) at 25 ml/kg of hemorrhage after splenectomy. Thus, in conscious dogs, as expected, the spleen contributes significantly to the maintenance of arterial pressure during hemorrhage. Surprisingly, despite the greater fall in arterial pressure with hemorrhage after splenectomy, which should have caused more intense reflex peripheral vasoconstriction, the rise in peripheral resistance was actually less.

37.9

REGULATION OF THE CHICKEN PINEAL BY THE SUPERIOR CERVICAL GANGLIA. Vincent M. Cassone* and Michael Menaker. Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Adult hens were cannulated 5-9 days in light/dark cycles (LD) and/or constant darkness (DD). Blood samples were taken every 4 hours. Plasma melatonin titres were determined by radioimmunoassay. Intact hens exhibited melatonin rhythms in both LD and DD. Neonatal superior cervical ganglionectomy (SCGX) had little effect on adult melatonin rhythms in LD but in DD the rhythms were disrupted. Daily infusions of norepinephrine (NE) for 12 of every 24 hours decreased plasma melatonin titres and restored a 24-hour rhythm in SCGX hens in DD.

After each experiment hens were killed; their pineals were removed and assayed by HPLC-EC for NE, dopamine (DA), serotonin (5-HT) and 5-hydroxy-indole acetic acid (5-HIAA). SCGX resulted in a 90% depletion of pineal norepinephrine; DA content was reduced to undetectable levels. Pineal 5-HT content was decreased (9.57±1.93 ng vs. 6.12±2.96 ng/pineal; $p<0.05$) as was 5-HIAA (7.46±2.34 ng vs. 2.49±1.5 ng/pineal; $p<0.01$).

The chicken pineal contains circadian oscillators that persist in vitro[1]. The results reported here suggest that noradenergic fibres from the SCG may modulate the pineal's inherent rhythmicity. Norepinephrine released during the bird's day may synchronize oscillators within the pineal by inhibiting them.

[1]Takahashi, J., Hamm, H., Menaker, M. (1980) PNAS 77(4):2319-2322.

(Supported by NIH HD13162 and PHS Training Grant GM07257.)

37.10

EFFECTS OF VERAPAMIL ON CARDIAC CHRONOTROPIC RESPONSE TO VAGAL STIMULATION IN PIGLETS. John C. Lee. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061

The purpose of this study was to assess the effects of verapamil on the chronotropic response to vagal stimulation (VS) in anesthetized piglets. After bilateral vagotomy, the administration of verapamil (100 µg/kg loading dose, followed by 2 µg/kg/min infusion) resulted in a significant reduction in both heart rate (HR, $P < 0.05$) and mean arterial blood pressure (MABP, $P < 0.001$). Cardiac frequency response to VS was obtained by stimulating the distal right vagus at selected frequencies (5, 10, 15, and 20 HZ) with constant voltage (10V) and duration (2 mSec). HR was measured after 15 sec at each level of stimulation. The changes in HR following VS were significantly reduced after verapamil (at 20 HZ, $\Delta HR = -52.5 \pm 6.1$ bpm) as compared to its control values (at 20 HZ, $\Delta HR = -86.7 \pm 4.5$ bpm). The vagolytic action of atropine (0.5 mg/kg), however, was not affected by verapamil. These data suggest that verapamil may influence the parasympathetic control of the heart in piglets. (Supported in part by AHD project no. 7824650.)

RESPIRATORY PHYSIOLOGY

38.1

RECOVERY OF PULMONARY BLOODFLOW DISTRIBUTION IN PATIENTS WITH COPD BREATHING DIFFERENT OXYGEN MIXTURES. John F. Metcalf and R. Srinivasan.* Medical University of South Carolina, Charleston, South Carolina, 29403.

Date from patients with chronic obstructive pulmonary disease and normal controls breathing different oxygen concentrations of 40% or less were analyzed using a four compartmental model. A two dimensional search procedure recovered a distribution of bloodflow to a shunt compartment, a low ventilation-perfusion compartment and normal alveolar compartment. The bloodflow to the shunt compartment did not significantly differ in the patients with COPD from the normal controls. The magnitude of this shunt component, 1-2%, is consistent with bronchial bloodflow and indicates that anatomic shunt does not play an important role in COPD. In all cases, venous admixutre while breathing 100% O_2 was greater than shunt. The average difference of 5.8%[2] can be explained by absorption atelectasis and incomplete denetrogenation. Large amounts of bloodflow was estimated for the low ventilation perfusion compartment inpatients with COPD, ranging from 15 to 82%. This bloodflow correlated well with the degree of hypercapnea. The model accurately predicts changes in arterial oxygen pressure resulting from changes in inspired oxygen concentration.
(Supported in part by USPHS NIH Grant HL 22932.)

38.2

A REBREATHING (RB) METHOD FOR MEASUREMENT OF CARDIOPULMONARY FUNCTION IN SMALL ANIMALS. M. Friedman, W.M. Mentz,* E.E. Lawson. University of North Carolina, Chapel Hill, NC 27514.

Inert gas RB has been used in large mammals to measure pulmonary capillary blood flow (Qc), diffusing capacity (D_LCO), O_2 consumption ($\dot{V}O_2$), FRC, and volume of the lung tissue and capillaries (VTPC), a measure of total lung H_2O. 20 piglets (2-20 days of age, wt. 1.4-4.8 kg) were anesthetized with chloralose, urethane and ketamine, underwent tracheostomy and mechanically ventilated. The RB apparatus consisted of a 150 ml bag-in-bottle system (2 ml dead space). At FRC, the animal was rebreathed for 20 s at a rate of 30/min with 30 ml/kg of a gas mixture containing 0.3% $C^{18}O$, 0.9% C_2H_2, 10% He, 21% O_2, bal N_2. Gases were sampled at the airway opening by a mass spectrometer and the data analyzed on-line (J. Appl. Physiol. 48:66-71, 1980). The lungs were then removed for estimation of wet-dry lung wt. (W-D). The regressions of RB parameters on body wt. and average coefficients of variation (CV) for the RB parameters were:

Parameter	Regression Equation	r	CV
Qc (L/min)	-0.07 + 0.27 (kg)	0.89	8%
D_LCO (ml/min/mmHg)	0.15 + 0.39 (kg)	0.94	8%
FRC (ml)	17.63 + 19.49 (kg)	0.76	5%
$\dot{V}O_2$ (ml/min)	3.30 + 10.16 (kg)	0.93	5%
VTPC (ml)	-6.13 + 18.60 (kg)	0.90	11%

The correlation of VTPC and W-D was 0.86. Thus, RB in small animals yield rapid, reproducible and accurate estimates of several cardiopulmonary parameters including lung H_2O.

38.3

MICROVASCULAR PRESSURE AFTER HISTAMINE MEASURED BY THE MICROPUNCTURE AND VENOUS OCCLUSION METHODS IN THE ISOLATED DOG LUNG. J. Bhattacharya, Y. Nagasaka*, M. Gropper* and N.C. Staub. Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, CA 94143.

We have determined the principal site of histamine vasoconstriction in the pulmonary circulation and have compared lung microvascular pressures measured by the venous occlusion and the micropuncture techniques (FED.PROC. 41:1685, 1982). We perfused 4 isolated dog lung lobes with constant blood flow (300 ml/min) and at constant venous (10 cmH_2O) and alveolar (7 cmH_2O) pressures. Both before and 20 min after continuous histamine infusion (25-50 µg/min), during which flow resistance rose 82%, we simultaneously measured pressure in 20-40 µm diameter venules by direct micropuncture and occlusion pressure by clamping venous outflow. The data are summarized in the table below (mean ± S.D.).

	PRESSURES (cmH_2O)		
	Pulmonary Artery	Venule	Occlusion
Control	16.5±0.7	10.6±0.7	12.4±1.0
Histamine	21.8±2.0	16.4±2.7	17.2±0.8

By both methods of measurement histamine raised microvascular and arterial pressures comparably. The pressure drop increased downstream but decreased upstream. In the dog lung histamine constricts veins. The slight vasodilatation upstream is probably due to passive distension. (Supported by HL25548).

38.4

ZONE II AND ZONE III PULMONARY VASCULAR RESISTANCE. (PVR) S.L. SooHoo*, R. Graham*, and H.S. Goldberg. Division of Pulmonary Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

Conventionally it is thought that PVR is less in zone III than zone II. To test this hypothesis we measured pressure-flow (P-Q) relationships in 6 isolated canine lobes perfused with autologous blood, inflated to a static transpulmonary pressure of 5 cmH_2O. Pulmonary venous pressure (P_{pv}) was set at each of 3 levels; < 0, 10, and 20 cm H_2O. For the first two conditions, the lobe is in zone II and zone III for the third. The slopes of P-Q curves between 100% and 25% of maximal flow were 54.6±10.1 SD (ml/min)/cmH_2O, 56.3±15.9, and 61.7±22.4; and were not statistically different. Extrapolation of the slopes to zero flow gave average pulmonary arterial closing pressures (P_{pa_i}) of 15.6±1.6 cmH_2O ($P_{pv} < 0$), 15.9±1.4 (P_{pv}= 10cmH_2O), and 20.6±1.3 (P_{pv}=20cmH_2O). The last P_{pa_i} value is significantly different from the first two, which are not significantly different from each other. These results show incremental or flow resistance does not change significantly between zone II and III and that for $P_{pv} < P_{pa_i}$, the pertinent back pressure to flow is P_{pa_i}. Since calculation of PVR is dependent on the back pressure used, and incremental resistance does not change, the apparent PVR decrease from zone II to III is due to the larger underestimation of back pressure in zone II than in zone III where $P_{pv} < P_{pa_i}$. For $P_{pv} > P_{pa_i}$ there is no underestimation of back pressure, but there is no significant alteration in PVR. (Supported by James Irving Foundation, The Sidney Stern Memorial Trust, and Herzog Fund.)

38.5

QUANTITATIVE RECOVERY OF VENOUS GAS EMBOLI. D.L.Sherrill*, R.W. Virtue, A.J.Lechner[1], and J.Friedman*. Dept.Anesthesiology, U. Colorado Health Sci. Ctr., Denver, CO 80262, and [1]Dept.Physiology, St. Louis U. Sch. Medicine, St. Louis, MO 63104.

A computer controlled quadrupole mass spectrometer was used to measure total expired volumes of test gases injected as 2.0 ml/kg boluses into the airways (AW) or right ventricles (RV) of 4 mongrel dogs. Flow signals from a hot wire anemometer or mass flow meter were linearized, summed and multiplied by gas concentrations every 20 msec for inspired and expired volumes of each breath; test gases (N_2O, Ar, N_2, Ne) reflected a 5-fold range of solubilities. Integrations of the mass spectrometer volume outputs were verified for each gas with 40 ml injections into a ventilated 1-liter anesthesia bag; recoveries were 95-100%. Tracheotomized dogs were ventilated with 100% O_2 under Na-pentobarbital (30mg/kg,i.v.) and succinyl choline anesthesia;Swan Ganz catheters were used for RV injections and to measure cardiac output and P_{ap}. The % recoveries of test gases in the dogs were: N_2O: AW = 63.0±3.2, RV = 62.6±3.4; Ar: AW = 78.1±2.3, RV = 79.2±2.7; N_2: AW = 79.0±2.6, RV = 53.0±2.6; Ne: AW = 89.7±2.4, RV = 79.9±3.8. As expected, the AW % recoveries increased with decreasing solubility, and represent maximal values obtainable for RV emboli, due to reabsorption of alveolar gases into the blood following their excretion from pulmonary capillaries.The RV % recoveries vary due to reabsorption and to variable rates of bubble solubilization within blood vessels. While trapping occurred in small vessels, bubbles from RV emboli did not pass directly into the pulmonary veins.

38.6

LUNG BLOOD VOLUME IN DOGS SUBJECTED TO PEEP. Milena L. Lewis. Veterans Administration Medical Center, New York,N.Y. 10010

In anesthetized dogs(mean wt.20Kg) cardiac output(CO),lung blood volume(PBV), right ventricular ejection fraction(RVEF)& volumes were determined by an external isotope dilution method. Right(RV) & left(LV) ventricular filling pressures, pulmonary artery(PA), tracheal(T), & esophageal pressures were measured & integrated over a respiratory cycle. Dogs were studied at zero, 10 & 20cm PEEP, & during recovery. CO fell 19% ($p<0.05$) at 10cm, & 25%($p<0.05$) at 20cm PEEP, from a control level of 3.9 L/min, & returned to 99% of control. RVEF did not change significantly at either level of PEEP. RV systolic pressure-volume ratio rose at 10cm PEEP($p<0.01$), returning toward control at 20cm. Thus evidence of impaired RV contractility was not obtained. Transmural RV & LV filling pressures did not change significantly; P_T exceeded LV filling pressure during PEEP; pressures in the PA rose pari passu with P_T. PA diastolic pressure exceeded P_T at each level of PEEP in each dog, indicating continued diastolic flow. PBV at 10cm was not different from control, rose by 20% at 20cm ($p<0.05$) & by 30%($p<0.01$) during recovery. Since PBV did not fall but rose under Zone 2 conditions, we conclude that P_T transmitted to alveolar capillaries effectively increases upstream blood volume, shifting arterial vessels upward on their compliance curve. Thus results in an increased head of driving pressure through less compressed (presumably extra-alveolar) vessels.

38.7

EFFECTS OF MICROFILARIA-INDUCED PULMONARY HYPERTENSION ON PULMONARY ARTERY PROPERTIES. Samuel Chacko and Robert Cox, University of Pennsylvania and The Graduate Hospital, Philadelphia, PA 19104.

Segments of extralobar and intralobar pulmonary arteries (PA) were obtained from control (C) and heart worm (HW) affected dogs, and used for studies of mechanics and composition. Length-force measurements were made on rings under passive (0-Ca^{+2} and 2 mM EGTA) and active conditions (100 mM K^+). Other rings were used for connective tissue, water and electrolyte analyses. Mean pulmonary artery pressure was significantly elevated in the HW animals: C = 10 ± 2 and HW = 24 ± 7 mmHg. Hypertrophy was found in all HW arteries. Passive stiffness of arteries from HW was increased compared to C. Except for the main PA, the total collagen and elastin content was decreased while their ratio was increased in HW arteries. Maximum active force (K^+) was increased in extralobar HW arteries but not in intralobar ones. The cell content of all HW arteries except the main PA was increased. Values of active force development based upon cellular cross-sectional area were increased in extralobar HW arteries. No differences were found in water content or distribution but K^+ and Na^+ content of HW arteries were generally increased. These results suggest that microfilaria-induced pulmonary hypertension produces selective nonuniform alterations in pulmonary arteries which are similar to those occuring in systemic hypertension. (Supported by HL-23779).

38.8

MECHANISM OF CARDIAC DEPRESSION BY ALBUMIN. J. Kay* and D.L. Traber. Dept. Anesthesiology, UTMB, Galveston, TX 77550.

Seven sheep were instrumented for CI, PAP, MAP, and LAP and given 0.5 grams/kg of salt poor albumin over 5 min. Parameters were followed for 30 minutes. Ca^{++}, COP, and ABG's were measured. SVRI, PVRI and LVSWI were calculated. Equal volumes N.S. were given to the same sheep as controls.

Albumin significantly ($p<0.05$) increased MAP for 6 min, PAP for 20 min, LAP for 30 min, SVRI for 10 min, PVRI over the 3 to 10 min interval and COP for 30 min. CI decreased over 3 to 10 min as did Ca^{++} for 30 min. HR and LVSWI did not change. Except for LAP, all changes were different from the N.S. group ($p<0.05$).

Ibuprofen, 14 mg/kg, prior to albumin blunted the albumin response. In conclusion, albumin produces myocardial depression. This appears to be due to both decreased Ca^{++} levels and increases in R and L heart afterload probably mediated by prostaglandins.

38.9

PRESSURE IN THE TRAPPED GAS SPACE DURING LUNG INFLATION. D.G. Frazer, C.E. Turick, J.J. Morgan, G.N. Franz, E.L. Petsonk. DRDS, ALOSH, NIOSH, CDC, DHHS, and Dept. of Physiol., WVU, Morgantown, WV. 26506

Gas was trapped in excised rat lungs by ventilating them at a slow inflation-deflation rate. Lungs were then ventilated upside down in a saline filled plethysmograph (CA), see fig 1. Inflation-deflation was achieved by increasing-decreasing transpulmonary pressure, PL, as chamber (CB) was lowered-raised. In this system the sum of the gas volumes in CB and the lung, V_{TOT}, remained constant during an inflation-deflation cycle. When there was trapped gas in the lung V_{TOT} consisted of two parts: free gas in the lung and chamber, and trapped gas in the lung (V_{tg}). Results showed that during lung inflation tracheal (free) gas pressure, Pao, was > 0.0; i.e., the gas in the free gas space was compressed with respect to the gas in the trapped gas space. This is consistent with previous results which indicated that free gas diffuses through menisci into the trapped gas space during lung inflation. (Supported in part by DOE contract# DE-AT-21-79 MC 11284)

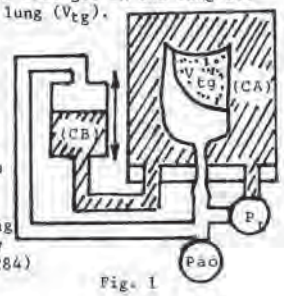


Fig. 1

38.10

COMPARATIVE ASPECTS OF THE DYNAMICS OF BREATHING IN NEWBORN MAMMALS. Jacopo P. Mortola, McGill Univ., Dept. of Physiology, Montreal, Canada H3G 1Y6.

Static and dynamic properties of the respiratory system have been studied in anesthetized tracheostomized newborns of six species, ranging in size from rats to piglets. Compliance (C_{RS}), total resistance (R_{RS}), expiratory time constant (τ) have been measured in the paralyzed passively ventilated animals. C_{RS} is found to be proportional to $BW^{0.80}$ and R_{RS} to $BW^{-0.75}$; τ is independent of body size, the shortest value being in kittens and guinea pigs, and around 0.14 s in the other species. Including the upper airway resistance, τ becomes approximately 0.22 s. This value is similar to the expiratory time of the fastest breathing species, therefore in the smallest species the high breathing rate can be regarded as a mechanism to raise end expiratory level. On a few occasions, dynamic lung compliance and pulmonary resistance, measured in spontaneously breathing kittens, puppies and piglets, were respectively smaller and larger than C_{RS} and R_{RS}, suggesting that the hysteresis of the pressure-volume curve may be substantial. R_{RS} was almost linear within the volume and flow range investigated, the Rohrer's constant K_2 being always less than 2.5% of K_1. The Reynolds' number increases with body size ($\alpha BW^{0.51}$) more than predictable from the changes in tracheal diameter, since the tracheal flow velocity is not an interspecific constant. In all the species the respiratory work seems to be very close to the theoretical minimum. (Supported by the Medical Research Council of Canada).

38.11

PLEURAL LIQUID PRESSURE (Ppl) MEASURED THROUGH AN IMPLANTED RIB CAPSULE IN ANESTHETIZED DOGS. J.P. Wiener-Kronish*, M.A. Gropper* and S.J. Lai-Fook. Cardiovasc. Res. Inst., Depts. of Physiol. and Med., University of California and S.F.G.H., San Francisco, CA 94143.

In 6 anesthetized dogs (28 ± 3.3 kg), we bonded two liquid-filled capsules (6 mm dia.) into the 4th and 6th ribs through holes drilled to the parietal pleura approximately 3 and 12 cm in height (h) above the most dependent part of the lung. We punctured the parietal pleura with 18 gauge blunt liquid-filled needles to allow continuity between capsular and pleural liquids. We measured Ppl (cmH_2O) via the capsule at end-expiration during spontaneous breathing in the supine and prone positions. No pneumothoraces were detectable at post-mortem. The data are summarized in the table (mean ± S.D.).

Supine			Prone		
h	Ppl	ΔPpl/Δh	h	Ppl	ΔPpl/Δh
2.3±2.0	-1.2±1.1	.46±.17	3.9±2.1	-3.0±1.8	.23±.26
11.8±2.1	-6.2±1.2		13.0±2.8	-5.0±1.8	

Prone, the vertical gradient of pleural pressure averaged less than when supine and showed more variability; possibly due to chest deformation. The supine gradient is similar to that reported using liquid-filled catheters in the pleural space (JAP 21:1500, 1966). The data do not support the concept of a pleural liquid pressure gradient equal to 1 cmH_2O/cm height. [Supported in part by HL25816 (PPG) and HL29054].

38.12

CONTROL OF CHEST WALL DISTORTIONS DURING LOADED BREATHING. E. Ringel*, S. Loring, J. Mead and R.H. Ingram, Jr. Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA 02115.

In 5 men, we recorded chest and abdomen motions, pleural (Ppl) and transdiaphragmatic (Pdi) pressures, and sternocleidomastoid (SCM) and abdominal wall (ABD) EMG signals during inspiration against a fixed orifice resistor. Uncoached inspiratory efforts (UIE) to target pleural pressures produced large tidal volumes, and an initial lateral (LAT) increase followed by enlargement of anteroposterior (AP) chest wall. Greater time lags between AP and LAT motion and greater SCM EMGs occurred with greater ΔPpl. Predominantly diaphragmatic inspirations (ΔPpl≈ΔPdi) resulted in small tidal volumes, absent SCM and ABD EMG activity, and decreased AP and increased LAT diameters. Non-diaphragmatic inspiration (ΔPdi≈0) resulted in intermediate tidal volumes, greater SCM EMG, and a chest wall pattern similar to that of UIE. Inspiration with tensed abdominal muscles (and probably intercostals as well) resulted in high ΔPdi, ΔPpl comparable to UIE, large SCM EMG, normal or large tidal volumes, and virtually no lateral chest wall motion. This effect was independent of ABD diameter. We conclude that various voluntary activation patterns of abdominal, diaphragmatic, accessory, and lower chest wall musculature can profoundly affect shape and motion of the rib cage during loaded breathing. Supported by NIH Grants HL 16463, HL 07010 and HL 00943.

38.13

MEASUREMENT OF COMPLIANCE IN SACRIFICED RABBITS. J. Toyoshima*, B. Shon* and J. Seidenfeld* (SPON: B. Burrows). Veterans Administration Medical Center, Tucson AZ 85723

Lung compliances (C_L) normal rabbits were measured under different conditions to find a method suitable for use in the development of an animal model of interstitial lung disease with consistent physiological and pathological correlations to human disease. New Zealand White rabbits (mean weight=2.5 kgs) were prepared with lungs excised (EL, n=3), chests open (OC, anterior thorax removed, n=10) or chests intact (IC, endotracheal intubation, n=8). Volume histories were standardized by fully inflating and deflating the lungs 3 times. Fully inflated lungs were deflated by 5cc increments and pressure changes recorded by a direct writing 4 channel recorder. C_L was taken as the slope of the linear portion of the pressure-volume curve. EL values ($\bar{x}$=9.2 ±0.7 cc/cm water) were significantly higher than either OC ($\bar{x}$=5.5±1.0) or IC ($\bar{x}$=5.0±1.0) values. IC compliance values obtained from rabbits exposed to sublethal doses (250mg/100cc distilled water) of aerosolized paraquat were significantly lowered ($\bar{x}$=1.7±0.6, n=7) and correlated with increased pathological changes in lung tissue sections, a decreased percent of lavaged pulmonary macrophages and increased lung weight. The clear difference between normal and abnormal lungs imply C_L measurement can be used as an indicator of disease. The IC method is the most efficient in terms of time and effort, and the similarity of IC and OC results indicate IC is the method of choice. (Supported by VA funds)

38.14

SENSITIVITY OF FORCED EXPIRATORY TESTS TO EMPHYSEMA IN RATS. Joe L. Mauderly and W. C. Griffith*. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185

Previous studies suggested that expiratory driving pressure might influence the sensitivity of maximal expiratory flow-volume (MEFV) tests and that partial expiratory flow-volume (PEFV) tests might be more sensitive than MEFV to emphysema in rats. Tests were performed before and 4 weeks after instillation of elastase (9 rats) or saline (10 rats). Non-forced tests included lung volumes, dynamic and quasistatic lung mechanics, single-breath N_2 washout and morphometrics. PEFV was done by inflation to 15 cm H_2O transpulmonary pressure (P_{tp}) and deflation at -40 cm H_2O reservoir pressure (P_{res}). MEFV was done by inflation to 30 cm H_2O P_{tp} and deflation at -20, -40, -50, -60 and -80 cm H_2O P_{res}. Group differences between baseline and post-treatment values were compared. Elastase significantly changed lung volumes, quasistatic compliance, gas distribution, closing capacity, morphometrics and all forced expiratory parameters. There was no difference in sensitivities of PEFV and MEFV. As predicted, MEFV flows at higher and lower lung volumes tended to be more sensitive at higher and lower P_{res}, respectively; however, there were no statistically significant P_{res}-related differences in sensitivity of MEFV indices. Thus, sensitivity of MEFV to emphysema in rats was not significantly altered by manipulating driving pressure. (Research performed under U.S. Department of Energy Contract No. DE-AC04-76EV1013.)

38.15

INFLUENCE OF LUNG VOLUME (V_L) HISTORY AND INCREASED SURFACE FORCES ON COLLATERAL RESISTANCE (Rcoll). R. Kikuchi* and J. Hildebrandt. Virginia Mason Res. Ctr., Seattle, WA 98101.

Primary sites of Rcoll and its dependence on V_L and recoil (P) remain uncertain. We studied excised canine lower lobes during static inflation (I) and deflation (D) by negative Ppl in controls (peak P=30cm H_2O) and after stiffening by ventilation for 1 hr at 15°C (peak P=38-40). A double lumen catheter was sealed into a branch off the main bronchus. Air was injected through one lumen ($\dot{V}$coll) to maintain segmental pressure (Ps) monitored through the other lumen at 2 cm H_2O, then Rcoll= Ps/$\dot{V}$coll. Time constants (Tcoll) of segments discharging through Rcoll were obtained from exponential decays of Ps after stopping $\dot{V}$coll. Rcoll vs.V_L showed little hysteresis (H), in contrast to Rcoll vs P (R higher during I). Gcoll=1/Rcoll was nearly linear vs V_L:Gcoll≈K(V_L-Vo). Stiff lungs had about 2X the slope (K) of controls but similar Vo (about 20% TLC). Thus, in each state, Gcoll depended primarily on V_L, but a changed state (stiff) may have enlarged existing channels or recruited new ones. Dependence of Gcoll only on V_L, despite 2-3 fold differences in P, occurred because H for G vs. P was similar to H for V_L vs P, suggesting that most of Rcoll also resides at the parenchymal level. Unlike Rcoll, Tcoll exhibited more H when plotted vs V_L than vs. P. Thus, H for effective segmental compliance (C_S=Tcoll/Rcoll) was large in either plot (C_S lower during I). C_S on D limbs paralleled lobe compliance. Tcoll was much reduced in stiff lobes. Rcoll vs. V_L and P somewhat resembles bronchial diameter behavior. Support: NIH HL14854.

38.16

SULFURIC ACID AND FLY ASH INHALATION: EFFECTS ON TRACHEAL MUCOUS CLEARANCE, THE MUCUS BLANKET, AND AIRWAY FLUIDS. Ronald K. Wolff and Rogene F. Henderson*. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185

Guinea pigs were exposed for 6 hours to 1 or 10 mg H_2SO_4/m^3 alone or in combination with 70 mg fly ash/m^3. Tracheal mucous velocities were measured and lung damage was evaluated by analysis of enzymes and cytology of lung washings at 1 day and 1 week after exposure. Tracheal mucous velocities were slowed at 1 day after exposures to 1 mg H_2SO_4/m^3, either with or without fly ash ($p < 0.1$ and $p < 0.05$, respectively). No significant changes were found at other times or concentrations, although a tendency towards increased clearance was noted at high concentrations of H_2SO_4. Scanning electron micrographs showed a thickening of the mucus blanket and a more patchy distribution of mucus 1 day after exposures to H_2SO_4. Most changes resolved after 1 week. Minor changes were found in airway fluid; the only significant change was an increase in protein 1 day after fly ash exposures, both with and without H_2SO_4 ($p < 0.05$), indicating increased alveolar permeability. These studies showed subtle responses to acute, high level exposures: mucous clearance was affected by H_2SO_4, not by ash; and airway permeability was affected by ash, not by H_2SO_4. (Research supported by U.S. Department of Energy and Environmental Protection Agency under Contract No. DE-AC04-76EV01013 and interagency agreement EPA-IAG-DS-E61.)

38.17

INVITRO EFFECT OF ALUMINA AND ZINC OXIDE PARTICLES ON DIPALMITOYL LECITHIN. Kenneth C. Weber and Jane Ma*. NIOSH, Morgantown, WV. 26505

Dipalmitoyl lecithin (8 μgr), obtained from Calbiochem, was dissolved in chloroform and spread on the surface of a Kimray-Greenfield surfactometer filled with approximately 42 cc of 10^{-4}M EDTA and .01 M phosphate buffer (pH 7). The surface tension vs surface area was recorded for four cycles at a speed of 5.5 min per cycle with the surface area changing from 100% (52.5 cm^2) to 15%. To determine the effects of particle surface area on dipalmitoyl lecithin in the air-liquid interface, alumina (3.04 and 81.4 m^2/g and 90 mesh) and zinc oxide (0.62 and 3.9 m^2/g) particles and zinc powder (200 mesh) (obtained from Duke Standards) were independently and in increasing quantities sprinkled onto the air-liquid surface. The surface tension vs surface area was recorded for an additional four cycles after each addition of particles. In each case, the amount of material required to reduce the surface tension-surface area hysteresis increased as the particle surface area per g decreased. For respirable size alumina and zinc oxide particles, the hysteresis decreased by greater 90% with less than 5 mg added to the surface. For non respirable size particles (90 and 200 mesh) it required more than 40 mg. The minimum surface tension always increased with the addition of particles, but the changes did not always correspond to the surface area per g of the particles.

38.18

TECHNIQUE FOR PRODUCTION OF HOLLOW CASTS OF THE CENTRAL AIRWAYS. C.J. Pedersen†, J. W. Watson†, and A.C. Jackson. Calif. Primate Res. Ctr. Univ. of Calif., Davis, CA. 95616.

We have developed a method for producing ridged hollow casts of the central airways. Wood's metal (melting point 70°C) is poured into the airways at the trachea of an excised canine lung air dried at a transpulmonary pressure of 30 cm H_2O. The tissue is dissolved and removed. The resulting negative cast is carefully trimmed leaving only airways with a diameter of 2 mm or greater. The resulting negative cast is electroplated with copper which provides a ridged jacket encasing the woods metal. Heating this structure to 100°C liquifies the wood's metal allowing the majority of it to flow out. Residual amounts are readily evacuated by a high pressure steam hose attached to the trachea. Comparisons were made between airway diameters from in vivo bronchograms (D_B) and direct measurements of the cast (D_C). Linear regression analysis of these data indicated a high degree of correlation (D_C = .98 D_B + .03, r = .99). We conclude that using this technique can produce positive casts that accurately represent the airways. Supported by NIH grants HL-25631 and HL-26606, and EPA grant 807661.

38.19

CONTINUOUS ON-LINE MEASUREMENT OF INERT GASES IN ARTERIAL AND MIXED VENOUS BLOOD. Gwen E. Gale* and Peter D. Wagner. Dept. of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093

Physiologically "inert" gases are useful for studying gas exchange as their solubilities do not vary with partial pressure and they can be detected in trace quantities. Until now, measurement of these gases in blood has required an intermediate step of equilibration with gas, limiting experiments by sampling and analysis time. We have developed a method for real time measurement of these gases in both arterial ($\bar{a}$) and mixed venous ($\bar{v}$) blood using a quadrupole mass spectrometer (MS). Both $\bar{a}$ and $\bar{v}$ blood from an anesthetized dog are pumped continuously at ≤15 ml/min either through a cuvette or a bypass line. Flow to the cuvette is automatically switched between $\bar{a}$ and $\bar{v}$ at an adjustable rate. The cuvette (Fed.Proc. 40:593, 1981) has a volume of 4 μl and a 25 μm silastic membrane through which gases diffuse to the MS inlet. The gases are detected without overlap at these mass/charge ratios: SF_6:127, krypton:84, freon-12:85, enflurane:117, diethyl ether:59 and acetone:58. All gases have signal to noise ratios >20 to 1. The signals obtained are insensitive to flows >8 ml/min and 95% response times are 10-15 seconds. This approach provides continuous measurements of: 1) steady state inert gas retentions, 2) the time course of changes in retentions in response to experimental perturbations, 3) transient changes in gas concentrations in order to estimate the distributions of tissue and gas volume in the lungs in relation to ventilation and perfusion. (Supported by HL 17731 and HL 07212)

38.20

LUNG ANTIOXIDANT PROFILE IN MONKEYS EXPOSED CHRONICALLY TO CIGARETTE SMOKE. Anthony J. DeLucia, J.R. Dunbar,* J.H. Whitaker,* M.G. Read,* and L.R. Bryant.* *East Tennessee St. Univ., Johnson City, TN 37614.

Cigarette smoke (CS) and phagocytic cells recruited to the lungs of smokers contain a wide variety of potent oxidants. Both sources put a premium upon antioxidant pathways in the lungs of smokers. After 4-8 years of CS exposure of male stumptailed macaques (Macaca Arctoides), 7 low-dose (L) animals (5,000-8,000 cig.), 6 high-dose (H) animals (11,000-21,000 cig.), and 7 controls (C) were killed, allowing measurement of the tripeptide glutathione and other sulfhydryls and antioxidant enzymes in lung tissue. The left lung was homogenized and assayed for total (TSH) and nonprotein (NPSH) sulfhydryls. A 9000 x g. supernatant was prepared for assay of the following enzymes: Glucose-6-phosphate (G6PD) and 6-phosphogluconate dehydrogenases (6PGD), glutathione reductase (GR), glutathione peroxidase (GP), and glutathione-S-transferase (GST). Lung SH pools were not significantly different among CS-exposed and control animals; however, average levels of TSH, oxidized glutathione, and mixed disulfides of protein SH and NPSH were 5-25% higher for both smoking groups. Enzyme activities were also increased by CS.:

	G6PD	6PGD	GR	GP	GST	
H	9±3 (150)	32±9 (118)	79±6 (123)*	20±8 (143)	152±58	(124)*
L	6±1 (100)	35±7 (130)*	70±9 (109)	21±6 (150)*	110±47	(88)
C	6±3	27±10	64±11	14±3	124±23	

Values given as mean ± S.D.; per cent of C in parentheses; (P<.05)*

The increased metabolic activities observed in lungs of CS-exposed groups may relate to greater need for lung antioxidant protection in smokers due to the continuous insult of that tissue by exogenous (CS-derived) and endogenous (phagocytic oxidative metabolites) oxidants. (Supported by the Tobacco and Health Research Institute)

38.21

PULMONARY SURFACTANT CHANGES FROM STELLATE GANGLION STIMULATION. David L. Beckman and Daniel J. Crittenden.* East Carolina University, Greenville, NC 27834

Previous studies have shown that stellate ganglion stimulation (SGS) in cats resulted in decreased lung compliance, increased surface tension and decreased cholesterol (CHOL) relative to disaturated phosphatidylcholine (DSPC). These changes after 1 to 6 min SGS were prevented by alpha receptor blockade. Maintenance of proper proportions of CHOL to DSPC may facilitate respreading of the surface film. In the present study cats were exposed to SGS for 10 or 30 sec and also pretreated with the alpha agonist phenylephrine (PHE) (0.1-0.2 mg/kg SC) 30 min prior to lung lavage. CHOL in controls was 0.23 ± .03 (SE) mg/g wet lung (n=5), in 10 sec SGS 0.34 ± .02 (n=5; p<.01), in 30 sec SGS 0.19±.05 (n=5), and in the PHE cats 0.21±.03 (n=3). Release of DSPC and possibly CHOL from type II cells has been shown to be under beta receptor influence, and the present data suggest that CHOL release is maximal within the first 10 to 20 sec of sympathetic discharge. Return of CHOL to normal values after 30 sec SGS suggests that SGS may also activate mechanisms tending to deplete alveolar CHOL. Similar pulmonary changes are associated with the sympathetic discharge from cerebral traumatization. The present results suggest that the CHOL/DSPC ratio is influenced by sympathetic stimulation, with possible implications for lung compliance. (Supported in part by NIH grant 1F32HL06254-01.)

38.22

PHOSPHATIDYLGLYCEROL IS PROBABLY NOT NECESSARY FOR NORMAL LUNG SURFACTANT FUNCTION. Osvaldo S. Beppu*, John A. Clements, and Jon Goerke. Cardiovascular Research Inst., and Depts. of Ped. and Physiol., Univ. of Calif., San Francisco, CA 94143

We have investigated the effects of substituting phosphatidylinositol (PI) for phosphatidylglycerol (PG) on the functional properties of rabbit lung surfactant. We gave oral 10% glucose solution for 3 days to 11 rabbits and 10% inositol to 12 others. Lung lavage composition (% total phospholipid±SEM) was normal in both groups, except for PG which was higher in the glucose group (5.6±0.3 vs 0.3±0.1), and for PI which was higher in the inositol group (8.5±0.4 vs 3.2±0.7). Arterial blood gases (PaO_2, $PaCO_2$, $P(A-a)DO_2$ and pH) breathing air and 100% O_2 were normal in both groups. Pressure-volume curves of excised lungs gave normal values for pressure at 40% TLC on deflation for both air- and saline-inflated lungs from both groups. The time course of surfactant adsorption to an air-water interface was similar in both groups, requiring 6.9±1.8 sec (mean±SEM) to reach a surface pressure of 30 $mN \cdot m^{-1}$ in glucose-treated rabbits and 4.2±1.1 sec in inositol-treated animals. Maximum surface pressure after compression was 66.0±0.8 $mN \cdot m^{-1}$ (mean±SEM, max=70 at 37C) in the glucose group and 67.1±1.3 $mN \cdot m^{-1}$ in the inositol group.

The results suggest that PI may substitute for PG in maintaining normal arterial blood gases, normal pressure-volume relationships and normal in vitro surfactant properties. (Supported in part by NHLBI-24075 from the NIH. JAC is a Career Investigator of the American Heart Association).

38.23
REPLACEMENT OF DIFFUSIVE BY CONVECTIVE GAS TRANSPORT IN THE DEVELOPING HEN'S EGG. A.H.J. Visschedijk and H. Rahn. State University of New York at Buffalo, Buffalo, N. Y. 14214.

Comparison of diffusive and convective transport equations for O_2, CO_2 or H_2O in the gas-phase shows that for the same gas flux ($ml \cdot day^{-1}$) and the same pressure difference (torr) one can substitute an effective ventilation, $\dot{V}_A$ ($ml \cdot day^{-1}$) for a given diffusive conductance, G, ($ml \cdot day^{-1} \cdot torr^{-1}$). The theoretical relation is: $\dot{V}_A = (RT) \cdot G$. Thus a ventilation of 865 $ml \cdot day^{-1}$ (BTPS) is required for each unit of G. This relation was tested in the developing chick embryo (day 15 to 19) by continuously ventilating the air space beneath the shell while the egg was maintained at 37.8°C in air or submersed. During this exposure normal development, oxygen uptake, and gas tensions were maintained, verifying the theoretical prediction.

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38.24
EFFECT OF HEIGHT ON SPECIFIC DIFFUSING CAPACITY OF THE LUNGS FOR CO (D_L/V_A) IN HEALTHY NON-SMOKERS. Edith Rosenberg, Department of Physiology and Biophysics, College of Medicine, Howard University, Washington, D.C. 20059

The single breath measurement of diffusing capacity is a simple, non-invasive test of the extent of the alveolar capillary membrane available for gas exchange but failure to establish reproducable norms has limited its usefulness. It seems likely that D_L/V_A will be a more reproducable expression of the same measurement (Lung 157:23-29, 1979). This study is part of an attempt to establish normal values for D_L/V_A. Data from fifty-four normal women and forty-three normal men who had never smoked and had $FEV_1/FEV \geq 79\%$ was examined. They were part of a multidisciplinary investigation of genetic and environmental factors in COPD conducted by Bernice H. Cohen of the Department of Epidemiology, Johns Hopkins School of Hygiene and Public Health. The mean of two measurements of D_L/V_A during one study from each subject was used. Regression of D_L/V_A vs age and height, revealed that D_L/V_A appeared to vary inversely with height as well as with age. Examination of the data by decades showed clear decreases of D_L/V_A with increases in height. In women between 30 and 39 years of age the decrease in D_L/V_A was 6% per inch of increase in height ($p < .025$). For men between 20 and 39 years the decrease in D_L/V_A per inch increase in height was 4.8 % D_L/V_A ($p < .005$). The effect of height on D_L/V_A suggests that in tall people a larger fraction of the total gas exchange surface is in the alveolar duct region than in short people.

38.25
EFFECT OF LOWERED P_{50} ON ALVEOLAR END-CAPILLARY EQUILIBRATION DURING NORMOXIC AND HYPOXIC TREADMILL EXERCISE IN THE DOG. A.J. Suggett*, P.T. Schumacker*, P.D. Wagner and J.B. West. Dept. of Med., U. of Calif., San Diego, La Jolla, CA 92093

Theoretical work from this laboratory has predicted that a leftward shift of the oxyhemoglobin dissociation curve may improve systemic O_2 transport at high $\dot{V}O_2$ at high altitude when diffusion limitation to O_2 equilibration in the lung is present. This is due to the ability of a left-shifted curve to reduce the contribution of diffusion to the alveolar-arterial O_2 difference. To test these predictions experimentally, we studied gas exchange in male, tracheostomized dogs at rest and during graded exercise (9 km/hr; 0,5,10 and 15% incline) on a treadmill. Studies were conducted at an FI_{O_2} of 0.21, .12 or 0.10, with measurements made at normal P_{50} (27-29 torr) and after the Hb-O_2 curve was left-shifted (P_{50}=17-19 torr) with Na cyanate. PaO_2 did not fall from resting levels during exercise, even at low FI_{O_2}. $(A-a)DO_2$ was small at rest (4-9 torr) and did not increase significantly during exercise at any FI_{O_2} level. Left-shift of the dissociation curve improved arterial saturation as expected, but did not change PaO_2 or $(A-a)DO_2$ as a function of $\dot{V}_{O_2}$. Since little diffusion limitation for O_2 was present, even at high $\dot{V}_{O_2}$ (60-70 ml/kg·min) with low PAO_2, lowered P_{50} did not improve gas exchanging efficiency. Hence, further study of the effects of decreased P_{50} on gas exchange may require a model exhibiting greater diffusion limitation for O_2 during hypoxic exercise. (Supported by Grants HL 27410, HL 17731 and MRC, London)

38.26
EVALUATION OF REGIONAL DISTRIBUTION UPON GAS INHALATION TESTS FOR MEASUREMENT OF CARDIO-PULMONARY FUNCTION. A. F. Wilson and J. H. Roum, Department of Medicine, University of California, Irvine, Orange, CA 92668
SPON: V. D. Minh

The major problem associated with measurement of cardiopulmonary function by gas inhalation techniques is the unknown effects of regional distribution of ventilation, blood flow ($\dot{Q}c$), diffusion, (DLCO) and tissue volume (Vt) upon gas absorption. This problem would presumably be greatest with a single breath (SB) technique, which utilizes most of the expirate. Since it is generally accepted that multiple single breath (MSB) techniques either overcome or allow evaluation of this problem, a comparison of the two techniques would provide a basis for interpretation of the limitations of SB. MSB requires analysis of multiple breaths in which only breath holding time varies. SB is based upon the fact that DLCO and $\dot{Q}c$ are inversely proportional to the rate of change of lung volume during constant expiratory flow. MSB and SB measurements were made using a mass spectrometer, rolling seal spirometer, and a mini-computer. MSB and SB data was collected in 6 normal males; MSB was analyzed at several isovolumes; As lung volumes declined from 90 to 30% of vital capacity, DLCO and Qc increased about 40% and Vt decreased. MSB and SB values were identical at 50-70% of vital capacity. These data indicate that $\dot{Q}c$ and DLCO are regionally distributed similarly but not identically throughout the lungs and that values for SB and MSB are comparable at certain lung volumes.

38.27
INFLUENCE OF TEMPERATURE AND HEMOGLOBIN SATURATION ON PARTITION COEFFICIENTS FOR THREE GASES USED FOR INERT GAS ELIMINATION STUDIES. Robertson HT, HP McKenna* and MP Hlastala. Univ. Wash. Seattle, WA 98195.

Ostwald partition coefficients (λ) for mongrel dog blood were measured for acetylene (λ_{37°=.911), Freon-22 (λ_{37°=.886) and Forane (λ_{37°=1.281) at extraction temperatures of 32°, 37° or 43°C with both oxygenated and deoxygenated blood. Gas extractions were performed with room air for the oxygenated samples and nitrogen for the deoxygenated samples. Inert gas concentrations were measured with a quadrupole mass spectrometer, and results were expressed as λ_T(ml gas at temp T) · (ml blood)$^{-1}$. The temperature data were linear when plotted as ln λ versus $\frac{1}{T}$(°K). For λ measured at 37°C, conversion to appropriate inert gas partial pressures for an animal at 40° requires a correction factor (K_T) of 1.052 for acetylene, 1.107 for Freon-22, and 1.166 for Forane. K_S, the ratio of the partition coefficient of an inert gas in desaturated blood to that in oxygenated blood, was 1.03 for acetylene, 1.19 for Freon-22 and 1.12 for Forane. K_S demonstrated no significant temperature dependence, and was not different from unity for any of the three inert gases dissolved in saline. While K_T and K_S do not significantly alter the location of inert gas data points on the λ axis, excretion values must be corrected by $1/K_T$. Retention and excretion values for high K_S gases will be artificially elevated by decreases in $S\bar{v}O_2$.

38.28
ABNORMALITIES OF VENTILATION AND DEAD SPACE IN CHRONIC HEART FAILURE -- A POSSIBLE CAUSE OF DYSPNEA. Stanley A. Rubin*, Harvey V. Brown*, Mark Nathan*, Deirdre Siemienczuk*, Howard Goldberg, H.J.C. Swan. Cedars-Sinai Medical Center, Los Angeles, Ca. 90048

Increased minute ventilation ($\dot{V}_E$) is found in patients with heart failure, and the need to increase $\dot{V}_E$ has been proposed as one of the mechanisms that contributes to the sensation of dyspnea. Therefore, we investigated ventilation, gas exchange and hemodynamics in 19 severe chronic heart failure patients at rest and during cycle ergometery in order to define the factors which lead to increased $\dot{V}_E$. At rest, we found an increased dead space to tidal volume fraction (V_D/V_T) of 0.52±0.08 (mean±SD), while $\dot{V}_E$ was increased at rest to 12.5±4.1 $l \cdot min^{-1}$ and arterial carbon dioxide tension ($PaCO_2$) was maintained at 37±6 torr. Cardiac index (CI) was decreased to 1.9±0.4 $l \cdot min^{-1} \cdot m^{-2}$ and pulmonary capillary wedge pressure (PCW) was increased to 21±9 mmHg. When $\dot{V}_E$ increased during exercise in order to meet the needs of metabolic carbon dioxide elimination (609±306 $ml \cdot min^{-1}$), V_D/V_T remained abnormally increased at 0.44±0.07, while $PaCO_2$ fell to 32±7 torr. CI and PCW remained persistently abnormal. Therefore, in order to maintain carbon dioxide balance, ventilation is increased in heart failure patients at rest and during exercise because of increased dead space. Additional increases in minute ventilation occur during exercise, probably from hemodynamic abnormalities, and this causes hypocapnia. Although we do not know the exact cause of increased dead space in heart failure, it is an important ventilatory abnormality which may contribute to dyspnea.

38.29

ENDOTRACHEAL INTUBATION OF THE RAT USING A LARYNGOSCOPE WITH AN INFANT (SIZE 0) STRAIGHT BLADE. *C.F. Schaefer, D.J. Brackett, P. Downs,* J.L. Scates,* P. Tompkins,* and M.F. Wilson. Depts. of Anesth. & Med., U. Okla. Hlth. Sci.Ctr. & VAMC, Oklahoma City, OK 73190*

The desire to use modern gas anesthetics for surgery in the rat prompted us to develop a simple, reliable method of endotracheal intubation for the rat. Many helpful ideas were found in the literature, but every technique required construction of some device to perform the intubation. We found that an off-the-shelf device, the laryngoscope fitted with an infant-sized straight blade, enabled us to visualize the rat's cords and insert the cannula (9.5 cm length of beveled PE 240 tubing) with relative ease. We routinely use enflurane (E) in oxygen and nitrous oxide (1:1). Induction is achieved within 15-20 min. in a chamber supplied with 5% E. The rat is then quickly removed, held on its back with its jaws open and tongue extended by one person while the other person inserts the tube using the laryngoscope in the usual way. A rodent respirator is used to deliver maintenance anesthesia (2% E). To date over 200 successful rat intubations have been made with this technique using the modern potent halogenated agents which provide stable, repeatable and adjustable stages of anesthesia, rapid recovery and remarkable safety. (Supported by the Anesth. Res. Fund and the Veterans Administration Research Service)

38.30

VARIATIONS OF SINGLE BREATH CO_2 EXCRETION CURVES DURING ANESTHESIA AND SURGERY. Frances E. Noe*, Albert J. Whitty and Kenneth R. Davies*. Sinai Hospital of Detroit, Detroit, MI 48235

Microcomputer-generated single breath curves derived from the gas flow and CO_2 concentration curves recorded during anesthesia and surgery show distinct changes from the normal awake curve and these changes can be related to the ventilation and perfusion of the lung. The initial published study of these curves showed changes with hyperventilation and hypoventilation of normal awake subjects and awake patients with chronic emphysema and obesity. Online microcomputer cross-product integration of expired air flow rate and CO_2 concentration from an infrared analyzer at a sampling rate of 40 Hertz is used to generate a curve of continuous CO_2 volume from the expired breath. The curves are normalized to permit comparison of curve forms. Inverse curves show the variations in dead space of the lungs with changes in ventilation and perfusion. Series of curves will be presented with plots of air flow volume with time and CO_2 excretion with time and with air flow volume from normal awake spontaneously breathing subjects and from anesthetized mechanically ventilated human breaths. Curves will be shown from patients before and after cardiac bypass, and with occlusion of the right pulmonary artery, and with acute hypotension. (Supported in part by Michigan Heart Association).

38.31

SAMPLING SITE FOR "MIXED VENOUS" BLOOD IN DOGS — PULMONARY ARTERY OR RIGHT VENTRICLE? Paul F. Beeman* and Harold I. Modell. Virginia Mason Research Ctr, Seattle, WA 98101

It is generally assumed that pulmonary arterial blood best represents mixed venous blood. Under some conditions (e.g. during high +Gz stress) sampling from this site is difficult. Shapiro et al. (Anesthesiology 40:291, 1974) have shown that pulmonary capillary (PC) blood may be drawn back into samples at this site in dogs. To determine if the right ventricle (RV) is as good a sampling site as the pulmonary artery (PA), a 7 Fr Swan-Ganz catheter was placed in the PA, and a similar sized catheter with multiple side holes was placed in the RV of 6 pentobarbital anesthetized, adult mongrel dogs. Catheter placement was determined by monitoring pressure profiles, and care was taken to position the Swan-Ganz tip just beyond the pulmonary valve. Ten PA-RV pairs of blood samples were drawn from each animal for blood gas comparison. The samples in each pair were drawn sequentially, and the sampling order (PA-RV, RV-PA) was alternated with each pair. Blood gas analysis on each sample was done in duplicate, calibration being checked after each sample. Data were compared by paired t-test. Po_2 ranged from 35 to 50 Torr, Pco_2 from 35 to 55 Torr, and pH from 7.3 to 7.4. Mean PA Po_2 was 0.8 Torr higher than mean PV Po_2 ($P<.005$), but no statistically significant differences in Pco_2 or pH were detected. No physiological importance could be assigned to the small Po_2 difference. Because RV sampling minimizes chance of the PC blood contamination described by Shapiro et al., it may be the sampling site of choice in dogs. Whether this is also true in other species remains to be determined. (Supported by AFOSR Contract F49620-81-C-0055)

38.32

CSF IONS AND ACID BASE BALANCE DURING MODERATE AND SEVERE HYPERCHLOREMIC ACIDOSIS IN AWAKE RATS. E.E. Nattie. Depart. of Physiology, Dartmouth Medical School, Hanover, N.H. 03755.

CSF acid-base regulation depends on changes in CSF strong ions. This paper tests the hypothesis that if plasma Cl^- is increased sufficiently in an HCl acidosis, the CSF Cl^- will increase and be accompanied by an equal and opposite change in CSF HCO_3^-. Unanesthetized rats were dialyzed ip over 6 hrs with solutions of 150mM NaCl with 30 or 50mM HCl. Plasma Cl^- increased 8 to 20 mmol/l while plasma HCO_3^- decreased 7 to 17 mmol/l. Plasma Na^+ was unchanged but plasma osmolality increased. The hematocrit increased from 43 to 53 due to loss of plasma to the acidified peritoneal space. In CSF, the Cl^- increased 2 to 10 mmol/l but the CSF HCO_3^- decreased only 0.4 to 2.3 mmol/l. The CSF Na^+ increased 1 to 6 mmol/l and the CSF osmolality increased as in plasma. In this particular case the increase in plasma Cl^- is associated with an increase in CSF Cl^- that becomes more prominent as the plasma Cl^- is greater. However, the CSF HCO_3^- changes little even though the plasma HCO_3^- decreased up to 17 mmol/l and the CSF Na^+ increases even though the plasma Na^+ was unchanged. These results differ from reports using iv HCl. Possible explanations include: 1) more severe hyperchloremia, 2)the increased Hct, 3)the increase in osmolality. (Supported by HL 18351, 00634, 28066)

38.33

INDOMETHACIN REDUCES CEREBRAL BLOOD FLOW IN FETAL LAMBS: A MECHANISM FOR STIMULATING FETAL BREATHING MOVEMENTS. A. Roger Hohimer, Bryan S. Richardson*, John M. Bissonnette, Cynthia M. Machida*, Sharon J. Knopp*. Oregon Health Sciences University, Portland, Oregon 97201.

Indomethacin (Indo), a prostaglandin synthetase inhibitor, stimulates virtually continuous fetal breathing movements (FBM) in fetal sheep. In order to investigate the mechanisms which might mediate this drug's effect, we studied 13 fetal sheep (122-132 days gestation) before and after a 4 hour infusion of Indo (15 mg/kg bolus, 2 mg/kg/hr). We measured axillary artery (a) and saggital vein (v)(10 animals) blood gases, pH's, oxygen contents ($C\ O_2$, mM). CBF (ml/min·100g) was measured with microspheres.

	CBF	pHa	PaO_2	$PaCO_2$	CaO_2	pHv	$PvCO_2$	CvO_2
Control	187	7.39	18.6	47.4	3.15	7.35	51.2	2.28
	±19	±.01	±0.7	±0.5	±0.17	±.01	±0.6	±0.15
Indo	141	7.33	17.2	48.0	2.72	7.31	52.9	1.42
	±18	±.02	±.8	±0.9	±0.18	±0.01	±0.9	±0.11
P<	.01	.01	.001	ns	.02	.01	.05	.001

Indo induces an arterial metabolic acidosis and a reduction in CBF without a significant change in cerebral metabolic rate. Both of these changes elevate the hydrogen ion concentration in the fetal brain and may stimulate central chemoreceptors and thereby contribute to the stimulation of FBM caused by Indo. (Supported by HD 11251 and HD 10034 from USPHS and MRC of Canada)

38.34

EFFECT OF METABOLIC ALKALOSIS ON CAROTID CHEMOSENSORY AND VENTILATORY RESPONSES TO ACUTE HYPOXIA IN THE CAT. M. Pokorski,* A. Mokashi* and S. Lahiri. University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

Acute metabolic acid-base changes of arterial blood are known to influence ventilation. Whether this effect is mediated through peripheral or central chemoreception mechanisms has long been debated. One experimental approach to settle this question is to investigate the relationship between carotid chemoreceptor activity and ventilation simultaneously in the same experiment. The rationale is that ventilation would be a unique function of carotid chemoreceptor activity if the effect were mediated entirely through peripheral chemoreceptors. We investigated this relationship measuring steady-state carotid chemosensory discharge rates and ventilation at five levels of PaO_2 ranging from 450 to 35 Torr before and after the induction of alkalosis in nine anesthetized cats. Intravenous infusion of $NaHCO_3$ (6 mmol·kg^{-1}) at a constant arterial PCO_2 of 37±2 Torr increased the arterial pH from 7.317±0.026 to 7.582±0.030. This alkalosis was associated with decreases in both chemosensory discharge rates and ventilation at all levels of PaO_2. At any level of carotid chemoreceptor activity due to different levels of PaO_2, ventilation was lower during alkalosis than the control by the same magnitude. Thus, the ventilatory effect of alkalosis is not a single function of carotid chemoreceptor activity: a part of the ventilatory effect is independent of the carotid chemoreceptor response to metabolic alkalosis. Supported in part by grants HL-19737-06 & HL-08899-18.

38.35

PULMONARY DEAFFERENTATION USING A SINGLE STAGE SURGICAL PROCEDURE. P.S. Clifford*, R.L. Coon, J.H. von Colditz*, E.J. Zuperku and J.P. Kampine. Medical College of Wisconsin and Wood VA Center, Milwaukee, WI 53193

This study was undertaken to determine the feasibility of producing a chronically lung-denervated animal using a single stage surgical procedure. The left vagus was exposed through a left thoracotomy and the pulmonary branches were sectioned. Manipulation of the heart and right superior and middle lobes provided access to the right vagus which was transected just caudal to the azygos vein. 5 beagle dogs were denervated (DD) and 5 served as controls (CD). The recovery of the DD was uneventful with only minor, transient gastrointestinal problems. To evaluate the Hering-Breuer inflation reflex (HBIR), the dogs were anesthetized, intubated and connected to a solenoid-actuated device programmed to allow the dog to take 9 spontaneous breaths. At the tenth breath the airways were connected to a constant pressure reservoir. The HBIR was quantified by measurement of the duration of expiration (TE) as a function of tracheal pressure (TP). Based on pooled data, the regression line for the CD is TE=9.039 TP + 0.153 (r^2=0.78) and for the DD, TE=0.360 TP + 4.118 (r^2=0.09). The slopes of these lines are significantly different ($p<.01$). The described procedure involves a relatively uncomplicated surgical technique which has been shown to abolish the HBIR with minimal post-surgical complications. Laryngeal control of respiratory frequency and TE should be unaltered by this intervention. (Supported by the VA).

38.36

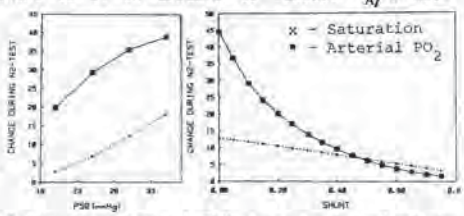
CHARACTERISTICS OF SLOWLY ADAPTING AIRWAY STRETCH RECEPTORS (SARs) IN THE OPOSSUM. J. P. Farber, J. T. Fisher, and G. Sant'Ambrogio, University of Texas Medical Branch, Galveston, Texas 77550.

Location and discharge properties were examined in SARs of 4 adult opossums. Animals were anesthetized using sodium pentobarbital, paralyzed using gallamine, and artificially ventilated with the chest open. Of 37 receptors studied, 30% were located by probing in the trachea while 70% were found to have bronchial locations (27% of bronchial receptors were found in the contralateral lung). While 91% of tracheal receptors showed activity at a transpulmonary pressure (Ptp) corresponding to the FRC, only 54% of bronchial receptors exhibited this characteristic. Of the 13 receptors whose discharge was initiated above the FRC, 12 (92%) were bronchial. Static receptor discharge was determined at Ptp values between 0 and 20 cmH_2O; the average discharge of receptors active at FRC was greater than the higher threshold receptors (i.e. 100 ± 7.1 SE pulses/sec vs. 45 ± 5.4 SE pulses/sec at 20 cmH_2O). Inhalation of 10% CO_2 in air inhibited all 4 tracheal and 6 of 7 bronchial receptors tested. Thus, the opossum, a marsupial mammal shows a distribution of SARs comparable to that of placental mammals. The proportion of contralateral bronchial receptors was large in comparison with other mammals, and static discharge rates in several receptors were very high (up to 176 pulse/sec at 20 cmH_2O). (Supported in part by grants HL-20122 and HL-00619 from NIH).

38.37

EFFECT OF CARDIAC SHUNT AND ALTERED P50 ON THE RESPONSE TO THE FOUR BREATH NITROGEN TEST (N_2-TEST) IN MONGREL DOGS. J.H. von Colditz*, R.L. Coon, S.B. Litwin*, and J.P. Kampine. Depts. of Physiology, Anesthesiology and Thoracic Surgery, Med. Col. Wis., Milwaukee, WI 53193

The N_2-Test is used clinically to assess hypoxic sensitivity in man. In a previous abstract we reported that chronic shunt hypoxemia reduced hypoxic sensitivity in mongrel dogs, as measured by the N_2 test (Fed. Proc. 41:988). To determine what effect shunt and altered P50 had our results a computer model was developed to simulate the N2-test. O_2 Sat, PO2, and O_2-CON were determined using Easton's equation for the O_2-dissociation curve (J. Theor. Biol. 76:335). CvO_2 was assumed remain constant during the test. A pulmonary shunt of 5% is assumed to reflect $\dot{V}_A/\dot{Q}$ mismatch. The following results were obtained:



The N_2-Test may not be an appropriate measure of hypoxic sensitivity when abnormal P50 or a cardiac shunt is suspected. (Supported by the VA).

38.38

·LEUKOTRIENE C_4 (LTC_4) AND INTRAPULMONARY RAPIDLY ADAPTING RECEPTORS (RAR) IN GUINEA PIGS. D.R. Bergren*, D. Myers* and J. Gustafson* (SPON: M.H. Laughlin). Oral Roberts University, Tulsa, OK 74136

We administered LTC_4 by means of aerosol and by i.v. injection while recording RARs. LTC_4 (100ng, i.v.) increased activity of 4 RARs in 4 guinea pigs from 17±24* immediately before injection to 165±64 imp at the peak 1 min nerve activity after injection ($p<0.0025$). That peak was 3.8±3.2 min. Tracheal pressure (P_t) increased concurrently with nerve activity from 7.5±0.4 to 14.7±7.6cm H_2O ($p<0.1$). We gave 3 min aerosols of both saline and LTC_4 to 8 RARs in 8 guinea pigs. We monitored RAR activity and P_t for at least 10 minutes. RAR activity exposed to saline was 135±67 and 801 ± 492 imp/10 min exposed to LTC_4 ($p<0.001$). The peak P_t with saline exposure was 5.9±1.1 cm H_2O. Peak P_t with LTC_4 exposure was 7.2±1.1 ($p<0.025$). However, RAR activity did not correspond to peak P_t with LTC_4 aerosol exposures as did i.v. injection of LTC_4. The pattern of RAR in response to LTC_4 aerosol was a large burst of activity delayed in time (5.1 min. ± 4.1). Due to the latency of increased RAR activity which occurred in the absence of changes in P_t, possibly LTC_4 acts not only on airway smooth muscle directly but acts to degranulate mast cells which in turn release mediators which may stimulate RARs directly.

*± equals standard deviation. +-1 ug/ml

(LTC_4 graciously provided by J. Rokach, Ph.D., Merck Frosst Canada Inc.; and sponsored in part by a grant from the American Lung Association).

38.39

EFFECT OF CHEMICAL STIMULATION AND PHARMACOLOGICAL AGENTS ON THE ELECTRICAL ACTIVITY OF UPPER AIRWAY MUSCLES AND THE DIAPHRAGM IN AWAKE UNSEDATED CATS. M.A. Haxhiu*, J. Mitra*, J. Salamone* and N.S. Cherniack. Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

The effect of hypercapnia and nicotine administration on the electrical activity of the genioglossus (GG), posterior cricoarytenoid (PCA), and the diaphragm (D) was studied in six awake unsedated cats with chronically implanted electrodes. Hypercapnia (inhalation of 3.4% and 7% CO_2 in O_2) increased the phasic electrical activity occurring during inspiration in all three muscles; and also increased the tonic activity of the GG. When total inspiratory activity was expressed as a percent of its control value, activity increased proportionately in the PCA and the D. At 3.4% CO_2 PCA increased by a mean of 43% ± 8 (SE), and D activity by 40% ± 17; at 7% CO_2 PCA increased 106% ± 19 and D 101% ± 18. The change in total activity of the GG was significantly greater than that of the other two muscles, particularly at greater levels of chemical drive (at 3.4% CO_2 percent of change of GG was 91% ± 33, and at 7% CO_2, 620% ± 79). Administration of nicotine either subcutaneously or intravenously in doses of 10 - 200 µg also increased GG activity more (504% ± 86) than that of the PCA (90% ± 36) or D (28% ± 10). The results of this study suggest that the response of the GG to chemical stimuli differs from that of the PCA and D. In addition, the results indicate that pharmacological stimuli can preferentially activate some of the upper airway muscles and may decrease the incidence of obstructive apnea.

38.40

VENTILATORY RESPONSE OF NORMAL AND OBESE RATS TO HYPERCAPNIA AND HYPOXIA. E.H. Schlenker, M. Goldman* and I.A. Mardini*. Depts. of Physiology and Pharmacology and Biology, University of South Dakota, Vermillion, SD 57069

Ventilatory responses to 5% and 10% CO_2 in O_2, and to 13% and 10% O_2 in N_2 were compared in unanesthetized obese rats, who had been injected neonatally with aspartic acid (AS), and control rats (C). The minute ventilation ($\dot{V}E$) in C increased from 123.2±23.8 on air to 171.3±40.5 on 5% CO_2 ($P < .01$) and to 205.1±44.8 ml/sec on 10% CO_2 ($P < .001$). This change resulted mainly from a 34% increase in tidal volume (VT) on 5% CO_2 ($P < .01$) and a 59% increase on 10% CO_2 ($P < .001$) with no significant change in frequency (f). Inspiratory flow rate (VT/TI) increased 106% on 10% CO_2 ($P < .01$). The AS responses were comparable. VE increased from 86.9±13.9 on air to 146.3±29.1 ml/sec on 10% CO_2 ($P < .001$) with a significant increase in both VT, 46% ($P < .01$) and f, 15% ($P < .01$). The increase in VI/TI was 70% ($P < .01$). Exposure of C to 10% O_2 in N_2 resulted in an increase in VE from 105.2±39.5 on air to 201±71.9 ml/sec ($P < .01$). This change was due to an increase in both VT, 28% ($P < .02$), and f, 50% ($P < .001$). $\dot{V}E$, VT and f at 13% O_2 were not significantly different from the control values. VT/TI increased 98% on 10% O_2 ($P < .05$). The AS response to hypoxia was minimal. None of the parameters showed any significant change except that VT increased 25% at 10% O_2 ($P < .02$). AS rats appear to have a blunted response to hypoxia, whereas the response to hypercapnia is present and almost normal.

38.41

DIAPHRAGM FATIGUE: AN ANIMAL MODEL. P.A. Koen*, N.S. Arora*, R.H. McBrayer* and R.F. Planas* (SPON: M.C. Conrad) Eastern Virginia Medical School, Norfolk, VA and University of Virginia, Charlottesville, VA.

Diaphragmatic muscle fatigue was studied in six adult mongrel dogs whose weight was 22 ± 2 kg (mean ± SE). Transdiaphragmatic pressure (Pdi) was measured at 20 hz, 40 hz, 60 hz and 120 hz by supramaximal stimulation of one phrenic nerve both before and after fatigue. The phrenic nerve was stimulated by positioning a bipolar platinum electrode under fluoroscopic guidance near a section of the phrenic nerve which lies on the external border of the inferior vena cava just above the diaphragm. The diaphragm of the dogs was fatigued by repeated stimulations using the following sequence: four seconds of tetanus at 20 hz, four seconds of rest, four seconds of tetanus at 120 hz and four seconds of rest. The stimulation pattern was repeated over a 15-minute period while the airway remained unoccluded. There were no significant changes ($p > 0.10$) in blood gases from baseline (PaO_2 = 84 ± 5, $PaCO_2$ = 29 ± 2, pH = 7.42 ± 0.01) to the end of the fatiguing sequence (PaO_2 = 87 ± 5, $PaCO_2$ = 29 ± 3, pH = 7.42 ± 0.02). Pdi prior to stimulation at 60, 40, and 20 hz were 95 ± 3, 89 ± 3 and 71 ± 3% of the 120 hz value. After 15 minutes of cyclic stimulation, Pdi was reduced ($p < 0.02$) by 29% at 120 hz, 24% at 60 hz, 22% at 40 hz and 24% at 20 hz. We conclude that the canine diaphragm can be fatigued by repeated stimulations and that the fatigue occurs at all frequencies of stimulation.

38.42

CORRELATION OF PULMONARY RESPONSIVENESS TO AEROSOLIZED METHACHOLINE AND Na_2PtCl_6 IN CYNOMOLGUS MONKEYS. W.J. MOORMAN*, R.E. BIAGINI* and T.R. Lewis* (SPON: A. Vinegar). NIOSH, Exptl. Tox. Br., Cincinnati, OH 45226

Responsiveness to inhaled aerosols of methacholine is a commonly used method for assessment of airways irritability and asthma in humans. Workers in precious metal refineries demonstrate pulmonary symptoms suggestive of asthma, presumably due to exposure to soluble platinum salts. Physiologic dysfunction precedes immunologic evidence of disease, suggesting an initial pharmacologic mechanism. Using a model for the screening of occupational asthmogens, 25 cynomolgus monkeys were evaluated and compared for pulmonary responsiveness to inhaled aerosols of methacholine hydrochloride and Na_2PtCl_6. The pulmonary function variables evaluated were average pulmonary flow resistance ($R_{ave.\ flow}$), dynamic compliance ($C_{L\ dyn}$) and maximum expiratory flow volume (MEFV) curves. Both agents produced dramatic dose-dependent increases in $R_{ave.\ flow}$ with decreases in $C_{L\ dyn}$ and MEFV performance. Analyses of the correlation between dose-responses to the two agents suggest a moderate association between airway irritability and Na_2PtCl_6 induced bronchoconstriction (based on R_L). These results indicate that acute pharmacologic pulmonary responses are significant in addition to classical immune mechanisms in the susceptibility to occupational asthma.

NEONATAL CIRCULATION

39.1

THE EFFECTS OF RESPIRATORY ACIDOSIS ON CEREBRAL BLOOD FLOW (CBF) IN THE NEWBORN DOG. Michael DeVoe, M.D.*, Uma Kotagal, M.D.*, (Spon. L.I. Kleinman, M.D.), University of Cincinnati Medical Center, Cincinnati, Ohio, 45267.

The partial pressure of CO_2 in the blood is known to be a potent factor controlling CBF. Previous studies in adult dogs have demonstrated a linear increase in CBF as pCO_2 increased from 40 to 80 torr with no further increases in CBF as pCO_2 increased beyond 80 torr. In order to determine the response of CBF in the newborn to a change in pCO_2 we studied 5 newborn dogs who were paralyzed and ventilated, and CBF (ml/100g/min) and cardiac output (ml/kg/min) measurements were made using the radioactive microsphere technique. pCO_2 levels of 40, 60, 80 and 100 torr were attained in a random order by varying ventilation. The results of the study are shown in the following table.

	pCO_2=40	pCO_2=60	pCO_2=80	pCO_2=100
CBF	26±7	54±10	84±15	115±36
pH	7.36±.06	7.25±.05	7.13±.04	7.01±.04
BE	-1.0±.03	-2.4±1.9	-4.4±1.5	-6.2±1.3
C.O.	251±64	242±52	276±75	276±69

These results suggest that the newborn animal increases CBF at a constant rate up to pCO_2 levels of 100 torr, unlike the adult. This is accomplished by a significant drop in cerebral vascular resistance, as cardiac output remains unchanged. Further studies in progress are extending this CBF response to pCO_2 levels of 140 torr.

39.2

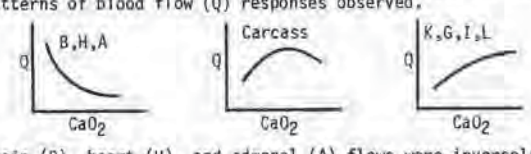
NIFEDIPINE INCREASES CARDIAC OUTPUT BUT DOES NOT ALTER PULMONARY ARTERY PRESSURE DURING HYPOXIA IN NEWBORN PIGLETS. Gregory J. Redding* (Spon: Alan Hodson). Univ. of Washington, Seattle, WA 98195

We reported that Nifedipine (N), a calcium channel blocker, prevents hypoxic pulmonary vasoconstriction (HPV) in newborn piglets when given prior to exposures to hypoxic gas. (Physiologist, 24(4):93, 1981). We now describe the pulmonary hemodynamic effects of N when administered during acute isocapnic hypoxia. Heart rate (HR), cardiac output (CO), mean pulmonary and systemic artery pressures ($\bar{P}_{pa}$,$\bar{P}_{sa}$), arterial blood gas tensions, and calculated total pulmonary and systemic vascular resistance (TPR,SVR) were recorded in 4 awake catheterized piglets before and after 4 and 8 minutes of exposure to hypoxia. HPV was measured without interventions during the first exposure to hypoxia. 15 mcg/kg/min of N was infused during the last 4 minutes of a subsequent hypoxic exposure and the response to N was recorded after 8 minutes of hypoxia. N produced no changes in $\bar{P}sa$, $\bar{P}pa$, PaO2, PaCO2, or pH during hypoxia. HR was increased by 27±21% and CO by 45±40% by N during hypoxia compared to HR and CO during 8 minutes of hypoxia alone. N produced a 33±12% fall in both TPR and SVR during hypoxia. We conclude that N produces a non-selective fall in vascular resistance in both the systemic and pulmonary vascular beds during hypoxia in newborns, and that this effect occurs primarily by increasing cardiac output. Supported by MCH Grant #000955.

39.3

BLOOD FLOW TO NEONATAL ORGANS AS A FUNCTION OF ARTERIAL BLOOD OXYGEN CONCENTRATION (CaO_2). Daniel I. Edelstone, Mary E. Paulone,* Robert Mirro,* Daniel R. Lattanzi,* and Ian R. Holzman. Univ. of Pittsburgh, Pittsburgh, PA 15213

The purpose of the present studies was to determine the relationship of neonatal organ blood flows to a continuum of O_2 availability. In 12 chronically-catheterized unanesthetized lambs, ages 3-9 days, organ blood flows were measured while CaO_2 was varied over the range of 3 to 18 ml O_2/dl blood. Blood flows were calculated with the radionuclide labelled microsphere technique. The figures below indicate the three patterns of blood flow (Q) responses observed.



Brain (B), heart (H), and adrenal (A) flows were inversely related to CaO_2 such that oxygen delivery ($Q \cdot CaO_2$) to those organs remained relatively constant. Carcass flow increased initially and then declined as hypoxemia became more severe. Because of this biphasic flow response, O_2 delivery to the carcass was kept constant until CaO_2 was reduced by more than 50%. In contrast, blood flows and O_2 deliveries to the kidney (K), gastrointestinal tract organs (GI), and liver (L) decreased during hypoxemia. (Supported by NIH Grant HD-16368).

39.4

ARACHIDONIC ACID AND THE PERINATAL PULMONARY PRESSOR RESPONSE TO HYPOXIA. S. Cassin, M.L. Tod and H. Kuck*. Dept. of Physiol., Univ. of Florida, Gainesville, FL. 32610.

Effects of arachidonic acid (AA) on the pulmonary pressor response to hypoxia were studied in ventilated term fetal sheep. Following delivery by cesarean section, the fetal left pulmonary artery was perfused, at constant flow, with blood from the inferior vena cava. Blood flow through the right pulmonary artery (PA) and ductus arteriosus was left undisturbed. After surgery was completed, the cord was tied and the fetus ventilated with air enriched with O_2 (FI_{O2}= 0.30). Pulmonary, left atrial and systemic pressures as well as pulmonary flow along with blood gases and $[H^+]$ were monitored. Two minute infusions (N=19) of AA (0.29±0.1 mg/kg. min) directly into the PA produced 70% increases in pulmonary vascular resistance (PVR). Hypoxia (FI_{O2}=.07) for 3 min produced a 50% increases in PVR. Infusions of AA, 1 min after initiation of hypoxia, resulted in a 105% increase in PVR. The pressor response to AA, but not to hypoxia, was depressed (peak PVR increased 28%) following infusion of thromboxane synthetase inhibitor OKY-1581 and eliminated following indomethacin (2mg/kg). These results suggest that: 1) perinatal pulmonary vasculature does not produce primarily a pulmonary dilator substance(s) from exogenous AA; 2) hypoxia may not alter the metabolic conversion of AA; and 3) the pressor response observed may be due in part to thromboxanes, $PGF_{2\alpha}$ and/or intermediate cyclic endoperoxides. (Supported in part by American Heart, Fla, Affiliate, Inc, AG-616 and NIHHL10834)

39.5
BLOOD VOLUME AND ITS MEASUREMENT IN THE CHRONICALLY CATHETERIZED SHEEP FETUS. Robert A. Brace, Div, Perinatal Biology, Loma Linda Univ, Loma Linda, CA, 92350.

Single and double indicator dilution measurements of the circulating blood volume were made in chronically catheterized sheep fetuses averaging 131 days gestation. The ^{51}Cr-labeled red cell volume averaged 110.3 ml/kg with a range of ±15% of the mean (n = 23). The ^{125}I-albumin (n = 7) and ^{125}I-fibrinogen (n = 5) blood volumes averaged 126.2 ±3.7 (SE) and 124.5 ±3.1 ml/kg respectively. The double indicator fetal blood volume averaged (n = 12) 120.6 ±2.4 ml/kg; however this value is too high due to the assumptions used when determining plasma volumes. In addition, following either a 15% fetal hemorrhage or intravenous epinephrine infusion, there was no release of red cells into the circulation. Labeled maternal red cells were removed from the fetal circulation at an average rate of 18%/hr, whereas labeled autologous fetal cells remained in the circulation. Finally, there were spontaneous changes in fetal blood volume including an average decrease of 2.3 ±0.3% during uterine contractures (n = 10). In summary, it appears that labeled fetal red cells provide the most accurate estimate of circulating fetal blood volume because of problems in extrapolating the plasma label back to zero time. In addition, changes in blood volume can be accurately calculated from either hematocrit or hemoglobin content because there appeared to be no red cell reservoirs in the fetuses. Supported by NIH Grant HD 13949.

METABOLISM

40.1
TURNOVER, RECYCLING, AND OXIDATION OF GLUCOSE IN FASTING NORTHERN ELEPHANT SEAL PUPS. Edward O. Keith* and C. Leo Ortiz. Thimann Laboratories, UCSC, Santa Cruz CA 95064

Using several microdiffusion methods, and radioactive and nonmetabolizable tracers, we have studied the utilization of glucose carbon from the blood of northern elephant seal pups. The pups had been fasting naturally since weaning, approx. 1-2 months prior to experimental study. Adult seals also fast naturally while breeding at Ano Nuevo State Reserve, about 31 km north of Santa Cruz. The rate of appearance of $^{14}CO_2$ after injection with ^{14}C-glucose was measured by acidifying 0.5 ml of whole blood in the outer chamber of a Conway microdiffusion cell, and trapping the released $^{14}CO_2$ in glass discs saturated with NaOH, located in the center of the cell. The glass discs were then counted by liquid scintillation and ^{14}C d.p.m. determined. The rate of $^{14}CO_2$ appearance was less than the rate of ^{14}C-glucose removal from the blood. These data, and the rates of ^{3}H-glucose removal and $^{3}H_2O$ appearance, support the hypothesis that elephant seals recycle glucose carbon in large quantities, and do not oxidize glucose carbon rapidly while fasting, perhaps due to low blood insulin values. The rate of ^{3}H-2-deoxyglucose (2DG) removal from the blood is slightly, but not significantly, greater than the rate of ^{3}H-glucose removal, indicating that 2DG may be an efficacious tracer for in vivo glucose metabolism studies.

40.2
REGULATION OF AXONAL OXIDO-REDUCTION POTENTIALITY OF SHEEP MEDULLA OBLONGATA BY ELECTRO INDUCED AXONAL TRANSPORT. K.S. Swami*, V. Mohanachari, P.V.S.L. Narasimham and K. Indira. Department of Zoology, S.V.University, Tirupati-517 502, India.

The axonal transport of neural components seems to be the basis of neuronal metabolic integrity. This aspect has been studied in sheep medulla oblongata (M.O). The levels of activity SDH, MDH, GDH, LDH, AAT, AlAT and AChE were studied and the axonal transport was induced by exposing M.O to an electric gradient of 5 volts D.C/Cm for 10 min along the long axis. The graphical plots of activity levels of enzymes along the length of M.O exhibited positive slopes for SDH, MDH, LDH and AlAT while the GDH, AAT and AChE showed negative slopes. The positive and negative slopes indicate cathodal and anodal enzyme migration while the slope values denote the respective charge density. The prevalent charge density of the neuronal enzyme correspond to the activity levels of enzymes in the control axoplasm. The operative oxido-reduction systems of neurons can be enhanced by induced cathodal shifting of oxido-reductases and consequently the neuronal metabolic energy production can be stepped up by this new technique.

40.3
REGIONAL CEREBRAL METABOLIC RATE FOR GLUCOSE DURING OXYGEN INDUCED CONVULSIONS. D. Torbati* and C.J. Lambertsen. Inst. for Envir. Med., Univ. of Pennsylvania, Phila., PA 19104

Rats exposed to 5 atmospheres absolute oxygen (ATA-O_2) showed a statistically significant increase in regional cerebral metabolic rate for glucose (rCMRgl) during an early preconvulsive period (Torbati et al., Undersea Biomed. Res., 8(1):52, 1981). In the present study, the rCMRgl was autoradiographically measured in 27 brain structures in 12 rats during oxygen-induced convulsions and in 10 controls. Rats were cannulated in the femoral vein and artery, and cortical electrodes were implanted. After a week's recovery, rats were exposed to 5 ATA-O_2. ^{14}C-2-deoxyglucose was intravenously injected during the appearance of the first clinical convulsions. Thirty min. afterwards, the rats were sacrificed, decompressed and the brains removed and treated for autoradiography. Significantly elevated rCMRgl values were found in Globus pallidus (81%); Substantia nigra (57%); Septum (40%); Hippocampus (30%); Hypothalamus (26%) and cerebellar cortex (23%). Significant reduced values were found in Inferior colliculus (-39%); Superior olivary (-31%); Medial geniculate (-20%) and cerebral cortex (-18%). The results indicate that the rCMRgl in different brain structures during oxygen-induced convulsion is not uniform. The elevated rCMRgl in subcortical structures may be involved in seizure production, while the observed reductions may be related to impaired functional activity during brain oxygen toxicity. (Supported by NIH grant HL-08899-16 and ONR contract N00014-7-C-0248).

40.4
METABOLIC ADJUSTMENTS TO DIVING AND RECOVERY IN THE AFRICAN LUNGFISH. J.F. DUNN*, M. GUPPY*, W. DAVISON* and P.W. HOCHACHKA. Zoology Dept, Univ. of British Columbia, Vancouver B.C., Canada V6T 2A9

The metabolic potentials of the heart, brain, white muscle and liver in the African lungfish were estimated on the basis of enzymatic data and the metabolic effects of an experimentally extended submergence were monitored using metabolite measurements. Heart appeared to be the most oxidative tissue but also had the greatest anaerobic potential. The brain displayed relatively low oxidative capabilities while, metabolically, the white muscle was almost inert. Blood-tissue lactate and glucose gradients indicated (1) that the heart and brain released lactate throughout submergence, (2) that by 12 hours of submersion the brain and heart were probably obtaining all their required glucose from the blood, and (3) that the liver released glucose throughout submergence. After 12 hours of recovery the lactate concentrations returned to low levels but the glycogen stores were not repleted. (This project was largely carried out at the Univ. of Nairobi and was funded by NSERC of Canada).

40.5

THE ROLE OF TEMPERATURE-INDEPENDENT METABOLISM IN THE FOSSORIAL LIZARD, ANNIELLA PULCHRA. Margaret Fusari* (SPON. C.L.Ortiz). College VIII, UCSC, Santa Cruz, Ca. 95064

The fossorial California legless lizard, like several other secretive species of lizard, was shown to exhibit temperature-independent aerobic metabolism at ambient temperatures above and below its preferred temperature range of 15-25°C (mean body temperature = 21°C).

The Q_{10}s for oxygen consumption were 1.0 (6 - 13°C), 2.6 (13 - 20°C), and 1.4 (25 - 30°C).

The oxygen consumptions measured at 25 and 30°C were 76% and 63% (respectively) of values predicted for lizards of comparable body mass (approximately 4g).

The body temperature of the lizards in sand will approximate the temperature of the medium to within one °C due to the high thermal conductivities of sand. The lizards must thermoregulate by seeking suitable sand temperatures. Likewise the osmoregulation of the lizards depends upon their seeking suitable soil moisture contents either to decrease the evaporative water loss or to provide water for drinking from the soil interstices.

The significance of the reduced metabolism probably lies in conservation of both energy and water and possibly in the dissipation of heat loads.

40.6

THE EFFECT OF EXPOSURE TO HIGH ALTITUDE ON AORTIC ENZYMES AND METABOLITES IN RABBITS. T. Zemplenyi*, D.L. Tidwell*, and K. Fronek. University of S. California, Los Angeles, CA. and University of California, San Diego, La Jolla, CA. 92093

Local arterial hypoxia and the associated metabolic changes may contribute to atherogenesis. The effect of generalized hypoxia has been little investigated in this regard. Eight male, 3 month old, New Zealand rabbits were kept for 6 additional months at the White Mtn. U.C. Research Station, elevation 12,400 ft. Eight male rabbits served as controls. Intima-media homogenates from the thoracic (TA) and abdominal (AA) aortas were assayed for LDH, pyruvate kinase (PK), lipoamide dehydrogenase, MDH, ß-glucuronidase, and NAGA activity. Aortic lactate, glucose and malate were also measured. Only PK showed significant rise in activity (in milliunits, mU) in both high altitude thoracic aortas and abdominal aortas, on the DNA, dry weight (DW) and protein (PR) bases. On the other hand, both lysosomal enzymes i.e., ß-glucuronidase and ß-glucosaminidase (NAGA), revealed a significantly lower activity in aortas of high altitude rabbits. There was no difference in glucose, lactate level and LDH in the aortas of high altitude and control groups. PK is considered a quantitative index for glycolytic activity. In the rabbit high altitude appears to enhance aortic glycolysis by stimulating the activity of this rate limiting enzyme. In spite of the low oxygen tension the aortas of high altitude rabbits did not present signs of chronic hypoxia.

(Supported by NIH Grant HL-20564)

40.7

EFFECTS OF VITAMIN B6 DEPRIVATION IN MEAL FED RATS. J.L. Hoover-Plow and Y.N. Sinha*. San Diego State Univ., San Diego, CA 92182 and Scripps Clinic, La Jolla, CA 92307

Weanling male rats were trained to consume in one 4 hr meal a day the control diet (20% casein, 54.2% sucrose, 20% corn oil, cellulose 2%, salt mix 3.5%, B6 deficient vitamin mix 0.3%, and 6 mg pyridoxine/Kg diet). Rats (9-12/group) were then fed one of 3 treatments for 7 days: (1) control diet minus B6 (-B6); (2) control diet (B6); or (3) control diet pair-fed with -B6 (B6PF). Xanthurenic acid (XA) excretion was negligible in B6PF. In -B6 XA was 2x greater 24 hr after a tryptophan load. Body weight (BW) gain and food efficiency (FE) were not different between -B6 and B6. However, in B6PF BW gain and FE were significantly ($p<.05$) lower than both -B6 and B6. Serum glucose (12-16 hr after meal) and % body fat were similar in all 3 groups. Pituitary weight (mg) was significantly ($p<.05$) higher in B6 than in -B6 or B6PF, but pituitary weight as %BW was higher in B6PF than -B6 or B6. No significant differences were observed for serum growth hormone (GH), pituitary GH, serum prolactin (PRL) or pituitary PRL among the 3 groups. When meal fed, differences between B6 deprived and unrestricted control rats in BW gain, FE, and body composition--parameters which alter carbohydrate and lipid metabolism--were minimized. Thus meal fed rats that are B6 deprived for a short term may be a useful model for examining the effects of B6 deprivation on carbohydrate and lipid metabolism. (Supported by a SDSU Foundation Grant and NIH Grant CA-18664)

40.8

CHANGES IN THE LIPID COMPOSITION OF THE STEELHEAD TROUT, SALMO GAIRDNERI, ASSOCIATED WITH SMOLT TRANSFORMATION. Mark A. Sheridan and William V. Allen. Humboldt St. Univ., Arcata, Ca. 95521.

The total lipid content of the steelhead trout serum, liver, light muscle and dark muscle (measured between Jan. 25, 1982 to April 19, 1982) was depleted during smolt transformation. Serum lipids dropped 47% from 26±2 (S.E.M.) mg/ml to 14±2 mg/ml. Liver lipids were depleted from 48±3 mg/g to 30±4 mg/g, representing a 38% decrease. Light muscle lipid concentration dropped slowly until March 19. Between March 19 and March 31, the decrease was dramatic, dropping from 56±7 mg/g (March 19) to 27±8 mg/g (March 31). The overall seasonal drop in light muscle lipid concentration was 66%. Mesenteric fat lipid concentration fluctuated little from 535 mg/g. Of those tissues depleted of lipid, the triacylglycerol (TGL) fraction was reduced by the greatest extent. The large stores of TGL in the dark muscle, liver and mesenteric fat strongly implicate these tissues as lipid depot organs. (Supported in part by NOAA, through California Sea Grant Program Grant No. NA80AA-D-00120, Proj. R/F-79.

40.9

PROSTAGLANDINS AND TRIGLYCERIDE PRODUCTION IN RANA PIPIENS TISSUES. K. D. Doolittle*, G. A. Eiceman*, and C. A. Herman, New Mexico State University, Las Cruces, N.M. 88003.

Amphibians may have the potential to use several fatty acids as prostaglandin (PG) precursors. Tissue levels of eicosatrienoic acid (ETA), arachidonic acid (AA), and eicosapentaenoic acid (EPA) were quantitatively identified in the total lipids and triglyceride (TG) fractions of frog heart and urinary bladder using gas chromatography (GC) and gas chromatography /mass spectroscopy (GC/MS). The time courses of radiolabelled substrate conversion to PG's and TG's were measured by aqueous sampling and TLC methods. At 60 minutes, the bladder synthesized 6% PGE_2 and 25% TG, while the heart synthesized 10% PGE_2, 7% PGI_2, and less than 2% TG, using AA. Equal incorporation into TG but less conversion to PG's was observed using ETA or EPA as substrate. Incorporation of exogenous substrate into TG was specific to the urinary bladder, and was blocked by .28 mM indomethacin. The labelled fatty acids incorporated into TG were identified by GC-radioactive detector and the structures confirmed by GC/MS analysis. In addition, all three fatty acids are endogenous TG components. Since frogs store large quantities of urine in the bladder, the highly effective incorporation of these substrate fatty acids into TG may be important as a general absorbtive mechanism. Supported by NSF Grant PCM-8021878, a N.M. Heart Assoc. Grant-In-Aid, and a Sigma Xi Grant-In-Aid.

40.10

UREA HYDROLYSIS AND RELATED METABOLIC STUDIES IN BLACK-TAILED PRAIRIE DOGS (CYNOMYS LUDOVICIANUS). G.M. Wallace* and E.W. Pfeiffer* (SPON: D.L. Kilgore). Univ. of Montana, Missoula, MT 59801

Urea hydrolysis was compared in winter among fed (C), food and water deprived (FWD), and water deprived (WD) prairie dogs by measuring the percent recovery of an injected ^{14}C-urea tracer in the animals' expired air and excreted urine. Antibiotics were administered via gavage to investigate their affect on urea hydrolysis. Plasma and urine urea concentrations, 24 hour urea excretion, respiratory exchange ratio (R), and oxygen consumption (VO_2) were compared among groups.

FWD and WD animals expired significantly less $^{14}CO_2$ than did C animals (3.4 & 3.7 vs. 8.9%, respectively). Drug-treatment had no affect on ^{14}C recovery in the animals' air or urine. FWD animals had lower plasma urea concentration (6.1 mM) and lower 24 hour urea excretion (0.07g) than did C animals (8.1 mM, and 0.34 g, respectively). R values indicated that fat was the principle fuel source with values of 0.72, 0.66, and 0.71 for C, FWD, and WD groups, respectively. VO_2 and body temperature (T_B) were reduced in FWD (3.4 ml $O_2/Kg^{0.73}/hr$, 34.7°C) and WD (2.9 ml $O_2/Kg^{0.73}/hr$, 32.8°C) compared to controls (4.1 ml $O_2/Kg^{0.73}/hr$, 36.2°C). These results suggest that urea production is reduced in deprived prairie dogs. (Supported by the Zoology Department, University of Montana, Missoula, MT.

40.11

PROTEIN CONSERVATION DURING STARVATION: POSSIBLE ROLE OF LIPID FUELS. Michael N. Goodman, Bradford Lowell* and Neil B. Ruderman. Boston University School of Medicine, Boston, MA 02118.

Older and/or obese rats conserve body protein during prolonged starvation. To determine the basis for this, protein synthesis and degradation in skeletal muscle were evaluated in the isolated perfused hindquarters of 8 and 16 week old control rats and 16 week old obese rats in both the fed state and when starved for 2,5,10 and 11 days. An early event in starvation was a decline in muscle protein synthesis. This occurred in all groups, although it occurred more slowly in the older and obese rats. A later response to starvation was an increase in muscle proteolysis. This occurred between 2 and 5 days in 8 week old rats. In 16 week old rats it did not occur until between 5 and 10 days, and it was preceded by a period of decreased proteolysis. In 16 week old obese rats, a decrease in proteolysis persisted for upwards of 10 days. When starved rats were treated with either nicotinic acid, an inhibitor of lipolysis, or with methyl-tetradecylglycidate, an inhibitor of fatty acid oxidation, muscle protein synthesis diminished markedly and proteolysis increased. The data suggest that the ability of older or obese rats to conserve body protein during starvation is due, in part, to both a better maintenance of muscle protein synthesis and curtailment of proteolysis. This adaptation seems to correlate with the availability of lipid fuels.

40.12

THE INFLUENCE OF DEXAMETHASONE ON WHOLE BODY PROTEIN DYNAMICS. E.C. Fisher*, W.J. Evans*, R.A. Hoerr*, V.R. Young*, D.M. Bier* and D.E. Matthews* (SPON: H.G. Knuttgen). Exercise Biochemistry Laboratory, Boston, MA 02215 and Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

The purpose of this investigation was to assess the influence of a synthetic glucocorticoid on whole body protein metabolism. Four healthy men (age 20-25) participated for a 15-day period, while consuming a standard, eucaloric diet providing protein in the amount of 1 gm·kg^{-1}·day-1. All urine and stools were collected over the 15-day period for assessment of N balance. Days 10-12, the subjects were administered 2 mg of dexamethasone (DEX) daily. Two days prior to and one day following DEX treatment, the subjects received a primed, constant intravenous infusion of 1-^{13}C-leucine, ^{15}N-alanine, and ^{18}O-urea over a 3-hr period. Isotopic plateau of all three labeled substrates was achieved within the three hour period. Leucine flux was unchanged as a result of DEX treatment (.191 ± .014 mg·kg^{-1}·min^{-1} pre-DEX vs. .193 ± .016 mg·kg^{-1} min^{-1}). Alanine flux increased significantly by 19% from a pre-treatment mean of .460 ± .082 mg·kg^{-1}·min^{-1} to .549 ± .088 mg·kg^{-1}·min^{-1}. Urea enrichment from only two subjects measured indicate a consistent reduction in flux. These data indicate that a small dose of a potent glucocorticoid given for a 3-day period causes no change in whole body leucine flux, while it increases alanine N flux.

40.13

EFFECT OF ATHEROGENIC DIET AND DIAZEPAM ON PLASMA AND ADRENAL CATECHOLAMINES DURING REGRESSION OF INDUCED ATHEROGENESIS IN ROOSTERS. **H.Y.C. Wong, N.B. Thos*, S.K. Cheng*, and D.T. Ng*. Dept. of Physiol., Howard Univ. Col. of Med., Washington, D.C. 20059 and Dept. of Anesthesiology, USUHS, Bethesda, MD. 20814.**

We have studied effects of an atherogenic diet (AD) and diazepam on plasma and adrenal catecholamine levels during regression of induced atherosclerosis in roosters. After it was produced in birds by an AD, consisting of 2% cholesterol + 5% cottonseed oil added to mash, they were divided into the following groups: I. Controls on plain mash (PM); II. PM + 0.4 mg/kg of diazepam (D); III. AD only; and IV. AD + D. D was orally administered daily for 13 weeks after atheromatosis was produced. Prior to sacrificing birds, plasma samples were collected. These samples were vortexed, centrifuged and 200 ul of clear supernatant were frozen. Upon sacrificing birds, adrenals were rapidly removed and placed in ice then frozen along with blood samples until assayed for catecholamines by the methods of DaPrada and Zurcher (Life Sci., 19: 116, 1976) and Weise and Kopin (Life Sci., 19: 1673, 1976). After 13 weeks of daily administration of D, the following data were obtained: (1) There were no significant differences in plasma norepinephrine (pg/cc) between groups I, II and III. However, when group IV was compared to group 1, it was significantly lower ($p < 0.05$). Epinephrine levels of groups II, III and IV were significantly lower than group 1 ($p < 0.05$); and (2) No significant changes in catecholamines were noted in the adrenal glands. **(Supported by a grant from USPHS 2S06RR0816-11).**

41.1

ROLE OF CALCIUM INFLUX IN PULMONARY ADRENERGIC RESPONSIVENESS. M.S. Polkow* and R.J. Porcelli. (Spon. G.C. Smaldone). VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

The effect of nifedipine (NF)(3.0nM,IV)on pulmonary pressor responses was studied in isolated perfused cat lungs. NF in the normotensive animal had a small effect on pulmonary vascular resistance (-2.3±1.1%ΔRpv*,n=17). Animals were pretreated with NF and %ΔRpv to various agonists studied @ 2',30' & 60' p NF.

Agents	n	Control	2' pNF	30' pNF	60' pNF
NE (6nM)	6	47±6	19±3*	30±4*	30±3*
↑[H$^+$](10% CO_2)	5	41±4	21±4*	24±4*	25±4*
Hypx (8% O_2)	8	55±6	26±7*	33±6*	41±4
AII (0.4nM)	8	91±18	39±7*	42±6*	54±12
Hist (4nM)	5	91±9	31±11*	71±10	59±19

NF had sustained effects on NE &↑[H$^+$],reversible effects on Hypx, AII and Hist while having no effect on 5HT (not shown). In another group of studies NF ↑Rpv (torr/ml/min) from 0.64± 0.12 to 0.83±0.16 and when NF was superimposed it ↓Rpv to 0.71±0.12* (n=3). ↑[H$^+$](0.52±0.05 to 0.67±0.03,NF:0.57±0.04*, n=3) & Hypx (0.41±0.06 to 0.60±0.08, NF:0.48±0.07*,n=6) both showed the same pattern. Hist and 5HT did not. These studies demonstrate; 1)that NF attenuates and partially reverses only α-adrenergic stimulation and such agents utilize a specific and common Ca^{++} channel; 2)stimulation of the pulmonary α-adrenergic mechanism involves either another Ca^{++} influx channel or intracellular Ca^{++} release, lastly; 3)NF may be useful in the treatment of specific forms of pulmonary hypertension. *p<0.05 (Supp: Veterans Administration and NHLBI #23210)

41.2

ROLE OF INCREASED β-ACTIVITY IN REVERSING ESTABLISHED ACUTE PULMONARY HYPERTENSION. H. Velez* and R.J. Porcelli (Spon. W.M. Foster). VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

Previous studies have shown ↑β-activity to block the pulmonary α-adrenergic responses to norepinephrine (NE,6nM) Histamine (Hist,4nM), Hypoxia (8%O_2) and Hypercapnia (↑[H$^+$](6nM) (JAP.43:612,1977). The present study focuses on the ability of ↑β-activity using isoproterenol (Iso) to reverse sustained vasoconstriction (Vc) in the isolated blood perfused cat lung. Vc was established using the above pressor agents as well as phenylephrine (PE,1nM) and angiotensin (AII,0.4nM). Iso (6nM) reduced pulmonary vascular resistances (Rpv) by 20±5%* in all the normotensive animals tested (n=15). Furthermore, once Vc was established Iso became a more effective vasodilator with each agent given:

	NE	PE	HIST	HYPX	↑[H$^+$]	AII	HIST
	n = (11)	(8)	(12)	(14)	(8)	(6)	(11)
%↑Rpv	28±5	8±1	40±7	26±6	12±2	7±2	37±9
%↓Rpv (Iso)	-32±5	-17±4	-54±10	-38±7	-18±3	20±5	-49±17

These studies demonstrate that:1) Iso has significant vasodilating capability in the normal pulmonary circulation & becomes more effective with acute pulmonary hypertension; 2) although β-activity only prevents certain pressor responses, viz 5HT, it seems to reverse all forms of acute hypertension, suggesting 3)β-receptor activity may not only interact and modify α-receptor activity, but may play a fundamental role in the regulation of smooth muscle activity at the level of the contractile mechanism *p<0.05. (Supported by the Veterans Administration and NHLBI #23210).

41.3

THE ROLE OF β-ACTIVITY IN THE PULMONARY VASCULAR TACHYPHYLAXIS TO HISTAMINE(HIST)AND EPINEPHRINE(EPI). M.V. Cutaia* and R.J. Porcelli, VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

We have reported specific alterations in pulmonary vascular responsiveness after repetitive exposure; Hist(.33-2nM/ml)and Epi(.26-7nM/ml) show decreased responses over time (Physiologist,24(4):74). The present study investigated this finding (isolated perfused cat lung) by repeating cumulative dose response curves (DR),I→IV @ 1 hr intervals; the last curve preceded by specific receptor blockade. For Epi and Hist, β-blockade with propranolol (Pro, 45nM/ml) was used. Cimetidine (Ci, 72nM/ml) was used for H_2 blockade. DR curve slopes, maximum responses and ED 50's were compared:

	Epi(Pro),n=8:		Hist(Pro),n=8:		Hist(Ci),n=9:	
	Slope	Max.Resp.	Slope	Max.Resp.	Slope	Max.Resp.
DR I	58±17	70±20	108±19	181±27	115±21	201±32
DR IV	14±3*	19±4*	35±5*	51±9*	58±9*	77±12*
DR V	39±10	50±6	102±17	169±17	58±12*	80±14*

ED 50's for curve IV in the Hist group was shifted (right) while for Epi there was no change. β-blockade (DR V) restored the responses to Epi and Hist, while H_2-blockade did not. In conclusion, 1)the tachyphylaxis to Epi after repetitive exposure represents a shift in the functional balance between αßß receptors towards β activity; 2)restoration of vascular responsiveness to Hist with Pro demonstrates the functional link between Hist and the adrenergic system; 3)Hist tachyphylaxis does not involve an H_2 vasodilator receptor. *p<.05

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41.4

THE EFFECT OF INCREASED [H+] ON PULMONARY ARTERIAL SMOOTH MUSCLE REACTIVITY STUDIED IN VITRO. E.H. Bronstein* and R.J. Porcelli (Spon. W.M. Foster). VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

Previous studies have shown that the catecholamines have a specific [H+](45±2nM/L) at which maximum responsiveness occurs in vivo (Jap.31:679.1971). The present study investigates if optimal reactivity is also associated with a specific [H+] in vitro. Pulmonary arterial vessel segments were isometrically suspended in tissue baths and cumulative dose response curves developed. Only one agent at a specific [H+] was studied on each segment. The following table summarizes the effect of +[H+] on the maximum response (Δmg tension/mg tissue) of these vessel segments to norepi(NE,$0.06\text{-}6\cdot10^3$ nM/ml),Histamine(Hist, $0.09\text{-}9\cdot10^3$ nM/ml),5HT($0.06\text{-}6\cdot10^3$ nM/ml) and KCl(10-640 mM/ml):

[H+]nM/L=	44±5	(n)	53±2	(n)	60±3	(n)	69±5	(n)
NE	280±39	(22)	289±74	(14)	658±180*	(7)	680±144*	(7)
HIST	378±41	(24)	465±57	(16)	510±93	(10)	778±182*	(8)
5HT	352±63	(25)	478±128	(15)	185±59	(9)	570±115	(9)
KCl	535±78	(30)	467±95	(14)	679±91	(12)	634±161	(7)

These data demonstrate: 1)the [H+] for optimal pulmonary reactivity in vitro (69±5 nM/L) markedly differs from in vivo (45±2 nM/L); 2)+[H+](pH=7.40 to pH 7.13) either +H_1 - α-receptor activity or +β - H_2 activity (NE & Hist), while not significantly changing 5HT receptor activity, and; 3)this +[H+] does not seem to alter membrane potential or its effect on the smooth muscle contractile mechanism (KCl). *p<0.05

(Supp. by Vet. Admin. & NHLBI # 23210)

41.5

ETHANOL (ETOH) INDUCES SEVERE PULMONARY VASOCONSTRICTION IN CHRONICALLY INSTRUMENTED NEONATAL LAMBS. Willa H. Drummond* and Hugh H. Shrager.* (SPON: S. Cassin). Univ. of Florida, College of Medicine, Gainesville, FL. 32610

We infused 95% ETOH at a dose of 0.03 ml/kg/min for 10 min into the inferior vena cava (IVC) of 5 lambs, age 11-30 d, previously instrumented with aortic, pulmonary artery, (PA) left atrial (LA) and IVC catheters and an electromagnetic flow transducer around the PA. Initial arterial blood gas was normal in all lambs. Mild hypoxemia developed after ETOH (PaO_2 89 ± 2 vs 73 ± 11 torr (p=.1), but rose on FIO_2 1.00 to 317 ± 34 torr. $PaCO_2$ and pH did not change. PAP rose from 17.1 ± 1.4 to 41 ± 2.3 mmHg (p<<.001) after 5 min. of ETOH. PAP remained high (39 ± 1.5 mmHg) during hyperoxia/ETOH. SAP rose from 70.8 ± 2.4 to 84 ± 6.2 mmHg (p=.1). LAP and cardiac output did not change significantly. Thus, pulmonary vascular resistance (PVR) rose 212% from .16 ± .03 to 0.50 ± .14 mmHg/ml/kg. min (p<.05) while SVR increased 57% from 0.7 ± .1 to 1.1 ± .3 mmHg/ml/kg min (p=.2). The ratio PVR/SVR rose significantly (p<.001) from base .23 ± .02 to .46 ± .04, and .45 ± .08 under room air/ETOH and oxygen/ETOH conditions respectively. Blood ETOH levels measured in a different group of lambs were 54 ± 5.5mg/100ml after 10 minute infusion of this ETOH dose. Thus, we conclude that intravenous infusion of low doses of 95% ETOH into lambs causes pulmonary vasoconstriction which is more marked than its vasoconstrictor effects on the systemic circulation. This effect appears independent of blood oxygen tension.

41.6

EFFECT OF VASOACTIVE INTESTINAL PEPTIDE AND SUBSTANCE P ON THE IMMATURE PULMONARY CIRCULATION. James E. Lock*, Thomas J. Kulik*, Dana E. Johnson*, and Stanley Einzig. University of Minnesota, Minneapolis, MN 55455

Vasoactive intestinal peptide (VIP) and Substance P (SP) may be neurotransmitters in a peptidergic nervous system. Both occur in pulmonary nerves, but their effects on newborn pulmonary vascular resistance (PVR) are unknown. We placed flow probes around L and R pulmonary arteries (PA) of 3-18 day old lambs; the aorta (Ao) and either L or RPA were catheterized 7 days later. VIP and SP were injected into the L or RPA of awake lambs in normoxia and hypoxia. L and RPA flows, Ao and PA pressures (AoP, PAP) were recorded continuously. Direct effects on PVR of VIP or SP result in changes in the ratio of injected lung flow over total PA flow (Q_{inj}/Q_T). VIP (n=8) is a potent direct PA and systemic vasodilator (threshold (T) = 0.1μg/kg):Q_{inj}/Q_T rose (0.49 to 0.52*); AoP fell (84 to 77 mmHg*); cardiac output (CO) rose (1.2 to 1.4L/min*). These effects were unchanged by pretreatment with propranolol (1 mg/kg). SP (n=6) consistently raised AoP*(T=1μg/kg) but inconsistently raised Q_{inj}/Q_T and CO. SP no longer raised AoP after phenoxybenzamine (2 mg/kg) and may have actually decreased AoP. We conclude that VIP is as potent a PA vasodilator as PGI_2, and thus may help maintain PVR homeostasis. It was not, however, a selective PA (as opposed to systemic) dilator. SP had little effect on PVR, but in the newborn is a systemic constrictor whose actions are at least partly mediated by alpha receptors. *p<0.05.

41.7

Arachidonic Acid Infusions Increase Plasma 6-keto-$PGF_{1\alpha}$ and Block Hypoxic Pulmonary Vasoconstriction. M.Moon*, R.J.Lemen, S.Quan*, M.Whitten.* University of Arizona, Tucson AZ 85724.

We studied transpulmonary prostaglandin (PG) metabolism before, during and after hypoxia (10 dogs) and arachidonic acid (AA) infusions (1 μg/kg/min) plus hypoxia (5 dogs). Plasma 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TxB_2), PGE_2 and $PGF_{2\alpha}$ were measured by radioimmunoassay from the pulmonary artery (PA) and aorta (AO). Hypoxia (H) (10% O_2 in N_2) alone did not alter PG metabolism, but increased mean PA pressure from 10.5 ± 1.0 to 18.2 ± 1.7 mm Hg (p<0.05).

	Site	Before	AA	AA-H	After
Pressure	PA	11.3±1.4	10.8±1.1	12.6±1.7**	11.8±0.55
(mm Hg)	AO	103±6.8	77.8±37	91.2±15	97.8±18.9
6-keto-$PGF_{1\alpha}$	PA	356±42	722±79*	961±110*	1053±43*
(pg/ml)	AO	567±65	1037±113*	1372±170*	927±38*
TxB_2	PA	728±114	1069±110	1991±418	1217±131
(pg/ml)	AO	888±82	1328±92	1089±116	995±103
$PGF_{2\alpha}$	PA	591±80	1150±193	1090±172	1409±127*
(pg/ml)	AO	569±48	1062±159	884±120	876±104
PGE_2	PA	709±99	1441±370	715±81	1568±139*
(pg/ml)	AO	491±66	1213±148*	679±114	947±99

* p<0.05 compared to Before data.
** p<0.05 compared to ten animals receiving H alone.
These data indicate that AA infusion (1 μg/kg/min) stimulates PGI_2 production and attenuates hypoxic pulmonary pressor activity. Supported in part by NIH Grant # HL23773.

41.8

IMMEDIATE PULMONARY VASCULAR AND AIRWAYS RESPONSES AFTER INTRAVENOUS LEUKOTRIENE (LT) D_4 INJECTIONS IN AWAKE SHEEP. M.L. Ogletree, J.R. Snapper and K.L. Brigham. Pulmonary Circulation Center, Vanderbilt Univ., Nashville, TN 37232

We studied lung responses to synthetic LTD_4 (U-63,305, gift from the Upjohn Co.) in adult sheep with chronic lung lymph fistulas, intravascular catheters, pleural silastic envelopes and tracheostomies. After a stable baseline period, we injected LTD_4 (1 to 10 μg, i.v. bolus) and monitored hemodynamics, lymph flow and lung mechanics. Results are summarized below (mean ± SEM, N=7; * p < 0.05).

	P_{PA} (cmH_2O)	P_{LA} (cmH_2O)	P_{Aorta} (mmHg)	C. O. (L/min)	C_{dyn} (%)
Baseline	19±2	3±2	80±3	4.8±0.3	100
LTD_4(3μg)	50±4*	-5±1*	66±3*	4.4±0.2*	70±3*

The transient pulmonary hypertension was accompanied by maximally decreased dynamic compliance (C_{dyn}) 17±4 sec after LTD_4 injection. As pulmonary artery pressure (P_{PA}) returned to baseline, left atrial (P_{LA}) and aortic pressures increased to 8±1* cmH_2O and 96±5 mmHg, respectively. PGH_2-analog (PGH, 10μg, i.v.) caused moderate pulmonary hypertension without affecting cardiac output (C.O.) or aortic pressure. LTD_4 injections did not change lung lymph flow but did increase lymph thromboxane (Tx) B_2 concentration from 0.05± 0.01 to 0.12± 0.05* ng/ml. Meclofenamate abolished changes in P_{PA} and C_{dyn} and prevented TxB_2 release after LTD_4 but did not prevent delayed increases in P_{LA} and P_{Aorta} nor responses to PGH. These results indicate that LTD_4 causes cardiac, lung vascular and airways responses. Some, but not all of the responses may be mediated by local release of TxA_2. (Supported by HL 19153, HL 26198 and HL 27274)

41.9

TRANSIENT PROSTACYCLIN PRODUCTION WITH ELEVATED PRESSURE AND FLOW IN ISOLATED DOG LUNGS. M.L. Ellsworth*, T.J. Gregory* and J.C. Newell. Biomed. Eng., Rensselaer Polytech., Troy NY 12181

We studied the effect of an abrupt increase in perfusion pressure and flow on the production of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) in isolated, left lower lobes perfused in situ with pulsatile flow. When flow was increased from 55±7 to 308±23 ml/min, mean pulmonary arterial pressure (Ppa) increased from 8.6±1.1 to 27.2±5.2 Torr, followed by a slow decline to 23.7±3.6 Torr at 5 min. Concentrations of the PGI_2 metabolite (6-Keto $PGF_{1\alpha}$) and the TXA_2 metabolite (TXB_2) were determined by RIA in pulmonary venous blood. The initial increase in Ppa was associated with a decrease in 6-Keto $PGF_{1\alpha}$ by 0.08±0.07 ng/ml and an increase in TXB_2 concentration by 0.03±0.03 ng/ml. By 5 min these changes were reversed, and the level of 6-Keto $PGF_{1\alpha}$ was 0.40±0.28 ng/ml higher and the level of TXB_2 was 0.10±0.06 ng/ml lower than the low flow values. The ratio of 6-Keto $PGF_{1\alpha}$ to TXB_2 was initially decreased, but at 5 min was 44% higher than at low flow. Veno-arterial differences showed an initial production of both metabolites by the lung, [Δ(6-Keto $PGF_{1\alpha}$)=.13±.26 ng/ml, Δ(TXB_2)=.05±.05 ng/ml] which then became consumption, [Δ(6-Keto $PGF_{1\alpha}$)=-.42±.24 ng/ml, Δ(TXB_2)=-.13±.05 ng/ml]. We suggest that the decline in Ppa is due to the release of PGI_2 from the endothelial cells in response to abrupt vascular stretch. The increase in PGI_2 exceeded that of TXA_2, resulting in a net increase in the PGI_2/TXA_2 ratio, and a consequent decline in Ppa. Supported by NIH Grant HL18630.

41.10

PROSTACYCLIN PRODUCTION DURING STEADY AND PULSATILE PERFUSION OF ISOLATED DOG LUNGS. T.J. Gregory*, M.L. Ellsworth* and J.C. Newell. Biomed. Eng., Rensselaer Polytech., Troy NY 12181

We compared the effect of steady and pulsatile perfusion on the production of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) in isolated, in situ left lower lobes during normoxic ventilation. Concentrations of the PGI_2 metabolite, 6-Keto $PGF_{1\alpha}$, and the TXA_2 metabolite, TXB_2, in pulmonary venous blood were measured by RIA. During steady perfusion, when flow was increased from 75±2 ml/min to 303±1 ml/min, mean pulmonary arterial pressure increased from 7.8±0.6 Torr to 13.6±0.7 Torr. Thirty sec after the flow increase, 6-Keto $PGF_{1\alpha}$ increased by 0.24±0.13 ng/ml and TXB_2 increased by 0.07±0.06 ng/ml. Five min later the levels of both metabolites were unchanged. Perfusion was then made pulsatile at the same mean flow, and mean pulmonary arterial pressure did not change. Thirty sec later, the concentration of 6-Keto $PGF_{1\alpha}$ was increased by an additional .08±.05 ng/ml, and TXB_2 was increased by an additional .05±.01 ng/ml. After 5 min the concentration of 6-Keto $PGF_{1\alpha}$ was further increased by 0.21±0.09 ng/ml but TXB_2 decreased by 0.08±0.01 ng/ml. Thus during steady perfusion, increasing flow caused transient synthesis and release of both 6-Keto $PGF_{1\alpha}$ and TXB_2. The increases in 6-Keto $PGF_{1\alpha}$ were sustained during pulsatile perfusion, but those in TXB_2 were not. We conclude that vascular stretch, produced by increases in flow or by pulsatile perfusion, causes synthesis and release of PGI_2 which may attenuate increases in pulmonary perfusion pressure.
Supported by NIH grant HL18630.

41.11

HYPOXIA ALTERS BLOOD COAGULATION DURING ACUTE DECOMPRESSION IN HUMANS. H. O'Brodovich*, M. Andrew*, G.W. Gray*, and G. Coates*. (SPON: N. Jones). Dept's of Paediatrics and Radiology, McMaster University, Hamilton, Canada L8S 3Z5 and DCIEM, Downsview, Canada.

Since microembolization increases lung vascular permeability activation of the coagulation cascade may contribute to high altitude pulmonary edema. Maher described alterations in blood coagulation during acute decompression in humans (JAP: 41: 702, 1976). We investigated whether these changes were due to hypobaria or hypoxia. On 3 separate occasions 5 healthy adults (27 to 42 years) were exposed to 2 hrs of: 1) hypoxic hypobaria (410 torr); 2) normoxic hypobaria (410 torr breathing supplemental O_2); 3) hypoxic normobaria (F_IO_2=0.11). Venous blood was drawn from separate sites into either EDTA, o-phenanthroline, or sodium citrate plus EACA utilizing a two syringe technique before, during and following the 2 hr intervention. The P.T.T. shortened by 3 seconds during hypoxic hypobaria and hypoxic normobaria, but was unchanged during normoxic hypobaria. The P.T., hematocrit, and concentrations of fibrinogen, bradykinin and total protein were not affected in any study. After 1½ hr of hypoxic normobaria one subject was too ill to continue. At that time platelet count decreased by 100,000/mm^3, and the P.T.T. shortened by 10 seconds. We conclude that alterations in the coagulation system during acute decompression result from hypoxia and these changes are exaggerated in some individuals.
(Supported by the M.R.C.)

41.12

HYPOXIA ALTERS MEMBRANE POTENTIALS IN RAT MAIN PULMONARY ARTERY SMOOTH MUSCLE: A POSSIBLE CALCIUM MECHANISM. O. Hottenstein, W. Mitzner, and G. G. Bierkamper*. LNMT, Johns Hopkins Univ. School of Hygiene, Baltimore, MD 21205

Acute hypoxia has been hypothesized to directly depolarize pulmonary vascular smooth muscle cells (VSM). This study tested whether hypoxia reversibly depolarizes the membrane potentials (Em) of VSM and examined how changes in external (Ca++) or Ca++ antagonism might alter the Em of VSM during hypoxia. Main pulmonary arteries (MPA) were taken from male, Sprague-Dawley rats (300-380g) and denuded of endothelia and adventitia. Utilizing a cross-sectional population design we made intracellular recordings in a Krebs-Henseleit medium with strict control of temp. (37°C), pH (7.40), PCO_2 (36 torr), and PO_2 (420 torr). In 3mM Ca++ the average resting Em of the MPA VSM was -52 ± 1.8 mV (n=6 vessels, 136 cells). The Em recorded during hypoxia (PO_2=67 torr) were significantly less negative than during control and post-hypoxic periods except for vessels with 0.5mM Ca++. Hypoxia depolarized Em by 31% for 3mM Ca++ vessels, whereas only 10% depolarization occurred in the presence of 0.5mM Ca++. Upon reoxygenation reversal of the depolarizations occured in all but the 0.5mM Ca++ vessels. Nitrendipine (10^{-6}M) a Ca++ entry blocker prevented the hypoxic depolarization. High magnesium replacement for Ca++ also blocked the response. These results suggest that MPA VSM are directly sensitive to hypoxia and depolarize by a calcium dependent mechanism which may be functionally important in the mechanism of hypoxic pulmonary vasoconstriction.

41.13

AGE DEPENDENT EFFECTS OF CHRONIC HYPOXIA (CH) ON PULMONARY VASCULAR REACTIVITY (%ΔRpv). M.A. Lowen*, M.J. Bergman* and R.J. Porcelli. (Spon. G.C. Smaldone) VAMC @ Northport & SUNY @ Stony Brook, NY 11794.

The relation between age and the ability of CH to alter pulmonary vascular reactivity was studied in the isolated perfused lungs of 7 week old (7 wk) and 28 week old (28 wk) rats, exposed to 2 weeks (CH2) and 4 weeks (CH4) of 10% O_2. CH2 and CH4 had no effect on %ΔRpv to KCl in either the 7 wk (C:42±14%; CH2:28±5%; CH4:32±12%) or 28 wk group (C:108±19%; CH2:118±17%; CH4:140±16%), although the latter showed greated responsiveness at each stage*. %ΔRpv to 5HT in 7 wk rats at CH2 (78±24*) and CH4 (63±14) were elevated over control (43±13). The 28 wk rats showed the same pattern (C:33±9; CH2:94±17*; CH4:67±14). The %ΔRpv(constrictors and dilators)to histamine during hypoxia for each group are as follows:

7 wk	C	CH2	CH4	28 wk	C	CH2	CH4
Constrict:	34±8	42	19±2	Constrict:	78±38	27±8	13±3
n:	(7)	(1)	(3)	n:	(3)	(9)	(5)
Dilate:	-29±8	-26±6	-30±5	Dilate:	-27±12	-9±1	-10
n:	(3)	(5)	(6)	n:	(2)	(2)	(1)

These data demonstrate: 1)CH has no effect on membrane potential or its effect on smooth muscle contraction (KCl) in either 7 wk or 28 wk rats; 2)CH2 and CH4 increase 5HT receptor activity regardless of age, and; 3)CH in 7 wk rats functionally alters H_1 to H_2 receptors while in 28 wk rats CH only reduced histamine's vasoactivity. *p<0.05 (Supported by Veterans Administration and NHLBI # 23210).

41.14

INHIBITION OF THE HYPOXIC PULMONARY PRESSOR RESPONSE BY VASODILATOR DRUGS IN THE CONSCIOUS RAT. Hilary S. Stanbrook*, Kenneth G. Morris*, and Ivan F. McMurtry. Univ. of Colo., Denver, CO 80262.

An effective treatment of hypoxic pulmonary hypertension with no undesirable side effects has yet to be established. We compared the effects of 3 potentially useful vasodilator drugs; nifedipine (Nf), verapamil (Vp), both calcium channel blockers, and hydralazine (Hyd), a direct vasodilator, on the hypoxic pulmonary pressor response of the chronically instrumented, conscious rat. The rats were placed in a plexiglass chamber flushed with room air and control measurements of systemic arterial pressure (SAP), heart rate (HR), cardiac output (CO), pulmonary artery pressure (PAP) and blood gas tensions made. Hypoxia was established by passing 12% oxygen through the chamber and hemodynamic measurements were repeated. Hypoxia (PaO_2 35 mmHg) significantly increased PAP, CO and TPR and decreased SAP and SVR. Twenty minutes after i.p. injection of Nf (10mg/kg), Vp (10mg/kg) or Hyd (1mg/kg) there was a small fall in SAP and the hypoxic pressor response was significantly reduced to 35% of the before-drug response. However, of the 3 drugs used, Nf appeared not to produce the decreased CO and increased TPR often elicited by Vp and Hyd. These results demonstrate that Nf, Vp and Hyd effectively inhibit hypoxic pulmonary vasoconstriction but Nf appears to be the drug which is best tolerated. We speculate therefore, that Nf may be of potential use in the treatment of chronic hypoxic pulmonary hypertension.(Supported by the Parker B. Francis & Cystic Fibrosis Foundations.

41.15

CHANGES IN PULMONARY ARTERIOLES IN HYPOXIC PULMONARY HYPERTENSION IN THE RAT. Herta M. Tremer*, Sidney S. Sobin, John D. Hardy* and Hugo P. Chiodi. University of Southern California, School of Medicine, Los Angeles, CA 90033.

In an electron microscopic study of pulmonary arterioles 15-30 μm in diameter in young adult female and male rats, the results of hypoxic exposure were studied. Hypoxia was produced by 10% oxygen at one atmosphere or hypobaria at one-half atmosphere. Within one hour there were characteristic changes limited to these arterioles consisting of blebs beneath the endothelial cell, swelling of the basal lamina and edema of the arteriolar wall with dissection of wall elements. These progressively intensify and at 24 hours an increased number of fibroblasts are found abluminally especially in septal areas. Continuing hypoxia results in transformation of the fibroblasts through a transitional form in which myofilaments, dense bodies, caveoli and basal laminae develop producing a typical mature smooth muscle cell during the course of four to seven days. The larger arteries, capillaries and venules show none of these reactions. Pulmonary hypertension develops rapidly with increased blood hemoglobin and hematocrit in both female and male with increased right ventricular weight. The progressive ultrastructural changes suggest that they may be sequential and cascade-dependent phenomena. (Supported in part by USPHS NIH Grant HL-23224 and Amer. Heart Assoc., Greater Los Angeles Affiliate Grant C-218)

41.16

SITE AND SENSITIVITY FOR HYPOXIC PULMONARY VASOCONSTRICTION (HPV). B.E. Marshall and C. Marshall*. Dept. Anesthesiology, Univ. Penn. Med. Sch. Phila., PA. 19104.

This study describes the effects of alveolar (PAO_2) and pulmonary artery ($P\bar{v}O_2$) oxygen tensions on HPV. Rat lungs were ventilated (180 ml/min) and perfused (15 ml/min) in vitro. Temperature (37°C) and PCO_2 (40 mmHg) were constant. Pulmonary artery pressure was measured when FIO_2 was changed from 0.21 to 0.10, 0.06, 0.04, 0.03, 0.02 or zero for 6 mins. while $P\bar{v}O_2$ was maintained constant at 10, 40, or 140 mmHg by equilibration in a membrane oxygenator containing 21, 6 or zero percent oxygen. Responses (R%) to HPV were expressed as percent of maximum response. A sigmoid stimulus-response surface was defined where:

$$R\% = \frac{100\ (P\bar{v}O_2)^{-1.029}\ (PAO_2)^{-1.623}}{0.0001175 + (P\bar{v}O_2)^{-1.029}\ (PAO_2)^{-1.623}}$$

For regression r=0.86, and when PAO_2 and $P\bar{v}O_2$=30.2 mmHg response is 50% with 95% confidence interval of ±4%. Iso-response lines were constructed and assuming linear diffusion the sensor region is located 60% of the distance from the blood to the alveolar gas surface and the oxygen tension at this site (PsO_2) defines the response where : $R\%=100\ (PsO_2)^{-2.692} / (0.0001039 + (PsO_2)^{-2.292})$. Therefore PAO_2 and $P\bar{v}O_2$ together determine the PsO_2 that stimulated HPV. (supported by NIH GM-29268)

41.17

PROLONGED CHRONIC HYPOXIC PULMONARY HYPERTENSION AND RECOVERY IN THE RAT. John D. Hardy*, Sidney S. Sobin, Herta M. Tremer* and Hugo P. Chiodi. University of Southern California, School of Medicine, Los Angeles, CA 90033.

Chronic hypobaric (0.5 atm) hypoxic pulmonary hypertension (HPH) in the female rat was followed from 1-9 months and during recovery for 1-12 months. Hemoglobin and hematocrit peaked at 25 g% and ± 70%, respectively, at one month. Right ventricular mass and pulmonary artery pressure (anesthetized) did not increase significantly after one month exposure. Electron microscopic study of pulmonary arterioles 15-30 μm diameter showed little change in neomuscularized arterioles after 2-4 months except for thickened elastic lamina and increased wall thickness with increased collagen. Termination of hypoxia resulted in rapid fall in hemoglobin and hematocrit to normal and reduction in pulmonary artery pressure to above normal levels, all within one month. Platelet initiated thrombotic occlusion occurs regularly in arterioles. The smooth muscle cell of neomuscularized arterioles dedifferentiates into a cell with interrupted basal lamina, without myofilaments, and a cytoplasm both glassy and reduced in proportion to the nucleus. Dedifferentiation of vascular smooth muscle in 15-30 μm diameter pulmonary arterioles is the most striking feature of recovery from HPH. (Supported in part by USPHS NIH Grant HL-23224 and Amer. Heart Assoc., Greater Los Angeles Affiliate Grant C-218)

41.18

EFFECT OF ALVEOLAR HYPOXIA ON REGIONAL PULMONARY PERFUSION. P.H. Neumann‡ C.M. Kivlen‡ A. Johnson, F.L. Minnear and A.B. Malik. Albany Medical College, Albany, New York 12208

We examined the effects of varying levels of alveolar hypoxia on the regional distribution of pulmonary blood flow (rPBF) in control-ventilated sheep. rPBF was measured in the prone animal using 15 um diam. labelled microspheres during baseline and at two levels of hypoxemia (PaO_2 44 and 20 Torr). During the baseline period, rPBF was uniform (14±4 % pulmonary blood flow/g bloodless dry lung wt in the upper lung and 16±2 % of PBF/g in the dependent lung). During hypoxia, however, the flow increased in the upper lung (20±3 % of PBF/g) while it decreased in the dependent lung (10±2 % of PBF/g). The degree of flow distribution was dependent on the severity of hypoxia. The flow distribution was not associated with significant increases in pulmonary blood flow (2.0±.4 → 2.4±.5 → 2.6±.1 l/min); but, the shift was associated with increases in mean pulmonary arterial pressure (18.2±1.2 → 21.7±1.1 → 29.0±3.8 mm Hg). The results indicate a shift in regional pulmonary perfusion to the upper lung which appears to depend on the level of pulmonary hypertension. The upward shift may be due to vessel recruitment or to vasodilation. (HL-17355, HL-27016, New Investigator Award HL-29273 to F.L.M., and T32-GM-07033).

41.19

17 BETA-ESTRADIOL ATTENUATES THE PULMONARY VASOCONSTRICTOR RESPONSE TO HYPOXIA. Randall C. Wetzel* and Jimmie T. Sylvester, The Johns Hopkins Medical Institutions, Baltimore, MD. 21205.

We have previously demonstrated that the steady state hypoxic pulmonary constrictor response in isolated, in situ lungs of post pubertal sheep perfused with autologous blood is less in the female as compared to the male. Furthermore, the response of castrated male sheep is not different from non-castrated males. To test the possibility that the attenuation of the response in the female is due to the presence of estrogens, we compared the response in treated (10 mg, 17 β estradiol in oil at least 24 hours prior to perfusion) and untreated (control) 6-month old male sheep castrated at birth. The pulmonary artery pressure flow relationship was determined at each level of PIO_2 (200-0 torr) every 5 minutes until a steady state was achieved. To generate stimulus response curves, the pulmonary artery pressure at a flow of 50 cc/kg/min (Ppa50) was determined and plotted against the PIO_2. At PIO_2's of 200 and 0 torr, the Ppa50's were not significantly different between controls and treated animals. However at maximal pulmonary artery vasoconstriction, PIO_2=30, the treated animals had a significantly lower mean Ppa50: 23±4 vs 35±4 in controls. The entire curve was not different from that demonstrated for females. These results suggest that 17 β estradiol, or one of its metabolites is responsible for the attenuation of the hypoxic response of the pulmonary vasculature seen in females.

41.20

STIMULUS FOR HYPOXIC PULMONARY VASOCONSTRICTION (HPV) IS PRE-CAPILLARY C. Marshall* and B.E. Marshall. Univ. Penn. Med. Sch., Phila., PA. 19104.

This study was undertaken to identify the sensor site for HPV. Rat lungs were suspended in a humidified chamber. Perfusate, oxygenated with a Kolobow membrane (PMO_2), was pumped either through the PA for forward flow or the left auricle for reverse flow. Ventilation was 180 ml/min and perfusion 15 ml/min so $PAO_2 = PIO_2$. All gas mixtures contained $FCO_2 = 0.055$. After a baseline with $PAO_2 = 150$ mmHg & $PMO_2 = 45$ mmHg, the perfusate was equilibrated with either zero, 0.06 or 0.21 FIO_2. The FAO_2 was then alternated between 0.21 or 0.06 for six minutes for each PMO_2 in random order. PAP increased with hypoxic ventilation. The results summarized below compare the PAP increases when FMO_2 was 0.21 or 0.06 as ratios of the increase observed with zero FMO_2.

	Reverse perfusion		Forward perfusion	
	21/0	6/0	21/0	6/0
mean	1.03	1.04	0.44*	0.68*
SE ±	0.14	0.12	0.04	0.03

(* different from 1.0 $p < 0.05$)

Therefore the sensor is 1) pre-capillary: ratio = 1 with reverse flow, 2) alveolar gas and pulmonary artery perfusate influence its response: ratio < 1 with forward flow. (supported by NIH GM-29628).

42.1

FURTHER STUDIES ON THE COLD TOLERANCE OF THE PYROCHROID BEETLE DENDROIDES CANADENSIS. John G. Duman and Kathleen L. Horwath. Biol. Dept., University of Notre Dame, Notre Dame, IN. 46556

Previous studies showed that overwintering larvae of the beetle, Dendroides canadensis, were freeze tolerant (able to survive the freezing of their extracellular body fluids) during the winters of '77-'78 and '78-'79. Involved in this cold tolerance were, (1) high concentrations of polyols (glycerol and sorbitol), (2) hemolymph ice nucleating proteins which function to inhibit supercooling and thus prevent lethal intracellular ice formation and (3) thermal hysteresis producing antifreeze proteins. Recent studies have shown that during the winters of '80-'81 and '81-'82 Dendroides did not become freeze tolerant but instead supercooled to temperatures near -30. High levels of polyols and antifreeze proteins were still produced, but the ice nucleator proteins were not present thus allowing supercooling. The lower lethal temperatures of larvae during both freeze tolerant and susceptible winters were approximately the same. This is the first known instance of such a switch in the overwintering mechanism of an insect.

This change from freeze tolerance to freeze susceptibility with extensive supercooling makes the antifreezes (polyols and protein antifreezes) of critical importance. An antifreeze protein with a molecular weight of 14,500 was purified from Dendroides hemolymph. The most notable aspect of the composition of this protein is that 22% of the amino acid residues are cysteine.

42.2

INDICES OF TEMPERATURE ACCLIMATION AND THE TIME COURSE OF THEIR EVENTS IN CHANNEL CATFISH. J.D. Kent*, M. Koban*, J. Cameron*, and C.L. Prosser. Univ. of Illinois, Urbana, IL

Channel catfish acclimated to 15°C were transferred to 25° (n=39) and at various times over a 21 day period, livers were excised and assayed for cytochrome oxidase (CO), citrate synthase (CS), hydroxyacyl-CoA dehydrogenase (HADH), glucose-6-phosphate dehydrogenase (G6PDH), and lactate dehydrogenase (LDH). During the first 24-72 hrs at 25°, all enzyme activities (based on DNA) increased above 15° acclimated steady state (SS) values by 10-135%, followed by rapid decreases in CO, CS, HADH, and G6PDH activities so that by day 6-10, their values were comparable to 25° SS activities. The mean activity decrease of 40% for these enzymes thereafter remained invariant for the duration of the experiment. In contrast, LDH activity did not vary appreciably from the 15° value. the 15°→25° transfer increased the DNA:protein ratio (6.8±1.7 → 12.6±.2), decreased hepato-somatic index (1.9±.2 → .8±.1), and decreased total liver protein content (mg) (155±21 → 81±5). Total liver DNA and water contents did not change. Electron microscopy revealed decreased cell volume and mitochondrial numbers following the 15°→25° transfer. Hence, increased ambient temperature decreased liver mass and total protein but this was not due to decreased cell numbers. When enzyme activities are expressed on a protein basis, the acclimation phenomenon is not as pronounced as on a DNA basis. This suggests that for thermal acclimation studies, basing enzyme activity data on DNA or on total liver may be more meaningful. (Supported by NSF)

42.3

THE PHYSIOLOGICAL ROLE OF LOW MOLECULAR WEIGHT ANTIFREEZE GLYCOPEPTIDES IN POLAR FISHES. S.M. O'Grady*, J.C. Ellory* and A.L. DeVries. University of Illinois, Urbana, IL 61801

Antarctic fishes synthesize 8 glycopeptides that have unique antifreeze properties. The large glycopeptides (1-5; MW 10,500-33,700 daltons) have the greatest activity, while low molecular weight glycopeptides (6,7&8; MW 2600-8000 daltons) show 1/3 the antifreeze activity of 1-5. In this study, we examine the freezing susceptability of intestinal fluid in polar fishes. Polar fishes live in ice-laden sea water so intestinal fluid should freeze since the osmotic concentration is not high enough to depress the freezing point below the environmental temperature (-2°C). Freezing points of intestinal fluid and bile are 0.9°C lower than melting points suggesting the presence of antifreeze glycopeptides in both fluids. The presence of low molecular weight antifreeze glycopeptides in bile and intestinal fluid was confirmed by ion exchange chromatography followed by polyacrylamide gel electrophoresis and amino acid analysis. Removal of the gall bladder and occlusion of the common bile duct eliminates the transport of antifreeze glycopeptides into the intestine suggesting that they enter by biliary secretion. We conclude that low molecular weight antifreeze glycopeptides are required to prevent freezing of intestinal fluid, and provide the first clear evidence that these glycopeptides have a specific biological function in polar fishes. (Supported by NSF-DPP 78-23462)

42.4

METABOLIC EFFECTS OF RAPID, SMALL TEMPERATURE CHANGES IN CALM CARP. Brenda P. Moffitt[1], Dept. of Rehabilitation Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

Carp (Cyprinus carpio L.)were acclimated to 19.0°C and habituated to a metabolic chamber. At 19.0°C the metabolic rate (MR) was 27.1 ± 1.1 ml $O_2 \cdot hr^{-1} \cdot kg^{-0.77}$, the heart rate ($f_H$) 18.3 ± 0.34 beats.$min^{-1}$ and the ventilation rate (f_V) 21.8 ± 0.90 ventilations.min^{-1}. Rapid changes in ambient temperature (T_a) of ± 2.0°, ± 4.0° or ± 6.0°C elicited rapid, temperature dependent changes in MR and f_H, both of which returned rapidly to the resting rate when T_a was restored to 19.0°C. Neither parameter showed any major compensatory changes while exposed to altered T_a. f_V was altered significantly when T_a was changed ± 4.0° or ± 6.0°C. The ± 2.0°C changes caused consistent but not significant changes in f_V. When the T_a was lowered both f_H and f_V responded simultaneously, but were temporarily dissociated when the T_a was raised, f_V increasing at once, but f_H responding after a delay of up to 3.5 min. The responses of MR and f_H to rapid thermal change had Q_{10}'s of 2.61 and 2.4 respectively, which are similar to Q_{10} values obtained for long term acclimation. In calm carp, small or moderate shifts in body temperature produce metabolic alterations which are comparable to those produced by long term acclimation. (Supported in part by PSC-CUNY grant # 13327).

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42.5

PHYSIOLOGICAL THERMOREGULATION BY "WATERPROOF" FROGS AT HIGH TEMPERATURES. Vaughan H. Shoemaker. Univ. of California, Riverside, CA 92521

The arboreal frogs Chiromantis xerampelina and Phyllomedusa sauvagei are unusual among amphibians in their ability to restrict evaporation from the skin. However, both species inhabit regions where evaporative cooling may be necessary on hot days. Individuals (ca. 40 g) of both species were studied in a wind tunnel where ambient temperature (T_a), wind velocity and humidity could be varied independently. Body temperature (T_b) and evaporative water loss (EWL) were monitored. At T_a below 38°C, T_b closely approximated T_a and EWL was low (<2 mg/min). At T_a above 40°C, frogs of both species regulated T_b between 39 and 40°C. At a variety of rates of convective heat input the frogs varied EWL between 2 and 20 mg min^{-1} to match heat gain and thus regulated T_b. Moreover, humidity could be varied while convective input was constant without affecting EWL or the regulated temperature. Thus these frogs are able to vary the resistance of the integument to water loss greatly and with remarkable precision. Secretions from the mucus glands were observed during periods when frogs were maintaining T_b below T_a, and these glands are probably operating in a manner analagous to sweat glands in the control of evaporative heat loss. The lowest resistances observed (10 sec/cm) were much higher than the calculated boundary layer resistance. (Supported in part by NSF Grant DEB 77-25291.)

42.6

METABOLIC AND BIOCHEMICAL CORRELATES OF THERMAL ACCLIMATION IN THE ROUGH-SKINNED NEWT, TARICHA GRANULOSA. Martin E. Feder. The University of Chicago, Chicago, IL 60637

To determine whether the biochemical changes often proposed as mechanisms of thermal acclimation occur in urodele amphibians, rough-skinned newts (Taricha granulosa) were acclimated to 5° and 20° C. No thermal acclimation was evident in organismal oxygen consumption when measured at 5° C. At 20° C measurement temperature, warm acclimation resulted in a 25% reduction in oxygen consumption. The apparent K_m's of lactate dehydrogenase and malate dehydrogenase decreased after cold acclimation; acclimation did not affect the V_{max} of these enzymes. The changes in apparent K_m's, however, corresponded poorly with the pattern of metabolic acclimation. Gel electrophoresis of these enzymes and also isocitrate dehydrogenase revealed no differences between cold and warm acclimated salamanders that were attributable to acclimation. Cold acclimation increased the proportion of unsaturated fatty acids in lipid extracted from liver, but produced no significant changes in the proportions of the major phospholipids. Newts had unexpectedly high levels of arachidic acid and sphingomyelin. In general, Taricha granulosa resembles other anamniote vertebrates in the consequences of thermal acclimation. (Supported in part by NSF Grant DEB 78-23896).

42.7

TEMPERATURE REGULATION IN HAWAIIAN BROWN NODDIES (*Anous stolidus pileatus*). H. I. Ellis*, M. Maskrey*, T. N. Pettit*, and G. C. Whittow. Dept. Physiology, School of Medicine, Univ. of Hawaii, Honolulu, Hawaii 96822.

Physiological responses of the Brown Noddy, a dark sea bird limited in distribution to low latitudes, were measured at different ambient temperatures (T_a). As in many dark, hot-climate birds, daytime "basal" metabolism ($\dot{H}_b$) is relatively low: only 88% of that expected by weight. Unlike many such birds, minimal thermal conductance (C_m) is also low: 75% of expected. Low $\dot{H}_b$ and C_m combine to produce a broad zone of thermoneutrality (ca. 20°-37°C). Body temperature is allowed to drop gradually from 41°C near T_a=37°C to about 37.5°C near T_a=0°C. Brown Noddies dissipate 150% of their metabolic heat production by evaporation, when heat-stressed. Respiratory frequency is about 20/min over a wide temperature range until heat-stressed panting increases it sevenfold. (Supported by Univ. of Hawaii Sea Grant #N1/R-14.)

42.8

THERMOREGULATION DURING SWIMMING IN THE MINK, A SEMI-AQUATIC MAMMAL. Terrie M. Williams. Rutgers University, New Brunswick, N.J. 08903.

Thermal energy budgets of adult minks (*Mustela vison*, Schreber) resting in water and swimming against a current in a water flume were determined from total heat production, heat storage and thermal conductance. Aquatic activity by minks resulted in high levels of heat loss which generally exceeded metabolic heat production within five minutes of immersion. This imbalance was manifested as changes in core body temperature (T_b) and was a function of swimming speed, the duration of immersion, and seasonal changes in pelage. Negative heat storage, determined from decreases in T_b, was greatest at intermediate swimming speeds (.29 - .36 m $\cdot$ s^{-1}). The highest values of negative storage measured for female minks in summer pelage was -12.28 cal $\cdot$ g^{-1} $\cdot$ h^{-1}; 64% greater than the peak value for animals in winter pelage. Over the range of 0 to .97 m $\cdot$ s^{-1} minimum thermal conductance of winter and summer pelt covered models increased with water speed, and was consistently greater for the summer pelt. Insulatory value of the pelts was dependent upon the integrity of an air layer pervading the fur. The inability of these animals to remain homeothermic in water coincides with limited swimming bouts observed for wild minks. (Supported in part by NSF Grant PCM 80-11158.)

42.9

THE ROLE OF NASAL COUNTERCURRENT HEAT EXCHANGE IN REDUCTION OF EVAPORATIVE WATER LOSS IN NORTHERN ELEPHANT SEALS. Anthony C. Huntley, Daniel P. Costa, Robert D. Rubin*, C. Leo Ortiz. Center for Coastal Marine Studies, University of California, Santa Cruz, CA 95064.

Northern elephant seals abstain from both food and water for one to three months during the terrestrial reproductive period. In weaned, fasting pups, water is derived almost entirely from fat oxidation and the primary avenue of water loss is respiratory evaporation (EWL). This study demonstrates the importance of temporal countercurrent heat exchange in the nasal passages in reducing the EWL. In resting animals, the temperature of expired air (T_e) averaged 22.5°C, 13.2°C below core body temperature (T_b). This cooling effect reduces respiratory water loss by approximately 70% under naturally occurring ambient temperature (T_a) and humidity conditions. The measured EWL for a 100 kg pup was 247 ml H_2O/day, which is somewhat higher than the value calculated from the above data for an animal of similar size (162 ml/day). Nevertheless, in the absence of nasal heat exchange (T_e=T_b) EWL would be 480 ml/day, thus exceeding the calculated metabolic water production of 334 ml/day. This mechanism is based in part on the large nasal turbinate surface area found in elephant seals, which is estimated to be 720 cm^2 in pups and 3140 cm^2 in adult males. Both water savings and total heat exchange surface area are comparable on a mass specific basis to other mammals which employ such water conserving mechanisms. (NIH: AM06093-01; MBRS RR08132-08).

42.10

MAXIMUM OXYGEN CONSUMPTION OF EXERCISING HARBOR SEALS. R. Elsner and S. Ashwell-Erickson*. Institutes of Marine Science and Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

Three young harbor seals (*Phoca vitulina richardsi*), mean body weight 25 kg and about neutrally buoyant, were trained to carry lead weights in pockets of a canvas jacket while treading water (temperature 15-18°C) in an upright cylindrical tank. The weight supported could be incrementally changed between rest periods. The work load (lead weight in water) varied from 0.8 to 8.0 kg. A safety line prevented drowning. Open circuit respirometry permitted determinations of oxygen consumption ($\dot{V}O_2$) during rest and exercise. Steady values were attained during 5-10 min periods and showed increasing $\dot{V}O_2$ with increasing work up to the level of $\dot{V}O_{2\ max}$. Achievement of $\dot{V}O_{2\ max}$ was determined by leveling of the $\dot{V}O_2$ versus work load plot, further increase in work failing to elevate $\dot{V}O_2$. Signs of exercise distress relating to difficulty in maintaining flotation then became apparent. $\dot{V}O_{2\ max}$ was similar in all seals and averaged 32.8 ml/kg$\cdot$min. The typically elevated mean resting $\dot{V}O_2$ was 8.8 ml/kg$\cdot$min, thus the aerobic scope was about 4x. These values of $\dot{V}O_{2\ max}$ and aerobic scope are lower than have been determined in both wild and domestic mammals of similar body weight. (Supported in part by NIH Research Grant HL-23950 and Sea Grant.)

42.11

BIOENERGETICS OF LACTATION IN THE NORTHERN FUR SEAL. Daniel P. Costa*, Steven D. Feldkamp*, Roger L. Gentry* (SPON. A.B. Hastings). Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, CA 92093.

Fur seal cows fast while nursing their pups for 7 days after parturition. They then go to sea and feed, but periodically return to nurse their pups onshore. The energy expended while lactating, the amount of milk transferred to the pup and the change in milk composition was measured during the initial 7 day post-partum suckling period. During this time pups gained 3.0% $\pm$ 0.6sd (n=15) of their body mass per day, while cows lost 4.1% $\pm$ 1.6sd of their body mass per day. Pups gained 13.1% $\pm$ 3sd of the mass lost by cows (n=15). Metabolism of fasting, lactating cows was 96.4 $\pm$ 13.8 kcal/kg-day determined from metabolic water production measured by tritiated water turnover. Protein catabolism determined from the turnover of 14-C urea was 1.78 $\pm$ .24 kcal/kg-day, only 1.9% of total metabolism. Milk ingested by 5 suckling pups estimated from tritiated water turnover averaged 560 $\pm$ 189 ml/day. Milk fat declined from 46% $\pm$ 2.7 (n=7) to 38.7% $\pm$ 3.8 (n=9) after 7 days of lactation and fasting. Whereas, milk water increased from 42.7% $\pm$ 2.7 to 46.3% $\pm$ 4.3. (funded by NIH fellowship #AM 06093-01. NIH grant #17773) and NOAA contract #81-ABC-00016).

42.12

IN VITRO PHARMACOLOGICAL STIMULATION OF EQUINE SWEAT GLANDS SHOWS EQUINE SWEATING IS PREDOMINANTLY UNDER β-ADRENERGIC CONTROL. Jan Bijman* and Paul M. Quinton. Division of Biomedical Sciences, University of California, Riverside, CA 92521-0121.

Pharmacologically induced sweating of in vitro incubated sweat glands from the donkey was used to examine the control of secretion from these glands. Small skin biopsies (Φ 2mm) were taken from the neck region of the animals and mounted in a cuvet. Sweat of individual glands was collected under H_2O saturated mineral oil to determine the sweat rate (maximal 7.7 $\pm$ 0.1 nl/gl/min). No sweating was observed with even high doses of cholinergic agents (acetylcholine or mecholyl). Maximal sweat rates were obtained with the adrenergic catecholamines epinephrine and nor-epinephrine and the β-adrenergic agonists isoproterenol and terbutaline. The α-adrenergic agonists methoxamine and phenylephrine gave low responses, respectively 15% and 35% of the maximal sweat rates. None of these responses could be prevented with the cholinergic antagonist atropine nor with the α-adrenergic antagonist phentolamine. In all cases sweating is prevented by the β-adrenergic antagonist propanolol (5×10^{-5} M). The responses to methoxamine and phenylephrine were attributable to a β-adrenergic component of the α-adrenergic agonists. These results suggest that sweating in equines is under β-adrenergic control. This work is supported by grants from Getty Oil Co., Gillette Co., and the NIH #AM 00324 and #AM 20356.

43.1

SUSTAINED ELEVATION OF PLASMA ACTH INCREASES SALT INTAKE AND BLOOD PRESSURE IN RATS. Rudy A. Bernard, Timothy W. Priehs*, Gregory D. Fink* and Raymond F. Nachreiner*. Michigan State University, East Lansing, MI 48824

ACTH (Acthar, Armour) was injected by a subcutaneously implanted minipump (Alza) at the rate of .21 U (1ul)/hr for a daily dose of 5 U/day. Voluntary salt intake was measured by presenting the rats with 2 drinking bottles, one containing distilled H2O, the other 0.5 M (3%) NaCl solution and weighing the bottles daily. A chronic intra-arterial catheter, exteriorized and cemented at the top of the head, was used to measure mean blood pressure (MBP) and obtain blood samples without handling the animals. The catheters were installed in half of the rats 7 days after infusion was begun. Plasma ACTH and corticosterone (CORT) were measured by radioimmunoassay. Intake of salt solution averaged 5±2 mg (n=7) before ACTH injection, rose significantly by the 2nd day and achieved a 10-fold increase by the 5th day and thereafter to 50±2 mg/day. The controls (n=6) averaged 4±2 mg/day. H2O intake more than doubled, going from 51±3 mg/day to 119±5 mg/day, whereas the controls averaged 56±5 mg/day. Nine to thirteen days after ACTH infusion was begun, the experimentals (n=3) had MBP=138±10 mm Hg ($p<.001$), ACTH=1141±355 pg/ml ($p<.001$) and CORT=150±81 ng/ml ($p<.05$), whereas the control values were MBP=108±10 mm Hg, ACTH=274±108 pg/ml and CORT=75±25 ng/ml. These results lay the basis for a stress-related model of hypertension. (Supported in part by Michigan Heart Association.)

43.2

COMPARATIVE EFFECTS OF MINERALOCORTICOID EXCESS IN THE SHEEP AND PIG. David F. Bohr and John Mitchell.* Univ. of Mich., Ann Arbor, 48109

Hemodynamic, electrolyte, and endocrine changes were monitored in sheep and pigs following subcutaneous implantation of deoxycorticosterone acetate (DOCA, 100mg/kg). In both species there was a significant increase in mean arterial pressure within 48 hours, reaching a plateau approximately 40% above normal, somewhat earlier in the sheep (2 weeks) than in the pig (3-4 weeks). Both animals evidenced renal sodium retention for 3 days; then escape occurred, returning sodium balance to normal. Both had a minimal and persistent hypernatremia which occurred within the first week. Water balance was positive for only the first three days following DOCA implantation. Beginning on the third or fourth day, water turnover increased, but water balance remained at control levels. Plasma renin activity fell to unmeasurable levels within 10 days. Strikingly different effects were produced by DOCA on potassium metabolism in the two species. The sheep had a marked kaliuresis and supplemental potassium had to be given to prevent a lethal hypokalemia. In contrast, the pig evidenced a mild, transient urinary potassium retention, yet also became hypokalemic. It is concluded that, in the pig, there may be an intracellular shift of potassium, and that mineralocorticoids affect renal handling of sodium and potassium by independent mechanisms. (Supported by NIH Grant HL 18575.)

43.3

EFFECTS OF DESOXYCORTICOSTERONE AND SALT ON ARTERIAL WALL CHANGES IN TWO-KIDNEY DOCA HYPERTENSION. Robert H. Cox, Bockus Research Institute, Graduate Hospital and Department of Physiology, University of Pennsylvania, Phila., PA 19146.

The effects of DOCA and salt on hypertension related changes in arterial wall mechanics and composition were assessed in rats. Half of the animals received a DOCA implant, and half did not. Of the former, half were on a low salt (DL) and half on a high salt (DH). Of the latter, half were untreated (CU) and half received saline (CS). After 16 weeks, systolic pressures were: CU = 133 ± 3, CS = 147 ± 4, DL = 139 ± 5 and DH = 167 ± 6 mmHg. Passive stiffness of carotid and tail arteries of DH rats only was increased. Maximum active force (100 mM K^+ and 1 mM norepinephrine) of DH carotids only were increased. Total connective tissue content of DH thoracic aortae and carotids only was decreased. The K^+ content of arteries from DH was increased. No significant differences existed in the properties of arteries from DL and CU animals. Likewise, no significant differences existed in arteries from CU and CS animals. Two kidney DOCA-low salt treated rats did not develop hypertension or hypertension-related arterial wall changes. Saline-treated, control rats developed mild hypertension but no arterial wall changes. These results suggest that DOCA does not contribute directly to the arterial wall changes in this model. (Supported by HL-28476).

43.4

INTESTINAL HEMODYNAMICS IN PERINEPHRITIC HYPERTENSIVE DOGS. D.R. Bell* and H.W. Overbeck. University of Alabama in Birmingham, Birmingham, AL 35294

In pentobarbital anesthetized dogs with chronic perinephritic hypertension the status of blood flow and resistance differs among vascular beds: There is normal blood flow with elevated resistance in the limb, and elevated blood flow with normal resistance in the ileum. In the present study we investigated the mechanism of the elevated ileal flow, by measuring blood flow, pressure, O_2 consumption, reactive hyperemia, pressure-flow relationships, resistance at maximal vasodilation, and non-entrapment of 9μ microspheres in isolated, perfused ilea of 9 dogs with chronic (> four weeks duration) one-kidney perinephritic hypertension (H; $\bar{P}_A$=163mmHg) and 9 normotensive one-kidney sham-wrapped dogs (C; $\bar{P}_A$=107mmHg). In H, ileal blood flow was elevated by 33% (ml/min/100g; M±SD:55.3±9.5 (H); 41.5±3.8(C), $p<0.001$), but ileal vascular resistance was unchanged (mmHg/ml/min/100g: 3.36±0.73(H); 3.40±0.46(C)). We noted no significant differences in ileal O_2 consumption, reactive hyperemia, pressure-flow relationship, or resistance at maximal vasodilation. However, we found shunting of microspheres to be a positive function of perfusion pressure ($p<0.02$). Thus, the increased ileal blood flow in H may represent "shunt" flow through pressure-dependent channels. Increases in wall-to-lumen ratio apparently do not develop, suggesting that the "shunts" may be upstream to the resistance (and exchange) vessels protecting them from elevated pressure/flow. (Supported in part by USPHS, NIH Grant HL-23312).

43.5

EFFECT OF PLASMA SUPERNATE FROM DOGS WITH ONE-KIDNEY, ONE WRAPPED HYPERTENSION ON SHORT CIRCUIT CURRENT IN THE TOAD BLADDER J.S. Chen, M.B. Pamnani, S.J. Huot, W.T. Link*, D.L. Clough* and F.J. Haddy. Uniformed Services University, Bethesda, MD 20814

Previous studies, using two different bioassay systems, suggest the presence of a ouabain-like agent in the plasma of animals with experimental low renin hypertension (Hypertension 3(Suppl 2):96, 1981; Physiologist 24:6, 1981). We here present data from a third bioassay system. Supernates of boiled plasma (PS) from eight pairs of dogs were studied in toad urinary bladders. One member of the pair had one-kidney, one wrapped hypertension of five weeks duration (1K,1W) and the other had a one-kidney, sham wrap operation five weeks earlier and was normotensive (1K,SW). When short circuit current (SCC) became steady, PS from 1K,1W was added to both sides of the experimental bladder and PS from 1K,SW was added to both sides of the control bladder. SCC promptly (~10 sec) decreased, reaching a minimum in about 5 min. The maximal decreases were 17.5% and 8.6%, respectively (Δ=8.9±1.4% $P<.005$). In the two bladders, PS also decreased total tissue conductances by 6.8% and 1.3%, respectively (Δ=5.5±1.9%, $P<0.025$) and membrane potentials by 11.5% and 7.5%, respectively (Δ=4.0±1.3%, $P<0.025$). The addition of ouabain (2 mM) reduced the conductances to the same levels in the two bladders. Since these effects are similar to those exerted by ouabain, they suggest the presence of a ouabain-like humoral agent in the plasma of the dog with 1K,1W hypertension.

43.6

REVERSAL OF REDUCED RENAL MASS HYPERTENSION (HT) IN RATS: EFFECT ON VASCULAR Na^+-K^+ PUMP ACTIVITY AND CIRCULATING OUABAIN-LIKE FACTOR. S.J. Huot, M. Jagusiak*, F.J. Haddy and M.B. Pamnani. Dept. of Physiology, USUHS, Bethesda, MD 20814

We have reported that reduction of 70-80% renal mass (RRM) and saline drinking increases the level of a ouabain-like humoral factor (OLHF), suppresses vascular Na^+-K^+ pump activity (ouabain-sensitive ^{86}Rb uptake) and leads to HT in rats. In this study, effect of withdrawal of saline as drinking fluid on blood pressure, vascular Na^+-K^+ pump activity and plasma level of OLHF in RRM HT rats was investigated. RRM male Wistar rats were fed a low sodium (0.02%) diet and divided into pairs of control(C) distilled water drinking and experimental (E) 1% saline drinking rats. After four weeks of sustained HT in the E rats and a similar time period in the paired C rats, E rats were switched to distilled water for drinking. Systolic blood pressure (SBP) was then monitored for seven days, following which the rats were anesthetized, tail arteries removed for measurement of ouabain-sensitive (OS) and ouabain-insensitive (OI) ^{86}Rb uptakes, and blood collected for preparation of boiled plasma supernates to assay for OLHF. SBP of E rats decreased significantly ($P<.01$, n=10) 24 hours after substitution of distilled water for drinking. There were no significant differences in OS or OI ^{86}Rb uptakes by tail arteries or in the plasma level of OLHF in C and E rats. These data show that saline withdrawal reverses RRM HT, depressed vascular pump activity, and elevated OLHF suggesting a causal relationship between HT and OLHF.

43.7

EFFECT OF DIETARY SODIUM AND POTASSIUM IN SUBTOTALLY NEPHRECTOMIZED RATS. M.M. Huth*, N.C. Trippodo, and E.D. Frohlich. Alton Ochsner Medical Foundation, New Orleans, LA 70121.

High potassium (K) and/or low sodium (Na) intake have been shown to attenuate the rise in arterial pressure in some experimental forms of hypertension. To study the effects of altered dietary Na and K in the development of hypertension in subtotally nephrectomized (Nx) rats (right Nx plus 66% left Nx), 4 groups, each containing 8 male Wistar rats, were fed daily: 1, normal Na (4mEq) and K (5mEq); 2, normal Na (4mEq) and high K (10mEq); 3, low Na (< 0.1mEq) and normal K (5mEq); 4, low Na (< 0.1mEq) and high K (10mEq). After 21 days, mean arterial pressures (MAP), measured in conscious rats, were: 166±5*, 131±12, 132±7 and 119±4 mmHg in the 4 groups, respectively, (*$p<0.01$, as compared with the other 3 groups). Heart rates were 348±13, 335±27, 399±15 and 345±11 beats/min, respectively. In a second experiment, 2 groups of similarly subtotally Nx rats were fed either high Na (6.5mEq) and high K (6.6mEq) or high Na (8.6mEq) and low K (1.8mEq) daily (n=16 per group). After 21 days there were no differences in MAP (146±5 and 144±5 mmHg, respectively), nor in heart rate (380±11 and 372±9 beats/min, respectively). The results suggest that both high K and low Na attenuate the rise in MAP in subtotally nephrectomized rats. However, the K effect on MAP may not occur if Na intake is increased or the dietary Na/K ratio is greater than 1.0. Supported in part by HL22261

43.8

INCREASED HINDQUARTERS RESISTANCE AND THE ASSOCIATED MICROVASCULAR CHANGES IN SKELETAL MUSCLE DURING THE ACUTE ONSET OF RENAL HYPERTENSION IN RATS. G.A. Meininger and H.J. Granger. Microcirculation Research Institute and Dept. of Medical Physiology, Texas A&M University, College Station, Texas 77843.

Changes in the cremaster muscle microcirculation were correlated with simultaneous changes in mean arterial pressure (MAP) and hindquarters resistance (HQR) during the onset of two-kidney one-clip renal hypertension by placing doppler flow probes on the lower aorta and left renal artery in urethane-chloralose anesthetized rats. Cremasters with intact innervation and circulation were suspended in a Krebs bath for measurement of vessel diameters at three arteriole and venule branch levels. Control diameters ($\bar{X}$±SEM) for first (1A), second (2A) and third (3A) order arterioles were 143±6μm, 83±7μm and 23±4μm; and for first (1V), second (2V) and third (3V) order venules were 221±5μm, 104±16μm and 40±6μm. Rats were made acutely hypertensive by inflating a balloon occluder on the left renal artery to reduce resting renal artery flow by 50%. A 3-hour period of renal artery stenosis was characterized by increases in MAP (30%) and HQR (14%). Vessel diameters increased (8%) for 1A and 2A, but diameters of 3A decreased (30%) and slowly returned to the prestenosis control diameter. Venule diameters did not change at any branch level. Our results suggest that during the acute onset of renal hypertension, skeletal muscle microvascular changes vary for different microvascular levels and vasoconstriction is confined to smaller arterioles. (Supported by HL 25387)

43.9

HEMODYNAMIC STUDIES ON THE HYPERREACTIVITY TO BRADIKININ IN CONSCIOUS RENAL HYPERTENSIVE RATS. E.M. Krieger, L.C. Michelini and E.D. Moreira. School of Medicine, USP, Ribeirão Preto, SP, BRAZIL.

We have shown that one-kidney, one clip renal hypertensive rats (RHR) have a marked hyperreactivity to intraaortically administration of bradykinin (BK). In the present study cardiac output (CO) was measured in the unrestrained conscious rats by means of the thermodilution method to study the role of the heart and of the peripheral resistance (PR) in the enhanced responsiveness to BK. The control parameters in 8 conscious RHR (256±5g) and in 8 conscious normotensive control rats (NRC, 245±3g) were, respectively: BP 233±8/152±6(193±5) and 141±3/94±3(117±2); CO: 49±3 and 50±4 ml/min/100g; HR: 417±10 and 451±10 beats/min; PR: 4.05±0.28 and 2.41±0.17. Equivalent pressure falls of 10-15 and 23-33 mmHg were produced by i.a. infusion of BK and NP, respectively. In 5 of 8 NCR the reduction of PR was the main cause of the hypotensive responses to BK and NP. The same was observed for BK in the RHR; however for NP the fall in CO was the most important factor. Conclusions: 1) In the RHR with hyperreactivity to intra-arterial administration of BK the hypotensive responses are due mainly to a decrease in PR, as is the case for the NRC. 2) BP reduction caused by normal doses of NP in the RHR are produced mainly by reduction in CO, rather than by decrease in PR as seen in the NCR.

Financial support by FAPESP, FINEP and CNPq.

43.10

NEUROGENIC FACTORS IN RENOVASCULAR HYPERTENSION. H. Suzuki*, K.B. Brosnihan, R.C. Speth* and C.M. Ferrario, Cleveland Clinic Research Division, Cleveland, OH 44106.

Because neurogenic factors may play a key role in both the initiation and maintenance of hypertension, we measured the levels of norepinephrine (NE), epinephrine (Epi) and angiotensin II (Ang II) in plasma and in cisterna magna cerebrospinal fluid (CSF) of 6 conscious dogs before and during a 28 day period of developing II-kidney, I clip hypertension (IIK-IC) using a procedure described previously (Masaki et al., Clin Sci Mol Med 52: 163, 1977). The early phase (< 7 days) of hypertension (MAP: 117 ± 4 mmHg) was accompanied by tachycardia and increases in plasma and CSF NE. Although plasma renin activity (PRA) rose, there were no changes in plasma or CSF Ang II levels. Seven days after occlusion of the renal artery, blood pressure rose further (135 ± 10 mmHg) while heart rate fell below control. At this time, PRA, plasma Ang II and CSF Ang II were elevated markedly; these changes were accompanied by a decrease in both plasma and CSF levels of NE below control. Since activation of the renal pressor system is associated with time-related changes in the concentration of NE and Ang II in both plasma and CSF, these observations may be indicative of an early involvement of central and peripheral neurogenic factors in the pathogenesis of renovascular hypertension. (Supported in part by Grants HL-6835 and HL-24100 from NHLBI, NIH).

43.11

The role of kidneys in the development of genetic hypertension in rats. Miklos Gellai, Susan Sundstrom*, Department of Physiology, Dartmouth Medical School, Hanover, NH 03756

We assessed the possible role of kidneys in the development of hypertension in the spontaneously hypertensive (SH) rat. Measurements of arterial pressure (AP), glomerular filtration rate (GFR, by inutest clearance) and clearance of PAH (C_{PAH}) were performed on at least 5 SH and Wistar-Kyoto (WKY) normotensive control rats at 4,6 and 8 weeks of age. In order to avoid the depressing effect of anesthesia and post-surgical stress the experiments were performed on trained, chronically catheterized, conscious rats. The results (mean ± SE) were:

AGE (weeks)		4	6	8
AP	WKY	99± 4	106± 3	116± 4
(mm Hg)	SH	106± 6	145± 9†	150± 10†
GFR	WKY	1115± 69	1123± 65	1040±103
(ul/min·100g)	SH	1107± 88	1129±113	1095±163
C_{PAH}	WKY	3396±311	3172±175	3258±335
(ul/min·100g)	SH	3558±311	3136±281	3395±306

†$p<.01$ for comparison between SH and WKY rats.

At 4 weeks of age no significant difference was observed in any of measured parameters between SH and WKY rats. Despite significant increase of AP in the SH rats with age, GFR, C_{PAH} and sodium handling remained unchanged. Thus impairment of these functions appears not to play a causal role in the development of hypertension in the SH rat. (Supported by NIH & NH Heart.)

44.1

AIRWAY DYNAMICS DURING HIGH FREQUENCY VENTILATION (HFV): A CINERADIOGRAPHIC STUDY. M. Gavriely*, J. Solway*, S. Loring, R.H. Ingram, Jr., R. Brown, A. Slutsky and J. Drazen. Harvard School of Public Health, Brigham & Women's Hospital, and West Roxbury V.A. Hospital, Boston, MA 02115

Radial expansion and contraction of airways and the ensuing "shunt capacitance" effect has been suggested as a possible explanation to the relative inefficiency of HFV at higher frequencies, and during peripheral bronchoconstriction. To assess the magnitude of airway wall motion during HFV, we took cineradiographs of the airways of 4 anesthetized, paralyzed, and tracheotomized dogs (18-25 Kg) after coating them with tantalum dust. The animals were studied at frequencies (f) of 2, 6, & 12 Hz and tidal volumes (V_T) of 50 & 100 ml, before and immediately after histamine exposure by means of aerosol inhalation. We found radial displacement of the walls of the trachea and central airways to vary from 10% of the initial diameter with low f and V_T to 25% with f=12 Hz, V_T=100 ml. Histamine exposure resulted in substantially increased airways diameter changes of 20 to 50%, respectively. We conclude that the combination of central airway wall compliance and the impedance of the peripheral airways may cause partial shunting of the volume delivered during HFV. This phenomenon may have an important limiting effect on the efficiency of small V_T, HFV in higher frequencies, especially in situations where peripheral resistance or airway wall compliance are increased. Supported by EAOG and NHLBI grants HL00549, HL26566, HL00943, and Parker B. Francis Fdn.

44.2

SIGNIFICANCE OF MEAN AIRWAY PRESSURE DURING HIGH FREQUENCY VENTILATION (HFV). B. Simon*, G. Weinmann*, and W. Mitzner. The Johns Hopkins University, Baltimore, Maryland 21205.

We have tested how well the measured mean airway pressure (Paw) corresponds to mean alveolar pressure (Palv) during HFV. Paw was measured from small diameter side arms in six tubes with different internal diameters ranging from 12 to 24mm. Palv was determined by clamping the ventilatory tube close to the animal and measuring the equilibration pressure. With a tidal volume of 90ml we found that above 10Hz Paw always underestimated the Palv. The magnitude of this underestimation increased with increasing gas velocity, whether caused by increasing frequency or decreasing tube diameter. This Bernoulli effect was accurately predicted by the calculated kinetic energy in the measuring tube ($\frac{1}{2}\rho v^2$). However, not all of the underestimation could be explained by the Bernoulli effect. For example, at 20Hz and 90ml tidal volume, the Bernoulli effect in a 22mm tube is less than 0.5cm H_2O. Yet the Paw still underestimated Palv by 7cm H_2O. The magnitude of this "non-Bernoulli" effect also increases with increasing frequency, but the causes of it remain unclear. It may possibly result from different inspiratory and expiratory impedances of the lung airways. Whatever the reason, it is clear that for frequencies above 10Hz Paw becomes an increasingly more unreliable indicator of Palv and lung volume. Also, since all HFV systems must regulate Paw, the lung will be inflated as frequency increases, unless Paw is set lower. Supp: GM07309, HL-26532, and Parker B. Francis Foundation

44.3

CARDIOVASCULAR BEAT FREQUENCIES DURING HIGH FREQUENCY VENTILATION (HFV). W. Mitzner, F. Gioia*, R. Wetzel*, G. Weinmann* & W. Ehrlich. The Johns Hopkins Medical Inst., Baltimore, MD., 21205.

Previous studies have shown minimal changes in mean vascular pressures and cardiac output during HFV. However, during HFV in 3 pediatric patients we have observed periodic oscillations of the pressure pulsations in the aorta (Pao), pulmonary artery (Ppa) and right atrium (Pra). To study these oscillations, dogs were instrumented to measure Pra, Pao, Ppa, left atrial pressure (Pla) and either aortic or pulmonary artery stroke volume, and subjected to a jet type high frequency ventilator. We found that oscillations in all the cardiovascular variables occurred when the ventilator frequency (f) was close to the heart rate (HR), and the period of the phasic oscillations was equal to $|(f-HR)|^{-1}$. The oscillations were often of sufficient magnitude to completely obliterate the pulse pressure in Pra, Ppa, & Pla. These cardiovascular beats could also be obtained when the ventilator frequency was close to integral multiples of the HR, up to the 7th harmonic. The mechanism underlying these cardiovascular beats is not clear, but since their magnitude was attenuated by opening the chest, variations in pleural pressure (Ppl) must play an important role. Phasic fluctuations in Ppl affect both cardiac preload and afterload and thus would be enhanced at frequencies close to the HR. Supported by: HL-26532 and Parker B. Francis Foundation.

44.4

GAS CONCENTRATION PROFILES ALONG THE AIRWAYS OF DOG LUNGS DURING HIGH FREQUENCY VENTILATION. P. Scheid, T. Kaethner*, J. Kohl*, J. Piiper. Dept. Physiol., Max-Planck-Inst. exp. Med.,D-3400 Göttingen, FRG.

Anesthetized, intubated dogs at low tidal volume (40-50 ml) high frequency (1200 min^{-1}) ventilation (HFV) were equilibrated with 1 % He and SF_6. After partial wash-out from the lungs, HFV was stopped, and a deep expirogram was produced at constant, low expired flow in which He and SF_6 were recorded by mass spectrometry. The initial steep concentration rise in the expirogram ("Phase II") was identical for He and SF_6, and so were the Fowler dead space values. The slope of the alveolar plateau ("Phase III") was larger for SF_6 than for He, but both were significantly smaller than those obtained in the same animal after conventional mechanical ventilation (CMV). The data suggest that during HFV, gas transport along the conducting airways, giving rise to Phase II in the expirogram, is not limited by diffusion. Diffusion limitation, on the other hand, contributes to incompleteness of alveolar gas mixing, whose extent is, however, less for HFV than for CMV.

44.5

FACTORS DETERMINING GAS TRANSPORT IN HIGH FREQUENCY VENTILATION. M.J. Jaeger and U. Kurzweg*, Depts. of Physiology and Eng. Sc., University of Florida, Gainesville, Fl 32610.

The factors that determine the rate of transport of gases during high frequency ventilation were analysed in a model consisting of a tube (16 mm ID; 80 cm long) open on one end to atmosphere and connected at the other end to a variable speed (f) variable stroke pump. O_2 was injected at flow rates ($\dot{V}O_2$) varying between .5 and 2.5 L/ min into the tube through a needle located near the pump. The N_2 fraction (F_{N2}) was measured by continuous sampling from a needle stuck into the tube 18 cm the from pump. The tidal volume of air (V_T) entering and leaving the orifice of the tube was determined with a hotwire anemometer. With the pump at rest, F_{N2} was zero; with the pump running, air was transported upstream, against the continuous stream of O_2, and F_{N2} rose. F_{N2} was found to be proportional to V_T and inversely proportional to $\dot{V}O_2$; it was weakly dependent on frequency. Thus, with V_T = 50 ml and $\dot{V}O_2$ = .5 L/min, F_{N2} rose from .59 to .64 when f changed from 5 to 21 cps. On the contrary, F_{N2} rose from .02 to .75 when V_T increased from 10 to 120 ml. F_{N2} rose monotonically along the tube as the probe was moved from the pump toward the orifice of the tube.

44.6

TOPOGRAPHY OF $\dot{V}_A/\dot{Q}$ DURING HIGH FREQUENCY VENTILATION. T.C. Amis*, J.W. Watson*, W. Hornof* and A.C. Jackson. Depts. of Med. and Radiol. Sci. and Primate Res. Ctr., School of Vet. Med., Univ. of Calif., Davis, CA. 95616.

Recent interest in mechanisms contributing to maintenance of pulmonary gas exchange during high frequency (2-40 Hz) low tidal volume mechanical ventilation prompted us to examine the vertical distribution of $\dot{V}_A/\dot{Q}$ in 5 supine dogs (8.6 to 15.9 kg). The dogs were anesthetized with sodium pentabarbital (28.6 mg/kg), paralyzed with pancuronium bromide (0.2 mg/kg) and ventilated with positive pressure mechanical ventilation (PPV-10-15 breaths/min., 10-12 ml/kg) or HFV (5-25 Hz, 2.2-3.4 ml/kg) using a piston driven high frequency oscillator. Frequency and tidal volume levels for HFV were chosen to provide levels of CO_2 output equal to, greater than or less than that measured during PPV (5.7 to 7.3 ml/min/kg). $\dot{V}_A/\dot{Q}$ topography was assessed using continuous intravenous infusion and inhalation of radioactive Krypton-81m and a gamma camera (Searle LFOV). $\dot{V}_A/\dot{Q}$ decreased from superior to inferior lung regions in all dogs with both PPV and HFV; however the vertical gradient was less with HFV at all frequencies. Comparison of normalized inhaled Krypton-81m regional count rates suggested that better matching of $\dot{V}_A/\dot{Q}$ during HFV compared to PPV might be related to decreased ventilation of superior zones. Supported by NIH grant HL-26606.

44.7

Frequency (f) and tidal volume (V_t) dependence of CO_2 elimination ($\dot{V}CO_2$) in rabbits during high frequency ventilation (HFV). J. W. Watson† and A. C. Jackson. Primate Res. Ctr., Univ. of Calif., Davis, CA. 95616.

We examined the relationship between $\dot{V}CO_2$, f and V_t in 7 New Zealand white rabbits weighing 2.3 to 3.2 Kgs. We utilized a piston type ventilator with a bias flow of 5 l/min. $\dot{V}CO_2$ was estimated by measuring CO_2 content of the bias flow output. Flow was measured by a pneumotachometer that was shown to be linear up to 100 l/min. Inadequacies in pneumotachometer frequency response were compensated for and V_t was computed from these measurements. Equipment dead space from bias flow ports to the end of the tracheostomy tube was 12 ml. Minimal pulmonary dead space was estimated to be 5 ml from measurements of an airway cast. Measurements of $\dot{V}CO_2$ were made for constant V_t's of 3, 6, 12 and 18 ml at frequencies between 2 and 40 Hz. We found that $\dot{V}CO_2$ increased with frequency at a given V_t at low frequencies but at high frequencies CO_2 output was independent of frequency or decreased with increasing frequency. This "optimal frequency" varied from 10 Hz with a V_t of 18 ml to 18 Hz with a V_t of 6 ml. At low frequencies $\dot{V}CO_2$ appeared to be dependent upon flow amplitude with a strong tidal volume dependence at a given flow amplitude. At high frequencies $\dot{V}CO_2$ was dependent only upon V_t. These results are in contrast to what we have found in the dog where $\dot{V}CO_2$ is primarily dependent upon flow amplitude over the same frequency range (Fed. Proc. 41:1747, 1982). Supported by NIH grant HL-26606.

44.8

LUNG UPTAKE OF $C^{18}O$ DURING HIGH FREQUENCY OSCILLATION. Neil R. MacIntyre* and Mitchell Friedman. Duke Univ, Durham, NC 27710, and Univ. of North Carolina, Chapel Hill, NC 27514.

Because high frequency oscillations (HFO) have been shown to produce normal O_2 and CO_2 transport in the lungs, we reasoned that HFO might also be useful in delivering and sampling carbon monoxide (CO) in the lungs. To study this, we gave 30 cc boluses of 10% Helium (He) and .3% carbon monoxide ($C^{18}O$) and continuously measured airway gas concentrations for 30 sec. in 4 dogs attached to a closed system piston oscillator (rate=30Hz, piston displacement=35cc) at function residual capacity (FRC). From the He equilibration values (usually reached in 5-6 sec.) FRC was calculated. From the $C^{18}O$ disappearance after He equilibration, a log decay constant was calculated. In each dog these values were compared with the FRC and the log decay constant for $C^{18}O$ uptake as determined by the standard breath hold technique. FRC and the log decay rate for $C^{18}O$ by the HFO technique were respectively 342±74cc and -.076±.011 sec^{-1} (mean, SEM). With the breath hold technique, these values were 361±47cc and -.14±.005 sec^{-1}. Our results show that CO uptake during HFO can be rapidly and reproducibly measured. The slower apparent decay of CO with HFO than with the breath hold technique may be: 1)a function of the different lung volumes used for each test, 2)a reflection of physiologic changes in the lung during HFO, or 3)a result of the lung not behaving as a single compartment system during HFO.

44.9

AEROSOL PENETRATION DURING HIGH FREQUENCY VENTILATION. H. Maeda*, W. Mitzner, S. Watanabe*, and H. Wagner. The Johns Hopkins Medical Institutions, Baltimore, Md. 21205.

It has been known that effective alveolar ventilation can be maintained with tidal volume less than the dead space volume under high frequency ventilation (HFV). An explanation of this phenomena is given in terms of the combined effects of diffusion and convection. We investigated penetration of non-diffusible aerosols to assess the extent of convection during HFV in anesthetized dogs. 0.9u aerosol labeled with high specific activity of Tc-99m was generated with an ultrasonic nebulizer. A continuous flow of oxygen (2 to 3ml/sec) containing the aerosol was established into the trachea about 7 cm from the carina through an endotracheal tube. Oscillations were generated by a piston pump at frequencies of 10 and 20 Hz with a tidal volume of 15ml, which was about one quarter of physiological dead space. Deposition of aerosol in the lung was detected by a gamma camera. We found that in the lung periphery the radioactivity increased linearly with time. This result shows that transfer of aerosol into the peripheral lung regions is possible, and that aerosol penetrates almost with the same speed as radioactive gases under these conditions, suggesting an important role of convective flow during HFV with small tidal volume.

44.10

AN IMPROVED MODEL OF GAS TRANSPORT DURING HFV. M.C.K. Khoo*, A.S. Slutsky, J.M. Drazen, J. Solway*, N. Gavriely*, and R.D. Kamm*. West Roxbury VA and Brigham & Women's Hosp, M.I.T. and Harvard School of Public Health, Boston, MA.

Whereas a previously described theoretical model of gas mixing during HFV (Science 209:1609, 1980) predicted the sole dependence of CO_2 elimination ($\dot{V}CO_2$) on the product of frequency and tidal volume, subsequent experiments have shown an additional dependence on V_T ("V_T-effect")(J.C.I. 68:1475, 1981). We have modified our previous theoretical model in the following ways: (1) a frame of reference that moves with the oscillatory flow is assumed, thus taking into account the movement of gas across adjacent regions of changing cross-sectional area. This modifies the effective diffusivity and accounts for about 10% of the "V_T-effect"; (2) during each exhalation a portion of the CO_2 concentration (FCO_2) profile established by augmented dispersion is swept away at the airway opening (AO) by a fresh gas bias flow (BF) which traverses the airway. This accounts for about 90% of the "V_T-effect" and (3) as $\dot{V}CO_2$ increases, FCO_2 in the BF increases leading to a decreasing alveolar-BF FCO_2 difference and thus may limit the rise in $\dot{V}CO_2$. With these modifications the model predicts a substantial "V_T-effect" and that a large percentage of the total resistance to CO_2 transport resides in the first 6 generations of canine lung. This result is consistent with published observations (Fed Proc. 41(5):1692, 1982). (Supported by VA, HL26566 and Parker B. Francis Foundation.)

44.11

EFFECT OF BIAS FLOW RATE ON CO2 REMOVAL DURING HIGH FREQUENCY VENTILATION IN HUMAN SUBJECTS. J. Solway*, N. Gavriely*, J.M. Drazen, J.L. Lehr*, P.A. Drinker*, A. Saari*, A.S. Slutsky, and T.H. Rossing*. Brigham and Women's Hospital, Harvard School of Public Health, and West Roxbury VAH, Boston, MA.

In many experimental configurations, CO2 is removed during low tidal volume, high frequency ventilation (HFV) by a fresh gas bias flow (BF). Although rapid bias flow rates ($\dot{V}_{BF} \geq 30$ L/min) were used previously to maintain very low bias flow CO2 concentration, the influence of $\dot{V}_{BF}$ on HFV efficacy has not been studied. We reasoned that the rise in BF CO2 concentration which would accompany a decrease in $\dot{V}_{BF}$ to 10-15 L/min should result in minor decrease in HFV efficacy. To test this prediction, we studied 4 patients with chronic tracheostomies during HFV with various $\dot{V}_{BF}$s. We found that for any frequency (0.5-20 Hz) - tidal volume (20-40 ml) combination, CO2 removal rate did not change significantly as $\dot{V}_{BF}$ was varied over the range 12-27 L/min. We conclude that during these conditions of HFV, CO2 removal is not substantially impaired by decreasing bias flow rate to as low as 12 L/min. Use of lower $\dot{V}_{BF}$ might have important practical design and safety advantages for clinical application of HFV. (Supported by Parker B. Francis Fnd., HL26566, HL00549, and the V.A.)

44.12

HIGH FREQUENCY CHEST WALL COMPRESSION AS A METHOD OF ASSISTED MECHANICAL VENTILATION IN OBSTRUCTED HYPERCAPNEIC DOGS. D. Gross*, V.Vartian*, H.Menami*, H.K.Chang, A.Zidulka*(SPON:M.King) Department of Respiration and Anesthesia, Montreal General Hospital, McGill University, Montreal, Quebec.

High frequency chest wall compression (HFCWC) was studied in 6 spontaneously breathing (SB) anesthetized dogs (24-33 Kg) who had airflow obstruction created by metal beads inserted in the airways. HFCWC was achieved by a piston pump rapidly oscillating the pressure in a modified double blood pressure cuff wrapped around the lower thorax. Every 30 min. spontaneous ventilation was alternated with HFCWC (at 3, 5 and 8 Hz) while the dogs continued to breathe spontaneously. At the end of each 30 min. arterial blood gases were measured. Thereafter, a screen pneumotachometer was attached transiently and by integrating flow, spontaneous tidal volume (Vts) and oscillatory tidal volume (Vto) were measured as well as spontaneous respiratory frequency (fs).

HFCWC reduced the elevated $PaCO_2$ during spontaneous ventilation from 52.3 ± 4.3 to 29.0 ± 3.4 mmHg during 5 Hz($p<.01$), from 53.6 ± 4.9 to 36.0 ± 6.5 mmHg during 8 Hz($p<.05$), and from 49.1 ± 4.4 to 35.4 ± 2.4 mmHg during 3 Hz($p<.005$). Values of PaO_2 remained unchanged despite a decrease in spontaneous minute ventilation. We concluded that HFCWC can assist in eliminating CO_2 in obstructed hypercapneic dogs. (Supported by the Canadian Thoracic Society).

45.1

PLANT CELLS, EMBRYOS, GROWTH AND DEVELOPMENT IN SPACE. Abraham D. Krikorian . Dept. of Biochemistry, State University of N.Y., Stony Brook, N.Y. 11794

A progress report will be given on work aimed at developing plant test systems at different levels of initial organization which, being capable of further growth and development, may be exposed to a hypogravity environment. Behavior and responses of such systems may then be examined, compared and contrasted with their performance at 1 g. While in the first instance some investigations need to focus upon more partial responses, e.g. tropisms, nastic movements, cell division, metabolism and somatic development, all these need to be seen in terms of their consequences for the development of the whole organism. It is for these reasons that our preferred experimental systems are being developed at different levels, i.e. free protoplasts which can constitute new cellulose walls and eventually grow into plants, free somatic cells of angiosperms which by division under aseptic conditions may be induced to form somatic embryos that can develop into plantlets, and finally at the level of plants as they develop from seeds. Base-line data on cell division rates, behavior of cells in growing points, chromosome replication and distribution, and phenotypic clonal fidelity have been emphasized recently. Such information will be useful in tests aiming to disclose thresholds of sensitivity for significant morphogenetic events at the sub-cellular, cellular or at the apical/growing region and organ level. (Supported by NASA Grant NSG7270).

45.2

EFFECT OF CLINOSTAT AND CULTURAL CONDITIONS ON THE SEED TO SEED GROWTH OF ARABIDOPSIS AND CARDAMINE: INITIAL RESULTS. Takashi Hoshizaki . JPL, Pasadena, CA 91109

A clinostat simulates weightlessness for plants by slowly rotating plants around a horizontal axis. Though not ideal, it is a practical device to study on Earth the effects of weightlessness on plants by simulation. About half of Arabidopsis thaliana (L.) Heynh. plants grown on clinostats die prior to flowering. The survivors do not differ in appearance from controls but produce seeds less viable than controls. In a Soviet space experiment, only one A. thaliana plant was reported to survive. This plant was still in the vegetative stage on the 56th day of the experiment while ground controls were flowering and setting seeds. In this and other studies one or more stages of A. thaliana life cycle has been completed in space. However the growth of plants through all stages of development in a single sequential episode has not been accomplished. It has been hypothesized that the difficulty of growing plants may be more from cultural than from gravitational conditions. Arabidopsis thaliana (L.) Heynh. and Cardamine oligosperma Nutt. were chosen for study for their short life cycle and small size. The initial results of clinostatting and varying moisture levels, light intensity and gas exchange rates on the growth, flowering and seed pod formation are discussed. A follow-on space experiment is planned. (Supported by NASA Contract NAS 7-100.)

45.3

THE FIRST PLANTS TO FLY ON SHUTTLE. Allan H. Brown and David K. Chapman. Univ. of Penna. and Univ. City Sci. Ctr., Philadelphia, PA 19104-4288

The first plants to journey for scientific purposes into earth orbit on board the NASA Shuttle, Columbia, were sunflower seedlings. Heflex Bioengineering Test (HBT) objectives were those that could establish more confidently the most appropriate soil moisture content to be used for plant culture in microgravity during a botanical experiment, "Heflex," scheduled for flight on the NASA/ESA Spacelab-1 Mission in late 1983. Seedling growth rates have been found strongly dependent on soil moisture content at 1 g and, both from theory and from reports of difficulties encountered when plant growth was tested in a Soviet space vehicle, it seemed possible that seedling growth response to soil moisture as a test variable might not be the same on earth and in near weightlessness. In our third attempt to accomplish HBT all flight test objectives were achieved on STS-2. After the flight a ground control (same temperature profile and same test duration) was performed. Principal conclusion: the 70% soil moisture content originally planned for use with "Heflex" was found acceptable in microgravity as well as on earth. (Supported by NASA Grants NGR 39-030-010 and NGR 39-010-149 and Contracts NAS 9-15531 and NAS 9-15340)

45.4

RESPONSE OF YOUNG PLANT SEEDLINGS TO SPACE FLIGHT ON STS-3. Joe R. Cowles, H. W. Scheld*, Carol Peterson* and Richard LeMay*. Department of Biology, University of Houston, Houston, TX 77004.

Pre-germinated pine seedlings and oat and mung bean seeds were prepared for flight on STS-3. The experimental packages (flight and ground control), were placed in mini-plant growth units (PGU) equipped with lights, day:night cycling and some temperature regulation. Observations after the 8-day flight revealed that pine seedlings grew and developed similar to ground controls. Oat and mung bean seeds germinated and also grew well in space. The most noticeable difference between flight and ground control seedlings was the number of oat and mung bean roots which grew upward out of the root support in the flight seedlings and certain orientation difficulties experienced by the mung bean. Quantitation of lignin in stem sections of all three species showed very little difference in lignin content between flight and ground control tissues. Protein content and PAL and peroxidase activity also was determined in the pine tissues. The upper hypocotyl sections showed an increased amount of protein and a corresponding decresse in PAL and peroxidase activity. These results along with certain laboratory studies will be discussed. (Supported in part by NASA grants NAS 2-1165 and NSG 9042)

45.5

A COMPARATIVE STUDY OF MONOCOT AND DICOT ROOT DEVELOPMENT IN NORMAL (EARTH) AND HYPOGRAVITY (SPACE) ENVIRONMENTS. Robert D. Slocum and Arthur W. Galston. Dept. of Biology, Yale Univ., New Haven, CT 06511 USA

A major unanswered question pertaining to the growth and development of plant organs in hypo- or microgravity environments is whether organelles and tissues which are thought to be involved in gravity perception and growth orientation develop normally. We have investigated this and other parameters of root development in oat (Avena sativa) and mung bean (Phaseolus aureus) seedlings grown under a 14/10 hr. L/D cycle in sealed growth chambers carried aboard the space shuttle during a recent mission (STS 3) of eight days duration. Plants grown continuously in the hypogravity environment of the shuttle were fixed in the field shortly after landing and were compared with parallel ground controls. In all cases, gross root tissue morphology was normal at the light microscope level, but flight-grown tissues were slightly plasmolyzed. The subcellular morphology of root tissues and, particularly, root cap cells, which contain dense, multigranular amyloplasts that rapidly sediment in response to changes in the direction of mass acceleration and are implicated in root graviperception, were also examined using electron microscopy. General cell and organelle ultrastructure of the cap cells was similar in both sets of plants, thus gravity appears to play a minimal role in their development.

45.6

GRAVITROPIC BASIS OF LEAF BLADE NASTIC CURVATURES. Alice B. Hayes. Loyola University, Chicago, Illinois 60611.

The curvatures produced in leaf blades by auxin treatment have been described as nastic curvatures because the initial differential growth was always enhanced on the lower side regardless of the side of application. We now know, however, that blades can show differential growth of either the upper or the lower side depending upon conditions of treatment. Therefore, the dorsiventrality of the blade influences but does not limit the direction of curvature. Although auxin supply appears to be the limiting factor with respect to the production of curvature, gravitational orientation is an important factor in determining the direction of curvature. The dorsiventral directionality of response to growth regulators and the response to changes in orientation to gravity suggest that blade curvatures are analogous to negative or positive gravitropism. Only growth regulators associated with auxin transport or ethylene production show a directional response. Cytokinins modify the response, but gibberellic and abscisic acid do not produce, promote, or inhibit leaf blade responses. Studies of the time course of ethylene production and the effects of ethylenegenic or ethylene-inhibiting compounds show that neither blade hyponasty or epinasty can be accounted for by ethylene alone. Petiole responses, however, are not directional, and the leaf angle changes produced by rotation or auxin treatment can be accounted for by ethylene production.

45.7

MECHANICAL STRESS REGULATION OF GROWTH AND PHOTOSYNTHETIC PRODUCTIVITY OF GLYCINE MAX MERR. CV. WELLS II UNDER DIFFERENT ENVIRONMENTAL REGIMES. Thalia Pappas* and Cary A. Mitchell. PURDUE UNIVERSITY, West Lafayette, IN 47907

Brief seismo- or thigmo-treatments applied twice daily to vegetative soybean plants as gyratory shaking or manual stem rubbing, respectively, significantly retarded plant growth and photosynthetic productivity. Plants grown in a greenhouse in the summer under one-third to one-half of full sun showed a greater degree of sensitivity to mechanical stress than did plants grown under full sun. Growth dynamics analysis of plants grown in a controlled environment favoring mechanical responsivity indicated a decrease in relative growth rate (RGR) of shaken plants, indicating a stress-induced inhibition of photosynthetic productivity. Changes in net assimilation rate (NAR) and leaf area ratio (LAR) are being assessed to determine which of these RGR components contribute to the observed changes in RGR. Shorter-term measurements of leaf gas exchange are being conducted to complement growth dynamics analysis (supported by NASA grant NSG 7278).

45.8

POLYAMINE FORMATION BY ARGININE DECARBOXYLASE AS A TRANSDUCER OF HORMONAL, ENVIRONMENTAL AND STRESS STIMULI IN HIGHER PLANTS. **Arthur W. Galston, Hector E. Flores and Ravindar Kaur-Sawhney* Dept. of Biol, Yale Univ., New Haven, CT. 06511.**

Recent evidence implicates polyamines such as putrescine (Put), spermidine and spermine in the control of cell division and aspects of growth, morphogenesis and senescence in higher plants. Put is formed preferentially in many plants through a pathway involving arginine decarboxylase (ADC) and agmatine as an intermediate. In dark-grown pea plants, we find that ADC in both the photostimulated buds and photoinhibited stems is regulated by light absorbed by the photochromic pigment phytochrome. It is also controlled by the hormone gibberellin, which can reverse the effects of red light. In dwarf pea plants grown in the light, gibberellin application promotes ADC activity within 3 hrs, and maximally at 9 hrs. A second peak near 30 hrs indicates circadian control correlating with mitotic cycles. In peeled oat and other cereal leaves, sharp rises in ADC activity and Put titer occur within 2-3 hrs following osmotic shock (0.6M sorbitol) or pH stress (pH<5). Both responses are sensitive to cycloheximide and α-difluoromethylarginine, a specific enzyme activated inhibitor of ADC. Analogous inhibition of ornithine decarboxylase (ODC) produces no effect on the stress-induced rise in Put, despite the presence of ODC activity in the leaves. (Aided by NASA Grant No. NSG-7290 to A.W.G.)

45.9

THE INTERACTIONS OF THIGMIC, GRAVITIC AND DROUGHT PERTURBATIONS ON CALLOSE AND ETHYLENE PRODUCTION IN PLANTS. M. J. Jaffe, Wake Forest University, Winston-Salem, NC 27109

Thigmic or gravitic perturbation of corn seedlings induce callose deposition in the shoot. The inhibitor of protein glycosylation, 2-deoxy-D-glucose (DDG) inhibits both callose deposition and the morphological responses. Data will be presented which shows the result of the interaction of the two environmental stimuli. Thigmic or drought perturbation of tomato plants induces callose deposition. Interactive experiments will also be described in this system. The use of computer assisted image analysis is used to implement the experiments. (Supported by grants from NASA, NSF and BARD).

45.10

DIFFERENTIAL EFFECTS OF GIBBERELLIC ACID ON DEVELOPMENT OF THE THREE ORGANS OF THE MARINE, GIANT COENOCYTE, CAULERPA PROLIFERA. William P. Jacobs and Wendy Davis. Biology Department, Princeton University, Princeton, N. J. 08544.

A gravity effect on morphogenesis was recently discovered in this alga (Jacobs and Olson, 1980). Sedimenting organelles are being investigated as the likely gravity-sensors and hormones as likely transducers. The hormone gibberellic acid (GA), known from fungi and angiosperms, has not been identified in algae, although GA-like activity was reported in bioassays of Caulerpa extracts (Augier, 1974). The addition of GA at hormonal levels to the usual culture medium resulted in increased elongation of the horizontally growing rhizome, no change in blade elongation or rate of initiation, and an increase in the rate of initiation of rhizoids. Such a differential effect on development of the 3 organs produced by this giant single cell increases the probability that a GA-like hormone is involved in the morphogenetic gravity response. (Supported in part by NASA Grant NSG-7280.)

CALCIUM AND CARDIAC MUSCLE CONTRACTION

46.1

FREQUENCY, CONTRACTILE FORCE AND CALCIUM LOAD. Hiroshi Kotake* and Mario Vassalle. Department of Physiology, SUNY, Downstate Medical Center, 450 Clarkson Ave., Brooklyn NY. 11203.

The relationship between drive rate and contractile force was studied in Purkinje fibers perfused in vitro under different calcium loads. Both electrical and mechanical activities were recorded. The following results were obtained. 1) When the rate was increased from 30-45/min to 60, 120, 180 and 240/min the contractile force decreased and then increased. 2) On return to the basal rate, the force increased transiently above control. 3) The initial fall of force during and the subsequent rebound after a faster drive were greater the faster the rate. 4) Increasing $[Ca]_o$ from 2.7 to 5.4, 10.8 and 16.2 mM changed the response in that the initial fall became less and was substituted by an increase in force depending on the $[Ca]_o$ and the rate of drive. 5) At the same time, the force rebound decreased and it could be substituted by a fall in force. 6) Increasing $[K]_o$ during exposure to high $[Ca]_o$ reversed the effects toward the patterns seen in Tyrode solution. 7) During exposure to high $[Ca]_o$, tetrodotoxin acted similarly to high K. 8) Lowering $[Na]_o$ with or without simultaneous increase in $[Ca]_o$ tends to modify the response to faster rates as high $[Ca]_o$. 9) In the low $[Ca]_o$, the initial fall in force with faster drives was still present but the rebound increase in force was relatively greater than at normal calcium. It is concluded that cellular calcium is responsible for or modulates some of the changes in force related to a change in rate. (Supported by a NIH grant HL27038).

46.2

CONTRACTILE RESPONSES IN AMPHIBIAN ATRIAL AND VENTRICULAR PACEMAKER PREPARATIONS. P.L. Morales*, J.R. López, C. Caputo. C.B.B., IVIC, Apartado 1827, Caracas 1010A, Venezuela.

Atrial and ventricular pacemaker preparations were dissected from the tropical toad Leptodactylus insularis. The isolated pacemakers were mounted in a lucite chamber that allowed rapid changes of the experimental solutions (< 0.4 sec). The treatment of spontaneously beating pacemakers with high potassium solutions (90 mM) induced contractures, which in the presence of normal calcium and high sodium, relaxed spontaneously with a time course of about 152 ± 2 sec. This time course was reduced to 126 ± 3 in the absence of extracellular calcium. Exposure of the preparation to high concentration of external divalent cations (Ca^{2+}, Sr^{2+}, Ba^{2+}) caused contractile responses whose amplitude and duration depended on the divalent cation and its concentration. The largest response was obtained with Ba^{2+}, which contrary to what happened with Ca^{2+} and Sr^{2+} induced contractures, that did not relax spontaneously, and required withdrawl of external Ba^{2+} to do so. After the spontaneous relaxation phase of K^+ contractures, obtained in the absence of external Ca^{2+}, the addition of Ba^{2+}, Sr^{2+} or Ca^{2+} in the presence of high K^+ also caused transients responses. Caffeine produced similar effects. These results may be interpreted either that, Ba^{2+}, Sr^{2+} and Ca^{2+}, enter the fibers and interact directly with the contractile proteins or that they may act to induce further release of calcium from some intracellular store which is also sensitive to caffeine. (Supported by CONICIT, S1-1148).

46.3

ACTIVATION OF A CAFFEINE-SENSITIVE COMPARTMENT IN THE RABBIT SEPTUM. Terrell L. Rich* and Glenn A. Langer. Department of Physiology and Cardiovascular Research Laboratories, UCLA School of Medicine, Los Angeles, California 90024.

These studies were directed to the activation of a caffeine-sensitive compartment in the arterially perfused interventricular rabbit septum. When the heart was perfused with HEPES-buffered perfusate, pH=7.40, the addition of 20 mM caffeine had no effect on ^{47}Ca uptake rate or on ^{45}Ca efflux rate. If the sarcolemmal (SL) Na^+,K^+-ATPase is inhibited by ouabain addition to the perfusate during a ^{47}Ca load there is a linear, ouabain concentration-dependent increase of the ^{47}Ca uptake rate upon addition of caffeine. The addition of 1-3 μM vanadate to the perfusate also activates caffeine-sensitivity in that the ^{47}Ca uptake rate is augmented by caffeine in the presence of vanadate. Caffeine has no effect on ^{45}Ca washout rate in either ouabain- or vanadate-treated muscles. It is proposed that under HEPES perfusion conditions the sarcoplasmic reticulum (SR) may not be activated, with Ca exchange limited to a rapidly exchangeable SL compartment. These kinetics are similar to those found in tissue culture under similar perfusion conditions. It is further proposed that as Ca flux is increased by ouabain the SR is activated and its inhibition by caffeine therefore becomes manifest as a retention of Ca by the cell. Low dose vanadate may inhibit the SL Ca pump, therefore diverting Ca pumping to the SR and making it sensitive to caffeine inhibition.

(Supported in part by USPHS HL 28539-01 and AHA-GLAA 694 G2-2.)

46.4

EARLY TRANSIENT DEPLETION OF $[Ca]_o$ DURING SINGLE BEATS OF RABBIT VENTRICULAR MUSCLE MEASURED WITH Ca SELECTIVE MICRO-ELECTRODES. Donald M. Bers. Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024.

Extracellular Ca activity was monitored with double-barreled Ca-selective microelectrodes (with tip diameters 4-15 μm) in rabbit papillary muscles. One barrel was silanized and backfilled with Simon's neutral Ca exchange resin (ETH 1001) and a 1 mM Ca buffer. The other barrel was filled with 140 mM NaCl or 1 M KCl and served as the reference barrel. During individual beats, isolated or during steady stimulation an early transient depletion of extracellular [Ca] is recorded. This decrease of $[Ca]_o$ begins before significant tension develops. The percentage depletion of $[Ca]_o$ decreases as $[Ca]_o$ increases (with consequent increase of tension) suggestive of saturation. (In one experiment the depletion was ∿50 μM at 2.0 mM Ca_o, ∿25 μM at 0.5 mM Ca_o and ∿15 μM at 0.2 mM Ca_o.) On the other hand the magnitude of the depletion appeared to increase in parallel with the increase of tension produced by increasing the stimulation frequency. This early Ca depletion can be virtually eliminated by 1 mM Co (0.2 mM Ca) and is decreased by $4x10^{-7}$M verapamil. The magnitude of Ca_o depletion clearly depends upon the exact position of the electrode, but should always be a minimum estimate of depletion occurring at the sarcolemmal surface. If this depletion represents Ca entering the cells in a single beat, the amount of Ca is indeed nearly that required for tension development. (Supported by American Heart Association, Greater Los Angeles Affiliate, and USPHS HL 11351-15.)

46.5

MEASUREMENT OF ACTIVATION-RELATED EXTRACELLULAR Ca^{++} CONCENTRATION CHANGES IN ATRIAL MUSCLE WITH ANTIPYRYLAZO III. Don Hilgemann*, Michael Delay*, Julio Vergara* and Glenn Langer. Department of Physiology UCLA Los Angeles, California 90024.

Free Ca concentration in a restricted extracellular space in cardiac muscle ($[Ca^{++}]$) was monitored using external antipyrylazo III (AP). Left atria of guinea pigs were superfused rapidly with a physiological salt solution (100% O_2/10 mM HEPES/pH 28°C/0.3 mM AP). In the presence of 4 mM Mg, AP is an advantageous Ca indicator in the range from 0.1 to 0.6 mM. A patch of muscle was illuminated, and transmitted light intensities were monitored at selected wavelengths to allow separation of Ca-related from movement-related transmission changes. When the total bath Ca concentration is rapidly changed, $[Ca^{++}]$ takes several minutes to equilibrate, and contractile force follows closely this time course. From such curves $[Ca^{++}]$ signals are readily calibrated for each muscle. Effects of 2 min stimulation at 2 Hz after rest are described: 1) Changes are close to the noise level (∿10 μM) under control conditions. 2) With 10^{-7}M isoproterenol estimated mean $[Ca^{++}]$ drops by 15 to 50 μM over several beats; replenishment during rest takes minutes. 3) With addition of 10^{-7}M ryanodine under these conditions a rapid positive force staircase develops, and mean $[Ca^{++}]$ drops by about 100 μM in just 5 to 7 beats with little subsequent change. Results demonstrate with a new method that transsarcolemmal Ca movements at excitation can be substantial while diastolic efflux is slow.

(Supported by the Castera Foundation.)

46.6

Ca^{2+}-BINDING TO PHOSPHOLIPASE TREATED CARDIAC SARCOLEMMA: SPECIFICITY OF POLYMYXIN B (PxB) VS. La^{3+} AS A Ca^{2+} DISPLACER J.M. Burt and G.A. Langer. Univ. Calif. Los Angeles, CA 90024.

Sarcolemmal anionic phospholipids (AP) represent potential Ca^{2+}-binding sites which may be important in cardiac contractility. PxB is a positively charged protein (1200 MW) which has been postulated to bind specifically to AP. If true, this protein may represent a probe for the role of AP in cardiac contractility. PxB displaces Ca^{2+} from isolated cardiac sarcolemma in a dose dependent, reversible manner. Whereas La^{3+} (1 mM) displaces 0.198±0.02 SEM nmole Ca/μg membrane protein and 0.211±0.035nmole Ca/nmole membrane PO_4(hydrolyzed from extracted lipids), PxB (0.1mM) displaces 0.083±0.01nmole Ca/μg protein and 0.106±0.01 nmole Ca/nmole PO_4. Treatment of the membranes with Phospholipase C (PLC) does not affect the La^{3+} or PxB displaceable Ca/μg protein in spite of a reduction in total detectable PO_4. This probably reflects the preference of PLC for non-anionic phospholipids. Treatment of the membranes with Phospholipase D, which increases the quantity of AP in the membrane, results in a 1.3 fold increase in La^{3+} displaceable Ca/μg protein (to 0.262±0.02, $p<.05$) and a 2.5 fold increase in La^{3+} displaceable Ca/nmole PO_4 (to 0.542±0.10, $p<.05$). PxB displaceable Ca^{2+} increases 2 fold with respect to protein (to 0.163±0.02, $p<.02$) and 2.6 fold with respect to PO_4 (to 0.281±0.05, $p<.05$). These data indicate that PxB can be used as a probe for anionic phospholipid Ca^{2+}-binding sites. (Support: American Heart Association, Greater Los Angeles Affiliate #652-F2, USPHS HL 11351-15 and Castera Foundation.)

46.7

EFFECT OF DIVALENT AND TRIVALENT IONS ON Na^+/Ca^{2+} EXCHANGE IN CARDIAC SARCOLEMMAL VESICLES. Terry L. Trosper* and Kenneth D. Philipson. UCLA School of Medicine, Los Angeles, CA 90024.

Several divalent and trivalent ions inhibit Na^+/Ca^{2+} exchange in canine cardiac sarcolemmal vesicles, as measured by inhibition of initial rates of uptake of ^{45}Ca in vesicles containing 140 mM Na^+. The relative order of effectiveness of the ions (La > Nd > Tm > Y > Cd > Sr > Ba > Mn > Mg) differs from that for their displacement of bound Ca^{2+} from sarcolemma and their inhibition of excitation-contraction coupling in rat papillary muscle. Inhibition of initial rates of Na^+-dependent Ca^{2+} uptake by the lanthanides is significant at micromolar concentrations in the presence of 20 μM Ca^{2+}; this range is more than an order of magnitude lower than divalent concentrations causing marked inhibition. Divalent ion effectiveness is related to the ionic radius as compared to that of Ca^{2+}. The lanthanides are also potent inhibitors of Na^+-independent Ca^{2+} efflux from sarcolemmal vesicles, in the same concentration range as for uptake, and they inhibit Na^+-induced efflux. However, effects on initial efflux rates were not investigated. Very low concentrations (1-6 x 10^{-7}M) of some lanthanides stimulate Na^+/Ca^{2+} exchange slightly, e.g., by 10 to 20%.

(Supported by an Investigative Group Award from the American Heart Association, Greater Los Angeles Affiliate.)

46.8

Na^+-Ca^{2+} EXCHANGE IN CANINE CARDIAC SARCOLEMMAL VESICLES (SLV): KINETIC PROPERTIES AND ROLE OF COUNTERIONS. Raymond F. Kauffman.*(SPON: K. D. Kurz). Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285

SLV were prepared by the method of Jones et al. (J. Biol. Chem. 255:9971, 1980). Na^+-Ca^{2+} exchange across the membrane of SLV loaded with NaCl was monitored in a KCl medium by dual wavelength spectroscopy in the presence of Arsenazo III. The $K_{0.5}(Ca^{2+})$ for Na^+-Ca^{2+} exchange was 12.0 ± 1.9 μM (mean ± S.E.M.) and this value was not altered by 2μM valinomycin. By contrast, valinomycin increased V_{max}, from 13.0 ± 1.0 to 25.8 ± 1.7 nmol/sec/mg protein. The $K_{0.5}(Ca^{2+})$ and V_{max} were essentially unaltered (12.0 ± 1.2μM and 15.0 ± 0.8 nmol/sec/mg protein, respectively) when choline-chloride was used in place of KCl in the uptake medium. In an isoosmotic sucrose medium $K_{0.5}(Ca^{2+})$ was approximately 15-fold lower (0.70 ± 0.06 μM), while V_{max} was unchanged. The present data indicate that 1) V_{max} is limited by the permeability of the membrane to counterions (thus confirming the electrogenic nature of the exchange), and 2) $K_{0.5}(Ca^{2+})$ is a property of the Na^+-Ca^{2+} exchanger in these vesicles rather than a reflection of a Ca^{2+}-dependent increase in K^+-permeability. The low $K_{0.5}(Ca^{2+})$ in the sucrose medium demonstrates high affinity of Na^+-Ca^{2+} exchange for Ca^{2+}--higher $K_{0.5}$ values observed in KCl media are apparently due to cation competition and/or effects of high ionic strength.

47.1

A COMPARISON OF THE SUBCELLULAR DISTRIBUTION OF ^{125}I-INSULIN AND ^{125}I-ASIALOFETUIN IN PERFUSED RAT LIVER. Walter F. Ward, Dept. of Physiology, Univ. of Texas Health Science Center at San Antonio, Texas, 78284.

Insulin appears to be internalized by liver cells through an endocytotic process. A logical sequence of events would then include fusion of the endocytotic vesicle with a lysosome resulting in degradation of the insulin molecule. Asialoglycoproteins are known to be internalized by an endocytotic process which leads to fusion with a lysosome and degradation of the molecule. Asialoglycoproteins should therefore provide a model system for investigating the fate of the insulin molecule. Isolated perfused rat livers were exposed to either ^{125}I- insulin or ^{125}I-asialofetuin. The mitochondrial-lysosomal fraction was isolated and subjected to density gradient centrifugation in a Percoll-sucrose medium. It was observed that the density gradient profiles of ^{125}I-asialofetuin corresponded to the profile for lysosomal marker enzyme, n-acetyl-β-D-glucosaminidase, with an additional peak in the plasma membrane (5'-nucleotidase) region of the gradient. ^{125}I-insulin distribution corresponded to the distribution of 5'-nucleotidase and even under varying perfusion conditions could not be found in the lysosomal fractions. It would appear that either lysosomal degradation of insulin is extremely rapid or that lysosomes play a minor role in comparison to other insulin degrading enzymes. (Support: AM 29797).

47.2

GLUCAGON AND INSULIN RECEPTORS IN CULTURED HEPATOCYTES. EVIDENCE FOR REGULATION BY INSULIN AND GLUCOSE. Joseph C. Dunbar* and Paolo Cremonesi.* (Spon: P.P. Foa) Wayne State Univ., Detroit, MI 48201

Glucagon and insulin receptor populations are altered in many different states. To determine the direct effect of insulin and/or glucagon, hepatocytes were isolated from female Wistar-Furth rats and cultured in TC 199 in the presence or absence of hormones. Following 24 hours culture, there was a decrease in glucagon and an increase in insulin receptor binding compared to pre-incubation levels. Fetal calf serum (fcs) was necessary to maintain binding following culture. When fcs was omitted from the media both insulin and glucagon binding was decreased. When hepatocytes were cultured in the presence of added insulin and/or glucagon, insulin increased glucagon and decreased insulin (down regulation) receptor binding. Added glucagon decreased the binding to both insulin and glucagon receptors. When both glucagon and insulin were added there was a decrease in the insulin and an increase in glucagon binding. High glucose (5mg/Ml) in the culture media decreased glucagon binding but had no effect on insulin binding. Added amino acids did not alter insulin or glucagon binding. Analysis of the data indicated that binding changes were due to changes in receptor number rather than affinity. This study confirms the direct hormone regulation of its own receptor as well as other hormone receptor populations. (Supported in part by USPHS-NIH Grant RR 08167)

47.3

SODIUM-CALCIUM EXCHANGE IN HAMSTER INSULINOMA PLASMA MEMBRANE. Malcolm M. Bersohn, Kenneth D. Philipson University of California at Los Angeles, CA 90024

Insulin release from isolated pancreatic islets and from insulinoma cells has been linked to increased uptake of Ca into the cells as a result of several different secretory stimuli. To investigate the possible role of Na-Ca exchange in regulating intracellular Ca in beta cells, we isolated plasma membranes from a transplantable Syrian hamster insulinoma by differential and sucrose-gradient centrifugation. Purification of plasma membrane marker enzymes Na,K-ATPase, adenylate cyclase, and adenosine monophosphatase was approximately 6-fold, and the preparation was substantially free of endoplasmic reticulum and mitochondria. We measured Na-Ca exchange using plasma membrane vesicles loaded with 140 mM Na, then diluted into 140 mM KCl with ^{45}Ca, as previously described for heart tissue (Am. J. Physiol. 242:C288-C295, 1982). Na-dependent Ca uptake was linear for about 10 sec and then slowed, reaching a plateau at 2-4 min. Na-independent Ca uptake was less than 1/3 of the Na-dependent Ca uptake under most conditions and had a different time course, reaching half maximal value in 5-10 sec and then increasing very slowly. Using inside Na-dependent Ca uptake at 10 sec to measure initial rates, K_m for Ca was 21 μM and V_{max} was 0.27 nmol·mg protein^{-1}·sec^{-1}. Na-Ca exchange may be an important mechanism for regulation of intracellular Ca and stimulus secretion coupling in beta cells. (Supported by Am. Diabetes Assoc., Southern California Affiliate.)

47.4

ACTION OF INSULIN ON ERYTHROCYTE HEXOSE MONOPHOSPHATE SHUNT ACTIVITY. Janice L. Podolski* and Akira Omachi. Univ. of Illinois at the Medical Center, Chicago, IL 60680

Although insulin is not required for glucose transport into human erythrocytes, the recent identification of insulin receptors on these cells suggests that this hormone may influence red cell metabolism. Hexose monophosphate shunt (HMP) activity was determined by the conversion of D-[1-^{14}C]glucose to $^{14}CO_2$. Freshly drawn, unwashed packed cells from normal male donors were suspended in Tris-Ringer's buffer containing 16.7 mM glucose (Hct 54%;WBC count≤500/mm^3). The suspensions were incubated in the presence or absence of insulin (931 μU/ml) under anaerobic (100% N_2) or aerobic (100% O_2) conditions at 37° for 3 hours. Mean suspension pH was 7.55. Hyamine hydroxide, a CO_2 trapping agent, was present in a sidearm during the incubation. HMP activity in μmole/ml cell/hr (Mean±SEM) was increased by insulin (0.0234±0.0019) when compared to paired controls (0.0211±0.0019) under anaerobic conditions ($p<0.05$;N=4). Under aerobic conditions, there was no significant effect of this hormone (0.0468±0.0219) compared to controls (0.0490±0.0188). Glucose consumption, as measured by glucose disappearance from the medium in μmole/ml cell/hr, was not altered by insulin from control anaerobic (2.07±0.14) or aerobic (2.18±0.29) values. These results suggest that insulin does have an effect on human erythrocyte HMP activity under anaerobic conditions.

47.5

RELATIONSHIP BETWEEN HISTAMINE SYNTHESIS AND AORTIC ALBUMIN ACCUMULATION IN EXPERIMENTAL DIABETES. STRICKBERGER, S. ADAM* and THEODORE M. HOLLIS. Department of Biology. The Pennsylvania State University, University Park, PA 16802.

Diabetes constitutes one independent risk factor of atherosclerosis. In experimental diabetes, aortic albumin accumulation has been examined in relation to alterations in aortic histamine synthesis. Diabetes was induced in rats by streptozotocin (60 mg/kg, i.v.), with animals held for 4 weeks following overt manifestation of diabetes, i.e. plasma glucose concentrations greater than 275 mg/dl. During the 4th week, some received alpha-hydrazinohistidine (alpha-HH, 25 mg/kg, i.p. @ 12 h). Animals were injected with fluorescein isothiocyanate conjugated to rat serum albumin (FITCRSA), and the intima-media mass transfer rate of FITCRSA was determined. Mean data (cm/sec X 10^{-6}) are as follows: control, 6.84±2.39; nondiabetic-alphaHH, 2.89±0.18; diabetic, 21.30±4.72; diabetic-alphaHH, 4.01±0.26. This alphaHH dose completely blocks alterations in histamine synthesis observed in diabetic aortic endothelial and smooth muscle cells. The overall data are consistent with the hypothesis that de novo histamine synthesis mediated via histidine decarboxylase plays a significant role in mediating increased arterial wall macromolecule permeability, a process constituting an initial event in atherogenesis which may contribute to the atherogenicity of diabetes as well. (Supported by USPHS, NIH grant HL 20460)

47.6

CHANGES IN INSULIN-SENSITIVITY IN TRAINED ATHLETES UPON CESSATION OF TRAINING. R. Burstein*, D. MacDougall*, C. Toews* and C. Polychronakos*(SPON: N.L. Jones). McMaster University, Hamilton, Ontario, L8S 4K1, McGill University, Montreal.

This study was designed to investigate possible changes in insulin sensitivity (IS) with cessation of training. Six endurance trained athletes were studied at 12, 60 h and 7 days following cessation of training. In vivo IS was established by a glucose clamp technique (Greenfield et al. Diabetes 30, 1981) and expressed as the metabolic clearance rate of glucose (MCR) in ml. plasma cleared kg^{-1} min^{-1}. At 12 h after the last training session the mean MCR was 15.6±1.8 compared with 7.8±1.2($p<0.001$) in age, weight-matched sedentary controls. The MCR decreased to 10.1±1.0 after 60 h and decreased significantly to 8.5±0.5($p<0.05$) after 7 days of detraining. In vitro IS was measured by determining the insulin binding of fractionated young erythrocytes by the method of Polychronakos et al.(Clin. Invest. Med.4,145,1981). Insulin binding was 10.4±0.9% at 12 h and decreased significantly to 8.1±0.7%/4x10^9 cells after 60 h of detraining ($p<0.01$). In conclusion: 1) detraining of endurance athletes resulted in a rapid decrease in IS. After 7 days,glucose MCR reached values indistinguishable from sedentary controls. 2) changes in IS observed may be partially mediated by alterations in insulin binding to receptors. 3) since the high IS observed with endurance athletes on the initial test disappeared shortly after cessation of training, it is probably an acute effect of the last exercise bout rather than a chronic effect of training.

47.7

COMPUTER-CONTROLLED BASAL HORMONE REPLACEMENT DURING SOMATOSTATIN. G. Pacini* and R.N. Bergman. USC, Los Angeles, 90033.

To investigate the role of hormones in controlling glucose (G) metabolism, endogenous insulin (I) and glucagon (GN) secretion are inhibited with somatostatin (SRIF;0.8 µg/kg.min), and I and GN are infused intraportally. Intraportal hormone infusion rates must be determined to re-establish euglycemia and basal glucose turnover (Ra). We have adapted our minimal model glucose clamp (DIABETES 31;432, 1982) to re-establish basal conditions with intraportal hormone replacement during SRIF. AUTOMATIC HORMONE REPLACEMENT: The minimal model was implemented on a minicomputer during experiments. I was infused intraportally at a constant rate (50 µU/min.kg). GN was infused at variable rates calculated to control endogenous Ra and maintain euglycemia. Ra was assumed proportional to GN infusion [GNI]: (Ra[mg/min]=6 GNI/[Portal plasma flow]. AJP 236:E246,1979). RESULTS: NO HORMONE REPLACEMENT(n=4). With SRIF alone, G fell from basal (84±2) to 65±6 mg/dl in 60 min ($p<0.025$); hypoglycemia was maintained thereafter. HORMONE REPLACEMENT(n=5). Basal: G=89±4 mg/dl; I=8±2µU/ml; Ra=3.6±0.7 mg/kg.min. Automatic control(150 min): G(84±3 mg/dl)and Ra(4.1±2) were the same as the basal values ($p>0.1$ and 0.4). Ra predicted by the computer during experiments was highly correlated with Ra measured a posteriori with tracers ($r=0.89$, $p<0.025$). GNI averaged 1.9±0.3 ng/kg.min, but tended to rise as the potency of glucagon waned due to evanescence. CONCLUSIONS: This automated method maintains glucose concentration and glucose production constant during SRIF infusion, and can be used to re-establish basal condtions during intraportal hormone replacement experiments. The computer model predicts hepatic glucose production, without tracers, while glucose is automatically regulated. (AM 29867).

47.8

INSULIN RELEASE IN AGING: DYNAMICS OF RELEASE TO GLUCOSE AND GLYCERALDEHYDE. Loren G. Lipson*, Francis H. Premdas* and Joseph M. Molina* (SPON: R. N. Bergman). Univ. of Southern California School of Medicine, Los Angeles, California 90033

Insulin release to glucose is diminished from islets of old rats. The mechanism for this decrease in release is not clear but has been postulated to be related to the decreased activity of adenylate cyclase and decreased glucose oxidation in islets from older rats. To gain further insight into the physiology of this age-related decrease in insulin release, we investigated dynamic insulin releasefrom islets of older and young rats to D-glucose and D-glyceraldehyde. Simultaneous experiments were performed on islets from 2 and 12 month old rats to determine the insulin release response to 2.8 mM D-glucose and 16.7 mM D-glucose or 8.0 mM D-glyceraldehyde. The dynamics of release were determined using the technique of islet perifusion at 37C with the resulting perifusate being then assayed for insulin by radioimmunoassay. Insulin secretion to 2.8 mM glucose was similar in the two groups of islets. Dynamic insulin secretion to 16.7 mM glucose was diminished by 42% from islets of older animals and the first phase of release was not present. When D-glyceraldehyde was used, insulin secretion was similar from batches of islets from both older and young rats and the biphasic nature of the release process was present in both groups of islets. From these and previous results, the diminished insulin release from islets of older rats involves changes in a rate-limiting step in stimulus-secretion coupling between glucose and the trioses.

47.9

CHEMOTACTIC ACTIVITY OF COLLAGENASE: A POTENTIAL CAUSE OF ISLET TRANSPLANT FAILURE. John M. Ham, Weldon B. Jolley, Kathleen Knierim and David B. Hinshaw. Jerry L. Pettis Mem. VA Hospital and Loma Linda Univ. School of Medicine, Dept. of Surgery, Loma Linda, California 92354.

The involvement of macrophages in inflammatory and immune processes has been demonstrated. Attempts to minimize macrophage activity in or near allografts would seem to be beneficial. Worthington Type IV collagenase has been widely used in freeing islets of Langerhans from exocrine tissue. We have been concerned that collagenase or the products of the action of this enzyme on collagen would be inflammatory in nature and cause an accumulation of macrophages at the site of islet transplantation which could impair islet function. To test this, the chemotactic response of rabbit peritoneal macrophages was studied comparing two distinct methods of islet isolation. Islets were isolated using a non-enzymatic or a collagenase technique. Supernatants from both techniques were used as chemoattractants in Boyden Chambers. Results demonstrate increased response by collagenase isolated islets over non-enzymatically isolated islets or over culture media controls. Collagenase technique; N=16, 187 cells/10 fields, non-enzyme technique; N=16, 45 cells/10 fields, culture media; 56 cell/10 fields ($p<0.001$). The macrophage response to collagenase alone was dose dependent from 1 µg/ml to 100 µg/ml. The results suggest that part of the difficulty encountered in islet transplantation may in part be attributed to the use of collagenase in isolation procedures.

ADH, SALT AND WATER

48.1

HORMONAL AND WATER AND ELECTROLYTE CHANGES IN MAN DURING 14 DAYS AT 31 ATMOSPHERES. J. R. Claybaugh, S. K. Hong, N. Matsuda,* N. Matsui* and H. Nakayama*. Tripler Army Medical Center, Honolulu, HI 96859; State Univ. of N.Y. at Buffalo, NY 14214; Japan Marine Sci. and Tech. Ctr., Yokosuka, Japan.

Four Japanese male subjects were studied during 3 control days at 1 ATA, 3 days of compression to 31 ATA (1000 ft sea water), 14 days at 31 ATA, 12 days of decompression, and 3 post-dive control days. The chamber was maintained at a thermal neutral temperature at 1 ATA (26°C) and at 31 ATA (31°C). During the 31 ATA exposure, urine flow was consistently increased about 500 ml/day ($P<0.05$) and characterized by about 500 ml/day increase in Cosm ($P<0.05$), both increases occurring during the overnight collection periods. Although C_{H_2O} did not change, the $-C_{H_2O}$/Cosm decreased ($P<0.05$) at 31 ATA. The urinary excretion rate of aldosterone increased from 1 ATA levels of 2.7±0.3 to 4.3±0.9 µg/day ($P<0.01$) at 31 ATA, remaining about 3.8 µg/day ($P<0.05$) until decompression. Urinary ADH excretion decreased from 50±7 to 33±3 mU/day ($P<0.01$) upon compression to 31 ATA, and continued to decrease through decompression. Plasma renin activity was increased by twofold ($P<0.01$) and plasma aldosterone by about 35% (N.S.) during 31 ATA exposure. The mechanism for the osmotic, nocturnal diuresis remains unclear. (Supported by U. S. Army Health Services Command, USPHS Grant HL-14414, U. S. Dept. of Commerce NOAA Grants 04-8-M01-102 and 04-7-158-44129, and The Science and Technology Agency of the Office of the Prime Minister, Government of Japan).

48.2

PLASMA VASOPRESSIN RESPONSE TO CONTINUOUS HEMORRHAGE IN CONSCIOUS, CARDIAC-DENERVATED DOGS. K. L. Goetz, B. C. Wang, W. D. Sundet*, P. G. Geer*, and M. O. K. Hakumaki*. St. Luke's Hospital and Foundation, Kansas City, MO 64111

The increase in plasma levels of vasopressin (AVP) that occurs after sufficient blood loss has been attributed to reflex effects from atrial volume receptors and sinoaortic baroreceptors, but the relative importance of these different receptors during hemorrhage on conscious dogs has not been reported. We investigated this question by hemorrhaging 7 sham-operated (SO) and 7 cardiac-denervated (CD) conscious dogs until 30 ml of blood per kg body weight had been removed. In SO dogs plasma AVP increased significantly from a control value of 3.5 ± 0.7 pg/ml to 12.4 ± 2.6, 113.2 ± 24.1, and 296.2 ± 54.4 pg/ml at 10, 20, and 30 ml/kg blood loss, respectively. Control levels of AVP were comparable in the CD dogs (3.2 ± 0.6 pg/ml), but the response to hemorrhage was markedly attenuated in these dogs, reaching only 5.7 ± 1.6, 11.8 ± 4.5, and 39.8 ± 13.0 pg/ml at 10, 20, and 30 ml/kg blood loss, respectively. Mean aortic pressure was not changed after 10 ml/kg blood loss in either group but decreased significantly at 20 and 30 ml/kg hemorrhage in both groups. These results suggest that cardiac volume receptors play a dominant role in modulating the secretion of AVP during hemorrhage in conscious dogs.

48.3

EFFECT OF CARDIAC DENERVATION ON THE PLASMA VASOPRESSIN RESPONSE TO INTRAVENOUS HYPERTONIC SALINE OR TO CHRONIC DEHYDRATION IN CONSCIOUS DOGS. B. C. Wang, W. D. Sundet*, M. O. K. Hekumäki*, C. E. Ralph*, and K. L. Goetz. St. Luke's Hospital and Foundation, Kansas City, MO 64111

Plasma levels of vasopressin (AVP) increase when plasma osmolality is increased by chronic water deprivation or by iv infusion of hypertonic saline. These maneuvers, however, also cause contraction or expansion of the blood volume which could affect AVP release via reflexes from cardiac volume receptors. To quantitate the influence from cardiac volume receptors, we measured plasma AVP during water deprivation for 96 hr and after the administration of hypertonic saline (1M NaCl, 0.1 ml/kg/min iv, for 2 hr) in sham-operated (SO) and cardiac-denervated (CD) conscious dogs. A significant positive linear correlation between plasma AVP and plasma osmolality was found in each group of experiments. The osmotic thresholds for AVP release were between 278 and 280 mOsm/kg H_2O and did not differ statistically. Although the AVP/osmolality slope changed in response to volume changes in the SO dogs (0.390 during water deprivation vs 0.228 during hypertonic saline), the slopes were unchanged during these interventions in the CD dogs (0.288 during water deprivation vs 0.291 during hypertonic saline). These data suggest that cardiac receptors effectively alter the plasma AVP/osmolality relationship during changes in extracellular fluid volume.

48.4

CHANGES IN SODIUM AND POTASSIUM EXCRETION, PRA AND HEART RATE WITH VASOPRESSIN INFUSION IN THE ACUTELY HYPOPHYSECTOMIZED DOG. R.E. Shade. U. of S. Carolina Sch. of Med., Columbia, SC 29208

This study was designed to determine if changes in plasma vasopressin within the normal range for renal H_2O conservation would significantly alter renal electrolyte excretion. Five male dogs anesthetized with 6% urethane-0.6% chloralose were hypophysectomized, and the right kidney was prepared with an electromagnetic flow probe. Two control clearance periods were followed by vasopressin infused at rates calculated to increase plasma levels by 2, 4, and 8 μU/ml.

△AVP:	C	2	4	8	R
Uosm (mOsm/Kg · H_2O)	74±7	180±38	465±69	621±56	187±44
PRA (ng/ml · hr)	42±7	33±5	25±4	12±5	38±12
$U_{Na}V$ (μEq/min)	6±2	8±3	23±8	48±20	25±11
$U_K V$ (μEq/min)	14±2	25±4	35±4	53±12	47±10
HR (BPM)	147±10	129±9	114±10	113±12	125±14

Vasopressin infusion resulted in dose-related increases in urine osmolality and potassium excretion and decreases in plasma renin activity and heart rate. Only the 4 and 8 μU/ml increments in vasopressin increased sodium excretion. There were no significant changes in GFR or RBF. Physiological increases in plasma vasopressin result in a significant kaliuresis and natriuresis in the anesthetized, hypophysectomized dog. The changes in sodium excretion are not due to changes in aldosterone since PRA at all times was high compared to conscious PRA levels. The increased sodium excretions, however, may be due to decreases in circulating angiotensin II. (Supported by NIH Grant HL 25998.)

48.5

NON-EPISODIC RELEASE OF VASOPRESSIN FOLLOWING CAROTID OCCLUSION AND HYPERTONIC SALINE IN ANESTHETIZED CATS. Richard P. Menninger and Donald C. Bolser*. University of South Florida, Tampa, Florida 33612

It has been suggested that release of vasopressin (AVP) may not be tightly coupled to electrical activity of supraoptic neurosecretory cells following hypertonic saline in concious dogs (Weitzman et al. Am.J.Physiol. 233: E32-E36, 1977). The present studies were conducted on pentobarbital anesthetized cats and dogs. Samples for AVP assay were collected at 0, 3, 6 and 10 minutes. In cats, 0.5ml of 3% NaCl injected into a carotid artery in 4 seconds increased plasma AVP 107% at 3 min. and 21% at 6 min. post-injection. One minute of bilateral carotid occlusion in aortic nerve sectioned cats elevated plasma AVP 72%, 88% and 54% at 3, 6 and 10 minutes post-occlusion, while heart rate and mean arterial pressures (MAP) returned to pre-occlusion levels within 3 minutes. In four of five vagotomized dogs subjected to one minute of bilateral carotid occlusion, plasma AVP was elevated 112%, 53% and 18% at 3, 6 and 10 minutes post-occlusion. Heart rate and MAP returned to control within 3 minutes. These data give no evidence of episodic secretion of AVP. Moreover, it would appear from the early rise in AVP that the data are consistent with the notion that hormone release is coupled fairly tightly to neurosecretory cell firing rate in anesthetized animals.
Supported by NIH Grant 29497.

48.6

ROLE OF VOLUME AND OSMOLALITY IN THE INCREASE IN PLASMA VASOPRESSIN IN DEHYDRATED DOGS. C.E. Wade, L.C. Keil, and D.J. Ramsay. Department of Physiology, University of California San Francisco, CA 94143 & Department of Clinical Investigation, Letterman Army Medical Center, Presidio of San Francisco, CA 94129, and Ames Research Center, Moffett Field, CA 94035.

Plasma vasopressin concentrations (pAVP) are elevated during dehydration due to alterations in extracellular fluid (ECF) volume and tonicity. The contribution of the reduction in volume and increase in plasma osmolality (pOsm) to the rise in pAVP was assessed in six dogs with bilateral carotid loops following 24 h of fluid but not food deprivation. Dehydration significantly ($P<0.05$) increased plasma osmolality from 297 ± 1 to 315 ± 2 mOsm/kg, decreased body weight from 20.8 ± 1.2 to 20.3 ± 1.2 kg, and elevated pAVP from 1.5 ± 0.4 to 5.8 ± 0.9 pg/ml. Volume replacement was assessed from the change in body weight and achieved by intravenous infusion of 0.15 M saline. Plasma vasopressin concentration was significantly reduced by 1.3 ± 0.2 pg/ml and pOsm unchanged following volume replacement. The contribution of the increase in pOsm was assessed by bilateral intracarotid infusions of water at 0.6 ml/min/artery which lowered jugular venous pOsm to 296 ± 4 mOsm/kg, similar to euhydrated values, but did not reduce systemic pOsm. Plasma vasopressin levels were significantly reduced 3.2 ± 1.1 pg/ml. Thus, following 24 h of fluid deprivation in dogs, the rise in pAVP is due to changes in both ECF volume and tonicity. The increase in tonicity plays a greater role in the elevation of pAVP, than the reduction in volume.
Supported by NIH grant #HL-06268 and AM-06704.

48.7

THE LONG TERM EFFECTS OF HYPOPROTEINEMIA ON RENAL FUNCTION AND FLUID VOLUMES. R.D. Manning, Jr. and A.C. Guyton. Univ. Miss. Med. Ctr., Jackson, MS 39216

Plasma protein concentration (PPC) was decreased in 8 unanesthetized dogs over a period of 2 weeks by daily plasmapheresis to determine the chronic effects of hypoproteinemia on the ability of the kidney to excrete sodium and water. Control values of PPC, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were 6.9 gm/dl, 69.0 ml/min and 173.1 ml/min, respectively. When PPC was reduced to 4.0 gm/dl, GFR increased slightly to 72.4 ml/min; ERPF decreased to 156.5 ml/min; plasma renin activity (PRA) increased 7 fold from a control value of 0.5 ng AI/ml/hr; plasma aldosterone concentration (PA) increased 3 fold from a control value of 3.4 ng/dl; and iothalmate space, sodium balance and water balance increased. When PPC was further reduced to 2.5 gm/dl, mean arterial pressure decreased markedly; GFR decreased to 48.7 ml/min; ERPF decreased to 118.5 ml/min; PRA was elevated 6 fold; PA increased 5 fold; and iothalmate space, sodium balance and water balance remained elevated. We conclude that long term decreases in PPC result in significant decreases in GFR and ERPF and a positive sodium and water balance. The effects of hypoproteinemia on renal function may be mediated in part by a decrease in mean arterial pressure and increases in PRA and PA. (Supported by NIH grant HL-11678).

48.8

ATRIAL NATRIURETIC FACTOR CAUSES NATRIURESIS IN IMMATURE RATS. Helmut Braunlich*, and Sidney Solomon. U. of New Mexico, Dept. of Physiology, Albuquerque, New Mexico 87131.

Recent studies have shown that atria contain a natriuretic factor, but it is not certain that this factor has physiological significance. Past studies have also shown that when infant rats are volume expanded with blood from a sibling, no diuretic or natriuretic response is found. If the donor blood is from a mature animal, diuresis and natriuresis is produced. Mature and 25 day old infant rats were treated with submaximal doses of atrial factor and the renal responses compared. In infants urinary volume increased between 3 and 9 fold over the eighty minutes following administration of the agent. The increase in sodium excretion was somewhat greater than the increased diuresis. In mature animals there was slightly greater response than in rat pups. Injection of ventricular extract had no effect in either population of rats. Comparable studies are being done on fifteen day old rats. These results can be interpreted to indicate that the atrial factor has physiological significance. The factor is either absent or not released in infant rats. These conclusions are consistent with those previously made on the basis of indirect evidence. (Supported by a grant from NSF PCM 78-15383).

48.9

RELATION OF CNS, PANCREATIC AND β RECEPTOR MEDIATED CELL UPTAKE OF POTASSIUM (K) IN K-LOADED NEPHRECTOMIZED DOGS.
N. Hiatt, L. W. Chapman, J. A. Sheinkopf, Med. Res. Inst., Cedars-Sinai Med. Ctr., Los Angeles, CA. 90048

We have reported, separately, that insulin, β receptors, and the CNS induce transfer of infused K into cells. We report the interrelation of these in protecting against hyperkalemia. 2mEq KCl/kg/hr was infused to 35 anesthetized nephrectomized dogs until ECG evidence of lethal hyperkalemic cardiotoxicity. Plasma insulin (PI) was determined by RIA. The dogs were divided into six groups. In group I, control (n=6), 70% of infused K was transferred to cells with PI increasing by 58.8 μU/ml. In group II, pancreatomized (panx) (n=7), 67% of infused K was transferred. In group III, (n=7) receiving 0.3 mg propranolol/kg/hr(prop), only 33% of infused K was transferred with a 39 μU/ml increase in PI. In group IV, (n=5)-cerebral artery ligation-the K transferred was 42% with PI increasing by 70 μU/ml. In group V, (n=5)-vessel ligation and prop-61% of infused K was transferred with a 38 μU/ml increase in PI. Group VI-vessel ligation, prop, panx-transferred only 10% of the infused K. The data indicate that β receptor mediated K transfer is more powerful than either the insulin or CNS K transfer mechanisms and suggest that β blockade enhances insulin mediated K transfer at physiologic PI concentrations.

48.10

SODIUM EXCRETION AND ALDOSTERONE IN DE- AND RE-HYDRATION IN DOGS. T.N. Thrasher, C.E. Wade and D.J. Ramsay. Dept Physiol, UCSF,CA 94143 and Letterman Army Med Ctr SF,CA 94129

Mckinley et al(1981) recently reported that sheep, rabbits and rats all show increased sodium excretion and negative Na balance during dehydration. This study was designed to examine the control of Na excretion during dehydration and rehydration in dogs. Balance studies were conducted on 7 dogs including daily measurements of plasma renin activity (PRA), arginine vasopressin(AVP) aldosterone(aldo),Na,K,and osmolality. Following 24h dehydration plasma Na, osmolality,AVP and PRA all increased. However,plasma K fell from 4.5±.1 to 4.1±.2 mEq/l and aldo was unchanged (5.5±1.2 and 6.5±1.0 ng/dl). During the 24h of dehydration, Na balance became significantly negative at -41.7 mEq/24h,whereas K balance was not affected. Sixty min following voluntary rehydration, plasma aldo increased from 6.5±9.2 to 17.2± 3.7 ng/dl, although PRA and plasma K were unchanged. However, intestinal absorption of the ingested water was associated with a fall in plasma Na of 9.4±1.8 mEq. These results demonstrate a clear dissociation between PRA, K and the increase in plasma aldo suggesting that the response may be due to the rapid fall in plasma Na. In the next 24h,all dogs showed a marked positive Na balance of +20.8mEq, although water balance had returned to normal. These results show that Na excretion is increased during dehydration and this may contribute to the maintenance of plasma osmolality. Furthermore,after rehydration the increase in plasma aldo is not due to PRA or plasma K. Supported by NIH grant HL 18862.

48.11

RENAL HANDLING OF Na^+ AND K^+ IN THE SAND RAT AND ALBINO RAT. Armand Gold, Shlomo Samueloff*, and Jonathan Adler*. Dept. Physiol. & Biophys., Howard U. Coll. Med., Washington, D. C. and Dept. Physiol., Hadassah Med. Sch., Jerusalem, Israel

The natural diet of the sand rat is the salt bush. The albino rat under control conditions is fed standard lab chow and tap water. Experiments were carried out on both species given the following "high" and "control" salt diets: (1) sand rats, fresh salt bush (12); (2) sand rats, lab chow and tap water (16); (3) albino rats, lab chow and 1.8% (11); (4) albino rats, lab chow and tap water (15).

	(1)S-HI	(2)S-CON	(3)A-HI	(4)A-CON
GFR, ml/min/g KW	.73±.10	.57±.06	.71±.10	.94±.10
P Na^+, uEq/ml	166±3	163±2	163±5	148±5
F Na^+, uEq/min/g KW	121±9	93±5	116±10	139±9
E Na^+, uEq/min/g KW	.26±.05	.13±.02	.44±.14	.07±.01
P K^+, uEq/ml	7.4±1.1	4.9±.3	5.6±.5	8.1±.5
F K^+, uEq/min/g KW	5.4±.8	2.8±.2	4.0±.5	7.6±.6
E K^+, uEq/min/g KW	.08±.02	.14±.03	.13±.03	.17±.03

Clear differences in Na^+ and K^+ handling in sand rats on "high" versus "control" salt diets were observed: in the former, greater filtered Na^+ load and even greater Na^+ excretion; greater filtered K^+ load but lower K^+ excretion. In albino rats Na^+ excretion was extremely rapid with "high" salt despite similar Na^+ filtration rates with both diets. Conversely, K^+ excretion was similar with both diets, although K^+ filtration was greater with "high" salt. The results suggest dissimilar renal mechanisms in the two species for handling Na^+ and K^+.

48.12

PROXIMAL TUBULE SODIUM REABSORPTION IN NEWBORN AND ADULT DOGS DURING SALINE EXPANSION. L.I. Kleinman, R.O. Banks, T.A. Disney*, University of Cincinnati College of Medicine

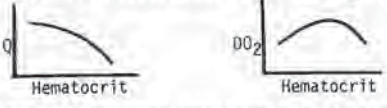
Studies were carried out in 23 anesthetized neonatal and 16 adult dogs to compare the effects of saline volume expansion (SVE) on renal tubular Na reabsorption. Proximal and distal tubule function was estimated by the distal nephron blockade technique using ethacrynic acid and amiloride. In non-expanded animals proximal tubule fractional Na reabsorption (PFR Na) was greater in adults (.77) than in newborns (.66, p<.01) but distal nephron Na fractional reabsorption (DFR Na) was less in adults (.22) than in puppies (.33, p<.01). During saline infusion, which increased extracellular volume by approximately 30% for both age groups, fractional Na reabsorption was .91 for adults but only .98 for puppies (p<.01). However, PFR Na was greater in adults (.64) than in puppies (.48, p<.01) and DFR Na was much greater in puppies (.51) than in adults (.26, p<.01). Fractional K excretion during SVE was similar in adults and newborns, although the fraction of filtered K escaping proximal tubule reabsorption was greater in newborns (.72) than in adults (.53, p<.01) indicating greater net K reabsorption in the distal nephron of the puppy. These results indicate that in response to SVE there is a greater proximal tubule natriuresis in newborns than in adults but overall renal Na excretion is less in the newborn due to enhanced Na reabsorption in the neonatal distal nephron, particularly in Henle's Loop.

SPLANCHNIC CIRCULATION

49.1

BLOOD FLOW AND O_2 DELIVERY TO FETAL GASTROINTESTINAL ORGANS AS A FUNCTION OF ARTERIAL HEMATOCRIT. Fred D. Fumia,* Daniel I. Edelstone, and Ian R. Holzman. Univ. of Pittsburgh, Pittsburgh, PA 15213

In 11 chronically-catheterized fetal lambs (0.7-0.9 of gestation), we measured blood flow (Q) and O_2 delivery (DO_2) to the fetal stomach, small intestine, and colon. Q and DO_2 were measured at the basal hematocrit (32±3(SD)%) and after the hematocrit was varied over a range of 12% to 47% by isovolemic exchange transfusion with plasma or fetal red blood cells obtained freshly from a donor fetus. Q was calculated with the radionuclide-labelled microsphere technique, and DO_2 was calculated as Q times the arterial blood O_2 concentration. The figures below show the relationship of Q and DO_2 to hematocrit.



Increases in hematocrit were associated with decreases in flow to the stomach, small intestine, and colon. These flow-hematocrit relationships were such that O_2 delivery to each organ was maximal at a hematocrit of about 35%. Our results indicate that the hematocrit normally found in the undisturbed fetal lamb in utero is optimal for providing maximal oxygen delivery to the gastrointestinal organs. (Supported by NIH Grant HD-16368).

49.2

ADRENERGIC SENSITIVITY OF DIFFERENT SIZE GASTRIC SUBMUCOSAL ARTERIOLES. N. Oren-Wolman* and P.H. Guth, CURE, VA Wadsworth & Physiology Dept., UCLA, Los Angeles, CA 90073.

In the mesentery, smaller arterioles are more sensitive to norepinephrine (NE) vasoconstriction than larger ones. Is this true of gastric submucosal arterioles (GSA), the vessels that control blood flow to the mucosa? Rats were anesthetized, the stomach exteriorized, and the gastric wall transilluminated. Muscle and serosa were removed from a small area exposing the submucosa to direct viewing and videotaping via a closed circuit TV microscopy system. The response of 4 different size GSA (2 per rat) to topical application of NE was measured using an image shearing videomonitor. Results (for 2 groups): diameter expressed as % of control diameter (CD), mean±SE, n=7-14.

NE ($\times 10^{-7}$M)	CD: 10-19 (μm)			CD: 60-79 (μm)		
	S	E	PS	S	E	PS
1	83±11	105±12	126± 9	74±10	85±12	99±10
2.2	44± 7	83± 9	111± 9	49± 6	69± 9	99± 7
4.6	31± 3	83±10	122±11	36± 4	67± 8	113± 9

S = maximum constriction; E = escape of vessel diameter toward control despite continued presence of NE; PS = post stimulation diameter first min after NE removal. Arterioles 20-39 μm and 40-59 μm showed similar constriction responses. Larger GSA dilated significantly less than smaller ones in the post-stimulatory period (analysis of variance, p<.05), but not significantly less during escape. Conclusion: Unlike mesenteric arterioles, different size GSA did not show any difference in sensitivity to NE vasoconstriction.

49.3

PERITONEAL DIALYSIS SOLUTIONS AND THE SPLANCHNIC CIRCULATION. D. Neil Granger, Michael A. Perry, Michele Ulrich, and Peter R. Kvietys, Dept. of Physiology, Univ. South Alabama, Mobile, AL 36688

Commercial solutions for peritoneal dialysis are vasoactive when topically applied to arterioles of cremaster muscle and cecum in the rat (*Kid. Int.* 15:630 1979). We chose to examine the effects of 1.5% and 4.25% dextrose-Dianeal solutions on blood flow in mesentery (Mes), parietal peritoneum (PP), omentum (Om), spleen, liver, stomach, pancreas, small bowel (whole wall), and the intestinal serosa (Ser) in the cat. Blood flow to the tissues was estimated using radio-labelled microspheres. Table 1 summarizes the results from tissues affected by the dialysis solutions.

Table 1: Blood Flow (ml/min x 100g)

	Mes	PP	Om	Ser
C	5.4	6.2	6.3	9.6
1.5%	12.1*	10.0*	11.0*	35.1*
4.25%	18.3*	14.7*	16.0*	103.2*

*p<.05, c = control

Blood flow to the other tissues were unaffected by the dialysis solutions. Our results indicate that 1.5% and 4.25% dextrose-Dianeal solutions significantly improve blood perfusion to several tissues in the abdominal cavity. The results also suggest that hyperosmolality is the mechanism for the vasodilation produced by the dialysis solutions. (Supported by HL26429.)

49.4

RELATIONSHIP OF BLOOD FLOW AND OXYGEN CONSUMPTION TO ISCHEMIC INJURY IN THE SMALL INTESTINE. Gregory B. Bulkley†, Peter R. Kvietys, Michael A. Perry, and D.N. Granger. University of South Alabama, Mobile, Alabama 36688 and †Johns Hopkins Univ.

Intestinal oxygen consumption has been shown to be independent of blood flow at flows ≥ 30 ml/min x 100g, and flow-dependent at flows below this. We investigated the effect of this relationship on ischemic intestinal injury in anesthetized dogs. Isolated jejunal segments were autoperfused from the femoral artery through an electromagnetic flowmeter. Blood flow and arteriovenous oxygen difference were monitored continuously and oxygen uptake calculated. The lumen of each segment was perfused with warm Tyrode's solution and the clearance of ^{125}I-albumin from blood to lumen measured as an index of early mucosal injury. Blood flow was varied with a screw clamp on the arterial cannula. As in previous studies, oxygen consumption was constant at blood flows ≥ 30 ml/min x 100g due to compensating increases in oxygen extraction, and markedly flow-dependent at lower flow levels. Correspondingly, reductions in blood flow to levels at or above this threshold caused no increase in albumin clearance, even after 2 hrs of ischemia and 1 hr of reperfusion. Reductions of flow substantially below that threshold caused profound increases in albumin clearance, the magnitudes of which were directly related to the degree of ischemia. The capability of the small intestine to increase oxygen extraction and thereby maintain oxygen consumption results in protection from tissue injury during even prolonged periods of mild to moderate ischemia.

49.5

SYMPATHOADRENAL STIMULATION AND BLOOD FLOW DISTRIBUTION IN THE WALL OF THE GASTROINTESTINAL TRACT IN THE ANESTHETIZED DOG. C.C. Chou, L.C. Yu*, and Y.M. Yu*. Depts. of Physiol. and Med., Michigan State Univ., E. Lansing, MI 48824.

Utilizing the radioactive microsphere method (^{141}Ce and ^{85}Sr, 15 um diameter), we measured total blood flow (TF in ml/min-g), and total vascular resistance (TR, mmHg/ml/min-g) of the wall of the stomach, duodenum, jejunum, ileum and colon, and percentage of TF (%TF) perfusing the mucosa-submucosa and muscularis layers, before and during the following four experimental perturbations: bilateral carotid artery occlusion (CAO) which raised aortic pressure (AP) from 112 to 150 mm Hg (N=7), acute hemorrhage (H) to lower AP from 120 to 60 mm Hg (N=8), intravenous infusion of epinephrine (16 ug/min) (Epi) which raised AP from 121 to 176 mm Hg (N=7), and intravenous infusion of norepinephrine (16 ug/min) (NE) which raised AP from 119 to 172 mm Hg. Flow measurements were made when AP was at a steady level. Significant changes from controls are as follows. CAO increased TF, did not alter TR of all 5 organs, and increased only the %TF perfusing the colonic muscularis. H decreased TF and increased TR of all 5 organs but only increased %TF perfusing the gastric muscularis. Epi increased only colonic TR, tended to increase TF of the other 4 organs, and did not alter %TF of 5 organs. NE decreased TF, increased TR and increased %TF to the muscularis of all 5 organs. Conclusion: Sympathoadrenal stimulation tends to redistribute flow away from the mucosa. (Supported by NHLI Grant HL-15231)

CELL MEMBRANES, TRANSPORT AND RECEPTORS

50.1

ISOLATION AND CHARACTERIZATION OF HUMAN PERITONEAL MAST CELLS. P. A. Schaefer*, S. J. Littler*, W. Wessels*, G. Drzewiecki*, and E. Middleton, Jr.* (SPON: R. A. Klocke). SUNYAB and Buffalo General Hospital, Buffalo, NY 14203

Human mast cells (HMC) have been isolated from peritoneal lavage fluids and partially purified using isopycnic density centrifugation techniques with Percoll (Pharmacia). This method is non-destructive and does not require enzymatic digestion, as used with other human tissues (lung). Initial purification attempts yielded 30 to 50% HMC, as assessed by toluidine blue staining. Buoyant densities of the HMC's ranged from 1.05 to 1.08 gm/L, in contrast with the rat mast cell's (RMC) density of 1.11 gm/L. Structural details were examined by both light and electron microscopy. Cell suspensions exceeded histamine concentrations of 100 ng/10^6 cells. HMC's released histamine in response to concanavalin A (10-100 ug/mL) and anti-human IgE (5-80 ug/mL), but not with compound 48/80 (0.2-1.0 ug/mL). Release was inhibited up to 85% by 5 and 50 uM quercetin, comparable to results with RMC and human basophils. Suspensions of purified HMC's from serosal surfaces, unmodified by enzymatic treatment, represent a new source of HMC for study of their immunologic and biochemical characteristics. (Supported in part by NIH grant AI 14198 and by Margaret Duffy and Robert Cameron Troup Memorial Fund)

50.2

ANALYSIS OF MAST CELL PERIGRANULAR MEMBRANES: IDENTIFICATION OF A HIGH-AFFINITY CA,MG-ATPASE. L. M. Amende, G. N. Catravas* and M. A. Donlon*. AFRRI, Bethesda, MD 20814.

Perigranular membranes from rat peritoneal mast cells were isolated and analyzed for membrane marker enzymes (5' nucleotidase, succinic dehydrogenase, monoamine oxidase, and glucose-6-phosphatase) and Ca,Mg-ATPase activity. Membrane marker enzyme analysis indicated minimal (3%) contamination with plasma membranes and mitochondrial membranes, with slightly higher contamination by Golgi membranes (10%). The perigranular membranes were found to contain a low-affinity ATPase maximally stimulated by 1 mM Mg or 5 mM Ca, with maximal activity (3 nmole P_i/min/mg protein) at 1 mM ATP. This enzyme activity was found to be insensitive to 5 μM vanadate, 2 mM ouabain, and 20 μg/ml oligomycin, although 5 mM N-ethylmaleimide inhibited activity by 40%. A high-affinity Ca,Mg-ATPase was also detected, which required low magnesium concentrations (<20 μM) for maximal activity, and was inhibited by CDTA, a magnesium chelator. Maximal enzyme activity was 1.5 nmole P_i/min/mg protein at 8 μM Ca, with a K_a of 2.5 μM Ca. The high-affinity Ca,Mg-ATPase is associated with calcium transport in other cell types where its activity is necessary to maintain low intracellular calcium levels. It is likely that the enzyme functions in a similar manner in the mast cell. (L.M.A. is an NRC-AFRRI fellow.)

50.3

COMPARISON OF RENAL AND CARDIAC NA,K-ATPASE SENSITIVITY TO VANADATE. David Clough* (SPON. Motilal Pamnani). Dept. of Physiology, Uniformed Services Unversity, Bethesda, MD 20814

Orthovanadate (VO_4) has been shown to produce positive inotropy and diuresis in rats. Although the mechanism(s) of these effects is uncertain, inhibition of the Na,K-pump has been implicated in the kidney. In the present study we compared the sensitivity to VO_4 of Na,K-ATPase from rat kidney cortex (C), medulla (M) and left ventricle (LV) in microsomes treated with deoxycholate and NaI. Na,K-ATPase (total minus Mg-ATPase) activity was assayed at 37°C in media containing (mM): 120 NaCl, 4 KCl, 4 $MgCl_2$, 2 Na_2ATP (Boehringer Mannheim), 0.5 EGTA and 40 Tris-HCl (pH 7.5). Control Na,K-ATPase activities (absence of added VO_4) of M, C, and LV, in umoles Pi/mg/hr were: 129.5±34, 103.0±20.6, and 7.8±0.6 respectively. VO_4 inhibition curves were obtained with 8 different $[VO_4]$'s ranging from $5x10^{-9}$ to $5x10^{-5}$ M. The values were expressed as fractional activity (activity with VO_4/control) and I_{50}'s were obtained from the individual curves. Fractional activities for M and C were significantly less than for LV at $[VO_4]$'s of $5x10^{-7}$M and above ($P<.05$, N=6), however, M and C were not significantly different at any of the $[VO_4]$'s. The I_{50} was significantly greater for LV ($13.6±2.4x10^{-7}$M) than for M ($4.8±1.4x10^{-7}$M) or C ($5.0±1.0x10^{-7}$ M, N=6, $P<.05$) but there was no significant difference between the I_{50} for M and C. These results suggest that renal Na,K-ATPase is more sensitive than ventricular Na,K-ATPase to inhibition by VO_4 and would therefore be more likely to be inhibited in vivo.

50.4

Ca^{2+} -MODULATION OF THE Na^+ -APTase OF GUINEA-PIG KIDNEY PLASMA MEMBRANES. F. Proverbio, T. Proverbio* and R. Marín*. Centro de Biofísica y Bioquímica, IVIC, Apartado 1827, Caracas 1010 A, Venezuela.

Basolateral plasma membranes from guinea-pig kidney cortex cells, show a Mg^{2+} -dependent, Ouabain insensitive, Na^+ -stimulated ATPase activity, which is considered to be associated to the mechanism of Na^+ extrusion accompanied by Cl^- and water, present in these cells. The Na^+ -ATPase activity can be increased by the addition of μM quantities of Ca^{2+} to the assay medium. The activating effect of Ca^{2+} on the Na^+ -ATPase is on Vmax, and not on the Ka of the system for Na^+, which is 8mM. The Ca^{2+} involved in this effect, seems to be associated to the membranes in two different ways: as a labile or as a stable component. The labile component can be easily deactivated by incubating the membranes in a medium without Ca^{2+}. The stable component can be deactivated only by incubation of the membranes for 3 hours in a medium with 2mM EDTA or EGTA. Once deactivated, both components can be very easily activated by Ca^{2+}. The Ca^{2+} modulation of the Na^+ -ATPase activity may be taken as an indication of a Ca^{2+} regulating role on the way the kidney proximal tubular cells handle their Na^+ content. (Supported in part by CONICIT, Grant DF S1-1245).

50.5

A MODEL FOR OXYGEN UPTAKE BY RBCs. Guillermo Gutierrez*, Harold H. Rotman and David R. Dantzker. Univ. of Michigan, Ann Arbor, MI 48109

$d[O_2Hb]/dt=k'_c[O_2][Hb]-k_c[O_2Hb]$ (1) describes RBC oxygenation where k'_c and k_c are determined with rapid reaction techniques. It does describe the initial reaction, but near equilibrium, it gives not the S-shaped dissociation curve, but a rectangular hyperbola $[O_2Hb]=[O_2][Hb]_{total}/([O_2]+k_c/k'_c)$ (2). Adair's hypothesis is mathematically cumbersome due to its 8 velocity constants. Hill's equation, $[O_2Hb]=[Hb]_t[O_2]^n/([O_2]^n+k_c/k'_c)$ (3) gives excellent fit to the dissociation curve when n=2.6, and if one point on it is chosen, a value for the ratio k_c/k'_c can then be calculated from it. Its kinetic enunciation is $d[O_2Hb]/dt=k'_c[O_2]^n[Hb]-k_c[O_2Hb]$ (4), and using data obtained with a rapid reaction apparatus, we calculate k'_c from the initial slope and $k'_c=(d[O_2Hb]/dt)(1/[O_2]^n)(1/[Hb])$ (5). The off reaction rate (k_c) is now calculated from k'_c and the ratio k_c/k'_c. With this approach and data from published work (Rotman et al, 1980) we get $k'_c=5787$ and $k_c=1.1$. Equation (4) is now solved numerically with these rate constants and experimental values for the initial $[O_2]$ and [Hb]. For the first 50 msec, the resulting curve is very close to the experimental one, and is closer still to that calculated from (1), assuming $k'_c=143$ and $k_c=11$. In addition, the steady state solutions fall within 2% of the experimental Hb-O_2 curve. We conclude that equation (4) is a good predictor of RBC O_2 uptake from onset to equilibrium.

50.6

Methylation of erythrocyte membranes: inhibition by the anti-sickling drug procaine. Gloria A. Green * (Sponsor A. Dunn) USC School of Medicine, Biochemistry Dept., L.A., CA 90033.

Since it has been previously demonstrated that procaine retards the generation of irreversibly sickled cells in vitro and restores deformability to sickle erythrocytes, studies were initiated to determine the molecular mechanisms involved in this drug action. Experiments were carried out which examined the influence of procaine on methyl esterification of erythrocyte membrane proteins catalyzed by endogenous protein methylase II in normal (AA) red cells. Erythrocytes preincubated in 0.1 mM procaine followed by methylation with tritiated methionine showed a 50% decrease in total ^3H methyl groups incorporated into the membrane compared to controls. In pulse-chase experiments, erythrocytes were pulsed with 4 uM tritiated methionine (no procaine) and incubated with 4 mM "cold" methionine for 1-3 hrs (± procaine). Results show that samples incubated in the presence of procaine retained a greater fraction of total and alkali labile ^3H methyl groups incorporated into the membrane compared to controls indicating that procaine inhibits methyl group turnover. SDS-electrophoresis of membranes methylated after preincubation with procaine revealed that total ^3H methyl incorporation into band 4.5 (comigrating with glycophorin), and a 94 K protein is significantly reduced. These results suggest that the anti-sickling effects induced by procaine may proceed by a mechanism in which the drug alters post synthetic modification of membrane components. Supported by Greater L.A. American Heart Assoc. 707F2-1.

50.7

A23187 INDUCED INHIBITION OF ANION EXCHANGE IN ERYTHROCYTES: STUDIES ON THE MECHANISM OF ACTION. Victor M. Mancha*and W.R. Galey. Department of Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

A23187 in the presence of Ca^{++} induces Ca^{++} accumulation, ATP depletion, intracellular K^+ loss, cellular water loss, transformation of discocytes to spherechinocytes and increased membrane rigidity in normal human erythrocytes (AM J Pathol 1980, 101:543-556). We have shown that this ionophore for calcium also decreases anion exchange as seen by a 27% decrease in $^{35}SO_4^=$ efflux. Dreher et al, have shown that the increased membrane rigidity is dependent upon not only Ca^{++} accumulation by the cell but the subsequent K^+ loss and cell volume decrease. We have performed experiments to determine whether the inhibition of anion exchange is also dependent on these two factors and thereby potentially associated with the decreased membrane fluidity. By using a high K^+ buffer we were able to prevent the A23187 induced K^+ loss and cell volume change. We monitored the rate of $^{35}SO_4^=$ efflux and found that the previously observed decrease in anion exchange was maintained. These results suggest that the decrease in $^{35}SO_4^=$ efflux in normal human erythrocytes is not dependant on K^+ loss and cell shrinkage and therefore probably not dependant on membrane fluidity changes. Hence, the inhibition of anion exchange by A23187 must be due to some other effect of intracellular calcium. Supported in part by NIH grant RR08139.

50.8

ANESTHETICS INHIBIT BICARBONATE-CHLORIDE EXCHANGE ACCORDING TO THE MEYER-OVERTON RULE OF ANESTHESIA. Esther I.Chow*, Rugo Zhang*, and Robert E. Forster. University of Pennsylvania, Philadelphia, PA 19104.

HCO_3/Cl^- exchange rate of human erythrocytes at 37°C was studied (Chow, et al, J. Gen. Physiol. 68:633, 1976) in the presence of different concentrations of uncharged anesthetics: halothane, ether, chloroform, pentanol, hexanol, heptanol, octanol, α-chloralose, and charged anesthetics: pentobarbital, chlorpromazine, and tetracaine. The inhibition constant K_i, or the concentration for 50% inhibition of the HCO_3/Cl^- transport, is positively correlated with the concentration of an anesthestic that blocks transmission in the peripheral nerve. K_i is inversely proportional to the membrane solubility of the anesthetics (Meyer-Overton rule of anesthesia). About 5 times the nerve blocking concentration is needed for 50% inhibition of the exchange rate. This study shows that over 2 orders of magnitude of solubility in the cell membrane, a constant concentration of dissolved anesthetics produces the same inhibition of anion exchange, presumably producing the same decrease in turnover rate of the carrier protein (Band 3 protein). (supported by NIH grant HL-19737).

50.9

PHOSPHATE COMPOUNDS IN RED CELLS OF EMU AND RHEA.
G. R. Bartlett. Lab. Compar. Biochem., San Diego, CA 92109

Three striking features of water-soluble phosphates in bird red cells are: 1)fluctuating ATP in the embryo, 2) rapidly increasing inositol pentaphosphate (IP5) after hatch, and 3) a brief appearance of a high concentration of 2,3-diphosphoglycerate (DPG) in the late embryo. This has been a consistent pattern in examples from four orders: Galliformes, Anseriformes, Charadriformes and Columbiformes. The nonflying ratites separated from other birds early in their evolution and loss of ability to fly led to major anatomical and physiological differences from present flying birds. To see what changes might have occurred in the red cells we selected two members of the order, emu and rhea, with specimens supplied courtesy of the San Diego Zoo. A remarkable finding was the absence of DPG in both birds at any stage of development. Changes in ATP were similar to other birds, very high in the early embryo, low in the late and up again after hatch. It has been reported that red cells of the adult ostrich are unique in having more inositol tetraphosphate (IP4) than penta. Only traces of IP4 were found in red cells of the emu at any age while the adult rhea had about 1/4 as much IP4 as IP5. Total red cell inositol polyphosphate was substantially lower than in the flying birds. Supported in part by NIH Grant HLB-6950.

50.10

EFFECT OF AMBIENT OXYGEN ON ERYTHROCYTE ORGANIC PHOSPHATE LEVELS IN THE CHICK EMBRYO. M.K. Stock*, R.L. Ingermann* and J. Metcalfe. Oregon Hlth. Sci. Univ., Portland, OR 97201.

We examined the mechanism underlying the developmental changes in red blood cell (RBC) adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) concentrations in the chick embryo. Results below (mole ATP or DPG/mole hemoglobin tetramer) show that incubation of embryos in 70% O_2 attenuates both the decrease in RBC [ATP] and the increase in [DPG] typical of normoxic (21% O_2) controls. Embryos incubated 17 d in 70% O_2 then switched to 21% O_2 for 24 h exhibit a 30% decline in RBC [ATP] and a 200% increase in [DPG] compared to 18 d controls. Conversely, [ATP] increases 77% and [DPG] decreases 34% in RBCs from embryos incubated 17 d in 21% O_2 then switched to 70% O_2 for 24 h. Embryos incubated 13 d in 21% O_2 then switched to 15% O_2 for 24 h show a 35% decrease in [ATP] and a 141% increase in [DPG]. We conclude that intraerythrocytic [ATP] is directly related and [DPG] is inversely related to ambient oxygen concentration, and that [DPG] in embryos from normoxic eggs is low on day 14 due to environmental factors rather than to a lack of synthetic capability. (Supported by PHS HD-10034, HD-07084 and the Oregon Heart Association.)

		DAY 10	DAY 12	DAY 14	DAY 16	DAY 18	DAY 19
[ATP]	70% O_2	3.08	2.14	2.21	2.00	1.64	1.86
	21% O_2	2.96	2.25	2.18	1.52	0.75	0.69
[DPG]	70% O_2	0.10	0.12	<0.01	0.08	0.18	0.26
	21% O_2	0.03	0.14	0.16	1.06	1.69	1.97

50.11

ERYTHROCYTE METABOLISM: SOME PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF PIG RED CELL HEXOKINASE. Frank Kearse* and Earl Dixon, School of Veterinary Medicine, Tuskegee Institute, AL 36088.

Previous studies in this laboratory demonstrated the presence of two Type III hexokinase isozymes in porcine erythrocytes. The prevalence of the isozyme specie is an age-dependent phenomenon. The red cells of juvenile pigs (less than six months old) possess an isozyme that has an apparent Km (glucose) value which is lower than that of adult pigs (0.012 and 0.042 mM respectively). Currently, studies are designed to further investigate the physiological and biochemical differences between the two isozymes. D-Mannoheptulose, a structural analog of glucose, was observed to differentially inhibit the isozymes. The apparent Ki values (Mannoheptulose) of the juvenile isozyme was found to be 0.113 ± 0.006 mM while the corresponding value for the adult isozyme was 0.074 ± 0.008 mM. The thermo-labilities of the isozymes were studies at 40 °C. The adult isozyme maintained 27 percent of its original activity after 60 minutes of incubation at 40 °C under these conditions. The juvenile isozyme maintained 20 percent of its original activity under identical conditions. These data further support evidence that two Type III hexokinase isozymes are present in the mature red cells of the pig during development. (Supported by USPHS Grant # RR09198; (2S06 RR08091-10) MBS and CSRS Fund, P. L. 95-113 Section 1445).

50.12

ANTILEISHMANIAL ACTIVITY OF THIOSEMICARBAZONE H: INHIBITION OF ADENOSINE RECEPTOR BINDING. Brian D. Hansen*, Jose Perez-Arbelo* and Peter K. Chiang* (SPON: P.S. Aronson). Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20012

An azacycloheptane derivative of a 2-acetylpyridine thiosemicarbazone (H) was tested for antileishmanial activity and effect upon the binding of adenosine (an important precursor for the parasite synthesis of purine nucleotides) to receptors on the plasma membrane. Promastigotes of Leishmania braziliensis panamensis (WR008) were exposed to H at varying concentrations and incubation times and the I^{50} and the T^{50} determined (0.1 µg/ml and 14 hr respectively). The cells were then exposed to H (1 µg/ml for 0, 2, 30 and 60 min) followed by an additional 2 min incubation in the presence of radiolabeled adenosine, 2-deoxyglucose and aminoisobutyric acid. Only adenosine uptake was significantly reduced. In fact, pre-exposure of the cells to the drug for only 2 min reduced the subsequent 2 min adenosine uptake by 40%. Studies were also conducted to determine the effect of the drug on the binding of adenosine to membrane receptors. H significantly reduced the binding of a radiolabeled adenosine agonist (N^6-cyclohexyladenosine) and antagonist (1,3-diethyl-8-phenylxanthine). These data suggest that thiosemicarbazone H may exert its antileishmanial effect by inhibiting the binding of adenosine to adenosine membrane receptors.

50.13

SEROLOGICAL AND BIOCHEMICAL STUDIES ON TUNICATE PLASMA LECTINS. Gerardo R. Vasta and John J. Marchalonis. MUSC, Charleston, SC 29425

Plasma from Clavelina picta, Didemnum candidum, Amaroucium stellatum, Ciona intestinalis, Halocynthia pyriformis, Boltenia ovipera, Botryllus schlosseri, Styela partita, S. plicata, and S. monterreyensis were investigated for the presence of lectins on the basis of agglutination of a panel of untreated and enzyme treated erythrocytes, crossed absorption studies and the inhibition of agglutination with glycosubstances. All species possesed detectable lectins, and all but one, multiple lectins. The specificities of the lectins, which included D-galactose (D. candidum), sialic acids (H. pyriformis and S. plicata) and complex carbohydrate structures, were not correlated with taxonomical relationships of the species studied. H. pyriformis major lectin (affinity purified on formaldehyde fixed horse erythrocytes) and D. candidum major lectin (affinity purified on asialo-fetuin-Sepharose 4B) exhibited single subunits of apparent molecular weights 45,000 and 16,000 daltons respectively, as assessed by sodium dodecyl sulphate polyacrylamide (10% acrylamide, 0.2% bisacrylamide) gel electrophoresis under reducing conditions (1% 2-mercapthoethanol). Amino acid compositions were determined in a Durrum amino acid analyzer after hydrolysis in 6N HCl for 24h at 37°C in tubes sealed under vacuum. Although different in specificity and subunit molecular weight, H. pyriformis and D. candidum lectins showed a high degree of relatedness on the basis of amino acid composition as determined by the SAQ statistical parameter.

50.14

IDENTIFICATION OF A D.I.C.-PROMOTING FACTOR IN HUMAN ASCITES. Carol A. Fisher*, William Inouye*, Ernest Rosato*, L. Henry Edmunds, Jr., Alden H. Harken and V. Paul Addonizio. U of PA, Philadelphia, PA 19104.

Peritoneal-venous shunts are associated with a significant incidence of disseminated intravascular coagulation (D.I.C.). This study identifies a site of origin, a pathogenic mechanism, and the hemostatic pathway which accounts for the thrombogenicity of human ascites. Ficoll-Hypaque column chromatography and ultracentrifugation were utilized to prepare four fractions from human ascites: Cellular, consisting of monocytes and lymphocytes; a low speed cell-free fluid; a high speed supernatant; and the precipitate from the high speed centrifugation. The cells demonstrated an ability to shorten a one stage clotting time by 60% relative to saline and endotoxin controls. Similarly, low speed cell-free fluid shortened the clotting time of pooled normal plasma by 65%; was also effective in factor VIII deficient plasma; but had no effect on factor VII deficient plasma and little effect on platelet aggregation and release. The high speed supernatant was demonstrably less thrombogenic. The resuspended precipitate shortened the clotting time of pooled normal plasma by 64% and of factor VIII deficient plasma from infinity to 86 sec. In contrast, this material was ineffective on factor VII deficient plasma. We conclude that the thrombotic factor exists in suspension and is thromboplastin-like in its behavior, operating through the extrinsic (VII dependent) pathway of coagulation. (Supported by NIH grants HL 19055, HL 22315.)

50.15

DEVELOPMENTAL IMMATURITY OF THE CONTACT SYSTEM PROLONGS THE PARTIAL THROMBOPLASTIN TIME (PTT) IN PREMATURE LAMBS.
M. Andrew* and M.E. Towell* (SPON: E.J.M. Campbell). Dept's of Paediatrics and Obstetrics, McMaster University, Hamilton Canada L8N 3Z5

Newborns have a prolonged PTT primarily due to low levels of contact factors: XII, XI, prekallikrein (PK), and high molecular weight kininogen (HMW-K) (Andrew NEJM 305:1130, 1981). Lowest levels are in the most immature infants. Although proteolytic cascades are activated at birth (factors may be consumed) we hypothesized that immaturity itself contributes to low contact factor levels. Utilizing epidural anaesthesia we inserted silastic catheters into the femoral artery of both the fetus and ewe at 108-119 days gestation (n=8). Two ml of blood was drawn into Na-EACA at initial surgery and 1 week after normal term vaginal delivery. Clotting tests were performed including a PTT with contact product which contains XIa (PTTcp). Factor levels are % of non-pregnant adult sheep (mean (SE)).

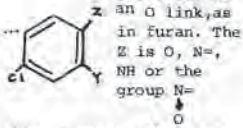
	PTT (sec)	PTTcp (sec)	TT (sec)	XII (%)	XI (%)	PK (%)	HMW-K (%)
in utero	106(24)	31(1)	18(2)	42(13)	73(17)	19(10)	73(14)
1 week	28(2)	20(1)	17(1)	87(7)	177(31)	145(25)	119(4)
pregnant ewe	28(1)	24(1)	17(1)	94(16)	160(38)	107(7)	97(32)
adult sheep	36(1)		17(.4)	100	100	100	100

We conclude that developmental immaturity of the contact system contributes to the prolonged PTT at birth.
(Supported by the MRC of Canada).

50.16

CHEMICALLY INDUCED ACNE: STUDY OF A RECEPTOR HYPOTHESIS AND POSSIBLE RELATIONSHIP TO VITAMIN A. Richard P. Spencer, Univ. Connecticut Health Center, Farmington, CT 06032.

A type of chemically induced acne in workers is related to compounds reported to be isosteric to 2,3,7,8-tetrachlorodibenzo-p-dioxan and the furan. Analysis of literature data (Science 194:627, 1976) suggests that the requisite homology likely involves the benzene ring and the o-position relative to the usual upper Cl. The compounds are known to induce hepatic aryl hydrocarbon hydroxylase and to compete for cytosol binding sites. Plots of potency (P) for inducing the enzyme in chicken embryo liver, versus the binding affinity (A) are described (over 4 orders of magnitude) by: $\log P = \log 0.56 + 3 \cdot \log A$. The molecular configuration bears some resemblence to part of the vitamin A molecule. It is known that 13-cis-retinoic acid (vitamin A congener) has a beneficial effect on acne. We can hypothesize that the chemical products compete for an acceptor site on the sebaceous glands to stimulate production. In such a model, the vitamin A compounds would have the opposite effect (either directly on the same sites or by a general effect on the sebaceous glands).



The Y can be an O link, as in furan. The Z is O, N=, NH or the group N=O

(Supported by USPHS CA 17802, National Cancer Institute).

50.17

TRANSFER OF NUCLEOPROTEINS BETWEEN AND INTO CELLS. Anwar A. Hakim and Charles E. Joseph. Univ. Ill. Med. Ctr. Chicago, Ill 60612. and Univ. Southern Calif. School of Dentistry, Los Angeles, Calif. 90007.

To modify cell genetical expression, the modifier must be introduced into the cell by either fusion or by intracellular injectins. The present studies report on a third alternative. In two groups, 5 per group, of Falcon plastic cell culture dishes, 2 platinum electrodes were firmly fixed against the bottom at opposite sides of each dish. Human gingival fibroblasts (HGF) were seeded at 10^4 per ml in MEM and were allowed to proliferate for 5 days at 22°C. At confluency, the medium was removed and a fresh MEM containing 1 uCi ^{3}H-thymidine/ml was added. The cultures were reincubated for 4 additional Hrs. The monolayers were washed, then seeded with human mammar carcinoma (HMCC-GM) cells in MEM. In parallel, HMCC-GM were seeded into MEM-containing lysates of ^{3}H-thymidine-labeled HGF. Eight electric pulses (1.0 kV/cm, 1/10 sec duration at 15 sec intervals were applied to the experimental group. The control group was not pulsed. The cells in both groups were allowed to proliferate for 96 Hrs. The medium and the cells were harvested. After washing the HMCC-GM cells were cloned and allowed to proliferate for 96 additional Hrs. Whether on HGF-monolayers or cultured in presence of ^{3}H-labeled-HGF-lysates, control HMCC-GM grew in soft agar forming colonies, were oncogenic in athymic mice, and their lysates did not contain radioactivity. Whereas, the experimental cells, did not grow in soft agar, they grew under standard conditions, they were non-oncogenic and their lysates contained radioactivity.

RENAL/EPITHELIAL PHYSIOLOGY

51.1

SIDEDNESS OF LACTATE AND GLUCOSE TRANSPORT AND METABOLISM IN LLC-PK1 CELLS. James M. Mullin, Linda Fluk and Arnost Kleinzeller. Dept. Physiology, Univ. Pennsyl., Phila., Pa. USA

Further studies of ^{14}C-L-lactate metabolism have shown an absence of gluconeogenesis in confluent LLC-PK1 cultures on collagen-coated filters, as was previously shown using culture dishes (Am. J. Physiol. 242:C41, '82). In Ussing-type chambers the cells metabolized 1mM L-lactate similarly, whether it was presented luminally or antiluminally. In both cases the principal metabolites were alanine, glutamate and (transiently) glycerol. No evidence for a Na^+-lactate cotransport system was found. Glucose, a substrate actively transported by LLC-PK1 cells, was also metabolized similarly whether presented antiluminally or luminally.

However in questioning the ability of an LLC-PK1 monolayer to adequately keep small nutrient molecules in one or the other extracellular compartment, the following results were obtained: 1) Unlike the luminal membrane, mutual inhibition for uptake of glucose and alpha-methyl-D-glucoside (AMG) is not seen at the antiluminal membrane, and AMG uptake here is quite slow; 2) 0.1 mM phlorizin (luminal) decreases the uptake of 1mM AMG (antiluminal); 3) Incubated simultaneously, the antiluminal to luminal flux of 1mM ^{3}H-mannitol is 40% greater than that of 1mM ^{14}C-AMG. Taken together such findings suggest that transcellular or paracellular transport of a substrate followed by uptake at the unintended membrane is a factor in transport studies with LLC-PK1 monolayers in Ussing-type chambers. (Supported by grants NIH-AM12619 and the Whitehall Fndn.)

51.2

Interrelationships between Urate and p-Aminohippurate (PAH) Transport in Dog Kidney Plasma Membrane Vesicles. Peter D. Holohan*, Charles R. Ross, (Spon: R.L.Terjung) SUNY/Upstate Medical Center, Syracuse, N.Y. 13210

The dog kidney demonstrates bidirectional transport of urate. The direction of net transport, as in man, is reabsorption. The question under study was whether or not PAH and urate share a common transport mechanism, and if so, why does one show secretion and the other reabsorption. The mutual effects of PAH and urate on their transport in isolated brush border and basolateral membrane vesicles was tested using a rapid filtration assay. In brush border vesicles, cis concentrations of PAH inhibited [^{14}C]urate uptake while trans concentrations stimulated both [^{14}C] urate influx and efflux. An entirely analogous situation was found when the effects of urate on [^{3}H] PAH was studied. In the basolateral membrane however, cis concentrations of PAH did not inhibit nor did trans concentration stimulate [^{14}C] urate transport. Similarly, PAH transport was unaffected by a concentration series of urate. Interestingly, probenecid proved to be an inhibitor of PAH and urate in both membranes. Our interpretation of these data is that in the dog kidney PAH and urate share a common transporter in the brush border membrane but are transported independently at the basolateral membrane; suggesting that it is the events at the basolateral membrane which control transepithelial transport. (Supported by PHS Grant #HL 02835).

51.3

IDENTIFICATION OF THE D-GLUCOSE TRANSPORTER IN ISOLATED MICROVILLI FROM HUMAN PLACENTA. Rolf L. Ingermann* and John M. Bissonnette. Oregon Health Sci. Univ., Portland, OR 97201

We have previously shown that [^{3}H] cytochalasin B (CB) (an inhibitor of D-glucose transport) binds with high affinity to isolated microvilli from the maternal surface of the human placenta. A portion of this CB binding can be displaced by D-glucose, presumably at a site on the glucose transporter. An appreciable amount of these binding sites exist in the microvilli (17 pmols/mg microvillus protein)(Fed. Proc. 41:1414, 1982). This suggests that the recently described photoaffinity labeling of membrane proteins with [^{3}H] CB to identify the D-glucose transporter in chick embryo fibroblasts (Pessin et al. PNAS 79:2286, 1982) and human erythrocytes (Carter-Su et al. JBC 257:5419, 1982), may be applicable to placental microvilli. Isolated microvilli 1 mg protein/ml were incubated with 0.3 μM [^{3}H] CB in the presence of either 250 mM L-glucose or D-glucose. The microvilli were irradiated with a 150 watt xenon lamp for times ranging from 0 to 30 min. The microvilli were pelleted, washed, and solubilized in urea-sodium dodecyl sulfate. The membrane proteins were resolved by PAGE electrophoresis. The gel was cut into 3 mm sections and counted. A peak of [^{3}H] CB activity in the gel slices containing proteins of Mr 51,000 to 61,000 was seen for membranes irradiated in the presence of both D or L-glucose but the peak was less in the presence of D-glucose. We conclude that this methodology is satisfactory for identification of the D-glucose transporter in human placenta.(Supported by HD07084 and MRF of Oregon.)

51.4

Heterogeneity of "brush-border" membranes prepared from rabbit renal cortex. A.K. Mircheff, H.E. Ives*, V.J. Yee*, and D.G. Warnock. Department of Physiology and Biophysics, USC, Los Angeles, CA 90033, and Department of Medicine, UCSF, San Francisco, CA 94143.

Brush-border membranes prepared by Mg^{++}-precipitation contain small amounts (less than 5% recovery) of marker enzymes characteristic of basolateral membranes (BLM) and intracellular organelles (BBA 647:169, 1981). It is not known whether these enzymes are present in BBM or represent contamination by other membranes. We analyzed the distribution in BBM of alkaline phosphatase (AP), K^+-Stimulated Phosphatase (BLM) and NADPH Cytochrome C reductase (ER, Golgi) with sorbitol density gradients followed by countercurrent distribution with dextran/polyethylene glycol (CCD). Centrifugation on a continuous density gradient yielded 30% of AP centered at a density of 43% sorbitol and 60% of AP centered at 55% sorbitol. A separate BLM population had an intermediate density. The major AP-containing membrane population was subjected to CCD, and was separated into 2 subpopulations, each containing 50% of the recovered AP activity. One subpopulation was broadly distributed, was not enriched relative to protein, and overlapped most of the NADPH Cytochrome C reductase activity. The other AP-containing subpopulation was enriched 1.5-fold relative to protein, and overlapped the residual K^+-Stimulated Phosphatase activity. Conclusions: 1) BBM prepared by Mg^{++}-precipitation contain several membrane subpopulations which can be resolved by density gradients and CCD. 2) Alkaline phosphatase is heterogeneously distributed and may not be an ideal marker for BBM. (Supported by NIH grants AM-28408, AM-19407, AM-00668, and AM-07219).

51.5

ISOLATION OF APICAL AND BASOLATERAL PLASMA MEMBRANE VESICLES FROM BOVINE TRACHEAL EPITHELIUM J.E. Langridge- Smith,* M. Field, and W.P. Dubinsky,* Univ. of Texas, Houston, TX 77025 and Univ. of Chicago, Chicago, IL 60637

A method is presented for the separation of apical (AM) and basolateral (BLM) membrane vesicles from bovine tracheal epithelium. Fresh tracheae are washed in cold oxygenated Ringer's solution, stripped of cartilage and incubated in isotonic buffered sucrose (pH 7.8) containing 2 mM EGTA and 1 mM dithiothreitol at 0°C for 15'. Epithelial scrapings are then collected and homogenized in 50 mM mannitol--5 mM HEPES (pH 7.4) with a teflon pestle homogenizer. The post-mitochondrial supernatant is centrifuged at 35,000xg for 40' yielding a fraction enriched in both Na,K-ATPase (BLM marker) and alkaline phosphatase (AM marker). This fraction is re-suspended in buffered 100 mM mannitol containing 10 mM $MgCl_2$ and incubated on ice for 1 hour. Low speed centrifugation (1500xg for 12') sediments a fraction enriched in BLMs. An AM fraction is obtained by centrifugation (100,000xg for 15') of the Mg-treated supernatant which yields 19-fold enrichment of alkaline phosphatase (49.0 ± 5.6 μmol mg^{-1} hr^{-1}; n = 4). The Na,K-ATPase of the BLMs is enriched 4-fold (10.8 ± 0.23 μmol mg^{-1} hr^{-1}; n = 4). The AM fraction exhibits time-dependent transport of ^{22}Na into an osmotically active space which is completely inhibited by amiloride (1 mM) but insensitive to furosemide. Uptake is also anion dependent with the following selectivity: SCN >Cl> Gluconate. (Supported in part by USPHS grants AM21345 and AM27377).

51.6

INHIBITION OF RENAL HYDROLYSIS OF ABSORBED PROTEIN BY NH_4Cl AND CHLOROQUINE. M.J.F. Camargo*, E. Eich* and T. Maack. Dept. of Physiol., Cornell Univ. Med. Coll., New York 10021.

Filtered proteins are absorbed by the renal tubular cells by endocytosis and hydrolysed within lysosomes to aminoacids which are then returned to the circulation (Maack et. al. Kid. Internat. 16:251,1979). We tested whether the acid pH of lysosomes is essential for the normal metabolism of absorbed proteins. Rate of hydrolysis of absorbed cytochrome C (CYT C) was measured by perfusing isolated rat kidneys pre-loaded in vivo with methylated $^{14}CH_3$-CYT C and determining the rate of efflux of radioactivity to the perfusate (P) as previously described (op. cit.) The pH of lysosomes was raised by adding NH_4Cl or chloroquine (CQ) to P. Chromatographic analysis showed that the radioactivity present in kidneys eluted as $^{14}CH_3$-CYT C while that released to P eluted as $^{14}CH_3$-lysine. In control perfusions, 77±4.5% SE (n=4) of the radioactivity initially present in kidney was released to P after 60 min of perfusion. This value decreased markedly ($p < 0.001$) to 20.2± 3.3% (n=4) in the presence of 10 mM NH_4Cl and to 27.8±1.4% (n=4) with 0.1 mM CQ. Catabolism of CYT C was completely blocked by 0.5 mM CQ. The effect of NH_4Cl and CQ was reversible upon removal of these weak bases from P. Results demonstrate that alkalinization of intralysosomal pH reversibly inhibits hydrolysis of absorbed CYT C and indicate that the acid milieu of lysosomes is essential for the normal disposal of endocytosed proteins by the renal tubular epithelium.

51.7

NEPHRON SITE OF THE RESTORATION OF THE PHOSPHATURIC EFFECT OF PARATHYROID HORMONE (PTH) BY NICOTINAMIDE. J. A. Haas,* T. J. Berndt,* A. Haramati, F. G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester, MN 55905

Nicotinamide (Niam) reverses the increased phosphate reabsorption and resistance to the phosphaturic effect of PTH induced by phosphate deprivation. The present study was performed to localize the nephron site of action of Niam and to compare the phosphaturic effect of PTH in the presence and absence of Niam. Clearance and free-flow micropuncture samples were obtained from thyroparathyroidectomized rats stabilized on low (0.07%) Pi diet injected with Niam (1 g/kg, i.p.) or vehicle two hours prior to anesthesia. Following control collection, PTH (1 μ/kg/min) was given. *significant.

	Fractional Delivery of Phosphate (%)	
	Late Proximal Tubule	Urine
Vehicle	6.1 ± 2.2	0.8 ± 0.2
Niam	36.0 ± 4.6*	2.6 ± 1.1
Vehicle	6.1 ± 2.2	0.8 ± 0.2
PTH (n = 9)	30.8 ± 3.9*	3.5 ± 0.6*
Niam	36.0 ± 4.6	2.6 ± 1.1
PTH (n = 6)	39.2 ± 6.4	15.9 ± 3.5*

In phosphate deprivation, Niam increases phosphate delivery from the early proximal tubule. Resistance to and restoration of the phosphaturic effect of PTH by Niam occurs at a nephron segment beyond the late proximal tubule.

51.8

EFFECT OF GLUCOCORTICOIDS ON AMMONIAGENESIS AND GLUTAMINE UTILIZING ENZYMES OF ADRENALECTOMIZED RAT KIDNEY. C. J. Welbourne and T. C. Welbourne. Dept. Physiol. & Biophys., LSUMC Shreveport, LA 71130

Glucocorticoids administered to intact rats stimulate NH_4^+ excretion and production from glutamine similar to that observed in metabolic acidosis. We investigated the effect of triamcinolone on the capacity of two ammoniagenic pathways-mitochondrial phosphate dependent glutaminase (PDG) and the brush border γ-glutamyltransferase (γ-GT). Adrenalectomized male Sprague Dawley rats were maintained on 0.9% NaCl for 1 week and then given either 50 μg triamcinolone kg^{-1} per 24h or vechicle alone. After 24h the animals were anesthesized, blood samples drawn and their kidneys homogenized and assayed for total PDG and γ-GT capacity using standard assays; capacity is expressed as μmole product min^{-1} per gram protein. Results: ADX decreases plasma total CO_2, 23 ± 1 mM, which returns to normal 26 ± 2 mM in ADX + steroid treated rats; $UV + NH^+_4$ doubled, 1,204 ± 168 vs 444 ± 84 μmole $24h^{-1}$ rat^{-1} despite a rise in urine pH, 7.3 ± 1 vs 7.1 ± 0.1; UV_{ϵ} fell from 990 ± 120 to 610 ± 90 μmole $24h^{-1}$. PDG and γ-GT activity both decreased with triamcinolone; PDG fell from 49 ± 5 to 39 ± 3 μmole min^{-1} g^{-1} while γ-GT dropped from 160 ± 10 to 125 ± 7 μmole min^{-1} g^{-1}. Conclusion: Glucocorticoids acutely stimulate UV_{NH_4} without increasing the capacity of either glutaminase. The reduction in these activities may reflect structural alterations and subsequent autophagy induce by glucocorticoids.

51.9

CYCLIC-AMP STIMULATION OF HCO_3 SECRETION BY FROG CHOROID PLEXUS. *Yoshitaka Saito* and Ernest M. Wright. Department of Physiology, UCLA School of Medicine, Los Angeles, Ca. 90024.*

We have studied the effect of ion substitutions, hormones, diuretics and cyclic nucleotides on short-circuit current (SCC) across the bullfrog choroid plexus. In HCO_3 Ringer's solution, the potential difference, SCC and electrical resistance were 0.8mV (the ventricular side negative), 5μA/cm^2 and 160ohms·cm^2, respectively. Ion substitution experiments showed that this negative SCC is due to net HCO_3 transport toward the cerebrospinal fluid (CSF). Theophylline (1-30mM), dibutyryl cAMP (1-5mM), isoproterenol (0.01-1mM), prostaglandin E_1 (0.56mM), ACTH (1mM) and forskolin (0.05-1mM) all significantly increased the SCC. The pH of the serosal bathing fluid became more acid after addition of theophylline while the ventricular bathing fluid became more alkaline. Cl substitution with gluconate reduced by 50% the magnitude of theophylline induced change in SCC (ΔSCC) while the response was abolished to < 12% by Na-free and HCO_3-free solutions. Ouabain (0.01-1mM), acetazolamide (0.1μM-1mM), furosemide (1μM-1mM), stilbene compounds (SITS and DIDS: 0.01-1mM) and harmaline (0.01-10mM) inhibited both SCC and ΔSCC. We conclude from these results that HCO_3 secretion by the choroid plexus into CSF is controlled by hormones which stimulate the adenylate cyclase system.

(Supported by USPHS grant NS09666).

51.10

MECHANISM FOR GENERATION AND MAINTENANCE OF ELEVATED RENAL CORTICAL CO_2 TENSION. Akhil Bidani, Edward Crandall, and Thomas DuBose, Jr. University of Texas Medical Branch at Galveston, TX 77550 and University of California at Los Angeles, CA 90024

Recent studies employing PCO_2 microelectrodes have demonstrated CO_2 tensions in the renal cortex (60-65 mm Hg) that are significantly greater than systemic arterial PCO_2 (JCI 63:338). Three sources for CO_2 generation have been proposed: 1) luminal production from $H^+ + HCO_3^-$ 2) addition of HCO_3^- to peritubular plasma with disequilibrium for $H^+/HCO_3^-/CO_2$, and 3) metabolic CO_2 production. None of these mechanisms has adequately explained the findings. The purpose of this study was to develop a mathematical model for the reactions and transport process involved in proximal tubule HCO_3^- reabsorption. Steady-state calculations of pH, PCO_2 and HCO_3^- employed mass balance considerations for the luminal, cellular, and vascular compartments, and assumed metabolic CO_2 production from 0.3 to 1.0 mM/L RBF. To demonstrate close agreement between measured and calculated values it was necessary to impose a vascular-to-vascular diffusive gas transfer system with an exchange efficiency of 70-90%. The model suggests that the level of renal cortical PCO_2 is a function of the magnitude of metabolic CO_2 production and the efficiency of CO_2 trapping. We propose that vascular-vascular exchange of CO_2 gas coupled with metabolic CO_2 production provides an adequate explanation for the level of CO_2 tension demonstrated previously. NIH Grants AM-30603 and HL-25669.

51.11

ABSENCE OF CARBONIC ANHYDRASE ON THE INITIAL EXCHANGE VESSELS OF THE RABBIT KIDNEY P.M. Effros, S. Nioka*, G. Mason*, P. Silverman*, E. Reid*, L. Mellander* Division of Respiratory Physiol. and Med., Harbor UCLA Med. Center, Torrance, CA 90509

Although there is abundant carbonic anhydrase associated with pulmonary and skeletal muscle capillaries, we have not detected any on some of the exchange vessels of the kidney. When rabbit kidneys are perfused with solutions not containing c.a., $^{14}CO_2$ transit from artery to vein was significantly more rapid than that of $H^{14}CO_3^-$: initial recoveries (R) of $^{14}CO_2$ (compared to ^{125}I-albumin) exceeded those of $H^{14}CO_3^-$ by a factor of 4 ($R[CO_2]=.67\pm.04$, $R[HCO_3^-]=.15\pm.04$ SEM, n=10). This difference between the $^{14}CO_2$ and $H^{14}CO_3^-$ curves was eliminated with c.a. infusions (200 mg/l). The differences persisted with infusions of the c.a. inhibitor acetazolamide (20-100 mg/l) but the transit of $^{14}CO_2$ through the organ was slowed ($R[CO_2]=.43\pm.05$). This effect of acetazolamide did not seem to be due to a change in tissue pH which was $7.30\pm.06$ before and $7.32\pm.03$ after inhibition (measured with ^{14}C-5,5-dimethyl-2,4-oxazolidinedione transient injections). These data suggest that c.a. is absent on the arterial end of the capillaries but present in more venous exchange vessels. Histochemical studies have noted the absence of c.a. in glomerular vessels in all mammals, but its presence in post-glomerular capillaries of some species. In vivo studies suggest that red cell c.a. does not ensure HCO_3^--CO_2 equilibration in renal vessels. (NIH grant HL28255).

51.12

RENAL OXYGEN CONSUMPTION DURING HYPOXEMIA. R.W. Gotshall, D.S. Miles and W.R. Sexson*. Wright State Univ., Dayton, OH 45435.

To determine the extent to which hypoxemia affects renal O_2 consumption ($R\dot{V}O_2$; μM/min/100g), $R\dot{V}O_2$ was calculated and renal arterial-venous O_2 difference [(a-v)O_2; vol%], glomerular filtration rate (GFR; ml/min/100g), and renal blood flow (ml/min/100g) were measured in single kidneys of 7 anesthetized dogs breathing progressively lower fractional inspired O_2 (FIO_2).

FIO_2 =	20%	14%	12%	10%	8%	20%
PaO_2	77	46*	40*	31*	24*	74
$R\dot{V}O_2$	466	505	502	444	381*	424
(a-v)O_2	1.9	2.1	1.9	2.4	2.3	2.5
GFR	75	72	77	65	58*	64
RBF	525	556	565	456	426*	461

These data indicate (*P<0.05) that hypoxemia does not reduce $R\dot{V}O_2$ directly. It is only at very low PaO_2's that RBF, GFR and, subsequently, tubular oxygen requirements are reduced, which proportionately decreases $R\dot{V}O_2$. Proportional changes in RBF and $R\dot{V}O_2$ are indicated by the unchanging (a-v)O_2 [(a-v)O_2 = $R\dot{V}O_2$/RBF]. The low renal (a-v)O_2 together with the high RBF indicate that renal O_2 delivery is relatively greater than other organs (whole body(a-v) O_2=5 vol%; coronary (a-v)O_2=15 vol%). Thus, $R\dot{V}O_2$ cannot be compromised by simply reducing PaO_2. Only by markedly reducing RBF alone or in conjunction with a decreased PaO_2 can renal O_2 delivery be reduced to compromise renal function. (Supported by Miami Valley Chapter AHA).

51.13

RENAL BLOOD FLOW DISTRIBUTION AND "REDISTRIBUTION". A CRITICAL ANALYSIS IN RATS USING 15μ AND 10μ MICROSPHERES. Daniel Casellas*, Bernard Jover* and Albert Mimran* (SPON: L.G.Navar). Dept Medicine, Montpellier, 34059 France.

The suitability of the microsphere (M) method to assess intrarenal blood flow distribution (IRBFD) and the concept of renal blood flow (RBF) "redistribution" were reexamined in 227 anesthetized rats using the reference sample method and mixtures of differently labeled 15μ and 10μ M. The apparent IRBFD was calculated for both M sizes (IRBFD15, IRBFD10) as the dimensionless ratio: outer half cortical/ inner half cortical blood flow. There was a good agreement between 15μ and 10μ values for cardiac output (CO, 60-400ml/min.kg) and RBF (0.3-11ml/min.g). For all RBF values, 15μ M were more concentrated than 10μ M in outer half cortex. There was a good correlation (r=0.78, P< 0.001) between IRBFD15 (range 1.45-5.57) and IRBFD10 (range 1.41-2.49). However, IRBFD15 inversely correlated with RBF (r=-0.83, P< 0.0001) and directly correlated with renal resistance (r=0.64, P< 0.001); IRBFD10 was less related to these parameters (r=-0.44, NS and r=0.55, P< 0.02 respectively). In conclusion, 15μ M are subject to steric hindrance with increased renal resistance, while the cortical distribution of 10μ M remains stable regardless of renal resistance or RBF. These findings indicate that IRBFD does not vary greatly during spontaneous variations in total RBF.

51.14

RESPONSE OF ISOLATED RENAL ARTERIES TO ADENOSINE AND INOSINE. R.J. Sinclair, J.R. Randall* and C.E. Jones. Department of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas, 76107.

The responses of isolated renal arteries to purine nucleosides were tested by progressively increasing concentrations of adenosine (ADO) or inosine (INO) in the bathing medium. Vessel rings (0.7-1.3 mm o.d.) from various sites in the canine kidney, including the interlobar and arcuate arteries, were aerated with 95% O_2/5% CO_2, and isometric tension was monitored. Rings were equilibrated at 1 g, and all vessels were treated with ADO (0.25-11 μM) and INO (50-800 μM). In addition, the effect of aminophylline on the responses to ADO and INO was tested. In most vessels ADO elicited relaxation. In these vessels the maximal relaxation response to ADO was 1.59 times that to INO. Also, the ED_{50} to ADO was 0.25 μM, while that to INO was 183 μM. Interestingly, in two vessels ADO produced a clear contraction. In all vessels it was noted that aminophylline completely blocked the response to ADO but did not affect the response to INO. These observations suggest that the dominant response to both purines is relaxation and that INO may act through a receptor distinct from the ADO receptor such as previously reported in coronary arteries.

(Supported in NIH Grant HL-29731 and ICOM Grant 34982).

51.15
EFFECTS OF FLURAZAPAM AND CHLORDIAZAPOXIDE ON THE KIDNEY OF THE RAT. Susan Hathaway*, Donald R. Britton* and Sidney Solomon. U. of New Mexico, Albuquerque, New Mexico 87131.

Flurazapam (FLR) and chlordiazapoxide (CDX) at a concentration of 1 mM in a final volume of 0.1 ml were injected into the left renal artery or, at a concentration of 10 mg/kg BW, into the femoral vein. Urine volume, glomerular filtration rat (GFR) and urinary sodium and potassium excretion ($U_{Na}V$ and U_KV) were measured at 30 and 60 minutes after injection and compared to baseline, pre-injection values. Preliminary results indicate that following CDX injected into the renal artery, volume, GFR and U_KV were decreased from baseline ($p < .05$) in the right kidney 60 minutes after injection. No change was observed in the left (denervated) kidney following CDX, which suggests that the decreases seen in the right kidney are mediated through the renal nerves. This is further supported by the decreases in GFR and U_KV observed in both innervated kidneys following CDX administration through the femoral vein. FLR had no effect at either the 1mM or 10 mg/kg BW concentration, however, preliminary results suggest that at higher renal artery concentrations (1 and 3 mg), decreases in urine volume, GFR and $U_{Na}V$ do occur at the left kidney. This suggests a more direct renal action of FLR. (Supported by a grant from NSF PCM 78-15383)

51.16
EFFECT OF GLUCAGON ON SEGMENTAL RESISTANCE AND THE GLOMERULAR FILTRATION COEFFICIENT (K_f) IN DOGS. Gary R. Marchand. Physiology Department, Duluth, Mn. 55812

Glucagon is a vasodilator that increases glomerular filtration rate in dogs. The present experiments were done to identify the resistance vessels affected and determine whether K_f is increased by glucagon. Therefore, the determinants of GFR were measured in 7 dogs before and during glucagon (.25-.5 µg/kg/min, iv). Glomerular pressure was estimated from single nephron stop-flow pressure. Bowman's space pressure was estimated from free-flow tubule pressure. Blood was collected from efferent arterioles and glomerular plasma flow calculated from single nephron GFR and filtration fraction. K_f and mean filtration pressure were calculated from the average of each determinant. Glucagon significantly increased total GFR and plasma flow. SNGFR increased 24 ± 11 nl/min. Glomerular plasma flow was not increased significantly (246 ± 22 to 273 ± 24 nl/min). Glomerular pressure increased 19 ± 5 mmHg and Bowman's space pressure increased 16 ± 6 mmHg. The transcapillary pressure difference was not changed significantly. Efferent arteriole pressure increased from 16 ± 2 to 29 ± 3 mmHg. Although afferent resistance was reduced significantly (.35 ± .03 to .23 ± .03) total resistance was not (.73 ± .09 to .63 ± .07). K_f increased from 5.0 to 6.6 nl/min/mmHg per glomerulus, whereas mean filtration pressure was unchanged (15.4 to 15.5 mmHg). (Supported by NIH grant #HL25390)

51.17
INHIBITION OF ENDOTHELIAL CELL PROSTAGLANDIN RELEASE BY ANGIOTENSIN-CONVERTING ENZYME INHIBITORS. K. Pomerantz, J.A. Oliver*, R.R. Sciacca*, L. Witte*, & P.J. Cannon. Columbia University Medical Center, New York, N.Y. 10032.

Angiotensin-converting enzyme inhibitors (CEI) stimulate prostacyclin (PGI_2) and PGE_2 production by isolated glomeruli and renomedullary interstitial cells (Galler, M., et al. J. Pharm. Expt. Ther. 220:123, 1982, & Zusman, R., Clin. Res. 29:362A, 1981). Since angiotensin converting enzyme is primarily localized in vascular endothelium, we tested the effects of captopril and teprotide on basal, bradykinin (BK), and angiotensin II (AII)-induced PGI_2 and PGE_2 release by primary cultures of post-confluent bovine aortic endothelial cells. Endothelial cells were exposed to captopril or teprotide (25 µM) for 15 min at 37° C prior to the addition of media alone or media with BK or AII (1.0 µg/ml) for 30 min at 25°C. Media were assayed for 6-keto-$PGF_{1\alpha}$ (6KF) and PGE_2 by radioimmunoassay. Under basal conditions, endothelial cells released 6x more 6KF than PGE_2 (2.54 ± 0.79 vs 0.43 ± 0.06 ng/ml, $p<0.01$). BK significantly increased 6KF and PGE_2 3x and 1.5x (7.92 ± 0.20 & 0.63 ± 0.07, $p<0.01$); AII significantly increased 6KF and PGE_2 1.3-1.8x (4.56 ± 0.38 & 0.57 ± 0.08, $p<0.01$). Both captopril and teprotide significantly inhibited basal, BK, and AII-induced 6KF and PGE_2 release by 19-45 % ($p<0.01$). In contrast to the stimulatory effects of CEI on renal PG release, our data indicate that CEI reduces basal and peptide-hormone stimulated PG release by bovine aortic endothelial cells. Supported by NIH Grant HL 21006.

51.18
ANGIOTENSIN I INDUCED VASODILATION IN THE ILIAC ARTERY OF THE DOG. M.J. Fiksen-Olsen*, P.C. Houck, S.L. Britton, R.K. Zalups* and J.C. Romero. Department of Physiology, Mayo Foundation, Rochester, MN 55905.

The effects of angiotensin I (AI), angiotensin II (AII) and bradykinin (BK) on blood flow in the iliac artery was examined in 7 pentobarbital anesthetized dogs during the development phase of 1 clip 2 kidney hypertension (6 to 9 days post clipping). These agonists were administered as bolus injections directly into the iliac artery (160 - 1280 pM AI, 40 - 160 pM AII, 1.25 - 40 pM BK) prior to and following i.v. administration of captopril, 50 µg/kg bolus plus 50 µg/kg/hr. In 19 normal pentobarbital anesthetized dogs, AI and AII vasoconstricted both the iliac and renal vasculature and BK vasodilated the iliac vasculature. Captopril inhibited the AI vasoconstriction and potentiated the BK vasodilation in the normal dog, but did not alter the AII vasoconstriction. Like the normal dogs, AII vasoconstricted and BK vasodilated the iliac vasculature of our clipped dogs, however, in contrast to the normal dogs, AI produced dose dependent increases (vasodilation) in iliac blood flow prior to any systemic changes in blood pressure. Bolus injections of AI in the renal vasculature of the clipped dogs still produced vasoconstrictions. In the clipped dogs, captopril had no effect on the iliac vascular response to AI or AII, but potentiated the iliac response to BK. These data suggest that the AI vasodilation in the iliac vasculature of our clipped dogs is not related to its conversion to AII. The mechanism of this AI vasodilation remains to be elucidated.

51.19
EFFECTS OF CEREBROVENTRICULAR INFUSION OF ANGIOTENSIN II ON WATER INTAKE, URINARY OUTPUT AND BRAIN ^{125}I-ANGIOTENSIN II BINDING IN RATS. R. Singh*, A. Husain*, C. M. Ferrario and R. C. Speth*. Cleveland Clinic, Cleveland, OH 44106.

Induction of drinking by intraventricularly administered Angiotensin II (Ang II) is putatively mediated by brain Ang II receptors. The effects of a 6 day Ang II infusion in the lateral ventricle (IVT) was studied in male Sprague-Dawley rats (400-450 g) using subcutaneously implanted osmotic pumps. Ang II was infused in 6 rats (500 ng/µl/hr) while 6 other rats received only 0.9% saline vehicle (1 µl/hr). In the IVT Ang II group, mean water intake increased from 32 to 109 ml/day and urine volume from 15 to 72 ml/day during days 1-5 of infusion. The control group did not show any change in either water intake (34 vs 31 ml/day) or in urine volume (15 vs 15.7 ml/day). The density of ^{125}I-Ang II binding sites in the hypothalamus-thalamus-septum-midbrain region and the affinity of binding sites for ^{125}I-Ang II, did not differ between the two groups. The persistent dipsogenic response coupled with the lack of alteration in brain ^{125}I-Ang II binding site density suggests that continued high levels of Ang II does not cause down regulation of brain Ang II receptors. Supported by USPHS grant (HL-27568).

51.20
ANGIOTENSIN-INDUCED RELEASE OF VASOTOCIN (AVT) IN CONSCIOUS CHICKENS. T. I. Koike, K. Goto*, H. L. Neldon*, and D. W. McKay*. Dept. Physiology-Biophysics, Univ. Ark. Med. Sci., Little Rock, AR 72205.

Systemic administration of angiotensin (ANG II) arouses thirst in chickens suggesting that ANG II may stimulate release of AVT. White Leghorn cockerels were infused iv with 0.154 M NaCl for 30 min, thence with saline alone or saline + (Asp^1 Val^5)ANG II for another 30 min (0,2,20,&200 ng/kg/min; N=6/gr). Prior to infusion, immunoreactive AVT in plasma (pAVT) ranged from 1.8±0.20 to 2.6±0.27µU/ml ($p>0.05$) and did not change with saline infusion. pAVT's in ANG II-infused groups were 139% (2 ng/min) $p<0.05$), 178% (20 ng/min, $p<0.01$), and 400% (200 ng/min, $p<0.01$) of pre-infusion values. The effect of ANG II on osmotic release of AVT was studied by infusing 0.1 M NaCl for 30 min, then 1.0 M NaCl for 60 min without (N=6) or with (N=6) ANG II (200 ng/kg/min). In controls, pAVT increased only during 1.0 M NaCl infusion whereas in ANG II-infused birds pAVT increased during both 0.1 M (~2.6X, $p<0.01$) and 1.0 M NaCl infusion (~6X, $p<0.01$). The slopes of the regression lines relating pAVT to osmolality (pOsm) in control (0.467) and ANG II-infused (0.401) groups were similar but the X-intercept of the latter (pOsm=295 mOsm/kg) was lower than in controls (314 mOsm/kg) ($p<0.05$). The results indicate that systemic ANG II stimulates release of, and lowers the threshhold for osmotic release of AVT in chickens. Supported in part by American Heart Association, Arkansas Affiliate.

51.21

MECHANISM OF ACTION OF ARGININE-VASOTOCIN AND LYSINE-VASOTOCIN ON SURFACE EXUDATION BY A SLUG BODY WALL IN VITRO. Arthur W. Martin, D. Luchtel* and I. Deyrup-Olsen. University of Washington, Seattle, WA 98195

The exudations of the body surfaces of terrestrial slugs (Ariolimax columbianus and other species) were found to contain large particles such as dextran (5,000,000) hemocyanin (9,000,000) and carbon (0.1 μm) (Deyrup-Olsen and Martin, Comp. Biochem. Physiol. in press, 1982). We had anticipated that the particle penetration might be intercellular. However, our ultrastructural observations indicate that the cellular junctions are tight, and that a specific cell type, which we have termed channel cell, is the site at which particles and large amounts of fluid traverse the slug epidermis.

This effector pathway has proved to be highly sensitive to the peptides arginine-vasotocin (AVT) and leucine-8-vasotocin (LVT) (Sawyer, et al, 9th Internat'l Sym. Comp. Endocrinology, 1981), responding with markedly enhanced output of fluid and particles. The fluid responses are increased when the body wall is placed in contact with water, and inhibited in hypertonic media. Progressive dehydration of the slugs inhibited the response as well, and suggested the development of an inhibitory agent active at the effector site. Since the actions of the peptides are blocked by atropine we infer that they act through a synapse on a peripheral cholinergic neurone.

51.22

EFFECTS OF ANTIDIURETIC HORMONE ON MEMBRANE POTENTIALS, RESISTANCES AND CONDUCTANCES OF TOAD URINARY BLADDER. Allan G. Ramsay. The M.I. Bassett Hospital, Cooperstown, NY 13326, and the College of Physicians and Surgeons, Columbia University, New York, NY 10032.

This study was undertaken to quantitate the changes in membrane, paracellular and epithelial resistances and conductances that occur in toad urinary bladder after serosal exposure to vasopressin. The second purpose was to clarify the effect of hormone on membrane potentials. Microelectrode methods were used. Vasopressin decreased the voltage divider ratio from 1.74 to 1.02. There was a decrease in apical membrane resistance from 1795 to 1243 ohm·cm^2, and an increase in apical conductance from 586 to 885 μMhos·cm^2. Epithelial resistance decreased from 2177 to 1881 ohm·cm^2 and conductance increased from 458 to 542 μMhos·cm^2. Vasopressin had no effect on basal-lateral and paracellular resistances and conductances. The hormone increased basal-lateral potentials from 17.6 to 33 mV. This increment was the major contribution to the increase in epithelial potential from 39.8 to 57.1 mV. There was no significant change in apical potential. We conclude that vasopressin increases Na^+ conductance of the apical membrane, thereby delivering a greater Na^+ load to the basal-lateral rheogenic pump. The consequent increase in pump activity without change in basal-lateral resistance results in a significant increase in basal-lateral potential. (Supported by the Stephen C. Clark Research Fund and the Walter H. D. Killough Fund.)

51.23

HEMODYNAMIC AND NATRIURETIC EFFECTS OF ATRIAL EXTRACT IN THE ISOLATED PERFUSED RAT KIDNEY. H.D. Kleinert*, M.J.F.Camargo*, J.E.Sealey*, J.H.Laragh* and T.Maack. Cardiovascular Center and Dept. of Physiol., Cornell Univ. Med. Coll., NY, NY 10021

Atrial extracts (AE) elicit a potent and transitory natriuresis in the intact rat. To investigate direct AE renal actions we determined its effects on renal resistance (RR), GRF, filtration fraction (FF), fractional (FE) and absolute (UV) excretion of Na and K in the isolated perfused rat kidney (IK) with adequate, stable renal functions, as previously described (Maack, AJP 238: F-71, 1980). IK were perfused in a closed-circuit system with 60 ml of perfusate at constant pressure. After two 10' control (C) periods, 100 μl of 1/10 (w/v) homogenate of rat atria or ventricles (VE) were added to the perfusate. Three 10' experimental (E) periods followed. Results are mean ± SE (n=4 IK), compared by paired t test. AE increased RR slightly by 24% (E=2.96±.20 mmHg/ml min^{-1}, C=2.39±.21, $p<0.05$). AE increased GFR markedly ($p<0.001$) by 75% (E=1.0±03 ml/min, C=.57±.11), FF by 214% (E=.03±.003, C=.014±.003) FE_{H_2O} by 324% (E=.137±.015, C=.042±.004), $U_{Na}V$ by 831% (E=9.31±.68 μEq/min, C=1.12±.39), FE_{Na} by 480% (E=.065±.004, C=.013±.003), U_KV by 659% (E=2.11±.26) μEq/min, C=.32±.16) and FE_K by 410% (E=.459±.072, C=.112±.053). The action of AE lasted for at least 30 min. VE had no significant effects ($p>0.05$, n=4). These results demonstrate a direct natriuretic and hemodynamic effect of AE. The marked increase in GFR and FF suggests that AE preferentially constricts efferent arterioles and/or causes redistribution of flow in the IK.

51.24

WATER IMMERSION IN THE CONSCIOUS RHESUS MONKEY. B.A. Benjamin,* M.S. Shapiro,* N.S. Bricker, and H.A. Sandler. NASA/AMES Moffett Field, CA 94035

Conscious Rhesus monkeys (M. mullata) were studied to develope a model capable of evaluating cardiovascular and renal function during water immersion. Experiments were carried out in 9 chair conditioned animals. Two monkeys were instrumented to obtain left ventricular pressure, aortic flow, and ECG. Two animals were studied anesthetized (nembutal 25 mg/kg), 5 were conscious uninstrumented. The monkeys were determined to be in metabolic balance (salt and water) for 2 days prior to the study. The protocol consisted of a 90 min. control period and 2 hours of immersion to the neck in a thermoneutral bath. Blood samples were taken hourly before, during, and after immersion. In the conscious monkeys immersion caused urine flow to increase from 0.9 to 2.4 ml/min. and sodium excreation to increase from 12 to 39 uEq/min. Blood pressure and heart rate did not change while cardiac output increased 30% and end diastolic pressure increased from 1.5 to 9 mm Hg. Plasma cortisol did not change during the immersion. In the anesthetized studies immersion caused urine flow to increase from 0.6 to 2.0 ml/min., sodium excreation to increase from 5.1 to 70 uEq/min., and blood pressure to increase 25 mm Hg. Control urine flow was greater in the conscious than in anesthetized studies. On the basis of these findings water immersion in the conscious Rhesus monkey provides changes similar to that seen in man. (Supported by NASA contract NCC2-104.)

51.25

NON-ISOTOPIC ESTIMATE OF BODY WATER AND ITS SPACES IN HYPERTONIC EXPANSION. Antonios H. Tzamaloukas* (Spon: K. D. Gardner, Jr.). V.A. Medical Center, Albuquerque, NM 87108.

Mass conservation equations for sodium and chloride were combined to compute initial ECF fraction of body water, and apparent sodium volume of distribution was used to measure body water in five anuric dogs infused with 0.85m NaCl solution in two steps. ECF space was also measured by i.v. $Na_2{}^{35}SO_4$. ECF Na and Cl concentrations were corrected for the water infused and ECF Cl concentration was corrected for the discrepancy between apparent volumes of distribution of Na and Cl. Results: Apparent Na volume of distribution: 0.505±0.058 of body weight. Initial ECF fraction: 0.247±0.047, $^{35}SO_4$ space 0.232±0.025 of body weight (NS). Regression of $^{35}SO_4$ space on ECF fraction: y=0.5+1.04 1/1, ($r = 0.911$, $p < 0.01$). Initial ECF fraction of body water was at the beginning of the second expansion higher (appropriately) than at the beginning of the first expansion in four dogs. In one dog, a slight decrease in ECF fraction was computed. Conclusion: ECF fraction of body water can be reasonably approximated by corrected Na and Cl mass equations during expansion with hypertonic saline. (Supported in part by a V. A. RAG Grant).

51.26

THE RENAL EXCRETION OF ENTERALLY ADMINISTERED CHROMIUM-51 (III) CHLORIDE IN THE DOG. David L. Donaldson*, Cindy C. Smith* and Andy A. Yunice. Univ. of Oklahoma Health Sci. Ctr. and V.A. Hospital, Oklahoma City, Oklahoma 73190.

The trace element chromium is excreted primarily by the kidney. Previous studies have suggested that there is a significant tubular reabsorption of ultrafilterable plasma chromium (III) (UFPCr). Cr-51 chloride (5 Ci=50 ug) was administered by nasogastric tube to Nembutal - anesthetized dogs (n=5) following an overnight fast. Normal saline was infused as an initial bolus of 500 ml over 30 min followed by a continuous infusion of 2 ml/min. Between 2 and 8 hrs following administration of the isotope, urine samples were collected at 20-30 min intervals. Heparinized plasma was collected at the midpoint of each interval. Blood pressure, urinary flow rate, and creatinine clearance were stable for each animal during this time period. UFPCr-51 was determined by a rapid ultrafiltration technique (Amicon MPS-1 Micropartition System using YMB membranes) which retained >99% of plasma proteins. UFPCr-51 comprised between 3 and 20% of the total plasma Cr-51. Ultrafiltration of urine specimens or repeat ultrafiltration of plasma ultrafiltrates yielded 95-100% recoveries. Total plasma Cr-51 and UFPCr-51 reached peak levels 4-6 hours following administration of the isotope and declined slowly thereafter. The clearance of UFPCr-51 was found to be consistently equal to that of the creatinine, indicating that there is no net tubular reabsorption of UFPCr-51 species. (Support - USPHS Grant #1 KO1 AM99891-01)

51.27

INDICATIONS IN SHEEP (S) OF A KALIURETIC REGULATORY FACTOR OTHER THAN PLASMA POTASSIUM (P_K) OR PLASMA ALDOSTERONE.
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Previous reports (Kid. Int. 16:833,1979; 21:285,1981; Fed. Proc. 39:1079, 1980) showed that neither changes in P_K or Paldo decisively regulated meal dependent changes in K excretion (U_KV) in S. We now examine these factors' role in regulating U_KV in 2 conscious, adult ewes during repletion or depletion of K. Treatments were: (1) normal - S regularly fed a daily meal providing 600 meq K/day, (2) K replete - S regularly fed the daily meal and given an additional 600 mM KCl per day for 2 days via ruminal (fistula) infusion, (3) K depleted - S fasted 4 days. Mean ± SD baseline values for P_K (meq/l) and U_KV (ueq/min) for the treatments were: (1) 4.16 ± 0.2, 426 ± 134; (2) 4.53 ± .35, 680 ± 64; (3) 3.61 ± .07, 23 ± 11. After baseline measurements, 50 mM KCl was infused i.v. over 30 min to elevate P_K by 2-3 meq/l above baseline. Although P_K varied over the same range (4-7 meq/l) during K infusion in all 3 groups, U_KV was in the range 390-981 (normal), 632-1236 (K replete), 18-321 (K depleted). Repetition of the K replete and K depleted experiments with i.v. aldo (20 ug/hr) did not alter the basal P_K or U_KV values or diminish the difference in U_KV between groups during KCl loading. Thus, large differences in K intake led to large, corresponding diferences in U_KV which were independent of P_K (during K loading) and which existed despite identical rates of aldosterone infusion. It appears that kaliuretic regulatory factors other than P_K and aldo exist in sheep.

51.28

UNIMPAIRED POTASSIUM REGULATION DURING BETA-BLOCKADE WITH PROPRANOLOL.
D.B. Young, M.R. Smith,Jr.*, U. Tipayamontri, and R.H. Read. Univ. of Mississippi Med. Ctr., Jackson, MS 39211.

To determine if the β-adrenergic system is involved in long-term K control, the ability of dogs to respond to a K challenge with and without β-blockade was compared. Both groups had a 3 day control period followed by 10 days of 200 mEq/day KCl infusion. Group I(N=5,23.3±1.3 kg) was untreated, group II(N=6,24.6±1.4 kg) received continuous iv propranolol, 7.5 mg/kg/day, starting 4 days prior to the control period. Control plasma K averaged 4.16±.09 and 4.35±.11 mEq/L in the untreated and treated groups, and increased to 4.90±.11 and 4.92±.08 mEq/L in the two groups after 10 days of KCl infusion. The untreated and treated groups' aldosterone concentrations were 8.8±2.0 and 6.5±1.1 ng% in the control period while after KCl infusion the values were 18±4(untreated) and 9.2±2.8 (treated) ng%. Fasting insulin concentration remained higher throughout the study in the untreated group, the control values being 15±5(untreated) and 6±1(treated) μU/ml while after KCl infusion the values were 19±11(untreated) and 6±1(treated) μU/ml. The responses of other measured variables (PRA, plasma glucose, arterial pH and HCO_3^-) to KCl infusion were not affected by β-blockade. In summary, although β-blockade with propranolol did attenuate the rise in aldosterone in response to KCl infusion, and did reduce fasting insulin throughout the study, β-blockade did not impair the ability of the K control system to respond to a 10 days period of high K intake. (Supported by NIH grants HL 21435 and HL 11678.)

51.29

CELLULAR MECHANISMS OF POTASSIUM TRANSPORT BY RABBIT DESCENDING COLON.
R.D. McCabe#, P.L. Smith and L.P. Sullivan. U. of Kansas Med. Ctr., Kansas City, Ks. 66103

Transepithelial fluxes of K were measured under short circuit conditions in isolated segments of rabbit descending colon to determine: (1) the K concentration dependence of unidirectional K fluxes (J^K); and (2) the effects of Ba which blocks K conductance in both excitable and epithelial tissues. Both the mucosal(m)-to-serosal(s) and s-to-m fluxes of K contained saturable and nonsaturable components. Ouabain (10^{-3}M) added to the serosal bathing solution abolished the saturable component of J^K_{sm} resulting in a linear relation between J^K_{sm} and [K] between 1 and 60mM (r^2=1.00). The K permeability (P_K) calculated from the slope of this line was 0.026 cm/hr. Subsequent addition of DNP (10^{-4}M) to both solutions abolished the saturable component of J^K_{ms}, which ouabain had not abolished, and increased P_K. In seperate studies the effects of Ba were examined. The addition of Ba (2mM) to the serosal bathing solution produced significant decreases in J^K_{ms} and I^{SC} and significant increases in J^K_{sm} and G_t resulting in an increase in net K secretion. However Ba had no significant effect on unidirectional or net Cl fluxes. These results provide further evidence for active uptake processes across both the luminal and basolateral membranes of colonic epithelium cells. However, they do not support the presence of a K selective shunt pathway. In addition, these results suggest that the basolateral membrane contains a Ba sensitive permeability. (Supported in part by BRSG SO7 RR05373 and AM 15883.)

51.30

ABSENCE OF Na^+ AND Cl^- COTRANSPORT IN RAT SUBMANDIBULAR GLAND?
Geeta Lingam* and Amar K. Sen., Dept. of Pharmacology, University of Toronto, Toronto M5S 1A8.

Na^+ and Cl^- cotransport processes have been identified in several tissues. In the salivary glands, one possible model proposed to explain fluid secretion, is a Na/Cl cotransport mechanism similar to that in the shark rectal gland (Silva et al., Am.J. Physiol.1977.233:F298-F306). Our studies on the rat submandibular gland (SMG), however, argue against the possibility of the existence of a coupled Na/Cl entry mechanism. The rates of uptake of ^{22}Na and ^{36}Cl were measured in rat SMG slices. Carbachol (10^{-4}M) increased the rate of ^{22}Na but not ^{36}Cl uptake, an effect that could be blocked completely by atropine (10^{-3}M) but unaffected by ouabain (10^{-4}M). Similar increases in ^{22}Na uptake were observed in the presence of carb when Cl^- in the Krebs was wholly substituted by either a permeant ion such as NO_3^- or a relatively impermeant ion like isethionate. Moreover, the time course of ^{22}Na was unchanged in the unstimulated state (i.e. absence of carb) in Cl^- free NO_3^- Krebs. Similarly, ^{36}Cl uptake in both gradient and equilibrium conditions was unchanged in the presence of Na^+ free choline substituted Krebs. The present study indicates that 'true' Na/Cl cotransport may not be present in the rat SMG either in the unstimulated state or when stimulation is induced by a muscarinic agonist such as carbachol, since Na^+ and Cl^- uptake occur in the absence of the other ion species. (Supported by MRC Grant MT-2485).

51.31

RELATIONS AMONG CELL Na, BASOLATERAL MEMBRANE CONDUCTANCE AND ACTIVE Na TRANSPORT BY NECTURUS URINARY BLADDER.
S.R. Thomas*, S.M. Thompson*and S.G. Schultz. University of Texas Medical School, Houston, Texas 77030

"Instantaneous" transepithelial current-voltage (I-V) relations of Necturus urinary bladder were determined during impalement of an absorptive cell with a microelectrode before and after addition of amiloride to the mucosal solution as described by Thompson et al. (J. Membrane Biol. 66:41-54, 1982). These studies were carried out in the presence of 5, 15 and 45 mM Na in the mucosal solution. Among the results of these studies are: (1) The relation between the Na current across the apical membrane and the electrical potential difference across that barrier conforms to the "constant field flux" equation over a wide range; (2) The steady-state short-circuit current is a linear function of the chord conductance of the apical membrane to Na indicating that the electrochemical potential difference for Na across that barrier is maintained constant and is independent of the rate of Na transport; (3) The intracellular Na activity determined from the reversal potential of the I-V relations (6 mM) is independent of the mucosal Na concentration and the short-circuit current; and (4) The slope conductance of the basolateral membrane increases linearly with the short-circuit current.

Possible explanations for the findings that pump activity increases in spite of the fact that intracellular Na activity is constant and that basolateral membrane conductance parallels the increase in pump activity will be discussed.

51.32

ASYMMETRY OF CANINE TRACHEAL EPITHELIUM: NONELECTROLYTE PERMEABILITIES OF APICAL AND BASOLATERAL CELL MEMBRANES.
S.F. Paul Man and Alan B.R. Thomson*. Dept. of Medicine, U. of Alberta, Edmonton, AB, Canada

We wished to determine if the apical and basolateral cell membranes of canine tracheal epithelium had different nonelectrolyte permeabilities. In 8 pairs of tissues (surface areas = 2 cm^2) with matched resistance, the unidirectional fluxes, from mucosa to serosa and from serosa to mucosa, of ^{14}C-sucrose, ^3H-inulin or ^3H-polyethylene glycol were similar (P = NS). The spaces occupied by the isotope compounds, and the wet and dry weights of the tissue were measured after 3 hours of incubation. When the compounds were introduced to the mucosal fluid, sucrose and polyethylene glycol or inulin spaces were similar and that of sucrose was 19.8 ± 3.7 μl/100 (mean ± SE) mg dry tissue weight. When the isotopes were added to the serosal fluid, sucrose had a tissue space of 527.0 ± 59.7 μl/100 mg of dry tissue weight. Also, the sucrose space was larger than that of inulin, P<0.01. Together, the mucosal and serosal sucrose spaces accounted for the entire difference between the wet and dry weights of the tissues. We conclude that the apical cell membrane is impermeable to these nonelectrolytes. However, the basolateral cell membrane is freely permeable and is not the restriction for the transepithelial movement of these compounds.

51.33

LACK OF INFLUENCE OF VASOPRESSIN ON IMMERSION DIURESIS IN MEN AND WOMEN. S.E. Kravik*, L.C. Keil, J. E. Silver*, N. Wong*, W.A. Spaul*, and J.E. Greenleaf. Biomedical Research Division, NASA, Ames Research Center, Moffett Field, CA 94035

To investigate fluid, electrolyte, and hormonal interactions during immersion, 6 men (20-35 yr) and 3 women (23-27 yr) were immersed to the neck (NI) in water (34.5°C) for 6 hours after overnight food and fluid restriction. Three hundred ml fluid were consumed 2 hr before NI, and all subjects refused drinking water during NI. Mean water balance was -1,285 ±S.E. 104 ml/6hr in the men and -1,076 ±197 ml/6hr in the women during immersion; diuresis was 1,061 ±160 ml/6hr and 1,016 ±113 ml/6hr, respectively. Plasma vasopressin concentration [pVP] went from 0.7 to 3.0 pg/ml ($P<0.05$) at 6 hr NI and from 0.3 to 0.8 pg/ml ($P<0.05$) in men and women, respectively. During the first 60 min of immersion, plasma volume increased by 7.8 ±1.6% ($P<0.05$) in the men and by 5.6 ±2.1% (NS) in the women. Serum osmolality was constant in the men (292 ±1 mosmol/kg) and women (287 ±1 mosmol/kg) throughout NI. Plasma renin activity decreased progressively in both groups. These data indicate that [pVP] is not suppressed during NI and suggest other factors are necessary to explain the regulation of immersion diuresis.

MEMBRANE PROPERTIES OF MUSCLE AND NERVE

52.1

EQUILIBRIUM CALCIUM-CALCIUM EXCHANGE IN CARDIAC SARCOLEMMAL VESICLES. Robert S. Slaughter*, John L. Sutko* and John P. Reeves. Roche Institute of Molecular Biology, Nutley, NJ and University of Texas Health Science Center, Dallas, TX

Ca-Ca exchange activity was measured as the uptake of ^{45}Ca by sarcolemmal vesicles preloaded with an equal concentration of ^{40}Ca; all experiments were carried out using intra- and extravesicular media of identical composition. Vesicles maintained at 60°C showed identical rates of inactivation for both Ca-Ca and Na-Ca exchange activities indicating that both processes were mediated by the same transport system. The rate of Ca-Ca exchange in 0.3 M sucrose was stimulated more than 2-fold by 8 mM NaCl, KCl, LiCl or RbCl; CsCl and choline Cl were less effective. Unlike the other cations tested, Na inhibited Ca-Ca exchange at concentrations greater than 32 mM and markedly reduced the total quantity of exchangeable Ca; the latter result suggests that Na and Ca compete for intravesicular binding sites. Maximal stimulation of Ca-Ca exchange required the presence of monovalent cations on both sides of the vesicle membrane. Ca-Ca exchange activity showed a sharp optimum at pH 7.0 in the absence of monovalent cations, but increased monotonically with increasing pH (6.0-9.0) in the presence of 8 mM NaCl. Rb-stimulated Ca-Ca exchange was not associated with an increased flux of ^{86}Rb across the vesicle membrane. The results suggest that the monovalent cations interact allosterically with the exchange carrier to stimulate Ca-Ca exchange but are not transported during the exchange process.

52.2

UNITARY SODIUM CURRENTS IN MAMMALIAN VENTRICULAR CELLS. D.L. Kunze and A.M. Brown. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550

We studied the properties of single Na channels in isolated rat ventricular myocytes using the gigaseal patch clamp method at bandwidths of 0.5 to 1 kHz. The myocytes were dispersed enzymatically and the experiments were done at room temperature. Results were obtained from cell-attached and inside out patches. Resting potentials of these cells were -40 mV to -60 mV, but full-blown action potentials could be produced by anodal break from pulses that hyperpolarized the membrane to -90 to -120 mV. Unitary currents were observed in the first several msec following depolarizing steps to between -50 and -20 mV. At later times during the step, activity was absent. Multiple levels were common indicating more than one channel per patch. After correction for step transients, summed currents of 10-100 traces had an appearance that was similar to the macroscopic Na current at equivalent potentials. Unit amplitudes were about 1.1 pA at -50 mV. The unitary Na currents appear to be similar to those reported for myoballs (Sigworth & Neher, 1980).

Supported by NIH grants NS-11453 and HL-25145.

52.3

pH-DEPENDENT CHANGES OF THE SLOW RESPONSE IN DEPOLARIZED RABBIT MYOCARDIUM. Jose Geraldo Mill, Dalton V. Vassallo e Antonio Paes de Carvalho. Departamento de Ciencias Fisiologicas CBM, UFES, 29000 Vitoria, ES, Brazil.

This study describes the electrical activity changes of myocardial cells when intracellular (pHi) and extracellular pH (pHe) are altered. Trabeculae were isolated from the left atrium of rabbits and perfused in Tyrode solution(K=2.7mM) or in a depolarizing solution (K=12mM, Ba=1mM). The last solution was used to induce slow response(SR). The solutions had its pH in the range between 6.6 and 7.7. The pH values were adjusted with bicarbonate buffer(used to change pHe and pHi simultaneously) or HEPES buffer(used to change only pHe). Intracellular alkalinization was obtained by adding 10mM NH_4Cl to HEPES-buffered solution(pH=7.2). Electrical activity was recorded with intracellular glass microelectrodes. The results showed that the rapid changes in pHe or pHi did not affect significantly the fast upstroke of the normal action potential. SR was highly depressed by decrease in the pHi and enhanced by its increase while changes in pHe(6.8-7.4) did not affect the SR. Spontaneous SR firing was induced by increasing pHi(bicarbonate-buffered solution pH=7.4 to 7.7, or with HEPES-buffered solution + NH_4^+, pH=7.2). These results show that the slow channels are much more affect by pH changes than rapid channels.pHi is a factor that modifies the excitability of the slow channels in depolarized myocardium.

CNPq

52.4

ACTIVATION OF Ca CURRENTS. Y. Tsuda*, D.L. Wilson* and A.M. Brown. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550

Turn-on and turn-off of Ca currents were studied in isolated Helix neurons. The combined suction pipette-microelectrode system was used for voltage clamp and internal perfusion. Ca currents were isolated after suppression of Na currents by Tris substitution for Na ion, and suppression of K currents by Cs substitution for K ion and the use of TEA and 4-AP. Linear components of leakage and capacitance were subtracted using equivalent hyperpolarizing voltage steps. Asymmetry currents were measured following Co substitution for Ca ion and were subtracted from Ca currents. Temperatures were regulated from 24-14°C. Turn-on occurred after a delay and was initially fitted by the Hodgkin-Huxley activation variable m, raised to the second power. Turn-off or tail currents were fit by a sum of three exponentials, the faster two, designated f and s, being due to Ca currents. Tau_f was 1/4 to 1/8 tau_s and tau_s was between tau_m and $tau_m/2$. Tau_f was too fast to account for the delayed turn-on at small depolarizations. These results imply that three exponentials have been uncovered at a given voltage and this implies a 4-state model for activation of I_{Ca}. Cooling slowed turn-on greatly but turn-off rates were uneffected. The temperature effect may be explained by making the leading forward rate constant more temperature sensitive than the other rate constants.

Supported by NIH grants NS-11453 and HL-25145.

52.5

THE POTENTIAL DEPENDENCY OF K, Na, AND Cl MEMBRANE PERMEABILITIES IN STEADY STATE DEPENDS ON METHOD OF DEPOLARIZATION. Alfred Strickholm. Physiology Section, Medical Sciences Program, Indiana University, Bloomington, IN 47405

The steady state or zero current membrane permeabilities of K, Na, and Cl in medial giant axons of crayfish (Procambarus clarkii) was determined as a function of membrane potential (Biophys. J. 35, 677). Membrane potential alteration was done by two different methods: (1) raising external K_o by one to one replacement of Na, and (2) allowing a slow intracellular depletion of K_i to occur with a concurrent increase in Na_i and Cl_i. When depolarizing by raising external K_o from its resting value (5.4 mM), a marked transition in membrane ion conductances or permeabilities occurs around K_o = 12 to 15 mM (V_m = -60 to -65 mV) which involves largely Na and Cl. The data suggests that two different permeability conformational states exist for the resting and depolarized axon. In contrast, for an equivalent depolarization produced by changing intracellular ions, no permeability transitions are observed until membrane potential is around -20 to -30 mV. In addition, permeabilities measured by methods (1) and (2) are pH dependent but in very different ways. These experiments thus show that other factors such as local ion atmospheres at the inner or outer membrane surface, in addition to membrane potential, contribute to ionic permeability control. (Supported in part by a grant in aid from the Indiana Affiliate of the American Heart Association)

52.6

ELECTROGENIC RESPONSE OBTAINED IN THE LARVAL MUSCLE OF DROSOPHILA, Kageyuki Yamaoka* and Kazuo Ikeda. City of Hope Research Institute, Duarte, CA 91010.

Since the insect muscle is highly sensitive to oxygen, membrane properties must be measured under proper respiratory conditions. Experiments neglecting this fact often result in misleading conclusions. The longitudinal ventro-lateral muscles of the larvae (6a & 7A; Crossley, 1965) have been reported to be electrically inexcitable (Jan and Jan, 1976; Suzuki and Kano, 1977). The present report demonstrates the oxygen dependency of the resting and active membrane and concluded that a voltage dependent, regenerative action potential is the normal response of this muscle upon stimulation. The muscle was exposed by dissection along the dorsal midline in saline solution and the tracheae were connected to a respirator. A resting potential of -80mV could be maintained for over 1.5 hrs. when air was supplied through the respirator. Under these conditions, the muscle responded with an electrogenic response characterized by a double-peaked configuration when stimulated by direct current injection or by the nerve. When the air supply was stopped, the membrane gradually depolarized to -30mV over a one-hour period, then stabilized at this level thereafter. As the membrane depolarized, the amplitude of the response decreased, until, at about -50mV, the electrogenic component disappeared entirely. (Supported by USPHS NIH grant NS-18856.)

52.7

IONIC CURRENTS IN PERFUSED SKELETAL MUSCLE FIBRES OF A TROPICAL TOAD. II.DELAYED CURRENTS. Argibay,J.,N.Arispe & L.Rojas. Esc. Biología Univ. Central de Venezuela.Apdo.21201,Caracas Venezuela.

Potassium currents were studied in single fibres from sartorius muscle of the toad Bufo marinus using a voltage clamp method similar to that employed by Hille & Campbell,1976,which allows internal perfusion. Sodium currents were blocked with TTX and stability of the delayed currents was obtained using a recently developed internal perfusate where aspartate is the main anion.Potassium currents develop to a maximum and then decay as a consecuence of inactivation and ionic accumulation. To study the kinetics of accumulation the effect of the frequency of pulsing on the decaying phase was observed.Intervals of 2 min between pulses eliminates the effect of accumulation on the peak currents in experiments lasting several hours.Delayed currents do not totally inactivate in depolarization maintained for 1 min .Time constants of inactivation seems to be voltage independent.Delayed channels are highly selective to K^+ and activate at -50 mV. I-V relation for leak currents between -50 mV to -200 mV is linear.They are the sum of a chloride current component and an inwardly rectifying Cs-sensitive current. (Supported by CDCH grants # 373-72 & 2783).

Hille,B. & Campbell,D.T.(1976),J.Gen.Physiol.67 ,265-293.

52.8

IONIC CURRENTS IN PERFUSED SKELETAL MUSCLE FIBRES OF A TROPICAL TOAD.I.EARLY CURRENTS. Arispe N.,J.Argibay & L.Rojas. Esc. Biología Univ. Central de Venezuela. Apdo.21201 Caracas,Venezuela.

Single fibres from the sartorius muscle of the toad Bufo marinus were used to study ionic currents using a fast voltage clamp method , similar to that employed by Hille & Campbell, 1976,which allows internal perfusion.These fibres normally have a resting potential of -70mV and a threshold at -50mV(Argibay & Colina).Sodium currents were analized following H-H formulations.Activation of I_{Na} measured as g_{Na}/g_{Namax} occurs at -50mV and shows a slight decay after a maximum for positive values of Vm.Inactivation ,as I_{Na}/I_{Namax},is fully developed at -50mV.Time constans for inactivation is maximum at -60mV.Internal perfusion with CsF blocks delayed currents but produces shifts in the equilibrium potential of the early channel due to sodium accumulation.Delayed channels are very unstable when perfusion is performed with fluoride.This unstabilities and equilibrium potential shifts were solved replacing fluoride by aspartate in the internal perfusate.Late peaks of inward current probably associated with activity at the tubular system were occasionally observed for small depolarizations. (Supported by CDCH grants 373-72 & 2783).

Hille,B. & Campbell,D.T.(1976),J.Gen.Physiol.67,265-293.
Argibay,J. & Colina,V.L.(1980)Proc.Int.Union PhysiolSc.14,302.

52.9

THE INFLUENCE OF MICRO-INJECTED CYCLIC AMP PROTEIN KINASE CATALYTIC SUBUNIT ON THE SODIUM EFFLUX IN SINGLE BARNACLE MUSCLE FIBERS. E.E. Bittar, G. Chambers* and E. Fischer*. Dept. of Physiology, Univ. of Wisconsin, Madison, WI and Dept. of Biochemistry, Univ. of Washington, Seattle, WA.

Injection of pure catalytic subunit (CSU) of bovine heart muscle cAMP-protein kinase type 2 into barnacle muscle fibers causes a stimulation of the Na efflux in ouabain-poisoned fibers that is always sustained rather than transitory. Stimulation is seen with as little as 10^{-7}M-CSU, this being a concentration which before dilution by the myoplasm equals the cAMP-PK content of skeletal muscle. The response of the ouabain-insensitive Na efflux to injected CSU depends on $[Ca]_e$ but is unaffected by prior application of 10^{-4}M-verapamil. Injection of Gpp(NH)p or 'dialyzed' cholera toxin following peak stimulation by CSU leads to a further sustained stimulatory response. Injection of CSU following peak stimulation by Gpp(NH)p or cholera toxin also leads to a sustained stimulatory response. Injection of protein kinase inhibitor partially reverses the response to CSU. The same is true of protein kinase type 2 regulatory subunit but only when injected before CSU. Mg^{2+}, whether injected before or after CSU, fully reverses the response. Fe & Zn cause a partial reversal. Imipramine but not trifluoperazine is able to reduce the response to CSU. Taken together, these results are in line with the view that CSU acts as a modulator of the ouabain-insensitive Na efflux & that Mg, Fe & Zn are inhibitors of the catalytic reaction (& possibly the membrane substrate phosphorylation step).

53.1

CHANGES IN OSTEOBLASTIC ACTIVITY DUE TO SIMULATED WEIGHTLESS CONDITIONS. Stephen B. Doty[1] and Emily Morey-Holton[2] 1.Columbia University,NY,NY, 10032 2.NASA, Ames Research Center, Moffett Field,CA. 94035

One result of spaceflight or simulated weightless conditions is a dramatic reduction in new bone formation. Using the hypokinetic, orthostatic rat model designed by Morey-Holton, we have studied the morphological and histochemical changes which occur in osteoblasts during reduced bone formation. We have evidence that two major changes occur in osteoblasts following 15 days of hypokinetic treatment. First, there is a reduction in cell-cell contact between adjacent osteoblasts and also between osteoblasts and the connective tissue cells of the periosteum and endosteum. Secondly, there is a reduction in cell membrane associated alkaline phosphatase activity. This reduced enzyme activity reflects the reduction in bone formation rates. It is not possible, at present, to determine which of these two changes was the primary factor in reducing new bone formation.

(Supported in part by NASA grant NAGW-238)

53.2

CALCIUM TRANSPORT FROM THE INTESTINE AND INTO BONE IN A RAT MODEL SIMULATING WEIGHTLESSNESS. D.D. Bikle, R.K. Globus* and E.R. Morey. 239-17 A.R.C. NASA Moffett Field, CA 95035

The objective of this study was to determine whether a defect in active transport of calcium in the duodenum was related to decreased bone formation due to simulated weightlessness. Rats were suspended at a 40° angle causing the hindlimbs to be totally non-weightbearing and the forelimbs lightly loaded. Rats were suspended for up to 15 days and pair fed control groups were maintained. ^{45}Calcium was injected into the ligated duodenal loop in situ 15 minutes prior to sacrifice. Blood, tibia, vertebra and humerus were obtained for total calcium and ^{45}calcium analyses. Intestinal calcium transport did not appear to be significantly altered by suspension. However, by 5 days of suspension a significant decrease in accumulation of ^{45}calcium into tibia and vertebra was observed. A trend of decreasing ash weight was established in tibia and vertebra by the fifth day of suspension achieving statistical significance by 10 days (calculated as mg ash weight per gram body weight). In contrast to the tibia, the humerus failed to demonstrate a significant ash weight decrease even after 15 days of suspension. Results from the simulated weightlessness model suggest that transport of calcium from intestine into bone is decreased within 5 days of suspension. This deficiency appears to be associated with a progressive decrease in total mass of non-weightbearing bones. (Supported by NASA grant NAG W #236).

53.3

IS SUPPRESSION OF BONE FORMATION DURING SIMULATED WEIGHTLESSNESS GRADUAL AND RELATED TO GLUCOCORTICOID LEVELS? Emily R. Morey-Holton, Martin D. Bomalaski*, and Thomas J. Wronski*. NASA-Ames Research Center, Moffett Field, CA 94035.

Following 18.5 days of spaceflight, young rats routinely exhibit about a 45% suppression of periosteal bone formation at the tibiofibular junction. Similar information has been reported on an animal model designed to simulate some aspects of weightlessness. To determine whether the suppression of bone formation was gradual and related to serum corticosterone levels, two series of experiments were conducted. The first series examined temporal variations in bone formation during simulated weightlessness. Growing rats were suspended head-down and periosteal bone formation rate and corticosterone levels were measured at various time periods. Bone formation was suppressed by about 30% after 1 week and approximately 50% after 2 weeks of suspension. Serum corticosterone levels were not above control values. The second series investigated the relationship between cold exposure and periosteal bone formation rate. Exposure to 4°C caused approximately a 40% decrease in bone formation within 1 week and about a 25% decrement during the second week. Corticosterone levels were elevated in cold-stressed rats. Data from these experiments suggest 1) suppression of bone formation is continuous throughout 2 weeks of suspension on the rat model, 2) serum corticosterone levels are elevated above control values during cold exposure but not during suspension, and 3) suppression of bone formation provoked by unloading the rear limbs is not due solely to an elevation of serum glucocorticoids.

53.4

BONE MINERAL ANALYSIS OF RAT VERTEBRA FOLLOWING SPACE FLIGHT: COSMOS 1129. E. Paul France*, Clarence M. Oloff* and Leon E. Kazarian* (SPON: Russell Burton). AFAMRL, WPAFB, OH, 45433

The mission of COSMOS 1129 was to investigate how organisms adapt to microgravity then readapt to earth gravity following 18.5 days in earth orbit. This study describes comparative mineral/element content of vertebral centra in Flight (F) and Synchronous (S) rats. S and F rats were sacrificed on a predetermined readaptation schedule following spacecraft recovery (R) at R+0, R+6, and R+29 days. The results compared to ground based synchronous control animals. 947 cleaned individual vertebral centra were placed in plastic vials, stored in sterile water and frozen. At the start of the experiment each sample was visually examined and a coded narrative description recorded. Each sample was dried, weighed, digested in nitric acid and analyzed for 12 minerals/elements. Ca, PO_4, K, Na, Ba, Sr, Ci, Fl, Mg, Pb, Mn and Y using standard analytical techniques. Bone mineral content is correlated with ash weight. In this paper comparative mineral/element content data between F, S, and recovery time are shown. The use of the rat model for further understanding osteopenia is discussed.

53.5

SOME SKELETAL EFFECTS OF LAND-BASED MODELS FOR HYPOGRAVITY IN THE RHESUS MONKEY MANDIBLE: EFFECTS OF CHRONIC TETRACYCLINE TREATMENT vs POSTCRANIAL IMMOBILIZATION. David J. Simmons*, Brent Grazman*, Shih-Lieh Chang*, Jean E. Russell*, K.C. Smith*, Noel Nussbaum* and Clarence M. Oloff*. Washington University, St. Louis, Mo. 63110, and Wright Patterson AFB, Dayton, Ohio 45433

Land based models of hypogravity induce changes in the histomorphology of the rat and primate skeletons which are thought to be largely similar to those observed in man after spaceflight(=O-G). We recognize, however, that the head-down tilt (HDT)and postcranial immobilization (PI) models do not always produce the specific spaceflight-like changes in the maturation of the mineral-matrix moieties in the non-weight bearing bones (ex. mandibles of rats flown in space). Herein, we descibe how chronic tetracycline (T) injections in Rhesus monkeys (50mg/kg/d for 365d) can produce HDT-/PI-like effects on mandibular bone histology and chemistry. T did not alter the total bone calcium (Ca), inorganic phosphorus (Pi), or Ca/Pi ratios in the PMI region, but gradient density studies showed that the least mature bone fraction (1.3-1.7 sp.gr.) had a higher than normal Ca content(=PI effect). Unlike the effects of PI, T did not decrease the ratios of bone Ca/or Pi/hydroxyproline. By microradiography, those bones (lingula region) also showed a normal percent distribution of lowly, moderately, and highly mineralized osteons, but had significantly increased numbers of T-labeled highly mineralized osteons. This study suggests that chronic T-injection schedules impair the maturation of mineral & matrix moieties, as well as tissue remodeling processes. (Supported in part by NASA)

53.6

EFFECT OF IMMOBILIZATION ON THE ATP, AMP, ADP, NAD^+ CREATIN~P AND Pi CONTENT IN SKELETAL MUSCLES. István Sziklai and József Gróf. Univ.Med.School, Szeged and Semmelweis Univ.Med.School and Hung. Acad. Sci., Budapest, Hungary

The ATP, ADP, AMP, NAD^+, Creatin-P /CrP/ and Pi contents of slow, oxidative soleus muscle and fast, glycolytic gastrocnemius muscle of rabbit have been determined by analytical isotachophoresis during developement of inactivity atrophy caused by limb immobilization from 1 to 6 weeks. The ATP and CrP contents of the normal glycolytic muscle seemed to be higher /ATP:3.8; CrP:7.81 µmol/g wet wt./ then of the oxidative /ATP:2.58; CrP:5.14 µmol/g wet wt/ The NAD^+ content of the normal oxidative muscle was nearly two times higher then of the glycolytic. The minimal level of the ATP content was measured after 1 week of immobilization /Δ %:5o/, while the AMP and NAD^+ content reached maximum value /Δ %: 1ooo and 3oo resp. in the slow muscle; 2ooo and 15o resp in the gastrocnemius muscle/ in both muscle at same time. The CrP level of both muscles decreased after 2 weeks /Δ %:65/, the Pi after 1 week in fast, 4 wks in slow muscle/Δ %:6o/ to their minimum value. The group-specific differences in the energy-supply of skeletal muscles decreased during inactivity probably because of the lack of Pi as substrate in the synthesis of high energy phosphates.

53.7

ALTERATIONS IN MITOCHONDRIA AND SARCOPLASMIC RETICULUM FROM HEART AND SKELETAL MUSCLE OF HORIZONTALLY CASTED PRIMATES. Louis A. Sordahl and H. L. Stone. Univ. Tex. Med. Br., Galveston, TX 77550 and Univ. Oklahoma Med. Ctr., Oklahoma City, OK 73190.

Weightlessness (zero G) produces a series of physiological changes collectively termed cardiovascular deconditioning (CVD). Recent studies indicate that horizontally-casted primates constitute an animal model of CVD. Mitochondria (HM) and sarcoplasmic reticulum (SR) were isolated from hearts of control and 30-day casted primates. Skeletal muscle mitochondria (SM) were also prepared from control and experimental gastronemius. HM from casted animals had essentially the same respiratory activity and ADP:O ratios as controls while SM from casted animals exhibited significant decreases in respiratory activity compared to controls. Cardiac SR from casted animals had >50% decreases in rates of Ca^{2+} binding and uptake compared to controls. Significant decreases in total SR Ca^{2+} bound were also observed in experimental compared to control preparations. The decreased SM function in casted primates can be interpreted as a phenomena of disuse atrophy as contrasted to the enhanced SM activity seen in exercise-training. Although preliminary, the significant depression in cardiac SR functions suggest altered Ca^{2+} homeostasis in the casted primate heart which may be a factor in CVD. (Supported by NASA Grant NSG-2282).

53.8

Effect of Suspension Hypokinesia/Hypodynamia on Glucocorticoid Receptor Levels in Rat Hindlimb Muscles. J.M. Steffen and X.J. Musacchia. Dept. Physiol. Biophys., Univ. Louisville School Med., Louisville, Ky. 40292

Suspension hypokinesia/hypodynamia (H/H) results in differential atrophy of hindlimb muscles in rats (soleus > gastrocnemius = plantaris > EDL). Data from humans and rats under conditions of weightlessness indicate a role for glucocorticoids in disuse atrophy. Glucocorticoid receptor levels were assessed in muscles from control and 7 day suspended rats to evaluate a role for glucocorticoids. Muscles were homogenized and the 100,000 x g supernatants incubated for 20hr with ^{3}H-dexamethasone (0.4 to 16nM) in the absence and presence of a 100-fold excess of unlabeled dexamethasone at 4°C. Incubations were terminated by absorption of free steroids with dextran-coated charcoal and aliquots counted for total or nonspecific binding. Specific binding to receptor sites was the difference between total and nonspecific binding. Specific binding correlated (r = 0.98) with the fast twitch oxidative glycolytic fiber composition of control muscles (soleus = 10±5 fmol/mg cyt. prot.; gastrocnemius = 35±7 fmol/mg cyt. prot.; plantaris = 60±8 fmol/mg cyt. prot.; EDL = 70±8 fmol/mg cyt prot.). k_d values were similar in the individual muscles. Suspension produced elevation in receptor levels corresponding with extent of atrophy (soleus = 130% gastrocnemius = 55%; plantaris = 43%; EDL = no change). These studies indicate glucocorticoid involvement in muscle atrophy resulting from H/H. (Supported by NASA grants NSG 2523 and NAGW-70).

53.9

INFLUENCE OF SUSPENSION-HYPOKINESIA ON RAT SKELETAL MUSCLE. G.H. Templeton, J. Manton, P. Silver, M. Glasburg and J. Sutko. Univ. of Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235

Rats suspended in the head-down tilt position (Holton model for hypokinesia) were studied for changes in their soleus and gastrocnemius muscles. Suspended rats gained weight at same rate as control rats. Analyzing pyrophosphate-polyacrylamide gel electrophoretic patterns, rat soleus muscles showed no significant change in myosin content after one week of hind-limb suspension but a significant decrease after both 2 and 3 weeks as determined using one way analyses of variance and the Neuman-Keul's multiple comparison test. A significant decline in slow-twitch myosin was responsible for the decline in total myosin. In rats suspended for 2 weeks and allowed to recover for one week, total myosin and its slow and fast components were not significantly different from control rats. In a continuation of an histochemical analysis, progressive changes in denervation-like atrophy in the gastrocnemius muscles paralleled changes in soleus muscles previously reported by us. After one week of rat suspension, 6 rats showed normal gastrocnemius muscles. After two weeks, atrophy of both Type I and II fibers were noted and scattered necrotic fibers were seen. After three weeks, 5 rats showed Type I atrophy and both esterase and DPNH positive fibers. Three additional rats showed more advanced Type I atrophy as well as Type II atrophy. (Supported in part by NASA Grant NAGW-140)

53.10

SYNTHESIS OF AMINO ACIDS IN WEIGHT BEARING AND NON-WEIGHT BEARING LEG MUSCLES OF SUSPENDED RATS. Marc E. Tischler* and Stephen Jaspers* (SPON: R.W.Gore). Univ. of Arizona, Tucson, AZ, 85724

Young female rats, whose hindquarters were suspended for 6 days without weight bearing (SNWB), showed atrophy of the soleus but normal growth of the extensor digitorum longus (EDL) muscles, relative to these muscles from suspended weight bearing (SWB) rats. We also tested whether suspension hypokinesia might alter net synthesis of alanine (ala) and glutamine (gln), which are released by muscle to facilitate the removal of nitrogenous waste. Although net synthesis of ala was unaffected, the net synthesis of gln was slower in the incubated soleus muscles of the SNWB than of the SWB rats. No differences were seen for the EDL muscles. Since glucocorticoid hormones normally promote the synthesis of gln by muscle, we examined whether this response might be changed in soleii of SNWB rats. As seen with normal rats, the synthesis of gln in soleii of SWB and EDL muscles of SWB and SNWB rats was slower if the animals were adrenalectomized (Adx) prior to suspension. Administration of cortisol to Adx rats during 6 days of suspension offset this decrease. In contrast, synthesis of gln was similar in soleus muscles of intact, Adx and Adx cortisol-treated SNWB rats, even though Adx led to diminished ala synthesis as it did in muscles of SWB rats. These results suggest that in soleii of hypokinetic rats, the ability of cortisol to induce glutamine synthetase may be absent. (Supported by NASA Grant NAGW-227).

53.11

WEIGHTLESSNESS HYPOKINESIA: SIGNIFICANCE OF MOTOR UNIT STUDIES. Douglas G. Stuart and Roger M. Enoka.* University of Arizona Tucson, AZ 85724.

A considerable body of knowledge exists delineating the effect of weightlessness-induced atrophy on muscle properties. Muscle, however, represents but one component of the segmental motor apparatus, albeit the effector organ. In view of the demonstrated morphological and concomitant physiological interactions between muscle and the central nervous system, it seemed appropriate to examine the implications of disuse for the functional unit of muscle, that is, the segmental motor control system. The latter comprises four basic elements; motoneurons, muscle unit, muscle sensory receptor, and muscle receptor connections with spinal neurons. Within this context we are interested in ascertaining the effects of suspension hypokinesia- and limb immobilization (pinning)- induced disuse upon intrinsic motoneuron properties (input resistance, rheobase, after hyperpolarization and axonal conduction velocity), twitch contraction times and fatigability of the four muscle unit types (FF, FI, FR and S), sensitivity of muscle spindles and tendon organs, and the efficacy of proprioceptive and descending input to spinal neurons. Preliminary results will be presented on rat muscle units of soleus, extensor digitorum longus and tibialis posterior which address the issues of preferential (type S > FF, FI, FR) vs variable time-course disuse effects, representative sampling, and muscle stretch as an atrophy retardant. (Supported by NASA 68383)

53.12

VO_2 KINETICS DURING SUBMAXIMAL EXERCISE FOLLOWING SIMULATED WEIGHTLESSNESS. V. A. Convertino and H. Sandler. Biomedical Res. Div., NASA-Ames Res. Center, Moffett Field, CA 94035

The purpose of this study was to determine the effects of simulated weightlessness on changes in oxygen uptake (VO_2), O_2 deficit, steady-state VO_2, and recovery VO_2 during the performance of constant-load exercise. Five male subjects (36-40 yr) underwent 7 days of continuous bedrest (BR) in the headdown (-6°) position. Two days before (pre) and the day after (post) BR each subject performed one submaximal exercise test in the supine (SUP) and one in the upright (UP) position consisting of 5 min rest, 5 min cycle ergometer exercise at 700 kgm/min and 10 min of recovery from exercise. VO_2 was measured continuously in all tests from 2-liter aliquot air samples collected every 30 s. Following BR, steady-state VO_2 was unchanged in SUP and UP exercise. In the SUP position, BR did not change exercise VO_2, O_2 deficit, or recovery VO_2. However, compared to pre BR, total VO_2 decreased ($P < 0.05$) from 7.41 ± 0.11 ℓ to 7.23 ± 0.17 ℓ, O_2 deficit increased ($P < 0.05$) from 1.10 ± 0.05 ℓ to 1.36 ± 0.07 ℓ, and recovery VO_2 increased ($P < 0.05$) from 5.22 ± 0.12 ℓ to 5.47 ± 0.17 ℓ during the post BR UP test. The elevation in O_2 deficit during post BR UP exercise resulted from a significant increase in the VO_2 time constant. Despite the ability to attain similar steady-state VO_2, simulated weightlessness results in a reduction of total VO_2 capacity and an increase in the O_2 deficit during submaximal constant-load exercise. This change in VO_2 kinetics is induced by re-exposure to the upright (+1 G_z) environment.

53.13

HANDGRIP AND GENERAL MUSCULAR STRENGTH AND ENDURANCE DURING PROLONGED BED REST WITH ISOMETRIC AND ISOTONIC LEG EXERCISE TRAINING. J.C. Starr*, J.E. Greenleaf, W. Van Beaumont, and V.A. Convertino. Biomedical Research Division, NASA, Ames Research Center, Moffett Field, CA 94035.

Maximal grip strength and endurance (at 40% max strength) were measured in 7 men (19-21 yr) 1-2 days before and on the first recovery day during three 2-wk bed rest (BR) periods, each separated by a 3-wk recovery period. In the 3 BR periods they performed isometric exercise (IME) for 1 hr/day (250 kcal/hr), isotonic exercise (ITE) for 1 hr/day (780 kcal/hr), or no exercise (NOE) at 90 kcal/hr. Mean max grip strength was unchanged after all 3 BR periods. Mean ±SE grip endurance was unchanged after IME (147±7 to 136±8 sec, -7.5%, NS) and ITE (138±8 to 143±14 sec, +3.6%, NS) training, but was reduced from 148±7 to 119± 10 sec (-19.6%, P<0.05) after NOE. Our results and those from the literature indicate: (i) IME and ITE training during BR do not change max grip strength, but they prevent loss of grip endurance; (ii) max strength of all other major muscle groups decreases in proportion to the length of BR to 60 days; (iii) max strength reduction of trunk, thigh, and leg muscle groups is about twice that of hand, forearm, and arm muscle groups during BR; (iv) ITE during BR greatly reduces losses of strength in all major muscle groups; and (v) changes in max strength after space flight, BR, or water immersion deconditioning cannot be predicted from changes in submaximal or maximal oxygen uptake values.

53.14

EFFECTS OF AGE AND SEX ON HORMONAL RESPONSES TO WEIGHTLESSNESS SIMULATION. F. LaRochelle, C. Leach, and J. Danellis. NASA/Johnson Space Center, Houston, TX 77058 and NASA/Ames Research Center, Moffett Field, CA 94035

As the age of experienced astronauts increases and as more women become Shuttle crewmembers, the need to study the effects of age and sex on the physiologic responses to space flight becomes more apparent. In this study we examined the effects of horizontal bedrest on the excretion of catecholamines, aldosterone, and cortisol. The subjects were 35-65 yr old and grouped by age and sex. Responses were assessed by biochemical and radioimmunoassays of 24-hr urine samples collected continuously throughout the studies. In the 35-45 yr groups the excretion of epinephrine increased (3 and 68% respectively) while decreases were seen in the 45-55 and 55-65 yr old groups. Norepinephrine excretion decreased (5-27%) in all groups during bedrest. Aldosterone excretion increased in the 35-45 and 45-55 yr age group of both males (19 and 6% respectively) and females (47 and 9%). A slight decrease was seen in the 55-65 yr males (6%) while the excretion in females was unchanged. During bedrest cortisol excretion increased in the youngest groups of both men (12%) and women (13%), but decreased in the respective 55-65 yr groups (6 and 5%). In the two groups of intermediate age (45-55 yrs) the excretion in females was decreased (15%) while males increased their excretion (19%). These data indicate that hormone measurements may be of value in explaining variations in stress tolerance due to age and/or sex during space flight.

53.15

INCREASED HEMATURIA FOLLOWING HYPERGRAVIC EXPOSURE IN MIDDLE-AGED WOMEN. D. J. Goldwater*, D. B. O'Hara*, and H. Sandler. NASA-Ames Research Center, Moffett Field, CA 94035

To study the effects of simulated weightlessness on orthostatic tolerance of middle-aged women, 8 females (45-55 yr) underwent acceleration (+G_z) and lower body negative pressure (LBNP) before and after 10 days of horizontal bedrest (BR). These subjects passed a screening exam, including creatinine (Cr), BUN, and urinalysis (UA). All women gave a negative history for renal disease. All subjects underwent duplicate testing during a 9 day control period consisting of 1.5 G_z, 2 G_z, and 3 G_z exposures, in addition to LBNP. The + 1.5 G_z run and + 2 G_z run were modeled after projected Shuttle reentry profiles. The + 3 G_z run was used as a physiological acceleration tolerance test. The 1.5 G exposure was repeated on BR day 7, 2 G on BR day 8, and 3 G on BR day 9. Three of 8 women had hematuria from 1 to 5 RBC's/high power field and routine UA after entry into the study. Menstruation was not a factor. In 2 subjects, LBNP increased hematuria. Acceleration levels of + 2 G_z or greater produced 3 to 5-fold increases in hematuria in these women. Within 1 day post +G_z or LBNP, hematuria subsided to basal levels. Horizontal BR per se did not affect the degree of hematuria. Individual serum Cr and BUN indicated no change in renal function. Acceleration of predisposed individuals may produce alterations in glomerular capillary permeability or changes similar to those responsible for hematuria in runners.

53.16

INSULIN SENSITIVITY OF EXERCISE TRAINED RATS FOLLOWING CONFINEMENT IN SMALL SPACE FLIGHT SIZE CAGES. Carl E. Mondon, Constantine B. Dolkas* and Gerald M. Reaven*. NASA Ames Res. Ctr., Moffet Field, CA 94035 and VA Medical Ctr., Palo Alto, CA 94304

Previous studies in man and laboratory animals have shown resistance to insulin-induced glucose uptake under conditions of total bed rest or limitation of skeletal muscle activity and increased sensitivity to insulin with exercise training. To determine whether enhanced insulin sensitivity in exercise trained (ET) rats persists following small cage confinement, oral glucose tolerance tests (OGTT) were given to control and resting ET rats before and after placement in small space flight size cages (11x4x4½ in) for 7 days. ET rats ran over 6 miles/day after 4 weeks training and show less gain in body weight than control rats despite 36% greater food intake. The product of the area of the insulin and glucose curves of the OGTT (IG index) provides a measure of insulin sensitivity and values taken before confinement were significantly lower ($p < .001$) in resting ET rats than in younger control rats of comparable body weight (42 ± 3 vs $86 \pm 8 \times 10^3$). After 7 days confinement, the IG index was not significantly different from initial values for ET rats (49 vs 42) and was slightly lower in control rats (66 vs 86). These findings suggest that increased insulin sensitivity in ET rats persists 7 days after cessation of running activity, and ET may be beneficial in minimizing loss of insulin sensitivity during decreased muscle activity induced by hypogravity in space flight. (Funded by NASA and US Veterans Administration.)

53.17

SHORT TERM (1 AND 3 DAY) CARDIOVASCULAR ADJUSTMENTS TO SUSPENSION ANTIORTHOSTASIS IN RATS. X.J. Musacchia and J.M. Steffen. Dept. Physiol./Biophys., School Med., Univ. of Louisville, Louisville, KY 40292

Antiorthostasis (AO) results in responses reflective of thoracic vessel loading; based on initial findings such as fluid and electrolyte shifts (diuresis and natriuresis) in AO but not in orthostatic (O) rats. This study aims at obtaining supportive evidence for cardiovascular responses, e.g. blood pressure and related parameters, in light of the original hypothesis. Tilting rats rapidly head-up from horizontal (O) or head-down (AO) positions was used to assess cardiovascular sensitivities. O and AO rats were used after 1 or 3 days of suspension. Rats were: control (C), pre tilted O and AO, tilted O and AO (rapid head-up 70-80°) and post tilted (to original postures). MAP in C rats was 107 ± 3 mmHg. On day 1, MAPs increased (e.g. 115-120 mmHg) in both O and AO rats; on day 3 only AO subjects continued to show marked elevations, e.g. 135-145 mmHg whereas O rats returned to C levels. When tilted head-up, both O and AO rats on day 1 showed marked elevation in blood pressures which returned toward pre-tilt values. On day 3, AO rats appeared to lose this (adaptive ?) response. These results support the concept that fluid volume (diuresis) and electrolyte concentration (natriuresis) changes may result from stimulation of central cardiovascular volume receptors. (Supported by NASA, NSG-2325).

53.18

DAILY RHYTHMS OF ACTIVITY AND TEMPERATURE OF *Macaca nemistrina*. Frank M. Sulzman and Sharon A. Sickles*. Dept. Biol. Sci., SUNY, Binghamton, NY 13901

In preparation for the joint US-USSR Biorhythm experiment that will be flown on COSMOS'83, we have examined activity and temperature rhythms of pig-tailed macaques (*Macaca nemistrina*). Four young male monkeys (4-7 kg) were maintained in LD 16:8 at 25°C in specially designed restraint chairs. Activity was monitored via a pressure sensitive milar device that was attached to the restraint chair. Temperature was monitored at four sites: colon, axilla, ankle and ear. These variables showed prominent day-night variations, and except for ankle temperature, all had highest values during the daytime. Ankle temperature had an inverted pattern, suggesting that heat loss from the feet is an important contributor to the rhtyhm of core body temperature. These patterns show that the regulation of the daily rhythm of body temperature involves anatomical sites that are utilized in a temporally distinct fashion. (Supported in part by NASA Grant NAS 210621)

53.19

TOTAL LDH ACTIVITY AND ITS ISOENZYME PATTERNS DURING BED REST WITH EXERCISE TRAINING. L.T. Juhos*, H.L. Young*, and J.E. Greenleaf. Biomedical Research Division, NASA, Ames Research Center, Moffett Field, CA 94035

Plasma total lactate dehydrogenase activity (LDH_T) and isoenzyme profiles were quantitated in 7 men (19-21 yr) 1-2 days before, during, and 1-3 days after three 2-week bed rest (BR) periods, each separated by a 3-week recovery period. In the three BR periods they performed isometric exercise (IME) for 1 hr/day (250 kcal/hr), isotonic exercise (ITE) for 1 hr/day (780 kcal/hr), or no exercise (NOE) at 90 kcal/hr. Compared with control and recovery results, LDH_T was reduced ($P<0.001$) during BR; this reduction was independent of ITE and IME. Reductions in LDH_3 and LDH_4 accounted for the reduction in LDH_T. While there were some exercise specific responses of the isoenzymes, LDH_5 made no contribution to the reduction of LDH_T. We conclude that reduced hydrostatic pressure during BR overrides the effects of exercise resulting in a decrease of LDH_T.

ENVIRONMENTAL PHYSIOLOGY II

54.1

THERMOREGULATORY RESPONSE TO MAXIMAL EXERCISE IN PATIENTS WITH SEVERE HEART FAILURE. F.G. Shellock*, S.A. Rubin*, H.J.C. Swan. Cedars-Sinai Medical Center, L.A., CA 90048

Heat dissipation is presumed to be impaired in heart failure (HF) patients due to their constricted cutaneous circulation and depressed cardiac output. We studied core temperature (Tc) and hemodynamics during rest (R) and maximal (50 watts) upright bicycle exercise (E) in 8 pts with severe HF and five normal (N) subjects in a 20-22°C, 50% RH environment. Tc was measured in the pulmonary artery with a sensitive (accuracy±0.01°C) thermistor catheter. Resting forearm skin temperature (Ts) was also measured in 10 HF pts and 10 N subjects using a radiometer.

	HR (min^{-1})	MAP (mmHg)	PCW (mmHg)	CO (L/min)	$\dot{V}O_2$ (ml/min)	Tc (°C)
N-R	81±8	96±20	4±3	4.5±1.0	258±14	37.11±0.40
N-E	111±27*	121±34*	11±4*	10.1±1.2*	993±61	37.34±0.39*
HF-R	91±12	84±12	19±9	4.3±1.2	289±63	37.01±0.29
HF-E	118±20*	93±19	30±9*	6.0±1.4*	830±124*	36.68±0.31*

(HR=heart rate; MAP=mean arterial pressure; PCW=pulmonary capillary wedge pressure, CO=cardiac output; $\dot{V}O_2$=oxygen uptake; $\bar{X}$±SD; E vs R, *$p<0.05$). Tc significantly decreased during E in the HF pts compared to a significant increase in Tc in the N group. Resting Ts was significantly lower than N ($p<0.001$) in the HF pts. Femoral vein temperature measured in 2 HF pts during E did not increase above Tc. We conclude that the Tc decrease observed in exercising HF pts is caused by a redistribution of body heat, that is, there is a mixing of core blood with the cooler contents of the periphery (skin and muscle).

54.2

EXERCISE HEAT-TRAINING EFFECTS ON THE ANTERIOR HYPOTHALAMIC RESPONSE TO NOREPINEPHRINE IN THE RAT John V. Christman* and Carl V. Gisolfi. Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa. 52242.

This study was performed to determine whether the increase in sensitivity of the anterior hypothalamus (AH) to norepinephrine (NE) injection which occurs with exercise conditioning in heat is due to central and/or peripheral changes. Bilateral guide cannulae were implanted and a NE-sensitive site was located (drop in colonic temperature (Tc) of at least .8°C in response to 10 µg NE injection). A work-heat tolerance test was performed before and after 3 weeks of conditioning for 25-40 min/day at either 22°C (C) or 35°C (H). After conditioning, NE doses produced the following reductions in Tc, normalized to each animal's preconditioning response to 10 µg NE (mean normalized response ± SEM):

	2 µg	5 µg	10 µg	20 µg	40 µg
C (n=7)	.5 ± .1	.8 ± .1	1.2 ± .1	1.6 ± .2	1.8 ± .2
H (n=6)	1.0 ± .3	1.5 ± .3	1.8 ± .1	3.0 ± .2	2.9 ± .4

The maximal response to the NE doses was greater in the H compared to the C animals. However, the dose producing one half the maximal response was not different. These observations indicate that the increased response to central injection of NE in rats chronically exercised in the heat is due to a peripheral adaptation.

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54.3

FUNCTIONAL RECOVERY OF INTERSCAPULAR BROWN ADIPOSE TISSUE (IBAT) IN COLD-ACCLIMATED (CA) RATS AFTER CHEMICAL SYMPATHECTOMY WITH 6-HYDROXYDOPAMINE. Chi-Chung Chan* and Florent Depocas, Environmental Physiology Group, National Research Council of Canada, Ottawa, Ontario, Canada.

Earlier studies from this laboratory have shown that content of noradrenaline (NA) and activity of dopamine-β-hydroxylase in IBAT of CA rats had recovered to 40% and 80% of control values respectively 7 days after treatment with 6-hydroxydopamine (6-OHDA) (10 mg/kg, s.c.) whereas cold-induced increase in blood flow to this tissue had recovered completely at that time. Full recovery of function in spite of reduced stores of NA could be due to pre- or postsynaptic sensitization. To examine these possibilities, calorigenic responses to infused noradrenaline were obtained in anesthetized CA rats and the dose of NA at half maximal responses (ED_{50}) was determined. ED_{50} was 3.38 ± 0.28 $ng \cdot min^{-1} \cdot g^{-0.74}$ in control rats and was decreased to 1.23 ± 0.05 and 1.88 ± 0.20 in rats treated with 6-OHDA 1 day and 7 days prior to NA tests respectively. ED_{50} was not significantly further reduced by additional treatment with either desmethylimipramine (1 mg/kg) or 6-OHDA (10 mg/kg). It is suggested that sensitization of responses to NA in the 7-day 6-OHDA-treated rats is of presynaptic origin and that the complete recovery of function observed at this stage is not a consequence of postsynaptic sensitization but possibly a result of enhanced sympathetic activity and reduced neuronal uptake.

54.4

EFFECTS OF IONTOPHORETICALLY APPLIED MET- AND LEU-ENKEPHALIN ON HYPOTHALAMIC THERMOSENSITIVE NEURONS IN RATS. Alexander L. Beckman and Steven K. Salzman*. Alfred I. duPont Institute, Wilmington, De. 19899

Met- and leu-enkephalin, naturally occurring opioid neuropeptides, have been reported to produce an increase in body temperature following intraventricular microinjection. This study was designed to determine whether the enkephalin-induced thermal responses could be attributed to a direct action on the firing rate of thermosensitive neurons in the preoptic/anterior hypothalamic area (POA). Male Sprague Dawley rats were anesthetized with urethane and implanted, in a stereotaxic instrument, with a bilateral pair of water-perfused stainless steel thermodes (rostral to the POA) and a stainless steel, thermistor reentrant tube (contralateral to the POA single unit recording site). Single unit recording and current-balanced iontophoretic application of met- and leu-enkephalin (0.03 M, pH 4) was accomplished with five-barrel glass micropipettes. The enkephalins decreased the firing rate of warm-sensitive and temperature-insensitive cells, and increased the firing rate of the one cold-sensitive cell encountered thus far. These preliminary results suggest that the thermal responses produced by central microinjection of enkephalins are mediated by the action of these compounds on POA thermosensitive neurons. (Supported by N.I.D.A. grant DA-02254 and the A. I. duPont Institute)

54.5

BEHAVIORAL AND PHYSIOLOGICAL THERMOREGULATORY RESPONSES FOLLOWING CENTRAL ADMINISTRATION OF THYROTROPIN-RELEASING HORMONE IN GROUND SQUIRRELS. Toni L. Stanton* and Alexander L. Beckman. A.I. duPont Inst., Wilmington, DE. 19899.

Thyrotropin-releasing hormone (TRH) has been shown to alter body temperature (T_b) in several mammalian species following central administration, prompting the suggestion that TRH has a role in thermoregulation. In the awake ground squirrel, TRH produces dose-related decreases in metabolic rate and T_b when microinjected into the dorsal hippocampus (HPC). To investigate the possibility that TRH-induced hypothermia is associated with a decrease in thermoregulatory set point, animals were trained to bar-press for radiant heat reinforcement in a -10 C environment. During experimental testing, the animals were microinjected with TRH (25-100 ng/ul) in either the HPC or preoptic/anterior hypothalamic area (POA) through chronic bilateral cannulae. Cumulative bar presses were obtained on a computer-generated display. T_b, sensed in the brain, was recorded continuously. Following TRH administration in the POA of 2 animals and the HPC of one animal, a 28-50% decrease in mean bar-press rate occurred during the period in which T_b was falling, when compared to a comparable time period immediately prior to the microinjection. These preliminary data indicate that TRH produces hypothermia in the ground squirrel by lowering the thermoregulatory set point. The results thereby support the hypothesis that TRH is involved in thermoregulatory control processes. (Supported by NSF grant# BNS 78-19002 and the A.I. duPont Institute.)

54.6

EFFECT OF LESIONING OF CUTANEOUS TEMPERATURE RECEPTORS ON HYPOTHALAMIC THERMOSENSITIVITY FOR HEAT PRODUCTION IN THE RAT. M.E. Heath,* J.C. Crabtree,* and H.T. Hammel. PRL, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

Experiments were conducted to determine the extent to which input from cutaneous temperature receptors of the face and trunk affect the hypothalamic temperature (Thy) threshold and sensitivity for heat production. The rate of O_2 consumption in response to different Thy were measured using a paramagnetic O_2 analyzer in an open circuit system while rats were in ambient temperatures (Ta) of 20°C (cool) and 25°C (neutral). Skin and rectal temperatures were also monitored. The relation between Thy and O_2 consumption was determined for three rats when they were a) normal, b) after their trunk was shaved, c) after lesioning of trunk cutaneous nerves, and d) after also lesioning the cutaneous nerves of the face. In normal rats the Thy threshold for heat production was elevated in 20°C as compared to 25°C but sensitivity was similar at both Ta. Shaving the trunk of the rats raised their Thy threshold in the cool environment indicating that trunk skin temperature does affect thermoregulatory responses. Lesioning the cutaneous nerves of the trunk reduced Thy sensitivity. When the cutaneous nerves of the face as well as trunk were lesioned, Thy threshold and sensitivity for heat production were the same at an Ta of 20°C as they were at 25°C. (Supported by PHS 5 F32 NS06516-02.)

54.7

THE EFFECT OF A SINGLE ACUTE EXPOSURE TO HIGH AMBIENT TEMPERATURE ON PULMONARY SURFACTANT OF THE RABBIT. B.A. Sturbaum, R.V. Kotas, and M.A. Moxley*. Oral Roberts Univ. Sch. Med. and Tulsa Med. Coll. Tulsa, OK. 74171.

Rabbits were exposed to 50°C ambient temperature for 45-50 min. Blood taken before and after heat exposure showed no differences in PO_2, PCO_2, pH, hematocrit, and total red blood cells per mm^3, but did show a significant decrease ($p < 0.001$) in white blood cells per mm^3. After exposure animals were sacrificed and lungs lavaged. Total phospholipid (PL), phosphatidylcholine (PC), disaturated phosphatidycholine (DSPC) content and surface tension of the lavage fluid were determined. Results are (* = significance; ± = SEM):

	CONTROLS	ACUTE HEAT	
PL µmol/ml lavage	0.159 ± 0.033	0.276 ± 0.077	
PC µmol/ml lavage	0.106 ± 0.027	0.184 ± 0.055	
DSPC µmol/ml lavage	0.078 ± 0.019	0.104 ± 0.032	
PC as % of PL	65.2 ± 3.105	65.6 ± 2.066	
DSPC as % of PC	74.3 ± 5.266	56.6 ± 3.037*	p<0.02
DSPC as % of PL	48.1 ± 2.307	37.1 ± 1.894*	p<0.01
Surface Tension dynes/cm	8.7 ± 1.59	19.2 ± 1.05*	p<0.001

The results suggest that high ambient temperatures over a 45-50 minute period were associated with decreased circulating leucocytes and decreased pulmonary surfactant activity which may lead to respiratory distress.

54.8

PULMONARY RESPONSES TO EXPOSURE TO 0.24 PPM OZONE AT ELEVATED TEMPERATURE. D.H. Horstman, W. McDonnell,* S. Salaam.* U.S.EPA, Chapel Hill, NC 27514

High ozone levels in the environment are usually associated with elevated ambient temperature. The purpose of this study was to evaluate pulmonary decrements resulting from exposure to an ozone concentration and ambient temperature representative of a typically polluted environment. Healthy, young males (N=41) were randomly divided and exposed for 2.5 hrs to 0.24 ppm ozone at either 22°C/40% RH (NORM, n=21) or 30°C/60% RH (WARM, n=20). During the first 2 hrs of exposure alternating 15 min periods of rest and heavy exercise ($\dot{V}_E$ = 35 l/m^2 BSA·min) were used. Forced expiratory spirometry (FVC, FEV1, FEF25-75) was performed, specific airway resistance (SRaw) measured and symptoms evaluated before exposure and after the final exercise. After WARM exposure, FVC, FEV1 and FEF25-75 were reduced by 708 ± 109 ml, 781 ± 109 ml and 1.23 ± 0.19 l/sec, respectively, while after NORM exposure decreases for these same variables were 530 ± 81 ml, 588 ± 96 ml and 0.93 ± 0.17 l/sec, respectively. SRaw increased 0.65 ± 0.22 and 0.56 ± 0.11 cm H_2O·sec following WARM and NORM exposures, respectively. None of these difference between WARM and NORM were statistically significant ($P > 0.05$). Cough, pain on deep inspiration and shortness of breath increased similarly following both exposures. In conclusion, when compared with normal indoor conditions, there was the suggestion of greater decrements in pulmonary function resulting from ozone exposure at slightly elevated temperature and relative humidity.

54.9

EFFECT OF DEHYDRATION ON THERMOREGULATION IN RESTING DOGS AT HIGH AMBIENT TEMPERATURES. M.A. Baker. Div. of Biomedical Sci. and Dept. of Biology, University of California, Riverside, CA 92521.

We measured rectal temperature (T_r), respiratory frequency (f), respiratory evaporation (E_{resp}) and cardiac output (C.O.) in large dogs (mean wt. 30kg) at rest at ambient temperatures (T_a) between 25° and 45°C. Dogs were studied when they were hydrated ad lib and when they were dehydrated by removal of drinking water. Dehydration resulted in a 10% loss of body weight and an increase in plasma osmolality from 297 to 326 mosm/kg. During experiments, the dogs rested for 45 min at 25°C and then T_a was raised to 35°, 40° and 45°C and held at each T_a for 45 min. Measurements were made during the last 5 min at each T_a. Dehydrated animals had higher body temperatures, lower f and E_{resp} and lower C.O. than hydrated animals at all T_a's. T_r averaged over the four T_a's was 0.38°C higher in dehydrated animals. Respiratory frequency in dehydrated animals was 85% lower than in hydrated andimals at 25°C and 40% to 50% lower at 35°, 40° and 45°C. E_{resp} in dehydrated animals was reduced by 79% at 25°C and by 30% to 45% at higher T_a's. The average reduction in C.O. in dehydrated animals at all T_a's was 30%. The changes in E_{resp} and f in dehydrated dogs exposed to heat are similar to those seen in other panting mammals. Reduced C.O. in dehydrated dogs probably reflects reduced blood flow to respiratory muscles and to the nasal and oral passages, since blood flow to these regions correlates strongly with E_{resp}. (Supported by NSF Grant BNS-7901006)

54.10

INTESTINAL ABSORPTION IN HEAT ACCLIMATED DESERT RODENTS. M.K. Yousef and D.T. Chu.* Dept. Biol. Sci., Univ. of Nevada, Las Vegas, NV 89154.

Gastrointestinal functions of heat acclimated wild desert rodents have received little attention. Such studies are relevant to our understanding of caloric and nutritional requirements of small mammals during acclimation or acclimatization to heat stress. The objectives of this study were to measure intestinal mucosal uptake and serosal transport of glucose using the everted intestinal sac technique in heat acclimated kangaroo rats (D. merriami), wood rats (N. lepida) and Antelope ground squirrels (A. leucurus). Also the relative changes in body organs were studied. Heat acclimation significantly increased intestinal serosal transport of glucose in all three species, and had no effect on mucosal uptake. Intestinal fluid uptake decreased in heat acclimated ground squirrels and did not change in the other two species. Although body weights did not change in the heat acclimated animals, the relative intestinal weight was decreased in all species. The intestinal length was not influenced by heat acclimation. The data suggest that heat acclimation increased the absorptive capacity of the intestines. The increased intestinal serosal transfer of glucose in all three species represent a compensatory mechanism for the known decreased food intake during heat acclimation.

54.11

ACTIVITY OF THE SYMPATHETIC INNERVATION AND ULTRASTRUCTURAL CHANGES IN THE ARTERIES FROM RABBITS RAISED AT HIGH ALTITUDE. K. Fronek and O. Mathieu*. University of California, San Diego, La Jolla, CA. 92093.

We studied the effect of high altitude hypoxia on the indices of the sympathetic innervation activity and the amount of mitochondria in abdominal aortas (AA) and iliac arteries (ILA) of rabbits raised at 12,470ft (HA) and compared with corresponding arteries from matched sea level controls (C). The threshold dose to NE was significantly higher in AA and ILA of the HA rabbits as compared with the C, as well as the ED_{50}. There was no significant difference in the maximal contractile force in either vessel. The ^{3}H-NE accumulation was found to be significantly elevated, and NE content lower, in the arteries from HA rabbits, indicating high activity of the adrenergic neurons. When tyramine (TY) was used to deplete NE from adrenergic terminals, the NE accumulation data obtained in the TY treated rabbits were in good agreement with those of HA rabbits. The volume density of mitochondria in the smooth muscle cell cytoplasm evaluated morphometrically was almost doubled in the media of arteries from HA rabbits as compared with C. In conclusion the activity of adrenergic neurons was significantly elevated in arteries from HA rabbits while the maximal contraction unchanged. Furthermore, the aerobic capacity of the smooth muscle cells in the arterial wall of HA rabbits was significantly increased.

(Supported by NIH Grant HL-20654)

SHOCK I

55.1

CARDIOVASCULAR MECHANISMS OF ANAPHYLACTIC SHOCK. W.R. Taylor, H.J. Silverman*, R.A. Wise*, S. Permutt, P.L. Smith*, and E.R. Bleecker. The Johns Hopkins Med. Inst., at Baltimore City Hospitals, Baltimore, MD 21224.

In anesthetized dogs on right heart by-pass, we examined circulatory and ventricular mechanics during anaphylactic shock. Purified Ascaris suum antigen was used to induce anaphylaxis. Measurements were made during a control period, during shock, and after cardiac output was restored by giving volume. The following measurements were made: slope of the left ventricular function curve(LVF), mean arterial pressure (PAO), mean systemic (i.e. stop-flow) pressure(PMS), and venous compliance(VC). Resistance to venous return(RVR) was calculated by dividing PMS by the cardiac output. Results were:

	CONTROL	SHOCK	CARDIAC OUTPUT RESTORED
LVF	1.84 ± 0.02	—	2.26 ± 0.43
PAO	74.2 ± 2.8	34.9 ± 5.5*	55.2 ± 5.6*
PMS	6.2 ± 0.3	2.8 ± 0.6*	11.6 ± 1.8*
RVR	2.9 ± 0.2	—	5.2 ± 0.6*
VC	55.9 ± 12.6	—	18.5 ± 4.4*

*p<0.05 vs control

985 ± 241 ml of volume was required to restore cardiac output. We conclude that during anaphylactic shock: (1) Left ventricular function is normal; (2) There is arterial vasodilation; (3) The fall in cardiac output is due to a decrease in the driving pressure for venous return and an increase in the RVR; (4) The fall in PMS is probably due to an increase in the unstressed volume of the circulation.

55.2

PREVENTION OF ANAPHYLACTIC SHOCK BY ISOPROTERENOL IN AN IN VIVO CANINE MODEL OF ANAPHYLAXIS. H. Silverman*, W.R. Taylor, P.L. Smith*, L. Lichtenstein*, A. Sobotka*, and E.R. Bleecker. The Johns Hopkins Med. Inst., at Baltimore City Hospitals, Baltimore, MD 21224.

In vitro studies have shown that B-adrenergic agents inhibit mediator release via an increase in cAMP levels. To determine whether B-adrenergic stimulation blocks in vivo anaphylaxis, 14 dogs with positive skin tests to Ascaris suum antigen(Ag) were assigned to 2 groups: Group I-control, received only Ag, Group II-received a continuous infusion of 0.3μg/kg/min of Isoproterenol(Iso), beginning 30 min before Ag injection. Results ($\bar{x} \pm$ SEM) are shown for mean arterial blood pressure (Pa, mmHg) and cardiac output (CO,l/min) both before and 5 min. after Ag injection (peak of reaction).

		GROUP I	GROUP II
Pa	Baseline	121.7 ± 12.6	90.7 ± 7.2
	5 min	66.4 ± 21.0	74.9 ± 10.4
CO	Baseline	4.20 ± 0.25	8.83 ± 1.27
	5 min	2.66 ± 0.70	7.86 ± 1.77

There were significant falls in Pa and CO in Group I ($p<.05$), while dogs pre-treated with Iso showed no significant fall in Pa or CO. This protective effect of Iso was not due to its hemodynamic properties as it did not cause any significant increase in Pa or CO when given 6 min after Ag injection in 7 other dogs. We conclude that B-adrenergic stimulation prevents systemic anaphylaxis in an in vivo preparation. This effect may be due to prevention of mediator release.

55.3

ALTERATIONS IN THE RATE OF REMOVAL OF GLYCEROL FOLLOWING ENDOTOXIN ADMINISTRATION. O.P. McGuinness* and J.J. Spitzer, LSU Med. Ctr., N.O., LA 70112.

Following E. coli endotoxin (ET) administration plasma glycerol (gly) concentration is markedly elevated, which has been assumed to indicate enhanced lipolysis. Alternatively, the rate of removal of gly may also be slower which would be reflected by decreased gly clearance. In order to determine the nature of the altered gly metabolism, the clearance of gly was determined in anesthetized dogs following the administration of either ET (0.3 mg/kg; n=7) or saline (N=8) during the early (15-135 min) the late (260-380 min) post treatment time periods. Gly clearance was determined by a stepwise infusion of glycerol at known infusion rates (4.3, 8.7, 17.4 μmole/kg/min). Gly clearance was calculated as the reciprocal of the slope of the linear correlation between gly infusion rate and the change in steady state arterial gly concentration. Gly clearance was markedly decreased in the ET-treated group, i.e. during the early phase, clearance of gly in the ET and saline groups was 20.8 + 0.3 and 31.6 ± 0.3 and during the late phase 19.2 ± 0.3 and 31.6 ± 0.2 ml/kg/min respectively. A negative correlation was observed between the initial fall in blood pressure, or the initial percent change in cardiac output, and plasma gly clearance (r= -0.885 and r= -0.653 respectively). Thus, impaired gly removal following ET is - at least partially - responsible for the elevated plasma gly. The diminished gly removal is proportional to the severity of the cardiovascular response to ET.

55.4

INCREASED SKELETAL MUSCLE AND HEPATIC CELLULAR CALCIUM DURING ENDOTOXIN SHOCK. M.M. Sayeed, M.D. Karlstad*, and A. Doroba*, Dept. of Physiol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Increased accumulation of calcium into cells has been implicated in endotoxin shock pathogenesis. We have quantitated total exchangeable calcium in skeletal muscle and liver cells during endotoxin shock. Fasted male Holtzman rats (250g) were injected IV with saline or 20 mg/kg S. enteritidis endotoxin. After 5 hrs livers and soleus muscles were removed. Blood was analyzed for glucose and lactate levels. Liver slices (0.3mm thick) or soleus muscles were incubated for 1 hr at 37°C in oxygenated Krebs-Ringer bicarbonate (KRB) containing ^{45}Ca. Radiolabeled tissues were then successively washed in radioisotope-free KRB at 2°C. ^{45}Ca released in media and that remaining in tissues after completing the washes was counted to assess ^{45}Ca efflux from the cellular compartment. Extrapolation of the cellular efflux to time zero yielded total exchangeable cellular calcium in units of DPM x 10^3/g. Blood lactate, plasma glucose and cellular calcium (mean±SE) were:

	Glucose (mg%)	Lactate (mM)	Exchangeable Calcium Muscle	Liver
Saline	90±3(14)	0.8±0.2(18)	156±7(6)	202±20(6)
Endotoxin	63±7(17)*	2.3±0.2(27)*	202±16(7)*	266±23(6)*

( ),# of animals;* P<0.005 Compared to saline group.

These data show a significant increase in cellular exchangeable calcium in skeletal muscle and liver during endotoxin shock which is characterized by hyperlactic acidemia and hypoglycemia. (Supp. by the William G. Potts Research Funds).

55.5

DECREASED BASAL AND INSULIN-STIMULATED AMINO ACID UPTAKE BY SKELETAL MUSCLE DURING ENDOTOXIN SHOCK. M.D. Karlstad*, R.M. Raymond, A. Doroba*, and M.M. Sayeed. Dept. of Physiology and Surgery, Loyola Univ., Stritch Sch. Med., Maywood, IL 60153

The present study was designed to determine insulin's effect on the uptake of 2-amino-[1-^{14}C]isobutyric acid (AIB) by skeletal muscle during S. enteritidis endotoxin shock. Male fasted rats, 140-160 g, were anesthetized with ether and injected IV with saline or 20 mg/kg endotoxin. To measure AIB uptake, intact soleus muscles, 78-80 mg, were removed 5 hrs post-injection and incubated for 3 hrs in Krebs-Ringer bicarbonate (pH 7.4) containing labeled AIB. Incubations were carried out aerobically (95%O_2-5%CO_2) at 37°C in the absence (Basal) or presence of 1, 10, or 100 mU/ml insulin. Mean (±SE) values of plasma glucose, blood lactate and AIB uptake were:

	Glucose (mg %)	Lactate (mM)	AIB Uptake (nmoles/g dry wt/180 min) Basal	Insulin mU/ml 1	10	100
Saline	89.8 ±2.1(55)	0.65 ±0.06(44)	22.23 ±0.47(30)	31.71 ±0.98(8)	32.11 ±0.83(13)	33.74 ±0.92(9)
Endotoxin	61.1* ±3.7(41)	2.09* ±0.15(28)	18.45* ±0.33(32)	21.67* ±0.59(13)	24.40* 0.96(8)	25.33* 0.79(11)

( ), # of animals; *=P<0.001 Compared with saline group.

Decreased plasma glucose and elevated blood lactate levels were indicative of endotoxin shock. The data indicate a depression of basal AIB uptake and a decreased ability of insulin to stimulate AIB uptake by skeletal muscle during endotoxin shock. (Supp. by the William G. Potts Research Funds.)

55.6

DEPRESSED SURVIVAL RATE AND METABOLIC RESPONSES AFTER ENDOTOXIN CHALLENGE IN THE CONSCIOUS VASOPRESSIN-DEFICIENT RAT. D.J. Brackett, C.F. Schaefer, P. Tompkins and M.F. Wilson. VAMC, Depts Med. and Anesthesiol., OUHSC, Okla. City, OK 73104.

We have reported the importance of vasopressin (VP) in maintenance of cardiovascular function during early endotoxin (E) shock and that prophylactic corticosteroid treatment (Rx) improved the early hemodynamic response to E in VP-deficient rats. This study evaluated the 24 hr survival rate (SR), pH, pO_2, pCO_2, glucose (G), and hematocrit responses to E in Rx and unRx male homozygous Brattleboro rats (B) lacking VP. Results were compared with Rx and unRx Sprague-Dawley rats (SD) having intact VP systems. Cannulae were placed in the carotid artery and jugular vein. After recovery, Methylprednisolone (300 mg/kg)(Upjohn Co.) or an equivalent amount of saline was given followed 15 min later by E (20 mg/kg)(Difco). Arterial blood (0.4 ml) was taken from each group (grp)(6/grp) before E and 60 and 240 min after E. An additional 4 grps (10/grp) were tested for SR. Nine of 10 unRx B did not survive the E challenge for 2 hr. Before death unRx B presented acidosis and hemoconcentration (H) with no increase in G. Rx B were similar in response to Rx SD with an increase in G and no abnormal pH alteration or H. SR in both Rx grps was 80%. None of the unRx SD survived 15 hr. At 4 hr after E unRx SD presented acidosis, H, and hypoglycemia. These results indicate early death resulting from E in VP-deficient rats is due in part to inadequate respiratory compensation to the initial E insult accompanied by rapid H. These deleterious events are significantly alleviated by Rx.

55.7

DOES HYPOGLYCEMIA BREED ENDOTOXEMIA? James P. Filkins, Dept. of Physiology, Loyola Univ. Medical Center, Maywood, IL 60153

Profound, progressive hypoglycemia is a constant occurrence in endotoxin shock and contributes to depressed RES clearance of exogenous endotoxin. This study evaluated the premise that hypoglycemia per se interferes with physiological host defense against gut-released endotoxin and thus breeds an endogenous endotoxemia. Fasted male Holtzman rats were treated with saline (Euglycemic Controls EG) or insulin (2U,sc) to produce hypoglycemia (HG). Plasma samples were obtained from portal (P) and systemic (S) blood for analysis of endotoxin using both the Limulus gelation test (LGT) and bioassays in lead-sensitized rats. Plasma glucose (PG, mg/dl) was determined with YSI Model 23A Glucose Analyzer.

Group	PG P	PG S	+ LGT P	+ LGT S	Bioassay Lethality P	Bioassay Lethality S
EG	130±4 (41)	120±3 (42)	11/28	8/28	6/20	1/20
HG	15±2 (49)	11±2 (54)	37/38	22/28	18/20	15/20

Hypoglycemia resulted in increased portal and systemic endotoxemia as determined both by the LGT and bioassay. These results support the concept that hypoglycemic depression of the RES clearance of endotoxin breeds endogenous endotoxemia, which in turn may further exacerbate the pathogenesis of endotoxic shock. (Supp. by The Earl M. Bane Charitable Trust Fund)

55.8

BENEFICIAL EFFECTS OF AUTOTRANSPLANTED MUSCLE TISSUE (AMT) ON SURVIVAL FOLLOWING SPLENECTOMY (SPLY) AND SEPSIS. Irshad H. Chaudry, Sarah A. Schleck*, Mark G. Clemens, Masanori Ohkawa* and Arthur E. Baue. Dept. of Surgery, Yale Univ. School of Medicine, New Haven, CT 06510.

Since autotransplanted splenic tissue (AST) improves the survival of animals following SPLY and sepsis, we investigated whether the protective effect offered by the AST was specific to the spleen or whether it was due to a non-specific stimulation of the RES. To determine this, 4 groups of rats were studied: Group 1 - sham; Group 2 - SPLY; Group 3 - received 100 mg heterotopic AST in an omental pocket after SPLY and Group 4 - received 100 mg heterotopic AMT (from the abdominal wall) in an omental pocket after SPLY. Seven days thereafter, the cecum was ligated and punctured and removed 16 hrs later in all groups. The survival rates (5 days) in Groups 1, 2, 3 and 4 were 60%, 40%, 84% and 85%, respectively (Groups 3 and 4 $p<0.05$ compared to Group 2). Thus, survival after SPLY can be improved by placing either a fragment of the spleen or the muscle in the omental pocket. Additional studies have shown that AMT as well as AST stimulated the RES function. Since both tissues appeared necrotic at 7 days following autotransplantation, the RES stimulation and improved survival following SPLY and sepsis appears to be due to the presence of a necrotic tissue irrespective of the organ. Thus, non-specific immunostimulation may bear promise for improving the survival rates following SPLY and sepsis.

55.9

PRETREATMENT WITH NALOXONE (NAL) ATTENUATES THE CARDIOVASCULAR EFFECTS OF ENDOTOXIC SHOCK IN DOGS. E. Ganes,* N.J. Gurll, D.G. Reynolds, and T. Vargish.* University of Iowa College of Medicine, Iowa City, IA 52242

We have previously shown that opiate receptor blockade with NAL reverses the decreases in mean arterial pressure (MAP), cardiac output (CO), and left ventricular contractility (LV dp/dt max) found during E. coli endotoxic shock in dogs. To investigate whether these effects require an established shock state, we anesthetized adult mongrel dogs with pentobarbital. The dogs were treated with NAL 2 mg/kg plus 2 mg/kg/hr i.v. from t=0 to 120 min (n=5) or 0.9% NaCl m equivalent volumes as a control (CON, n=5). E. coli endotoxin 1.5 m/kg (LD_{80}) was given i.v. at t=15. NAL attenuated the fall in MAP and LV dp/dt max:

		t=0	15	20	60 min
MAP	CON	144±7	140±4	51±5	90±4
(mmHg)	NAL	146±6	152±7	99±11*	99±9
CO	CON	167±15	169±20	43±2	101±15
(ml/kg/min)	NAL	176±15	202±28	57±4	110±14
LV dp/dt max	CON	2.2±0.3	2.1±0.2	0.8±0.1	1.4±0.1
(mmHg X 10^3/sec)	NAL	2.1±0.2	2.5±0.4	1.4±0.2*	1.9±0.2

*p<.05 versus CON

4 NAL but only 1 CON dogs survived 72 hrs. NAL pretreatment attenuates the cardiovascular depression in endotoxic shock with improved survival. This lends support to the role of endorphins acting on opiate receptors in the pathophysiology of cardiovascular shock.

55.10

MYOCARDIAL PROTECTION DURING ENDOTOXEMIA: NALOXONE VERSUS IBUPROFEN. M. E. Soulsby, E. R. Jacobs* and B. H. Perlmutter*. Univ. Arkansas Medical Sciences, Little Rock, AR 72205.

Mongrel dogs were anesthetized with pentobarbital, paralyzed with pancuronium bromide, ventilated on room air, administered 4 mg/kg E. Coli endotoxin, and treated with either ibuprofen or naloxone (25 mg/kg). After 3 hrs, animals were sacrificed by removal of heart, from which a microsomal fraction was isolated by differential centrifugation. Ca^{+2}-Mg^{+2} ATPase activity and calcium uptake were monitored simultaneously using $^{45}CaCl_2$. Microsomes from hearts of control animals were able to actively sequester calcium at a rate of 1.20 ±0.06 μmoles/mg protein/min while hydrolyzing ATP at a rate of 1.20±0.06 μmoles P_i/mg protein/min. Microsomes isolated from hearts of animals receiving endotoxin only were able to sequester calcium at a rate of 0.3±0.07 μm/mg/min while hydrolyzing ATP at a rate of only 0.62±0.06 μm P_i/mg/min. Microsomes isolated from hearts receiving endotoxin followed by ibuprofen were able to sequester calcium at a rate of 1.4±0.16 mm/mg/min while hydrolyzing ATP at a rate of 0.98±0.07 μm P_i/mg/min, rates not significantly different from control. Microsomes isolated from hearts of animals receiving endotoxin followed by naloxone were able to hydrolyze ATP at a rate of 0.94±0.10 μm P_i/mg/min ($p>0.05$), but calcium uptake was only 0.40±0.08 μm/mg/min ($p<0.01$). Data indicate that ibuprofen overcomes the negative inotropic myocardial influence of endotoxemia while naloxone is associated with an uncoupling of ATP-ase activity from calcium uptake. Supported in part by AHA.

55.11

THYROTROPIN RELEASING HORMONE (TRH) IN PRIMATE ENDOTOXIC SHOCK. D.G. Reynolds, N.J. Gurll, J. Holaday*, and E. Ganes*. Univ. of Iowa College of Med., Iowa City, IA. 52242 and Walter Reed Army Institute of Research, Washington, DC. 20012

Endorphins acting on opiate receptors have been implicated in the pathophysiology of cardiovascular shock because naloxone, an opiate receptor antagonist, improves cardiovascular function and survival in several shock paradigms. TRH has anti-endorphin like effects but not via opiate receptor blockade. Adult cynomulgus monkeys were anesthetized with N_2O/O_2 and instrumented to measure mean arterial pressure (MAP) and left ventricular contractility (LV dp/dt max) to see if TRH would be effective in shock. E. coli endotoxin 5 mg/kg (LD_{80}) was given i.v. at t=0. The monkeys were treated when MAP reached its nadir at t=10-75 min with TRH 2 mg/kg plus 2 mg/kg·hr i.v. (n=5) or 0.9% NaCl as a control (CON, n=5). TRH significantly (p<.05 by analysis of variance) improved cardiovascular function over CON:

		t=0	60	90	120 min
MAP	CON	68±4	56±7	60±7	70±4
(mmHg)	TRH	59±7	81±3	87±4	91±7
LV dp/dt max	CON	1.7±0.4	2.1±0.8	2.4±0.7	2.2±0.7
(mmHg x 10^3/sec)	TRH	1.9±0.6	3.3±0.7	3.2±0.7	3.0±0.7

There were no differences between CON and TRH in survival. TRH improvement of cardiovascular function in shock and its lack of antinociceptive effects makes it an attractive alternative to naloxone in the treatment of endotoxic shock. (Supported by U.S. Army contract DAMD 17-81-C-1177).

55.12

COMPARISON OF DIFFERENT STEROID/ANTIBIOTIC PROTOCOLS ON RECOVERY FROM LD_{100} E. COLI-INDUCED SHOCK. L.B. Hinshaw, B.K. Beller*, L.T. Archer*, D.J. Flournoy*, and R.B. Passey*. VA Medical Center and Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma 73104.

Twenty-four dogs were given the corticosteroid methylprednisolone sodium succinate (MPSS) by "intermittent bolus administration" or "continuous infusion" in combination with the aminoglycoside antibiotic gentamicin sulfate (GS) or netilmicin (N) as therapy for LD_{100} E. coli-induced shock. Adult dogs of either sex were anesthetized and aseptically catheterized for infusing E. coli and drugs, sampling blood, and monitoring hemodynamic changes. Animals were divided into four experimental groups: Group I, E. coli only; Group II, E. coli + MPSS (constant inf.) + GS; Group III, E. coli + MPSS (bolus) + GS; Group IV, E. coli + MPSS (constant inf.) + N. LD_{100} E. coli (1.0 - 1.2 x 10^{10}/kg) was infused for one hour. MPSS was begun at +15 minutes and then administered over 6 hours by "continuous infusion" or "intermittent bolus" (total dose 60 mg/kg). Intermittent infusions of GS or N were initiated after E. coli infusion was completed (total doses/6 hrs: GS = 10.5 mg/kg; N = 31.5 mg/kg). The number of dogs surviving after seven days/total number of dogs in the group were as follows: Group I = 0/5; Group II = 6/6; Group III = 4/7; Group IV = 5/6. (This research was supported by the Veterans Administration Research Service).

CONTROL OF BREATHING: GENERAL

56.1

PROGESTERONE INDUCES HYPERVENTILATION IN THE GUINEA PIG. J.D. Hosenpud*, M.V. Hart*, M.J. Morton* and A.R. Hohimer. Oregon Health Sciences University, Portland, Oregon 97201

Hyperventilation (H) occurs during human pregnancy but has been difficult to demonstrate in other species. We investigated arterial blood gases (ABG) at end-gestation and during chronic progesterone (Prog) administration in the guinea pig (GP) to determine whether H occurs during GP pregnancy, and what role Prog might play. ABG were obtained from aortic catheters in awake, mildly restrained animals. ABG in term animals (Preg) and female controls (C) were:

(Mean±SD)	pH	P_{CO_2}	$[HCO_3^-]$	P_{O_2}
Preg (n=5)	7.51±.05	31.2±2.9	24.6±2.5	79.6±3.8
C (n=6)	7.41±.05	37.5±5.8	22.9±3.2	79.2±5.5
P	<.005	<.025	N.S.	N.S.

Virgin female GPs received Prog-filled subcutaneous silastic implants in order to achieve chronic serum levels seen during pregnancy. Weight-matched C GPs were dosed with cholesterol implants. Prog levels at 2 and 4 weeks were 564.3 ng/ml and 139.9 ng/ml, respectively, in the Prog GPs. ABG were:

Prog (n=8)	7.43±.04	31.0±3.7	19.9±2.5	81.2±4.3
C (n=10)	7.40±.05	36.2±3.7	21.8±1.6	80.2±6.1
P	N.S.	<.005	<.05	N.S.

CONCLUSION: Hyperventilation is present during GP pregnancy and in virgin animals chronically administered Prog. Increased Prog may be the mechanism for H in pregnant GPs; but the extent of compensatory metabolic acidosis may be separately influenced by pregnancy.

56.2

REFLEX LENGTHENING OF EXPIRATION DURING HFV AT CONSTANT LUNG VOLUME IN ANESTHETIZED DOGS. Robert Banzett, John Lehr* and Barbara Geffroy*, Harvard School of Public Health, Boston, MA 02115

Thompson et al. (JAP, 51:1484-7) reported that high frequency ventilation (HFV) produces apnea in barbiturate anesthetized dogs. They did not, however, measure or control mean lung volume. Kafer et al. (Physiologist) attributed apnea produced by HFV in halothane anesthetized dogs entirely to an increase in mean lung volume (Hering-Breuer reflex). We tested the response of 8 barbiturate anesthetized dogs to HFV (30-60 cc at 15 Hz) while measuring and controlling lung volume. We found that HFV lengthened expiratory time (Te) in 5 of 8 dogs, even when mean expiratory lung volume was not allowed to rise and blood gases were constant. Maximum increases in Te in individual dogs ranged from 50% to apnea. This response occurred in the first breath. Inspiratory time and peak diaphragm electrical activity were unaffected. In some dogs abdominal muscle activity was inhibited. If lung volume was allowed to rise during HFV, Te was lengthened further. In all cases, vagotomy abolished the response of Te to HFV. We conclude that HFV reflexly lengthens expiration in some dogs and not in others. We could find no explanation for the difference in individuals in depth of anesthesia, body temperature, sex, health, size, or sensitivity of the Hering-Breuer reflex. (Supported by NIH HL19170, HL26566, and HL14580.)

56.3

ACTIVATION OF FETAL BREATHING. Immanuela R. Moss and Emile M. Scarpelli. Pediatric Pulmonary Division, Albert Einstein College of Medicine, Bronx, New York 10461.

We used acute fetuses in utero or in liquid bath to correlate electrophysiologically and behaviorally determined sleep/wake states with spontaneous breathing and with breathing activated by CO_2, naloxone and cooling. We placed electrodes for definition of sleep/wake states, an arterial catheter for injections and measurements of pressure, pHa, $PaCO_2$ and PaO_2, and both intratracheal and intraesophageal catheters for measurement of fetal breathing movements and swallowing. We recorded the onset, pattern and offset of breathing and estimated fetal respiratory responsivity (threshold; sensitivity) to CO_2 rebreathing by the ewe using our fetal CO_2 test. Spontaneous breathing occurred in either active sleep (AS) or with wakefulness (W). Activation of breathing by CO_2, naloxone or cooling either accompanied or followed the occurrence of W. Breathing threshold to CO_2 was lower when the transition was from AS to W than from quiet sleep to W. It was also lowered by cooling and naloxone. Offset of breathing was associated with the resumption of sleep, often preceding, otherwise accompanying it. These results are not in disagreement with those obtained in chronic fetal preparations, thus demonstrating the validity of acute experimentation. These studies provide evidence for the importance of wakefulness (arousal) to normal postnatal respiratory responsivity and for the possible potential therapeutic use of naloxone as a respiratory activator. (Supported by NIH grants HL 00688, HL 23995 and HL 07060).

56.4

ANALYSIS OF THE STOCHASTIC CHARACTERISTICS OF STEADY-STATE VENTILATORY RESPONSES IN MAN. Jens I. Jensen* and Susan A. Ward. Univ. Lab. Physiol., Oxford Univ., England, Inst. Physiol Aarhus Univ., Denmark and Dept. Anesthesiology, UCLA.

While the pattern of temporal variation in steady-state ventilatory responses should provide insight into the operation of the ventilatory controller, there have been few attempts to formally analyze such variation. We have therefore investigated the short-term, steady-state temporal variability of ventilation ($\dot{V}_E$) and its components (V_T, T_I, T_E) during eupnea and in response to increased central chemoreflex drive (hypercapnic hyperoxia) and to increased central and peripheral drives (hypercapnic hypoxia) both at rest and during moderate cycling. Three healthy adults were studied, each condition being repeated 2-3 times. Unbroken records (300 breaths) of the ventilatory variables were obtained breath-by-breath. The temporal characteristics of the responses were generally uniform: a decaying positive autocorrelation function; a power spectrum having high power at low frequencies; and the cumulated spectrum of the residuals after filtering with a first-order autoregressive filter not, or only just, exceeding the 95% confidence limits. The simplest, adequate description of the pattern of short-term temporal variation thus appears to be white noise superimposed upon a first-order autoregressive process. Therefore, under these experimental conditions, the ventilatory controller can be viewed as having a one-breath memory, with the noise most probably being incorporated at some point in its efferent pathways.

56.5

ALTERED VENTILATORY AND GAS EXCHANGE KINETICS IN CHRONIC AIRWAYS OBSTRUCTION (CAO). K. Wasserman, L.E. Nery*, J.A. Davis, D.J. Huntsman*, J.E. Hansen and B.J. Whipp. Harbor-UCLA Med. Center, Torrance, California 90509.

To characterize the role of CAO on the coupling between the ventilatory and cardiovascular ($\dot{Q}$) response to moderate exercise, we studied 9 patients with CAO and 6, age-matched controls. Cycle ergometry was repeated 4 times, from rest to sub-anaerobic threshold work rate for 8 min. $\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}O_2$ and HR were determined breath-by-breath and the mean response to the 4 transitions was obtained. The rapid, $\dot{Q}$-related increases (phase I) and the slower, exponential responses (phase II) were then determined. The CAO group had a smaller phase I response in $\dot{V}_E$ (3.4 vs 6.8 L/min), $\dot{V}CO_2$ (.10 vs .22 L/min), $\dot{V}O_2$ (.10 vs .24 L/min) and HR (6 vs 16 bpm) than the controls, the response of $\dot{V}_E$ being correlated with that of $\dot{V}O_2$ ($r = .88$) and HR ($r = .78$). This indicates a proportional reduction of $\dot{V}_E$ and $\dot{Q}$ responses during phase I in CAO, consistent with the hyperpnea being cardiodynamic. The HR response in phase II (t_{50}) was slower in CAO (68 vs 32 s) than the controls. The phase II time constants (τ) were also slower in CAO, for $\dot{V}_E$ (87 vs 65 s), $\dot{V}CO_2$ (79 vs 63 s) and $\dot{V}O_2$ (56 vs 39 s), but unrelated to the indices of bronchial obstruction. However the ratios $\tau\dot{V}O_2/\tau\dot{V}_E$ and $\tau\dot{V}CO_2/\tau\dot{V}_E$ were similar in both groups. Thus, the slowed phase II $\dot{V}_E$ kinetics appear unrelated to ventilatory mechanical limitation or attenuated chemosensitivity in the CAO group, but rather follow a slowed cardiovascular response.

56.6

CSF SAMPLING AND ANALYSIS FOR CHRONIC RESPIRATORY STUDIES IN AWAKE RATS. Y.L. Lai, P.M. Smith*, W.J.E. Lamm*, and J. Hildebrandt. Virginia Mason Res. Ctr., Seattle, WA 98101.

Several days before CSF sampling began, 22-G stainless steel or Teflon catheter was implanted in the cisterna magna of 20 anesthetized rats via a midline hole immediately rostral to the interparietal-occipital suture. Both CSF samples (slowly withdrawn) and blood samples from the same awake rats breathing air were collected in glass capillary tubes and pH, P_{CO_2} and P_{O_2} were determined immediately by Radiometer (See Table). About 140 µl of CSF was required to measure all three parameters, the last 20 µl being pushed into the electrode in small increments. Our data, along with literature values from awake goats and ponies, fit closely to the in vivo CSF CO_2 buffer line of 28.7 sl. (Lee et al. AJP 217:1035, 1969). Except for P_{CO_2}, we found no significant difference between the first sample and samples taken at least 4 hr later. CSF sampling did not significantly alter breathing patterns. Thus, it is possible to demonstrate the feasibility of CSF sampling in awake rats for respiratory studies. (Supported by NIH grant HL 20568).

	pH	P_{CO_2} (Torr)	$[HCO_3^-]$ (meq/l)	P_{O_2} (Torr)
CSF	7.400**	36.2	21.3*	88.8
(n=15)	±0.052	± 4.8	± 4.5	±33.0
Plasma	7.445**	34.8	23.7*	87.2
(n=17)	±0.021	± 2.5	± 1.9	±13.0

Means ± SD. *$P < 0.05$, **$P < 0.01$.

56.7

THE ASSESSMENT OF HYPOXIC VENTILATORY DRIVE. Fred H. Carlin* and Steven M. Horvath. Institute of Environmental Stress, University of California, Santa Barbara, CA 93106

The wide variations in hypoxic ventilatory drive may be due as much to the method used in the analysis of the experimental data as to the inherent variability from subject to subject. The commonly used method of assessing ventilatory drive is to find the best fit of the experimental data to the hyperbolic equation $V_E = Vo + A/(PO_2 - 32)$ where V_E is the minute volume, PO_2 the alveolar oxygen tension, C is a constant, i.e. a PO_2 of 32 where the ventilation is presumed to be infinite, Vo is the euoxic ventilation, and A is a measure of hypoxic drive. The value chosen for C as well as very small systematic errors in the measurement of PO_2 can greatly affect the value computed for A. The interaction between C and the measured PO_2 raises questions as to the significance of the parameter A as an accurate indicator of hypoxic drive. We propose an alternate method of assessing hypoxic ventilatory drive. The proposed analysis method computes two descriptors for each subject: a value So related to the hypoxic sensitivity, and a value Bo related to the hypoxic threshold. The analysis does not presume that ventilation reaches infinity for all subjects as does the hyperbolic analysis, nor is the sensitivity measurement affected by systematic errors in the measurement of PO_2.

56.8

EFFECT OF HYPOGLYCEMIA ON BRAIN PCO_2 IN FETAL LAMBS. John M. Bissonnette, Bryan S. Richardson*, A. Roger Hohimer, Cynthia M. Machida*, Sharon J. Knopp*. Oregon Health Sciences University, Portland, Oregon 97201.

We have previously reported that fetal hypoglycemia induced by fasting pregnant ewes for 24-36 hours results in a significant decrease in the incidence of fetal breathing movements. We have also shown that fetal breathing movements are influenced by central chemoreceptors (Physiologist 24:102, 1981). Since glucose is the sole substrate for fetal brain metabolism, hypoglycemia may alter the chemical environment of the medulla. Catheters were placed in the trachea, axillary artery and saggital vein of 8 lambs in utero. We also obtained well differentiated electrocortical activity (ECOG) from supradural leads in 4 of these lambs. Experiments were carried out 4-8 days postoperative. Fasting the ewe caused fetal arterial glucose to fall from 1.08 ± 0.13 (SEM) mM to 0.66 ± 0.07 mM and the incidence of fetal breathing fell from 38.6 ± 5.5 percent of the 3 hour observation period to 25.3 ± 5.7%. Low voltage ECOG fell from 66.7 ± 5.6 to 48.1 ± 4.2 percent with fetal hypoglycemia. Cerebral blood flow fell from 167 ± 21.3 ml·100 $grams^{-1}$·min^{-1} to 130 ± 10.1. Saggital vein Pco_2 decreased from 53.0 ± 1.0 torr to 49.2 ± 1.6 torr with fetal hypoglycemia. Fetal arterial Pco_2 had also dropped from 48.8 ± 1.0 to 45.4 ± 1.6 torr. We conclude that hypoglycemia produces a decrease in cerebral CO_2 tension which contributes to the depression of respiratory activity.
(Supported by HD 11251 from USPHS).

56.9

RESPONSES OF LARYNGEAL MECHANORECEPTORS TO TRANSMURAL PRESSURE IN THE UPPER AIRWAY. G. Sant'Ambrogio, O.P. Mathew*, J.T. Fisher, F.B. Sant'Ambrogio* and J. P. Farber. Departments of Physiology and Pediatrics. University of Texas Medical Branch, Galveston, Texas 77550.

Changes in upper airway pressure modify the pattern of breathing, primarily through afferents in the superior laryngeal nerve (SLN). In this study we examined the response of SLN-innervated laryngeal mechanoreceptors (LM) to alterations in transmural pressure. Three anesthetized dogs breathing through a tracheostomy were studied. Pressure was independently varied in the isolated upper airway. Single unit action potentials were recorded from the internal branch of the SLN. Twenty-eight LMs were challenged with both positive and negative pressure. Nineteen LMs increased discharge frequency with a slowly adapting behavior as pressure became more negative, 2 responded with a rapidly adapting response to a steady negative pressure while the remaining 7 were not affected. Positive pressure elicited a transient "on" and "off" response in 14 receptors, a slowly adapting discharge in 4 LMs, no response in 9 LMs and inhibition in the remaining receptor. When the motor supply to the larynx was intact the LMs showed an inspiratory modulation. Negative pressures in the upper airway induce decreases in breathing rate while positive pressure produce inconsistent ventilatory effects.[1] The response to negative pressure may arise from the observed stimulation of LMs. (Supported by NIH grant HL-20122).

1) J.A.P. 52: 445, 1982.

56.10

COMPONENTS OF OXYGEN EFFECTS ON CAROTID BODY CHEMOSENSORY ADAPTATION TO CO_2. S. Lahiri. University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

Although the same single chemosensory afferent from carotid body responds to both hypoxia and hypercapnia in an interactive pattern, it is possible to block the hypoxic response by metabolic inhibitors leaving the hypercapnic response in a modified form (Mulligan and Lahiri, 1982). In order to further investigate the role of oxygen in the chemoreceptor responses, we compared the effects of Antiomycin A and oligomycin with those of hypoxia on the time-dependent unsteady-state and steady-state responses to CO_2 stimulus of carotid chemoreceptors in the anesthetized cats. We confirmed the previous observation (Lahiri, Mulligan and Mokashi, 1982) that hypoxia augmented both initial and steady-state responses. After the initial response chemosensory discharge adapted down to a steady-state level which was greater at lower PaO2. The rate of adaptation from the peak activity appeared to be independent of PaO2. Antimycin A and oligomycin, the common effect of which is to decrease cellular phosphate potential, eliminated hypoxic chemoreception, augmented the initial peak response but not the final steady-state response to CO_2, and did not inhibit adaptation. Thus, unlike the steady-state response, augmentation of the initial response to CO_2 is independent of oxygen chemoreception. Since adaptation following the initial peak response occurs even in the absence of O_2 chemoreception and oxidative metabolism one may infer that the process is not a function of oxygen. (Supported in part by the NIH Grant HL-19737-06).

56.11

THE EFFECTS OF LOW-LEVEL OZONE EXPOSURE ON THE BREATHING PATTERN FOLLOWING SHIFTS IN TRANSRESPIRATORY PRESSURE IN AWAKE DOGS. M. H. Johnson,* J. R. Gillespie, Dept. Physiol. Sci., Univ. of Calif., Davis, Ca. 95616.

Low level ozone exposure has been shown to cause small airway epithelial lesions in a variety of experimental animals. We hypothesized that these lesions may cause injury to pulmonary mechanoreceptors leading to changes in pattern of breathing (depth and/or frequency). To test this hypothesis, we measured the ventilatory response of 8 awake beagle dogs to transrespiratory pressure (P_{RS}) changes of -5, -10, +5 and +10 cm H_2O. We compared their tidal volume (V_T), frequency (f), inspiratory and expiratory periods (T_i and T_e respectively) before and immediately after exposure to 0.40 ppm ozone for 7 days continuous exposure. Following ozone exposure, we found a significant decrease ($p < 0.05$) in V_T during the control breathing ($P_{RS} = 0$) period and after pressure breathing (lung inflation; $P_{RS} = +5, +10$ cm H_2O). There was no significant change in f, T_i or T_e following exposure. Minute ventilation ($\dot{V}$), however, also decreased.

There were no significant changes in breathing pattern associated with deflation of the lung following ozone exposure. We conclude that the stretch receptors of the lung are affected by the ozone induced small airway lesion. This finding correlates well with the known location of these receptors and the ozone-lesion in the small airways. (Supported in part by: U.S.E.P.A. Grant 807661 and NIEHS Grant ES00628).

56.12

REINVESTIGATION OF THE EFFECTS OF MUSCLE AFFERENTS ON THE RESPIRATORY CENTRE COMPLEX. P.Gill-Kumar* and C.B.G. Campbell*. Walter Reed Army Institute of Research, Washington, D.C. 20012.

The effects of electrical stimulation of cut central ends of gastrocnemius nerves (GNS) and calf muscle stretch (CMS) on neural respiratory output were studied in anesthetised, paralysed cats with vagi and carotid sinus nerves cut. Peak integrated phrenic activity (VTn), inspiratory time (TI), and expiratory time were measured. RESULTS (1) As reported earlier (Eldridge et al, 1981) respiratory stimulation due to CMS was preceded by a short inhibition (3 out of 4 cats), and increase in VTn was always associated with decrease in TI and TE. (2) GNS caused no initial respiratory inhibition (5 cats); increase in VTn was usually associated with decrease in TE; TI however decreased significantly only in 1 cat and slightly in another (out of 6) (3) Both GNS and CMS produced increase in arterial pressure. It is concluded that; (1) Initial respiratory inhibition and decrease in TI produced by CMS are due to activation of some non-muscle afferents by the manual procedure used. (2) The effects of GNS on TI and TE are best explained by the hypothesis that there are two distinct central mechanisms for the termination of inspiration and expiration.
(P.Gill-Kumar is a senior research associate of the National Research Council.)

LUNG FLUID BALANCE

57.1

MEASUREMENT OF VOLUME OF DISTRIBUTION AND MICROVASCULAR PERMEABILITY OF TRACER MOLECULES IN RABBIT LUNGS. Melinda S. Kwong & Edmund A. Egan, Depts. of Physiol. & Peds., SUNYAB and Children's Hospital, Buffalo, NY 14222

We have measured the accumulation of ^{57}Co-cyanocobalamin (B12), ^{125}I-cytochrome C, and ^{131}I-albumin in rabbit lungs at various times after bolus injection. Total lung tissue was counted in a well scintillation counter and lung blood volume calculated from ^{51}Cr-Red Blood Cell counts. Wet-dry weights were measured. We found that ^{57}Co-B-12 has an identical plasma decay curve to ^{14}C-sucrose after bolus injection and the volume of distribution of the two tracers in extravascular lung water is the same. ^{57}Co-B-12 equilibrates in the lung within 2 mins. after injection and has a volume of distribution of 37±6% of extravascular water. ^{125}I-Cyto-C has a $T_{\frac{1}{2}}$ of 7 mins. and equilibrates with a volume of distribution of 59±5% of extravascular water, (higher than ^{57}Co-B-12, $p<.01$). At 24 hrs. ^{131}I-albumin has a volume of distribution of 10% of extravascular water, (lower than the other tracers $p<.01$), and a $T_{\frac{1}{2}}$ of 50-60 mins. The volume of distribution of 57 Co-B-12 and ^{125}I-Cytochrome C are the same in heart, muscle, gut and brain tissue; only the lung appears to accumulate larger amounts of cytochrome C than expected. Labeled cyanocobalamin appears to be a convenient and accurate tracer to measure total extravascular, extracellular lung water; the use of protein tracer accumulation in lung to measure microvascular permeability requires definition of their individual apparent volumes of distribution. (Supported by HL 22552-03)

57.2

REMOVAL OF PORTAL SYSTEM CONTRIBUTION TO CAUDAL MEDIASTINAL NODE (CMN) EFFERENT LYMPH IN ANESTHETIZED SHEEP. P.J. Roos*, K.H. Albertine*, J.P. Wiener-Kronish*, P.L. Culver* and N.C. Staub. Cardiovasc. Res. Inst. and Depts. Physiol., Med. and Anat., Univ. of California, San Francisco, CA 94143.

The contribution of peritoneal fluid to CMN lymph can be reduced to low levels by superficial cauterization along the esophagus and diaphragm to destroy aberrant lymphatics (PHYSIOLOGIST 24:53, 1981). Baum (1912) showed transdiaphragmatic lymphatics from liver and spleen. In 2 anesthetized, ventilated sheep with CMN efferent lymph cannulas, we inflated a balloon to raise pressure in the inferior vena cava (Pivc) below the hepatic veins. There was no change in lymph flow ($\dot{L}$) or lymph/plasma protein concentration ratio (L/P). In 4 sheep, we raised Pivc above the hepatic veins for 1-1½h, before (1) and after (2) cauterization. The data are summarized in the table (mean ± S.D.).

	Pivc (cmH_2O)	$\dot{L}$ (ml/h)	L/P (total protein)
1. Baseline	4.1±4.2	4.6±1.2	.72±.05
↑ Pivc	19.1±4.9	12.8±3.5	.91±.03
2. Baseline	3.4±3.6	6.8±1.1	.76±.05
↑ Pivc	16.2±6.7	7.7±3.3	.84±.03

Some aberrant lymphatics come from the portal system (probably liver since L/P increased). Cauterization eliminates most of them. Control studies of CMN lymph are necessary when hepatic venous pressure is high or when abdominal organs are injured. [Supported by HL25816 (PPG), HL7156 (PFTG) and HL6168 (NRSA)].

57.3

LUNG DRY WEIGHT ESTIMATED BY HYDROXYPROLINE CONTENT IN SHEEP AND RABBITS WITH PULMONARY EDEMA. M. Julien*, J. Hoeffel*, S.C. Rall*, J.F. Murray and M.R. Flick. University of California, San Francisco, CA 94143

When gravimetric lung water is determined in animals with increased permeability edema, the dry, blood-free weight of the lung (dQl) is found to be higher than in controls. This has been attributed to blood protein leak into the extravascular space. The dry weight of these proteins may increase dQl, thereby lowering extravascular lung water (Qwl)/dQl ratio. Hydroxyproline (HP), an amino acid found in interstitial collagen but not in blood, should not be altered if proteins leak into the lung. We measured HP, Qwl and dQl in control and in oleic acid or endotoxin injured animals. Mean data (±SD):

	(4) SHEEP control	(11) edema	(5) RABBITS control	edema
Qwl/HP (gmH_2O/ng)	0.27±0.01	0.43±0.19	1.2±0.6	2.3±0.8
Qwl/dQl (gmH_2O/gm)	4.1±0.2	5.3±0.8	4.0±0.2	4.7±0.3
HP/dQl (ng/gm)	14.9±0.6	13.5±3.1	3.9±1.3	2.5±0.7

HP content of lungs was highly reproducible. In edema Qwl/HP increased more than Qwl/dQl in both sheep (60% vs 30%) and rabbits (90% vs 20%). Blood proteins add about 10% to sheep and 35% to rabbit dQl, causing underestimation of lung water in increased permeability edema.
(Supported by MRC Canada and NIH Grants HL-26913 and HL-19155)

57.4

THE EFFECTS OF HIGH FREQUENCY OSCILLATION (15 Hz) ON LYMPH FLOW, LYMPH PROTEIN FLUX AND LUNG WATER. K. Rehder, D. Martin* T.J. Knopp, and A.E. Taylor, Department of Anesthesiology, Mayo Clinic, Rochester, MN, and Department of Physiology, University of South Alabama, Mobile, AL.

Mongrel dogs were anesthetized with sodium pentobarbital (30mg/kg), the chest opened and a small prenodal lung lymphatic cannulated. Pulmonary arterial and left atrial pressure, cardiac output, total protein in lymph and plasma, and lung blood free wet/dry weight ratios were measured for controls (conventional mechanical ventilation) and at a high frequency oscillation of 15 Hertz using a stroke volume of 40-50mls. Lymph flow and lung water increased (68% and 20%,respectively) during high frequency oscillation and the lymph to plasma total protein concentration ratios (C_L/C_p) remained essentially unchanged. When left atrial pressure was increased, C_L/C_p decreased, indicating that pulmonary vascular permeability was not affected by the high frequency ventilation. The increased lung water and lymph flows associated with high frequency oscillation reflect either 1) an increased surface area for protein exchange, and/or 2) changes in Starling forces which favor filtration. The Starling force change may be a more negative interstitial pressure surrounding the perivascular spaces resulting in a slight interstitial volume expansion and an increased capillary filtration.
*D. Martin is a Parker B. Francis Pulmonary Fellow.
(Supported by HL21584 and HL22549.)

57.5

EFFECT OF HIGH FREQUENCY OSCILLATION (HFO) ON LUNG LYMPH FLOW. A.L. Jefferies*, P. Hamilton*, A.C. Bryan and H. O'Brodovich* (SPON: N. Jones). Dept. of Paediatrics, McMaster University, Hamilton, Canada L8N 3Z5 and Depts. of Anaesthesia and Paediatrics, Hospital for Sick Children, Toronto, Canada M5G 1X8

Since lymphatics possess valves and undergo rhythmic contractions, we hypothesized that lung lymph flow (Q_L) would not decrease during HFO. On 5 occasions, sheep and goats (21 to 30 kg) with lung lymph fistulas were anesthetized, paralyzed, intubated and ventilated according to the following protocol: 1) conventional ventilation (IPPV) at 25-35 breaths/min until baseline Q_L was stable for 1 hr; 2) HFO at 15 HZ for 1-2 hrs; 3) IPPV for ½ hr. PaO_2 was maintained above 150 torr. Every 15 min. vascular pressures, thermodilution cardiac output (CO), Q_L, and lymph and plasma total protein concentration were determined. Mean airway pressure proximal to the endotracheal tube was continuously monitored with a National semi-conductor transducer and confirmed by cross clamping the tubing. Baseline Q_L during initial IPPV was 4.0±1.0 ml/hr. Results are mean±SE.

	CO (L/min)	$\overline{PA}$ (Torr)	Wedge (Torr)	$PaCO_2$ (Torr)	Q_L % baseline	L/P Protein Ratio
IPPV	3.6±.2	21±2	12±3	37±2	100%	.59±.04
HFO	3.6±.2	22±3	15±3	48±5	114%± 9	.59±.04
IPPV	3.6±.1	21±3	15±1	36±3	120%±25	.50±.05

Mean airway pressure was 6-9 torr during HFO. We conclude HFO does not decrease Q_L under resting conditions.

57.6

EFFECT OF EDEMA AND HEIGHT ON BRONCHIAL DIAMETER AND SHAPE IN EXCISED DOG LUNG. K.C. Beck, A.M. Sutcliff*, J.T. Donaldson* and S.J. Lai-Fook. Mayo Clinic, Rochester, MN 55905.

Mechanical interactions between the artery (A) and bronchus (B) of a lung cause B to be pulled from a circular cross-section (CS) by A so that B's largest diameter is perpendicular to the tangent plane between B and A. We measured maximum and minimum (Dmx, Dmn) diameters of B from tantalum bronchograms as edema progressed in 10 isolated lung lobes at transpulmonary pressures (Ptp) of 6 and 25 cmH20 with arterial pressure constant at 10 cmH20. As lobe weight increased to 3X normal with edema, Dmx/Dmn decreased from 1.08 to 1.0 at both Ptp=6 and 25 cmH20, though estimated CS area fell only at Ptp=6. We conclude that peribronchial interstitial pressure (Pi) increased and became more uniform as edema progressed. To see if a hydrostatic gradient in Pi exists, we measured Dmn at points along the bronchial length with 5 lobes hanging, hilum nondependent (Dmn(nd)), then inverted, hilum dependent (Dmn(d)). If a gradient in Pi existed, we expected Dmn(nd) > Dmn(d) at points near the hilum. However, Dmn was unaffected by lobe inversion at Ptp=25, 10, 6 and 4 cmH20 in normal and edematous lobes. At Ptp=2, Dmn(nd) < Dmn(d) near the hilum in normal lobes and the difference increased with added edema; hence no evidence for a hydrostatic gradient in Pi. Bronchial distortion due to inversion was modeled using a finite element analysis, and was found to be consistent with distortions caused by lung weight. (Supported by HL-21584 from NHLBI).

57.7

PERIMICROVASCULAR PRESSURE CHANGE WITH LUNG INFLATION. Alan C. Jasper* and Howard S. Goldberg. Division of Pulmonary Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048.

We determined the change in extraalveolar perimicrovascular interstitial hydrostatic pressure (P_{is}) resulting from a step change in static lung inflation in eight isolated canine left lower lobes. The vasculature was flushed at low pressure with autologous plasma and the pulmonary artery and vein were connected in common to a reservoir of autologous plasma. At a transpulmonary pressure (P_L) of 5 cmH_2O, interstitial compliance (C_{is}) was determined. This was followed by a return to an isogravimetric state with intravascular pressure at 0 cm H_2O. The lung was then inflated in a single step to P_L = 10 cmH_2O. The rise in interstitial volume (ΔV_{is}) was determined gravimetrically and ΔP_{is} was calculated ($\Delta P_{is} = \Delta V_{is}/C_{is}$); ΔP_{is} = -2.19 ± 1.78 cmH_2O ($\bar{x}$ ± SD). The lobe was then inflated to P_L = 15 cmH_2O and ΔP_{is} was an additional -2.24 ± 1.49 cmH_2O. Each mean ΔP_{is} was significant, but the mean falls in P_{is} with each 5 cmH_2O ΔP_L were not significantly different from one another. Therefore, over the range of pressures studied, each 1.0 cmH_2O increase in P_L reduces extraalveolar perimicrovascular pressure by 0.44 cmH_2O. (Supported by the James Irvine Foundation and the Herzog Estate Fund.)

57.8

PERIVASCULAR FLUID-CUFF SIZE AND PROTEIN CONCENTRATION IN ISOLATED AND INTACT DOG LUNG LOBES INFLATED WITH PROTEIN SOLUTION. R.L. Conhaim, M.A. Gropper*, M.A. Matthay and N.C. Staub. Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, CA 94143.

To determine the effects of isolation on alveolar-airway barrier leakiness we compared 10 isolated and 9 intact closed-chest dog lung lobes which we degassed then inflated to 25, 50, 75 or 100% of capacity with 5% albumin solution labeled with Evan's blue dye. After 1h we froze the lobes and made color slides of 20 random blocks of each lobe. We measured relative cuff-to-blood vessel area (100/lobe) and cuff-to-airway dye concentration (10/lobe). We measured dye concentration at 620 nm (red) using a 50 µm diameter spot in a microspectrophotometer. The table summarizes the data (mean ± S.D.).

Volume (% TLC)	Cuff Size (Fraction of Vessel) Isolated	Intact	Dye Concentration (Fraction of Airway) Isolated	Intact
25	0.4±0.2	1.3±1.2	.32±.21	.37±.22
50	1.1±0.1	1.7±1.6	.45±.18	.33±.25
75	2.5±2.2	2.4±1.6	.31±.12	.32±.16
100	3.3±2.4	3.3±2.2	.44±.27	.44±.13

Cuff size was larger at the lowest inflation volume in intact lobes. Otherwise all results are the same between the groups. The alveolar-airway barrier appears to be equally leaky in both isolated and intact dog lungs. [Supported in part by HL25816 (PPG) and Parker B. Francis Fellowship].

57.9

LEAKAGE OF EDEMA FLUID INTO THE LARGE BRONCHI OF PERFUSED RABBIT LUNGS. G.R. Mason* and R.M. Effros. Division of Respiratory Physiol. and Med., Harbor UCLA Med. Center, Torrance, CA 90509.

It has been argued that pulmonary edema initially accumulates in the peribronchial and perivascular regions and that alveolar flooding only occurs after this fluid ruptures through a site in the small airways and flows back into alveoli. In this study, we filled the airways of rabbit lungs with from 40 to 60 ml of a physiological 5G% albumin solution and perfused them in situ with the same solution. This procedure prevented fluid entering airways from draining into alveoli. The airway solution contained ^{125}I-albumin and ^{14}C-sucrose, whereas the perfusate contained T-1824 labeled albumin. Left atrial pressures were increased to 15 cmH_2O and pulmonary artery pressures rose to 25 cmH_2O above airway pressure at a flow of 80 ml/min. After 1/2 hr of perfusion, the fluid in the airways was pumped into serial tubes. ^{125}I-albumin and ^{14}C-sucrose concentrations in the initial (large airway) samples were 20±5% (SEM,n=5) and 19±5% those originally placed in the airway fluid whereas these values were 64±5% and 56±5% in later (small airway) samples. T-1824 in large airway samples was 84±3% of that in the perfusate whereas this value was 40±4% in the small airway samples. These data suggest that proportionately more fluid leaks into the large than the small airways after elevation of left atrial pressure and this occurs through large defects which do not seive protein. (Support: GLAA:AHA and NIH HL18606).

57.10

ALVEOLAR LIQUID PRESSURE (Pliq) IN RABBIT LUNGS MADE EDEMATOUS BY OLEIC ACID. Stephen J. Lai-Fook and Mark R. Kaplowitz.* San Francisco General Hospital, University of California, San Francisco, CA 94110.

Is the reduced lung volume in oleic acid-induced edematous lungs due to uniform increase in alveolar surface tension or loss of ventilatable units? We studied 2 sets of rabbit lungs. In the control set (C) 5 excised lungs were made edematous by infusing 12 mls of normal saline into the alveoli via the trachea. In the second set (OA), edema was induced in 5 rabbits by i.v. injection of oleic acid (.10 gm/kg) and the lungs excised after 1 hr. Lungs were degassed, and inflated to a transpulmonary pressure (Ptp) of 25 cm H20. Pliq relative to pleural pressure was measured at constant deflation Ptp by the micropipet-servonulling method. Punctures using 5 µm pipets were made in nonatelectatic air-expanded lung regions. The table summarizes data (mean ± SD cmH20, n=5):

Ptp (cmH20) =	25	15	5
Pliq (C)	= 8.5 ± 2.5	2.0 ± .84	2.0 ± .11
Pliq (OA)	= 7.1 ± 2.0	2.2 ± 1.2	2.6 ± .74

There was no difference between Pliq in OA lungs and control. TLC (lung volume at 25 Ptp) was 25.7 ± 7.4 ml/kg body weight in OA lungs, less ($P<.05$) than TLC in control lungs (36.4 ± 5.9). Gross atelectasis was primary cause of volume decrease in lungs made edematous by oleic acid. Alveolar surface tension was not increased in the air-expanded lung regions.
(Supported by HL 29054 and HL 1071 from NHLBI.)

57.11

LIFETIME OF INDIVIDUAL DOMES IN MONOLAYERS OF PULMONARY ALVEOLAR EPITHELIAL CELLS. S.E.S. Brown*, B.E. Goodman, and E.D. Crandall, Univ. of California, Los Angeles, CA 90024

Type II alveolar epithelial cells, grown as monolayers on plastic in primary culture, form fluid-filled domes. We observed the kinetics of individual dome formation from initiation to collapse at 37°C. Dome diameter and height were measured and dome volume was calculated assuming that the domes are sections of spheres. Fluid flux Jv during dome growth was calculated from the change in volume with time. Dome growth was initially rapid, with full volume reached within 30-60 min and maintained for 60-90 min. Deflation then occurred rapidly (3-5 min), sometimes accompanied by small vortices of fluid movement in the medium above the dome. Complete deflation, with cells blended back into the surrounding monolayer, required an additional 20-30 min. Jv during initial dome formation was calculated to be 2.1 nl/cm^2-sec. These data are consistent with the hypothesis that domes result from active solute transport from medium to substratum, with water following passively. Fluid accumulates at first without and later with tension in the plane of the cells forming the dome. Rapid collapse of the dome may result from rupture of intercellular junctions as pressure increases within the dome. Interestingly, extrapolating our measured fluid flux to a large surface area yields Jv of 124 liters/day-$70m^2$, suggesting that adult mammalian alveolar epithelium may, under certain circumstances, contribute to the removal of large volumes of fluid from alveolar air spaces. (Supp: HL26223.)

57.12

Factors influencing clearance of aerosolized ^{99}Tc-diethylenetriaminepentaacetate (^{99}Tc-DTPA) from canine lungs. Norman W. Rizk*, John M. Luce*, David C. Price*, John Hoeffel *, and John F. Murray. Cardiovascular Research Institute, University of Ca., San Francisco. 94143.

To determine the factors influencing lung solute clearance, we administered aerosolized ^{99}Tc-DTPA (particle activity median aerodynamic diameter = 1.1μm) to 5 paralyzed intubated mechanically ventilated dogs and determined clearance half-times ($t_{½}$) with a gamma camera. We used a jet nebuliser-plate separator-balloon system to generate and deliver the aerosol in the following conditions: 1) control (CTL), with ventilatory frequency (f) = 15/min and tidal volume (Vt) = 15 ml/kg, 2) repeat control (RCTL), for reproducibility, 3) increased frequency (IF), f = 25/min and Vt = 10 ml/kg, 4) positive end-expiratory pressure (PEEP) of 10 cms. of H_2O, 5) unilateral pulmonary artery occlusion (PAO), and 6) bronchial artery occlusion (BAO). We then compared the clearance from both lungs of each dog in these conditions to control:

	CTL	RCTL	IF	PEEP	PAO	BAO
$t_{½}$ (min)	25±5	27±8	25±6	13±3	37±6* 19.9±3.4†	24±5
p		N.S.	N.S.	<.01	<0.05 N.S.	N.S.

*Clearance from occluded lungs; †from nonoccluded lungs.

We conclude that a) IF does not influence clearance, b) PEEP increases clearance, and c) with our method clearance depends upon pulmonary, not bronchial, blood flow. (Supported by Pulmonary Vascular SCOR HL-19155)

SYNAPTIC TRANSMISSION, NEURAL PEPTIDES AND NEUROCHEMISTRY

58.1

PROPERTIES AND SYNAPTIC RESPONSES OF THE INFERIOR MESENTERIC GANGLION OF THE RABBIT. David L. Kreulen. Dept. of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724

Intracellular recordings were made from principal ganglion cells of the inferior mesenteric ganglion (IMG) of the rabbit to determine cellular properties and characteristics of synaptic inputs. IMG's with associated mesentery and blood vessels were removed from male rabbits and pinned in an isolated organ tissue bath which was constantly superfused with oxygenated Krebs solution at 37°C. Nerve trunks were stimulated with bipolar platinum electrodes. The mean resting membrane potential was -50 mV (± SE 1.8 mV; n=35). Stimulation of the lumbar splanchnic, or lumbar colonic (LCN) or intermesenteric (IMN) nerves resulted in excitatory postsynaptic potentials (epsp's) in all cells. By grading the intensity of nerve stimulation, epsp's at more than one latency were usually recorded and at supramaximal intensities, cells often fired more than one action potential in response to a single shock. Of the cells tested, 72% received synaptic input from the LCN and 93% received input from the IMN. In addition, antidromic responses were recorded in 3% of the cells when LCN was stimulated and in 49% of the cells when IMN was stimulated. The mean conduction velocity of presynaptic fibers in the LCN was 0.5 M/sec (± SE 0.02 M/sec; n=7) and the mean synaptic delay was 7.5 msec (± SE 2.4 msec). These studies show that the IMG of the rabbit has some properties similar to these ganglia in other species. Support HL 27781.

58.2

SYNAPTIC HYPERSENSITIVITY IN VIRAL INFECTION. Jacob Zabara. Temple University, Phila., PA. 19140

The nature of brain hypersensitivity in viral infection has been investigated; Gajdusek et al. have presented evidence for presynaptic changes in serotoninergic neurons in the subacute spongiform virus encephalopathies. However, the locus of viral replication being in the postsynaptic cells, the nature of such presynaptic viral action remains unknown. It would be informative to observe the possible development of hypersensitivity in a relatively isolated synaptic region where electrophysiological techniques could monitor the entire course of the infective process. Thus, the infection of the superior cervical ganglion is investigated electrophysiologically by intraocular viral (pseudorabies) inoculation of the right eye of rats. Recordings are performed either in situ or after excision of the ganglion which is suspended in Ag-Ag Cl electrodes in a chamber containing a modified Ringer's solution and equilibrated with 95% O_2, 5% CO_2. The infected ganglion discharges autorhythmically in potentials which resemble slow EPSPs with a duration of 150-200 msec. in early stages of infection. As the viral replication process increases, the duration of the slow EPSP also increases, reaching many seconds of duration in late infective stages. This effect apparently is not due to a greater release of the neurotransmitter, Acetylcholine, since its application to the ganglion does not prolong the slow EPSP. Thus, these observations support the virological findings of brain hypersensitivity and indicate that the basis of this effect can arise from individual synaptic regions.(TURI Fund)

58.3

EXTRACELLULAR CONCENTRATIONS OF CALCIUM, CHLORIDE AND SODIUM IONS REGULATE THE FIRING ACTIVITY OF A SINGLE ISOLATED NEURON. Kok-Swang Tan* and Sheldon H. Roth* (SPON: F.L. Lorscheider) Department of Pharmacology, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

The rhythmical firing frequency of an isolated neuron (crayfish stretch receptor) at maintained stimulus (stretch) can be dramatically altered by small changes in extracellular sodium, calcium or chloride ion concentration. An increase in $[Na^+]_o$ or $[Ca^{++}]_o$ produced an initial depression of firing activity which slowly returned to just below control levels; a decrease in ion concentration produced enhancement. Na^+ replacement by choline resulted in a biphasic concentration-dependent response; 40 mM or less replacement produced enhancement of firing frequency; replacements greater than 40 mM resulted in depression. Replacement by Li^+ resulted in only depression, and appeared to be ~4 fold more effective than choline. Propionate or acetate replacement of Cl^- produced a concentration-dependent depression of firing activity; 100 mM replacement appeared to be optimal; concentration >100 mM resulted in less depression. Changes in extracellular Na^+ or Cl^- concentration produced a biphasic concentration-dependent alteration in firing activity. In contrast, the alteration by Ca^{++} replacement is monophasic. Compared on a molar basis, the firing activity of the neuron was most sensitive to changes in Ca^{++}, followed by Cl^- and Na^+. Supported by AHFMR and MRC.

58.4

MODULATION OF THE α_2-ADRENERGIC AUTOINHIBITION OF NOREPINEPHRINE (NE) RELEASE BY NEURONAL UPTAKE AND TISSUE PERFUSION. H. Fuder*, F. Bath*, R. Spemann* and H. Wiebelt* (SPON: M.N. Levy) Univ. of Mainz, 6500 Mainz, W.-Germany

The isolated rat heart with the right sympathetic nerves attached was used to investigate borderline conditions of the autoinhibition in a well perfused tissue. The hearts were perfused with 7 ml/min Tyrode's solution (aortic perfusion pressure, 62 - 83 mm Hg) and loaded with ^{3}H-(-)-NE (16 nM). The postganglionic nerves were repeatedly stimulated with trains of 1o pulses/min at 1 Hz (A) or 180 pulses/10 min at 3 Hz (B). Under A yohimbine 1 μM (YOH) failed to increase the stimulation-evoked overflow in the perfusate (SEO) but antagonized competitively the inhibition by oxymetazoline of SEO (pA_2 from Schild plot = - log K_B, 7.8). Under B YOH enhanced SEO by 15 - 26% of the expected control value depending on the amount of NE released. In the presence of cocaine (1 μM) plus propranolol (0.1 μM) YOH increased SEO by 27 - 58%. Reduction of the perfusion rate to 1.8 ml/min (aortic pressure, 9 - 15 mm Hg) enhanced the effect of YOH significantly to 77 - 134% (each group n = 4 - 5). Thus, in contrast to autoinhibition in incubated tissues lacking perfusion that in the perfused rat heart is absent or small, unless the synaptic NE concentration is increased by neuronal uptake inhibition or poor tissue perfusion. (Supported by Deutsche Forschungsgemeinschaft)

58.5
EFFECTS OF CHLORDECONE ON ACETYLCHOLINESTERASE ACTIVITY. Nuran M. Kumbaraci, Samuel Y. Kiel*, Chester J. Kovaleski*. Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, New Jersey 07030

Chlordecone (Kepone) is a pesticide which produces neurotoxic symptoms including tremors in humans and in experimental animals. To determine whether chlordecone impairs synaptic transmission its effects on the activity of the enzyme acetylcholinesterase were investigated *in vitro*. The Ellman colorimetric procedure was employed using electric eel acetylcholinesterase and acetylthiocholine iodide as the substrate. The rate of hydrolysis of the substrate by the enzyme was determined in the presence of 1-40 ppm chlordecone. Acetone was used in the preparation of solutions due to the limited solubility of chlordecone in water and was also included in the controls. Kinetic data were analyzed by Lineweaver-Burk and Eadie-Hofstee plots. No significant change was observed in the K_m value. However V_{max} decreased with increasing concentrations of chlordecone. For example, the addition of 5 ppm chordecone produced 10% decrease in V_{max}. These results imply that chlordecone is a non-competitive inhibitor of the enzyme acetylcholinesterase.

58.6
INTRACELLULAR RESPONSES OF CAUDATE NEURONS TO AMYGDALOID AND PIRIFORM CORTICAL STIMULI. Eve Andersen*, Chester Hull and Nathaniel Buchwald. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Recent studies have shown that cholecystokinin (CCK) cell bodies are found in the amygdala (Amy) and piriform cortex (Pir) and that CCK and CCK receptors occur in the caudate nucleus (Cd). There may also be a CCK projection from Amy to Cd. Horseradish peroxidase studies indicate a direct input from Amy to Cd. Our experiments were designed to study the electrophysiology of Amy and Pir projections to the caudate and then to study the action of CCK in these pathways. Intracellular responses were evoked in adult cats by precruciate cortical (Cx), Amy, and Pir stimuli. Cx stimulation evoked a large amplitude EPSP followed by an IPSP. Latency to onset of the EPSP was 7-10 msec. Stimulation of loci in the Amy produced an EPSP followed by an IPSP in 87% of the caudate cells tested. The EPSP was of smaller amplitude and of longer latency (20-25 msec) than that produced by Cx stimulation. Stimulation of the Pir in contrast, evoked a low amplitude IPSP in about 30% of the Cd cells. These results indicate that there may be functional projections from the amygdaloid complex and from the piriform cortex to the caudate and that these two inputs might utilize different transmitters.

Sponsored by USPHS Grants HD 05958 and HD 07032.

58.7
AGONIST ACTIONS OF LEVORPHANOL ON FROG SARTORIUS MUSCLE. T. E. Ary* and G. B. Frank, Department of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Intracellular microelectrode studies were conducted to examine the action of the narcotic analgesic levorphanol at an opiate receptor on excitable membranes of frog sartorius muscle. Initial studies showed that depressant effects on the max. rate of rise of the action potential begin to occur with exposure of muscle to bath concentrations $>10^{-5}$M for a 30 min period. Total depression of surface fibers occurred when the bath conc. was increased to 10^{-3}M. Bath conc. which were not sufficient to cause a large decrease in max. rate of rise over a 240 min. drug exposure period ($<10^{-4}$M) produced a large reduction of potassium conductance (gK) within the first 10 min. of exposure to drug thus increasing action potential duration. Higher bath concs., in addition, decrease conduction velocity. These initial findings demonstrate that low concs. of levorphanol reduce gK without affecting sodium conductance mechanisms (gNa), an effect not previously observed with other opiate agonist drugs in this preparation (Frank, J. Physiol. 252: 585, 1975); higher bath conc. reduce both gNa and gK. The potent and rapidly developing reduction of gK produced by levorphanol in low concs. shall be further investigated in order to determine whether this effect displays stereochemical specificity and to investigate the stereochemical nature of the interaction of this opiate agonist at the opiate receptor on excitable membranes. Supported by AHFMR and MRC.

58.8
CENTRAL CARDIOVASCULAR EFFECTS OF DYNORPHIN IN CONSCIOUS AND ANESTHETIZED RATS. A.I. Faden and G. Feuerstein*, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814.

The possible role of the endogenous opioid dynorphin (DY) in central cardiovascular control was investigated by evaluating the effects of DY 1-13 on mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) following microinjections into discrete brain nuclei - n. preopticus medialis (POM), periventricularis (HPV) and tractus solitarius (NTS). Injection sites were confirmed microscopically. In pentobarbital anesthetized rats, DY (6.4 nmol, 1.0 µl) in POM caused significant hypotension (-23 ± 1 mmHg) and bradycardia (-36 ± 8 beats/min); in HPV this dose of DY also significantly reduced MAP (-22 ± 6 mmHg), HR (-50 ± 15 beats/min), and RR (-23 ± 7 resp/min). By contrast, 6 nmol DY (0.1 µl) injected into NTS significantly increased MAP (13 ± 2 mmHg) without affecting HR or RR. In conscious animals intracerebroventricular injections of DY (6 or 60 nmol) caused dose dependent tachycardia (30 ± 13; 52 ± 17 beats/min respectively) without altering MAP. Similarly, DY (6 or 18 nmol) injected into POM of unanesthetized rats increased HR (52 ± 12; 77 ± 8 beats/min) but not MAP. From these data we suggest that DY may play a role in central cardiovascular control in both forebrain and hindbrain nuclei. The importance of conscious animal studies is emphasized by the different cardiovascular responses obtained in anesthetized and awake rats.

58.9
STRUCTURE-FUNCTION RELATIONSHIPS OF A PIGMENT-DISPERSING CRUSTACEAN NEUROPEPTIDE. John P. Riehm* and K. Ranga Rao. University of West Florida, Pensacola, FL 32504

This study examines the effects of sequence deletions and of chemical modifications on the melanophore pigment dispersing activity of a crustacean neuropeptide (DRPH, characterized by Fernlund in 1976 as: Asn-Ser-Gly-Met-Ile-Asn-Ser-Ile-Leu-Gly-Ile-Pro-Arg-Val-Met-Thr-Glu-Ala-NH_2). Peptides smaller than the tridecapeptide DRPH (6-18) did not show sustained biological activity. N-terminal extension of this peptide led to a steady increase in the activity, with the DRPH (1-18) showing the maximum activity. Carboxyl group modification had no effect, but acetylation, oxidation, cyanogen bromide, and trypsin caused a decrease in activity. Phenylglyoxal modification of Arg-13 in DRPH led to a 14-fold increase in activity. It is concluded that the N-terminus and the methionine residues are important for full activity and that the phenylglyoxal-induced potentiation is due to protection of the peptide from proteolysis in vivo.

58.10
CHROMOGRANIN A(CgA), A NEW PROBE OF SYMPATHOADRENAL FUNCTION: QUANTIFICATION BY RADIOIMMUNOASSAY(RIA). D.T.O'Connor*,R.P. Frigon*(SPON:R.C.Blantz).VA Med.Ctr./Univ. of California, San Diego, CA 92161

CgA, the major catecholamine storage vesicle(CSV)soluble protein, is released with catecholamines from CSVs by exocytosis, and is an index of exocytotic sympathoadrenal neurosecretion. We developed an RIA for CgA. CgA was purified from bovine adrenomedullary CSVs; a rabbit antiserum to CgA was obtained, at a working titer of 1:1,500. ^{125}I-CgA was prepared at 300,000 cpm/µg CgA, and was 70-85% immunoprecipitable.

Results: The double-antibody RIA to bovine CgA has the following characteristics: 1)Sensitivity and range: 10-100 ng/tube 2)Intra and inter-assay coefficients of variation: 4.8% and 13% 3)Interspecies crossreactivities(XR): sheep XR, but not human, dog,rat 4)Intra-CSV XR: no XR with dopamine-β-hydroxylase(DBH), catecholamines, ATP, ascorbate, or leucine enkephalin; partial and parallel XR with CgB(40.7%), CgC(8.6%), and CgD(1.2%). The RIA detected CgA in tissues with adrenergic function(adrenal medulla, sympathetic nerve, brain), as well as serum; mean serum CgA was 458±57 ng/ml; range = 228-786 ng/ml; n = 10.

We conclude: 1)CgA is an immunologically distinct molecule, to which an RIA has been developed; 2)The RIA is species-specific; 3)Within the CSV, DBH and small molecules(catecholamines, ATP, etc.)do not XR, while the minor chromogranins have partial XR; 4)The RIA detects CgA in several tissues, including serum. Thus, this new sympathoadrenal index molecule is accessible for study in intact animals.

58.11
COMPARISON OF BARBITURATE AND CALCIUM ANTAGONISTS ATTENUATION OF BRAIN FREE FATTY ACID LIBERATION DURING GLOBAL ISCHEMIA IN RATS. Gerald K. Shiu,* Joseph P. Nemmer,* and Edwin M. Nemoto. The Anesthesia and CCM Research Laboratories, University of Pittsburgh, Pittsburgh, PA 15261

We suggested that brain free fatty acid (FFA) liberation reflects the evolution of ischemic brain injury during complete global brain ischemia. Calcium activates brain lipases so we studied the effects of D-600 and flunarizine compared to pentobarbital on FFA liberation after decapitation ischemia in rats. Albino rats were decapitated after administration of: (1) 0.9% NaCl, 4 mls IP (controls); (2) D-600, 20 mg/kg, IP; or (3) flunarizine, 20 mg/kg, PO. Postdecapitation, their heads were incubated at 37°C for 0.5 to 60 min and their brains frozen in liquid N_2. Whole brain FFAs, palmitic, stearic, linoleic, oleic and arachidonic acids were quantitated by GC. Total FFA increased about 10-fold between 0.5 min and 60 min of ischemia from 253 ± 33 to 2,225 ± 80 nmoles/g of brain (mean ± SEM). Pentobarbital attenuated the rise in total FFA, stearic and arachidonic acids with less effect on palmitic and oleic acids. D-600 and flunarizine were less effective in attenuating FFA liberation. Stearic acid was attenuated for up to 30 min of ischemia. Both calcium antagonists unlike pentobarbital, did not affect FFA accumulation after 60 min of ischemia. Our results suggest that pentobarbital should have greater therapeutic effects than D-600 or flunarizine.

58.12
ATTENUATION OF FREEZE INJURY INTRACRANIAL HYPERTENSION BY D-600 IN CATS. Daisei Kaneko,* Edwin M. Nemoto, Thomas Gaisor,* Sharon DeCesare,* and Peter M. Winter.* The Anesthesia and CCM Research Laboratories, University of Pittsburgh, Pittsburgh, PA 15261

Our aim was to determine whether calcium antagonists would attenuate intracranial hypertension after freeze injury of the cat cerebral cortex. Twenty-two cats were mechanically ventilated on 0.5% halothane, 30% O_2, and 70% N_2O. Intracranial pressure (ICP) was continuously monitored via a subdural catheter. In 10 cats (group I), liquid N_2 was directly applied onto the skull for 5 min/kg body weight. In 10 other cats (group II), it was applied for 10 min onto the dura. When ICP rose above 20 torr, 5 cats were infused IV with 0.9% NaCl and 5 cats received D-600, 0.0125 mg/kg/min for up to 3 hours. Mean arterial pressure (MAP) was uncontrolled in the controls of group I, but kept at about 100 torr in the controls of group II. MAP in the D-600 treated cats was kept at about 100 torr. Preinsult, ICP was about 10 torr and MAP about 120 torr. In group I D-600 treated cats, MAP was lower than in controls and ICP ranged between 20 and 25 torr whereas in controls it rose to a peak of 55 torr at two hours. In group II D-600 cats, MAP was between 100 and 120 torr. ICP did not rise above 35 torr. In controls it rose to 85 torr at 2.5 hours. Cortical brain tissue water content rose from about 70% to 85% in both groups of cats and was unaffected by D-600.

NEUROENDOCRINOLOGY

59.1
INSULIN INFUSIONS DO NOT ALTER PUBERTY OR RESTRAIN FEEDING IN WEANLING RATS. J.K. Young, Dept. Anatomy Howard University, Washington, D.C. 20059

Hypothalamic restraint upon feeding and facilitation of gonadotropin release both appear to be initiated at the time of puberty (Endo 109:2032; Neuroendo 1:265). Yet, the factors initiating these changes are obscure. A body weight-related signal may be necessary for these changes (Science 200:1506) To see if insulin could be that signal, 30-day old female rats were infused intracerebroventricularly with 6.6 mU/kg/day (LI) or 100 mU/kg/day (HI) of insulin for 14 days, using osmotic minipumps. Neither dose affected timing of vaginal opening (VO) relative to saline (S): VO for S = 46.9 ± 1.1 days, LI = 45.5 ± 2.0 days, HI = 45.9 ± 1.7 days. Systemic infusion of 4.7 U/kg/day of insulin (I) likewise failed to affect VO: I = 44.1 ± 1.6 days, S = 42.6 ± 1.7 days. This dose did not affect daily food intake (FI) in weanlings, but when given to adults, did reduce FI relative to S ($F = 3.98$, $p<0.05$ for $df = 1,70$). I does not appear to modulate puberty; however, high FI of weanlings may be due to insensitivity to feeding restraining effects of insulin. Supported by NIH Grant 14378-02.

59.2
CORTICOIDS AND SEXUAL DIFFERENTIATION OF BRAIN STRUCTURE. L. Karaviti*, J. Schoonmaker*, J.E. Shryne*, and R.A. Gorski. Department of Anatomy and Laboratory of Neuroendocrinology, Brain Res. Inst., UCLA Sch. of Medicine, Los Angeles, CA 90024

The endocrine events that influence normal sexual differentiation begin in perinatal life. In previous studies from this laboratory we have identified a morphological sex difference in the rat hypothalamus: the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA). Because of the known effects of corticosterone in modifying neuronal development during perinatal life, a study of SDN-POA volume was undertaken following treatment perinatally with corticosterone (1 mg/0.5 ml oil) from day 11 of pregnancy until parturition. The pups continued to receive corticosterone (0.1 mg/0.05 ml oil) daily until day 4 or 8 postnatally when sacrificed and perfused for analysis of SDN-POA volume. Although there was a sex difference at both ages, treatment perinatally with corticosterone significantly ($p<0.001$) decreased the volume of the SDN-POA of males on day 8 when compared with that of appropriate male controls when expressed in terms of absolute volume or relative to brain weight, but had no effect upon the volume of treated females on days 4 or 8, or upon males on day 4. The difference in SDN-POA volume of 8 day old males may result either from corticoids inhibiting or delaying the development of the SDN-POA during the perinatal critical period either directly or through an indirect alteration of testicular function or androgen action. (Supported by USPHS NIH Grant HD-01182.)

59.3
PHOTOINDUCED CHANGES IN ESTRADIOL FEEDBACK IN THE FEMALE GOLDEN HAMSTER. Mary N. DiPinto* and Milton H. Stetson. University of Delaware, Newark, DE 19711.

Female golden hamsters maintained on long days (LD14:10) show a 4-day estrous cycle in which the LH surge occurs on the afternoon of proestrus. When cyclic females are ovariectomized the LH surge occurs daily. Administration of estradiol(E_2) enhances the magnitude of the LH surge in a dose dependent fashion. E_2 cannot augment the proestrous surge of LH, probably because endogenous levels of E_2 are already elevated. Short days, through their action on pineal melatonin(M) release, cause female hamsters to become anovulatory. These photo-induced anovulatory(PIA) females experience a daily afternoon surge of LH. A similar situation exists in long-day females rendered anovulatory by daily M injections (MIA females). We examine here the effects of short days (LD10:14) and M(15 ug/day, s.c.) on the positive feedback(PFB) response of LH to E_2. MIA and PIA hamsters were ovariectomized and a week later implanted with Silastic tubing that was either empty or contained 5mm of E_2. Blood samples were collected at 1400, 1700 and 2000h the next day and the LH measured by RIA. In neither the MIA nor PIA castrates was there a demonstrable PFB of E_2 on the daily LH surge. We conclude that the photoperiodic regulation of reproductive cyclicity in female hamsters involves a pineal-mediated suppression of E_2 PFB on the hypothalamus and/or anterior pituitary, resulting in suppression of the magnitude of the LH surge and transformation from estrous cyclicity to daily gonadotropin surges.

59.4
HYPOTHALAMIC DEAFFERENTATION ALTERS LH SECRETION IN MALE MICE. Arthur Coquelin* and Roger A. Gorski. Dept. of Anatomy and Lab. of Neuroendocrinology of the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Two distinct components characterize LH secretion in male mice: plasma LH remains low and relatively constant for long periods (basal), and dramatic elevations (≥ 50 ng/ml) occur intermittently (episodic). We examined the role of the medial basal hypothalamus (MBH) in regulating this pattern of LH release. Adult, CF-1 males received either complete deafferentation (9) or sham surgery (9) using a Halasz-type stereotaxic knife. Two weeks after deafferentation, sequential blood samples were collected at 5 min intervals for 6 hrs. Mouse red blood cells suspended in a human plasma protein fraction were returned following each sample. Plasma LH was measured by RIA. Control males exhibited 1-2 abrupt elevations above comparatively stable basal LH levels. After deafferentation the frequency of episodic LH release clearly increased although rapidly fluctuating basal titers made it difficult to identify all episodes.

Surgery	Peak Height	Nadir to Peak	Frequency
Sham	140 ± 13 ng/ml	102 ± 11 ng/ml	2 ± 0.4 in 6 h
Deafferented	133 ± 7	98 ± 12	9 ± 1.0

These results suggest that deafferentation releases the MBH or the pituitary from inhibition. The postulated inhibitory agent may prevent random discharge of LHRH from MBH neurons or it may reversibly suppress LH release from the pituitary. (Supported by NRSA HD06160 and NIH HD01182.)

59.5

EVIDENCE FOR SEPARATE NEGATIVE FEEDBACK ACTIONS OF ESTROGEN ON THE PITUITARY AND BRAIN IN THE OVARIECTOMIZED (OVX) MONKEY. R.F. Weick, V. Pitelka* and D.L. Thompson*. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The site(s) of negative feedback of estradiol on LH and FSH secretion in the rhesus monkey were studied by observing the responses to exogenous GnRH before and after estrogen treatment. A preliminary dose-response study in the 5 OVX animals used in this research showed the peak LH response to occur 15 min after GnRH injection and that 0.5 µg gave 50% of the maximum obtainable response. The animals were injected with this dose of GnRH before and (in 3 separate experiments) 3, 12 and 24 h after subcutaneous implantation of Silastic capsules containing 17β-estradiol. Serum estrogen levels averaged 217 pg/ml, resulting in LH levels (before GnRH injection) at 3, 12, and 24 h of 70%, 28%, and 37% of control values, respectively. Before estrogen treatment, the increase in serum LH levels 15 min following GnRH injection averaged 26.6 ng/ml (27%). Three h after estrogen implantation, the average response was 0.7 ng/ml, but after 12 and 24 h the responses were 41.8 and 43.6 ng/ml, respectively. The FSH responses to GnRH were smaller, but were similarly influenced by estrogen. We interpret these results to mean that estrogen acutely suppresses the ability of the pituitary to respond to GnRH. The capacity of the pituitary to respond to exogenous GnRH returns within 12 h. This occurs while circulating LH levels are low, presumably reflecting an inhibitory effect of the estrogen on hypothalamic secretion of endogenous GnRH. (Supported by MRC).

59.6

RELEASE OF LHRH FROM A RECHARGEABLE IMPLANT. Brian K. Davis. State University of New York, Stony Brook, NY 11794

Molecular sieving by crosslinked polymer gels has been demonstrated in our previous studies to provide a method for achieving slow, continuous delivery of a wide array of pharmacologically-active substances; significantly, they allow sustained, predictable administration of polar solutes, such as prostaglandins and protein hormones, from depots under the skin. As these implants require surgical placement in the bearer, an endeavor was made to develop a rechargeable device. An implant that could be recharged by subcutaneous injection might obviate the need for reimplantation. A polyvinylpyrrolidone-silastic implant (B. K. Davis, 1974, Prostaglandins, 7, 393) designed to be recharged has been tested in recent experiments using as implanted solute a radioactively labeled hypothalamic decapeptide, ^{125}I-LHRH. Release of the peptide from these implants during incubation under defined conditions, in vitro, was found to be reproducible after recharging. Moreover, ^{125}I-LHRH release occurred at a comparable rate with that found in vitro, following subcutaneous implantation in rats. These findings indicate the technical feasibility of developing a rechargeable implant, prepared from a non-biodegradable polymer.

59.7

PERIOVULATORY FSH SECRETION: EFFECTS OF A POTENT LHRH ANTAGONIST. T.P. Condon*, D. Heber*, J.M. Stewart*, C.H. Sawyer and D.I. Whitmoyer*. UCLA Med. School, Los Angeles, CA 90024.

The effects of a potent LHRH antagonist (Nac-L-Ala^{1},pCl-D-Phe^{2},D-Trp^{3,6}) LHRH (ALHRH) on periovulatory gonadotropin secretion were examined. Intact Sprague-Dawley rats were maintained under standardized lighting and temperature conditions (14:10 light:dark, lights on at 0600). Only animals showing two consecutive 4-day estrous cycles were used. Between 1200-1300 on proestrus (P), animals were implanted with intra-atrial cannulas under ether anesthesia. Hourly blood samples (0.75 ml) were taken from 1400 on P through 0900 on estrus (E). A reconstituted whole blood preparation consisting of 45-48% rat red blood cells suspended in 5% human plasma protein fraction was infused after each sample. ALHRH was dissolved in sesame oil and injected s.c. (100 µg/0.2 ml/animal). ALHRH given at 1300 on P completely blocked the LH surge, with levels remaining undetectable (<20 ng/ml) throughout sampling. Control rats displayed normal LH surges between 1700-1900 on P. However, administration of antagonist at 1300 failed to block completely the P primary FSH surge although peak values were greatly reduced when compared with controls (200 ± 26 vs 415 ± 62 ng/ml). Injection of ALHRH at 2300 after the P LH and primary FSH surges had occurred failed to alter the secondary FSH surge at 0400 on E (305 ± 14 ng/ml vs 311 ± 9 ng/ml for oil-injected controls). These data suggest that FSH secretion is not tightly coupled to LHRH. (Supported by NIH grants NS01162 and MH15345.)

59.8

GASTROPOD SOMATOSTATIN-LIKE IMMUNOREACTIVITY MAY BE A GROWTH HORMONE. Yvonne Grimm-Jørgensen, Susan M. Connolly* and David Pearson*. Univ. of Connecticut Health Center, Farmington, CT 06032 and City of Hope Research Institute, Duarte, CA 91010.

We have reported earlier that immunoreactive somatostatin (IRSRIF) is found in brain and hemolymph of Physa spp., that it does not migrate like vertebrate somatostatin during reverse phase thin layer chromatography, and that its concentration in the hemolymph is proportional to the rate of growth. We have hypothesized that gastropod IRSRIF is a growth hormone (Grimm-Jørgensen, Gen. Comp. Endocrinol., in press, (1982). To test this hypothesis, we have developed a specific and sensitive bioassay for Physa growth hormone activity. It measures growth hormone-induced release of labeled DOPA-rich proteins from in vitro incubated mantle edges and detects growth hormone activity from as little as ½ brain equivalents. Brain and hemolymph extracts from 150-200 animals were subjected to reverse phase HPLC and the IRSRIF and growth hormone contents of the collected fractions determined. Brain IRSRIF eluted as a single peak and the growth hormone activity co-eluted with the IRSRIF activity. The IRSRIF activity from the hemolymph eluted in several peaks. The elution time of one peak corresponded to that of brain IRSRIF. The elution times of all gastropod IRSRIFs differ from those of vertebrate somatostatin and Gillichthys urotensin II. These results suggest that the gastropod brain IRSRIF may be a gastropod growth hormone and that it may be secreted into the hemolymph. The origin of the other hemolymph IRSRIFs is not known. (NIH Grant 2R01 AM 20929)

59.9

VASOPRESSIN NEURONS IN RAT SUPRAOPTIC NUCLEUS: ANTIDROMIC IDENTIFICATION, LUCIFER YELLOW INJECTION AND IMMUNOCYTOCHEMICAL IDENTIFICATION IN THE HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANT. T.A. Reaves, Jr., A. Hou-Yu*, E.A. Zimmerman*and J.N. Hayward. University of North Carolina, Chapel Hill, N.C. 27514

In order to define the functional and morphological aspects of rat magnocellular vasopressin (VP) neurons, we developed a double-labeling technique utilizing intracellular recording, Lucifer Yellow (LY) injection and immunocytochemistry. We describe our initial results on the rat supraoptic nucleus (NSO) using an in vitro explant of the hypothalamo-neurohypophysial complex (Sladek & Knigge, '77). Pituitary antidromically identified NSO neurons were recorded intracellularly with 2% LY-filled glass micropipettes and iontophoretically-injected (5-10 nA, DC) with LY. Fixed explants were vibratome sectioned (30 uM) and the LY-identified neurons stained with indirect immunofluorescence method using a mouse monoclonal antibody to arginine vasopressin (clone III D-7) and a rhodamine tagged goat anti-mouse IgG secondary antibody. About half of our LY-injected cells were immunocytochemically identified as VP neurons. These cells were silent or displayed spontaneous firing activity ranging from slow to fast continuous activity through periodic bursting patterns. Bipolar or multipolar VP neurons were round or ovoid in morphology with multiple branching processes in the NSO and adjacent hypothalamus. We find no dye-coupling in the magnocellular VP neurons of the NSO. (Supported by NIH Grants NS-13411(TAR & JNH) & HL-24105, AM-20337(AH & EAZ))

60.1

RENIN SUBSTRATE IN HUMAN PLASMA. EVIDENCE FOR BINDING TO ALBUMIN. David B. Gordon. V.A.Med.Center Livermore,California 94550

Multiple forms of renin substrate in human plasma can be shown by electrophoretic separation on polyacrylamide gel. One major peak appears with an electrophoretic mobility of 0.75-0.85 x that of albumin, together with 3 or 4 minor peaks of slower mobility. The amount of renin substrate present in the minor peaks is only 3-5% of the total in normal plasma. Certain changes in procedure can cause an apparent splitting of the major peak into 2 components. These are a) increasing gel concentration (from 5 to 8%) and b) increasing sample size (from 50 µl to 200 µl). While the former maneuver would be expected to increase resolution, the latter would not. Ordinarily, plasma is diluted 1:1 by mixing with 0.5 M sucrose plus a dye prior to electrophoresis. A mixture with a higher proportion of plasma to sucrose can be used giving a higher protein concentration in the sample. Two findings suggest that the effect of increased sample size is due to binding of some of the renin substrate to albumin: 1) the faster peak approaches the mobility of albumin as sample size or protein concentration is increased and 2) the relative quantity of renin substrate in the faster peak increases as sample size or protein concentration is increased.

60.2

ROLE OF ANGIOTENSIN II IN MEDIATING RENAL HEMODYNAMIC EFFECTS OF ADENOSINE. John E. Hall and Joey P. Granger. Univ. Miss. School of Medicine, Jackson, MS 39216

The objective of this study was to quantitate interactions between adenosine (Ado) and angiotensin II (AII) in controlling renal segmental resistances and GFR. In 6 normal dogs, intrarenal Ado infusion (1.0 µmol/min) transiently decreased renal blood flow (RBF) and GFR to 73±4 and 72±6% of control, respectively, due mainly to increased preglomerular resistance (R_A). During continued Ado infusion, R_A returned toward control and RBF gradually increased to 122±7% of control, whereas GFR remained low due to a gradual fall in efferent arteriolar resistance (R_E). Blockade of AII formation with SQ-14225 almost abolished the transient decrease in RBF and increase in R_A, but did not prevent the sustained decrease in filtration fraction and R_E during Ado infusion. After AII formation was blocked with SQ-14225 and circulating AII was replaced by iv infusion of 20 ng/kg/min of AII, RBF decreased transiently to 78±4% of control during Ado infusion and then increased toward control; but in contrast to the response of normal dogs, the rise in R_A was sustained and Ado did not increase RBF above control when circulating AII was maintained constant. Maintenance of constant circulating AII did not prevent the Ado mediated decrease in R_E. These observations suggest that Ado mediated increases in R_A depend on AII. The renal vasodilator effect of Ado appears to be confined to efferent arterioles and is not markedly influenced by AII. (Supported by NIH grants HL-23502, HL-11678, and by the Mississippi Heart Association).

60.3

POSSIBLE ROLE OF CALMODULIN IN RENIN SECRETION FROM RAT KIDNEY SLICES. Paul C. Churchill and Monique C. Churchill. Wayne State University. Detroit, Michigan 48201

Much indirect evidence supports the hypothesis that the renin-secretory activity of juxtaglomerular cells is inversely related to intracellular Ca^{2+} concentration (Ca_i). Exactly how increases (decreases) in Ca_i inhibit (stimulate) renin secretion is unknown, but might involve changes in the concentration of Ca-calmodulin. The purpose of these experiments was to determine the effects of trifluoperazine (a calmodulin-inactivator) on renin secretion from rat kidney slices. As can be seen below, trifluoperazine stimulated renin secretion in a concentration-dependent manner. In preliminary experiments we have found that the inhibitory effect on renin secretion of K-depolarization, which is thought to be mediated by Ca influx, is antagonized by trifluoperazine. Taken together, these results are consistent with the hypothesis that changes in Ca-calmodulin concentration mediate the effect of changes in Ca_i on renin secretion. (NIH HL 24880)

Trifluoperazine (µM)	Secretory Rate (GU/g/30 min)
0	4.45 ± 0.48 (n=12)
1	4.65 ± 0.47 (n=12)
10	5.14 ± 0.36 (n=16)
20	7.62 ± 0.74 (n=12)
50	10.35 ± 1.11 (n=12)

60.4

EFFECTS OF CAPTOPRIL ON RENAL AND ADRENOCORTICAL RESPONSES TO SODIUM RESTRICTION IN MALE LONG EVANS RATS. A.McKeever*, I.W. Henderson* and J.A. Oliver*(SPON:H.Valtin).Zoology Dept., University,Sheffield S10 2TN, England.

Fluid and electrolyte balances of 12 rats(250-300g bw)were measured for a 3 day control period on a daily Na intake of 492±22µEq/100 bw. A Na-deficient regime (22 ±0.4 µEq/100g bw/24 h) was then introduced for 13 days:7 rats were given 30 mg Captopril/kg bw and 5 rats 1 ml water p.o. each day.

Captropril produced an immediate and sustained negative Na balance. Na excretory rates (µEq/100 g bw/24h) were reduced from 298.5 ± 19.8 on the normal Na diet, to 32.9 ± 7 in Na-restricted Captopril treated rats and to 4.3 ± 0.7 in Na restricted controls. Na restriction itself produced a progressive increase in urine production(ml/100g bw/24h) from 5.3 ±0.4 to 9.9 ± 1.1, while Captopril produced an immediate and sustained increase to 30.4 ± 5.8, an increase that preceded an increased fluid intake.Osmotically free water excretion increased with Captopril treatment.The width of the adrenocortical zona glomerulosa of the Captopril group was slightly narrower (38±10µm) than that of Na-replete rats (50±2), a value significantly less than that seen in the Na-deficient control animals(71±3).Peripheral aldosterone concentrations(ng/100ml plasma)were:Na-replete,36.1±2.7:Na-deficient,145.5±2.9:Captopril-treated Na-deficient,49.2±17.6.Captopril impairs the adrenocortical response to Na restriction; the consequent renal changes may also reflect primary intrarenal effects of interfering with the renin-angiotensin system.

60.5

EFFECT OF ADRENALECTOMY (ADX) ON PLASMA RENIN CONCENTRATION (PRC). W. J. Welch*, C. E. Ott and T. A. Kotchen*. Univ. of Kentucky, Lexington, KY 40536

Furosomide treatment and K depletion depress ascending loop solute transport and increase plasma renin activity (PRA). Saline infusion does not suppress PRA in K depleted animals suggesting that depressed loop transport increases PRA and obviates the macula densa signal to suppress PRA in response to saline infusion. Adrenal insufficiency decreases maximum urinary concentrating ability, also a function of loop solute transport. To determine if loop transport and renin are related in Adx, free water clearance and the renin response to saline infusion was compared in Adx and sham (Sh) operated rats. Anesthestized animals were infused with 0.45% NaCl (10% bw). Free water clearance was greater ($p < .001$) in Sh animals (2.86 ± .29 ml/100g/hr) than in Adx (0.56 ± .17). Adx animals drank 0.9% NaCl and were in more positive sodium balance than Sh animals that drank water. PRC was higher ($p < .001$) in Adx than Sh animals before NaCl infusion despite having a higher net sodium balance. Also PRC was not different after NaCl infusion in Adx, but was significantly decreased ($p < .01$) in sham animals. These results demonstrate that adrenalectomy without volume depletion stimulates PRC and that Adx animals are unable to suppress PRC with NaCl infusion and suggest that alterations in loop function in adrenalectomy could be responsible for the altered renin responses.

60.6

A DENERVATED NON-FILTERING KIDNEY PREPARATION IN THE RAT: A NEW MODEL FOR THE STUDY OF RENIN RELEASE. D. Villarreal*, J.O. Davis, R.H. Freeman, J.R. Dietz and A.M. Luger*. Univ. of Missouri, Columbia, MO 65212.

To examine the participation of the isolated renal vascular receptor in the control of renin secretion in the anesthetized rat, a denervated, non-filtering kidney model (DNFK) has been developed. The left kidney was exposed to a 2 hour period of total renal ischemia followed by ureteral ligation. Denervation was accomplished by stripping all visible nerves and painting the renal vessels with 5% phenol. Two days later lissamine green dye was injected I.V. and failed to appear in the surface renal tubules, indicating that glomerular filtration had ceased. H.E. and P.A.S. sections of these kidneys revealed focal to diffuse tubular necrosis with desquamation of epithelial cells as casts. Norepinephrine content of the left kidney was reduced 91% compared to the contralateral kidney. In another group of anesthetized rats with a single DNFK, 15 min of suprarenal aortic constriction increased plasma renin activity (PRA) from 3.4±0.6 to 11.5±1.6 ng/A-I/ml/hr ($P<0.001$). In a time control group, PRA was unchanged from 4.1±0.8 to 3.9±0.8 ng/A-I/ml/hr ($P>0.25$). It is concluded that in the rat the macula densa and the renal nerves are not essential for the hyperreninemia induced by a reduction in renal perfusion pressure. This study demonstrates that in the rat as in the dog (Blaine et al., Circ. Res. 27: 1081, 1970) the renal vascular receptor plays a crucial role in the control of renin release.

60.7

SUPPRESSION OF FUROSEMIDE-INDUCED INCREASES IN PLASMA RENIN ACTIVITY (PRA) BY AMILORIDE WITHOUT ATTENUATION OF NATRIURESIS IN CONSCIOUS RABBITS. Thomas C. Lee, Kenji Shimoda* and Morton H. Maxwell. Cedars-Sinai Med. Ctr. and UCLA Sch. of Med., Los Angeles, CA 90048

We previously reported that aprotinin, an estero-protease inhibitor, attenuated furosemide-induced increases in PRA and natriuresis in conscious rabbits. To test whether this effect may be mediated via an inhibition of renal kallikrein activity, we studied the effect of the kallikrein-inhibiting diuretic, amiloride, on furosemide-induced stimulation of renin secretion. Rabbits bearing cannulae implanted 2-3 days before were studied twice on separate days, once with and once without an infused dose of amiloride (A, 1 mg/kg, iv in 15') given prior to furosemide (F, 0.75 mg/kg, iv). F produced a rapid and persistent rise in PRA, but this effect was prevented by A (n=4, PRA=ng AI/dl/3 hrs, *p<0.05):

		0'	5'	15'	45'
PRA	F	2137	3921	3815	3937
	A+F	1910	1915*	1590*	1571*

A did not affect F-induced diuresis or natriuresis, but markedly suppressed kaliuresis without significantly affecting plasma potassium concentration. Neither F nor A, alone or in combination, affected mean arterial pressure. These results provide corroborative evidence in support of the hypothesis that renal kallikrein may function as an intrarenal activator of inactive renin. (Supported in part by NHLBI Grant #HL22069 and by AHA-GLAA Award #415IG14).

60.8

EFFECT OF APROTININ, A SERINE PROTEASE INHIBITOR ON RENIN RELEASE. S Seto*, V Kher*, AG Scicli and OA Carretero. Henry Ford Hospital, Detroit, MI 48202.

We studied the effect of aprotinin (Apt), a polyvalent serine protease inhibitor, on plasma renin activity (PRA). PRA in anesthetized rats infused with 5% dextrose for 30 min (time controls) increased from 18±4.4 ng Ang-I/ml/hr to 36±13.1, p<0.05; in rats infused with Apt (3,500 KIU/100g/30 min), PRA decreased (24±3.3 to 19±2.8, p<0.05, n=11). In unanesthetized rats, PRA did not change in time controls (4.9±0.9 to 6.5±0.6) or in the Apt treated group (5.0±0.09 to 4.2±0.06, p<0.05). At the end of the infusion in both anesthetized and unanesthetized groups, PRA was higher in the controls than in Apt treated groups (p<0.05), suggesting renin release inhibition. Isoproterenol (0.3 µg/100g/30 min) stimulated PRA was unaffected by Apt (control 6.2±1.6 to 16.7±3.8, Apt 4.8±04 to 15.7±1.6). A higher dose of Apt (32,000 KIU/100g/60 min) alone caused an increase in PRA (4.6±1.0 to 25.5±5.6, p<0.05), a decrease in mean BP (114±3 mmHg to 86±4, p<0.05), and an increase in hematocrit (49±1.6 to 55±3.5, p<0.05). The decrease in PRA produced by the lower dose of Apt may be due to the inhibition of serine protease(s) participating in the mechanism of renin release. Adrenergic-stimulated PRA appears to be independent of this mechanism. The increase in PRA produced by the higher dose of Apt is probably secondary to the changes in BP and hematocrit, which in turn may be due to the high cationic charge of Apt. Supported by NIH grants HL 15839 and HL 21092

60.9

Neural Control of Renin Secretion: Evidence for Prostaglandin Dependent and Independent Pathways. J.L. Osborn, U.C. Kopp, M.D. Thames and G.F. DiBona. Dept. of Physiology, Med. Coll. Wisconsin, Milwaukee, WI 53226 and Dept. of Int. Med., Univ. Iowa, Iowa City, IA 52242

Low frequency renal nerve stimulation (LFRNS; 0.5-1.0 Hz) increases renin secretion (RS) without changing renal blood flow (RBF). We investigated the role of renal prostaglandins (PG) in the RS response to LFRNS. In anesthetized dogs, LFRNS increased RS and PGE_2 secretion at 0.5, 1.0 and 2.0 Hz. RBF was unchanged at 0.5 and 1.0 Hz but decreased at 2.0 Hz. Thus, during LFRNS, RS may be mediated in part by PGE_2 secretion. Next, the relationship between neurally mediated PGE_2 secretion and RS was studied using PG synthetase blockade with indomethacin or meclofenamate. LFRNS at 0.5 Hz (N=10) increased RS by 79±16 ng/min before but not during intrarenal PG blockade. 0.5 Hz LFRNS had no effect on urinary sodium excretion ($U_{Na}V$) or RBF. In 10 dogs with intrarenal β_1-adrenoceptor blockade (atenolol, 0.5 µg/kg/min), LFRNS at 1.0 Hz increased RS by 740±255 ng/min before but only by 309±93 ng/min during PG blockade. These increases in RS were different (p<0.05). LFRNS (1.0 Hz) equally decreased $U_{Na}V$ before and during PG blockade. We conclude that during LFRNS at 0.5 Hz, RS is predominantly dependent on PG synthesis. LFRNS at 1.0 Hz increases RS both in PG dependent and PG independent pathways. We suggest that RS during 0.5-1.0 Hz LFRNS requires PG synthesis whereas at higher frequencies (>1.0 Hz) PG independent mechanisms predominate in mediating the response of RS.

60.10

EFFECTS OF ASPIRIN AND THEOPHYLLINE ON THE RENIN RESPONSE TO VOLUME CONTRACTION IN SHR. C. Rodriguez-Sargent*, J.L. Cangiano, E. Rivera*, R. Sanchez* and M. Martinez-Maldonado. VA Hospital, San Juan, PR 00936.

We previously showed that the renin response to volume contraction induced by furosemide (VC) in SHR is restored by indomethacin, a cyclooxygenase inhibitor. Indomethacin, however, is also known to inhibit phosphodiesterase. In the present study the effects of cyclooxygenase inhibition by indomethacin and of phosphodiesterase inhibition by theophylline on the renin response to VC were assessed in 16 adult SHR and 16 age-matched Wistar-Kyoto rats (WKY). Basal plasma renin activity was measured, after which 8 rats from each strain were treated with theophylline (4mg/kg/d) and the remaining rats with aspirin (20mg/kg/d) for 3 days. Furosemide (2mg/kg) was then administered to each rat and PRA again measured. Basal PRA was lower in SHR (4.7 ± 0.8) than in WKY (9.6 ± 0.7). VC increased PRA in WKY after treatment with either theophylline (17.0 ± 3.9) or aspirin (15.6 ± 2.1). In response to VC renin did not increase in theophylline-treated SHR (5.9 ± 0.9), but aspirin restored the renin response to VC in SHR (11.8 ± 0.9). We conclude: 1) the previously reported restoration of the renin response to VC by indomethacin in SHR is not mediated through phosphodiesterase inhibition and 2) restoration of the renin response to VC by aspirin in SHR suggests that this effect may be the result of prostaglandin synthesis inhibition.

60.11

STIMULATION OF RENIN BY KALLIKREIN AND KININ IN ISOLATED GLOMERULI. W.H. Beierwaltes, M.L. Arora* and O.A. Carretero. Henry Ford Hospital, Detroit, MI 48202.

The renal kallikrein-kinin system has been linked to renin release both in vivo and in vitro. We used rat isolated glomeruli as an in vitro model, free of tubular and hemodynamic signals and devoid of tubular kininases, to characterize the relationship between kallikrein, kinins and renin release. Glomeruli were isolated by a passive seiving technique and superfused with Krebs buffer. Serial effluent collections were measured for renin concentration by RIA; all values presented are in ng A-I/ml/hr. Concentrations of 0.3 or 3.0 µg/ml hog kallikrein increased renin from 2.36±.26 to 5.12±.99 (n=8, p<0.05) and 2.08±.30 to 8.02±2.06 (n=13, p<0.005), respectively after 30 min of superfusion. This response was blocked by 500 KIU/ml aprotinin (2.14±1.09 to 1.82±1.07, n=4). Administration of 10^{-6} and 10^{-5} bradykinin stimulated release of renin from 1.23±.22 to 7.27±2.35 (n=10, p<0.05) and 1.90±.54 to 8.50±2.67 (n=8, p<0.05) respectively, within 10 min of superfusion. The kininase-II inhibitor captopril at 10^{-5}M did not enhance the renin response to bradykinin. Time controls remained unchanged. These results suggest that, in the absence of hemodynamic, neural, and tubular influences, kallikrein and kinin can stimulate renin. The time course of their respective responses and the absence of kallikrein substrate suggests kallikrein and kinin stimulate renin through different mechanisms. (NIH HL-15839)

60.12

INHIBITION OF URINARY EXCRETION OF PROSTAGLANDINS AND KALLIKREIN IN ACUTE GLOMERULONEPHRITIS. J. Colina-Chourio, B. Rodríguez-Iturbe* and R. García-Ramírez*, Universidad del Zulia, Facultad de Medicina, Hospital Universitario, Maracaibo Venezuela.

Vasoconstrictor systems (α-adrenergic and renin-aldosterone) have been studied in acute glomerulonephritis, but vasodilator systems have not been analyzed to date. PGE_2, $PGF_{2\alpha}$ and kallikrein urinary excretion were studied in thirteen children with acute poststreptococcal glomerulonephritis (APSGN) with mild to moderate hypertension and low sodium diet. They were studied twice: within 48 hours of admission (acute phase, period A) and 4-6 weeks later (recovery phase, period B). Eighteen apparently healthy age and sex matched children on a 150 mEq Na^+/day diet were studied as controls. PGE_2 and kallikrein excretions were diminished during both periods of APSGN. $PGF_{2\alpha}$ excretion was also suppressed in period A but there were large individual variations during period B. There was no difference in PGE/PGF ratio between control and APSGN patients in both periods. There was no correlation between PGs and kallikrein excretions and serum and urinary electrolytes, serum and urinary osmolality, Cosm, urine flow, plasma renin activity, plasma and urinary aldosterone, blood pressure chenges and fluid retention. The results suggest a decreased urinary excretion of PGs and kallikrein in APSGN and that such alteration may play a contributory role in aggravating the fluid retention and hypertension of the APSGN. (Supported in part by CONICIT, Venezuela, Grant # SAL-SI-001).

61.1

PERSISTENT EFFECTS OF NEONATAL CARBON MONOXIDE EXPOSURE. David G. Penney and Audrey E. Stryker*. Dept. of Physiol., Wayne State Univ. School of Medicine, Detroit, MI 48201

To test the hypothesis that persistent cardiomegaly of adulthood (Toxicol. Appl. Pharm. 53: 271, 1980) is accompanied by altered cardiovascular function, newborn rats inhaled 500 ppm CO for 33 days in 2 experiments: 1) Hemodynamics were studied using an anesthetized open-chest preparation. Weights of left ventricle (LV) + interventricular septum (S), right ventricle (RV), and LV+S+RV were greater than predicted based on body weight (BW) at 87-97 and 166-176 days in females, and 98-108 and 177-183 days of age in males. LV+S weights were greater than controls in all but the oldest males. Heart rate (HR) was significantly higher in 3 of the 4 treated groups than in matched controls and stroke index (SI) was marginally depressed, whereas there were no differences in cardiac index (CI). LV end-diastolic pressure, stroke work, +dP/dt max., +dF/dt max., total vascular resistance and arterial systolic pressure (BP) were unaltered. 2) Resting HR and BP were obtained by auscultation in the conscious state. HR was significantly higher in both males and females from 78-200 days of age than in age and sex matched controls. There were no differences in BP. BW of males exposed to CO as neonates remained significantly below that of normal males until at least 300 days of age, whereas this was not the case with females. The data support the above mentioned hypothesis. Apparently tachycardia maintains normal CI at lower SI. (Supported by NIH Research Grant HL-22859)

61.2

THE EFFECTS OF PERINATAL CARBON MONOXIDE EXPOSURE ON HEART GROWTH IN THE NEONATAL RAT. Fred J. Clubb, Jr.* and Sanford P. Bishop*. Dept. of Path., UAB, Birmingham, AL 35294; David G. Penney and Michael S. Baylerian*. Dept. of Physiol., WSU, Detroit, MI 48201

In order to study the effect of increased hemodynamic stress on developing neonatal rat myocardial structure, pregnant Sprague-Dawley rats were maintained in room air (A) or exposed to 200 ppm carbon monoxide (CO) from 7 days (d) gestation through 28 d postpartum. At birth, 4, 6, 9, 12, 15, 21, and 28 d the following were studied: body weight (BW); total heart weight (HW); left ventricle + septum (LV) and right ventricle (RV) wet and dry weight; volume % myofibers from perfusion fixed hearts; and % binucleation (2N) and cell volume (CV) from isolated myocytes obtained by perfusion with Ca++ free media containing 0.1% collagenase. CO HW/BW was increased from birth (8.15 mg/g vs. 6.55 for A, $p<0.01$) to 28 d (5.70 mg/g vs. 4.79, $p<0.01$). CO had delayed 2N at 4 d (CO, LV 5.5 $\pm$0.3% SEM vs. 8.05 $\pm$0.6, $p<0.01$) and 6 d (CO, LV 23.7 $\pm$1.9% vs. 38.3 $\pm$2.1, $p<0.01$), but was not different at later times. At 28 d CV was less in CO than A (LV = 5992 $\pm$245 fl vs. 8621 $\pm$436, $p<0.01$). In summary, 28 d CO had increased HW/BW ratio, smaller CV, and no difference in water content or volume % myofibers. We conclude that perinatal CO exposure causes an increase in cardiac mass with smaller CV indicating sustained myocyte hyperplasia and increased cell number.

61.3

BIOCHEMICAL AND FUNCTIONAL INDICES OF CONTRACTILE POTENTIAL IN THE DEVELOPING RAT MYOCARDIUM. A.M. MacIntosh*, K.M. Baldwin, and R.E. Herrick*. UCI, Irvine, Calif. 92717

Several recent studies suggest that changes in myosin ATPase activity and contractility may be explained on the basis of a shift in the relative proportion of the cardiac myosin isozymes, V_1, V_2, V_3. Further, the isozyme pattern has been shown to change with age in the rodent myocardium. Therefore, this study was undertaken to investigate the cardiac myosin pattern during early development in the rat (5 to 75 days of age) and how this pattern correlates with cardiac myosin and myofibril ATPase activity as well as in situ derived functional indices of contractility (dp/dt). From 5 to 30 days of age the proportion of V_2 and V_3 isozymes progressively decreases. This corresponded to increases in both myosin and myofibril ATPase as well as dp/dt. From 30 to 75 days of age the proportion of V_2 and V_3 progressively increases, inspite of the fact that myofibril , myosin ATPase and dp/dt max remains relatively constant. These data suggest that the above biochemical and functional indices of contractile potential show similar trends only during the very early stages of neonatal development.

	10 day	30 day	75 day
Myofibril ATPase (µm/min/mg)	.163 ± .015	.230 ± .012	.230 ± .013
Myosin ATPase (µm/min/mg)	.654 ± .057	.746 ± .048	.809 ± .061
+ dp/dt (mmHg/sec)	2867 ± 215	6200 ± 408	6700 ± 215

61.4

CYCLIC NUCLEOTIDE ENZYMES IN DEVELOPING RAT HEART. R.T. Dowell, J.L. Haithcoat*, and H.M. Thirkill*. Dept. of Physiology, Univ. of Kansas Medical Ctr., Kansas City, KS. 66103.

Parallel transitions occur in rat heart growth, metabolism, and function during the perinatal period. Contributing cellular components were evaluated by measuring selected enzyme activities in LV homogenate and in isolated muscle and nonmuscle cells. Malate dehydrogenase activity (MDH) in homogenate increased by 50% during perinatal transition. MDH in adults was augmented in both muscle and nonmuscle cells, but to a greater extent in the nonmuscle fraction. In contrast to aerobic metabolism enzymes, adenylate cyclase activity (AC) in weanling LV homogenate was 12-fold greater than adult (21.7±4.8 vs 1.74±0.53;pmole/mg protein/min) and guanylate cyclase activity (GC) was enhanced 2-fold (2.48±1.18 vs 1.31±0.22;pmole/mg/min). Nonmuscle/muscle cell AC ratios were approx. 2.0 in weanling and adult LV. GC ratios were higher in adult (3.43±1.54) than in weanling LV (1.28±0.13). Despite elevated AC in weanlings, nearly identical cyclic AMP levels (1,520±120 vs 1,510±60;pmole/gm) were observed in weanling and adult LV, respectively. Cyclic GMP levels (pmole/gm) were nearly 2-fold greater in weanling (36±2) than in adult (21±1) LV. Thus, cAMP level may be regulated by a degradative mechanism (cAMP phosphodiesterase) while cGMP level is consistent with synthetic enzyme (GC) control. In turn, cGMP level modulates cAMP/cGMP ratio in weanling (43±3) and adult (76±4) LV in a manner that could allow divergent cellular adaptation. (Supported by NIH Grants HL 29456 and HD 16247).

61.5

SARCOMERE UNIFORMITY FROM RESTING SINGLE HEART CELLS. Kenneth P. Roos* and Allan J. Brady. Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024.

Striation patterns of isolated myocytes are directly imaged with a high resolution computer coupled optical system to provide discrete sarcomere positions (Roos, et al., Am. J. Physiol. 242:H68, 1982). Calcium tolerant isolated heart cells are enzymatically digested from adult rat myocardium (Brady et al., Nature 282:728, 1979). Small sub-volumes (86 µm long x .05 µm wide x 1.6 µm thick) of the A-I band striation patterns from these isolated cells are imaged with high-resolution, high-contrast Nomarski DIC immersion optics. The striation image is projected onto a 1728 element CCD photosensing array coupled to a PDP 11/34a digital computer. Optically discrete cell volumes have been sequentially imaged along lateral and focal planes from resting, unattached myocytes. Each frame of data is digitally image processed and discrete striation positions are determined within the imaged volume with a precision of up to ±.05 µm. Individual striations have been sequentially tracked through lateral and focal planes to provide planar striation maps. Gross structural information (nuclei, striation discontinuities) from correlative photomicrographs are precisely reconstructed in these planar striation maps. Discrete sarcomere uniformity can then be determined throughout an intact living heart cell to provide the means for improved understanding of myocardial kinetics.
(Supported by American Heart Association, GLAA to K. Roos and USPHS HL 11351-16 to A.J. Brady.)

61.6

REGIONAL DIFFERENCES IN THE RESPONSE OF THE ATRIAL PACEMAKER SYSTEM TO EPINEPHRINE, ACETYLCHOLINE AND KCl. Lou A. Roberts. Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430

Spontaneously beating atrial strips were used to evaluate regional differences in the chronotropic response to epinephrine (Epi), acetylcholine (ACh), and KCl. The right atrium was pinned into a rectangular shape and cut into three approximately equal strips, each containing part of the pacemaker region. Each strip was placed in a tissue bath attached to a glass hook and a Statham tension transducer. Electrical activity of the strips was monitored using bipolar Ag-AgCl electrodes. Strips were equilibrated for one hour in Tyrode bathing medium which was then replaced with a test medium. Dose response curves were obtained for all agents; repeatability of the response was established after one hour recovery in Tyrode medium. Qualitatively, the response of atrial strips to these agents resembled the response of entire right atria. In all regions, the chronotropic response was positive for Epi and negative for ACh and KCl. Variation among regions was lowest for the response to Epi. Strips from the posterior (low) pacemaker region responded with the greatest increase in rate to Epi (10^{-7} to 5×10^{-6} M) and the greatest decrease in rate to ACh (10^{-8} to 10^{-5}M). The anterior strip was depressed most by KCl (6-15 mM); the mid-pacemaker region was most resistant to KCl (active to above 20 mM). Atrial strips do indicate regional differences in pacemaker response and may prove useful in interpreting and predicting shifts in pacemaker location. (Supported in part by NIH Grant HL 21145.)

61.7

MEASUREMENT OF DIFFUSION DISTANCES FROM CAPILLARIES TO MITOCHONDRIA. Susan R. Kayar and Natalio Banchero. Univ. Colorado Medical School, Denver, CO 80262.

The distance over which oxygen diffuses from its source in capillaries (C) to its sink in mitochondria (M) is critical to an assessment of aerobic metabolism. We used a combination of morphometric methods to measure the distribution of distances from a C to the M surrounding it. In transmission electron micrographs in which C appeared in transverse section, we drew a series of concentric circles around a C such that concentric rings of equal area were formed. The radius of the largest circle in each set was approximately one-half the average intercapillary distance. The number of M per ring was recorded. Volume density of M per ring was calculated by the point-counting method, in which a grid of points was placed over the concentric circles and the relative number of points touching M was recorded. In tests on models this method had an accuracy of at least 95% when volume densities of M were greater than 0.10. From a test sample of 12 C from the right ventricular endocardium of an adult dog (C density = 1100 $C \cdot mm^{-2}$), we found that the highest volume density of M was 0.23 ± 0.01 at a distance of 8 μm (range 7-10 μm) from a C. This distance represents approximately 25% of the total O_2 diffusion distance for this tissue. Volume density of M decreased to 0.14 ± 0.01 at a distance of 12 μm from a C (40% of total O_2 diffusion distance), and was also 0.14 ± 0.01 (n = 43) in the "dead spaces", or regions farthest from any C. Supported by NIH grants HL06527 and HL18145.

NEURAL CONTROL OF CIRCULATION III

62.1

INHIBITION OF BARORECEPTOR AND HERING-BREUER REFLEXES BY NOXIOUS STIMULATION IN INTACT AND DECEREBRATE ANIMALS. C.Timo-Iaria, S.L.D.Cravo and C.A.B.Fraga. Institute of Biomedical Sciences, University of São Paulo, São Paulo SP, Brazil.

Cardiovascular and ventilatory adjustments are an integral part of behavioral patterns. Some homeostatic adjustments, such as the baroreceptor and Hering-Breuer reflexes, should be inhibited in many behaviors to allow centrally patterned hemodynamic and respiratory adjustments. The occurrence of such inhibition was investigated when physiological noxious stimulation of the paws (skin or bone) was performed in dogs (intact, anesthetized with pentobarbital) and cats (either intact, anesthetized or decerebrate, non-anesthetized). The response to stimulation was retraction of the stimulated member and increase in blood pressure and respiration. Noxious stimulation during 30 seconds consistently inhibited baroreceptor and Hering-Breuer reflexes elicited by electrical excitation of baroreceptor afferents and lung afferents in the central stump of one vagus in both intact and decerebrate animals. Stimulation of high threshold afferent fibers in one vagus also blocked the reflexes elicited by stimulation of the contralateral vagus. (Supported by the São Paulo State Research Foundation - FAPESP, National Research Council-CNPq, and FINEP)

62.2

CHRONIC SUCROSE INGESTION ELEVATES BLOOD PRESSURE BUT INHIBITS INSULIN SECRETION IN RATS. Ruben D. Buñag and Susumu Sasaki*. Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas, 66103.

Tail-cuff systolic pressures, in male weanling rats given 8% sucrose solution to drink instead of water, became appreciably elevated after 5 weeks. The pressure elevation was verified when phasic pressures were later recorded from the same rats directly from indwelling femoral catheters. Sympathetic overactivity was considered likely because sucrose-ingesting rats, compared with untreated controls, had faster heart rates and larger hypotensive responses to α-adrenergic blockade with phentolamine. Upon subsequently being anesthetized with urethane, pressor responses to electrical stimulation of the ventromedial hypothalamus were larger in sucrose-treated rats as were attendant increases in frequency of sympathetic neural firing. By contrast, pressor responses to injections of norepinephrine or tyramine were unaffected thereby indicating that cardiovascular sensitivity had not been enhanced by sucrose ingestion. In other rats given sucrose for 5 weeks, aside from hypertension and tachycardia, insulin secretion in response to intravenously-injected glucose was reduced. From these results we conclude that by sensitizing the ventromedial hypothalamus, sucrose ingestion increases sympathetic activity to elevate blood pressure and diminish insulin secretion. (Supported by NIH Research Grant AM 27660)

62.3

CAROTID SINUS BARORECEPTOR ACTIVITY IN THE NON-HUMAN PRIMATE. E. Tomomatsu*, L. Huffman and J.P. Gilmore. University of Nebraska College of Medicine, Omaha, Nebraska 68105

The impulse activity in single baroreceptor fibers of the carotid sinus nerve was studied in Macaca fascicularis monkeys during non-pulsatile perfusion of functionally isolated carotid sinus preparations. The results were compared with those obtained from dogs studied under the same experimental conditions. Curves relating the impulse frequency to the carotid sinus pressure were constructed. Under pentobarbital anesthesia, the mean arterial blood pressure of 8 monkeys (89.9 ± 4.0 mmHg) was lower than that of 10 dogs (114.1 ± 3.8 mmHg). Sixty-five baroreceptors from 8 monkeys showed a lower threshold pressure (58.8 ± 2.7 mmHg) than that of 68 fibers from 10 dogs (84.1 ± 3.9 mmHg). The gain of 33 baroreceptors from the monkey (0.21 ± 0.02 impulses/sec per mmHg) was lower than that of 35 baroreceptors from the dog (0.49 ± 0.03 impulses/sec per mmHg). The plateau pressure of 32 baroreceptors from the monkey (138.8 ± 4.7 mmHg) was lower than that of 33 baroreceptors from the dog (184.8 ± 4.7 mmHg). The impulse frequency at the plateau pressure of 32 baroreceptors from the monkey (27.2 ± 2.0 impulses/sec) was lower than that of 33 baroreceptors from the dog (57.2 ± 4.3 impulses/sec). The results provide direct evidence that the carotid sinus baroreceptors in the monkey are less sensitive to pressure changes than those in the dog. Supported by NIH grant HL-13427.

62.4

EFFECT OF VASOACTIVE DRUGS ON RABBIT AORTIC NERVE ACTIVITY. H.O. Stinnett, L.W. Sonnenberg*, and M.R. Magnusson*, Univ. of N. Dak., School of Medicine, Grand Forks, ND 58202.

In aortic denervated anesthetized rabbits, left aortic nerve (LAN) activity (CPS) was examined relative to mean arterial pressure (MAP). Protocol included 5.0 cm H_2O positive end expiratory pressure (PEEP) to reflexly and mechanically decrease aortic pressure and carotid occlusion (OCC) to reflexly increase pressure. Protocol was performed before and during administration of lysine vasopressin (ADH) or nitroglycerine (NTG). Drugs, infused at a constant rate, induced either a mild (5-10 mm Hg) pressor (ADH) or a depressor (NTG) response. Results included:

Condition	Baseline		OCC		PEEP	
(N=6)	MAP	CPS	MAP	CPS	MAP	CPS
No Drug	92	554	123	647	56	422
ADH	96	572	135*	674	58	427
NTG	80*	595	105*	670	45*	447

*Significantly ($p < 0.05$) different than MAP during no drug conditions. Standard errors omitted.

Regression analysis of results during no drug conditions demonstrated a significant ($p < 0.05$) and positive linear correlation ($r = 0.9981$) between nerve activity and MAP. ADH infusion did not alter this relationship. During NTG infusion the intercept was increased; indicating a change in the relationship. These experiments do not eliminate possible direct effect of drugs on the aortic baroreceptor. (Supported by American Heart Association, Dakota Affiliate Grant-in-Aid 79-606, and DA-G-04.)

62.5

DO THE INTRINSIC CARDIAC NERVES (ICN) DISPLAY TACHYPHYLAXIS TO NICOTINIC STIMULATION? W.W. Simpson*, T. Blomquist*, R.Anaya* and D.V. Priola. Univ. New Mexico, Sch. Med., Albuq.,NM 87131.

We have been studying the responses of the canine heart to stimulation of the ICN with nicotine (NIC) for the past several years. Tachyphylaxis (TPX) of the ICN may affect responses to NIC. These experiments were performed to resolve this issue. 15 dogs were anesthetized with sodium pentothal, placed on cardiopulmonary bypass and A-V nodal conduction measured with a surface electrode on the His bundle. Vagi were sectioned and positive effects of NIC eliminated by beta-blockade with timolol (4 mg). NIC (10-100 mcg) was used to stimulate the ICN. All agents were given intracoronary via an aortic catheter. Three protocols were used: I - 5 NIC injections separated by 3 min intervals; II - 5 NIC injections done successively as soon as A-V conduction time returned to control (45 sec - 1 min); III - as in II, but with a 10 min recovery time between each dose group. No significant TPX was observed with I; i.e., response to fifth dose was ca. 90% of initial response. Significant TPX was noted in II and III; i.e., response to fifth dose was only 2-20% of the initial response. The degree of TPX was directly related to dose and inversely to dose-interval. We conclude that the ICN can be stimulated with NIC without producing TPX so long as at least 3 min are allowed between successive doses. (Supported by NHLBI Grant No. HL-18517).

62.6

THE EFFECTS OF LEFT ATRIAL RECEPTOR STIMULATION ON RENAL SYMPATHETIC NERVE ACTIVITY IN DOGS WITH CHRONIC VOLUME OVERLOAD. Irving H. Zucker, Andrew J. Gorman, Kurtis G. Cornish and Michael Lang*. Univ. of Nebr. Coll. Med., Omaha, NE 68105

Left atrial receptors with vagal afferents are reset and become less sensitive in dogs with chronic high output congestive heart failure (Zucker et al., JCI 60:323-331, 1977). In the present study, we determined if the normal reflex inhibition of renal sympathetic nerve activity (RSNA) which occurs in response to left atrial distension (LAD) is altered in dogs with chronic volume overload (n=9) produced by an aorta-vena caval fistula (AVF). In addition, we examined the ability of the arterial baroreceptors to alter RSNA. In normal dogs (n=10), LAD decreased RSNA by 6.8±1.8%/ mmHg Δ left atrial pressure (LAP) ($p<.01$). After carotid sinus denervation (CSD), this inhibition was increased to 10.9±1.4% mmHg Δ LAP ($p<.01$). Vagotomy completely abolished any change in RSNA in response to LAD. In contrast, dogs with chronic AVF's demonstrated an increase in RSNA in response to LAD by 4.5± 3.9%/mmHg Δ LAP. The response of the AVF dogs was significantly different from the normal dogs ($p<.005$). This was also the case after CSD. Loading and unloading of the arterial baroreceptors with either norepinephrine or nitroprusside demonstrated that the modulation of RSNA by the baroreceptors was unaltered in both normal and AVF dogs. The present study demonstrates that chronic volume overload results in an abolition of the inhibitory effects of left atrial receptors and preservation of arterial baroreceptor function.

62.7

EFFECT OF NONHYPOTENSIVE HEMORRHAGE ON AORTIC PRESSURE, AORTIC BARORECEPTOR ACTIVITY, AND STROKE VOLUME IN ANESTHETIZED DOGS. M. O. K. Hakumäki*, W. D. Sundet*, B. C. Wang, and K. L. Goetz. St. Luke's Hospital and Foundation, Kansas City, MO 64111

It is a common observation that removal of relatively small amounts of blood (so-called "nonhypotensive hemorrhage") elicits a number of hemodynamic, humoral, and renal responses even though mean arterial blood pressure is unchanged during the experiment. The constancy of arterial blood pressure has allowed the assumption that arterial baroreceptor reflexes play no causal role in the concomitant physiological adjustments. We have tested this assumption in anesthetized dogs (morphine-pentobarbital) by hemorrhaging the animals (0.8 ml/kg/min for 30 min) while simultaneously recording aortic blood pressure, single fiber aortic baroreceptor activity, and stroke volume. Computer analysis of the data indicated that the number of baroreceptor neural impulses per cardiac cycle, as well as the average discharge rate per second, often decreased even though mean arterial pressure was constant during much of the hemorrhage. Stroke volume usually decreased. These data suggest that mean arterial pressure does not accurately reflect changes in arterial baroreceptor activity. Therefore, the hemodynamic and renal responses that occur during nonhypotensive hemorrhage may be elicited, at least in part, by changes in arterial baroreceptor activity.

62.8

NALOXONE INCREASES TOTAL PERIPHERAL RESISTANCE (TPR) IN THE CONSCIOUS RABBIT MADE HYPOTENSIVE BY HEMORRHAGE. James C. Schadt, Michael D. McKown*, Daniel P. McKown*, and Dean Franklin. Dalton Research Center, University of Missouri, Columbia, MO 65211

Studies in this laboratory have shown that α-adrenergic blockade reduces the pressor effect of naloxone by about 60% in hemorrhaged rabbits. This implies a mechanism involving vasoconstriction. We tested the hypothesis that naloxone increases mean arterial blood pressure (BP) by increasing TPR. Six rabbits, chronically instrumented with aortic flowprobes and arterial and venous catheters, were hemorrhaged until their $\overline{BP}$ fell to 30-35 mmHg. $\overline{BP}$ was held at this level for 30 min by additional bleeding or reinfusion. The rabbit was then given an IV injection of naloxone(N) (5 mg/kg) or saline(S). The effects on $\overline{BP}$, cardiac output (CO), heart rate (HR), and TPR are shown below.

		$\overline{BP}$ (mmHg)	CO (kHz)	HR (beats/min)	TPR (mmHg/kHz)
Control:	N	71±4	3.7±.4	206±9	20±2
	S	65±3	3.9±.3	206±8	17±1
Post-	N	34±3	1.7±.1	285±16	20±2
hemorrhage:	S	33±1	2.0±.2	278±10	17±1
2 min	N	84±5	2.1±.1	199±25	40±2
Postinject:	S	40±1	2.3±.2	280±12	18±1

Therefore, the increase in $\overline{BP}$ produced by naloxone in the conscious rabbit is due primarily to an increased peripheral resistance rather than increased cardiac output.

62.9

CORTICAL ACETYLCHOLINE EFFLUX IN THE RABBIT AS A FUNCTION OF HYPERCAPNIA, EEG STATE AND DECEREBRATION. D.M. Hudson, O.U. Scremin, R.R. Sonnenschein and D.J. Jenden. UCLA Sch. Med., Los Angeles, CA 90024

To evaluate further the proposed role of a cholinergic mechanism in cortical vasodilatation associated with hypercapnia and EEG arousal, we studied the rate of acetylcholine (ACh) efflux from cerebral cortex in these conditions, before and after precollicular decerebration, in mechanically ventilated rabbits under halothane - N_2O anesthesia. A lucite ring, sealed to the pial surface, was filled with mock CSF containing physostigmine to prevent degradation of ACh; atropine (topical or i.v.) was added to block presynaptic inhibition of ACh release. Samples of fluid were removed every 5-10 min for assay of ACh by gas chromatography/mass spectrometry. Under normocapnia ($ETCO_2$ = 4%), ACh efflux (pmoles/cm^2 · min) was 19 when EEG was in the non-aroused (high voltage) state, and 34 when EEG was in the aroused (low voltage) state. Hypercapnia ($ETCO_2$ = 8%) raised ACh efflux to 41 in both cases. After decerebration, ACh efflux was 3 pmoles/cm^2 · min during normocapnia and was unaffected by hypercapnia. The results parallel our previous observations on cerebral blood flow as a function of $ETCO_2$, EEG and decerebration. (Supported by NIH HL 17903 and MH 17691, and AMA-GLAA 437IG).

62.10

REFLEX AUTONOMIC INFLUENCES ON CARDIAC OUTPUT FROM AIRWAY RECEPTORS IN RABBITS. D. Fred Peterson and Jacque L. Bergman* Oral Roberts University Medical School, Tulsa, OK 74171

Nine rabbits, anesthetized with sodium pentobarbital, were instrumented to measure ECG, heart rate (HR), aortic blood pressure (BP), central venous pressure (CVP) and respiratory movements. Also, through a thoracotomy, an electromagnetic flow probe was placed around the ascending aorta for measurement of cardiac output (CO). Stroke volume (SV) was calculated. In six animals the chest was closed and evacuated so that the rabbit breathed spontaneously. The other rabbits were artificially ventilated. Two midtracheal cannulae allowed respiration with room air while passing cigarette smoke across the upper respiratory nociceptors and out through the nares. In 16 trials, as previously observed, smoke caused a fall in HR (-128±16 b/min) and a rise in BP (+24±3 mmHg). It also caused a rise in CVP (+1.8±.6 mmHg), a decrease in CO (-131±15 ml/min) and a large rise in calculated total peripheral resistance (TPR) (368%±66). SV (-14±11%) was not significantly affected. Changes during artificial ventilation were slightly less pronounced; HR (-106 b/min) and CO (-95 ml/min) fell while TPR rose (298%). SV (-18±11%) was not significant. After vagotomy in the spontaneously breathing trials HR fell only 29 b/min, CO (-80 ml/min) and SV (-32±8%) fell and TPR was up 129%. After vagotomy plus artificial ventilation changes persisted; HR (-12 b/min), CO (-69 ml/min) and SV (-35±4%) fell and TPR rose (132%). Thus, both vagal and cardiac sympathetic activity contribute directly or indirectly to changes in HR, CO, SV and TPR.

62.11

REFLEX MODULATION OF THORACIC, RENAL, AND SPLENIC SYMPATHETIC EFFERENT NERVE ACTIVITY BY ALTERATIONS IN SYMPATHETIC EFFERENT ACTIVITY TO THE CAROTID BARORECEPTORS. J.L. Seagard, C.A. Salter*, and J.P. Kampine. Dept. of Anesthesiology, Med. Col. Wis., Milwaukee, WI 53193

Increases in sympathetic efferent nerve activity (SENA) to the carotid sinus have been shown to increase baroreceptor sensitivity, resulting in enhanced baroreceptor discharge. Decreases in heart rate and blood pressure have been induced by electrical stimulation of sinus sympathetic efferent nerves, but reflex changes in overall SENA to other beds in response to physiolocially induced changes in sinus efferent activity have not been reported. This study examined reflex changes in thoracic, renal, and splenic SENA resulting from hypotension (Δ50mmHg), hypertension (Δ50mmHg), and hypoxia (PO_2<70 torr) before and after sympathetic efferent denervation of the carotid sinus baroreceptors, performed by section of the lower cervical sympathetic trunks. Sympathetic efferent denervation of the sinus produced sustained increases in SENA in all nerves studied. The reflex increases in thoracic, renal, and splenic SENA in response to hypotension and hypoxia, which also increased sinus SENA, were larger following sinus denervation. The reflex decreases in SENA in response to hypertension were not significantly different following sinus denervation. Thus, sympathetic activity to the sinus appears to tonically modulate baseline SENA and blunt reflex increases in thoracic, renal, and splenic SENA. (Supported by NIH GM29641-01 and the VA).

62.12

FUNCTIONAL MAPPING OF THE HEART WITH ^{14}C 2-DEOXYGLUCOSE DURING ELECTRICAL STIMULATION OF CARDIAC EFFERENT NERVES. D.R. Kostreva, J.A. Armour and J.P. Kampine. Depts. Anesthes. and Physiol, Med Col Wis, VA Med Ctr, Milw., Wis 53193 and Dept. Physiol, Dalhousie U., Halifax, Nova Scotia B3H 4H7.

A study was designed to determine if the ^{14}C deoxyglucose (DG) metabolic mapping technique of Sokoloff could be used to identify localized areas of the heart that are influenced by stimulation of specific cardiac efferent nerves. Dogs 3-4 kg were anesthetized with sodium pentobarbital (35 mg/kg i.v.). The left ventrolateral cervical cardiac nerve (VLCN) was cut and stimulated efferently using a constant current stimulator. Parameters of 10 Hz, 0.5 msec width and a current strength of 1-10 mA were used to evoke increases in amplitude and duration of the T-wave of the electrocardiogram. A single bolus injection of DG (Pathfinders Labs 100 uCi/kg) was administered i.v. After 45 minutes of periodic stimulation of the VLCN, the heart was frozen and sectioned at 20um increments for autoradiography. After 12 days of exposure the films were developed and examined to determine which areas of the heart had increased uptake of DG. Increases in DG uptake occurred only within the left heart. The greatest DG uptake was within the anterior and posterior papillary muscles and to a lesser degree the left ventricular endocardium. These results suggest that the DG technique may be useful for determining which regions of the heart are functionally influenced by specific cardiac efferent nerves. (Supported by Dept. Anesthes., Med Col Wis, VA and NIH RCDA HL00959).

62.13

EXCITATION OF FELINE THORACIC SPINAL NEURONS BY CARDIAC ARRHYTHMIAS. Robert W. Blair*, H. Rodney Holmes*, and Robert D. Foreman. Univ. Okla. Hlth. Sci. Ctr., Dept. of Physiology and Biophysics, Okla. City, OK 73190.

Sympathetic afferent fibers originating in the heart are sensitive to arrhythmia-induced mechanical changes. The purpose of this study was to determine whether this information regarding arrhythmias is transmitted to higher order spinal neurons. 28 cats were anesthetized with α-chloralose (40 mg/kg) and were paralyzed with pancuronium (0.15 ug/kg/min). Electrodes were placed in the right thalamus and both right and left medullary reticular formation to antidromically activate spinothalamic and spinoreticular neurons. With electrodes placed on the left atrium, hearts were paced 10-20 beats faster than the spontaneous rate. Single electrical pulses were delivered to the left ventricle late in diastole to produce an extrasystole; 25 repetitive cycles of stimuli were summed to construct histograms. Two of 17 (12%) neurons projecting to the thalamus, 8 of 26 (31%) spinoreticular neurons, and 10 of 23 (43%) spinal neurons (those not projecting to thalamus or reticular formation) responded to the arrhythmias. Responses were not abolished by vagotomy. Stimuli for cell responses usually appeared to be mechanical changes in the atria or ventricles due to the potentiated beat following the extrasystole. These results indicate that neurons in the spinal cord are sensitive to mechanical changes in the heart, although responses are often complex and not easily explained only by changes in left ventricular pressure and its derivative. (Supported by NIH grants HL27260, HL00557, HL07430, HL06318, and by a grant from the Oklahoma Heart Association).

62.14

ADRENERGIC CONTRACTILE RESPONSES AFTER REGIONAL LV SYMPATHECTOMY IN CONSCIOUS DOGS. J. Krasney, A. Orlick*, B. Sekovski* and F. J. Klocke. SUNY at Buffalo, NY 14214

Regional shifts in LV systolic pressure-length relationships were studied in 4 conscious dogs 11-18 days after regional LV sympathectomy (Sx) and in 3 sham Sx dogs. Sx was produced by topical application of phenol and verified by 98% reduction of local tissue norepinephrine (NE) levels. Sonomicrometric measurements of end-systolic segment length (ESL) were made in normally innervated (I) and Sx regions before and during transient aortic constriction. Before drug administration, ESL in I and Sx regions increased to 104 ± 0.6(SEM)% and 104 ± 0.7% of control as peak systolic pressure was raised from 140 ± 5 to 172 ± 7 mm Hg. During isoproterenol (ISO) ($0.025\ ug \cdot kg^{-1} \cdot min^{-1}$), ESL's before and during aortic constriction were less ($p<0.01$*) in the Sx region (96 ± 0.7%*, 101 ± 1.3%*) than in the I region (98 ± 0.4%, 105 ± 0.5%). Responses to NE infusion were similar. By contrast, tyramine (TYR) injection (650 ug) shortened ESL more in the I region (96 ± 0.4%*, 101 ± 0.8%*) than in the Sx region (100 ± 0.8%, 102 ± 0.6%). ISO and NE increased segment shortening velocities to a greater degree in Sx than I regions, whereas TYR produced a directionally opposite response. Sham Sx dogs showed none of the above regional differences. These data indicate functional impairment of the TYR response in the Sx region, and a local hypersensitivity in contractile response to both ISO and NE. (AHA 79-881, HLB 15194).

62.15

DETERMINATIONS OF RESETTING AND SENSITIVITY OF THE ARTERIAL BARORECEPTORS IN RESPONSE TO ACUTE SYSTEMIC PRESSURE CHANGES. L.B. Bell*, J.L. Seagard, F.A. Hopp*, and J.P. Kampine. Med. Col. of Wis. and Wood VA Med. Ctr., Milwaukee, WI. 53193

Studies using few fiber preparations from the carotid sinus nerve (CSN) or aortic depressor nerve (ADN) have shown that these nerves reset to increases or decreases in BP after 20 min without a change in sensitivity. This study was designed to determine if intact CSN and ADN reset in a similar fashion to few fiber preparations and if sensitivity changes occur during the 20 min resetting period. Either simultaneous multifiber nerve recordings of the CSN and ADN or few fiber recordings from the CSN were examined in pentothal anesthetized dogs. Peak aortic BP was isobarically adjusted to 80,120 and 160 mmHg in random order. Threshold (Th) and sensitivity responses were determined 5,10,15 and 20 mins after each pressure change. Thresholds in whole CSN and ADN as well as single fibers in the CSN were elevated within 5 min ($P<.01$) after increases in BP and depressed within 5 min ($P<.01$) after decreases in BP. No further significant changes in Th were observed over the next 15 min. The magnitude of the increase or decrease in Th was greater between 80 and 120 mmHg than 120 and 160 mmHg. Resetting of the single fibers followed the same patterns as that of the whole nerves. Sensitivity of single fibers of the CSN did not show any consistent changes with time during the resetting process. Therefore, resetting of the baroreceptors occurs within 5 mins with no change in sensitivity. (Supp. by NIH GM29641 and VA).

62.16

LOCALIZATION OF THE SPINAL MOTONEURONS MEDIATING A CARDIOSOMATIC REFLEX IN THE CAT. Warren E. Finn*, Kirby L. Jarolim* and Patricia M. Hedrick*. (Spon: E.L. Nelson) Oklahoma College of Osteopathic Medicine and Surgery, Tulsa, OK 74101

The activation of the cutaneous maximus muscles (CM) of the cat by stimulation of cardiac receptors via a sympathetic afferent pathway to the spinal cord has been reported in previous investigations (Schoen and Finn, 1979). The present study was conducted to establish the segmental distribution of the motoneuron pool influenced by cardiac mechanoreceptor stimulation. Retrograde transport of horseradish peroxidase (HRP) was used to localize the cell bodies of motoneurons supplying the CM. In 19 cats 50% HRP was injected directly into the CM fibers or the nerve to the CM was severed and placed in a capsule of 50% HRP. After an incubation period of 24-48 hours the animals were anesthetized and perfused. The spinal cords were removed and processed for visualization of HRP labelled motoneurons. Tetramethyl benzidine and benzidine dihydrochloride procedures were employed. The labelled motoneurons were observed at the caudal end of spinal segment C_6 and were found throughout segments C_7 and C_8 and did not overlap into T_1. The muscle injection procedure was more reliable than that of nerve encapsulation. Further studies of these sympathetic to somatic nerve relationships are currently under investigation as a model to study cardiosomatic reflex processes. (Support by AOA/NOF Grant #79-09-112)

62.17

ROLE OF SEPTAL AREA IN THE CARDIOVASCULAR REGULATION IN RATS. F.O. Guimarães Neto, N.E. Quedi*, L.C. Simas* and M.R. Covian. CFS, UFSC, Florianopolis, S.C., Brazil, 88.000

The effects of electrical stimulation (ES) of the septum on arterial blood pressure and cardiac frequency were observed in conscious and anesthetized rats (Nembutal 40 mg/kg, i. p.). The effects of blocking septal area by administering procaine HCl (4%, 3µl) was also studied in conscious rats. Chronic bipolar electrodes or cannulae were implanted stereotaxically in the lateral (LSN) and medial (MSN) septal nuclei. A catheter was introduced into the femoral artery for blood pressure recording. Septal nuclei were stimulated with currents of different durations, frequencies and intensities. ES of LSN in conscious rats produced hypertension with bradycardia while MSN stimulation elicited hypertension with tachycardia. In anesthetized rats, stimulation of LSN or MSN produced hypotension and bradycardia. LSN block resulted in a significant hypertension and tachycardia, but no alterations were observed after MSN block. These results indicate that septum influences the cardiovascular regulation either by inhibition or by excitation. Moreover, the present study demonstrates that anesthesia modifies the responses induced by ES of septal area. These data also suggest that LSN and MSN may play different roles in cardiovascular control. In conclusion, the LSN may be involved in the tonic regulation of arterial blood pressure by means of an apparently inhibitory action. (supported in part by CNPq).

62.18

EFFECT OF HYPEROXIA IN ESSENTIAL HYPERTENSION UPON THE TOTAL PERIPHERAL RESISTANCE AND THE RESPONSIVENESS TO CAROTID BARORECEPTOR INACTIVATION. M.Tafil, A.Trzebski, Department of Physiology, Medical Academy, Warsaw, Poland.

In 15 hypertensive subjects (HS) 21-23 years old and in the matched group of normotensives (NS) inactivation of the carotid baroreceptors (ICB) was produced by the 40 mmHg increase in the neck chamber pressure for 20s. Cardiac output (CO) and muscular blood flow (MBF) in the tigh were recorded by the impedance rheography. Total peripheral resistance (TPR), MBF resistance (MVR), gain of the heart reflex response (GHR) to ICB were calculated. Withdrawal of the arterial chemoreceptor drive and their stimulation were produced by inhalation of oxygen and nitrogen. Hyperoxia produced a mean fall of the diastolic blood pressure 14,2% and TPR 12,5% (P<0.001) in the HS and no change in the NS.

	MAP mmHg	CO l/min	TPR	MVR	Increased response to ICB in hyperoxia		
					Δ GHR	Δ TPR	Δ MVR
NS	91 ±1	6.1 ±0.4	0.82 ±0.03	4.10 ±1.3	+1.26±0.3 P<0.001	+0.03±0.04 n.s.	+0.14±0.1 n.s.
HS	111 ±1	5.4 ±0.2	1.20 ±0.03	0.66 ±0.7	+2.56±0.4 P<0.001	+0.21±0.03 P<0.001	+1.84±0.1 P<0.01

Hypoxia abolished all responses to ICB both in HS and NS. It is concluded that the increased tonic drive from the arterial chemoreceptors in essential hypertension(Trzebski et al. Cardiovasc.Res.1982,16,163) contributes to systemic vasoconstriction and to reduced response to ICB.

62.19

AUTONOMIC REGULATION OF HEART RATE DURING DYNAMIC EXERCISE: EVALUATION THROUGH ASSOCIATION WITH ISOMETRIC EXERCISE. L.Gallo Jr., B.C. Maciel, J.A. Marin Neto, J. Terra Filho, D.S.Amorim, and J.C. Manço. Seçao de Hemodinâmica e Funçao Pulmonar, Faculdade de Medicina, Ribeirão Preto, Brazil.

14 normal subjects performed standardized isometric exercise (I.E.) at rest and during 1 st, 2 nd, 3 rd and 4 th minute of dynamic exercise (D.E.) – 55 and 105 watts – in seated position, as well as at 1 st, 2 nd and 3 rd minute of recovery. Previous work in this Laboratory has shown that I.E. effected by sustained handgrip in a special dynamometer strain-gauge system (75% of maximal voluntary capacity, for 10 seconds) elicits tachycardia mediated almost exclusively by parasympathetic withdrawal. Mean heart rate (H.R.) increments in I.E. were 14 and 8 beats/min respectively at rest and during each minute of 55 W work load dynamic exercise. Correspondent changes during 105 W work load were 5 beats/min. These results lead to the conclusion that: 1) the first 10 sec increase in H.R. during D.E. is due to parasympathetic withdrawal; 2) the slow cardiac acceleration observed at the higher workload is not mediated by parasympathetic withdrawal; 3) standardized I.E. provides an useful method of assessing differential autonomic participation in reflex chronotropic changes, without resorting to pharmacological blockades. (Supported by FAPESP: P. 79/621 and P. 79/1798-3 and CNPq : PDE 2222-8-089/80).

PERIPHERAL CIRCULATION II

63.1

NONINVASIVE DOPPLER ULTRASOUND MEASUREMENTS OF CARDIAC OUTPUT AND COMMON CAROTID BLOOD FLOW IN CEREBROVASCULAR DISEASE PATIENTS AS A FUNCTION OF POSTURE AND EXERCISE. G. Cagle*, E.R. Greene*, I.P. Miranda*, F.G. Miranda* and P.A. Reilly*. (SPON: J.A. Loeppky). Lovelace Medical Foundation, Albuquerque, NM 87108

Noninvasive measurements of cardiac output (CO) and common carotid blood flow (CCBF) during postural changes and exercise should reflect the state of functional cerebral autoregulatory capacity. Beat-to-beat, noninvasive measurements of blood velocities and lumen diameters in the ascending aorta, right and left common carotid arteries were made in 7 normals and 12 patients with angiographically documented cerebrovascular disease (greater than 50% reduction in lumen diameter in the internal carotid). Heart rate (HR), CO, right and left common carotid blood flow (RCBF, LCBF) were calculated in the fifth minute of equilibration at supine rest, upright rest, upright 50 watt bicycle ergometer exercise and recovery. The patients demonstrated an increase in total CBF and CBF/CO ratio with each stage of increased CO. CBF values were .118 L/min, .133 L/min and .142 L/min with an increase in CBF/CO ratio of approximately 10% per stage. In contrast, normals reflected functional autoregulation by decreasing CBF values of .118 L/min, .093 L/min and .109 L/min at points of comparison, and decreased CBF/CO ratio of approximately 20% per stage. These data suggest that patients with hemodynamically significant cerebrovascular disease have decreased cerebral autoregulatory capacity during upright exercise, resulting in abnormal augmentation of cerebral blood flow with increases in CO.

63.2

LEG MUSCLE BLOOD FLOW DURING PROLONGED TREADMILL EXERCISE IN RATS. R.B. Armstrong, W.C. Mitchell*, R.O. Phelps*, C.B. Vandenakker*, J.A. White*, and M.H. Laughlin. Oral Roberts U., Tulsa, OK 74171

Blood flows (BF: $ml \cdot min^{-1} \cdot 100g^{-1}$) in 5 ankle plantar and 4 ankle dorsiflexor leg muscles were measured during preexercise (PE), at 0.5 (E.5), 1 (E1), 5 (E5), 15 (E15), 54 (E54), and 71 (E71) min of fast walking ($15\ m \cdot min^{-1}$), and 3 (P3) min postexercise. Microspheres were infused into the aorta via the right carotid artery, and reference samples were withdrawn from the aorta via the left renal artery. BF's to all muscles except white gastrocnemius (G_W) were elevated at E.5 ($P < 0.05$) and were proportional to the populations of fast-twitch-oxidative (FOG) fibers in the muscles ($P < 0.001$). Highest BF's at this time were in red tibialis anterior (TA_R) and red gastrocnemius (G_R) muscles (329 and 256, respectively). However, by E5 BF's to the muscles had decreased, and except for plantaris, were no different from PE. BF's then increased from E5 to E54. At E54 BF's (except G_W and flexor digitorum longus) were higher than at all other time points ($P < 0.05$) and were proportional to FOG populations ($P < 0.001$), with the highest BF's in TA_R and G_R (both 461). Thus, muscle BF's generally "overshoot" during the 1st min, then progressively rise with time during low-intensity treadmill exercise. By E71 the rats could not maintain the pace, and BF's were lower than at E54. By P3 all BF's decreased to PE or below. (Supported by NIH grants AM25472 and HL26963 and AHA-OK Affiliate funds)

63.3
CUTANEOUS VENODILATION IN RESPONSE TO SYSTEMIC HYPOXEMIA IN DOGS. Steven L. Britton, Department of Physiology, Medical College of Ohio, Toledo, Ohio 43699.

Experiments were performed in chloralose anesthetized dogs to test if the cutaneous veins (saphenous) participate in the cardiovascular response to systemic hypoxemia. The left saphenous vein was perfused orthogradely at constant flow with blood from the terminal aorta that was cooled to 32°C. Sixteen paralyzed (gallamine triethiodide) animals were ventilated with 50% O_2 in N_2 (or 100% O_2) during control conditions. Systemic hypoxemia was produced by ventilation with 10% O_2 in N_2 for four minute episodes. This treatment decreased arterial PO_2 to 49 ± 4 mmHg. In neurally intact animals hypoxemia caused a 29% decrease in saphenous vein perfusion pressure (interpreted as a dilation) and an 8% increase in aortic pressure. The hypoxemia-induced saphenous venous dilation was abolished by 1) section of the vagi and denervation of the carotid chemoreceptors, or 2) section of the lumbar sympathetic chain. The hypoxemia-induced dilation persisted after section of the vagi alone or the carotid chemoreceptors alone. These data demonstrate that systemic hypoxemia causes a neurally mediated dilation of the saphenous vein that is dependent upon intact vagi and/or carotid chemoreceptors. We conclude that the saphenous vein dilates as part of the integrated cardiovascular response to hypoxemia in anesthetized dogs. (Supported by The Northwestern Ohio Chapter of the American Heart Association and Biomedical Research Support Grant #94364).

63.4
SPINAL CORD VASODILATATION INDUCED BY DORSAL ROOT STIMULATION. Emilio E. Decima* and Oscar U. Scremin. UCLA School of Medicine. Los Angeles, CA 90024

Local blood flow in the ventral horn of the lumbar enlargement (VHBF) was measured with the H_2 clearance technique in decerebrated, low spinal cats. Average VHBF at normocapnia (72 measurements in 10 cats) was 43.2 $ml \cdot 100g^{-1} \cdot min^{-1}$. VHBF was dependent on blood CO_2 level and this phenomenon was not affected by a low thoracic spinal division. Dorsal root stimulation induced an average increase in VHBF of 128% over control that reached a maximum when slower conducting fibers were recruited but could still be elicited by selective stimulation of group I fibers. Measurements of tissue PO_2 in ventral horn during dorsal root stimulation showed a rapid increase with a tendency to return to control levels upon prolonged stimulation. This increase in ventral horn PO_2 was potentiated by hypercapnia. It is concluded that dorsal root afferents have the ability to vasodilate the spinal cord gray matter. The tissue hyperoxia during this vasodilation speaks against a role of metabolic factors in the initiation of the response. (Supported in part by Grants from NIH NS-1633-02, HL-17903 and AHA-GLAA 437IG.)

63.5
CEREBRAL BLOOD FLOW AND CNS OXYGEN CONSUMPTION DURING CHRONIC ETHANOL IN CONSCIOUS DOGS. H. Myers, K. Schalk*, W. Sargent, J. Peter*, E. Dunteman*, J. Hadlock*, and J. Beard. Southern Illinois University, School of Medicine, Carbondale, IL 62901

We have observed that conscious dogs have a significantly elevated jugular venous (JV) pO_2 90 min after ETOH (peak ETOH= 280mg/dl). We questioned whether this increase was due to cerebral vasodilation and/or reduced CNS O_2 extraction (ext), and whether it would change with long-term ETOH intake. Accordingly, we gave 6 instrumented dogs ETOH (2.5g/kg,25%v/v) or H_2O by gavage for 1 month. Cerebral blood flow (CBF, microsphere method), cardiac output (CO), blood pressure, arterial and JV pO_2, pCO_2 and pH, and body temperature (T°) were measured. O_2 ext and CNS O_2 consumption ($\dot{V}_{O_2}$) were calculated using corrected pO_2 values. CBF and CO were determined before ETOH or H_2O, at 90 min after the initial dose and at 90 min after the dose at weeks 2, 3 and 4. Other data were collected weekly before and 90 min after ETOH or H_2O. Compared to control, ETOH caused a significant ($P<.05$) increase in JV pO_2 and a decrease in pH and body T° at each measurement period. pCO_2 increased but not significantly. CBF increased at each period, ranging from a high of 45% during the first 2 weeks to a low of 29% at week 4. CNS O_2 ext decreased. The greatest reduction occurred during the first 2 weeks (↓50%); the least during week 4 (↓36%). CNS $\dot{V}_{O_2}$, while reduced (↓16%) early in the study, returned to near normal at week 4. H_2O dogs showed no significant changes during the study. These findings suggest evidence of adaptation to ETOH-induced CNS depression.

63.6
ASSESSMENT OF THE XENON-133 (Xe^{133}) INHALATION TECHNIQUE IN MEASURING REGIONAL CEREBRAL BLOOD FLOW (rCBF) DURING CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD). D.F. Watson,*R.M. Raymond,G.A.Rosenfeld*and O.H. Reichman.* Dept. of Surgery, Div. of Neurological Surgery, Loyola Univ. Med. Ctr.,Maywood, IL 60141 and The Veterans Administration Hospital, Hines, IL60141

The concept of inadequate gas diffusion across the alveolar membrane in patients with respiratory problems (COPD) is not new. The Xe^{133} inhalation technique of measuring (rCBF) requires adequate diffusion and equilibrium among body tissues. This study was completed to describe means of early detection of gas diffusion abnormalities in patients without classic symptoms of C.O.P.D. rCBF was measured with the Novo Laboratories inhalation cerebrograph. End tidal Xe^{133} air curve values were used in the estimate of arterial Xe^{133} concentration. These measurements were monitored by means of an in-line capnograph (Houston Instrument). Data were analyzed by Fouier analysis. Results from patients with C.O.P.D. exhibited decreasing arterial Xe^{133} concentrations during the equilibrium inhalation period (negative slope of end tidal Xe^{133} trough) and a delayed Xe^{133} washout from the vascular compartment. Patients and normal volunteers without respiratory problems showed typical end tidal Xe^{133} air curves (positive slope in trough and return to near normal baseline during Xe^{133} washout). These data demonstrate that the slope of the end tidal Xe^{133} air curve is a good indicator of pulmonary function (gas diffusion) and should be used in determining the appropriateness of using the Xe^{133} inhalation technique for measuring rCBF. (Supported by Dept. of Surgery)

COMPARATIVE PHYSIOLOGY: RESPIRATION AND ACID-BASE III

64.1
PULMONARY DESIGN IN THE MICROCHIROPTERAN, PIPISTRELLUS SUBFLAVUS. A.J. Lechner, J.M. Puccinelli*, and M.B. Laskowski. Dept.Physiology,St.Louis U.Sch. Medicine,St. Louis,MO 63104.

The Eastern Pipistrelle (P.subflavus) is typical of exceptionally small bats capable of a 30-fold range in aerobic metabolism as they arouse from deep hibernation and sustain foraging flight. This description of lung structure is the first in a series to examine adaptations within their oxygen transport system. Bats were collected from overwintering caves and kept in a humidified 5°C hibernaculum. Data are for males only as females had already departed these caves for spring nursing colonies. With body temperatures < 10°C and animals anesthetized (20-30µl Na-pentobarbital,i.p.), the lungs of 8 bats were fixed in situ by intratracheal instillation of 2% glutaraldehyde (20 cm H_2O) for 1 hr. Total lung volume,V_L, was measured by fluid displacement (±1% reproducibility) and then the lungs were processed for light and electron microscopy. In this group BW = 4.5±0.1g ($\bar{X}$±SEM) and V_L = 0.302±0.012ml, of which 0.192± 0.009ml was the right lung. Total alveolar surface area S_A = 240.5±17.5cm^2, capillary endothelial surface area S_C = 202.3± 17.9cm^2, S_A/BW = 53.1±3.5cm^2/g,and S_C/BW = 44.6±3.5cm^2/g. In 6 other bats, BW = 4.4±0.1g, total fresh lung weight = 44.9± 1.6mg, total lung DNA = 60.2±3.8µg, and total lung blood volume = 16.8±0.6µl. These values for V_L, S_A, S_C, and lung fresh weight agree well with predictions based on interspecific regressions against BW, but it remains to be determined if they persist annually, during which P.subflavus may double its BW before entering hibernation.

64.2
HYPOTHERMIA AND ACID BASE EQUILIBRIUM ON THE LOG C-pH DIAGRAM. N. B. Kindig*, G. F. Filley, H. Swan*. Webb-Waring Lung Institute, Denver, Colorado 80262

Complex interactions among CO_2 solubility and the equilibrium constants of CO_2, imidazole and water with temperature variation in closed biological systems are portrayed on a log C-pH diagram. Auxiliary diagrams explicitly depict the temperature dependence of each chemical equilibrium constant, and of solubility. When these are translated onto the log C-pH diagram, a clear picture of the effect of reduced temperature on equilibrium pH, PCO_2, and the state of ionization of imidazole protein emerges. Ionized imidazole and HCO_3^- remain nearly constant. pH and pK_{Im} increase equally. Even though solubility increases, the amount of dissolved CO_2 decreases because pK_{Im} increases faster than pK of the CO_2 system. PCO_2 falls because of both solubility and dissolved CO_2 trends, dissolved CO_2 dominating above 23°C and solubility below. The quantitative power of the diagram in dealing with ratios is demonstrated by showing that the length of a line on the diagram represents log PCO_2 = log $[CO_2]$ - log S, (PCO_2 = $[CO_2]$ /S). The length of another line which is constant at three equilibrium temperatures represents log $[OH^-]/[H^+]$. The diagram can portray open CO_2 systems with equal ease. A cutout scale can be superimposed permitting one to make surprisingly accurate determinations of the parameters which determine the acid-base state of body fluids in comparative physiological systems. (Supported also by Anesthesiology Dept., University of Colorado Health Sciences Center.)

64.3

OXYGEN DIFFUSING CAPACITY OF LIZARD LUNGS. K. Johansen and M.L. Glass*. Aarhus University, Dept. of Zoophysiology, Aarhus, Denmark and Max-Planck-Institut für experimentelle Medizin, Physiology Dept., Göttingen, FRG.

Pulmonary oxygen diffusing capacity (D_LO_2) is known for mammals (cf. Forster and Crandall, Ann. Rev. Physiol., 38, 69-95, 1976) and birds (Burger et al., Respir. Physiol., 36, 19-37, 1979). In contrast, D_LO_2 was unknown for reptile lungs. Therefore, we have determined D_LO_2 in the lizard Tupinambis teguixin (body weights 2-5 kg) using a rebreathing technique (Adaro et al., Respir. Physiol., 18, 43-63, 1973). Besides, pulmonary perfusion (Q_L) was determined by acetylene clearance. The animals were unrestrained and unanaesthetized. Both variables increased with body temperature and at 35°C the values were Q_L = 35 ml kg^{-1} min^{-1} and D_LO_2 = 4.29 µmol kg^{-1} min^{-1} $Torr^{-1}$. These values are low compared to those for similar-sized mammals and birds reflecting a smaller weight-specific O_2 uptake.

64.4

EFFECTS OF TEMPERATURE ON REGULATION OF ARTERIAL PO_2 IN THE TURTLE CHRYSEMYS PICTA BELLII. M.L. Glass*, R.G. Boutilier* and N. Heisler* (SPON: P. Scheid). Max-Planck-Institut für experimentelle Medizin, Department of Physiology, D-3400 Göttingen, FRG.

Pulmonary ventilation and O_2 uptake were measured in free diving fresh water turtles (Chrysemys picta bellii) at 10, 20 and 30°C. In addition, arterial PO_2, PCO_2 and pH were measured, and in vitro O_2 dissociation curves determined. The turtles were breathing room air or hypoxic gas mixtures. Arterial PO_2 of normoxic turtles was about 30 Torr at 10°C but twice as high at 20 and 30°C. A marked increase of ventilation with temperature mostly resulted from a reduction of dive duration. At 30°C ventilation about doubled as arterial PO_2 decreased from 60 Torr (normoxic value) to 30 Torr during hypoxia and increased more than ten-fold as arterial PO_2 approached 10 Torr. In comparison, the ventilatory responses to hypoxia occurred at much lower PO_2 values, when the turtles were at lower temperatures: at 10°C ventilation did not increase relative to normoxic control values until arterial PO_2 fell to about 5 Torr. The results indicate that ventilatory responses to hypoxia occur, when arterial PO_2 approaches P_{50} of the blood, which decreased from 28 Torr at 30°C to 5.5 Torr at 10°C.

64.5

ARTERIAL CHEMORECEPTOR INVOLVEMENT IN THE VENTILATORY RESPONSE TO EXERCISE IN THE HYPOXIC DUCK. F.M. Faraci*, J.P. Kiley, and M.R. Fedde. Dept. of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506.

The purpose of this study was to determine if arterial chemoreceptors contribute to the ventilatory response during exercise in hypoxic Pekin ducks (Anas platyrhynchos). Minute ventilation ($\dot{V}_I$) was recorded from ducks that breathed spontaneously from a gas stream flowing through a T-tube connected to a tracheal cannula. Both at rest and during running exercise (1.44 $km \cdot hr^{-1}$, 3° incline), we abruptly switched the inspired gas from either 21% or 12% oxygen to 100% oxygen for 45 seconds (O_2-test). In normoxia (PaO_2 = 98 Torr), $\dot{V}_I$ increased 3.8 times the resting value during exercise, while during exercise in hypoxia (PaO_2 = 55 Torr), $\dot{V}_I$ increased 4.3 times the resting level. At rest, breathing 100% oxygen reduced $\dot{V}_I$ 28% during normoxia and 66% during hypoxia. During exercise, breathing 100% oxygen decreased $\dot{V}_I$ by 16% and 34% in normoxic and hypoxic conditions, respectively. Thus, a small portion of the ventilatory response during exercise under normoxic conditions is due to arterial chemoreceptor input. During exercise in hypoxic conditions, arterial chemoreceptors provide a larger portion of the total drive to ventilation. (Supported, in part, by a grant from the American Heart Association, Kansas Affiliate, Inc.).

64.6

EFFECTS OF ACETAZOLAMIDE ON BRANCHIAL CO_2 EXCRETION IN THE MARINE CRAB CANCER PRODUCTUS. B.R. McMahon, L.E. Burnett and P.L. deFur*. Univ. of Calgary, Univ. of San Diego and Bamfield Marine Station.

Large Red Rock crabs C. productus injected (pericardial cavity) with sufficient Acetazolamide to achieve 2x10 -4M in hemolymph, or equivalent volume of SW for control, were maintained in 33‰ SW at 11°. Hemolymph samples taken prior to and 2, 6, 12h and 1, 2, 4 days following injection allowed assessment of oxygenation, acid-base and ionic status of hemolymph. Compared with controls, Acetazolamide treatment caused a slight transient acidosis and marked significant increase in both P_{CO_2} and C_{CO_2}. These effects peaked at 12-24h but persisted for 4 days. A second series of crabs were air exposed (emersion) for 4h following injection, then returned to seawater to study the removal of excess CO_2 following emersion. Hemolymph samples were taken prior to and during emersion and 15 min. and 2, 6, and 24h following reimmersion. Acetazolamide treated animals showed slightly greater acidosis and significantly greater increase in P_{CO_2} and C_{CO_2} during emersion. Control animals showed rapid loss of both P_{CO_2} and C_{CO_2} on reimmersion while treated animals retained significantly higher P_{CO_2} and showed no significant change in C_{CO_2}. We conclude that acetazolamide blocks branchial carbonic anhydrase, reducing mobilization of CO_2 at the gill and necessitating much larger P_{CO_2} gradients to ensure adequate CO_2 excretion. (Supported by NSERC A5672 to B.R.M.).

64.7

RELATIONSHIP BETWEEN HYPOXIC VENTILATORY RESPONSE AND HEMOGLOBIN O_2-AFFINITY IN BIRDS. D.F. Boggs and G.F. Birchard, Dartmouth Medical School, Hanover, N.H. 03755

Although PaO_2 is the likely stimulus for the hypoxic ventilatory response there are interspecific differences in the threshold PaO_2 initiating that response. A relationship between the PO_2 of the 'knee' of the oxyhemoglobin dissociation curve and the 'knee' of the hypoxic ventilatory response would seem physiologically efficient and evolutionarily probable. We investigated the hypoxic ventilatory response in 2 young rheas and 3 pheasants who had hemoglobins of quite different O_2 affinity; rhea P_{50}=30.5, pheasant P_{50}=42.2 (pH= 7.5, T=41°C). The PaO_2 threshold of the rhea's hypoxic ventilatory response was found to be roughly 20 torr lower than that of the pheasant. If, on the other hand, the response is plotted against SaO_2 instead of PaO_2 the curves for the two species are essentially the same, with ventilation beginning to increase at an SaO_2 of 80-85%. Experimental reductions in O_2 content do not stimulate ventilation in the duck (Boggs, unpublished), and it is unlikely that O_2 saturation could be sensed directly. Rather this correspondence between O_2 affinity and the hypoxic ventilatory response must reflect the process of natural selection favoring a low hypoxic threshold in species with a high affinity and a high threshold in species with a low affinity hemoglobin. (Supported by NHLBI grant #HL02888-25, Parker B. Francis Foundation Postdoctoral Fellowship, and NHLBI training grant #HL07449-02.)

64.8

METABOLIC AND HEMATOLOGIC EFFECTS OF PREGNANCY IN THE GARTER SNAKE, THAMNOPHIS SIRTALIS. Geoffrey F. Birchard, Craig P. Black, Gordon W. Schuett* and Virginia D. Black*. Dept. of Biology, Univ. of Toledo, Toledo, OH 43606 and Dept. of Pediatrics, Wayne State Univ. Medical School, Detroit MI 48202.

Pregnancy in mammals is known to result in significant changes in the maternal physiology. In order to access whether similar changes occur in other viviparous groups the Garter snake was studied. Oxygen consumption was measured in pregnant, postpartum, and nonpregnant female snakes as well as males and neonates. Hematocrit, hemoglobin concentration, and blood oxygen carrying capacity were also determined for all groups except neonates. Among adult snakes pregnant females showed a significantly higher weight specific metabolic rate than all other groups. No

	Pregnant	Postpartum	
cm^3 $O_2 \cdot h^{-1} \cdot g^{-1}$	0.095±.025	0.065±.01	p<.02

significant hematological differences were found between any groups. Analysis indicates that the oxygen consumption of fetal snakes is equal to or greater than neonatal snakes and that there may also be an increased maternal oxygen consumption. This maternal increase may be related to the cost of supporting the developing fetuses.

64.9
OXYGEN UPTAKE AND TRANSPORT IN BLUE CRABS HELD IN CONSTANT AND FLUCTUATING SALINITY. Thomas D. Sabourin. La. State Univ., Baton Rouge, LA 70803

Adult intermolt crabs (Callinectes sapidus) were stepwise acclimated to salinity at 22-25 °C. O_2 uptake and transport was investigated in steady state salinity and diurnally fluctuating salinity. Hemolymph protein and Cu , pH and in vitro P_{50} were higher in crabs at 10 o/oo than at 30 o/oo. No steady state salinity-related differences occurred in ventilation volume (V_w), O_2 extraction efficiency (E,%), O_2 uptake (VO_2), heart rate (F_H), stroke volume (SV), cardiac output (Q), post- and prebranchial O_2 tensions (PAO_2, PVO_2) and O_2 contents. Responses to salinity fluctuation were affected by acclimation salinity. During 30-10-30 o/oo fluctuations little change occurred in blood flow and O_2 content. V_W and VO_2 changed inversely with salinity. PAO_2 and PVO_2 and hemocyanin-O_2 affinity decreased. Salinity fluctuations of 10-30-10 o/oo elicited decreased F_H, SV, and Q as salinity was increased from 10 to 30 o/oo. After 96 h of fluctuation, mean F_H, SV and Q were slightly elevated over 0 h controls. E,%, VO_2, blood O_2 tension and content decreased during 10-30-10 o/oo fluctuations, while P_{50} remained constant. Protein and Cu were unaltered during 96 h of either salinity fluctuation. (Supported by the Petroleum Refiners Environmental Council of LA)

64.10
ATP METABOLISM IN MITOCHONDRIA OF Modiolus demissus GILL TISSUE. J. M. Burcham, S. H. Bishop, and L. L. Ellis, Iowa State University, Ames, IA 50011

Most marine bivalves show oxygen conformity with regard to metabolic rate. This may be due to poor respiratory control in the coupling of mitochondrial oxidative phosphorylation. Gill mitochondria from M. demissus (maintained in 1000 mOs ASW) isolated in isoosmotic sucrose media exhibit uncoupled respiration. Mitochondria appeared intact as judged by bouyant density centrifugation, lack of respiration by externally added NADH, lack of release of mitochondrial enzymes (succinate dehydrogenase, ornithine-δ-aminotransferase and proline oxidase) during mitochondria preparation, and ability to oxidize proline, succinate, and glutamate. However, the mitochondria synthesize ATP using an assay medium with endogenous substrate, ADP, Pi, glucose, and hexokinase. This ATP synthesis is blocked by rotenone. Addition of succinate plus malate or pyruvate plus 2-oxyglutarate increases ATP synthesis, and this increase is blocked by oligomycin. The mitochondria have a Mg^{+2}-ATPase activity which is sensitive to oligomycin but is not stimulated by 2,4-dinitrophenol. This data together with additional evidence suggests M. demissus gill mitochondria may carry on normal respiration which is partially uncoupled. (Supported in part by NSF grant PCM-80-22606)

64.11
SULFIDE TOLERANCE AND SULFIDE-DRIVEN CO_2 FIXATION IN TUBE WORMS AND CLAMS OF THE DEEP-SEA HYDROTHERMAL VENTS. Horst Felbeck, Mark Powell, Gabriele Wienhausen and George N. Somero*. Scripps Institution of Oceanography, La Jolla CA. 92093

The large pogonophoran tube worm (Riftia pachyptila) and clam (Calyptogena magnifica) of the deep-sea hydrothermal vent communities tolerate hydrogen sulfide (HS^-) at concentrations lethal for most animals. Whole organism, tissue and tissue homogenate respiration are not poisoned by 1 mM HS^-, but cytochrome-C oxidase activity is blocked by nM levels of HS^-. High concentrations of sulfide-binding proteins in tissues of these animals appear to protect cytochrome-C oxidase, and, thereby respiration from HS^- poisoning. HS^- is oxidized by symbiont bacteria in both animals, and the energy released is used in part to drive net fixation of CO_2 via the Calvin-Benson cycle. $^{14}CO_2$ labelling patterns suggest initial formation of a four-carbon intermediate (malate or oxaloacetate) which later is split to yield CO_2 for the ribulosebisphosphate carboxylase reaction. Rapid uptake of dissolved organic material by the worm and clam was found. The latter form of nutrition plus the synthesis of reduced carbon and nitrogen compounds by bacterial symbionts may contribute significantly to the nutritional demands of these organisms.

65.1
HUMAN METABOLIC RESPONSE TO COLD VARIES WITH AGE AND GENDER. Jeames A. Wagner and Steven M. Horvath, Institute of Environmental Stress, UCSB, Santa Barbara, CA 93106

Men and women between the ages of 20-29 yrs and 51-72 yrs, wearing bathing suits, rested for 2 h in 29°C and 10°C room temperatures. The mean metabolic rate ($W \cdot m^2$ or $ml \cdot kg\ LBM^{-1} \cdot min^{-1}$) of older women (n = 7) increased 39% within 15 min exposure to 10°C and 80% by the end of 2 h. The metabolic rate increases in younger women (n = 10) and older men (n = 10) were slowest (5% within 15 min, and 60 and 74%, respectively, after 2 h) and that of younger men was intermediate (18% after 15 min and 67% after 2 h). During 29°C exposures mean±SE rectal temperatures (T_{re}) was highest in younger women (36.8±0.1°C) and lowest in older men (36.4±0.1°C). During the 10°C exposures, older women maintained T_{re} at 36.9±0.1°C, whereas T_{re} of the other three groups declined 0.4°C by the end of 2 h. These data suggest that older men are more susceptible to cold ambients than younger people since they did not prevent a further decline in their initially low T_{re}. Despite greater insulation from body fat, the older women maintained a constant T_{re} at greater metabolic cost than men or younger women.

65.2
HIBERNATION INDUCTION TRIGGER ISOLATED FROM HIBERNATING WOODCHUCK ALBUMIN HAS OPIOID-LIKE ACTION ON BRAIN FUNCTION OF THE MONKEY. Wilma A. Spurrier, Peter R. Oeltgen,* John W. Walsh,* and David C. Randall. Div. Neuro. Surg. Loyola Univ. Med. Ctr. Maywood, Il. 60153 and Lexington V.A. Med. Ctr. Univ. Kentucky Med. Sch. Lexington, Kentucky 40536

Three male 6-8 kg rhesus monkeys, Macaca mulatta, had cannulas implanted into the lateral ventricles. The sequence of behavioral and physiological changes, following an infusion of 8 mg of hibernation induction trigger (HIT) in 400 ul of artificial CSF were: retching, yawning, lethargy, eye closure, head slumping and the appearance of an anesthetized state for 3-5 hours, hypothermia 1.5°C to 3°C, mean fall 2.6°C in cranial temperature, and bradycardia that lasted up to 8 hours with maximum decrease 43-50% in heart rate. For 12-18 hours post-infusion, no food was eaten and hypophagia lasted 5-7 days. Infusions of artificial CSF, active summer woodchuck serum albumin, or monkey serum albumin produced no changes. The opiate antagonists, naloxone and naltrexone, given before or after HIT abolished or reversed the modifications of brain function. The HIT may be an endogenous opiate-like substance unique to hibernators and responsible for entry into hibernation. These results suggest that primates, non-hibernators, also possess brain receptor sites that react with HIT to produce depression of behavioral and physiological activity. (Supported by Veterans Administration.)

65.3

ENDOGENOUS OPIOID MODULATION OF DAILY TORPOR IN PEROMYSCUS MANICULATUS. E.B. Pivorun. Clemson Univ., Clemson, S.C. 29631

Daily torpor represents a metabolic adaptation that counters the consequences of restricted food resources, thus effecting a profound analgesia and reduced energy expenditures. Since the opioids have been postulated to modulate this phenomenon, the effects of naloxone, an opiate antagonist, and metyrapone, a stimulator of B-endorphin release, were evaluated on the incidence, depth, and duration of daily torpor bouts (induced by food rationing) in Peromyscus maniculatus. Mice (implanted with telemeters) were injected IP with saline between 10-11PM (4-8 hrs after the end of a day's torpor bout but 8-11 hrs prior to the next day's bout. These saline injections were continued until each individual displayed a constant duration and depth of successive daily bouts. Naloxone (10-20mg/kg) or metyrapone (45mg/kg) was then injected IP between 10-11PM for 1-4 days; saline injections were then resumed. Naloxone precipitated an inhibitory effect manifested by a delayed onset, a shortening of the duration and an elevation of the minimum temperatures attained during torpor. Some individuals responded by a complete elimination of the torpor response. The inhibitory effects were short lived; saline injections resulted in subsequent bouts returning to normal. Metyrapone affected only those mice displaying short, shallow torpor bouts; in general deeper and longer bouts were initiated. These results suggest that the opiates are an important component of the control system regulating induced daily torpor.

65.4

KETONE BODY METABOLISM IN THE GROUND SQUIRREL, SPERMOPHILUS BELDINGI. B. L. Krilowicz*. (SPON: V. H. Shoemaker). Univ. of California, Riverside, CA 92521.

Torpid ground squirrels, Spermophilus beldingi and S. lateralis, and bats, Antrozous pallidus, show increased blood ß-hydoxybutyrate (B-HB) and acetoacetate levels when compared to fed non-hibernating controls. B-HB is the primary ketone found and its concentration in the blood increases 15-fold, 10-fold and 6-fold in torpid S. beldingi, S. lateralis and A. pallidus, respectively. This ketosis suggests that ketones are utilized during torpor, thus sparing glucose and protein. In vitro experiments were performed on heart slices from hibernating and fed non-hibernating S. beldingi to determine if a preference was exhibited for ketones. The slices were incubated at 38°C in media containing 150 mg% glucose or 150 mg% glucose and 1.5 mM B-HB, physiological concentrations of the substrates. Slices from both hibernators and fed non-hibernators incubated in ketones plus glucose derived 70-80% of their energy from metabolism of ketones. Metabolic rate and total substrate utilization expressed as cal g^{-1} hr^{-1} were similar for all four groups. The high blood ketone levels found during hibernation, coupled with the in vitro preference by heart slices for ketones suggests that ketones are an alternate energy source used by S. beldingi during torpor. (Supported in part by Chancellor's Patent Funds and Newell Research Awards.)

65.5

EFFECTS OF HIBERNATION ON LIVER SUCCINOXIDASE ACTIVITY IN HAMSTERS. Jane C. Roberts. Department of Biology, Creighton University, Omaha, NE. 68178.

In order to further examine the reduction in hepatic succinoxidase activity (SO) seen during hibernation, SO of hamster liver mitochondria (M) or homogenates (H) was assayed polarographically at 12, 25 and 37 C: 1) over the pH range 6.8 - 8.2, 2) over the substrate concentration [S] range of 0.09 - 3.03 mM, and 3) after mixing M or H from active with M or H from hibernating animals. Liver M from active hamsters showed no significant effect of pH on SO measured in the absence of ADP. In the presence of ADP, SO was relatively constant from pH 6.8 to 7.8, but decreased at higher pH. At 37 C, hibernator liver M showed a continuous rise in SO as pH decreased below 7.35 so that at pH 6.8 state 4, but not state 3, SO equaled that of M from active animals. This pH effect was less apparent at 25 C and was absent at 12 C, suggesting that the inhibition of SO in hibernation may be at least partially inactivated by the combined effects of increasing temperature and low pH. Lineweaver-Burke plots of SO at various [S] show that both Km and Vmax decrease in hibernation. Results of mixing experiments suggest the inhibition of SO in both H and M from hibernating animals is due to a factor(s) that is not free to influence SO of H or M from active hamsters. This might be accounted for by a tightly-bound inhibitor substance or a configurational change in the enzyme. Also, there appears to be no free "anti-hibernation" factor in H or M of active animals which reverses this inhibition. Supported in part by NSF Grant No. PCM80-21895.

65.6

EXERCISE TOLERANCE AND CEREBRAL FUNCTION AFTER ACUTE HEMODILUTION OF POLYCYTHEMIC MOUNTAIN CLIMBERS. F.H. Sarnquist*, R. Schoene*, and P. Hackett* (SPON: J.B. West). American Medical Research Expedition to Everest, Stanford University, Stanford, CA 94305 and Univ. of Washington, Seattle, WA 98195

We performed acute, isovolemic hemodilution on four well-acclimatized polycythemic mountain climbers+ to determine if a higher than average acclimatized hematocrit was detrimental to physical and mental performance in a hypoxic environment. The study was conducted at the Mt. Everest base camp (5400 meters above sea level) on the Khumbu glacier in Nepal. Graded exercise tests to exhaustion and tests of cerebral and psychomotor function were performed before and after hemodilution. We reduced the hematocrits of the subjects from a mean of 58.3% to 50.5%. This hematocrit reduction was not subjectively apparent to the subjects and their exercise tolerance and maximum oxygen uptake were unchanged. Heart rates at all levels of activity were slightly increased following hemodilution. There was a consistent but non-significant improvement in all the mental function test scores after hemodilution. These data fail to demonstrate any advantage to this degree of hematocrit reduction in healthy subjects. However, the fact that in this intensely hypoxic environment (P_B=400 torr) the higher hematocrit seemed to confer no advantage, even during exhaustive exercise, suggests that hematocrits above 50% are rarely, if ever, beneficial. (Supported by the American Lung Association)

+All gave informed consent to take part in this approved study.

65.7

RESPONSES TO SIMULATED DECOMPRESSION PROFILES WITH THE ADDITION OF 3% CO_2 TO THE INSPIRED AIR. Esar Shvartz, James G. Gaume*, Mark B. Hefner*, and Todd D. Savitt*. Douglas Aircraft Company, Long Beach, CA 90846.

Six decompression profiles were simulated by breathing low O_2 concentrations with and without the addition of 3% CO_2 to the inspired air, to determine its effectiveness in tolerance to hypoxia with particular emphasis on cerebral blood flow (CBF) measured noninvasively using a Bioimpedance Analyzer. Each profile consisted of: 10 minutes of simulated cabin altitude (6500 feet; 16.4% O_2); simulation of 36,000, 38,000, and 40,000 feet (4.7, 4.3, and 3.9% O_2) twice each time until time of useful consciousness (TUC); and simulation of 20.5, 22.5, and 25,000 feet (9.4, 8.6, and 7.8% O_2) with and without 3% CO_2 (in N_2). Breathing the low O_2 concentrations with CO_2, compared with no CO_2, resulted in the following responses: Lower heart rates (12 to 15%); higher arterial O_2 saturation, blood pressure and ventillation (10, 70, and 6%, respectively); lower calf blood flow and less calf venous pooling; and large improvements in TUC. CBF showed the same increases as found when measured invasively. The results show that at altitudes below 30,000 feet, the addition of 3% CO_2 to the inspired air improves hypoxia responses as shown at 5500 feet lower altitude. It was shown that CBF can be accuartely estimated in hypoxic conditions using impedance plethysmography.

65.8

IMPAIRED BLOOD-TO-ALVEOLAR TRANSFER OF CO_2 IN PULMONARY GAS EMBOLISM. Y.C. Lin, M.C.W. Lin*, J. Swilley*, K. Hata*, J.J. McNamara* and K.K. Shida*. Depts. of Physiology and Surgery, U. of Hawaii John A. Burns Sch. of Med., Honolulu, HI 96822.

Nitrogen uptake during compression and its elimination during subsequent decompression has been shown to be asymmetric. The reduced rate of N_2 elimination during decompression may be related to formation of intravenous gas bubbles and the resulting pulmonary gas embolism (PGE). A reduced blood N_2 delivery to the lungs during decompression has been demonstrated. It is also possible that blood-to-alveolar N_2 transfer is impaired in PGE. This possibility is tested in this study. We induced graded PGE in cats anesthetized with α-chloralose by infusion of air iv at rates ranging from 0.76 to 2.9 ml/min and volumes up to 1.6 ml/kg. PGE is expressed in the cat as pulmonary hypertension, systemic hypertension, decreased cardiac output with unchanged heart rate, and arterial hypoxemia, hypercapnia and acidosis. These changes are readily reversible within 15 min of cessation of air infusion. Alveolar CO_2 concentration decreases as systolic right ventricular pressure rises. Arterial-to-alveolar CO_2 gradient increases as a function of air volume infused. It is reasoned that blood-to-alveolar transfer of N_2 must also be impaired during PGE. The result of this study demonstrates the impaired gas diffusion across the alveoli contributes to the asymmetry of N_2 uptake and elimination during diving operation. (Supported in part by Sea Grant NA81AA-D-00070).

65.9

TEMPERATURE INDUCED CHANGES IN INERT GAS ELIMINATION. Gary W. Mack* and Y.C. Lin. Dept. of Physiology, University of Hawaii John A. Burns School of Medicine, Honolulu, HI 96822.

The rate of nitrogen elimination ($\dot{V}N_2$) during the first 30 min of O_2 breathing in the rat can be altered in response to temperature induced changes in cardiopulmonary function. $\dot{V}N_2$ and cardiac output ($\dot{Q}$) were determined on unanesthetized male rats at 15, 24 and 35 C. At 24 C the rats eliminated 11.6 +.9 ml N_2/kg b.w. equivalent to 63% of the total estimated body N_2 stores. Oxygen consumption ($\dot{V}O_2$) at 24 C averaged 25 ml O_2/min/kg while $\dot{Q}$, as determined by thermodilution, was 378 ml/min/kg. $\dot{V}N_2$ was significantly reduced ($p<.05$) to 8.5 + .6 ml N_2/kg when rats were exposed to 15 C. This 27% drop in $\dot{V}N_2$ coincided with a similar reduction in $\dot{Q}$ while $\dot{V}O_2$ had increased by 30%. At 35 C $\dot{V}N_2$, 10.8 + .9 ml N_2/kg, was not significantly different from the control conditions at 24 C. However, $\dot{V}O_2$ did not change significantly while $\dot{Q}$ rose to 561 ml/min/kg. Based on a perfusion limited system direct alteration in $\dot{Q}$ should be reflected in proportional changes in the rate of elimination of gases of low solubility. At 15 C this relationship held as $\dot{V}N_2$ and $\dot{Q}$ showed similar responses. Reduction in $\dot{V}N_2$ in the cold may be related to hypothermia and peripheral vasoconstriction. However, at 35 C $\dot{V}N_2$ showed no increase with elevated $\dot{Q}$ and body temperature. Increasing $\dot{Q}$ without directing it toward major N_2 stores, body fat, may have been responsible. The results indicate that hypothermia will prolong the duration of stay at each stage during decompression. (Supported in part by Sea Grant NA81AA-D-00070).

65.10

EFFECT OF LIGHT DESYNCHRONIZATION ON TESTIS LH RECEPTORS IN THE RAT. **O. Sanchez and J. W. Evans. University of California, Davis, CA 95616**

To evaluate the time course of the effect of desynchronization on LH receptors in rat testis, mature male Sprague-Dawley rats were exposed for 5, 13, 20 and 28 days to daily changing L:D schedules ranging from OL:24D to 24L:OD. A constant illumination of 8100 lux was maintained during the light periods. The number of LH receptor sites was determined in the 1,000 to 29,000 xg fraction of testis homogenates by Scatchard analysis of the bound and free components of varying titers of radiolabelled hormone incubated with a constant amount of homogenate. A significant reduction ($P < .01$) of 43 and 62% in the number of receptor sites was observed when the rats were exposed to the treatment for 20 and 28 days, as compared with control rats subjected to a constant 14L:10D schedule (9.19 pmol/mg and 6.32 pmol/mg vs. 16.37 pmol/mg). The changes in receptor numbers were correlated to changes in plasma corticosterone, testosterone and LH concentrations. Plasma LH was significantly increased ($P < .01$) and testosterone was significantly decreased ($P < .01$) on days 20 and 28. Plasma corticosterone was significantly increased ($P < .05$) on day 5 and returned to control values by day 20.

65.11

PHOTOPERIODIC AND TESTICULAR CONTROL OF SEASONAL CHANGES IN LOCOMOTOR ACTIVITY IN ADULT MALE MICROTUS MONTANUS, THE MONTANE VOLE. Carol N. Rowsemitt* (SPON: S.J. Fidone). University of Utah, Salt Lake City, Ut. 84112

In the field, some species of microtine rodents shift from nocturnal activity in summer to diurnal activity in winter. This shift can be induced in male Microtus montanus by a shift from 16L:8D to 8L:16D, while maintaining temperature constant at 20°C. An average of 43 days is required for predominantly diurnal activity to occur. In these seasonal breeders, testicular function controls the shift from nocturnal to diurnal activity in adult males. Castrates under 16L:8D are diurnal while sham-castrates are nocturnal. Under 8L:16D, diurnally-active castrates which received testosterone implants showed two different responses: some shifted to nocturnal activity, while others were active both night and day. These results demonstrate that photoperiod cues are transduced into endocrine terms which then alter the timing of activity in this species. (Supported in part by NSF Grant DEB-79-21059)

65.12

DAILY RHYTHMS OF PLASMA MELATONIN IN MARMOTS(Marmota flaviventris). G.L. Florant, W. Kirby* and L. Tamarkin*. Dept. of biology, Swarthmore Coll., Swarthmore, Pa. 19081 and IRP, NICHD, NIH, Bethesda, Md. 20205.

The pineal gland and its hormone melatonin regulate seasonal reproduction in a number of mammalian species. However, the mechanism by which reproductive function is regulated by melatonin is unclear. To determine if changes in the daylength results in changes in the daily melatonin profile, blood samples were obtained from euthermic marmots, in either short photoperiod lighting cycles(LD 4:20, LD 8:16, LD 10:14) or a long photoperiod(LD 16:8). Blood samples were collected via a chronic aortic catheter every 3 hrs for 2 consecutive days. Plasma melatonin concentration was determined by a RIA sensitive to 3.3 pg/ml. There was a daily rhythm of plasma melatonin secretion in short and long-day animals. In the short photoperiods, plasma melatonin concentration peaked between 24:00 and 03:00 hrs. The mean amplitude in all 3 photoperiods was 142.2 ± 60 pg/ml, with a mean duration of 10.6 ± 1.7 hrs. In a long photoperiod the peak melatonin concentration was 95 ± 20 pg/ml, and the duration was 5.5 ± 1.8 hrs. The daytime melatonin values for short and long-day animals were 32.2 ± 20 pg/ml and 33.5 ± 13 pg/ml respectively, and were not significantly different($P > .05$). Only the duration was significantly different between short and long-day animals. This result provides further support for the hypothesis that the duration of elevated plasma melatonin levels, not amplitude or daytime levels, is influenced by photoperiod.

BLOOD PRESSURE

66.1

AUSCULTATORY INDIRECT MEASUREMENT OF BLOOD PRESSURE IN DOGS. Jeff Harvey*, Herman Falsetti, Paul Cooper* and Dan Downing*, CV Center & Dept. of Med, Univ of Iowa, Iowa City, IA 52242

The blood pressure is an important indicator of the integrity of the cardiovascular system. Sustained hypertension is associated with development of cardiovascular, cerebral and renal disease. Although indirect measurement of blood pressure is performed routinely in humans, it is not measured routinely in animals. An indirect method of measuring blood pressure (cuff plus stethoscope) was evaluated in 70 dogs weighing 15-30 kg (17.5 ± 8.8 kg; $\bar{x}$ ± SD). The study had 2 goals: 1) to examine whether there is an optimal anatomical site to obtain an audible blood pressure, and 2) to verify that the measurement accurately reflects intraarterial pressure. A cuff, 12 cm wide was used. The measurements were most audible with the cuff on the upper foreleg of the dog and with the stethoscope placed in the medial epicondylar region just distal to the cuff. The cuff was inflated to greater than systolic pressure and allowed to deflate slowly. In lightly sedated dogs, systolic blood pressures averaged 145 ± 25 mmHg ($\bar{x}$ ± SD) and diastolic blood pressures averaged 84 ± 14 mmHg. Indirect measurements were compared to direct measurements (femoral arterial catheter). The correlation coefficient for systolic pressure was 0.96 and for diastolic pressure 0.97. This study demonstrates that indirect measurement of blood pressure in dogs is both practical and accurate.

66.2

SIMULTANEOUS MEASUREMENTS OF TAIL CUFF AND INTRA-ARTERIAL PRESSURES (IAP) IN 2-KIDNEY, 1-CLIP (2K-1C) HYPERTENSIVE RATS BEFORE AND AFTER CAPTOPRIL. Karen A. Stanek*, Thomas L. Smith, William R. Murphy*, and Thomas G. Coleman. University of Mississippi Medical Center, Jackson, MS 39216.

Angiotensin II (AII) levels are often elevated in the 2K-1C model of hypertension. This could result in excessive vasoconstriction of the tail artery thus producing artificially low tail-cuff pressure readings. Further, captopril administration might abolish this vasoconstriction and thus increase observed tail-cuff pressures. A .25 mm I.D. silver clip was placed on the left renal artery of 8 male Sprague-Dawley rats. Femoral catheters were inserted 7 weeks later and tail-cuff pressures and IAP were monitored simultaneously for three consecutive days. Tail-cuff pressure averaged 137±16 (S.D.) mmHg, while IAP averaged 150±18 mmHg. This difference was statistically significant using a paired t-test ($P<0.01$). Six rats received an acute dose of captopril (2 mg/kg, IA). After 15 minutes, the IAP decreased 7.1 mmHg from control while the tail-cuff pressures decreased 26.8 mmHg. These decreases were statistically different ($P<0.05$). We conclude that the tail-cuff method of measuring blood pressure in the 2K-1C model of hypertension yields values that are less than direct intra-arterial observations. This discrepancy may or may not be due to AII. When AII was removed in this model, the tail-cuff method appeared to be even less accurate as shown by the increased underestimation. Supported by Grants HL26412 and HL11678.

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66.3

ROLE OF KALLIKREIN IN THE HYPOTENSIVE EFFECT OF CAPTOPRIL AFTER SYMPATHETIC STIMULATION OF THE RAT SUBMANDIBULAR GLAND. TB Ørstavik*, OA Carretero, L Johansen* and AG Scicli. Univ of Oslo, Norway; Henry Ford Hospital, Detroit, MI.

After cervical sympathetic nerve stimulation (SNS), the rat submandibular gland (sub-gl) releases kallikrein (KK) into the circulation. We studied the effect of captopril on mean blood pressure (BP) in 48 hr nephrectomized rats with and without prior SNS of the sub-gl. Administration of captopril 10 min after SNS resulted in a BP decrease of 43±8.3 mmHg ($p<0.01$), while the BP did not decrease significantly in the rats without SNS (ΔBP -3.3±0.5; $p>0.05$). To confirm that the effect of captopril was due to a blockade of kinin destruction, we determined the effect of captopril after SNS in rats pretreated with either IgG from nonimmunized rabbits (normal-IgG), or IgG from rabbits immunized against kinins (antikinin-IgG) or KK (anti-KK-IgG). Normal-IgG did not significantly alter the hypotensive effect of captopril (ΔBP -30±7.7; $p<0.01$), while pretreatment with antikinin or anti-KK almost completely blocked its hypotensive effect (ΔBP -5.8±1.9 and -6.4±0.4, respectively). In the latter two groups, the decrease in BP was significantly smaller ($p<0.001$) than in the non-pretreated and pretreated with normal-IgG groups. These results suggest that kinins may be responsible in part for the antihypertensive effect of captopril in situations in which glandular KK is increased in blood. Supported in part by NIH grant HL 15839.

66.4

BLOOD PRESSURE REGULATION IN CHRONIC SPINAL DOGS. H. Mikami*, A.T. Kosoglov*, and C.M. Ferrario. Cleveland Clinic Research Division, Cleveland, Ohio 44106.

When the sympathetic nervous system (SNS) is rendered permanently ineffective, resting mean arterial pressure (MAP) is not modified because of compensation by other factors. To investigate the role of vasopressin (AVP) and angiotensin II in maintaining MAP in the absence of sympathetic tone, the spinal cord was transected at C-6 and 7 days later blood pressure was recorded before and after IV injection of [d(CH_2)$_5$ Tyr (ME) AVP] followed by Captopril 30 min later. The order of drug administration was reversed in a 2nd study performed 1 week later. On each occasion, dogs were studied under normal hydration (NH), after 36 hrs of water deprivation (WD) and again following a 24 hr IV infusion of 2.0 l of 0.9% saline (WL). Captopril caused significant hypotension in both the NH (-20%) and WD (-40%) states (but not in WL dogs) whether the blocker was given before or after treatment with the AVP antagonist. In the WD state, AVP blockade produced a fall in MAP only when Captopril had been given beforehand. These effects were not observed in NH and WL dogs. The data indicate a predominant involvement of the renin-angiotensin system (RAS) in maintaining the MAP of chronic spinal dogs in both NH and WD states. In the absence of a tonically active active SNS, vasopressin may support blood pressure in the WD state after but not before blockade of the RAS. (Supported in part by NHLBI grant HL-6835).

66.5

HEMODYNAMIC COMPARISON OF 2-KIDNEY, 1- (2K1C) or 2-CLIP (2K2C) and 1-KIDNEY, 1-CLIP (1K1C) HYPERTENSION IN RATS, R.A. Ferrone*, C.L. Heran*, and J.M. DeForrest, The Squibb Inst. for Medical Research, Princeton, N.J. 08540

In order to examine the hemodynamic manifestations of the renin-angiotensin system (RAS) in renovascular hypertension we examined arterial pressure (AP, mmHg), cardiac output (CO, ml/min/kg), peripheral vascular resistance (PVR, [mmHg/(ml/min/kg)]) organ blood flows (OBF) and vascular resistances (OVR) in 3 forms of hypertension. 2K1C rats were examined at a time when plasma renin activity (PRA) was elevated (+4 weeks) whereas 1K1C and 2K2C rats were examined during a 'normal' renin phase (+4 and +6 weeks, respectively). Hemodynamics were measured in conscious, Sprague-Dawley rats by combined Fick and radiomicrosphere techniques. MAPs were 174, 181 and 187, COs were 475, 502 and 435, PVRs were .39, .41 and .44 for 2K1C, 1K1C and 2K2C rats, respectively. There were no statistical differences among the groups for these parameters or for any OBF or OVR. The RAS was clearly active in 2K1C, since PRA was elevated (+ 100%) and since captopril (100 mg/kg/day) prevented the development of 2K1C but not of 1K1C hypertension. Although the RAS was important in the development of 2K2C hypertension (captopril also prevented its development) PRA was normal in this model from day 3 after surgery and so does not appear to be important in the maintenance phase. Thus the role of the RAS appears to be different in these models of renovascular hypertension but no differences in hemodynamics were manifested.

66.6

MECHANISMS PRODUCING HYPERTENSION AFTER CESSATION OF CAPTOPRIL IN THE 2-KIDNEY, 1-CLIP RAT. J.M. DeForrest, J.S. Creekmore, M.P. Ushay*, T.L. Waldron*, R.A. Ferrone*, and M.M. Asaad*. Dept. Pharmacology, Squibb Institute for Medical Research, P.O. Box 4000, Princeton, N.J. 08540

Previous studies from our laboratory have shown that continuous inhibition of angiotensin II formation with captopril (100 mg/kg/day, p.o.) completely prevented the development of 2-kidney, 1-clip (2KHT) hypertension in the rat. Interestingly, upon cessation of captopril treatment, systolic blood pressure (SBP) increased progressively to hypertensive levels. The present investigations studied the roles of the renin-angiotensin and the sympathetic nervous systems as well as sodium balance on the development of the hypertension after cessation of captopril. Neither plasma nor vascular wall renin activity were elevated during the first 3 weeks after captopril cessation, even though SBP was significantly elevated. Also, cumulative sodium balance measured for 6 weeks after captopril cessation, was not altered. Interestingly, either the sympatholytic agent guanethidine (45 mg/kg, b.i.d.) alone, or guanethidine plus adrenal medullectomy prevented the development of the hypertension after stoppage of captopril. These results suggest that both the renin-angiotensin and the sympathetic nervous system are jointly responsible for the rise in SBP after cessation of captopril in the 2KHT rat.

66.7

TWO NOVEL ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS: CGS 13934 AND CGS 13945. D-S. Chen*, R. Dotson*, R. Burrell*, G. Aberg* and B.E. Watkins. Research and Development Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

The present study characterizes in vivo effects of two non-thiol ACE inhibitors to reduce the pressor responses to intravenous angiotensin I (AI). CGS 13945 is an esterified derivative of the free acid compound CGS 13934. After i.v. administration to anesthetized male Wistar rats, CGS 13934, CGS 13945 or captopril produced dose-related inhibition of AI pressor responses; CGS 13934 was about one-third, while CGS 13945 was about one-seventh as potent as captopril. Intravenous CGS 13934 also produced dose-related AI inhibition in dogs, but CGS 13945 was ineffective. As it is assumed that CGS 13934 is the biologically active form, data indicate that CGS 13945 is effectively hydrolyzed in vivo in the rat but not in the dog. All three test compounds (p.o.) also produced dose-related inhibition of AI pressor responses in rats; captopril was 4 times as potent as CGS 13945 and 12 times as potent as CGS 13934. Thus CGS 13945 was better absorbed orally than CGS 13934. In the rat, captopril transiently inhibited AI responses while equipotent doses of CGS 13934 or CGS 13945 produced longer lasting ACE inhibition. In conclusion, CGS 13945 is an orally active ACE inhibitor which is less potent than captopril but has a longer duration of action. CGS 13945 was not active in the dog, presumably because of poor endogenous hydrolysis.

66.8

ANTIHYPERTENSIVE EFFECTS OF TWO NOVEL ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS: CGS 13934 AND CGS 13945. D. Miller*, R. Dotson*, M. Hopkins*, D. Van Orsdell* and B.E. Watkins. Research and Development Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

CGS 13945 is an esterified derivative of the free acid compound CGS 13934, which has been shown to inhibit the ACE. The current study profiles various antihypertensive aspects of these ACE inhibitors. Four consecutive daily doses (p.o.) of CGS 13934, CGS 13945 or captopril produced dose-related systolic pressure (SP) reductions in SHR, occurring optimally after the third daily treatment. The dose of 30 mg/kg/day of all test compounds lowered SP comparably. When tested at 3.0 mg/kg/day, captopril lowered SP less effectively than CGS 13945, but was more effective than CGS 13934. Acute administration of CGS 13934, CGS 13945 or captopril (up to 100 mg/kg, p.o.) in conscious SHR decreased mean arterial pressure (MAP) only slightly through 4 hr when dietary Na intake was unrestricted. However during active Na depletion (Na deficient chow + hydrochlorothiazide, 50 mg/kg/day), CGS 13945 and captopril produced similar marked reductions of MAP. CGS 13934 was still less effective to lower MAP acutely, presumably due to poor oral absorption. CGS 13945 (30 mg/kg/day, p.o.) also reduced MAP in Na deplete (but not in Na replete) normotensive rats. CGS 13945 (p.o.) did not lower MAP in Na replete or deplete renal hypertensive dogs, indicating that this species does not readily hydrolyze this ester to the active free acid form.

66.9

CONTRIBUTION OF ARGININE-VASOPRESSIN TO THE CENTRAL PRESSURE RESPONSE TO ANGIOTENSIN II (ANG II). Lisete C. Michelini*, Mahesh C. Khosla* and Carlos M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106.

Endogenous vasopressin (AVP) may interact with central autonomic nervous system factors in the regulation of cardiovascular function. With this in mind, we studied in 11 morphine-chloralose anesthetized dogs, the arterial pressor response produced by an Ang II infusion into the vertebral arteries (VA), before and after intracisternal pretreatment with an AVP antagonist (dPVDAVP). The dose-response curve to VA infusion of Ang II (range 2-20 ng/kg/min) was significantly shifted to the right of control after injection of the AVP-antagonist (~ 10 µg/kg) into the cisterna magna (CM), the ED at 20 mmHg being almost double after central AVP blockade. However, this effect of AVP blockade was confined only to the cardiovascular response mediated via the VA. When Ang II was injected into either a vein (47 ± 1 ng/kg) or directly into the CM (48 ± 2 ng/kg) of these same dogs, the increases in mean blood pressure were the same before and after AVP-antagonist treatment (IV: 30 ± 2 vs 31 ± 2 mmHg after; CM: 14 ± 2 vs 13 ± 3 mmHg after). These data indicate that endogenous brain AVP plays an important role in determining the magnitude of the centrally mediated VA cardiovascular response to Ang II which in the dog is known to involve the area postrema pressor pathway. (Supported in part by NHLBI grants, HL-6835 and HL-24100).

66.10

THE RENIN-ANGIOTENSIN SYSTEM IN PRESSURE-INDUCED MYOCARDIAL HYPERTROPHY. N.C. Gonzalez, V. Donoso* and M. Bailie, Univ. of Kansas Medical Center, Kansas City, Ks. 66103.

The objective of these experiments was to study a possible direct effect of the renin-angiotensin system (RAS) on the development of myocardial hypertrophy. Abdominal aortic coarctation (AC) and sham operation (SO) were performed in a) Intact, b) Adrenalectomized (Adx) and c) Adrenal Medullectomized (Mdx) male Sprague-Dawley rats. After AC, carotid blood pressure was (mmHg): 162±9 in group a, 162±6 in group b and 155±5 in group c. Myocardial hypertrophy developed in intact and in Mdx, but not in Adx rats. Ventricular weight (in mg/100g body weight) was: 338±12 in group a, 356±15 in group b and 287±8 in group c. Plasma renin activity (PRA) was not altered by AC in any of the three groups, but was increased threefold in both AC and SO Adx rats. Further experiments showed that this increase in PRA was accompanied by an even higher increase in plasma renin concentration (PRC) and by a decrease in plasma renin substrate concentration (PRS). Therefore, although the decrease in PRS of Adx may have limitedthe increase in PRA, it did not prevent it. The finding of elevated PRA (and possible elevated angiotensin II) in the absence of myocardial hypertrophy; and the development of pressure-induced hypertrophy without change in PRA do not support the idea of a possible direct effect on RAS on the development of myocardial hypertrophy.

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66.11

EFFECT OF ATRIAL EXTRACT ON HEMODYNAMICS IN RATS. M. Pamnani, S. Huot, M. Jagusiak*, W. Link*, and F.J. Haddy. Dept. of Physiology. Uniformed Services University, Bethesda, MD 20814

The hemodynamic effects of an atrial muscle extract which contains potent natriuretic activity have been studied in rats. Fresh rat atria were homogenized in saline, boiled for 5 min, and supernates of the boiled atrial extract (SA) separated. Similarly prepared supernates of boiled ventricular extract (SV) were used as controls. Infusion of SA (but not SV) into bilaterally nephrectomized (2 NX) hexamethonium (HEX) treated rats increased mean arterial pressure (BP) by 15% ($P<0.005$, N=6). However, the effect was transient, disappearing by the fourth minute of the infusion. Pressor responses to various doses of norepinephrine (NE) were not different following SA or SV infusion. In another study, NE (10 ng) mixed with .05 ml (I), .1 ml (II) and .15 ml (III) of SA or SV was given iv as bolus injection in 2 NX, HEX treated rats. Percent increases in mean BP were as follows:

SA + NE			SV + NE		
I	II	III	I	II	III
29*±1.6	35*±1.9	39*†±2.4	15±2.4	11±2.5	10±2.2

*$P<.01$ SA+NE vs respective SV+NE, †$P<.05$ SA+NE III vs SA+NE I Compared to SV+NE, SA+NE produced significantly greater pressor responses at all doses. The SA+NE pressor responses appeared to be dose-dependent. These findings suggest that the pressor effect of SA may be due to potentiation of NE. However, the potent diuretic effect of SA cannot be attributed to its transient pressor effect.

66.12

ARTERIAL PRESSURE REGULATION IN CONSCIOUS SODIUM DEPLETED RATS. Hélio C.Salgado, Leni H.Bonagamba*, Edson D.Moreira* and Eduardo M.Krieger. Dept. of Physiology, School of Medicine, USP, Ribeirao Preto, SP, BRAZIL.

Hemodynamic and reflex arterial pressure regulation were studied in rats fed normal (NS,1 mEq/day) or low (LS,0.1 mEq/day) sodium diet for 6 days associated with furosemide (5 mg/Kg, i.p.,in the 1st and 5th day). Thermodilution measurements showed that LS rats had a cardiac output 32% reduced, which was partially compensated by a 31% increase in peripheral resistance, resulting a 13% decreased mean arterial pressure (MAP). With cuffs implanted the day before, bilateral carotid occlusion for 20 sec promoted an acute hypertensive response in the LS rats that was 23% smaller than that observed in the NS rats. Sixty min after recovery from ether anesthesia sinus-aortic denervation showed almost the same relative increase in MAP (27% and 29%, respectively for LS and NS), but the LS rats did not achieve hypertensive levels (MAP=126 ± 5 mm Hg). The LS rats, required, respectively, 3.5 and 2.4 times larger dose of Nourepinephrine and Angiotensin II than the NS rats, to increase MAP 20-25 mm Hg. These data suggest that the blunted reflex sympathetic response of the LS rats could be due to a decreased pressure responsiveness involving the peripheral vasculature and the inotropic performance of the heart along with a significant role played by the aortic baroreceptor function during sodium depletion.

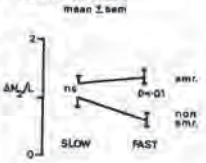
Financialsupport by FAPESP, FINEP and CNPq

MECHANICS OF BREATHING: GENERAL

67.1

A MODIFICATION OF THE SINGLE BREATH NITROGEN TEST DIFFERENTIATES NONSMOKERS FROM SMOKERS. T.S. Hurst*, B.L. Graham*, J.A. Dosman, D.J. Cotton, Department of Medicine, University of Saskatchewan, Saskatoon, Sask., Canada. S7N OXO.

We studied two groups: (1) ten symptom free lifetime nonsmokers (age 30 ± 6.8 years, mean ± 1 SD) and (2) twenty current smokers (age 33 ± 13.1 years). Spirometric variables and maximal expiratory flow volume curves breathing both air and a mixture of 80% helium-20% oxygen were similar between groups 1 and 2. Both groups performed single breath nitrogen tests by either (A) exhaling slowly (0.5 ℓ/s) from end inspiratory volume (EIV) to residual volume (RV) or, (B) exhaling maximally from EIV to RV. After either A or B subjects slowly inhaled oxygen to TLC and, without breath holding exhaled slowly back to RV. The phase III slope ($\Delta N_2/\ell$) was measured by linear least squares regression analysis. In nonsmokers $\Delta N_2/\ell$ was lower for maneuver B than for A ($p < .01$) while for smokers $\Delta N_2/\ell$ was higher for maneuver B than A ($p < .01$). While $\Delta N_2/\ell$ was similar between groups 1 and 2 for maneuver A, it was significantly different between the groups for maneuver B ($p < 0.01$). We conclude that $\Delta N_2/\ell$ measured following the rapid maneuver (B) separates a group of smokers with otherwise normal mean lung function variables from a group of normal nonsmokers, whereas the slow maneuver (A) does not.



67.2

NONINVASIVE QUANTITATION OF THREE-DIMENSIONAL LUNG GEOMETRY AND FUNCTION. Eric A. Hoffman, Lawrence J. Sinak,* Richard A. Robb, Lowell D. Harris,* and E. L. Ritman. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905

The Dynamic Spatial Reconstructor (DSR) was used to estimate total lung volume (air plus tissue) within intact living dogs. Six anesthetized dogs, ranging from 2.5 to 26 kg, were scanned supine and/or prone (suspended from the spine) at three static lung volumes (FRC to TLC). Regional roentgen attenuation values of the lung (CT numbers) were used to estimate regional differences in lung expansion occurring at each inflation step. Total lung volume as estimated using DSR image data matched the excision and water displacement volumes within 3% in all cases. Change in total lung volume as per DSR matched the known air volume injected (corrected for temperature and pressure) to within 3%. Regional air content of the lungs as per DSR image data, showed a gradient of decreasing air content of the lung (FRC) in the dependent direction ranging from 92% in the non-dependent region to 62% at the most dependent region of the supine lung. DSR data show that change in regional air content of the lung field with lung inflation is greater in the dependent than the nondependent regions of the supine dogs. Regional air content changes with lung inflation in the prone, spinal-suspended, position are approximately uniform along the vertical dimension. This is consistent with regional lung expansion data obtained via lung parenchymal marker measurements. (Supported in part by NIH HL-04664 and RR-00007)

67.9

ANTIPROTEASE ACTIVITY IN THE SHEEP LUNG INTERSTITIUM. G.D. Niehaus* and F.A.Blumenstock* (SPON: L. Grumbach). Albany Medical College, Albany, NY 12208.

Plasma and interstitial antiproteases protect the lung from proteolytic degradation. The source of interstitial antiproteases has not been well defined. To test whether interstitial antiproteases are plasma filtrates, the current study was designed to use the sheep lung lymph fistula preparation to compare the capability of plasma and lung lymph to inhibit a standardized protease (trypsin) activity. The trypsin inhibitory capacity (TIC) and total protein, albumin and globulin concentrations were measured in 14 hemodynamically stable sheep. Each mg of plasma protein inhibited 16.1±0.4 µg of trypsin. Albumin and globulin lymph-to-plasma ratios of 0.80±0.03 and 0.53±0.02, respectively, suggest that seiving of α_1 antitrypsin (55,000 M.W.) and α_2 macroglobulin (750-825,000 M.W.) should result in 25% fewer antiprotease molecules in the lymph, i.e. a lymph TIC of approximately 12.4 µg/mg of protein. The observed lymph TIC of 26.9±1.4 µg/mg was significantly ($p<0.001$) higher than the plasma TIC. We conclude that the lymph anti-proteases are not purely plasma filtrates, but are either products of active transmural transport or are produced in the lung. (Supported by an American Lung Assoc. grant)

67.10

RESPIRATORY SYSTEM IMPEDANCE OF NORMAL AND OZONE-EXPOSED RATS. M.I. Kotlikoff† J.W. Watson† and A.C. Jackson. Physio. Sci., and Primate Res. Ctr., School of Vet. Med., Univ. of Calif., Davis, CA 95616

The respiratory input impedance of tracheostomized rats was measured from 20-90 Hz at constant flow amplitudes (20ml/sec) in 25 normal rats, and in 11 rats exposed to .64ppm (UV) ozone for seven days. Ensemble averaged impedance curves from the two groups showed differences in the real and imaginary parts of impedance. The real part (effective resistance) was higher at all frequencies in the ozone-exposed rats, but the increase in resistance with frequency was similar. The imaginary part (effective reactance) was reduced at higher frequencies in the ozone group and, unlike the normals, never became positive. The difference in reactance was significant at frequencies above 50 Hz ($p<.05$), while the difference in resistance was not significant at any frequency. When the individual impedance curves were fitted to a lumped, six-parameter, shunt compliance model and the parameters from the two groups compared, a significant difference ($p<.05$) was observed in only the peripheral resistance parameter. We conclude that ozone exposure at this level causes changes in respiratory input impedance, that these changes consist primarily of decreased reactances at higher frequencies, and that these changes can, in general, be modeled by an increase in peripheral resistance. (Supported by NIH Grant ES-00628, and EPA Grant 807661.)

BEHAVIOR, PAIN AND TRAUMA

68.1

PLASMA MEDIATION OF BEHAVORIAL CHANGE OF MUSCLE METABOLISM. R. Jevning, J.P. O'Halloran*, and S. Guich*, U.C. Irvine, Irvine, Ca 92717

Previous measurement of the respiratory change of forearm tissue during the acute hypometabolism induced by the "transcendental meditation technique" (TM) indicated decline of CO_2 generation such that forearm RQ decreased (-95%) in 30 minutes. Concomitant and significantly correlated decrease of red blood cell metabolism was also recorded (Jevning, R. et al, Physiol and Behav, In Press). These changes were attributed primarily to altered muscle metabolism. The possibility of a plasma role in the mediation of some of these metabolic changes has now been studied in a model system. O_2 consumption and 14 CO_2 generation were measured in six 2 ml rat soleus muscle homogenate plasma mixtures (K.M.Baldwin, et al, J Appl Physiol 50:1272,1981), each utilizing 0.3 ml of plasma drawn during and for 30 minutes after 45 minutes of TM at 15 minute intervals in 6 subjects. Significant declines(mean±S.E.) of O_2 consumption (-20±8.4) and $^{14}CO_2$ generation (-28±9.2) were registered in the mixtures containing plasma drawn during TM followed by recovery in the mixtures prepared from plasma drawn during the post-TM period. Initial rates of O_2 consumption and 14 CO_2 generation (mean±S.E.) were respectively: 2950 ±430 ml (O_2/g wet wt.)/h and 287±46nmol/g/min. These data support a significant role for plasma factor(s) in the modulation of forearm (muscle) metabolism by TM and the usefulness of this model system in their depth biochemical investigation. (Research support: NIH Grant HL 27894.)

68.2

BEHAVORIALLY-INDUCED ELEVATION OF PLASMA ARGININE VASOPRESSIN (pAVP). J.P. O'Halloran*, R. Jevning and R. Skowski; U.C. Irvine, Irvine, CA 92717.

Previous studies of the "Transcendental Meditation" technique (TM) indicate that it is associated not only with acute hypometabolism, but also with significant long term alterations in behavorial tendencies and patterns as indexed by scores on standard personality inventories. We determined plasma levels of arginine vasopressin (AVP) a neuropeptide with behavior-modifying properties as well as blood osmolality and galvanic skin resistance (GSR) in 6 long term TM subjects and in 6 non-meditating individuals (ordinary rest group) at 15 minute intervals, 15 minutes before, during and 30 minutes after 45 minutes of practice (TM for the TM group and ordinary, non-stylized rest for the rest group). Blood samples were drawn at the time of day TM subjects routinely practice meditation, (8-10:30 AM). An additional single blood sample was taken from each TM subject at a time different from the routine TM practice time (12-1PM). pAVP was determined by specific RIA. pAVP values were 2.7-7.1 times greater ($P<.005$) in the TM group as compared to the ordinary rest group at all 7 points during the sampling period (including pre-and post practice). Plasma osmolality values for the two groups were within the normal range and did not differ significantly. The pAVP values for the additional, single samples were indistinguishable from those of the ordinary rest group. (GSR) measurements showed that both groups achieved a relaxed state. Elevation of pAVP in the TM group is probably not due to decreased hepatic clearance since hepatic blood flow does not differ consistently between TM and nonstylized rest (Am. J.Physiol. 235:R89,1978), but is likely caused by an increased rate of secretion of ADH from the posterior pituitary, independent of osmotic influences. The results also indicate that pAVP levels are not chronically elevated and suggests that the observed increase of pAVP in TM subjects is related to the time of induction of the meditative state. GSR data rule out contribution by psychologic stress. In consideration of the ability of systemically-administered AVP to modify behavior patterns in animals and humans, this striking effect may be associated with the numerous reported behavorial changes induced by TM. (Supported by NIH Grant RO1HL27894).

68.3

HUMAN POSTURAL LATERALITY FROM BIRTH TO ONE YEAR OLD. Santibáñez, Ibcia. Depto. de Fisiología y Biofísica. Fac. de Medicina, Universidad de Chile. Casilla 137-D. Santiago, Chile.

Three postural reflexes (vestibular acceleration response and palmar and plantar placing) were studied in a strictly selection sample (72 selection variables of normality) of 44 newborns (NB) and infants (I) from 1 to 12 months old. Each reflex was monthly controlled through a filmic and a direct observation protocholized behavioral study of latency and duration of the performance. The reflex placing responses were found to be consistently lateralized, left or right, in all the babies by 2 synchronous bilateral adequate stimuli, administered from birth up to 12 months. 18 of the babies completed monthly follow-ups for a year. The other 26 only had a partial follow-up during the first year. The Vestibular Acceleration Reflex (parachute reflex) appeared in the 4th month and due to its braquial component, presented the same lateralization as placing. Only one of the 44 babies presented a consistent left lateralized reflex response for both types of reflexes. These results demonstrate early lateralization in human babies and proposes a consistent and simple method for measuring human postural laterality in early stages of development. The dominant brainstem and basal ganglia mechanisms of these reflexes at early stages together with these results suggest the existence of an hypothetically lateralized mechanism of the participating structures of these reflexes.

68.4

IMMOBILIZATION STRESS AND BRAIN PROTEIN CONCENTRATION. Eric A. Stone. New York Univ. Sch. Med., New York, NY 10016

Previous unpublished studies in this laboratory have revealed small, nonsignificant increases in brain protein concentration in rats exposed to repeated immobilization stress. The present study was undertaken to determine whether these findings represent a reproducible phenomenon. Rats (male Sprague-Dawley, 180-200 g) were subjected to immobilization stress for 2.5 hrs daily by the method of Kvetnansky & Mikulaj (Endocrinol. 87:783,1970). Animals were killed after either 1 or 10 exposures to stress. Brains (brainstem and remainder) were weighed and assayed for protein by the Lowry procedure using bovine albumin as the standard. Acute immobilization was found to have no effect either on the wet weight or protein concentration of the tissues. Repeated stress produced no change in wet weight but caused a statistically significant increase in protein concentration amounting to 7.0%. Because of the small size of this effect the experiment was repeated two additional times. In the first replication a significant protein increase of 6.8% was obtained whereas in the second a nonsignificant increase of 2.6% was found. The results indicate that chronic immobilization stress does increase brain protein concentration but that the effect is small and is easily obscured by experimental error. The mechanism and significance of this change is not known although it may be related to the findings that various forms of chronic stress induce trophic effects in a number of peripheral organs. (Supported in part by USPHS grants MH 22768 and MH 08618).

68.5

MORPHINE INDUCES DOSE-DEPENDENT INCREASES IN STEREOTYPED HEAD AND PAW MOVEMENTS IN CATS. C.M. Harris,* J.R. Villablanca, I. de Andres* and J.W. Burgess.* Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

To quantify key components of the complex behavioral response to morphine (Villablanca et al., Brain Res., in press), we analyzed spontaneous behaviors of adult male cats before and after ip injection of morphine sulfate (M). Four doses (0.5, 1.0, 2.0 and 3.0 mg/kg) were given in random sequence, at least 2 weeks between doses, N=13, 5-7 cats/dose. Two-min samples were videotaped at 15-min intervals for one hour before and 5-6 hours after drug administration. Amount of walking, standing, sitting, crouching and lying, and frequency of stereotyped head and paw movements were scored on an event recorder. Percent-time spent in the various body postures did not discriminate among doses except for a tendency for more lying during the last 2 hours of sessions following the lowest dose. In contrast, both head movements (p=.0002) and paw movements (p=.0008) increased after drug. Paw movements increased with dose at a rate of 1.83 mvts/min/mg (r=0.99). Head movements increased at 7.53 mvts/min/mg (r=0.96), and were biased toward the right. In conclusion, although body postures may differentiate between the mania-inducing high doses not used here and the present dose range, phasic head and paw movements provide a fine discrimination for the discrete more specific changes produced by low doses. (Supported by USPHS HD-02518)

68.6

A NEW SKIN REFLEX "MULTI-PROJECTIONAL DOUBLE REFLEX" WHICH IS RELATED TO PAIN AND ACUPUNCTURE. MIN-HAING CHO and CHIN-YONG LEE CHO, 6116 Manatee Avenue West, Bradenton, Florida 33529

Liquid crystal thermography was used to visualize the temperature changes in the skin parts to be studied. If we stimulate the hand using electric current of I volt, I Hz, for 30 seconds, this will elevate the skin temperature of the outer ear from 33C to 37. If we place a small piece of ice on the knee cap for 15 seconds, the ear skin at the naviculor fossa, a different area from the above, show the same skin temperature elevation. We found the reverse, i.e., the stimulation of the ear skin causing the skin temperature elevation of the hand and knee area is also true. We have concluded that there is an autonomic nerve reflex that handle a stimulation incoming to one part of our body is projected to multiple site of our body. Sensory as well as motor functions are involved in this reflex and thus the new name "MULTI-PROJECTIONAL DOUBLE REFLEX".

68.7

DORSAL COLUMN POSTSYNAPTIC SPINOMEDULLARY (DCPS) NEURONS: A PHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS. G.W. Lu, G.J. Bennett*, N. Nishikawa* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

It is now known that in addition to primary afferents the spinal cord's dorsal columns (DC) also contain the axons of spinal neurons that ascend to the dorsal column nuclei. We labeled these cells in cats and monkeys by placing HRP on their severed axons within the DC. In both species the lumbosacral enlargements contained about 1,000 DCPS neurons located mainly in lamina IV of the dorsal horn. Intracellular HRP staining showed that most cat DCPS neurons had dendritic arbors that were rostrocaudally elongated (up to 2.3 mm) but relatively narrow mediolaterally (c. 0.5 mm). About half of over 100 antidromically identified cat DCPS cells responded only to innocuous tactile stimuli. The other half responded to both innocuous and noxious stimuli, having large receptive fields often encompassing entire paw and leg and receiving input from A but not C afferents. Intracellular recordings revealed a post-excitatory ipsp whose amplitude was diminished by intracellular hyperpolarization. The ipsp was frequency dependent and disappeared at stimulus frequencies above 20 Hz. The antidromic response of half of the intracellularly-recorded cells was followed by a synaptically-driven response that was probably activated by the antidromic invasion of the intraspinal terminals of DC primary afferents. We conclude that DCPS neurons constitute a physiologically-diverse, major projection system originating in the spinal dorsal horn.

68.8

NORMALIZATION OF DYSFUNCTIONS FOLLOWING THE ACUTE AND CHRONIC TRAUMATIC SPINAL CORD INJURY. N. Eric Naftchi. Lab. Biochem. Pharmacol., IRM, NYU Med Center, 10016

In 47 mongrel cats, intramuscular ketamine or intravenous pentobarbital was used for anesthesia. The cats were then traumatized by dropping a 20 gm weight from a height of 25 cm on the exposed dura at the fourth thoracic segment. Somatosensory evoked potentials (SEPs) were recorded before and after impact in order to ascertain the effectiveness of the trauma as well as the conduction return. The untreated control group comprised of 30 cats and was compared with another group of 17 cats which received similar impact at the T_4 level but was treated at different time intervals after spinal cord contusion with 0.075 mg clonidine I.V. After infusion of clonidine, angiotensin II was slowly infused to bring the B.P. to the stabilized pre-clonidine infusion level. The chronic cats were again infused with the same amounts of clonidine and angiotensin II twice a day until the SEPs returned. In all 17 cats treated with clonidine, SEPs did return. Six chronic cats treated from 14 to 48 days after paralysis walked with complete return of sensory and motor functions, including those of bladder and bowel. In the 30 traumatically spinal cord injured, untreated controls, tested for a period of one to four months, SEPs never returned as the animals progressed from the acute, flaccid phase to the chronic, spastic, and autonomically dysreflexic phase of paralysis. Preliminary results indicate that this approach is applicable to traumatic injuries of the brain and spinal cord in human.

68.9

STRESS AFFECTS THE SURVIVAL OF RATS WITH A MAMMARY ASCITES TUMOR. J.W. Lewis*, G.W Terman*, Y. Shavit*, R.P. Gale*, and J.C. Liebeskind. Depts. of Psychology and Medicine, University of California, Los Angeles, CA 90024

Prolonged, intermittent footshock stress causes the release of endogenous opioid peptides in the rat (Lewis et al., 1980). This same footshock procedure also results in a significant reduction in immune function indicated by a decreased responsiveness of T lymphocytes to mitogenic stimulation (Con A or PHA). The immunosuppression was prevented by an opiate antagonist drug, naltrexone, and thus appears to be mediated by opioid peptides released during stress (Shavit et al., 1982). The present study investigated the effect of this type of stress on the development of a mammary ascites tumor.

Three groups of Fischer 344 female rats were injected with 10^4 mammary tumor cells (13762 MAT/B); one group had received 10 min of intermittent footshock stress daily for 4 days preceding the injection, another group received the same stressor daily for 7 days following the injection, and the last group served as nonstressed controls. Footshock stress, administered either before or after tumor injection, decreased the number of surviving animals with rats stressed prior to tumor injection showing the lowest percentage of survival. Thus, the stress procedure that causes an opioid mediated immunosuppression, when chronically administered decreases resistance to mammary tumor acceptance and/or development. (Supported by a gift from the Brotman Foundation).

69.1

NETWORK THERMODYNAMIC MODELING OF ISOTONIC SOLUTE-COUPLED FLOW AND RECTIFICATION OF PASSIVE OSMOTIC FLOWS IN THE RAT ILEUM. Mark L. Fidelman* and Donald C. Mikulecky. Medical College of Virginia, Richmond, VA. 23298

The practical phenomenological equations as developed by Kedem and Katchalsky (1958) for describing coupled flows of volume and solute were applied to each membrane in a four membrane "leaky" epithelial model. A "sodium pump" is located in the basolateral cell membrane. Bulk fluid flow measurements were made with paired rat ileum segments, in vitro. A single set of membrane transport coefficients was found which could account for the experimentally observed values of both isotonic solute-coupled flow and the rectification of passive osmotically induced fluid flows. The primary features of the model are: 1) volume flow is primarily through the cellular pathway; 2) the apical membrane reflection coefficient is less than that of the basolateral membrane which permits large volume flows with only slight hypertonicity developed in the lateral intercellular spaces (LIS) and strongly couples the net volume flow through the cell with the net sodium flux produced by the Na pump; and 3) a low basement membrane reflection coefficient which drops the tonicity of transported fluid from slightly hypertonic in the LIS to isotonic in the serosal bath. The asymmetry produced in passive flows when osmotic differences are imposed in different directions is a result of differences in accumulation and depletion of solute in both the cell and LIS under the opposing osmotic conditions. (Supported in part by NIH Training Grant HL-07110.)

69.2

THE NEED FOR PROPER MODELLING OF BICARBONATE REABSORPTIVE PUMPS W.A. Brodsky, T.P. Schilb*, and J.H. Durham*. Mt. Sinai Sch. Med/CUNY and Brookdale Hosp. Med. Center

Because a particular bicarbonate reabsorptive pump model cannot account for the reduction of luminal acidification following the addition of a carbonic anhydrase inhibitor, it cannot be the acidification mechanism in turtle bladder or mammalian kidney. But this model is inherently flawed: (i) Its claimed mode of operation conflicts with laws governing the association reactions between ions and the conductance of electric current by different ions in aqueous solutions; and (ii) there is no explicit requirement for intracellular carbonic anhydrase, despite repeated demonstrations of this enzyme in cytosolic fractions of turtle bladder and renal epithelia. In a recently developed alternative model of the acidifying epithelial system, carbonic anhydrase is placed in the cytosol and an electrogenic bicarbonate ion pump in the apical membrane. After the pump is switched from the off to the on position, a significant part of the translocated bicarbonate ions interact with cellular protons to give at first a transient and later a steady state increase in the intracellular concentrations of H_2CO_3 and CO_2. Inhibition of intracellular carbonic anhydrase retards the rate of H_2CO_3 dehydration and consequently that of luminal acidification. With these and other properties, the currently-modelled bicarbonate reabsorptive mechanism is consistent with all of the available data on luminal acidification in turtle bladders and mammalian kidneys.
(NIH and NSF Grant Support)

69.3

ELECTRON PROBE MICROANALYSIS OF GASTRIC SURFACE EPITHELIAL CELLS: EFFECT OF PROSTAGLANDIN E_2 (PGE_2)

D. W. Rattner*, S. Ito*, W. Silen, C. P. Lechene

Electron Probe Microanalysis permits one to determine the elemental content of isolated cells and thus study transport processes. Enriched populations of isolated Guinea Pig gastric surface epithelial cells were prepared and cultured for 24 hours on fibronectin-coated vitreous carbon discs. The cells were kept as controls (C) or treated with 0.1% or 1% ethanol (ETOH) without or with 10^{-5} M PGE_2. The cells were then freeze dried in situ. Electron probe microanalysis of frozen dehydrated samples showed that ETOH caused a decrease in intracellular K concentration and an increase in intracellular Na concentration with a K/Na ratio = 4.04 (C) vs 2.22 (ETOH) $p< 0.01$. The addition of PGE_2 brought back the K/Na ratio to a value not significantly different from control (4.04 (C) vs 3.35 (PGE_2) N.S. Intracellular chlorine content was variable but was lower in cells treated with PGE_2 when compared to control, in spite of normalization of the K/Na ratio. These results suggest that i) PGE_2 restores the normal intracellular Na and K content following ETOH injury by mechanisms not elucidated in these experiments and 2) PGE_2 decreases cellular Cl content perhaps involving a Cl-HCO_3 exchange.

69.4

INTRACELLULAR CHLORIDE ACTIVITY IN CANINE TRACHEAL EPITHELIUM. Michael J. Welsh. Pulmonary Division, Department of Internal Medicine, University of Iowa, Iowa City, IA 52242

Tracheal epithelium secretes Cl via an electrogenic transport process. To examine the mechanisms involved, intracellular Cl activity $(Cl)_c$ was measured with Cl-selective microelectrodes under short-circuit conditions. When Cl secretion was minimal (indomethacin, 10^{-6} M mucosal solution), the apical membrane voltage (V_a) was -60±2 mV (n=7 tissues) and $(Cl)_c$ was 37±3 mM. Stimulation of Cl secretion (epinephrine, 10^{-6} M submucosal solution) depolarized V_a to -46±2 mV, but $(Cl)_c$ was not altered at 39±3 mM. These findings indicate that: a) Cl is accumulated across the basolateral membrane under nonsecreting and secreting conditions at activities 3.8 and 2.4 times, respectively, greater than predicted for a passive distribution; b) during secretion Cl may exit passively with an electrochemical driving force of 22 mV; c) stimulation of secretion enhances the rate of Cl entry since Cl transport increased without a change in $(Cl)_c$. In Na-free Ringers, $(Cl)_c$ was 17±2 mM (n=5), not significantly different from the 14±2 mM predicted for a passive distribution; subsequently, Na-Ringers increased $(Cl)_c$ to 34±2 mM. This indicates that Cl accumulation is dependent upon Na. Bumetanide (a loop diuretic) (10^{-4} M, submucosal solution) decreased Cl secretion and decreased $(Cl)_c$ indicating an inhibition of Cl entry. These findings suggest that Cl is accumulated intracellularly by a bumetanide-inhibitable, Na-coupled entry step at the basolateral membrane and that Cl may exit passively across the apical membrane.

69.5

INTRACELLULAR CL ACTIVITIES IN THE RABBIT DESCENDING COLON. W.K. WILLS, DEPARTMENT OF PHYSIOLOGY, YALE UNIVERSITY, NEW HAVEN , CT. 06510

The rabbit descending colon has been reported to actively absorb chloride in vitro, however the mechanism of this transport is unknown. To obtain information about the gradient for this ion, intracellular chloride activity (a_iCl) was measured with ion-sensitive microelectrodes using the recently modified Cl^- resin (Corning #477913). In pooled data from nine colons, a_iCl averaged 23 ± 1.7 mM (n=110 cells). The calculated equilibrium potential for chloride (E_{Cl}) was -38± 2.0 mV. Under open-circuit conditions, the apical and basolateral membrane potentials averaged -17.3± 5.3 mV and -53.4± 2.6 mV, respectively (cell interior negative). Under short-circuit conditions, a_iCl showed little change and was above equilibrium. After replacement of Cl^- by gluconate in the serosal solution a_iCl was 17± 2.8 mM (23 cells from 5 colons) whereas Cl^- replacement in the mucosal solution reduced a_iCl to 1.3 ±0.49 mM (47 cells from 5 colons; $p<.01$). In the absence of Cl^- a mean "residual" activity of 0.8 ±0.2 mM was measured. The findings support previous studies which demonstrated a low Cl^- conductance in the basolateral membrane and further suggest that Cl^- transport across the basolateral membrane may involve an electroneutral mechanism. (Supported by NIH grant AM 29962.)

69.6

MICROELECTRODE ASSESSMENT OF THE LUMINAL CELL MEMBRANE IONIC CONDUCTIVE PROPERTIES OF THE CORTICAL COLLECTING TUBULE (CCT). Roger G. O'Neil, Univ. of Texas Med. Sch., Houston, TX 77030.

Previous studies indicated that the apical cell border of the isolated rabbit CCT possessed an amiloride-sensitive Na conductance and a Ba-sensitive K conductance (Kid.Intern. 21:283, 1982; Fed. Proc. 41:1006, 1982). This analysis was extended by impaling cells with KCl-filled microelectrodes. The transepithelial voltage, V_{te}, basolateral cell membrane voltage, V^b, and apical cell membrane voltage, V^a, averaged -5.5 mV (lumen negative), -62.2 mV (cell negative) and -56.7 mV (cell negative) in control conditions (NaCl Ringer's 37°C, pH 7.4) and 0.9, -68.0, and -68.9mV, respectively, after addition of 50 uM amiloride to the perfusate (N=5). The fractional resistance (FR), estimated as the ratio of the change in V^a to V_{te} after a current pulse, averaged 0.53 and did not increase significantly after amiloride. In the continued presence of amiloride, luminal elevation of K from 5 to 50 mM, caused V^b and V^a to depolarize to -29.5 and -24.5 mV, respectively, and FR to decrease by 0.17 (N = 4). Alternatively, adding 5 mM Ba to the perfusate depolarized V^b and V^a to -32.7 and -31.6 mV, respectively, and increased FR to 0.95 (N=7). Similar changes were observed when the luminal pH was reduced to 4.0 (N = 4). In the continued presence of Ba or low luminal pH, the responses to luminal elevation of K were greatly reduced. It is concluded that the apical cell membrane possesses a low amiloride-sensitive Na conductance and a dominant Ba- and H-sensitive K conductance.

69.7

ION PERMEABILITY OF JEJUNAL BRUSH BORDER MEMBRANES MEASURED WITH A POTENTIAL SENSITIVE DYE AND WITH RADIOACTIVE TRACERS. Robert D. Gunther*, Richard Schell*, Sally Krasne* & Ernest M. Wright. Department of Physiology, University of California at Los Angeles, California 90024.

The fluorescent, carbocyanine dye, diS-C_3-(5) (3,3'-dipropylthiadicarbocyanine iodide), was used to measure the electrodiffusive permeability of isolated jejunal brush border membrane vesicles to several ions. The response of the dye was calibrated using: 1) potassium equilibrium potentials in the presence of the potassium ionophore valinomycin, and 2) sodium equilibrium potentials in the presence of the sodium ionophore ETH 1097 (N,N,N',N'-Tetrabenzyl-3,6-dioxaoctan-diamid). The response of the dye was linear with a 0.3% change in fluorescence intensity per mV. Biionic potentials were measured for different pairs of ions. The relative permeabilities of the ions were calculated from the membrane potential (fluorescence) data using the constant field equation. The permeability sequence, relative to sodium, was: F(0.04) < Na(1.0) < Cl(1.5) < NO_3(1.6) < K = Rb(2.4) < Cs = Br(2.6) < Li(3.8) < NH_4(11.5) < I(39.7). Preliminary data using radioactive tracer measurements indicated that the permeability coefficients for five of the above ions range between 6 and 20 nanoliters/(mg protein·sec) for sodium and rubidium respectively. These observations with both dye and tracers indicate that the jejunal brush border is a permselective barrier for monovalent ions.
(Supported by USPHS AM 19567, AM 07270 & HL 20254)

69.8

STIMULATION OF GLUCOSE ABSORPTION, CHLORIDE SECRETION, AND EPITHELIAL CYCLIC GMP CONCENTRATIONS IN THE RAT SMALL INTESTINE BY HEAT-STABLE TOXIN OF E. COLI. W.G. Marnane*, E. Perez-Lugo*, and Y.-H. Tai. Walter Reed Army Inst. of Res., Washington, D.C. 20012.

The effects of the heat-stable toxin of E. coli (ECST) on glucose and chloride transport under short-circuit conditions and cyclic GMP (cGMP) concentrations in the isolated villous tip and crypt cells in the rat small intestine were studied. ECST (20 MU/ml) increased glucose absorption and chloride secretion by 0.696 ± 0.210 umol/h/cm^2 and 3.44 ± 0.65 ueq/h/cm^2, respectively. The isolated villous tip and crypt cells were characterized by the enzyme activities of alkaline phosphatase and thymidine kinase:

	Alkaline Phosphatase nmol/mg prot./h	Thymidine Kinase pmol/mg prot./10 min
Villous Tip	2.19 ± .34 (11)	19.9 ± 6.8 (11)
Crypt	.16 ± .04 (11)	108.6 ± 13.6 (11)

ECST increased, in three min, the cGMP concentration in the villous tip cells from .24 ± .06 to 1.45 ± .50 pmol/mg prot./h ($p < .05$) whereas the cGMP concentration in the crypt cells was unchanged throughtout 20 min after the addition of ECST. The results suggest that the ECST-stimulated increase in chloride secretion is predominately regulated in the intestinal villous cells and is involved in interactions with the glucose absorptive system.

69.9

INCREASED POTENTIAL DIFFERENCE IN SWEAT DROPLETS OF CYSTIC FIBROSIS PATIENTS DUE TO DECREASED Cl PERMEABILITY. Paul M. Quinton and Jan Bijman*. Division of Biomedical Sciences, University of California, Riverside, CA 92521-0121.

Compared to controls, significantly larger potential differences (PD's) were recorded in vivo from sweat glands of Cystic Fibrosis (CF) patients, which probably are due to a decrease in chloride permeability in the CF sweat duct. Sweat secretion was induced by iontophoresis of 2% acetyl choline into an area of 0.02 cm^2 (200 μA for 5 min.) on the volar surface of the forearm. The electrical potential was measured as the difference between a microelectrode inserted into microdroplets of sweat which formed under oil and a saline agar bridge applied over an abrasion in the skin. The offset p.d. was measured by inserting the microelectrode in a drop of saline over a second abrasion at the site of stimulation. The PD and secretory rates for seven glands were recorded at 5 min. intervals in each subject studied. Independent of the sweating rates the PD in CF was always larger than in controls. Maximum recorded PD's were -67.5± 2.3 mV (n = 6) and -29.5±2.2 mV (n = 7) for CF and normal subjects respectively ($p < 0.001$; "n" = the number of subjects). Taken together with the general observation that the NaCl uptake rate is reduced in CF sweat glands, these results may be best explained by a reduced Cl permeability in CF ducts relative to normal. This work is supported by grants from Getty Oil Co., Gillette Co., and the NIH #AM 00324 and #AM 20356.

69.10

THE EFFECT OF Ba^{++} ON K^+ CONDUCTANCE ACROSS THE BASOLATERAL MEMBRANE OF TADPOLE SKIN EPITHELIAL CELLS. Stanley D. Hillyard Univ. of Nevada, Las Vegas, Las Vegas, NV 89154

The isolated skin of bullfrog tadpoles (Rana catesbeiana, metamorphic stages XVIII-XX) was mounted between Ussing chambers and bathed with 111 mM K^+ in the mucosal Ringer's while 111 mM Na^+ Ringer's bathed the serosal surface of the skin. When the ionophore-antibiotic nystatin was added to the mucosal Ringer's a short-circuit current (SCC) of 6.13 ± 0.48 μA/cm^2 could be measured across the skin. Since nystatin is known to form non-specific cation channels in cell membranes it is assumed that this SCC represents mucosal to serosal K^+ flux which is limited by K^+ channels in the basolateral membranes of the epithelial cells. This hypothesis is supported by the observation that the SCC could be completely inhibited by adding 10 mM Ba^{++}, a known blocker of K^+ channels, to the serosal Ringer's. In addition, Ba^{++} added to the mucosal Ringer's inhibited the SCC in a dose-dependent manner with complete inhibition being observed with 10 mM Ba^{++} in some preparations. The basolateral K^+ channels in this tissue can thus be blocked by Ba^{++} at either the extracellular or cytoplasmic surface of the membrane.

69.11

SODIUM TRANSPORT AT THE BLOOD-BRAIN BARRIER IN VIVO. A. Lorris Betz. University of Michigan, Ann Arbor, MI, 48109.

The brain capillary endothelium forms a continuous layer of cells that are sealed together by tight junctions. It has been proposed that this structure may contribute to production of the brain's interstitial fluid. Our laboratory has recently shown that Na^+,K^+-ATPase is located on the antiluminal and not the luminal membrane of brain capillary endothelial cells. The goal of the present study was to investigate other transport processes that might contribute to transendothelial Na^+ movement. Sodium uptake at the luminal membrane in vivo was studied using a modified single bolus injection technique in which the brain uptake of $^{22}Na^+$ was compared to that of the relatively impermeable molecule, ^{3}H-L-glucose. At a Na^+ concentration of 1.4 mM, Na^+ uptake was 1.73 ± 0.12 times greater than L-glucose uptake. This was reduced to 1.34 ± 0.08 at 140 mM Na^+, indicating saturable Na^+ uptake. Relative Na^+ extraction was not affected by pH but could be inhibited by amiloride (K_i = 3×10^{-7} M) and by 1 mM furosemide. The effects of these two inhibitors was additive. Our results suggest that there are two distinct transport systems which allow Na^+ to cross the luminal membrane of the brain capillary endothelial cell. These transport systems could play an important role in the movement of Na^+ from blood to brain. (Supported by NIH Grant HL26480).

69.12

CANINE LINGUAL EPITHELIUM HAS AMILORIDE-SENSITIVE ION CHANNELS. Sheella Mierson*, John A. DeSimone, Gerard L. Heck*, and Shirley K. DeSimone*. Department of Physiology, Medical College of Virginia, Richmond, VA 23298

The epithelium of the dorsal surface of the dog's tongue actively transports ions and contains amiloride-sensitive ion channels. This tissue was previously thought to be impermeable to electrolytes. When placed in a modified Ussing chamber with identical oxygenated solutions on both sides, the average transepithelial potential difference (PD) is 17.2 ± 2.4 mV serosa positive, short-circuit current (Isc) is 31.4 ± 6.7 μA/cm^2, and tissue resistance (R) is 576 ± 127 ohm-cm^2 (n=16). The in vitro preparation responds to salts, sugars, and acids in characteristic ways. Hyperosmotic NaCl solutions on the mucosal side cause increased PD and Isc and decreased R. The Isc is typically reduced to 0 for 30 mM NaCl solution, and increases for higher concentrations saturating between 0.5 and 1.0 M. This "hyperosmotic response" is reversible, is abolished by serosal ouabain (9×10^{-4} M), and is reduced by mucosal amiloride (10^{-4} M). If glucose or sucrose is added in increasing concentrations to 30 mM NaCl on the mucosal side, the PD and Isc increase at nearly constant R. The response to sugars is inhibited by amiloride, implying a substantial sodium current. Active currents in the presence of hyperosmotic solutions are not found in the ventral lingual epithelium. The response ranges of salts, sugars and acids coincide with those observed in gustation. (Supported in part by NIH Grants NS13767 and 5T32 HL07110)

70.1

ANTIHYPERTENSIVE EFFECTS OF KETANSERIN IN CHRONICALLY INSTRUMENTED CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS. Barbara L. Pegram*, Merrill B. Kardon and Edward D. Frohlich. Alton Ochsner Medical Foundation, New Orleans, LA 70121

Ketanserin (Ket) is an antihypertensive agent postulated to have antiserotonin and possible alpha adrenergic inhibitor properties. To assess the systemic hemodynamic effect of Ket, as well as the involvement of the adrenergic system, 7 conscious and unrestrained spontaneously hypertensive rats (SHR), previously instrumented with Doppler aortic flow probes and with iliac artery and jugular vein catheters for measurement of cardiac output (CO), arterial pressure (MAP) and drug infusion, respectively, were studied. CO, MAP, and heart rate (HR) responses to cumulative IV Ket (1-16 mg/kg) at 10 min intervals were 164±32, 85±4 and 109±2% of control, respectively, following Ket (16 mg/kg). Propranolol (1 mg/kg) given 20 min before Ket resulted in CO, MAP and HR of 93±9, 87±2 and 82±5% of control, respectively. Phenoxybenzamine (1 mg/kg; 20 min) had no effect on the responses to Ket other than obliteration of the initial rapid, but short-lived, 10-15 mmHg rise in MAP observed with higher doses of Ket. These results suggest that the hypotensive effect of Ket is produced by arteriolar vasodilation with little effect on the veins; the alpha adrenergic effect appears to be negligible. In contrast to other arteriolar vasodilators, however, the reduction in blood pressure is not accompanied by the normally expected reflex cardiac acceleration.

70.2

EFFECT OF ANTIDIURETIC HORMONE (ADH) ON VASCULAR Na^+-K^+ PUMP ACTIVITY. M. Jagusiak*, S.J. Huot, F.J. Haddy, M.B. Pamnani. Physiol. Dept., Uniformed Services Univ., Bethesda, MD 20814

The effect of ADH, 10^{-4}, 10^{-3}, 10^{-2}, 0,U of ADH/ml Krebs-Henseleit solution, on vascular Na^+-K^+ pump activity and in vitro blood vessel contractility (helical strips) was investigated using tail arteries from male Wistar rats. Ouabain-sensitive (OS) ^{86}Rb uptake, a measure of Na^+-K^+ pump activity, and ouabain-insensitive (OI) ^{86}Rb uptake, in part reflecting passive permeability, were measured. Compared to vessels not treated with ADH, the only significant differences were a decrease in OS and OI ^{86}Rb uptakes ($P<.05$, n=10) in vessels treated with 10^{-2}U/ml ADH. Contractility experiments showed that ADH (10^{-4}U/ml) produced no effect on tension, 10^{-3}U/ml caused a small but significant increase in tension whereas 10^{-2}U/ml produced a maximum tension response to ADH. The ^{86}Rb uptake study was next repeated in the presence of a vascular ADH antagonist [1(-β-mercapto-β,β-cyclopentamethylene propionic acid), 2-(methyl)tyrosine] arg^8-vasopressin (50 ng/ml) which completely blocked contractile responses. No significant difference in OS or OI ^{86}Rb uptakes were detected between ADH treated and untreated vessels. These studies show that the effect of 10^{-2}U/ml ADH on OS and OI ^{86}Rb uptakes was probably due to vasoconstriction and that ADH does not appear to directly effect vascular Na^+-K^+ pump activity. Furthermore, the suppressed Na^+-K^+ pump activity which has been observed in several low renin presumably volume dependent models of hypertension cannot be attributed to altered levels of ADH.

70.3

AGE-RELATED CHANGES IN BODY FLUID VOLUMES IN YOUNG SPONTANEOUSLY HYPERTENSIVE (SHR) AND WISTAR-KYOTO (WKY) RATS. Margaret M. Mullins. Wright State University School of Medicine, Dayton, OH 45435.

We have measured total body water (TBW, by dessication), extracellular fluid volume (ECF, $Na_2{}^{35}SO_4$ space) and plasma volume (PV, RISA space) in Inactin-anesthetized SHR and WKY aged 12 to 60 days, n=6-25 in each age-strain group. Interstitial fluid volume (ISF) was calculated as ECF − PV. Date were analyzed by analysis of covariance using a general linear model. Changes in TBW, ECF and ISF were largely a function of age (the regression coefficients of these volumes on age were not different between the strains). However, further analysis revealed that while these volumes were significantly different between the strains at ages 12 and 15 days, after 25 days no difference existed. On the other hand, there was significant difference between SHR and WKY in the slope of wet body weight versus age ($p<.001$). Further analysis indicated that there was no difference between the strains before 25 days of age, but that after that age WKY was significantly heavier than SHR. Thus, ECF is normalized in SHR and weight gain begins to slacken at the time when blood pressure becomes elevated and plasma aldosterone concentrations return to normal (Federation Proc.38: 1285, 1979). This normalization of ECF may be a result of decreased aldosterone-dependent volume retention or to diuresis induced by increasing blood pressure. (Supported in part by Miami Valley Heart Chapter, American Heart Association.)

70.4

FREQUENCY DISTRIBUTION OF RBC AND PLASMA MEAN TRANSIT TIMES IN WKY AND SHR CREMASTER MUSCLE MICROVESSELS. Carleton H. Baker and Frank R. Wilmoth.* Dept. of Physiol., College of Med. Univ. of So. Florida, Tampa, Fl. 33612

Fluorescent videomicroscopy was used to study the cremaster microcirculation of 14 WKY and 19 SHR rats. Rats were anesthetized with pentobarbital and their left cremaster muscles were spread over an optical port in a bath filled with a modified Krebs solution (ph=7.4, 34°C). The right femoral artery was cannulated for measurement of mean aortic pressure and injections of plasma indicator (FITC-dextran) or red blood cells (DTAF-RBC's). Mean arterial pressure was 85±3 mmHg for WKY rats and 110±5 mmHg for SHRs. Indicator passage was used to calculate mean transit times ($\bar{t}$) for several orders of microvessels. The $\bar{t}$ was similar for each indicator in first order arterioles. However, $\bar{t}$ for both indicators became protracted for both groups as the blood moved through successive arteriolar and venular branches. The frequency distribution of $\bar{t}$ demonstrated heterogeneity of transit routes which was different for plasma versus red cells. The distribution of mean transit times for DTAF-RBCs showed positive skewness for WKY in first, second and third order venules whereas the distribution of $\bar{t}$ for SHRs was negatively skewed in venules. These results suggest that $\bar{t}$ is protracted in SHR by a greater number of parallel circuits having lower velocities or are longer than those of WKY. (Supported by HL-18866 and Am. Heart Assn., Fl. Affiliate, Suncoast Chapter).

70.5

STUDIES ON THE TRANSITION FROM HIGH TO LOW RENIN STATE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). J.L. Cangiano, C. Rodriguez-Sargent, S. Opava-Stitzer, J. Cruz and M. Martinez-Maldonado. VA Hospital, San Juan, PR 00936.

We previously observed that plasma renin activity (PRA), plasma aldosterone concentration (PA) and renal Na^+-K^+-ATPase (ATPase) are increased in weanling SHR (5wks), while in adult SHR (16wks) PRA and PA are low, but ATPase is normal. In the present study PRA, PA and ATPase were measured in SHR during the transition from high to low renin state (8wks) and in control WKY. Extracellular fluid volume (ECFV) was measured by inulin space in SHR and WKY of both 5 and 8 weeks of age. Despite increased PRA, PA and ATPase in SHR at 5 weeks, ECFV was similar in SHR (40.3 ± 2.0 ml/100g) and WKY (43.9 ± 4.0). At 8 weeks PRA and PA had decreased in SHR (PRA: 11.8 ± 0.6-ng/ml/hr, PA: 16.8 ± 1.5 ng%) and were not different from values in WKY (PRA: 11.2 ± 1.2, PA: 15.7 ± 1.6). ATPase also decreased in SHR at 8 weeks (78 ± 8 μMPO4/mg pr/hr) and was not different from the activity in WKY (82 ± 9). Whereas PRA, PA and ATPase fell with age in SHR, no such changes were observed in WKY. ECFV was similar in SHR (33.9 ± 3.6) and WKY (36.8 ± 4.5) at 8 weeks. These results suggest that in SHR: 1) high PRA at 5 weeks is not the consequence of decreased ECFV 2) the transition from high to low renin state is not associated with a detectable expansion of ECFV 3) the decrease in ATPase with age may be influenced by aldosterone.

70.6

CENTRAL ANGIOTENSIN INVOLVEMENT IN HYPERTENSION SHOWN BY CAPTOPRIL/SARALASIN INTERACTION IN SHR. J.F. Stamler* and M. Ian Phillips. Department of Physiology, University of Florida, Gainesville, Florida 32610.

Evidence has accumulated for a role of brain angiotensin in the maintenance of hypertension in spontaneously hypertensive rats (SHR). Therapeutically, however, one requires an angiotensin blocking agent that could cross the blood brain barrier to lower brain AII activity. Captopril, an orally active converting enzyme inhibitor, appears to have that property. Captopril given i.v.t. (2 μg/2 μl saline) to adult SHR was more potent in reducing blood pressure than the same dose injected i.v. indicating its effects are partly central. Centrally injected Saralasin is known to decrease blood pressure in SHR, probably by binding to AII receptors. To test if Captopril inhibits central AII, we gave i.v.t. Saralasin and i.v. Captopril. 12 mg/kg i.v. reduced MAP from 143.2 ± 10.8 to 107 ± 8.7 mmHg. 20 μg/2 μl Saralasin alone reduced MAP 8 ± 2.34 mmHg but with Captopril this Saralasin effect was inhibited ($p<.05$ calculated on % change from baseline). Captopril did not inhibit 100 μg AII i.v.t. pressor action. Therefore, the central effect of Captopril is to reduce the amount of endogenous AII in the brain and thus less AII is available for Saralasin antagonism at the receptors. The data supports the concept of increased brain angiotensin activity in SHR contributing to their hypertension.

70.7

IMMUNOSUPPRESSION PREVENTS HYPERTENSION IN OKAMOTO SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Ali A. Khraibi*, Roger A. Norman, Jr., and David J. Dzielak*. Univ. Miss. Sch. Med., Jackson, MS 39216

Recent evidence suggests that hypertension in Okamoto SHR may be the result of an autoimmune disease. To test this hypothesis, 17 male SHR were given immunosuppressive therapy chronically, beginning at an age of 3.5 wks (cyclophosphamide, 1.5 mg/100g body wt/week). Beginning at an age of 5 weeks tail-cuff systolic pressure (SP) was monitored weekly in these 17 cyclophosphamide-treated SHR and in 14 control SHR. SP averaged 117±4.5 mmHg (mean±SE) in immunosuppressed, 5-week-old SHR compared to 120±4.4 mmHg in control SHR. By age 7 weeks, however, SP had risen to 154±3.0 mmHg in control SHR, while it averaged only 130±3.3 mmHg in cyclophosphamide-treated SHR. SP averaged 181±2.8 mmHg (n=14) in 15-week-old control SHR. However, SP in SHR given chronic immunosuppressive therapy had reached a plateau level of 143±3.0 mmHg (n=13) at this time. Average growth rate of cyclophosphamide-treated SHR was reduced. At 15 weeks of age the weight of control SHR averaged 286±3.9g, while treated SHR weighed 227±7.5g. This weight reduction may account for some of the blood pressure decrease, but control SHR were clearly hypertensive once their weight reached 130g. Chronic immunosuppression blocks development of or greatly reduces the degree of hypertension in Okamoto SHR. These data support the hypothesis that spontaneous hypertension may be an autoimmune disease. (Supported by NIH Grant HL-11678)

70.8

EFFECTS OF INCREASED CALCIUM DURING ACIDOSIS IN ISOLATED HYPERTENSIVE AND NORMOTENSIVE CARDIAC MUSCLE. K. Taubert, M. Amanam*, and T. Hair*. School of Pharmacy, University of the Pacific, Stockton, CA 95207

The effects of increasing extracellular calcium (Ca) during acidosis were examined in isolated, isometrically contracting left ventricular papillary muscles from 12 spontaneously hypertensive rats (SHR) and 12 age-matched normotensive controls (WKY). Muscles in well-oxygenated buffer containing 1.0 mMCa (control pH=7.4) were exposed to respiratory acidosis (pH 6.9), and Ca was raised to 2.5 mMCa after 1 hr acidosis. Both the max rate of tension development (+dT/dt) and the max rate of tension decline (-dT/dt) were monitored throughout the study. After 1 hr acidosis and immediately before Ca was raised, +dT/dt was depressed 22% in SHR and 30% in WKY (both $p<0.001$) while -dT/dt was decreased by 18% in SHR and 26% in WKY (both $p<0.02$). The differences in the magnitude of performance depression between the 2 groups were not significant. At the peak effect after Ca was elevated to 2.5 mM, +dT/dt was increased by 34% in SHR and 40% in WKY (both $p<0.001$), and -dT/dt was increased by 18% in SHR ($p<0.01$) and 45% in WKY ($p<0.001$). The magnitude of increase in +dT/dt was not different between SHR and WKY, but -dT/dt improved significantly more in the WKY than in the SHR ($p<0.05$). Since -dT/dt reflects the rate of Ca sequestration, these data suggest that after an increase in Ca, at least during acidosis, Ca reuptake during isometric relaxation is slower in hypertensive cardiac muscle when compared to normotensive cardiac muscle.

70.9

REPRODUCTIVE PERFORMANCE IN SPONTANEOUSLY HYPERTENSIVE RATS. Christina C. Lawrence* and Sarah D. Gray. Dept. of Human Physiology, Sch. of Med., Univ. of Calif., Davis, CA 95616

In our spontaneously hypertensive (SHR) and Wistar-Kyoto normotensive (WKY) breeding colonies we collected data on differences in reproductive capacity of the 2 strains, in order to determine pre- and postimplantation development and predict day of parturition for ongoing cardiovascular studies. Both strains had 4 day estrous cycles; however, a small but significant percent (2.4%, $p\leq.005$) of SHR cycles were 5 days long. Seven percent (4/54) of the SHR rats, but none (0/69) of the WKY, became acyclic (constant estrus) in the 11 month observation period ($p\leq.025$). Length of gestation (GL) for SHR (22.9 ± .09 days, $\bar{X}$ ± SEM) tended to be longer than that for WKY (21.8 ± .12). Litter size of SHR (7.7 ± .67) was smaller ($p\leq.05$) than for WKY (9.2 ± .41). Numbers of embryos recovered after flushing oviducts or uteri of pregnant females on day 4 or 5 of gestation were also different for the 2 strains, 7.0 ± .47 for SHR vs 9.2 ± .53 for WKY ($p\leq.005$), but we detected no difference in percentage of embryos that were degenerate or showed delayed development. These observations indicate that differences in litter size may be due to a difference in ovulation rate (OR). It is not known to what extent other characteristics were simultaneously segregated out when SHR were selectively bred for high blood pressure, but the differences in OR and GL between the 2 strains may be related to the high blood pressure itself or to differences in hormonal control. Supported by the California Heart Association.

70.10

EVOLUTION OF MALIGNANT HYPERTENSION INDUCED BY AORTIC LIGATION IN THE RAT. R.E. Chatelain*, B.N. Dardik*, C.M. Ferrario and J.R. Shainoff*. Cleveland Clinic Research Division, Cleveland OH 44106.

Aortic ligation (AL) in the rat produces either benign (BH) or malignant (MH) hypertension (Br J Exp Pathol 61:401, 1980). Three days after AL blood pressure and humoral factors (renin, aldosterone and corticosterone) are equally elevated in BH and MH rats. In MH, however, cross-linked fibrin-fibrinogen complexes are detected in the circulation while arterial lesions and focal parenchymal atrophy develop in the non-ischemic kidney above the AL. Renal damage results in increased BUN and plasma creatinine. Thus, impaired renal function appears to be the first evolutive divergency between MH and BH rats. From this period onward further elevations in humoral factors are present in MH whereas in BH, despite a sustained high blood pressure, humoral factors decrease. After 12 days, biochemical determinations of DNA, collagen and elastin in the aorta indicate marked impairments in the syntheses and contents of these elements in MH. On the contrary, in BH marked arterial hypertrophy and hyperplasia with connective tissue accumulation are observed. Thus, in MH impaired renal function and chronically elevated humoral factors may affect the vascular growth which occurs in response to high blood pressure. Defects in the arterial structure coupled with persistent intravascular coagulation may be of pathogenic importance for the development and progression of vascular disease. (Supported by the Ohio Kidney Foundation and NHLBI grant, HL-6835).

COMPARATIVE PHYSIOLOGY: CIRCULATION, MUSCLE AND LOCOMOTION II

71.1

SPLANCHNIC FUNCTION DURING VOLUNTARY DIVES IN WEDDELL SEALS. Randall Davis,* Michael Castellini,* Gerald Kooyman and Robert Maue.* Physiological Research Laboratory, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

Renal and hepatic function were studied during voluntary dives in Weddell seals by measuring the clearance rate of inulin and indocyanine green (ICG). Inulin is cleared exclusively by the kidneys and measures renal GFR. ICG is cleared by the liver and is blood flow dependent at concentrations used. Studies were conducted from a portable hut with a trap door placed over an isolated hole in the sea ice near McMurdo Station, Antarctica. An intravertebral extradural catheter was inserted percutaneously under light anesthesia in subadult seals weighing 130-200 kg. When released into the ice hole, the seals made voluntary dives, but always had to return to breathe. During studies lasting several days, foraging dives were made to depths of 250 meters. Serial blood samples were drawn after single injections of inulin and ICG and analyzed within 24 hrs. The results show a mean resting clearance rate of 2.6% · min^{-1} for inulin and 3.7% · min^{-1} for ICG. Clearance rates did not change from resting levels during short, aerobic dives, but decreased 90% during longer dives involving anaerobic metabolism. The plasma became very lipemic during aerobic feeding dives indicating that digestion was occurring. It appears that normal splanchnic function continues during natural aerobic dives. (Supported by NSF DPP79-23623 and USPHS HL 17731.)

71.2

ADRENERGIC EFFECTS ON TRANSMEMBRANE ACTION POTENTIALS RECORDED IN THE TELEOST HEART. John S. Cameron, Alphonse DeLucia, III* and A.L. Bassett. Univ. of Miami School of Medicine, Miami, FL 33101 and Albany Medical College, Albany, NY 12208.

Cellular electrophysiologic properties and adrenergic responses of atrial and ventricular muscle fibers from goldfish (Carrassius auratus; 1.5-4.5 g) were monitored using standard glass microelectrodes. Isolated, saline-superfused hearts were exposed to the sympathomimetic agonists isoproterenol (ISO), epinephrine (E), norepinephrine (NE) and phenylephrine (PHE) at concentrations of 10^{-8} to 10^{-4}M. At 22°C, these agents induced dose-dependent increases in spontaneous rate of depolarization, action potential amplitude (APA) and resting potential (RP) with relative potencies: ISO>E>NE>PHE; action potential duration at 90% repolarization (APD_{90}) was significantly shortened. ISO also increased the slope of phase 4 depolarization recorded in pacemaker fibers of the sinus venosus. Verapamil-sensitive delayed afterdepolarizations were observed in all parts of the heart; their relative incidence and amplitude were enhanced by ISO, E and NE. All responses were qualitatively similar in atrium and ventricle, although control values (mean±SE) for APA (83±3 mV), RP (64±2 mV) and APD_{90} (294±12 msec) were greatest in the latter chamber. Adrenergic affects were antagonized by propranolol (10^{-6}M), unaffected by phentolamine (10^{-6}M) and mimicked by low concentrations of nicotine and tyramine (10^{-7} to 10^{-5}M). These data indicate the presence of excitatory, β-adrenergic receptors in teleost myocardium, and suggest their electrophysiologic similarity to those of other vertebrates.

71.3

ADAPTATION TO HYDROSTATIC-PRESSURE-INDUCED INHIBITION OF ACTIN SELF-ASSEMBLY. Robert R. Swezey and George N. Somero, Scripps Institution of Oceanography, La Jolla, CA. 92093

Polymerization of skeletal muscle actin to thin filaments occurs with increases in both the entropy and the enthalpy of the system. Such entropy-driven processes are believed to result when hydrophobic interactions are the dominant forces involved with subunit binding. Physical chemical considerations suggest that entropy-driven reactions proceed with an increase in the total volume of the system, and will thus be inhibited by the application of hydrostatic pressure. Previous comparative studies showed that species adapted to different thermal and pressure regimes had varied their polymerization thermodynamics such that deep-sea and cold-water fishes had the lowest enthalpy and entropy values. These findings suggest that assembly of actins from the deep-sea and the cold-water fishes will be less affected by high pressures, and indeed polymerization of a deep-sea rattail fish (Coryphaenoides armatus) actin showed no significant pressure sensitivity up to 400atm (the normal physiological pressure for this species), whereas actin from chicken muscle was dramatically depolymerized at lower pressures.

71.4

SCALING MAXIMAL RUNNING SPEED AND MAXIMAL AEROBIC SPEED TO BODY MASS IN MAMMALS AND LIZARDS. Theodore Garland, Jr.* (SPON: A.F. Bennett) Univ. of California, Irvine, CA 92717

The available data on maximal running speeds (MRS) and maximal aerobic speeds (MAS = treadmill speed at which $\dot{V}O_2max$ is attained) of mammals and lizards are compared using analysis of covariance. Among 106 species of mammals (0.016-6000 kg), the scaling exponent for MRS is 0.17 ± 0.04 (± 95% confidence interval). MRS's of mammals average less than 3X greater than MAS's. Among 35 species of lizards (0.0013-50 kg), MRS scales with an exponent of 0.09 ± 0.06, and MRS's average 21X greater than MAS's. MRS's of mammals and lizards are similar, but MAS's of mammals average 10X greater than those of lizards. A more detailed analysis indicates that there is a curvilinear relationship between log MRS and log body mass among mammals, and a second degree polynomial fits the data significantly better than a simple linear regression. With respect to MRS, an optimal body mass of 119 kg is predicted, at which MRS is 56 km/h. Within orders of mammals (Artiodactyla, Carnivora, Rodentia) and within families of lizards (Iguanidae, Scincidae, Teiidae) MRS is mass independent. Differences in running ability among these orders and families are also apparent. In conclusion, running ability does not scale with an exponent of 0.25, as might be expected if animals were designed for elastic similarity. Taken as a whole, however, the data are consistent with theoretical expectations for geometrically similar animals. (Supported by N.S.F. Grant PCM81-02331 to A.F.B.).

71.5

ACTIVITY PHYSIOLOGY AND BEHAVIORAL CAPACITY OF TWO CONGENERIC LIZARDS. Albert F. Bennett, Raymond B. Huey, Henry John-Alder, & Kenneth A. Nagy*. U. Calif. Irvine, Irvine CA 92717; U. Washington, Seattle WA 98195; UCLA, Los Angeles CA 90024

Physiological and behavioral capacities of two lizards (Eremias lineoocellata and E. lugubris) that differ markedly in natural activity patterns were studied. E. lineoocellata, a sit-and-wait predator, has a greater initial velocity (1.29 vs 1.02 m/sec), burst speed (2.63 vs 1.76 m/sec), anaerobic scope (2.56 vs 2.12 mg lactate $g^{-1}h^{-1}$), and anaerobic capacity (1.81 vs 1.40 mg lactate/g). E. lugubris, a widely-foraging lizard, has greater endurance (>20 vs 7.4 min @ 0.5 km/h) and distance capacity (70.8 vs 29.9 m/15 min). It also has a greater $\dot{V}_{O_2max}$ (3.22 vs 2.49 ml O_2 $g^{-1}h^{-1}$), aerobic scope (2.98 vs 2.24 ml O_2 $g^{-1}h^{-1}$), hematocrit (30.1 vs 24.4%), and heart mass (0.28 vs 0.24% body mass). Muscle mass, enzymatic activity, myoglobin content, and contractile properties are not different between the species. Organismal physiological and behavioral capacities accord with natural activity patterns, but the structure and physiology of the skeletal muscle do not. (Supported by NSF Grants DEB81-09667 and PCM81-02331 and NIH K04 AM00351)

71.6

RHYTHMS OF MOVEMENT OF THE ELECTRIC FISH *GYMNOTUS CARAPO* UNDER INFLUENCE OF LIGHT AND TEMPERATURE. Fernando Pimentel-Souza, Mauro Schettino and Rodolfo Lautner-Jr. ICB, CP. 2486, Universidade Federal de Minas Gerais, 30.000 - Belo Horizonte, BRASIL.

How its movement varies under different illumination and must be influenced by season or temperature in Brazil? Direct observations have been used in lab and checked many times by a degree of accordance. *G. carapo* prefers illumination, that is so low as 0.01 lux and whose magnitude is checked in field. So, light variation in different lunar cycles might influence its vertical migration. In circadian periods the activity of the fish in group increases a little before sunset, decreasing only after the sunrise. But, when isolated, their movements increases only around sunset. Daily movement, when in group, has significant increase under continuous darkness and a decrease under continuous light. Under these last conditions, soon there is no more difference between diurnal and nocturnal activities, showing that endogenous rhythm lasts only a few days under continuous illumination. Thus, there is an absolute and differential influence of illumination. In relation to temperature it is observed a greater amount of movement in colder months and more aggressions in hotter months. (Supported in part by CNPq and FINEP).

COMPARATIVE PHYSIOLOGY: OSMOTIC AND IONIC REGULATION II

72.1

AMINO ACID UPTAKE INTO BIVALVE GILLS AND THE NA^+ GRADIENT HYPOTHESIS. S.H. Wright*, D.T. Manahan*, and G.C. Stephens. Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024, and Developmental and Cell Biology, U.C. Irvine.

Gills of marine bivalves can transport free amino acids (FAA) from external solution into large intracellular pools of FAA. This transport has been suggested to be a "coupled" process, energized by a Na-gradient. Calculations of the adequacy of the Na-electrochemical gradient in gill tissue to drive this transport were made, based on FAA concentration gradients against which net transport took place. Intact specimens of Mytilus edulis were placed in artificial seawater containing 16 FAA, 100 nM each. After one hour the concentration of each FAA was significantly reduced, as determined by High Performance Liquid Chromatographic (HPLC) analysis of medium samples. For example, the levels of glycine and alanine were reduced to 11 and 10 nM, respectively. In separate studies, the FAA pool in excised gills was determined; total pool concentration was approximately 93 mmoles/kg wet wt., with glycine and alanine concentrations of 16 and 8 mmoles/kg. We conclude that net transport of these substrates can occur against chemical gradients on the order of 10^6:1. Given an activity gradient for Na of approximately 20 ($320\ mM_{out}$:$15\ mM_{in}$), and a potential difference across the brush border of the gill of -60 mV, the Na-gradient hypothesis requires that a minimum of 4 Na ions be coupled to the transport of each molecule of glycine and alanine. Supported by NSF #'s PCM 78-09576, OCE 78-09017, and NIH # AM 19567.

72.2

CHANGES IN INTRACELLULAR ION CONCENTRATIONS, OXYGEN CONSUMPTION AND ULTRASTRUCTURE IN THE GILLS OF CRABS ACCLIMATED TO VARIOUS SALINITIES. I. Percy Zanders and María-José Martelo*, IVIC, CBB, Caracas 1010A, Venezuela.

The mangrove crab Goniopsis cruentata keeps its blood ion concentrations relatively constant when exposed to diverse seawater concentrations, ranging from 150 to 25% SW; as the gills constitute the main route for ionic exchanges between blood and medium, in this study we examined the effects of changes of external salinity on gill intracellular ionic levels, oxygen consumption and morphology. The intracellular space is three times greater in gills from 25% SW than in those from 150% SW; the intracellular Na^+, K^+ and Cl^- concentrations are proportionally smaller in the former than in the latter, the intracellular contents of these ions remaining roughly constant. The oxygen consumption is 30-40% higher in gills from 25% SW than in those from 100 or 150% SW. The gills and their epithelium, which consists of mitocondria-rich cells showing abundant apical membrane infoldings, show marked changes in their structure, in relation to external concentration; in 150% SW the gill tissue appears compact, without vacuoles, and shows dense arrays of tonofilaments or microtubules, which are absent from gills in 25% SW. In this latter medium, the gills swell and the infoldings increase in size and number, as do vacuoles in the cytoplasm. These histological changes correlate well with the observed physiological changes, suggesting that they may be involved in the efficient maintenance of constant blood ionic levels in this crab.

72.3

THE APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE STUDY OF AMINO ACID TRANSPORT IN A MARINE BIVALVE. D. T. Manahan*, S. H. Wright*, G. C. Stephens. University of California, Developmental and Cell Biology, Irvine, CA. 92717 and Department of Physiology, UCLA School of Medicine, Los Angeles, CA. 90024.

High performance liquid chromatography was used to measure the simultaneous accumulation of 16 amino acids from seawater into the mussel, Mytilus edulis. Specimens were placed in artificial seawater containing each amino acid at a concentration of 125 nM. The substrates included those amino acids commonly found in seawater (e.g., Asp, Glu, Ser, Gly, Ala). Medium samples were taken periodically for 1 hr. In all cases the concentration of each amino acid declined throughout the time-course of the experiment, down to concentrations as low as 5 nM. Influx of 4 amino acids was compared to net flux. In each case the influx of ^{14}C-labelled substrate, determined from the removal of isotope, accurately represented the net flux of that substrate as measured by direct chemical analysis. Rates of uptake of individual amino acids from concentrations of 100 nM ranged from 2.4 to 7.0 nmol (g dry flesh wt)$^{-1}$ min^{-1}. These studies show that M. edulis is capable of net uptake of dissolved amino acids from the very low concentrations characteristic of natural seawaters. This work was supported in part by NSF Grant PCM 78-09576.

72.4

ISOLATION OF BASOLATERAL AND APICAL MEMBRANES FROM EPITHELIAL CELLS OF CRAB GILL. David W. Towle, Cheryl A. Fletcher*, and Robert D. Fanelli*. Univ. of Richmond, Richmond, VA 23173

Sodium/ammonium exchange across crab gills may be regulated via control of basolateral NaK-ATPase pumping activity or apical sodium entry. In an effort to distinguish between these two possibilities, basolateral membranes (identified by NaK-ATPase activity) and apical membranes (identified by labelling whole gill with diazotised I-125 iodosulfanilic acid) were separately isolated from portions of gill lamellae rich in ion-transporting cells. Basolateral membranes, isolated by 20-40% sucrose density gradient centrifugation, were completely separated from apical membranes, and were shown to be essentially free of mitochondrial or lysosomal contamination. Apical membranes, isolated by 40-60% sucrose density gradient centrifugation, were enriched in alkaline phosphatase activity. Apical membranes also showed specific binding of the apical sodium transport inhibitor amiloride, determined by a new fluorescence binding assay. (Supported by Univ. of Richmond Faculty and Undergraduate Research Programs and by the Dickinson Memorial Research Award.)

COMPARATIVE PHYSIOLOGY: TEMPERATURE ADAPTATION AND ENERGETICS II

73.1

COMPARISON OF DOUBLY LABELED WATER AND TIME-ACTIVITY ESTIMATES OF AVIAN ENERGETICS. W. A. Buttemer, K. A. Nagy*, and W. W. Weathers. University of California, Davis, CA 95616

We estimated daily energy expenditure (DEE) of six free-ranging Budgerygahs (Melopsittacus undulatus) simultaneously with $^{3}HH^{18}O$ and a sophisticated time-activity-laboratory (TAL) model that incorporated continuous recordings of the bird's behavior (via a microprocessor) and known activity costs with careful assessment of the bird's thermal environment (based on operative temperature, Te, measured with unheated taxidermic mounts). In addition to Te, the following parameters were monitored at ten-minute intervals throughout the 24-hour cycle: wind speed, air temperature profile, and radiational fluxes and intensities. Our TAL estimates of DEE averaged 15 percent lower than $^{3}HH^{18}O$ estimates (80.4 versus 98.0 kJ/day). The discrepancy between the two methods apparently was due to the failure of Te, as measured with unheated taxidermic mounts, to fully account for convective heat exchange. (Supported by NSF grants PCM76-18314 and DEB80-22765, and U.S. Dept. Energy Contract DE-AM03-76-SF00012)

73.2

TROUT LIVER PHOSPHOLIPASE A_2 AND ITS ROLE IN MEMBRANE ADAPTATIONS TO TEMPERATURE. N.P. Neas* and J.R. Hazel. Ariz. State Univ., Tempe, AZ 85287.

Membrane phospholipids of rainbow trout undergo substitutions in fatty acyl components at different temperatures. It is unclear whether changes are due to a phospholipase-acyltransferase pathway or de novo synthesis of phospholipids with appropriate fatty acid composition. This study substantiates the presence of phospholipase A_2 (PLA_2), implicated in the former pathway, in liver microsomal membranes from both 20° and 5°-acclimated trout (WA and CA, respectively). PLA_2 removes a fatty acid from carbon #2 of a diacylphospholipid. The enzyme was assayed using a radiolabeled substrate of 1-acyl, 2-(^{3}H)-oleoyl phosphatidylcholine. Trout liver PLA_2 was stimulated by 0.1% Triton X-100 and exhibited Ca^{2+} dependence. About 20% of the total cellular activity was microsomal. Both WA and CA enzymes had a pH optimum of 8.0 when assayed at 20° and 9.0 when assayed at 5°. Below saturation, PLA_2 from CA fish had higher activity than WA fish when assays were done at either 5 or 20° (normal compensation). At saturating levels of substrate (Vm), activity was highest when measured in WA trout (WA=94.21; CA=12.18 nM [mg-hr]$^{-1}$). Km was independent of assay temperature, but elevated in WA fish (2463.01 µM) relative to CA fish (330.29 µM). These data indicate the possible contribution of this enzyme and the deacylation-reacylation pathway in membrane restructuring that occurs as a result of thermal acclimation. (Supported by NSF PCM#8003454).

73.3

METABOLIC CORRELATES OF WINTER ACCLIMATIZATION IN THE AMERICAN GOLDFINCH (CARDUELIS TRISTIS). William R. Dawson and Marshall E. Yacoe. Univ. of Michigan, Ann Arbor, MI 48109

Cold resistance of American goldfinches is significantly increased during winter in northern U.S. This involves a form of metabolic acclimatization featuring winter fattening and improved capacities for sustaining muscular thermogenesis at low T_a. To analyze further these improved thermogenic capacities, we have determined the activity at different seasons of three enzymes in the pectoralis muscles: phosphofructokinase (PFK), β-hydroxyacyl CoA dehydrogenase (HOAD), and citrate synthase (CS). The activities of PFK and CS do not differ significantly between winter and summer, implying essentially constant glycolytic and oxidative capacities. The latter are confirmed by the essentially constant oxygen uptake of muscle homogenates in the presence of exogenous substrates. On the other hand, HOAD shows significantly higher specific activity (mean + s.e. in µmoles·g muscle^{-1}·min^{-1} at 25°C) in winter (winter, 91.5 ± 9.6 vs summer, 43.8±4.3; $P < 0.001$), indicating enhanced capacity for β oxidation. This and competition experiments involving mitochondrial uptake of pyruvate in the presence of palmitoyl-L-carnitine suggest a potential mechanism in the American goldfinch for the preferential use of fatty acids as substrates in cold defense, as well as for conserving glucose. (Supported in part by NSF Grant DEB 80-21389).

73.4

THERMOREGULATORY BEHAVIOR OF COTTON RATS: PRIMARILY CONTROLLED BY SURFACE RATHER THAN CORE TEMPERATURE. Henry D. Prange and Polley A. McClure.* Medical Sciences Program/Physiology Section and Department of Biology, Indiana University, Bloomington, IN 47405

The core (visceral) and surface (subcutaneous) temperatures of cotton rats (*Sigmodon hispidus*) were monitored by radiotelemetry. The animals were allowed to move freely between cages placed in temperature-controlled chambers of 6°C and 46°C. The mean surface temperatures at which the animals exited the hot cage for the cold and the cold cage for the hot were 38.1°C and 33.8°C, respectively. Mean core temperatures of 38.5°C and 38.3°C at these exits were not significantly different. In many cases the core temperature was observed to change in a direction opposite to that suggested by the behavior: it was increasing as the animal moved into the hot cage or decreasing as entry into the cold cage occurred. In no case was physiological thermoregulation (shivering, salivation) observed. We conclude from these observations that behavioral thermoregulatory responses of this small mammal are primarily controlled by its surface temperature and that the limits for this mechanism lie within those for physiological thermoregulation. (Supported in part by a Biomedical Research Grant and by USPHS NIH Grant HD 13953)

73.5

POSSIBLE SIGNIFICANCE OF THE Δ9 AND Δ6 DESATURASES IN THE ACCLIMATION OF RAINBOW TROUT TO COLD TEMPERATURES. A.F. Hagar and J.R. Hazel. Ariz. State Univ., Tempe, AZ 85287

Probably the most ubiquitous response of poikilothermic organisms to a decrease in environmental temperature is an increased degree of unsaturation of the fatty acyl moities of membrane phospholipids. This change in membrane structure could be accomplished either by increasing the proportion of unsaturated fatty acids in the cellular free fatty acid pool or by selectively incorporating more of the highly unsaturated fatty acids. In this study the activities of the Δ9 and Δ6 desaturases from liver microsomes of 5^0 and 20^0 acclimated trout (CA and WA, respectively) were investigated. The enzymes were assayed by gas-liquid chromatography using 1-(^{14}C)-stearate and 1-(^{14}C)-linoleate, respectively. All assays were performed at 20^0 using 3 mg/ml of microsomal protein. The pH optima were determined to be 7.0 for the Δ9 desaturase and 6.8 for the Δ6 desaturase. The activity of both enzymes from both acclimation groups increased with increasing substrate concentration over the range of 100-500 μM. Whereas the Δ9 desaturase from WA fish was three times as active as that from CA fish (2.0 vs 0.6 nmoles·min^{-1}·mg protein^{-1}), acclimation temperature had no effect upon the activity of the Δ6 desaturase (v_o=≈0.3 nmoles·min^{-1}·mg protein^{-1}). The difference in the temperature dependency of the two desaturases may reflect an enhanced ability of CA fish to produce polyunsaturated fatty acids, thereby increasing the unsaturation index of the cellular free fatty acid pool. (Supported by NSF PCM#8003454)

73.6

A COMPARISON OF COSTS OF GROWTH AND MAINTENANCE IN DEVELOPING REPTILIAN AND AVIAN EMBRYOS. D.F. Hoyt, D. Roberts* and W. Kennedy*, Calif. State Polytechnic Univ., Pomona, CA, 91768

We tested four hypotheses: 1) different patterns of increase in O_2 consumption seen during development of embryos of different organisms result from different patterns of growth; 2) the O_2 consumed provides energy for growth and maintenance; 3) costs of growth (cal/g) are independent of rate of growth, and: 4) differences in rates of O_2 consumption between avian and reptilian embryos are only due to differences in incubation temperatures. Rates of O_2 consumption, mass and growth rate were determined for embryonic turtles (Chrysemmys concinna) and quail (C.coturnix). Costs of growth and maintenance are assumed to be equal to the regression coefficients obtained from multiple regressions of rate of O_2 consumption on growth rate and the three-quarters power of embryo mass, respectively. The fact that all regression coefficients are significantly different from zero supports hypotheses one and two. The costs of growth are the same for both species, inspite of a three-fold difference in length of incubation and growth rate. After correcting for differences in incubation temperature (Q_{10} assumed equal to 2.5) the rate of expenditure of energy on maintenance in the quail is about four-times that in the turtle. Thus, differences between the two taxa in rates of embryonic metabolism are only partly due to differences in incubation temperature. (Supported in part by NSF grant SPI 8026274).

73.7

TURTLE EGGS DEVELOP AND HATCH WHEN BURIED IN DRY SOIL. R.A. Ackerman, A. Ar[1], I. Sidis*[1] and A. Gasith*[1]. Iowa State Univ., Ames, Iowa, 50011 and [1]Tel Aviv Univ., Tel Aviv 69978.

The hard-shelled eggs of the pond turtle, Mauremys caspica are deposited in clutches of 7±1, in sphere-like nests excavated in soil (20-30°C) of water content from 0.1 to 20%, above maximum water level. Eggs have less contact with the substrate in wet soils than in dry soils where nest structure may break down. We incubated freshly laid eggs (10.7g±1.4SD) at Ti=20-33°C, buried individually in sand of constant water contents (0.1-10%H_2O). We measured hatchability, mass changes, incubation duration (I), hatchling mass (Mh), and shell water vapor conductance in air (GH_2O). I(days) correlates with Ti (°C):(I-72)·(Ti-20)=254(°C·day), where 254(°C·day) are needed to complete incubation. Eggs lost water only below 0.5%H_2O in substrate. Overall water loss measured in 0.1%H_2O ranged from 12% at 24°C to 18% at 33°C. Mortality increased below 24°C and above 30°C. Water content did not affect I or Mh(6.7 g±1.1SD). In sand of 2% H_2O or more, egg shells ruptured during incubation from water uptake and embryos died. GH_2O (mg·day^{-1}·torr^{-1}) was altered by hydration from 24(±12SD) in 0.1% H_2O to 58(±26SD) in 0.5%H_2O. Observed ΔPH_2O across the shell in 0.1% H_2O was 0.8torr±0.3SD, indicating that even dry soil impedes water vapor and gas transport. Eggs of Mauremys may be protected from dehydration by direct contact with dry substrates and from overhydration and asphyxia by nest geometry in wet substrates. Supported by US-Israel BSF #2407/81.

73.8

THEORETICAL ANALYSIS OF OXYGEN TRANSPORT AND DELIVERY DURING HYPOTHERMIA. D.C. Willford, E.P. Hill and W.Y. Moores.* UCSD and VA Hospital, La Jolla, CA 92093

With decreasing temperature, P_{50} (the P_{O_2} at 50% saturation) decreases, thereby potentially interfering with oxygen off-loading to tissues. On cooling from 37°C. to 25°C. at pH 7.4, the P_{50} decreases from normal 26.8 torr to 13.2 torr. Although oxygen consumption ($\dot{V}O_2$) decreases to about 39% of normal, cardiac output ($\dot{Q}$) decreases by almost the same degree, so that the arteriovenous O_2 content difference ($Ca-Cv=\dot{V}O_2/\dot{Q}$) remains constant at 5 ml/dl. Using the Hill equation with n=2.8 and P_{50}=13.2, we calculate that the PvO_2 must drop from normal 40 torr to 26.8 torr to supply 5 ml/dl to the tissues. Clinically induced hypothermia is usually accompanied by hemodilution to 50% normal hematocrit which would reduce the PvO_2 to 13.7 torr. Use of the 'ectothermic' acid base strategy (pH = 7.58 at 25°) further reduces the P_{50} to 10.8 torr and the PvO_2 to 10.9 torr. The effect of decreased P_{50} on oxygen transport would be partially offset by giving 100% oxygen (increasing PaO_2 to 500 torr and PvO_2 to 16.9 torr) and totally offset if $\dot{Q}$ were maintained at normal prehypothermic levels (PvO_2 = 41.5 torr). We conclude that significant mismatching could occur between hemoglobin O_2 delivery and tissue needs in some cases and that giving 100% O_2 does not guarantee normal tissue oxygenation, particularly in tissue beds with higher than normal oxygen extraction, or if regional blood flows are inadequate. (Supported by NIH and VA grants 5-T32-HL-07212, HL 17731 and KOH-HL-00418)

73.9

LIQUID VENTILATION:A METHOD OF INDUCED HYPOTHERMIA, D.L. Forman*, M.R. Wolfson*, T.H. Shaffer, Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140.

The physiological effects of hypothermia induced by liquid ventilation were studied in nine adult cats. The animals were stabilized on mechanical gas ventilation with 100% oxygen during a control period and then cooled for one hour using mechanical ventilation with liquid fluorocarbon. Fluorocarbon (RIMAR 101) liquid temperatures of 10°C, 20°C and 30°C were used in cooling the animal while rectal and subcutaneous body temperatures were measured. Three cooling rates of 9°/hr, 8°/hr and 4°/hr respectively, were produced while maintaining effective physiological gas exchange (Mean PaO_2 = 268±17SEM mmHg, mean $PaCO_2$ = 35±2SEM mmHg). Measurement of cardiovascular parameters after forty minutes of hypothermic liquid ventilation were found to be significantly altered. Cardiac output decreased 52±8SEM %, oxygen consumption decreased 48±6SEM %, heart rate decreased 47±1SEM % and mean blood pressure decreased 34±5SEM % as compared to control values. Decreases in cardiac output and stroke volume have previously been reported during normothermic liquid ventilation. Furthermore, similar results have also been reported for cardiac output, oxygen consumption, heart rate and mean blood pressure during hypothermia induced by other methods. The data presented here suggest that the physiological effects of liquid ventilation and hypothermia are similar and that this method may provide a viable alternative for rapid induction of hypothermia. (Supported in part by grant no. HL22843, and 5T32HL0741403).

74.1

LEFT VENTRICULAR PERFORMANCE DURING SPLANCHNIC ARTERY OCCLUSION SHOCK. Roy D. Goldfarb, Walter P. Tambolini* and Peter B. Weber*. Albany Medical College, Albany, NY 12208

Left ventricular contractile performance following splanchnic artery occlusion (SAO) shock was studied. We evaluated contractile performance by analyzing the left ventricular end systolic pressure-diameter relationship (Σ_{ES}). We have previously shown that Σ_{ES} is independent of large changes in preload, afterload, and heart rate but sensitive to changes in ventricular contractility. Following release from 2 hrs of SAO, 2 dogs survived and 6 expired immediately or prior to 4 hrs (termed "nonsurvivors", NS). The NS dogs exhibited a slight tachycardia, slight increase in total peripheral resistance, marked decreases in +dP/dT, -dP/dT, cardiac output, arterial blood pressure, stroke volume and stroke work. Ventricular performance (Σ_{ES}) was significantly depressed (50-70% of control) at all times following SAO release. In contrast, the surviving animals exhibited only a slight reduction in Σ_{ES} which was followed by a restoration to pre-SAO value. The dogs which expired immediately following SAO release exhibited a precipitous decline in Σ_{ES} from 41.3 $\pm$ 9.8 to 23.0 $\pm$ 4.9 mmHg/mm within minutes of SAO release. These results suggest that cardiovascular collapse of SAO shock is associated with an early and sustained loss of ventricular contractility. (Supported by grants from the National Institutes of Health HL-19977, GM-00947; and the Heart Association of Northeastern New York)

74.2

Cardiovascular failure following Puff adder (Bitis arietans) venom infusion in rats. R.C. Schaeffer,Jr. S-M. Chilton*, P. Teerapong*,T. Hadden*and R.W. Carlson. Depts. Med., Mt. Carmel Mercy Hospital and Wayne State University, Detroit, MI 48235

Male Sprague-Dawley rats (n=35,275-325g) in groups of 7 animals each, were given an iv infusion (30 min, 1 ml of 0.9% NaCl) that contained 3.0,2.5, 2.0 or 1.5 mg/kg of Bitis arietans venom. Another group (C) received 1 ml of 0.9% NaCl that contained no venom. Survival at 24 hrs was 7/7 for the C group 0/7 for 3.0; 1/7 for 2.5; 2/7 for 2.0 and 5/7 for 1.5 mg/kg groups, respectively. The magnitude of the hemodynamic, respiratory and metabolic effects correlated with the dose of venom. Venom (3.0 mg/kg) led to arterial hypotension (117±2 to 57±5 mm Hg, $\bar{x}$ ± S.E.M.) by end infusion (E) that persisted for 240 min or death. Arterial blood lactate slowly increased (1.2±0.1 to 4.7±0.4 mM,$p<0.01$) by E+240 min. Hematocrit initially increased (46±1 to 52±1%, $p<0.05$) but then declined to 39±1% by E+240 min. Estimated total protein decreased (6.0±0.1 to 4.7±0.1g%). Hemorrhages of the gut, myocardium and abdominal musculature were seen at necropsy. However, the modest hemodilution suggests that factors in addition to hypovolemia contribute to death. (Supported, in part, by MCREC grant #223-241)

74.3

COMPARISON OF VASOPRESSIN ANTAGONISM ON BLOOD PRESSURE IN INTACT AND CEREBELLECTOMIZED DOGS IN HEMORRHAGIC SHOCK. E. L. O'Leary*, B. C. Lutherer*, C. H. Chen, and L. O. Lutherer. Dept. of Physiology, Texas Tech Univ. HSC, Lubbock, TX 79430.

Our recent studies showed that bilateral lesions of the fastigial nucleus (FN) of the cerebellum prevents recovery of mean arterial pressure (MAP) normally seen in dogs after hemorrhagic hypotension. Others have now shown that stimulation of the FN increases secretion of vasopressin (AVP), which is important in MAP maintenance after hemorrhage. Therefore, we compared the relative effect of an AVP antagonist on the MAP response after hemorrhage in shamp-operated (SO) and cerebellectomized (Cx) dogs to determine the influence of the FN on AVP secretion under these circumstances. Dogs were bled (1 ml/min/kg) until MAP reached 50 mmHg and then observed for 180 min. SO dogs showed a linear increase in MAP during the first 45 min after hemorrhage (recovery phase) and then maintained this level of MAP from 45-180 min (maintenance phase) without any deaths. Treatment of intact dogs with a specific AVP antagonist 15 min before hemorrhage with hourly supplements did not change the initial rate of recovery of MAP but reduced the level of maintenance. Two of five dogs died. The MAP of Cx dogs was decreased in both phases, and all died. Treatment of Cx dogs with the AVP antagonist did not produce any further deficit in MAP response above that seen with Cx alone. These results confirm role of AVP in MAP maintenance after hemorrhage and suggest that much of the AVP response involves cerebellar output.

74.4

CARDIOVASCULAR COMPENSATORY RESPONSES IN RATS ANESTHETIZED WITH PENTOBARBITAL COMPARED TO CHLORALOSE-URETHANE. Robert F. Bond. Oral Roberts University, Tulsa, OK 74171

The purposes of this study were to evaluate: 1) a rat hemorrhagic shock model; and, 2) the cardiovascular compensatory responses under two anesthetizic regimes. Prior to hemorrhage, rats were anesthetized to Stage II plane 2 by i.p. administration of either 40 mg/Kg pentobarbital sodium (R_p) or a combination of 80 mg/Kg chloralose and 400 mg/Kg urethane (Rc-u). A two-step hemorrhagic shock protocol was employed which permitted two rats, one from each group, to be simultaneously bled to a mean arterial pressure (MAP) of 60 mmHg and 30 mmHg. Both were held at 30 mmHg by controlled bleeding and reinfusion until one of the pair decompensated to the point of 20% spontaneous uptake. The prehemorrhage control MAPs were higher in the R_p group (109±8 mmHg) than in the Rc-u group (86±7 mmHg); however, the blood loss necessary to lower MAPs to 60 mmHg and 30 mmHg were significantly higher in R_p (6.8±.9 ml/Kg and 12.6±1.4 ml/Kg) than in Rc-u (2.3±.4 ml/Kg and 9.2±.8 ml/Kg). In addition, the maximum shed volume during compensation at 30 mmHg MAP was significantly higher in R_p (23.1±.8 ml/Kg) when compared to Rc-u (17.6±.8 ml/Kg). In each of the 10 sets of experiments the R_p decompensated to 20% uptake before Rc-u. These data suggest that rats anesthetized with pentobarbital are better able to compensate for blood loss, but were less able to sustain this compensatory effort than the Rc-u group. (Supported by Oral Roberts University research funds.)

74.5

SKELETAL MUSCLE LACTATE/PYRUVATE METABOLISM DURING HEMORRHAGIC SHOCK IN THE DOG. T.E. Emerson, Jr., R.M. Raymond and D.A. Gibbons*. Mich. State Univ., East Lansing, MI 48824.

Previous data from this laboratory demonstrated that skeletal muscle glucose uptake increases initially in hemorrhagic shock and then returns to near control. Five dogs were anesthetized with Nembutal, anticoagulated with heparin, and ventilated spontaneously. The isolated, innervated, natural flow gracilis muscle preparation was used. Arterial and muscle venous [lactate], [pyruvate], [glucose] and other variables were determined before and hourly for 4 hrs. Arterial blood pressure was decreased to 50 mmHg by rapid hemorrhage and maintained for 4 hrs. Hemorrhagic shock caused a severe decrease in muscle blood flow, increase in muscle vascular resistance and local muscle hypoxia. As in our previously reported study, muscle glucose uptake increased initially following hemorrhage, peaked at 2-3 hrs of shock, and returned to near control level by the 4th hr. Changes in muscle venous plasma lactate/pyruvate ratio closely paralleled the changes in muscle glucose uptake. In general, changes in muscle lactate and pyruvate production paralleled changes in the lactate/pyruvate ratio, but there was some inconsistency between muscles and these data were not clear-cut. This preliminary study lends support to the hypothesis that the muscle venous plasma lactate/pyruvate ratio exhibits changes similar to changes in muscle glucose uptake during hemorrhagic shock. (Supported by NIH grant GM-26 394).

74.6

INSULIN RESISTANCE AND MAINTENANCE OF ENDOTOXIN'S "INSULIN-LIKE" AFFECT IN PROMOTING SKELETAL MUSCLE GLUCOSE UPTAKE DURING ENDOTOXIN SHOCK IN THE DOG. R.M. Raymond AND G.A.Rosenfeld* Dept. of Surgery and Physiology, Loyola University Medical Center, Maywood, IL 60153 and The Veterans Administration Hospital, Hines, IL 60141

Several reports have documented the insulin-like affect of endotoxin in promoting glucose uptake and/or oxidation by non-shocked skeletal muscle and adipose tissue. However, it is unclear whether endotoxin maintains its insulin-like activity during states of circulator shock. The present study was undertaken to clarify this point. Mongrel dogs of either sex weighing 20$\pm$ kg were anesthetized with pentobarbital sodium, anticoagulated with sodium heparin, intubated and allowed to breathe spontaneously. The isolated, innervated, constantly-perfused gracilus muscle was used. Drying of the muscle was prevented by bathing it with mineral oil and covering it with plastic wrap. Temperature of the muscle was maintained at 38°c. Shock was induced by a 5 minute I.V. infusion of either E. coli or S. enteritidis endotoxin (1mg/kg). Following shock induction, local intraarterial infusion of insulin (382 mU/min) or endotoxin (.382 mg/min) resulted in a 304% and 136% increase in muscle glucose uptake, respectively. Following one hour of shock, insulin infusion resulted in only a 30% increase in glucose uptake whereas endotoxin resulted in a 174% increase. These data demonstrate that endotoxin's insulin-like affect in promoting skeletal muscle glucose uptake is maintained during shock whereas insulin's affect is greatly attentuated.

74.7

IMPROVED CARDIOVASCULAR FUNCTION AND SURVIVAL USING THYROTROPIN RELEASING HORMONE (TRH) IN PRIMATE HEMORRHAGIC SHOCK.
N.J. Gurll, D.G. Reynolds, J. Holaday*, and E. Ganes*. Univ. of Iowa College of Med., Iowa City, IA. 52242 and Walter Reed Army Institute of Research, Washington, DC. 20012

TRH increases mean arterial pressure (MAP) in rats subjected to hemorrhagic shock. In order to investigate applicability to clinical shock, we studied TRH in cynomulgus monkeys anesthetized with N_2O/O_2 and instrumented to measure MAP and left ventricular contractility (LV dp/dt max). The monkeys were bled into a reservoir to achieve a MAP of 45 mmHg (t=0). MAP was maintained with the reservoir until t=60 min when the animals were treated with either TRH 2 mg/kg plus 2 mg/kg·hr i.v. (n=5) or an equivalent volume of 0.9% NaCl as a control (CON, n=5). Shed blood was reinfused at t=120 min. TRH significantly ($p<.05$ by analysis of variance) improved MAP and LV dp/dt max:

		t=60	90	120 min
MAP	CON	45	50±2	45±3
(mmHg)	TRH	45	69±3	72±4
LV dp/dt max	CON	1.5±0.2	1.7±0.3	1.6±0.3
(mmHg x 10^3/sec)	TRH	1.5±0.2	2.4±0.2	2.5±0.4

4/5 TRH and 1/5 CON monkeys survived 72 hr. The improved cardiovascular function and survival with TRH in primate hemorrhagic shock may be due to its anti-endorphin like effects. Clinical use may be attractive because TRH, unlike naloxone, has no effect on pain perception. (Supported by U.S. Army Contract DAMD 17-81-C-1177).

74.8

IMPROVED MITOCHONDRIAL FUNCTION FOLLOWING SEVERE HEMORRHAGIC SHOCK BY ATP-$MgCl_2$ TREATMENT.
Masanori Ohkawa*, Mark G. Clemens and Irshad H. Chaudry. Dept. of Surgery, Yale Univ. School of Medicine, New Haven, CT 06510.

Infusion of ATP-$MgCl_2$ following hemorrhagic shock has been shown to significantly improve the survival of animals. To determine the mechanism, hemorrhagic shock in 24 hrs fasted rats was produced by bleeding the rats and maintaining them at a BP of 40mmHg for 2 hrs. The rats then received IV either 0.5ml of saline or ATP-$MgCl_2$ (12.5umoles each) followed by the return of the remaining shed blood. One hr thereafter the rats were sacrificed, hepatic mitochondria isolated and adenine nucleotide translocase (ANT) activity measured using ^{14}C ADP. Mitochondiral respiratory control ratio (RCR) and ADP/O ratio using α-ketoglutarate were determined with a Clark O_2 electrode. The RCRs in sham-operated, saline and ATP-$MgCl_2$ treated rats were 2.86 ± 0.25, 2.19 ± 0.21 and 2.79 ± 0.20 (mean ± S.E.), respectively. The corresponding ADP/O ratios were 2.43 ± 0.05, 2.23 ± 0.06 and 2.73 ± 0.15, respectively. Similar trends were observed with succinate. Thus, the depressed RCR and ADP/O following hemorrhagic shock were restored by ATP-$MgCl_2$ infusion. Mitochondrial ANT activity was 36% of normal in saline-treated group but was 60% of normal in the ATP-$MgCl_2$ treated rats. These results lead us to conclude that ATP-$MgCl_2$ improves mitochondrial function after shock either directly or by improvement in the microcirculation. (Supported in part by USPHS grant 5 RO1 HL 1967307).

74.9

STUDIES ON THE CHARACTERIZATION OF ATP UPTAKE BY HEPATOCYTES.
Mark G. Clemens and Irshad H. Chaudry, Dept. of Surgery, Yale Univ. School of Medicine, New Haven, CT. 06510

Administration of ATP-$MgCl_2$ following shock or ischemia markedly elevates hepatocellular ATP levels. To help elucidate the mechanism, we studied the uptake of ATP by isolated hepatocytes. Collagenase dispersed hepatocytes were incubated in Krebs bicarbonate buffer supplemented with 10mM glucose, 2mM pyruvate, 2mM glutamine and 1.5% gelatin for 5 min in the presence of ^3H-ATP or adenosine (Ado). Uptake of the ^3H label was greater from ATP than from Ado at concentrations >10uM (ATP 149% of Ado at 50uM). This does not appear to be the result of leaky cells. Inhibition of 5'-nucleotidase with α-βmethylene ADP decreased uptake of ^3H from 10uM ATP by 60% while dipyridamole decreased uptake by 51%. The mitochondrial uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (5uM) produced a 32% decrease in ^3H uptake from 10uM ATP while the addition of 5mM $MgCl_2$ did not significantly affect ATP uptake. These results demonstrate that although ATP accumulation is very sensitive to inhibitors of Ado formation or transport, more rapid accumulation results when ATP rather than Ado is supplied. The ability of the mitochondria to synthesize ATP is also required for maximal rates of accumulation. Although $MgCl_2$ is necessary for the salutary effects of ATP treatment it does not appear to accelerate ATP uptake by isolated hepatocytes. (Supported in part by USPHS grant 5 RO1 HL 1967307).

74.10

DIRECT DOSE RESPONSE OF REFLEX ADRENAL MEDULLARY SECRETION TO ANGIOTENSIN II INFUSION IN HEMORRHAGED DOGS.
Elliott M. Badder*, Bernardo Duarte*, John F. Seaton* and Timothy S. Harrison. U. of Md., School of Med., Balto., Md. 21201 and Penn. State Univ., College of Med., HMS, Hershey, Pa. 17033

Reflex adrenal medullary epinephrine (E) secretion is inhibited in anephric dogs. Anesthetized anticoagulated (Na pentobarbital, 98% O_2/2% CO_2, controlled volume ventilation, Na heparin) mongrel dogs were prepared with monitoring arterial catheters and adrenal femoral venous shunts. Adrenal catecholamine secretion was determined from norepinephrine (NE),E concentrations in continuously aspirated aortic and adrenal vein samples multiplied by continuously determined (low flow, intravascular oscillating ball flow meter) adrenal vein flow. Three reference groups of dogs (N=5) were studied over 90 minutes at rest, after hemorrhage (25ml/kg) and after bilateral nephrectomy followed by hemorrhage. Study dogs, after bilateral nephrectomy, received angiotensin infusions (0.01, 0.1, 1.0, 10.0 mcg/kg/min) starting before and continuing 90 min. after hemorrhage. Results were analyzed by analysis of variance. At each study interval following bleeding, significant differences were noted between groups with highly significant log linear correlations of angiotensin dose to E ($p<.001$) and NE ($p<.001$) secretion rates. The results indicate a dose dependent relationship of reflex adrenal medullary secretion of E and NE to angiotensin II infusion.
(Supported in part by NIH grant #HL 18995-08)

TEACHING MATERIALS AND METHODS

75.1

TRENDS IN PHYSIOLOGY LABORATORY PROGRAMS — 1982.
Carl F. Rothe, Dept. Physiology, Indiana University School of Medicine, Indianapolis, IN 46223.

Trends in non-lecture teaching of physiology to medical students will be described based on a questionnaire sent to the members of the Association of Chairmen of Departments of Physiology. The results of this query will be published in The Physiology Teacher and compared to a similar study published in 1975. Topics of particular interest include:

(a) types of approaches used, such as integrated programs by organ systems or teaching programs organized around each discipline;
(b) the balance of lecture and non-lecture time;
(c) objectives to be met by the non-lecture part of the course;
(d) trends in laboratory emphasis;
(e) types of non-lecture programs, such as pre-assigned experiments, demonstrations, laboratory research, TV tapes and slide-tape material, problem solving sets, computer assisted instruction; and
(f) the role of "self-teaching" material in teaching physiology.

75.2

A MODEL OF THE CARDIOVASCULAR SYSTEM FOR EFFECTIVE TEACHING.
Carl F. Rothe, Dept. Physiology, Indiana University School of Medicine, Indianapolis, IN 46223.

A mechanical model of the circulatory system will be demonstrated. The model was described in 1962 (J. Appl. Physiol. 17: 156-158). More than 80 have been constructed for use in about 40 schools. The single ventricle fills passively and therefore the end-diastolic volume is a function of filling pressure and heart rate. Ejection is produced by a spring-loaded plunger and therefore the end-systolic volume is a function of arterial pressure. End-systolic and end-diastolic volumes may be estimated. Vigor of contraction is modified by changing the spring tension of the cam-driven plunger. Flow and arterial pressure are measured. Three heart rates (normal, bradycardia, and tachycardia) are available. Filling pressure and total peripheral resistance may be changed by the students. The inlet and outlet valves can be made stenotic or insufficient. The study of this simple mechanical model has been one of the most popular experiments for our medical, graduate and dental students as they investigate the interaction of factors influencing cardiac output and arterial pressure.

75.3

THE SELECTIVE LAB: AN ALTERNATIVE TO COOKBOOK EXPERIMENTS. Daniel Richardson, Dept. Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536

The purpose of a selective laboratory was to provide students with an opportunity for creativity and individual expression within the framework of a medical physiology course. The class was provided a list of 8 laboratory topics each with a set of guidelines. Students working individually or in teams of 2 to 4 selected one of the topics then designed and performed experiments on the topic. A faculty member was available for each topic to serve as an advisor and evaluate performance. The students prepared a report of their experiments in journal style. These constituted 10% of their total course grade. Average selective lab grades for our 1981 and 1982 classes were 88.3% and 88.0% respectively. Respective grades for the total course were 83.2% and 82.2%. These data show that selective lab grades are consistent among classes that are academicly matched. However among the students there was little correlation between selective lab and total course performance. Regression coefficients comparing these parameters for the 1981 and 1982 classes were 0.403 and 0.467, respectively. Evaluation by the students of the various components of the course showed that on a scale of 3 to 5 the selective lab received a class rating of 4.0 compared to a total course rating of 4.2. However the traditional lab component of the course received a rating of 3.6. Thus, in terms of laboratory experience our students preferred the selective lab over the traditional "cookbook" approach.

75.4

CV PATHOPHYSIOLOGY PROBLEMS IN SMALL GROUP TUTORIALS. Joel A. Michael and Allen A. Rovick. Rush Medical College, Chicago, IL 60612

Lectures do not allow much student-teacher interaction, nor do they facilitate the students' development of problem-solving skills. To do this, and to promote integration of separately taught (lecture) material, we have utilized small group sessions organized around sets of problems. Ideally, the instructor serves as a resource person but is available to guide or stimulate discussion when student input is inadequate or unable to generate a correct answer. The emphasis in these sessions is on the development of a logical approach to the ordering of information and the construction of a chain of cause and effect; this is applicable to both numerical and "diagnostic" problems. Some of the problem sets are based on human pathophysiology. For a CV session we utilized data from three patients from the Rush Cardiac Cath Lab (provided by Neil Ruggie MD, Director): two had valve problems and one had a shunt. The data permit the calculation of significant parameters describing CV performance. On the basis of the data and the calculations it is then possible to deduce the probable pathology that is present. Where necessary, students can be led through the logical development of an answer with a "Socratic" questioning method. The solution of clinical problems sparks student interest by illustrating the importance ("relevance") of physiology to medicine.

75.5

PROBLEM-BASED STUDENT CENTERED LEARNING OF THE CARDIOVASCULAR SYSTEM USING THE PROBLEM BASED LEARNING MODULE (PBLM). R.L. Coulson, Dept. of Physiology & Pharmacology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

Groups of six students, guided by a faculty tutor, work at solving the problems presented by a real patient (represented by a paper simulation (PBLM) which permits free inquiry and actual responses. Any action which could be performed on the patient by the physician can be performed on the simulation by the students. Students in groups derive a pooled background from diverse origins. Cues are perceived, multiple hypotheses are formulated, enqiury strategy is engaged, and data is used to modify, eliminate or include hypotheses which indicate diagnostic, therapeutic or management decisions. The tutor ensures that students adhere to the reasoning process which has been identified by research. At any stage in the process students (sometimes with the help of the tutor) identify areas of ignorance or incomplete understanding (learning issues) which prevent or obstruct coming to closure with the problem. These areas are noted on a blackboard by the group and used later as a guide to learning relevant information which can be brought to weigh against the problem at a later session of the group. First year medical students have as their objective learning basic mechanisms so their learning issues are oriented that way. Simultaneously, students gain practice in clinical reasoning, expand their medical vocabulary and perceive the relevance of the basic science they learn.

75.6

SIMULATED LABORATORY EXPERIMENTS IN MEDICAL PHYSIOLOGY USING A MATHEMATICAL MODEL OF THE HUMAN BODY. Thomas G. Coleman, University of Mississippi Medical Center, Jackson, MS, 39216 and James E. Randall, Indiana University, Bloomington, IN, 47405.

A mathematical model with digital computer solution has been developed to demonstrate interactions among organ systems: heart, peripheral circulation, cardiovascular reflexes, temperature regulation, muscle metabolism, acid-base balance, control of ventilation, gas exchange and transport, kidney function, blood volume regulation, and electrolyte and water homeostasis. The model can be used in an interactive manner to simulate laboratory experiments or patient encounters. The experimenter changes the numerical value of parameters of interest and the predicted consequences are computed and displayed as a function of time. Examples of theoretical investigations are: cardiovascular-respiratory-thermoregulatory interactions as in exercise and cardiovascular-electrolyte-fluid balance interactions with altered renal function. Twenty-six laboratory experiments and 13 simulated patients are currently described in a user's manual. The model is written in elementary FORTRAN for time-sharing installations and a microprocessor based version has also been created (Fed. Proc. 40:377, 1981). Supported in part by HL 11678.

75.7

HEARTSIM: A CARDIOVASCULAR COMPUTER SIMULATION WITH DIDACTIC FEEDBACK. Allen A. Rovick and Lisa Brenner*. Dept. Physiol. and Computer Based Ed., Rush Med Sch & Rush Univ., Chicago, IL 60612

Computer simulations allow students to perform difficult or impossible "experiments" and observe changes in a system's parameters. However, they also permit students to be "cook-book" learners who fail to learn important principles on-line. To insure that they take an active role we have used computer simulations in a "laboratory" where faculty could provoke student problem solving and integration. To also allow ad lib access to the simulations we have translated the MacMaster CV simulation (MacMan) for use on PLATO and have retained some of the benefit of faculty interaction by adding an instructional unit. Heartsim presents the students with a fixed protocol but compels them to predict in advance the qualitative changes that will occur to ten variables. This requires students to mentally organize their understanding of the relevant physiological relationships during the exercise. The simulation then runs, the qualitative outcome is entered alongside the students predictions. Where student errors are noted the program then explains the physiological basis for the model's output. Thus, student errors are automatically detected and corrected before s/he is allowed to proceed to the next step. Thus Heartsim provides guided learning within the problem-solving format of a computer simulation.

75.8

COMPUTER GENERATED FIGURES IN PHYSIOLOGY TEACHING: THE 'SMART VIDEO SLIDE'. Perry M. Hogan. Department of Physiology, School of Medicine, State University of New York at Buffalo, Buffalo, NY 14214

Cardiovascular teaching programs have been developed to take advantage of the graphical and interactive features of microprocessor based personal computers. Each program focuses on a single concept and may be used by the instructor as a teaching aid or by the student for purposes of self-study. In the aggregate, the programs demonstrate the versatility of the personal computer as a novel instructional instrument. Detailed pictorial and graphical video images are generated under program control at a rate sufficient to allow for both animation and simulation of physiological events. Further, the progress of a running program may be controlled dynamically through a variety of user initiated interface functions. For example the keyboard is used to select from a menu of program options, to respond to program interrogation, and to affect the course of a simulation by introducing select changes in relevant physiological variables. The outcome of a simulation is displayed graphically and may be examined using a moveable screen cursor under the control of game paddles or joystick. The latter devices are also used to modify the video display of pictorial images. In essence this approach expands the instructional content of a simple drawing by introducing motion, color, sound and multiple interactive modes, i.e. a computer based 'smart slide'. Programs will be available at the meeting for hands-on demonstration on an Apple Computer. (Supported by the School of Medicine, SUNYAB).

75.9

TEACHING CARDIOVASCULAR PHYSIOLOGY IN THE INTEGRATED FUNCTIONAL LABORATORY. Daniel L. Traber, James R.Walker*, and Monte A. Crawford*, The Integrated Functional Laboratory, The Univ. of TX Med. Br., Galveston, TX 77550

The Integrated Functional Laboratory is an independent course taught during the basic science years at our Medical School. (Physiologist 22:26-27, 1979). It is taught during the first and second year of the curriculum and consists of 24 experiments, 12 in each term. Of the 7 related to the cardiovascular system, some are taught simultaneously with the physiology course while others are taught during the second year just prior to the Medical Students entering into their clinical years. The rationale for the latter curricular inclusion is that the material serves as a refresher function prior to their clinical experience. Students are also taking pharmacology at this time. These 7 experiments relate to most of the aspects of physiology as they are traditionally taught cardiac muscle, the cardiac cycle, myocardial contractility, electrocardiogram during exercise, hypovolemic shock, and endotoxemia. The areas obviously not covered by the experiments but included nonetheless are the concepts of microvascular fluid flux and special circulations. The cardiovascular experiments are extremely popular with the students and they consistently do well on the National Board examination questions related to the cardiovascular system. Detailed descriptions of each experiment with their objectives will be given at the oral presentation.

75.10

TEACHING PHYSIOLOGY AT THE U.S. AIR FORCE ACADEMY. R.D. Reed. USAFA/DFB, USAF Academy, CO 80840.

Special attention must be paid to curriculum development at a military academy, where courses must provide not only training for future officers but also an undergraduate education comparable to other institutions. Many of our graduates go on to medical school or later graduate education. The Department of Biology at the USAF Academy has retained, for some time, a curriculum strongly oriented towards human biology. Recent changes led the author (course director for physiology) to review the place of physiology at the Academy. Until this year all students took an introduction to human physiology as their core biology course. Upper-level courses continuing this interest included human anatomy, biokinetics, comparative physiology, human physiology and occasional special-topics courses (e.g., animal surgery, space physiology and research methods). Last year the core biology course was changed to provide a well-balanced view of general biology (Starr and Taggart, Biology: The Unity and Diversity of Life). This change has helped to broaden the biological awareness of all Academy graduates, but it removed an initial introduction to physiology in any real depth. Upper-level courses are now being evaluated to ensure a well-integrated set of biology courses within our current limitation of 15 course offerings. The author will present the complete biology curriculum and the contents of Human Physiology as now taught. Whatever the results of current curriculum review, physiology should remain a prominent feature at this unique educational institution.

75.11

COMPUTER SIMULATIONS FOR PHYSIOLOGY TEACHING: ROLE OF GRAPHICS. H.I. Modell, A.J. Olszowka, J.L. Plewes and L.E. Farhi, Virginia Mason Res. Ctr., Seattle, WA 98101 and SUNY at Buffalo, Buffalo, NY 14214

Development of low cost, high resolution graphics has added a new dimension to computer simulations used for teaching. We have revised our earlier set of respiratory physiology models (Normal and Abnormal Lung Function, ATS, 1975) to include graphics for use on the Apple II computer. Two general schemes of employing graphics are available. The first is to provide schematic representation of the physiological process being studied, and the second is to present data derived from the model either by mimicking recorded data or by presenting plots of compiled data. The advantage of simulations over more traditional modes of presentation is that they may offer an active learning experience. The challenge in using graphics with models is to maintain the active learning component. By choosing to present plots of compiled data, it is easy to eliminate active student involvement to the point where the exercise is not substantially different from reading a textbook. Schematic diagrams or mimicked on-line recordings, however, provide visual aids to enhance conceptual understanding and maintain an active "laboratory" discovery process as long as data reduction remains with the student. In following this philosophy, we have avoided graphic presentation of compiled data and, instead, provide the student with graph paper and a list of questions to aid in reasoning from basic facts to general concepts. (Supported in part by AFOSR Contract F49620-81-C-0055 and SUNYAB School of Medicine)

75.12

NON-DIRECTIVE METHOD FOR TEACHING CARDIOVASCULAR PHYSIOLOGY. W.T.Beraldo. Department of Physiology and Biophysics, Federal University, Minas Gerais, Belo Horizonte, 30.000, Brazil.

The purpose of the teacher should be to create conditions, so that the student may learn. So, it will be unapparently obvious inference if the student does not learn it means that teacher may have failed in his mission or purpose. The professor should be able to contributed to the development of the student's personality, attitude and habits and should be able to stimulate the student interest in study and meditation. So, we decided to use the learning theory of Gestalt and the non-directive method of Rogers (Client-Centered Therapy, Boston, 1951) to teaching Physiology. The class was divided in discussion groups of 15 students seated in chairs disposed in a circle. The professor acted as "coordinator". The anwsers to the question were not given directly by the coordinator; he asked another correlated question in order to stimulate the discussion. When asked which anwser best expressed their thinking, they responded: did not study 2.6%; studied for the exam 23.4%; studied to learn 87%.

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Selected Topics on Microcirculation

A Refresher Course

Presented at the Meeting
of the
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Organized and edited by: B. R. Duling

Faculty: B. Zweifach, H. Lipowsky, J. Diana, C. Baylis,
H. Granger, N. Banchero, G. Bohlen

CHAPTER I

THE MICROCIRCULATION - OLD CONCEPTS AND NEW FACTS

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As the field of microcirculation has continued to develop over the past 50 years, its scope has been broadened with the availability of more sophisticated methods of measurement. Currently we find a re-examination of ideas of the past, many of which lost favor because of our inability to provide the data needed to corroborate or to disprove their validity. As we increase our fund of information and develop a keener insight into the truly basic features of microcirculatory behavior, it is apparent that large gaps of information still exist at the structural and organizational level.

Current concepts of the independent nature of microcirculatory behavior are based on a number of basic attributes: a) the network between the feeding arteriole and effluent venule has an overall modular design; b) blood flow within the tissue responds to local needs by adjustments of the vascular smooth muscle on both the arteriolar and venular side of the circuit; c) the unique properties of the endothelial barrier enable blood tissue exchange to occur along simple diffusion gradients.

Although these salient features were recognized by earlier workers in the field, the tools were lacking to come to grips with these complex entities. Most of the data on the microcirculation was observational and qualitative in nature. The general principles governing transcapillary exchange, the Starling Constitutive Equation for fluids and Fick's Law for the diffusion of hydrophilic solutes, were postulated without due consideration of the contributions of possible factors in the interstitium proper.

Many of the key unresolved issues of today are not basically different from those confronting workers in the field half a century ago. For example, with regard to local control of blood flow, the question of the existence of endothelial contractility has resurfaced, together with concern for the mechanisms that integrate vascular pressure adjustments as opposed to tissue metabolic needs. No agreement exists concerning the extent and mechanism of capillary recruitment during periods of increased tissue activity. With improved scanning electron microscope procedures, the possible role of the numerous pericytes on capillaries and especially on venular vessels is being given serious attention.

The move away from a simple pore concept of transcapillary permeability to a matrix type of barrier has brought back in vogue concern for the intercellular pathway as the major route involved in water and small solute exchange. The Starling concept of a balance between hydrostatic and colloid osmotic forces governing the exchange of fluid continues to be supported, but in a considerably more complex form involving time-dependent aspects and interstitial tissue forces. The origins of lymph fluid remain a controversial question. The mechanism for a gradient in vascular permeability along the course of the microvascular network indicating an increased perviousness to macromolecules on the venous side of the circulation has not been established.

The concept of microvascular heterogeneity has become an increasingly accepted explanation for the wide range of variation of microvessel function encountered even within a given tissue. These differences have been recognized for many years but their significance has puzzled scientists. Somehow, out of all this seeming chaos, order and precise functional needs are met. If some vessels carry less blood, others must carry more. Concepts of structurally discrete thoroughfare channels have been advanced for subcutaneous or mesenteric tissues to handle the continuous shifting of flow in the microcirculation whereas, in other muscular tissues, a functional type of shunting appears without anatomically distinctive shunt vessels. AVA type of connections between arterioles and venules were first described in the skin and gastrointestinal tract many years ago. Their control and functional involvement in other than temperature regulation remains largely unexplored.

The thinking of Nobel laureate August Krogh dominated the field in the 1930's. His monograph on the anatomy and physiology of the capillaries was the bible in the field. Krogh's pioneering work utilizing intravital microscopy convinced him that the all-important exchange functions of the terminal vascular network resided in local regulatory mechanisms attuned to the delivery of oxygen.

The contractile nature of all the microvessels including the capillaries was accepted. Histological work had led workers such as Vimtrup to rediscover the perivascular network of cells surrounding blood capillaries that had been

proposed by Charles Rouget as primitive muscle cells. Krogh championed the idea that these pericytes were the contractile elements of the capillary network and served to redistribute blood within the tissue.

Although endothelial type cells were found to undergo contraction in lower forms and in cold-blooded vertebrates, subsequent investigators were unable to demonstrate a similar function for endothelium in mammalian tissues. The absence of a response to vasoactive mediators led workers to conclude that capillary endothelial cells were non-contractile and that changes in capillary flow were brought about by active responses in the muscular feed arterioles and venules.

In recent years, with the advent of electron microscopy and immunofluorescent markers, evidence has begun to accumulate indicating that endothelial cells do contain actin and myosin filaments. These observations together with the action of kinases, prostaglandins and amines in increasing the permeability of endothelial vessels such as postcapillaries and venules has again brought into consideration the contractile potential of endothelial cells. Much of our thinking concerning the endothelial cell needs to be re-examined.

On the basis of visual inspection of small vessel behavior, utilizing intravital microscopy, the endothelal cell was considered more of an oddity than a typical cell with discrete metabolic functions. Its role in exchange was attributed to the thinness of the endothelial cells rather than to any intrinsic vital properties. The Clarks, in their pioneering studies on endothelial behavior in the rabbit ear chamber over extended periods of time, reinforced the view of the essentially passive nature of the adult endothelial vessels, except during periods of local injury and vessel proliferation.

The elegant work of Henry Dale and his associates led to the recognition of the release of local mediators such as histamines as a key event affecting capillary integrity and permeability during the inflammatory process. The role of such mediators in normal physiologic adjustments, however, has remained speculative and their mechanism of action has been poorly understood until recent demonstrations of specific receptor sites on endothelial cells for a wide array of biological mediators.

From the very earliest studies of undisturbed capillary networks in the wing of the bat and in transparent chambers inserted into the rabbit ear, Beatrice Carrier and the Clarks were impressed by the range and magnitude of spontaneous vasomotor excursions of the smallest arterioles as a basic mechanism for local distribution of blood. Subsequent studies by Webb and Nicoll served to develop the concept of precapillary sphincter activity for selective control of capillary perfusion. Here again, the unavailability of methods for quantifying such temporal events led to vasomotion being relegated as a curiosity rather than as a fundamental homeostatic process. Video and electronic recording combined with computer analysis have made it possible to analyze spontaneous vasomotion in relation to myogenic and metabolic mechanisms under both normal and abnormal conditions.

Unlike other cellular membranes, in which the cells have an active role in the selective permeability of the barrier, the capillary wall appears to behave as a passive filter, sieving off water soluble molecules on the basis of their molecular dimensions. Hence, there was a need to postulate either that the endothelial cell is different from other cells or that special structural considerations prevail in endothelial membranes. Thinness per se could not account for the unusual permeability properties of endothelium.

In view of the increased filtration rate and permeability to macromolecules encountered in perfusion with low calcium solutions and of the known tendency of calcium free media in vitro to lead to dissociation or uncoupling of cellular junctions, the concept of an intercellular pathway was developed as the equivalent of the small pore channels for transcapillary exchange. It was postulated that a weakening of inter-cellular adhesion forces led to the leakage of plasma proteins during the vascular response to injury. In synthetic membranes a population of pores is distributed randomly across the surface. In the capillary barrier, such pores are confined to the narrow slits between contiguous cell borders. Apparently, an increase in permeability is associated with an increased number of pores, as well as with large leaks or "holes" through which all elements of the blood can pass.

Current thinking includes an endocapillary layer as a barrier to the outward movement of materials from the bloodstream. This structure is believed to be a glycoprotein that carries a net negative charge. Older concepts, based on the events associated with tissue injury, envisaged an increased adhesiveness of the lumenal surface of the endothelial cell that entrapped particulate matter and dyes, and led Jansco to conclude that endothelial cells had phagocytic capabilities. In the light of more recent electron microscopy evidence, these reactions may include vesicular uptake of material. The coating of the endothelial surface observed by in vitro microscopy may be related to charge since cationizing various proteins interferes with their uptake by endothelial vesciles. Here again, our thinking has gone full circle over the years.

Another example of the vagaries imposed by the complexity of the microvascular system is the precapillary sphincter concept advanced some 50-60 years ago. Strategically situated at branch points, "Pfortnerzellen" reacted to stimulation by obstructing flow into the precapillary branch and

its consortium of capillaries. The existence of such configurations along the length of the terminal arterioles gave rise to the concept of "preferential channels" as the keystone around which the microcirculatory module is organized. Apparently not all tissues have clearly-defined modules of this kind and questions exist as to the universal distribution of precapillary sphincters for selective distribution of capillary perfusion.

Advancement in the field of microcirculation has gone through periods of sudden acceleration as new methods and tools have been applied. Intravital microscopy of mammalian tissues has provided more meaningful data as improved procedures were developed for general anesthesia, maintenance of blood gas levels, appropriately balanced electrolyte solutions for exteriorized tissues, control of body temperature, etc. The development of isotope-labelled tracers allowed for the monitoring of transcapillary exchange. A substantial leap forward came about with the advent of electron microscopy. Video recording and electronic instrumentation made it possible to quantify most of the basic attributes of the system to the point where well-characterized models of the microcirculation could be formulated. The availability of computer-assisted data analysis has made possible the handling of as many as 10-20 variables individually and collectively. Enormous opportunities exist today to carry the field of microcirculation into the forefront of physiology and medicine through the application of newly emerging tools in molecular biology and the extraordinary specificity of immunological probes.

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NOTES

Chapter 2

DETERMINANTS OF MICROVASCULAR BLOOD FLOW

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During the six decades following the pioneering studies of August Krogh (13), a conceptual framework has evolved in which the resistance to blood flow at the microcirculatory level is recognized as the resultant of a complex interaction between vascular and intravascular factors. Intravascular (blood rheological) factors encompass the relationship between blood velocity (shear rates), pressure gradients, hematocrit and leukocyte count, as it applies to single unbranched microvessels and at network branch points. The determinants of this relationship have been shown to be red cell deformability and aggregation, leukocyte deformability and leukocyte-endothelium adhesion; all of which act to specify the in vivo apparent viscosity of blood.

Vascular or topographical features may influence this relationship both directly or indirectly at any level of the hierarchy of microvessels from arterioles to venules. Direct topographical effects on the resistance to flow within an individual microvessel are derived from alterations in microvessel luminal diameter brought about by regulatory mechanisms. Also, the branching pattern of the network plays a major role in dictating the resistance to flow in a given microvessel by virtue of its effect on the local apportionment of the overall arteriovenous (A-P) pressure drop.

Thus, it is evident that the task of separating topographical and blood rheological factors which affect microvascular pressure-flow relationships is quite complex. To this end, numerous in vitro studies have sought to establish a frame of reference for the evaluation of the intrinsic properties of blood as they pertain to the in vivo environment (2,3,4,5,16). In this context, in vitro studies of blood rheology have demonstrated that the apparent viscosity of blood may increase dramatically with increasing hematocrit and also during red cell aggregation attendant to a decrease in shear rates. This departure from Newtonian fluid behavior (i.e. constant viscosity) has generally precluded the applicability of the Poiseulle equation, $Q = (\pi/128\eta)(\Delta P/L)D^4$, where $\Delta P/L$ is the pressure gradient, D is the luminal diameter and η is the apparent viscosity of blood.

From a rheological standpoint, recognition of the importance of the particulate nature of blood as a determinant of in vivo resistance was brought to light by the classical study of Fahreus (6). He showed that the hematocrit of blood flowing in a small bore tube diminishes in comparison to its value in the feeding reservoir as the diameter of the tube is reduced to a size comparable to that of the red blood cell. This effect has been attributed to alterations in the ratio of average red cell to plasma velocity, such that the dynamic tube hematocrit can be less than its feed value while maintaining conservation of red cell flux between the feeding and collecting reservoirs. This finding was followed by the discovery by Fahraeus and Lindqvist (7) that the apparent viscosity of blood was reduced as well for small bore tubes in comparison with values obtained in relatively large scale viscometric instruments.

In vivo observations on blood rheological behavior by Whittaker and Winton (19) provided the first indirect evidence on how such properties of blood may affect microvascular resistance. By perfusing the isolated hindlimb of the dog with suspensions of erythrocytes, they estimated the apparent viscosity of blood in this biological viscometer to be almost 50 percent less than that obtained in vitro using large bore tubes. While it was readily recognized by these authors that such behavior was attributable to the Fahraeus and Fahraeus-Lindqvist effects (i.e. reduced microvascular hematocrit and apparent viscosity, respectively) direct in vivo observations of these effects were not possible until recently.

With the development of techniques for the in vivo measurement of intravascular pressure and pressure drops (servo-null method), red cell velocity (two-slit technique) and optical methods for the measurement of hematocrit and vessel diameters, it is now possible to examine the details of the resistance to blood flow in the constituent vessels of the microvascular network. In the following sections, some of the major features of the in vivo rheological picture will be examined.

Topographical Considerations

Direct in situ measurements of intravascular pressure and red cell velocities have been obtained in many tissues and serve to elucidate the functional apportionment of pressure and flow throughout successive microvascular divisions. Efforts to evaluate these data have usually focused on the relationship between network structure (branching pattern) and hemodynamic parameters in

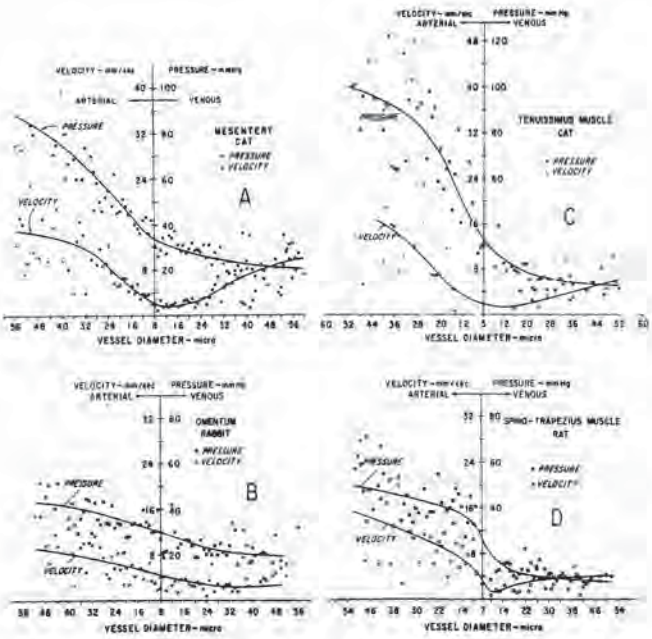


Fig. 1 Arteriovenous Distribution of Intravascular Pressures (Servo-null Method) and Red Cell Velocities (Two-slit Photometric Technique) as a Function of Microvessel Luminal Diameter. Diameter is indicated as an index of the position of a given vessel within the hierarchy throughout the network. (a) Mesentery (22), (b) omentum (22), (c) tenuissimum m. (8) and (d) spino-trapezius m. [unpublished data of B. W. Zweifach].

relation to the needs of the parenchymal tissue (i.e. convective transport and transcapillary exchange of water and solutes). To illustrate the basic features of network hemodynamics, the A-V distribution of intravascular pressures and red cell velocities are presented in Figure 1 for four apparently dissimilar networks, two splanchnic beds (mesentery and omentum (22)) and two skeletal muscles (spino-trapezius and tenuissimus (8)). These parameters are plotted as a function of microvessel luminal diameter (abscissa) which is taken as an index of position within the overall hierarchy of arterioles, pre-capillaries, etc. It is evident that each network presents a distribution of hemodynamic parameters which is characteristic of its topography, overall A-V pressure drop and blood rheological properties.

Upon consideration of the total throughput of each network, that is, the flow delivered by a large arteriole to a specific number of smaller microvessels, one may derive quantitative measures of the resistance to blood flow between successive network segments. For example, the mesenteric network shows that, as blood traverses the precapillary network, intravascular pressure declines most rapidly in the 15 to 35 μm vessels. From an anatomical standpoint, this segment can be identified with the arcading arcuate vessels characteristic of the modular network structure in the mesentery. The omentum, however, shows a maximum falloff in pressure at the true capillary level, which can be identified with the site of maximal vascularity in this nearly dichotomizing network (9). The two skeletal muscle networks exhibit a maximum network pressure drop near the true capillary level which may be associated with small arterioles which traverse the muscle fibers and the true capillaries which run parallel to the fibers (8).

Although such trends are indicative of the summated effects of the resistance to blood flow within individual vessels of the network, it is difficult to ascertain from such data the specific contributions of vessels within a given segment of the network. The problems of relating single vessel resistance to network resistance may be brought into clearer focus by examining the A-V distribution of individual vessel resistances obtained from the simultaneous measurements of upstream to downstream pressure drop (ΔP) and flow (Q). By analogy to Ohm's law, the resistance may be computed as $R = \Delta P/Q$. Presented in Figure 2a is the A-V distribution of R for the mesenteric network (14). It is evident that single vessel resistance rises about five decades as blood traverses the precapillary network, to achieve a maximum value at the true capillary level. This distribution of resistance, obtained in the resting or normal flow state, is indicative of the deployment of vessels of specific lengths with attendant blood rheological properties. Power law regressions of the form $R = aD^{-m}$ (solid curves) reveal exponents of 3.65 and 3.94 for arterioles and venules, respectively.

To further elucidate the dependency of resistance on luminal diameter, the resistance per unit of vessel length ($R/\underline{1}$) is presented in Figure 2b. The power law regressions of these data yield exponents of 4.04 and 3.94, for arteriolar and venular vessels, respectively. These results are quite remarkable in that, if blood behaved as a Newtonian fluid, with a fully developed flow (parabolic velocity profile) in a smooth wall tube of circular cross-section, then these regression exponents would be 4.00, as predicted by the Poiseuille relationship, where $R/\underline{1} = 128\eta/\pi D^4$. Evidently, by virtue of the large range in vessel diameters encountered as blood traverses the network, the fourth power relationship appears as the dominant factor in specifying intravascular resistance in the normal flow state. On a global or network scale, deviations from this behavior appear to be comparatively minor, and may be attributed to local variations in apparent viscosity as well as experimental errors.

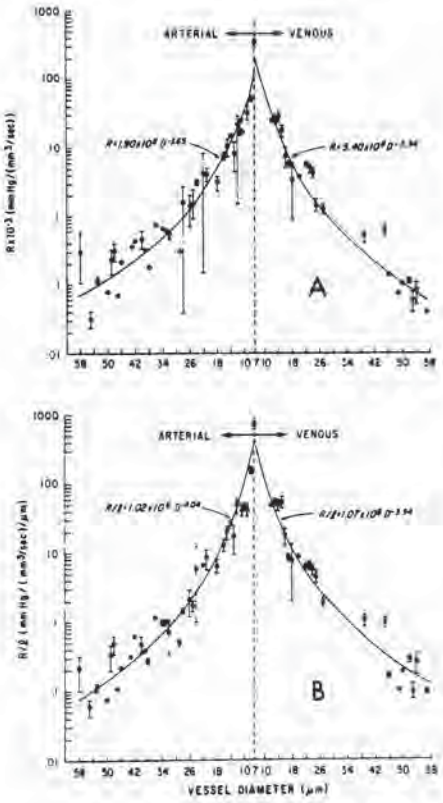


Fig. 2 A-V Distribution of Intravascular Resistance in Single Unbranched Vessels of the Mesentery. (a) Resistance, R, computed as the ratio of $Q/\Delta P$ and (b) the corresponding resistance per unit length, R/l (14).

Apparent Viscosity

The apparent viscosity of blood (η) flowing in individual mesenteric vessels has also been evaluated by computing η as that value which satisfies the Poiseuille relationship, $\eta = (\Delta P \cdot D^2) \div (k \cdot V_m \, l)$, where V_m is the mean (bulk) velocity of cells plus plasma and k is a constant dependent on the units employed (14). These data are given in Figure 3, where the A-V distribution of η is plotted as a function of luminal diameter (network position). Values of η averaged 3.6 ± 1.9 (SD) cP for arterioles and 5.2 ± 2.7 (SD) cP for venules. The elevated viscosities in the venules have been attributed to both higher hematocrits in venules of comparable diameter, as well as shear rate related phenomena (e.g. red cell aggregation and leukocyte-endothelium adhesion). Also, these values of η are considerably higher than those obtained for blood by the macrocirculatory whole organ approach (c.a. 2 cP in the dog hindlimb (19)) and correspond to values obtained *in vitro* for hematocrits of 40 to 50 percent (15). Inasmuch as hematocrits were not obtainable during these studies, the exact basis for comparison with *in vivo* or macrocirculatory studies is lacking.

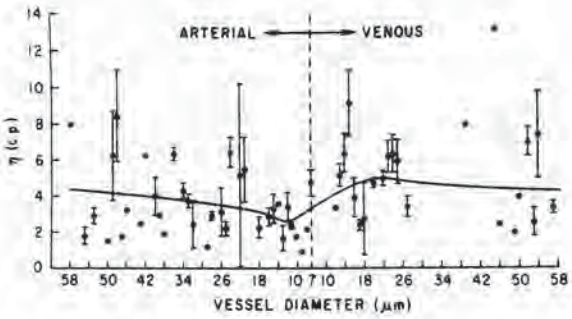


Fig. 3 A-V Distribution of the Apparent Viscosity Obtained from the Simultaneous Measurement of Pressure Drops and Flows in Individual Microvessels of the Mesentery (14).

Subsequent examinations of the variability of η with microvessel hematocrit (H_{micro}) have served to fill this void (15). Presented in Figure 4 is the variation of η with H_{micro} for simultaneous measurements of pressure drop, flow and hematocrit in small arterioles of the mesentery (cat). Microvessel hematocrit was measured by analysis of the light trnasmission characteristics of the vessels and was taken to be that value of hematocrit which one would obtain if the flow were instantaneously stopped and the packed cell fraction of erythrocytes measured by centrifugation. Also shown, for comparison, are measurements of η vs H_{micro} obtained *in vitro* in a cone-plate viscometer at comparable shear rates. This agreement between *in vivo* and *in vitro* data substantiate the hypothesis by Barbee and Cokelet (1), established *in vitro* for small bore tubes ranging in lumen from 29 μm to over 800 μm, that, given comparable hematocrits and shear rates, the apparent viscosity of blood will be the same regardless of the size of the tube or microvessel. Thus, the apparent viscosity deduced by macrocirculatory studies may be considerably lower due to the inability of such studies to evaluate the effective hematocrit at the microcirculatory level, as suggested by Whittaker and Winton (19). The relatively high values of η obtained *in situ* may also be biased somewhat since vessels with zero values of H_{micro} (plasma only) were not amenable to velocity and hence viscosity measurements, or low hematocrit (hence viscosity) vessels may have been inaccessible.

Microvessel Hematocrit

The importance of hematocrit in the transport of oxygen to tissues and as a determinant of the apparent viscosity of blood has prompted numerous macro- and microcirculatory studies aimed at evaluating its systemic and microvascular distributions. Essential to an understanding of these distribu-

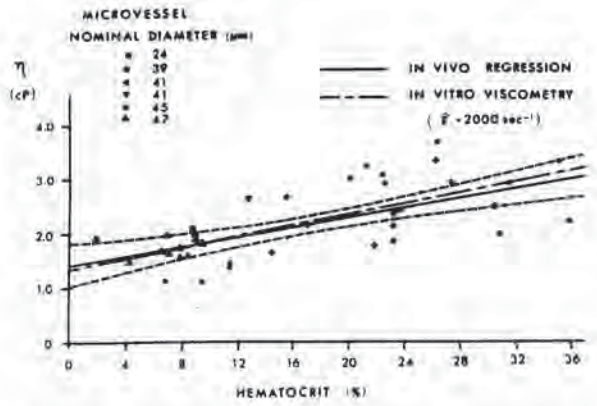


Fig. 4 Apparent Viscosity vs. Microvessel Hematocrit in Arterioles of the Indicated Sizes in the Mesentery of the Cat (bounded by ± 95 percent confidence limits). Also shown for comparison is the relationship obtained in a cone-plate viscometer at comparable shear rates. No significant difference between in vivo and in vitro determinations of viscosity is evident. From Lipowsky et al. (15).

tions is the recognition of the fact that two specific hematocrits are associated with the flow of blood: (a) the actual microvessel or dynamic hematocrit (H_{micro}), which corresponds to that value determined by instantaneously stopping the flow in a vessel and measuring the packed cell fraction, as by centrifugation; and (b) the discharge (H_{disch}) or outflow hematocrit, which is that value obtained by collecting the effluent of a vessel in a hypothetical "mixing cup" and determining its packed cell fraction (4). Differences between H_{micro} and H_{disch} arise principally due to the relative increase in the mean velocity of erythrocytes above that of the mean velocity of plasma, as suggested by the early studies of Fahraeus (6).

Measurements of the distribution of H_{micro} throughout successive microvascular divisions have demonstrated low capillary hematocrits ranging from 20 to 45 percent of $H_{systemic}$. For example, cinephotometric measurements of H_{micro} in capillaries of the omentum have revealed average values of 17.2 percent in the 8 μm capillaries, which corresponds to H_{micro}/H_{sys} equal to 0.45 (17). Based upon the micro-occlusion technique, mesenteric capillaries smaller than 10 μm diameter were found to average 8.2 percent (H_{micro}/H_{sys} = 0.23) (15), and comparable values of 10.4 percent have been shown in the cremaster muscle with H_{micro}/H_{sys} = 0.2 (11). Using a combination of micro-occlusion and optical density techniques, the entire arteriovenous distribution of H_{micro} has been obtained for the mesentery (cat) in the normal flow state (15) (Fig. 5).

In Figure 5, the microvessel hematocrit is normalized with respect to H_{sys} (average systemic hematocrit equals 35 percent). H_{micro} falls from about 31 percent in the 70 μm arterioles to 8.4 percent at the true-capillary level (D = 7 μm) with a subsequent rise in the venous circuitry to reach a value of 29 percent in the 70 μm venules. From these trends it appears that the minimum hematocrit occurs in the immediate post-capillary vessels, 12 μm diameter. For microvessels ranging in diameter from 10 to 60 μm, values of H_{micro}/H_{sys} averaged 0.46 ± 0.24 (SD) in arterioles and 0.53 ± 0.25 (SD) in venules.

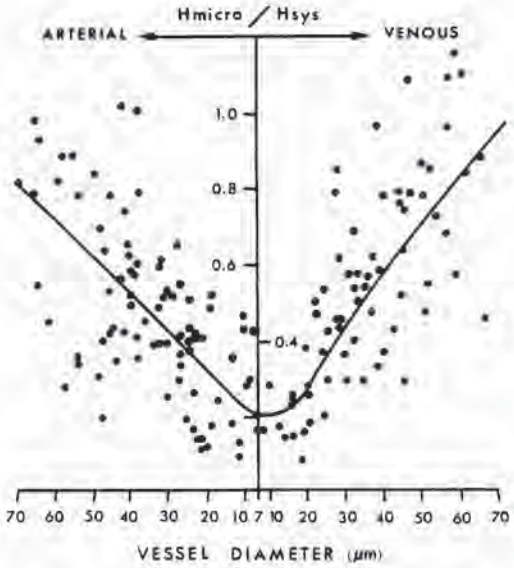


Fig. 5 Arteriovenous Distribution of Microvessel Hematocrit in the Mesentery of the Cat, Normalized with Respect to Systemic Values (from Lipowsky et al. (15)).

Branch Points and Bifurcations

The variability of hemodynamic parameters throughout the microvascular network, examined in the preceding sections on the basis of vessel caliber, has also been examined in light of the flow behavior of blood at a branching site. In most networks which have the configuration of a succession of branching tubes (e.g. muscle and splanchnic beds), the sequential division of arterioles into smaller diameter branches may be characterized as a bifurcation involving a parent and two daughter branches. Although substantial departures from this schema are present in many tissues (e.g. lung and liver), most analyses of branch flow processes have centered around bifurcations.

The reduction of intravascular pressure from parent to daughter branches in the arteriolar network has been measured for parent arterioles ranging in diameter from 50 to 20 μm (21). The magnitude of parent to daughter pressure drops was found to increase substantially with diminishing ratio of their respective diameters (D_d/D_p), ris-

ing from a 5 to 40 percent drop as D_d/D_p decreased from 0.9 to 0.1. In view of the technical difficulties in making such measurements without disturbing the flow field, more detailed information on this process has been derived from theoretical analyses. It has been shown that the specific geometry of the orifice of the side branch may greatly influence the pressure drop incurred as the flow enters the branch. Irregularities at the orifice, conceptualized as a pre-capillary sphincter (20) which may obstruct the inlet by as much as 80 percent, can produce pressure drops on the order of 20 to 30 times greater than those present without a constriction. Due to difficulties in evaluating these geometric factors by intravital microscopy, the relative contribution of this process to the overall A-V distribution of intravascular pressure remains obscure.

The apportionment of red cells at a bifurcation has also received considerable attention. The concept of plasma skimming introduced by Krogh (13) and the comparatively low capillary hematocrits have prompted an examination of the conservation laws governing red cell flux at a bifurcation for the two cases of a sizable arteriole feeding a capillary side branch, and the bifurcation of vessels of near capillary diameters. In the arteriole-capillary branch, non-uniformities in the radial distribution of hematocrit and the presence of a marginal zone of plasma along the wall of the arteriole has been ascribed as the principal factors to be considered in the dilution of the side branch hematocrit. Theoretical and *in vitro* studies of blood flow in small bore glass tubes have stressed the importance of daughter branch diameter and flow velocity as determinants of branch hematocrit. The larger the branch diameter and the greater the branch flow, the further into the parent stream the dividing streamline will protrude relative to the parent vessel plasma layer. Such behavior has been examined in the arteriolar network of the mesentery, where measurements of preferentially higher hematocrits have been correlated with higher velocities in a daughter branch (10).

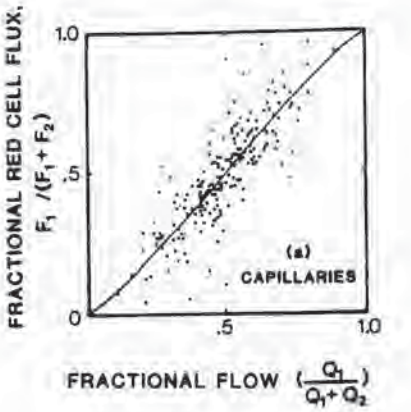
Detailed studies of blood cell distributions at a bifurcation with all branches comparable to capillary diameters, have emphasized the roles of both the balance of hydrodynamic forces and blood cell deformability (both erythrocytes and leukocytes) as a determinant of the cell concentrations in the daughter branch (18). The expression of these hemodynamic processes on the scale of the entire network has been examined both in the arterioles and venules of mesentery, and the capillary bifurcations of the cremaster muscle (12). These investigations have aimed to study the conservation of red cell flux at bifurcations, and their distributions as a function of the relative flow at a bifurcation must satisfy the following relationships for bulk flow of cells plus plasma in the daughter and parent vessels, Q_p and Q_d respectively, as well as for the volumetric flux of red cells, Q_{rbc}:

$$Q_{d_1} + Q_{d_2} = Q_p$$

and

$$Q_{rbc_{d_1}} + Q_{rbc_{d_2}} = Q_{rbc_p},$$

where the volumetric flux of red cells is given by the product of bulk flow and the discharge hematocrit (H_{disch}) for each branch, i.e. QH_{disch}. These relationships must be satisfied regardless of whether or not plasma skimming or other factors favor a preferential distribution of red cells into a branch. However, comparison of the ratio of QH_{disch} between daughter and parent branch, and the flow ratio Q_d/Q_p should reveal a linear relationship only if the red cells are uniformly distributed without a preferential shunting into a branch. Presented in Figure 6 are representative plots of these ratios of red cell flux versus bulk flow, from parent to daughter branches for the capillaries of the cremaster network (a) and the arterioles and venules of the mesenteric network (b). As shown in Figure 6, the deviation of the capillary distributions from a linear relationship is small, but significant, whereas the deviation from a linear relationship in the arterioles and venules was not statistically significant. Although it appears from these data that the effects of plasma skimming do not contribute greatly to the preferential distribution of blood at all network bifurcations, the effect of compounding this process through successive divisions remains to be determined.



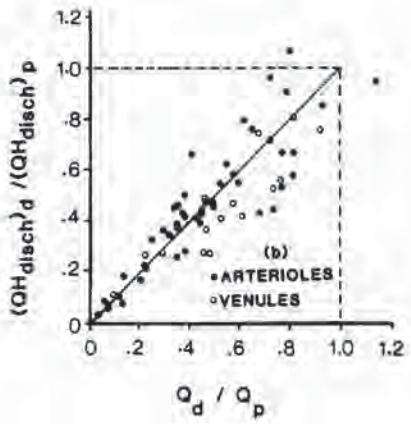


Fig. 6 Red Cell Flux at a Bifurcation. (a) Ratio of red cell flux ratio from daughter to parent vessel vs. the corresponding bulk flow ratio in the capillaries of the cremaster muscle. From Klitzman and Johnson (12). (b) Volumetric flux ratio, computed as the product of bulk flow and discharge hematocrit, between daughter and parent vs. the corresponding ratio of their bulk flows of cells plus plasma for arterioles and venules in mesentery (from Lipowsky, unpublished data).

Conclusions

The topographical and blood rheological determinants of microvascular perfusion discussed here are but a few of the many factors of importance in the normal flow state. Departures from these trends may be incurred under pathological conditions. This underscores the importance of understanding the physical principles governing hemodynamics. For example, in the low flow state, as in shock, one could reasonably expect red cell aggregation to modify microvascular perfusion due to increases in blood viscosity, and hence dramatically affect recovery. In experimental models of hypertension (e.g. SHR), the observed occurrence of a reduced number of arterioles might contribute to subtle blood rheological effects by altering the A-V distribution of hematocrit, and hence compromise tissue perfusion. Hence, although the investigation of these perfusion phenomena appears laborious and fraught with many technical difficulties, the potential for making substantial progress in understanding the disease process more than adequately justifies the effort.

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NOTES

Chapter 3

TRANSCAPILLARY WATER FLUX

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In most mammalian organisms approximately 70% of the total body weight is water which, for the body as a whole and organs in general, is compartmentalized into an extracellular and intracellular component. Such compartments are conveniently separated by the cellular membrane. The extracellular compartment is further divided into two general subcompartments: the intravascular subcompartment, which contains the circulating blood and its intrinsic constituents; and the interstitial subcompartment, which is the fluid and matrix phase interposed between the circulating blood and the cells. Each compartment and subcompartment contains a variety of inorganic and organic solutes which are dissolved in water. The organic solutes are primarily derived from food and the metabolic breakdown of food and are constantly moving into and out of the cells, organs and body. The inorganic solutes form a stable environment for the tissue cells, i.e., Claude Bernard's "milieu interieur". Most inorganic solute concentrations bathing tissue cells are maintained in a steady state and within narrow limits of normal. Any perturbation which permanently alters either fluid and/or solute concentration balance will ultimately lead to destruction and death of tissues in the living organism. In this discussion we will address fluid balance between the vascular and interstitial subcompartments and will not be concerned with those factors which are related to the regulation of intracellular water balance.

FUNCTIONAL MICROVASCULAR ANATOMY

Molecules must be brought to the exchange surface area. Thus, the regulation of blood flow or, more specifically, the regulation of the medium carrying the molecule to the microvascular exchange surface area, is an important component in the movement of molecules between blood and the interstitial fluid environment.

The microcirculation consists of the smallest vessels in the circulatory system. Anatomically, these vessels form the connection between the arterial and venous blood distributing systems. Physiologically, the microcirculation performs the ultimate function of the cardiovascular system; that is, it is responsible for the maintenance of optimal environment for cellular activity and is the only part of the cardiovascular system, in which exchange of water and solutes between blood and tissue fluid occurs. Thus, nutrients (for example, oxygen supplied through the capillary circulation of the lungs and glucose and fatty acids from the capillary circulation of the intestine) are delivered to the capillary circulations of all organs and exchanged with all tissue cells for cellular metabolism and survival. In the same manner, the by-products of cellular metabolism which are generally toxic in high concentrations (for example, CO_2 and hydrogen ion) are exchanged from tissue to blood at the capillary and delivered to the capillary circulation of the lungs and kidneys for final removal from the body.

BLOOD FLOW REGULATION

The heart and large arteries form a distributing system for delivering blood to the microvasculature. The flow of blood through the exchange vessels depends in part on their anatomy (the extent of branching and size of each branch, which vary considerably among different tissues and organs). A model of the microcirculatory unit is shown in Figure 1. In general, the arterial distributing system branches into terminal arteries, then arterioles, metarterioles, precapillary sphincter regions, and finally diverge into the capillary network. The capillaries drain into collecting venules which merge to form muscular venules, and these vessels, in turn, converge to become the initial segment of the venous circulation. The classification of vessels in the microvasculature is based primarily on vessel luminal diameter, but there are distinct morphological differences in all vessel types.

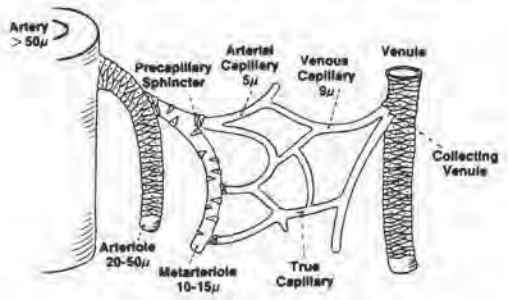

Fig. 1. General Anatomical Model of a Microcirculatory Unit.

Terminal arteries are usually greater than 50 microns in diameter. The wall structure of terminal arteries contains a continuous layer of endothelium, a prominent internal elastic lamina, and a surrounding sheath of two or more layers of vascular smooth muscle. Terminal arteries possess rich sympathetic neural innervation, and luminal diameter changes (thus blood flow) can be influenced by the level of neural discharge as well as circulating or local tissue vasodilator and vasoconstrictor substances.

Arterioles are usually from 20 to 50 microns in diameter. Their wall structure is similar to terminal arteries except that there is only a single layer of helicaly arranged vascular smooth muscle cells and there appear to be discrete points of connection between the inner layer of endothelium and vascular smooth muscle cells. Arterioles are sympathetically innervated and their luminal diameters (and thus blood flow) can also be influenced by the level of neural discharge and by vasoconstrictor and vasodilatory substances.

Metarterioles are usually between 8 to 15 microns in diameter. The wall structure of these vessels also contains a continuous layer of endothelium. The vascular smooth muscle cells are intermittent and constitute the major identifying marker for metarterioles. Innervation of metarterioles is controversial and their caliber appears to be influenced primarily by circulating or locally produced vasoactive substances.

At the origin of the capillary system there is a cuff of vascular smooth muscle cells, the precapillary sphincter region, which responds to local and systemic vasoactive agents. This region does not appear to be innervated. Contraction of these sphincters effectively isolates the downstream capillary from blood flowing through the parent metarteriole. Intermittent contraction and relaxation of the precapillary sphincter gives rise to intermittent blood flow through capillaries. This phenomenon is commonly seen when the microvasculature is observed by microscopy. Together, the arterioles, metarterioles, and precapillary sphincters constitute the resistance elements of the microcirculation. Vasoconstriction or vasodilation of these elements has profound effects on capillary blood flow and capillary surface area which are two crucial factors in the blood to tissue exchange of substances.

Capillaries are generally 4 to 5 microns in diameter on the arterial side and 7 to 10 microns in diameter on the venous side. The capillary walls are formed by thin portions of one or more endothelial cells rolled into a tube, and the junctions between cells form channels approximately 100 A in width which connect the lumen of the capillary with the outside (tissue side) basement membrane. Within the channel are one or more sites where the adjacent endothelial cell membranes come together, may even fuse, and form a tight-junction gap or seal. Such channels are generally considered the morphological counterpart of physiological "pores". Capillaries are not innervated and contain, so far as is known, no well delineated structure which can cause luminal diameter changes. Thus, capillaries are passive endothelial tubes whose primary and essential role is the site of exchange for nutrient and waste products. The large surface area of these vessels, which has been estimated at 5000 to 7000 square feet, facilitates material exchange.

Collecting venules are 10 to 15 microns in diameter. The walls of these vessels have no vascular smooth muscle cells, but contain a continuous layer of endothelium, some connective tissue, and a thin basal lamina. Collecting venules appear to be involved in fluid and solute exchange and are known to be affected by temperature extremes, inflammation and allergic reactions. They are not innervated and do not respond with diameter changes to vasoactive substances. Therefore, they have no effect on microvascular blood flow.

The final microvascular unit is the muscular venule, which is usually greater than 50 microns in diameter. The wall structure of these vessels is similar to terminal arterioles. Innervation appears to be somewhat less when compared with their arterial counterpart, but the vascular smooth muscle cells of these vessels will constrict and dilate in response to neural stimulation and vasoactive agents. Muscular venules appear to function as a reservoir where a substantial portion of the total blood volume may be either stored or released when activated by the appropriate stimuli.

To summarize this section, the idealized microcirculatory unit can be divided into three anatomical and functional segments. (a) Arterioles, metarterioles, and precapillary sphincters are the resistance vessels whose caliber can be increased or decreased by either neural or vasoactive substances. Luminal diameter changes in these vessels have profound effects on blood flow and exchange surface area, both of which are crucial factors in the exchange of fluid and solutes between blood and tissue cells. (b) The capillaries and collecting venules are the exchange vessels where water and solute transfer occurs. (c) Muscular venules have primarily a reservoir function, although vasoconstriction or vasodilation of these vessels will influence resistance to blood flow downstream from capillaries and thus affect capillary hydrostatic pressure (discussed below).

DIFFUSION VS. FILTRATION

The exchange of water across the capillary wall between blood and the interstitial space appears to occur primarily by the processes of diffusion and ultrafiltration. Diffusion of molecules in a solution occurs from random kinetic motions of the molecules. As with solutes in solution, water will diffuse from a region of

higher activity (concentration) of water molecules to one of lower activity in accordance with Fick's Law of free diffusion. Many measurements have been made of diffusion of water and solute and ultrafiltration (bulk flow) of water in capillaries as well as artificial membranes. The results indicate that the pathways for exchange are minute, fluid-filled channels which occupy a small fraction of the total area for exchange (less than 0.1%). It has been shown both experimentally and theoretically that the diffusion of water molecules back and forth across the capillary wall is very rapid (it has been calculated that a single water molecule could exchange with the interstitial water, back and forth across the capillary wall, some 80 times as it traverses the length of the capillary). Because the dimensions of the pores in the capillary walls are large with respect to the radius of the water molecule, the pores offer no barrier to diffusion and the net flow of water molecules from blood to tissue by the process of free diffusion is very small.

The major process for water exchange across the capillary wall is ultrafiltration or bulk flow of fluid in response to an osmotic or hydrostatic pressure difference. As stated by Onsager (1), bulk flow of fluid can be visualized as either a volume element of the liquid or the motion of adjacent portions of a liquid element moving through a pore, whereas diffusional flux relates to movement of a single water molecule which is but a single constituent of the fluid element. The exchange of water by diffusion (diffusional permeability, P_d) through capillary membranes, per unit activity gradient, is one thousandth as effective in producing net water flux as the bulk exchange of water induced by osmotic or hydrostatic pressure difference (osmotic permeability, P_f)). Further, bulk flow increases as the square of the total pore area while diffusion increases only in direct proportion of the pore area. Thus, as the pore area increases, the discrepancy between P_f and P_d becomes greatly magnified.

BARRIER FOR EXCHANGE

The ultimate function of the circulatory system, which is the transfer of nutrient substrate to tissue cells and the removal of waste products, is accomplished primarily by transport processes which occur across the endothelial cells of the capillaries. There is a close relationship between the ultrastructure of the capillary cells and their characteristics as a barrier to fluid and solute transport. Capillary ultrastructure differs among different organs and may even be different from region to region in the same organ. There is also evidence that there are differences in endothelial structure along a given capillary vessel. In spite of these complexities, capillary vessels have been classified according to their ultrastructure, and three distinct classifications have emerged.

(a) Capillaries with a continuous endothelium and continuous basement membrane. The gaps between the endothelial cells appear to have an average value of 40 Å in radius (or smaller) which have been generally described as a "small pore system". These vessels are found in cardiac, skeletal, and smooth muscle, testis, ovary, central nervous system, lung, skin, and thymus.

(b) Capillaries with a fenestrated endothelium and continuous basement membrane. The fenestrae (windows) in the endothelial barrier have been considered as the "pores" even though they are often seen with a membranous diaphragm covering them. The diameters of the fenestrae have been variously estimated as being between 25 Å to 150 Å for a variety of different tissues. Fenestrated capillaries are found in the kidney glomeruli, peritubular capillaries of the kidney, most endocrine glands, urinary bladder, choroid plexus, ciliary body of the eye, and the gastrointestinal tract.

(c) Capillaries (or, more properly, sinusoids) with an open discontinuous endothelial structure where the endothelial cells are separated by very large gaps. The basement membrane of these capillaries is also discontinuous in structure. The gaps or "pores" in these capillaries have been estimated to range in diameter from 200 Å to 800 Å. Discontinuous-type capillaries are found in the liver, bone marrow, and spleen.

The point to be emphasized here is that the different ultrastructural characteristics can be correlated with the rate at which water or fluid will traverse the barrier. Thus, fluid movement across the discontinous sinusoids of the liver would be greater than the fenestrated capillaries of the intestine which, in turn, would be greater than that which would occur in continuous-type capillaries of skeletal muscle. Direct measurements of filtration coefficients have borne this out in skeletal muscle, kidney glomerulus and intestine, while fluid movement across liver sinusoids has not been extensively investigated.

Although the existence of gaps or pores as the major pathway for fluid exchange has been emphasized in the above discussion, the capillary endothelium can be visualized as a mosaic consisting of multiple parallel pathways, as shown in Figure 2. Pathway 1 represents the movement of water directly across both plasma membranes and the intervening cytoplasm. Although definitive quantitative evidence is lacking for fluid movement through this pathway, it has been estimated by some investigators that 10 to 15 percent of the total water movement from blood to tissue may be via this route. Pathway 2 represents vesicular transport. Current notions are that vesicular transport is important in the movement of large molecules between blood and tissue. Although some fluid may be transported via this

pathway in association with large molecule movement it is most likely a negligible quantity. Pathway 3 is the interendothelial junction, described in detail above as the gap or pore which directly connects plasma and interstitial fluid. The schematic shown is similar to channels seen in the continuous type capillary endothelium. Current research strongly suggests that this is the primary route for fluid movement across the capillary wall.

Studies of the permeability of the capillary wall to macromolecules suggests that there are at least two populations of pores, large pores 300 to 1000 A in diameter, and small pores 40 to 100 A in diameter. The absolute number of large pores is considered to be very small relative to the number of small pores (1/40,000). Pathway 4 represents a "pore" formed by a fused chain of vesicles. Because such channels may have large diameters (500 A), thier potential contribution to fluid exchange could be substantial. Little is known, however, of the rate of formation of vesicular gaps or of their average diameter.

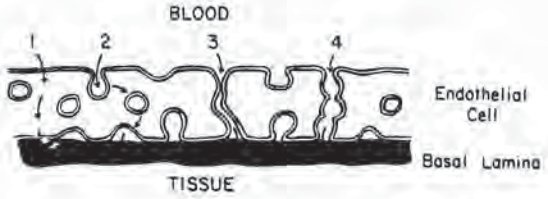

Fig. 2. Pathways for Transcapillary Fluid Exchange.

ULTRAFILTRATION, BULK FLOW, AND THE STARLING BALANCE OF FORCES IN THE CAPILLARY

The magnitude and direction of bulk fluid flow across the capillary endothelium results from a net hydrostatic or osmotic pressure difference across the capillary wall. There are two hydrostatic pressure forces and two osmotic forces acting simultaneously, and net fluid flow is determined by the magnitude and direction of these forces. It is important to emphasize that the permeability or porosity of the capillary wall, although a fundamental property which will influence the rate of fluid flux, has no influence on net transcapillary fluid movement if the osmotic and hydrostatic forces are in balance (that is, the net force across the capillary wall is zero).

Starling (2) was the first to recognize the forces related to net transcapillary fluid movement. The quantitative form of Starling's Law for transcapillary fluid movement is stated as:

$$FM = K_f (P_c + \pi_T - P_T - \pi_{pl}) \qquad (1)$$

where:

FM = net fluid movement across the capillary wall (ml/min)

K_f = capillary filtration coefficient (ml/min mm Hg), i.e., "water permeability" of the capillary wall

P_c = capillary hydrostatic pressure (mm Hg)

P_T = hydrostatic pressure in the interstitial fluid (mm Hg)

π_{pl} = plasma protein osmotic pressure (mm Hg)

π_T = tissue protein osmotic pressure (mm Hg)

If the sum of hydrostatic and osmotic forces (variables in parentheses) is positive, net fluid movement will be from the plasma in the capillary lumen to the interstitial space and this has been termed filtration. If the sum is negative, net fluid flow will be from the interstitial space into capillary lumen and this has been termed absorption. The forces are schematically shown in Figure 3 along with the idea that filtration designates net movement of fluid from plasma to tissue while absorption designates net fluid movement from tissue to plasma. Filtration in excess of absorption leads to accumulation of fluid in the tissue spaces and a clinical condition known as edema. Absorption in excess of filtration leads to loss of water from the tissue and a clinical condition known as dehydration.

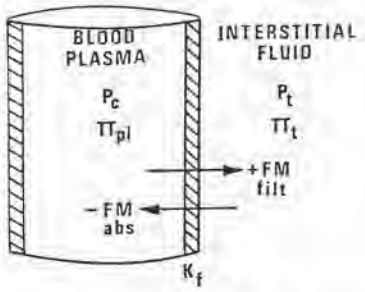

Fig. 3. General Schematic of the Capillary Forces Promoting Filtration and Absorption

The amount of fluid which traverses the capillary wall will be determined by: (a) the magnitude of the sum of the forces responsible for fluid exchange, i.e., by the quantity ($P_c + \pi_t - P_t + \pi_p$). For a given capillary porosity if the quantity is large, fluid movement will also be large, and if it is small, fluid movement will also be small; (b) the porosity (size and number of pores) of the capillary wall. It is apparent that, for a given osmotic or pressure difference across the wall, the larger the pore the more fluid will move across. Porosity is very often equated with permeability. Thus, with increased porosity of the capillary wall, it will be stated that permeability has increased. More rigorously, the term "hydraulic conductivity" (which is the rate of fluid movement per unit pressure difference across the wall) should be used instead of "water permeability". (c) The surface area of capillary wall available for fluid exchange in any given tissue. This factor is highly variable and will be discussed in more detail below. Briefly, the surface area available for fluid exchange is determined by the number of capillaries open to blood flow.

It is readily recognized that net fluid balance in the body must be maintained within narrow limits. The kidneys and associated hormones are in large measure responsible for whole body fluid homeostasis. As a functional part of the body fluid balance, the partition of blood plasma and interstitial fluid is also maintained in rather exact proportions by the mechanisms associated with the Starling balance of forces across the capillary. A full appreciation of this exquisite control system can only be realized from an understanding of the basic forces involved and how they interact. The following discussion is directed toward that objective. Each factor will be discussed individually and an attempt will be made to emphasize the relative importance of the factor in overall transcapillary fluid balance.

The four "forces" responsible for transcapillary fluid exchange are contained in parentheses in Equation 1.

CAPILLARY HYDROSTATIC PRESSURE (P_c)

The contraction of cardiac muscle imparts energy to the blood within the heart and creates a pressure energy. Such pressure energy is manifested first in the arterial system, creating arterial pressure, and later in the capillary system, creating capillary pressure as blood traverses the respective vascular segments. The pressure energy in the aorta has a mean value of approximately 100 mm Hg which is dissipated rapidly, falling to 40 mm Hg in the small artery and arteriolar systems as the viscous resistance to blood flow through vessels is overcome. In the capillary, the pressure is approximately 35 mm Hg at the capillary arterial end and 15 mm Hg at the capillary venous end. Thus, as in all systems, the continued blood flow through the resistance of the capillaries requires a pressure difference or gradient from the arterial to venous ends of the capillary. The mean pressure in most systemic capillaries is approximately 25 mm Hg. Notable exceptions to this value are mean capillary pressures found in the lung (10 mm Hg), liver (10 mm Hg), intestinal mucosa (16 mm Hg), and renal glomerulus (60 mm Hg).

Capillary hydrostatic pressure is a force for filtration, that is, it will move fluid from inside the capillary lumen to the interstitial space if unopposed by other forces. As a determinant of transcapillary fluid movement it is singly the most important and also the most variable because it can be altered on a moment to moment basis by a number of physiologic factors. These factors are: (a) arterial pressure (P_a), (b) venous pressure (P_v), (c) the resistance of the precapillary vessels (R_a), and (d) the resistance of the postcapillary vessels. Since all four of these factors can be influenced by metabolic, hormonal, and neural activity, it follows that capillary pressure has the potential of being altered both rapidly and continually. In fact, the clinical manifestation of excess fluid movement into tissue (edema) is most commonly the result of a sustained increase in capillary hydrostatic pressure by alteration in one or more of the four factors mentioned above. The relationship between the variables can be stated in the following formula for mean capillary hydrostatic pressure, as first recognized by Pappenheimer and Soto-Rivera (3).

$$P_c = \frac{P_a + (R_a/R_v)P_v}{1 + (R_a/R_v)} \qquad (2)$$

For example, suppose that P_a = 120 mm Hg, and it is found that the pre- to postcapillarry resistance ratio in skeletal muscle is 7/1. Under these conditions:

$$P_c = \frac{120 + 7\ (10)}{1 + 7} = 23.8 \text{ mm Hg}$$

This formulation would predict that localized arteriolar (precapillary) vasoconstriction would cause an increase in R_a/R_v (R_a gets larger) which would cause capillary pressure in the region to decrease. For example, with a ratio of 9 to 1, P_c in the equation would be

$$\frac{120 + 9(10)}{1 + 9} \quad \text{or 21 mm Hg}$$

Localized arteriolar vasodilation, alone, on the other hand, would cause capillary pressure to increase. An increase in venous pressure (P_v), alone, would result in an increase in capillary hydrostatic pressure. This is commonly the origin of the peripheral edema seen in patients with congestive heart failure, where venous pressure increases to very high levels (distended jugular veins caused by an increased venous volume and pressure are a classic sign of congestive heart failure). An increase in P_a, alone, would also result in an increased P_c. Although it is reasonably straightforward to see how one variable

might affect P_c when all others are maintained constant, it must be emphasized that, under physiologic conditions of altered neural, metabolic, or hormonal stimuli, all four factors could be altered simultaneously and the resultant change in capillary pressure would depend upon the magnitude of change in each variable. With current technology, it is not possible to directly measure or quantitate all these variables in the intact human. Fortunately, however, in pathological conditions it is generally possible to measure the one predominant variable which initiates edema formation (see discussion below).

INTERSTITIAL FLUID HYDROSTATIC PRESSURE OR TISSUE HYDROSTATIC PRESSURE (P_t)

This force is predominantly a force for moving fluid from tissue to capillary lumen, i.e., it is an absorptive force. It has been measured in a variety of tissues by several different methods, and the exact value of this variable remains controversial. Careful direct measurements with small micropipettes inserted into the tissue fluid have provided values for P_t of approximately + 2 mm Hg. Measurements using chronically implanted perforated capsules, or insertion of an absorbing "wick" into the tissue have provided pressure values which are subatmospheric ("negative") ranging from - 2 to - 8 mm Hg. From a didactic point of view, related to net transcapillary movement, it is not important whether the value for P_t is "negative" or positive. Since, under normal conditions, there is no net fluid movement between the interstitial and vascular subcompartments, it is only necessary that the absorptive forces ($P_t + \pi_{pl}$) be in balance with the filtration forces ($P_c + \pi_T$). Hence, whether P_t is positive or negative in value is of little overall consequence, since its value is small compared to the other factors.

PLASMA PROTEIN OSMOTIC PRESSURE (π_{pl})

Figure 4(A) shows general osmotic pressure relations which would be observed if the chamber on the right hand side of the membrane (side 1) is filled with pure water, and the chamber on the left hand size (side 2) is filled with one gram molecular weight of glucose dissolved in 1.0 Kg of water. Water movement down its activity (concentration) gradient from side 1 to side 2 could be stopped by a hydrostatic pressure of 22.4 atmospheres of pressure placed on the left hand side (side 2) of the membrane. By definition, the glucose solution then exerts an osmotic pressure of 22.4 ATM. In Figure 4(B), the same system has been set up except that human plasma is placed on side 2. In this example, a hydrostatic pressure of over 5000 mm Hg would be required to stop the movement of water from side 1 to side 2. The osmotic pressure of plasma is therefore approximately 5000 mm Hg. Osmotic pressures, of the magnitude generated in this example, are not expressed in the circulatory system, however, for the reasons shown in Figure 4(C). The capillary membrane is permeable to water, Na^+, Mg^{++}, K^+, Ca^{++}, and all other plasma constituents smaller than plasma proteins. These smaller molecules diffuse freely back and forth across the membrane. The net flow of fluid will be from side 1 to side 2 because side 2 has the plasma proteins and thus the greater number of molecules. A hydrostatic pressure of approximately 25 mm Hg would have to be placed on side 2 to stop the flow of fluid. This is exactly the situation. In most systemic capillaries, where the osmotic pressure of the plasma proteins (π_{pl}) exerts an absorptive force of 25 mm Hg, tending to move fluid from the interstitial compartment into the capillary. This is a real force and is equally as effective for producing fluid flow as a hydrostatic pressure of 25 mm Hg.

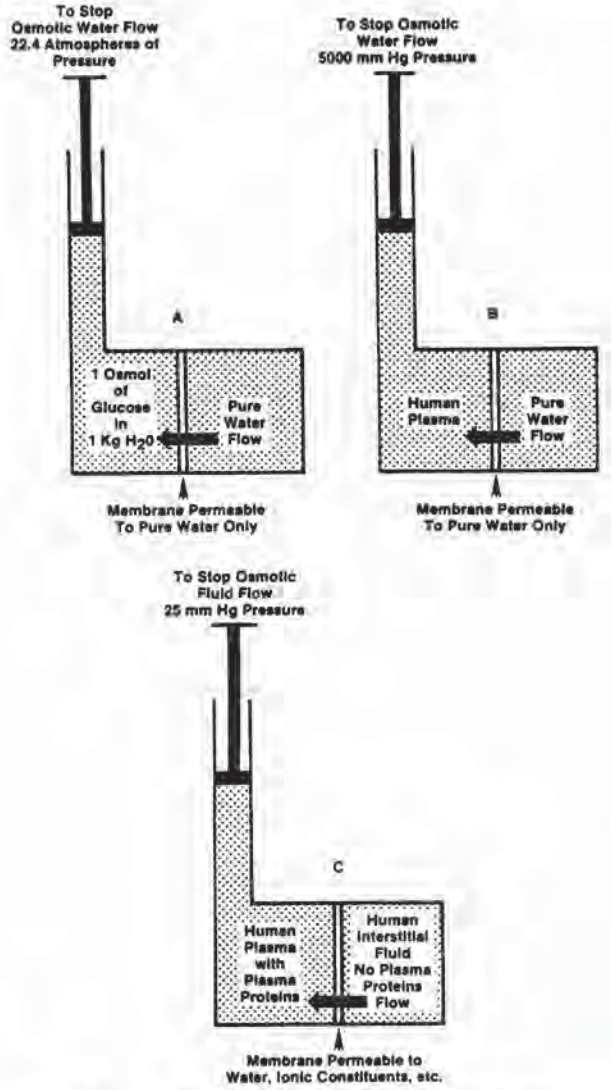

Fig. 4. An Operational Definition of Osmotic Pressure. See text for explanation.

For dilute aqueous solutions, the osmotic pressure can be calculated by the van't Hoff equation:

$$\pi = RTC \qquad (3)$$

where,

π = osmotic pressure
R = universal gas constant
T = absolute temperature (^{o}K)
C = concentration of solute in the solution (e.g., mol/l)

The average protein concentration in human plasma is approximately 7.0 grams/100 ml, which exerts both an actual and theoretical osmotic pressure of approximately 25 mm Hg. Although the mechanism is unknown, it has recently been shown that the relationship between protein osmotic pressure and protein concentration deviates significantly from van't Hoff's equation as proteins are concentrated. That is, if plasma protein concentration is doubled from 7 grams/100 ml to 14 grams/100 ml, the theoretical relation would predict an approximate doubling of osmotic pressure to 50 mm Hg. Direct measurements show, however, that the osmotic pressure of 14 g protein/100 ml fluid is almost 100 mm Hg. The importance of this mechanism for fluid homeostasis can be recognized. If protein-free fluid is quickly lost from the capillary, the proteins in the capillary lumen will be concentrated and produce an osmotic force which will function to attenuate further fluid loss from the vascular compartment.

TISSUE PROTEIN OSMOTIC PRESSURE (π_T)

The large plasma protein molecules traverse the capillary walls and gain access to the interstitial compartment where they exert an osmotic pressure effect. Movement of these macromolecules across the capillary barrier has been attributed to vesicular transport and its movement through some rather poorly defined "large pore" pathway. Tissue protein pressure is a force tending to move fluid from the capillary lumen to the interstitium. It is, therefore, a force for filtration, and the magnitude of the force is related to the concentration of protein in the interstitial compartment, which appears to be directly related to the protein permeability of the capillaries in the different organs. One would suspect that organs with the continuous type capillary ultrastructure (e.g., the skin) would be restrictive to protein movement, and that the concentration of protein in the interstitial compartment would be small. Similarly, organs with fenestrated capillaries (intestine) or the discontinuous type capillary ultrastructure (liver) would have more interstitial protein. The amount of interstitial protein increases in association with the size of the pores or gaps.

It is currently controversial as to whether the protein which is collected in the lymph draining an organ is truly "representative" of the concentration of protein in the interstitial fluid compartment. Although reasons for this controversy will not be addressed here, it is generally accepted that the higher the concentration of protein in the lymph, the more permeable the capillaries to protein and the higher will be the interstitial protein concentration. Table 1 presents a correlation between lymphatic protein concentration and the ultrastructure of the capillary barrier for a few organs. It should be remembered that plasma protein concentration is approximately 7.0 grams/100 ml.

Table 1. Lymph Protein Concentration in Different Organs

Organ	Concentration (g/100 ml)
Choroid plexus	0
Ciliary body	0
Muscle	2.0[1]
Skin	2.0
Lung	4.0
G.I. tract	4.1[2]
Heart	4.4[1]
Liver	6.2[3]

[1] Continuous endothelium

[2] Fenestrated endothelium

[3] Discountinuous endothelium

From Table 1 it can be seen that in some organs the tissue protein osmotic pressure as a force for filtration of fluid from plasma to tissue is negligible (choroid plexus and brain), whereas in others it may be substantial (10-15 mm Hg in lung and intestine, and approximately 20 mm Hg in liver).

Recent studies on the interstitial compartment indicate that tissue protein osmotic pressure, as with plasma protein osmotic pressure, is not a simple, straightforward function of just the protein concentration. The interstitial compartment is now viewed as a two phase gel system, composed of a matrix of mucopolysaccharides (hyaluronic acid, chondroitin, etc.) and glycoproteins from which plasma proteins are excluded, and a "free fluid" phase into which plasma proteins have access. The theoretical tissue protein osmotic pressure will depend upon the amount of the free fluid phase in the tissue and the amount of protein in that fluid phase. It has been shown, however, that there is a complex relation between the proteins and the constituents of the gel phase for in vivo systems (4). For example, the addition of plasma proteins to a solution with

hyaluronic acid shows that the osmotic pressure of the mixture is quite a bit higher than the algebraic sum of the osmotic pressure of the two constituents alone. This suggests that there is a physico-chemical interaction between the molecules to generate osmotic forces out of proportion to theoretical, ideal considerations. The complexities of such a system and its overall influence on in vivo transcapillary fluid flux have yet to be determined.

CAPILLARY FILTRATION COEFFICIENTS (K_f)

The last factor (it is not a force) in Equation 1 is the capillary filtration coefficient, K_f. K_f represents a combination of two factors which are related to the ultrastructure of the capillary as a barrier to ultrafiltration or bulk fluid flow. Conceptually, these two factors are: (a) the capillary surface area (A) available for exchange (with more surface area available there is potential for more fluid flux); and, (b) the hydraulic conductivity (L_p) of the capillary wall. Conceptually, the latter is a function of the porosity of the capillary wall. Hydraulic conductivity (the ability of fluid to move through a pore) will depend upon the size of the pore or gap. With large pores, hydraulic conductivity (or water permeability) is large since fluid flow is approximately related to the size of the pore by the radius to the fourth power (increasing the radius by 2x increases fluid flow 16x for the same pressure difference). Thus, net fluid flux across the capillary wall will be directly and substantially influenced by alterations in either L_p or A.

$$K_f = L_p \times A \qquad (4)$$

Total capillary surface area is determined primarily by the total number of capillaries available for flow. This is an extremely variable parameter which is discussed in more detail below. L_p, or hydraulic conductivity, appears to be a relatively stable parameter under normal circumstances. That is, the porosity or permeability of a given capillary does not grossly change under normal conditions although small changes may occur on a moment-to-moment basis in response to metabolic processes. Hydraulic conductivity may be grossly altered, however, under unphysiologic conditions such as trauma, inflammatory reactions, or burns, where the porosity of the capillary wall is increased.

THE STARLING BALANCE OF FORCES IN THE CAPILLARY

Each of the factors in the Starling relation (Equation 1) has been discussed above as an independent entity. Within the tissues, however, all the factors must be considered simultaneously to determine the rate and direction of net fluid movement. For purposes of this discussion we will consider that the porosity of the capillary wall does not change and the surface area of the capillary is constant (K_f). Under these conditions, a simple schematic diagram can be constructed to visualize the forces acting across the capillary wall and the net direction of fluid flow as it is related to these forces. The simplest physiological situation is shown in Figure 5. In this figure, the capillary is shown as a straight, tubular structure. The blood perfusing the capillary contains normal plasma proteins which exert an absorptive force of 25 mm Hg, tending to draw fluid from the interstitium into the capillary lumen. This is illustrated by a dashed line running through the center of the capillary. The hydrostatic capillary pressure, at the arterial side of the capillary, is shown in this example to be 34 mm Hg. As blood traverses the length of the capillary to the venous side, there is a pressure drop because of the resistance to fluid flow created by the capillary lumen. At the venous end of the capillary, in this example, the hydrostatic pressure, P_c, is (34 + 17)/2 = 25.5 mm Hg, which is a filtration force tending to move fluid from plasma to interstitium. Since the absorptive force and the filtration force are equal and act in opposite directions, there is no net fluid movement across the capillary wall. It should be noted, however, that the net transmural (across the wall) pressure gradient at the beginning of the capillary favors net filtration of fluid ($P_c > \pi_{pl}$), whereas, at the venous side, the net transmural pressure gradient favors net absorption of fluid ($\pi_{pl} < P_c$).

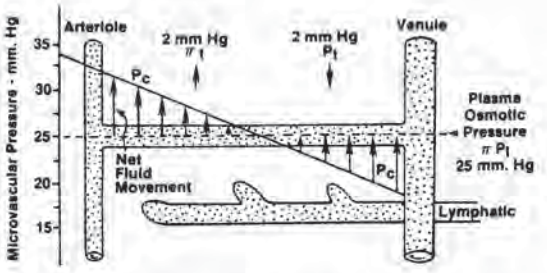

Fig. 5. General Schematic of the Capillary and Forces Which Govern Net Fluid Movement

In our example, the net force for filtration (arterial end) is $(P_c - \pi_{pl})/2 = (34 - 25)/2 = 4.5$ mm Hg. The small fraction of fluid which is not reabsorbed enters the lymphatics and returns to the systemic circulation via lymphatic channels, which ultimately discharge into the great veins near the heart. The classic view of transcapillary fluid movement (which is still generally accepted) is that normal interstitial fluid hydrostatic (P_I) and osmotic (π_T) pressures are equal and opposite at a value of + 2 mm Hg and - 2 mm Hg, respectively, and thus play no role in fluid movement. Inserting such values into Equation 1 yields:

$$FM = K_f (P_c - \pi_{pl} - P_T + \pi_T)$$

$$FM = K_f (25.5 - 25 - 2 + 2) = + 0.5\ K_f$$

or, there is a 0.5 mm Hg pressure which favors a small net filtration of fluid from plasma to tissue.

If, on the other hand, one uses the values for negative interstitial fluid hydrostatic pressure which can be found in the literature, the net effect of using a negative value is to change P_t from a force promoting absorption to a force promoting filtration. That is, a subatmospheric pressure tends to increase the transmural pressure gradient or, to view it another way, the negative pressure tends to "suck" fluid from the capillary intravascular compartment. If tissue fluid pressure is indeed negative, there are now three forces in Equation 1 which will promote fluid movement from plasma to tissue (P_c, P_T, and π_T). Since we know that, normally there is fluid balance (with only a small residual net filtration pressure), the three filtration forces are opposed only by the absorbing force of plasma protein osmotic pressure (π_{pl}). The three filtration forces, of necessity, cannot exceed the value for π_{pl} = 25 mm Hg except by some small amount. Since π_T = 2 mm Hg and $P_T = \pi - 4$ mm Hg, we must assume that P_c is only 19.5 mm Hg in order to balance the forces and leave a small residual filtration pressure. Thus,

$$FM = K_f (P_c - \pi_{pl} - P_T + \pi_T)$$

$$FM = K_f (19.5 - 25 - (-4) + 2) = +0.5\ K_f$$

Larger negative values for P_T must be accompanied by a lowering of values for P_c to maintain net fluid balance. It is worthy of note that manipulating any of the values in Equation 1 does not alter the basic and fundamental concept of the Starling balance of forces which simply states that, in a steady state, filtration forces and absorption forces are equal and opposite, and there is no net transcapillary fluid movement of any great magnitude. The relationship shown in Equation 1 can be usefully employed to predict the rate and direction of fluid transfer between the vascular and interstitial subcompartments in all physiological and pathological situations.

MECHANISMS OF EDEMA FORMATION

The pathophysiologic state in which an excessive, clinically observable accumulation of fluid occurs in the interstitial space is defined as edema. Qualitatively, the amount of fluid accumulation in humans is classified (especially for edema of the arms and legs) as 1+, 2+, 3+, 4+ "pitting" edema, with one being a small amount and four being a large amount of fluid. The "pitting" occurs because simple pressure with the finger on the affected area distorts the tissue made "soft" from fluid and leaves a "pit" which generally takes a few seconds to recoil back to normal. All cases of edema are related to alterations in forces or factors in the Starling relation, or to defects in the ability of the lymphatics to successfully clear the tissues of excessive interstitial fluid accumulation. A few examples are presented here, classified according to the Starling force which is affected.

A. Increased capillary hydrostatic pressure.

This generally results from an alteration on the venous side of the circulation. Examples are: (a) thrombophlebitis (a local blood clot in the venous vasculature), which increases local venous pressure and causes a compensatory rise in P_c upstream from the clot. Filtration caused by the increased P_c exceeds absorption and edema results. (b) Pulmonary hypertension; here, the resistance to blood flow in the pulmonary circuit is increased. With increased pulmonary vascular resistance, pulmonary artery pressure rises, followed by increased right ventricular end diastolic, right atrial and great vein pressures. The increased large vein pressures are transmitted to the systemic capillaries which, again, results in filtration in excess of absorption and edema. (c) Diseases of the heart, such as congestive heart failure and pericarditis, follow the same general format as that presented for pulmonary hypertension, with increased right or left ventricular end diastolic pressure leadng to an increased great vein and systemic capillary pressure which results in edema. (d) Cirrhosis of the liver and/or hepatitis will often cause either ascites (liver "sweating") or edema because of an obstruction to liver outflow, thus increasing porta venous and capillary pressures.

B. Decreased tissue hydrostatic pressure.

This is not important as an edemogenic stimulus. There are no known disease entities which decrease tissue hydrostatic pressure.

C. Decreased plasma protein osmotic pressure.

As the major force for absorption of fluid, any abnormality which reduces plasma protein concentration (hypoproteinemia) will tend to create an imbalance between filtration and absorption and lead to edema. (a) Nephrosis causes an excessive loss of protein in the urine (proteinuria), lowering the plasma protein osmotic reabsorbing force, and leads to edema. (b) Some gastroenteropathies lead to loss of plasma protein into the intestine, resulting in edema formation. (c) A disease not prevalent in the United States but very prevalent in third world countries is Kwashiorkor, or hunger edema. The poor diet does not provide enough substrate for plasma protein synthesis. Hypoproteinemia and generalized edema result. (d) Cirrhosis of the liver can result in the liver not synthesizing sufficient albumin to produce normal plasma protein concentratons. Here, also, the hypoproteinemia reduces the capillary absorbing force so that filtration exceeds absorption and edema results.

D. Increased tissue protein osmotic pressure.

Myxedema, caused by thyroid gland hypofunction, causes a progressive infiltration of mucoproteins and mucopolysaccharides into the interstitium of the skin. This results in an increased interstitial osmotic pressure, which is a

force promoting filtration in excess of absorption of fluid, and results in a generalized skin edema.

E. Increased porosity of the capillary wall.

This is a factor (K_f) in the Starling equation. Increased porosity (permeability) occurs in several pathophysiologic states, the most common of which are burns and inflammatory responses. Here, the capillary wall loses the capacity to function as an effective barrier for protein loss, as well as opening up large gaps through which fluid may move. Since capillary pressure may be either normal or elevated in such situations, large quantities of fluid are lost from the vascular compartment to the interstitial space. Phosgene or mustard gases (war gases) kill and maim by destroying capillary integrity.

F. Lymphatic obstruction.

Normally, there is a small amount of filtration in excess of absorption which is taken up by the lymphatics and returned to the systemic circulation. Burns, inflammation, tumors, and filaria are all potential causes of lymphatic obstruction which will result in clinically observable edema.

EDEMA PROTECTIVE MECHANISMS

Since the partition of fluid between the intravascular and interstitial subcompartments is of crucial importance to normal biological function, it would be expected that the "system" was designed with some margin of safety for the organism. Some protective mechanisms against edema formation are: (a) fluid removal by the lymphatics; (b) as fluid accumulates in the interstiitium from an edemogenic stress, there is a concomitant increase in tissue hydrostatic pressure (P_T) which opposes net filtraton of fluid; (c) a reduction in tissue protein osmotic pressure (π_T) occurs as fluid dilutes the tissue proteins. This reduces the filtration force of π_T and net fluid movement. (d) The large increase in plasma protein osmotic pressure as fluid is lost from the capillary tends to concentrate plasma proteins and augment their absorbing force. Net filtration of fluid into the tissue is thus reduced.

CAPILLARY SURFACE AREA AVAILABLE FOR FLUID EXCHANGE

Throughout this discussion, the capillary surface area available for fluid exchange has been alluded to but not specifically addressed. The reason for this is that, despite the extreme importance of this factor, it is an extremely complex and poorly understood variable. The total capillary surface area for exchange in the human is very large, with estimates ranging from 5000 to 7000 square feet. These estimates are based on the anatomicaly available vessels, which is a function of the total number of capillary vessels, or vascularization, of tissues. The anatomical "richness" or density of capillaries is variable from tissue to tissue. For example, the thyroid gland and the heart are rich in capillaries, with a high density per organ weight, while the cornea of the eye has a very sparse capillary supply. The epiphyseal cartilage and the lens of the eye lack any visible capillary supply, and nutrient exchange occurs by diffusive processes in surrounding fluids which are in contact with these tissues. Thus, there is wide anatomical variability in organ capillary surface area. In addition, capillary surface area available for exchange is physiologically variable. For example, in resting skeletal muscle perhaps 10% or less of the available capillaries are open for flow and exchange. In exercising skeletal muscle, metabolic vasodilation will open a large number of capillaries to blood flow, and surface area for exchange can be increased some two to five fold. The number of capillaries open for flow can be influenced by neural, hormonal, metabolic, and, in some tissues, myogenic factors. Little quantitative data are available on the regulation of capillary density or the factors which control it. This remains an area for quantitative investigation and, until the results are in, an important element in our understanding of transcapillary fluid exchange and microcirculatory function will be lacking.

REFLECTION COEFFICIENTS

The student reading today's literature on exchange of molecules across the microvasculature will be exposed to the concept of the reflection coefficient (σ). The definition of the reflection coefficient is the ratio of the observed osmotic pressure (π_{obs}) to the theoretical osmotic pressure (π_{theo}) for a given membrane and solute. That is, $\sigma = \pi_{obs}/\pi_{theo}$, where π_{obs} is a measured variable and π_{theo} is the osmotic pressure which would be produced across an ideal semipermeable membrane (i.e., a membrane permeable only to water). It turns out that the osmotic pressure generated across a permeable membrane by a given solute molecule is uniquely sensitive to the porosity of the membrane. If the membrane is ideally semipermeable and water, but not the solute molecule, can move through the pores, $\pi_{obs} = \pi_{theo}$, then the reflection coefficient is said to be 1. If the membrane is permeable to both water and solute, then the reflection coefficient will decrease to values less than 1 but greater than 0. $\sigma = 0$ when both water and solute molecules move across the membrane with equal freedom and an osmotic pressure cannot be generated. The mechanism by which a permeable membrane modifies the effective osmotic pressure of solute molecules is not known, although several attractive hypotheses have been advanced (5). For transcapillary fluid movement in mammalian tissues, the reflection coefficient is important only because it infuences the net osmotic pressure which can be generated by the protein molecules on either side of the capillary membrane. Albumin molecules contribute 66% of the total to the osmotic pressure of plasma proteins (there are many more albumin molecules in plasma than globulin,

fibrinogen, or α- or β-lipoproteins. We will not be concerned with the distribution of reflection coefficients for different plasma proteins which, if one were to be rigorously accurate, should be considered. The purpose of this discussion is to illustrate the effect of the membrane reflection coefficient on net transcapillary fluid movement through its alteration in protein osmotic pressure. In the brain, skeletal muscle, choroid plexus, ciliary body, and skin, the reflection coefficient is essentially one; that is, the full theoretical osmotic pressure can be generated by the plasma proteins. In these tissues, Equation 1 will hold without modification. In tissues such as intestine, heart, and liver, the porosity of the membrane modifies the effective osmotic pressure of the plasma proteins. Equation 1 is then modified with σ as follows:

$$FM = K_f[(P_c - P_t) + \sigma(\pi_t - \pi_{pl})]$$

Notice that, if the reflection coefficient is 0.6 (as has been found in the intestine), then the osmotic pressure produced by both the plasma proteins (π_{pl}) and tissue protein (π_t) is only 60% of the theoretical value. For the Starling balance of forces to be functional so that no net transcapillary fluid movement occurs, all forces must be reduced accordingly. For example, if we assume a theoretical value of 25 mm Hg for π_{pl}, 2 mm Hg for π_T, and a $\sigma = 0.6$, the values in Equation 1 must be modified to show that π_{pl} exerts an absorptive force of only 15.0 mm Hg (0.6 x 25) and π_T a filtration force of 1.2 mm Hg (0.6 x 2.0). Thus,

$$FM = K_f(P_c - \pi_{pl} + \pi_T - P_T)$$

$$FM = K_f(16.3 - 15 + 1.2 - 2) = + 0.5\ K_f$$

It can be seen that capillary hydrostatic pressure must be assumed to be a lower value in the intestine to maintain net transcapillary fluid balance.

Finally, it must be stated that a considerable amount of freedom has been used during this discussion of transcapillary water flux for purely didactic reasons and to achieve some semblance of brevity in an otherwise complex and extensive area of biological homeostasis. The values used in Equation 1 vary considerably from tissue to tissue, and some values have never been quantitatively substantiated (e.g., the values in the literature for π_T vary from + 1 to + 10). To review the literature for all the estimated values, for all organs or tissues, and for all forces and factors, would be a very large task indeed. The essential points to be made here are: (a) the general correlation between microvascular structure and function; (b) the general concept of transcapillary fluid balance as a function of the Starling balance of forces, and (c) a general understanding of the physiological and physicochemical mechanisms which relate to the forces and factors involved in fluid homeostasis between the vascular and interstitial subcompartments of the body.

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Chapter 4

TRANSPORT OF MOLECULES ACROSS THE GLOMERULAR PERITUBULAR RENAL CAPILLARIES

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The kidney provides a unique opportunity for the study of capillary permeability as a result of the fact that two anatomically and functionally distinct capillary beds are accessible for study by micromethods. These are the glomerular and peritubular capillary systems of the mammalian renal cortex. The direction of transcapillary fluid flux is determined, as in other tissues, by the magnitude of the Starling pressures acting across the capillary wall, the water permeability, and the capillary surface area. At the glomerulus the hydraulic pressure difference and water permeability are relatively high, ensuring a rapid rate of filtration. In contrast, net reabsorptive pressures normally persist throughout the entire peritubular capillary network. New and interesting findings about the nature of capillary permeability have shown that both capillary beds demonstrate permselective properties not only on the the basis of molecular size, but also an electrostatic barrier exists which preferentially restricts the transglomerular movement of anionic molecules, compared to similarly sized neutral or cationic macromolecules. An electrostatic barrier may also exist in peritubular capillaries.

The gross anatomy of the renal vascular supply is complex and there is marked heterogeneity of vascular organization within the kidney. In this presentation only the blood vessels of the renal cortex are considered, a region in which approximately 90% of the glomeruli are located. Most of the data described below were derived from experiments on the rat.

The glomerulus is unusual in that afferent and efferent arteriolar resistance vessels are arranged "in series" before and after the glomerular capillaries. The glomerulus shows a greater degree of organization than do most other capillary beds. The afferent arteriole splits to form a series of capillary loops which eventually join again to form the efferent arteriole. Lateral vessels form anastomoses between loops. An individual glomerulus is immediately surrounded by a Bowman's space, which represents the beginning of the nephron (1,2). A second and more anatomicaly "conventional" capillary system, the peritubular capillary, is located downstream to the glomerulus, interposed between the efferent arterioles and the venules. The peritubular capillaries form an extensive branching network which is located close to the cortical tubules (all the proximal tubules and some distal tubules) but with interstitium interposed between. The capillary plexus is continuous and individual nephrons are not invested with an individual peritubular blood supply. However, in the superficial cortex, some parts of the proximal convoluted tubule do receive the major part of their blood supply from the parent glomerulus (2,3).

Histologically, the glomerulus consists of three major components: firstly, the endothelial cells which lie on the blood side of the capillary wall and are separated by large gaps or "fenestrae". Unlike most other fenestrated capillaries, the glomerular endothelial fenestrae are not spanned by a thin membrane. Next comes the glomerular basement membrane (GBM), a gel supported by a fiber matrix; and thirdly, the epithelial cells whose bodies project into Bowman's space. Glomerular epithelial cells possess a highly organized arrangement of projections, the so-called "foot processes" which are in close contact with the surface of the GBM. The spaces between adjacent foot processes are narrow and uniform in diameter and are referred to as the "epithelial slit pores". These appear to be invested with a fine membrane (1,2). In addition, mesangial cells are located in the center of the glomerulus at the base of the individual capillary loops, usually in a subendothelial position, and possess contractile elements (1). This emphasizes another unique aspect of the glomerulus as a capillary bed, since it possesses an intrinsic contractile capability. The functional implications will be considered later. The ultrastructure of the peritubular capillary network is more akin to the peripheral capillaries, especially those of the viscera. On the blood side, the capillary endothelium is fenestrated like that of the glomerulus, and beneath the endothelium lies a continuous basement membrane layer. The fenestrae are bridged by a thin membrane. On the interstitial side, however, no organized epithelial cell layer is evident (2).

Functionally, as well as anatomicaly, the glomerular and peritubular microcirculations are quite distinct; the first step in the process of urine formation occurs at the glomerulus, i.e., the production of an ultrafiltrate of plasma. During passage down the nephron this ultrafiltrate is modified by various reabsorptive and secretory processes. Within the proximal tubules approximately 60% of the filtered sodium and water

are absorbed (as an isotonic solution) into the renal interstitium which surrounds the tubules. This absorbate may either leak back into the tubule or be taken up into the circulation by reabsorption into the peritubular capillaries (a small percentage of the tubular reabsorbate also enters the circulation via the lymphatics). Under normal antidiuretic conditions approximately 99% of the filtered fluid is eventually reabsorbed into the circulation.

The filtering and reabsorptive processes of the glomerular and peritubular capillaries, respectively, are determined by the Starling forces which operate across the capillary walls. Because of the unique anatomy of the kidney, which provides ready access to the capillaries and their environment, much information regarding the magnitude of these forces and the relative importance of the functional determinants of filtration and reabsorption has been derived from micropuncture studies, discussed below.

As described in detail in Chapter 3, the net rate of fluid flux across any capillary is determined in part by the imbalance between the hydraulic and oncotic pressure gradients across the capillary wall. The glomerulus is an exclusively filtering capillary, and the determinants of the total fluid filtered at one glomerulus in a unit of time (the single nephron glomerular filtration rate, SNGFR) are given in the Starling equation:

$$SNGFR = (\overline{\Delta P} - \Delta\Pi) \cdot K_f$$
$$= [(P_{GC} - P_{BS}) - (\Pi_{GC} - \Pi_{BS})] \cdot k \cdot s$$

where $\overline{\Delta P}$, the mean net transglomerular hydraulic pressure difference, is determined by the difference between the intraglomerular hydraulic pressure, P_{GC}, and the hydraulic pressure in Bowman's space, P_{BS}; $\Delta\Pi$, the mean net transglomerular oncotic pressure difference, is determined by the corresponding oncotic pressures; and the glomerular capillary ultrafiltration coefficient, K_f, is the sum of the glomerular water permeability, k, and the available filtration surface area, S.

Discovery of a mutant rat strain, the Munich-Wistar, which possesses glomerular capillaries on the immediately subcapsular surface of the kidney, has permitted direct measurement and thence quantitation of all the parameters that determine glomerular filatration. SNGFR is measured by computing the single nephron inulin clearance, the product of tubule fluid-to-plasma inulin ratio, and the fluid flow rate at the collection point in the tubule (fluid is collected by a "free-flow" collection technique using micropipettes inserted into the tubular lumen). Thus, it is possible to measure directly the rate of fluid flux from the glomerular capillary. The measurements of hydraulic pressures on either side of the filtering membrane are made by puncturing the glomerular capillary loops and the proximal tubule (since proximal tubule hydraulic pressure, P_T, has been shown to be equivalent to the hydraulic pressure measured in the Bowman's space of the same nephron (4)). The problems of measuring pulsatile pressures in small vessels (the diameter of a rat glomerular capillary loop is approximately 8 microns) are overcome by the use of the servonull pressure measuring device originally designed by Weiderhelm et al. (4). The corresponding oncotic pressures at the glomerulus are calculated from the measured protein concentrations in pre- and post-glomerular blood. Pre-glomerular blood is collected from the femoral artery, and post-glomerular blood from superficially located efferent arterioles or "star vessels", the latter by direct micropuncture. The plasma albumin-to-globulin ratio is approximately 1.0 in the rat, and the oncotic pressure may be calculated from the total protein concentration by the Landis-Pappenheimer equation (4). Because the glomerulus provides a very efficient barrier to the filtration of protein (discussed below), Π_{BS} may be regarded as negligible. The glomerular capillary ultrafiltration coefficient, K_f, may be calculated from the other measured variables in the Starling equation (4).

Direct measurements of glomerular capillary hydraulic pressure, P_{GC}, have demonstrated that this value averages approximately 40% of the arterial blood pressure (approximately 45 mm Hg), a value considerably lower than the indirect estimates previously assumed to be correct, but nevertheless, significantly higher than the hydraulic pressure in the peripheral capillary beds. The presence of the efferent arteriolar resistance vessel serves to maintain a high value of P_{GC}; indeed, variations in tone of the afferent and efferent arterioles allows the P_{GC} to be controlled independent of the arterial blood pressure within the autoregulatory range of the kidney (4). The measurement of P_{GC} represents an average value for the capillary network since it is not possible to puncture discrete sites along the glomerulus. A fall in the hydraulic pressure of the glomerular blood must occur from afferent to efferent ends of the network in order for forward flow of blood to occur. It seems likely, however, that this pressure drop is small, for reasons discussed elsewhere (4). The Bowman's space hydraulic pressure is low, approximately 10 mm Hg, and thus the transglomerular hydraulic pressure difference (ΔP) averages approximately 35 mm Hg (Figure 1a).

Measurements of pre- and post-glomerular protein concentrations have demonstrated that protein concentration rises as blood flows through the glomerular network, a consequence of the formation of a protein-free ultrafiltrate. Under control conditions in the rat, pre- and post-glomerular protein concentrations average 5-6 and 8-9 g/dl, respectively; thus, oncotic pressure rises along the glomerulus from a starting value of approximately 20 mm Hg (Π_A) to a value of approximately 35 mm Hg at the efferent end (Π_E). For reasons discussed elsewhere, the glomerular oncotic pressure profile is non-linear, rising most steeply at the afferent end of the glomerulus (Fig. 1, panel a).

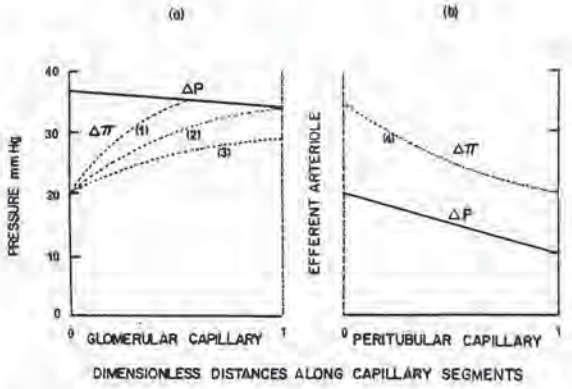

Figure 1. Hydrostatic and Oncotic Pressures in the Glomerulus. Several possible profiles of the transcapillary oncotic pressure difference, (broken lines) for a given transcapillary hydraulic pressure difference, ΔP (solid lines) along an idealized glomerular capillary (panel a) and peritubular capillary (panel b). See text for details.

It is not technically possible to sample blood from sites along the glomerulus, thus the oncotic pressure profile is computed, using modelling assumptions discussed elsewhere (4). Under control conditions in the normal Munich-Wistar rat, the transglomerular oncotic pressure difference ($\Delta\Pi$) rises to a value equal to and opposite to the corresponding hydraulic pressure ($\overline{\Delta P}$) by the time plasma reaches the efferent end of the glomerulus (Fig. 1a, curve 1). In this condition, referred to as filtration pressure equilibrium, no net ultrafiltration pressure therefore exists by the end of the glomerulus. At filtration pressure equilibrium it is not possible to construct a unique $\Delta\Pi$ profile from the measured values of pre- and post-glomerular oncotic pressure (Π_A and Π_E) since, as shown in Fig. 1a, curves 1 and 2 are but two of an infinite number of $\Delta\Pi$ profiles consistent with given values of Π_A and Π_E. Thus, at filtration pressure equilibrium it is not possible to derive an exact value of the mean net ultrafiltration pressure (P_{UF}), equal to the area between the ΔP and $\Delta\Pi$ curves. This in turn implies that an exact value of the glomerular capillary ultrafiltration coefficient, K_f, cannot be calculated either (from equation 1). However, if the rate of rise of Π along the glomerulus is assumed to be linear, it is possible to calculate a maximum value of P_{UF} and a minimum value of K_f (4). In some experimental (and chronic pathological) conditions (and in other species), filtration pressure disequilibrium may occur; here ΔP exceeds Π_E and thus a positive net ultrafiltration pressure occurs over the entire length of the glomerulus, as given by curve 3 in Fig. 1a. At filtration pressure disequilibrium, an exact $\Delta\Pi$ profile may be computed from the measured values of Π_A and Π_E, thus permitting precise calculations of P_{UF} and K_f (4).

Although not appearing explicitly in equation 1, the rate of plasma flow to the glomerulus (Q_A) will certainly influence the value of SNGFR since changes in Q_A will affect the rate of rise of $\Delta\Pi$ along the glomerulus. Increasing Q_A will attenuate the rate of rise of $\Delta\Pi$, thus increasing P_{UF} and also SNGFR (4). When Q_A is increased to supernormal values, the rate of rise of $\Delta\Pi$ is attenuated to such an extent that filtration pressure disequilibrium is created and, in this condition, the dependency of SNGFR on Q_A is diminished, i.e., smaller rises in SNGFR will result from a given increment in Q_A. This relationship between SNGFR and Q_A was predicted using mathematical models and subsequently confirmed in many experimental settings (4).

The technique of increasing Q_A to sufficiently high values to create filtration pressure equilibrium has been employed experimentally in the Munich-Wistar rat to enable calculation of exact values of the ultrafiltration coefficient, K_f. Values of K_f calculated in this manner range from approximately 0.06-0.08 nl/(sec · mm Hg); furthermore, K_f remains constant over a wide range of Q_A (4), suggesting that, at filtration pressure disequilibrium, changes in plasma flow rate "per se" do not influence K_f. Thus, a value of K_f of approximately 0.06-0.08 nl/(sec · mm Hg) is considered normal for the Munich-Wistar rat.

Micropuncture studies do not permit independent quantitation of the component variables which comprise K_f, i.e., filtration surface area (S) and effective water permeability (k). However, S has been estimated for the rat glomerulus, by the use of other techniques, to average approximately 2 to 3 x 10^{-3} cm^2 (4,5) and substitution of these values into the Starling equation yields values of glomerular water permeability, k, in the range of 30-40 nl/(s · mm Hg · cm^2). Thus, the water permeability of the rat glomerulus is between one and two orders of magnitude greater than reported for peripheral capillaries (4,5). This very high value of k therefore allows glomerular filtration to proceed at a rapid rate despite a mean driving pressure of only a few mm Hg (4).

Glomerular micropuncture studies have indicated that the mammalian glomerulus is a target for a number of hormones and that control of filtration may be exerted by acute variations in K_f. Table 1 summarizes the agents which have been shown, in acute studies, to reduce the value of K_f. Several studies have demonstrated that the potent vasoconstrictor angiotensin II (AII) has an acute action on K_f and "in vitro" observations have shown that isolated glomeruli and, indeed, isolated mesangial cells, contract in response to administered AII (6). Furthermore, the "in vivo" action of AII to reduce K_f may be reversed by simultaneous administration of verapamil or manganese, both agents known to antagonize Ca^{++} entry into smooth muscle cells. Taken together, these data are strongly suggestive that the AII-evoked fall in K_f is mediated by Ca^{++}-induced contraction

of mesangial cells and thence reduction in filtration surface area. Since the PGE_1 and ACh-evoked decreases in K_f are also abolished by verapamil it seems likely that these agents cause Ca^{++} dependent mesangial contraction.

There is evidence to suggest that not all agents act directly on mesangial cells; for example, neither PGE_2 nor PTH produce contraction of isolated rat mesangial cells. Further, the "in vivo" PGI_2-evoked fall in K_f may be prevented by prior inhibition of AII receptors with saralasin. It is also possible that factors affecting K_f may operate through the cyclic nucleotide system since dibutyrl cAMP has been shown to lower K_f, and several of the agents which reduce K_f, such as PTH and ADH, are known to cause increased glomerular cyclic nucleotide generation (6). Whatever the subcellular mechanisms causing K_f to fall in response to the various agents listed in Table 1, it is evident that the glomerulus possesses the unique ability to acutely vary both surface area and/or water permeability.

Table 1. Summary of Agents Which Acutely Reduce K_f

Agent	Possible Mediator
High calcium	direct
Angiotensin II	Ca^{++}
Prostacyclin	Ca^{++} (via AII)
Prostaglandin E_1	Ca^{++}
Acetylcholine	Ca^{++}
Bradykinin	?
Histamine	?
Dibutyrl cAMP	direct ?
Antidiuretic H (ADH)	cAMP ?
Parathyroid H (PTH)	cAMP or Ca^{++} ?

The relative importance of the various determinants of filtration, discussed above, will change according to whether filtration pressure equilibrium or disequilibrium exists. At filtration pressure equilibrium, the normal state for the Munich-Wistar rat, changes in plasma flow (Q_A) will lead to direct and proportional changes in SNGFR without change in single nephron filtration fraction (SNFF). The magnitude of the SNFF will be determined by the transglomerular hydraulic pressure difference ($\overline{\Delta P}$) and the pre-glomerular protein concentration (Π_A). At filtration pressure disequilibrium, however, the plasma flow dependence of filtration declines and the SNFF is now determined by ΔP, Π_A and K_f.

The peritubular capillaries operate in quite a different way, with net reabsorption rather than net filtration as observed in the glomerulus. A large body of evidence indicates that the absolute rate of proximal reabsorption (APR) is modulated by Starling pressures in the peritubular capillaries. The Starling equation, written in terms specific for the peritubular capillary is is given below:

$$APR = (\overline{\Delta P}' - \Delta\Pi') \cdot K_r \qquad (2)$$
$$[(P_c - P_i) - (\Pi_c - \Pi_i)] \cdot k' \cdot S'$$

where $\overline{\Delta P}'$ is the mean net transperitubular capillary hydraulic pressure difference, the difference between hydraulic pressures in the peritubular capillary (P_c) and renal interstitium (P_i); $\Delta\Pi$ is the transperitubular capillary oncotic pressure difference determined by the difference between capillary and interstitial oncotic pressures, Π_c and Π_i, respectively. Hydraulic pressure is measured by direct puncture of the peritubular capillaries. At the efferent arterioles, i.e., at the beginning of the peritubular capillary network, this pressure averages approximately 20 mm Hg. Hydraulic pressure declines approximately linearly with distance along the capillary, to a value of approximately 10 mm Hg in terminal peritubular capillaries (3,7). Peritubular oncotic pressure, Π_c, declines progressively along the length of the capillary since the entering reabsorbate dilutes the plasma proteins. The profile of Π_c is predicted to be non-linear and may be calculated from the measured efferent arteriolar protein concentration using a mathematical model (7).

Hydraulic and oncotic pressures in the interstitium are both low, averaging 3-5 and 2-3 mm Hg, measured by direct puncture of the subcapsular space and from the protein concentration of renal hilar lymph, respectively (7). As shown in Fig. 1b, the transperitubular capillary hydraulic pressure gradient, $\Delta P'$, is always lower than the corresponding oncotic pressure gradient, $\Delta\Pi'$, allowing a positive net reabsorptive pressure through to the end of the peritubular capillary network, i.e., reabsorption pressures are always at disequilibrium. This must hold since the

maximum possible dilution of Π_c would produce an oncotic pressure equivalent to Π_A, which is approximately 20 mm Hg, a value approximately 50% above the hydraulic pressure in the terminal peritubular capillary. By analogy with the glomerulus, the APR would therefore be expected to be relatively insensitive to changes in post-glomerular plasma flow rate, A_E (7). Reduction in Q_E to subnormal values will be predicted to significantly influence APR, as discussed elsewhere (7).

The value of the peritubular capillary reabsorptive coefficient, K_r, averages about 0.04 nl/(s·mm Hg) in control conditions. It is not at present known whether acute variations in K_r can control APR; however, aortic constriction, a maneuver known to cause increased renal AII and prostaglandin production, has no effect on the value of K_f (7). Nevertheless, it should be noted that, when peritubular capillaries are microperfused "in vivo" with AII solutions, reductions occur in capillary diameter, and it has been suggested that this represents an active contraction rather than a passive response to changes in interstitial or intratubular pressures (8).

Many different experimental approaches have demonstrated a major role of the post-glomerular protein concentration, and thence, Π_E, in the control of APR; however, some conflicting observations have been reported (7). Recent studies by Ichikawa and Brenner have highlighted the importance of peritubular capillary hydraulic pressures (P_c) in the control of APR, with control of P_c modulated by the efferent arterioles (7).

All capillaries exhibit a "sieve-like" property in that their permeability to large molecules decreases with increasing molecular weight (9). This "permselective" property has been studied at the glomerulus by the use of polydisperse tritiated dextrans, macromolecules homologous with respect to chemical structure and molecular shape, but whose molecular weight varies over a wide range. Comparison of the urinary excretion of given sized dextran to that of inulin (a substance which crosses the glomerular capillary as freely as does water) gives an index of the degree of restriction, if any, to the transglomerular transport of that dextran. This type of analysis relies on the assumption that the test dextran, once filtered, is not secreted or reabsorbed, exactly as is the case with inulin. This assumption has been directly tested and found to be valid by measuring the concentrations of the various molecular weight dextrans and inulin simultaneously in plasma and in Bowmans space or urine. The ratio of the clearance of a given molecular weight dextran to the clearance of inulin can then be computed (the fractional clearance). Fig. 2a, curve I, shows fractional clearance (FC) data calculated according to Equation 3.

$$FC = \frac{(U/P_D) \cdot V}{(U/P_{In}) \cdot V} \qquad (3)$$

U/P is the urine-to-plasma dextran (D) or inulin (In) concentration ratio and V is the urine flow rate (9). A fractional clearance value of 1.0 indicates that dextran clearance equals clearance of inulin, implying completely unrestricted transport of that dextran. In Fig. 2a, fractional clearance is plotted against effective molecular size (in Å) determined by gel chromatography as the Stokes-Einstein radius. It is evident that, with increasing size, fractional clearance decreases progressively and approaches zero for dextrans of radii greater than about 42 Å.

It is generally considered that some discrete series of "pores" exist somewhere in the glomerulus which are responsible for the behavior shown in Fig. 2. Some workers have suggested that the epithelial slit pores are the filtration barrier, and others claim that elements in the GBM are responsible. The endothelial fenestrations are obviously not candidates for this functon, as their diameters are too great and they do not possess a membrane.

The transport of macromolecules across any capillary may be determined by both diffusion and convection. In the case of the glomerulus, the movement of macromolecules across the wall of the glomerulus will be coupled to the transglomerular movement of water, i.e., a significant convective component of transport exists. Thus, the fractional clearance of large molecules will be influenced by the determinants of glomerular filtration, i.e., plasma flow rate and driving pressures in addition to the permeability, i.e., porous properties of the glomerulus (9).

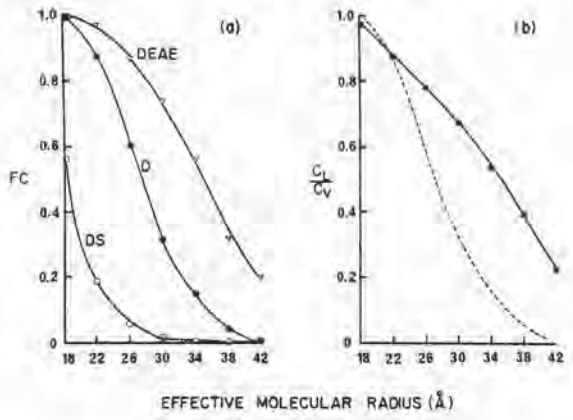

Figure 2. Transcapillary Movement of Macromolecules in the Kidney. Panel a shows fractional clearances (FC) across the glomerulus of neutral dextrans, D (solid circles), anionic dextran sulphate, DS (open circles), and cationic diethyl-

aminoethyl dextrans, DEAE (open triangles), plotted as functions of effective molecular radii for normal rats. Panel b shows the transperitubular concentration ratio of D in renal lymph (C_L) and renal venous plasma (C_V) plotted as a function of effective molecular radius (solid squares). The broken line gives the transglomerular FC profile for D, also shown in panel a. See text for details.

Charge can also influence the transcapillary flux of macromolecules. The clearance of albumin is about 1/20 that of a dextran molecule with a similar effective radius (36 Å). Albumin is a polyanion in physiological solution, whereas dextran is electrically neutral, suggesting that it is the charge difference between the two molecules that is operative in determining glomerular permselectivity to macromolecules. This possibility was tested by studying the transport of the anionic polymer, dextran sulphate (DS), and the results are shown in Fig. 2a. The fractional clearance of anionic dextran sulphate is lower than that for the equivalently sized neutral dextran. As with dextrans, dextran sulphate is neither secreted nor reabsorbed by the tubules and, since glomerular hemodynamics were similar in the two groups of rats studied, these data suggest that the glomerular capillary wall possesses some fixed, negatively charged components which electrostatically repel the tranaglomerular passage of anionic macromolecules (9). Other functional, histochemical and electron-microscope tracer studies have supported this possibility (9). Further, a cationic dextran species, DEAE, has been shown to pass across the glomerulus more readily than either anionic dextran sulphate of neutral dextran, as shown in Fig. 2a.

Less extensive studies have been perfomed to investigate the permselectivity properties of the peritubular capillaries, although it has been suggested that the peritubular capillaries are more permeable to macromolecules than is the glomerulus. At the glomerulus, the blood contains between 6-9 g/dl of protein, whereas the protein concentration of the fluid in Bowman's space averages a few micrograms/dl (4). In contrast, while the peritubular blood contains 9-6 g/dl of protein, the protein concentration in the compartment outside the capillary, the interstitium, averages approximately 1-2 g/dl (7). Deen and colleagues compared the renal lymph to renal venous plasma concentration ratios (C_L/C_V) of neutral dextrans and the data derived from these studies are shown in Fig. 2b (10). As at the glomerulus, the larger dextrans apparently cross the peritubular capillary into the lymph less readily than do the smaller dextran polymers, since the C_L/C_V ratio falls with increasing molecular radius. As shown by the broken line, the fractional clearance of dextran at the glomerulus falls below the C_L/C_V profile at the peritubular capillary for molecules of effective radius greater than approximately 30 A. However, this may not necessarily imply greater peritubular permeability, since the rate of removal of lymph and thus the rate of turnover of the interstitial fluid is very slow. Concentration ratios alone, therefore, may be misleading and it is more valuable to consider the membrane parameters, such as capillary surface area for diffusion, capillary permeability and σ, the reflection coefficient for transcapillary convection. The peritubular capillary reflection coefficient for large molecules is probably close to 1.0 since convective transport of large molecules out of the peritubular capillary will be absent because the direction of net fluid movement (APR) across the peritubular capillary is in the opposite (i.e., inward) direction. Thus, it seems probable that large molecules arrive in the interstitium solely by diffusion down a concentration gradient.

It has been suggested that the surface area of the peritubular capillary is about 20 times greater than that of the glomerulus; on the basis of this assumption and the permselectivity data previously reported for the glomerulus, Deen et al. suggest that the local diffusive permeability for peritubular capillaries is approxmately 10-100 times less than for the glomerulus (10).

Deen and colleagues also observed consistently lower values of the renal lymph to renal vein concentration ratio of albumin compared to neutral dextran of the same effective molecular radius. These observations are qualitatively in accordance with the glomerulus, suggesting preferential restriction to transcapillary passage of polyanions relative to neutral macromolecules. The differences in transperitubular concentration ratios for dextran and albumin are considerably less than the differences between corresponding concentration ratios at the glomerulus, however, indicating that peritubular capillaries may be less sensitive to molecular charge than glomerular capillaries. Peripheral capillaries may also exhibit charge selective properties. Anionic dextran sulphate passes less freely than neutral dextran across capillaries of the isolated rabbit ear (9). Also, tracer studies using cationic ferritin and alcian blue as electron-dense probes have demonstrated the presence of anionic sites on the endothelial side of the fenestrated capillaries of mouse pancreas and jejunum (11).

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NOTES

Chapter 5

LONG TERM ADAPTATION OF SKELETAL MUSCLE CAPILLARITY

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The most important physiological factor associated with the development of new capillaries in mammalian skeletal muscle is the process of normal body growth. The increases in body size and weight observed during normal maturation are accomplished by increases in the cross-sectional area of the muscle fibers and in the capillary density to fiber density ratio. Because the number of fibers in the total cross-section of a given skeletal muscle does not change appreciably during normal growth it can be concluded that the total number of capillaries in muscle increases when the capillary to fiber ratio (C/F) increases. Once the animals have reached adulthood the capillarity of skeletal muscle tends to remain constant. Apart from well known pathological conditions such as tumor vascularization and wound healing, increased tissue capillarity has been observed only in response to a few physiological and experimental stresses. Increased capillarity has been described in the skeletal muscle of humans and animals after a prolonged period of endurance physical training as well as in animals acclimated to hypoxia and cold, acting either alone or in combination. However, data in support of the contention that hypoxia leads to increased capillarity are often equivocal, and considerable controversy surrounds their interpretation. We show here methods of analysis which are important in reaching conclusions regarding the effects of physiological stresses on capillarity.

It is currently known that the normal variation in skeletal muscle capillarity associated with growth must be established for a given animal species before attempting to interpret data from animals exposed to different physiological and environmental stresses. This is particularly true if these stresses are capable of affecting body size and/or fiber cross-sectional area, which in turn affect muscle capillarity. Alternatively, comparisons can be made on age- and weight-matched control and experimental animals in which anatomical characteristics are similar. We have studied large groups of normal growing animals for which normal relationships between various parameters of capillarity were expressed as continuous functions of fiber cross-sectional area. The effects of environmental stresses were then analyzed as departures from the normal relationships using analysis of covariance.

For years investigators attempting to assess the capacity of skeletal muscle for oxygenation were beset by the lack of adequate methods for capillary visualization and tissue fixation. Histochemical techniques for endothelial staining in tissues fixed by nonchemical methods (rapid freezing), in which shrinkage is eliminated, and the use of electron microscopy have largely solved the problems of capillary visualization and quantification. However, the problem of capillary distribution in tissues and the effects of their spatial arrangement on oxygen diffusion distances is not yet completely understood. We use the myosin ATPase method for capillary visualization on frozen muscle samples and utilize the following parameters of capillarity: capillary density, CD (number of capillaries/mm^2); the capillary to fiber ratio, C/F; the number of capillaries around the fiber, CAF; and the measurement of fiber cross-sectional area, FCSA. It has been customary to assess the average area of tissue cross-section served by a capillary by calculating the inverse of CD. This value, however, provides no information on the actual shape of the area served by the capillary and, thus, the estimation of diffusion distances from the capillary into the fibers has remained elusive. Kayar et al. (5) recently published a series of formulae for estimating both the median (R_{50}) and the maximal (R_{95}) diffusion distances. The latter value is of much greater physiological significance since it is the longest diffusion distance that probably limits the supply of oxygen and may, by causing localized areas of hypoxia, impair muscle performance. These formulae are summarized in Table 1.

Table 1. Median (R_{50}) and Maximal (R_{95}) Diffusion Distances from Capillaries into Tissue Can Be Obtained from Values of Capillary Density (CD).

	Capillary Organization	Distance
R_{50}	Ordered array	$0.40\ \sqrt{1/CD}$
	Random array	$0.47\ \sqrt{1/CD}$
R_{95}	Triangular array	$0.55\ \sqrt{1/CD}$
	Quadrangular array	$0.60\ \sqrt{1/CD}$
	Hexagonal array	$0.71\ \sqrt{1/CD}$
	Random array	$0.98\ \sqrt{1/CD}$

CAPILLARY SUPPLY TO SKELETAL MUSCLE IN GROWING ANIMALS

In most mammalian species, skeletal muscle growth from birth to adulthood occurs mainly as a result of increased muscle fiber diameter as fibers accumulated more cytoplasm, a process known as normal hypertrophy. During normal growth the average FCSA increases linearly with body weight (1,2,7). Differences in muscle fiber size and weight among animal species of different sizes are due mainly to differences in the number of fibers composing the muscles, and thus, differences in FCSA among adult animals of different species are relatively minor. In growing rats and guinea pigs, the consequence of the increase in FCSA is a hyperbolic decrease in CD, increasing the diffusion distance which the oxygen molecule travels from the capillary into the tissue (Fig. 1). This decrease in CD with FCSA occurs despite a significant linear increase in the C/F ratio. Other species, dog, for example, show no significant change in CD with increasing age, body weight and FCSA, but the total number of capillaries in muscle increases, as demonstrated by the increasing values of C/F with FCSA (Fig. 1). Because CD does not change and because the pattern of capillary distribution in muscle cross-sections is the same, the diffusion distance in the skeletal muscle of the dog remains unaltered during growth (2,6). In small puppies, there is one capillary for each 25 fibers, i.e., C/F is around 0.04, whereas in the adult dog there are 4 capillaries per fiber (C/F about 4.0), a 100-fold increase in C/F ratios.

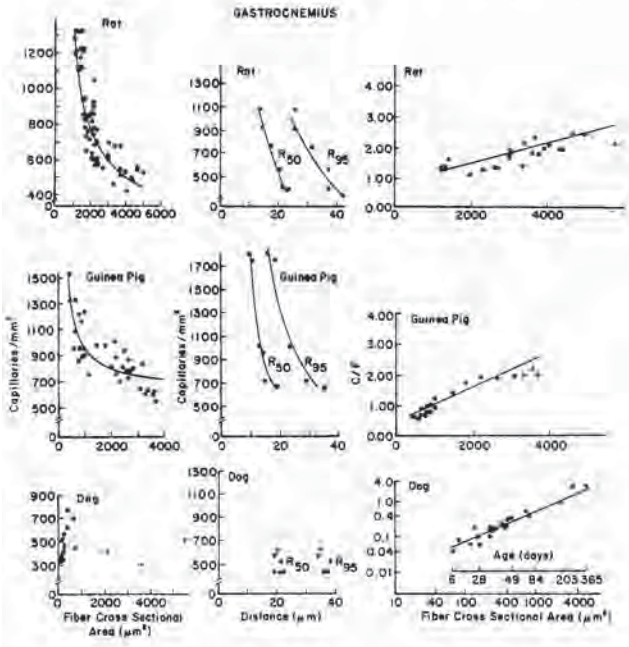


Fig. 1. Changes in capillarity and in median (R_{50}) and maximal (R_{95}) diffusion distances from capillary to tissues in the gastronemius muscle of three species during growth. In rats and guinea pigs, the increases in the total number of capillaries was insufficient to prevent a change in CD with growth, whereas, in the dog CD remained unchanged. Consequently, in rats and guinea pigs, diffusion distances increased as CD decreased but, in the dog, no change was found.

CAPILLARY SUPPLY TO SKELETAL MUSCLE IN ANIMALS ACCLIMATED TO HYPOXIA

For a number of years it was believed that adaptation to chronic hypoxia was accompanied by an increase in the capillary supply to skeletal muscle and other tissues. The teleological advantage of such changes in capillarity would be to facilitate diffusive oxygenation and to provide a relatively higher tissue PO_2 in the high altitude animal. However, when the original reports of increased capillarity are analyzed in the light of our current knowledge on capillarity in growing animals, a completely different conclusion is reached: chronic hypoxia does not affect skeletal muscle capillarity.

The original description by Valdivia (12) in the Peruvian high altitude guinea pig has never been substantiated by other investigators who have studied the same species using modern histochemical methods for capillary visualization. In 1958, Valdivia used India ink injection and fixed the medial head of the gastrocnemius with 10% formalin. His values for CD in the Andean guinea pig as well as those in sea level guinea pigs were much higher than any other value for CD in adult guinea pigs reported over the last 20 years, suggesting some inherent technical error, most likely significant tissue shrinkage. Shrinkage, however, is not the whole explanation. High values for CD, even if they were to occur, would probably be counterproductive. Large increases in CD lengthen mean transit times in the systemic capillaries which, in a situation like hypoxia where cardiac output is normal, would decrease PO_2 in the capillary blood, especially toward its venous end. We found no change in capillarity in Andean guinea pigs (9) and in guinea pigs exposed to severe environmental hypoxia (10). The change in capillarity with growth in thse two groups of hypoxic guinea pigs was similar to the normal changes found in sea level and in Denver (1610 m) animals (Fig. 2).

Similarly, the high values for CD measured by Cassin et al. (3), using a histochemical method for capillary visualization in Sprague-Dawley rats exposed to simulated altitude equivalent to 6150 meters for six weeks, can be explained by the small body weights (i.e., small FCSA) of the hypoxic rats (BW = 154 g), in comparison to the much larger rats (BW = 287 g) studied in normoxic conditions. Based on our data in the normoxic Sprague-Dawley rat, the differences in CD reported by Cassin et al. between hypoxic and normoxic rats were exclusively a reflection of differences in body weight (i.e., FCSA) and should not be attributed to the hypoxic stimulus. Sillau et al. (8)

found no evidence of increases in capillarity as a result of 3 and 6 weeks of hypoxia in growing Sprague-Dawley rats exposed to a simulated altitude equivalent to 5100 meters.

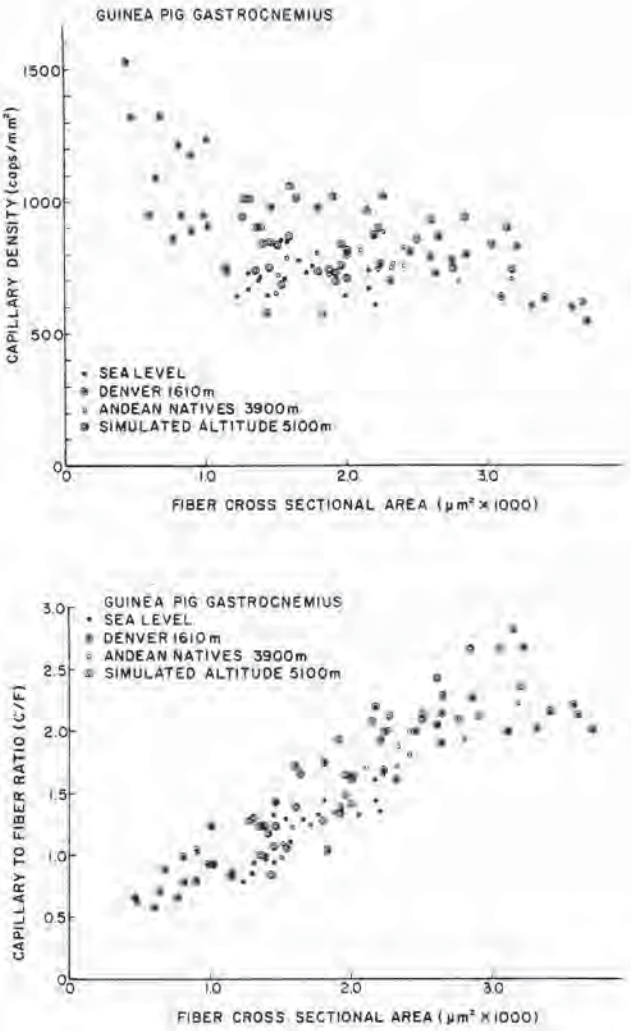


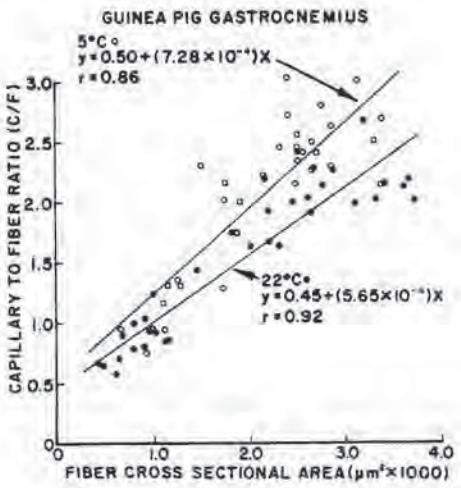
Fig. 2. Relationship between either CD or C/F and FCSA in the gastrocnemius muscle of four groups of guinea pigs studied at sea level, in Denver at 1610 m, at 3900 m (Andean natives), and at simulated altitude equivalent to 5100 m. CD decreased with FCSA in a hyperbolic fashion and no differences between groups were found. In all four groups, C/F increased with FCSA and no differences between groups were observed.

CAPILLARY SUPPLY TO SKELETAL MUSCLE IN ANIMALS ACCLIMATED TO COLD

Exposure of small animals to severe cold augments the cellular demands for oxygen, causing an increase in the basal metabolic rate and in cardiac output. This higher energy expenditure compensates for the increased heat loss of the animals and a normal core temperature can usually be maintained. Several biochemical factors have been invoked as responsible for these changes in metabolic activity and in the oxygen transport system. Acclimation to cold also causes an increase in skeletal muscle capillarity. In 1957, Heroux and St. Pierre (4), using the benzidine reaction to stain red blood cells in capillaries, reported greater CD in the skeletal muscles of the Sprague-Dawley rat exposed to 6°C. However, the cold acclimated rats had smaller muscle weights and FCSA's than the control rats, a common finding in cold acclimated animals. Thus, the increased CD measured by Heroux and St. Pierre could have resulted from the smaller FCSA and not necessarily from an absolute increase in capillary supply. Their data were difficult to interpret since their C/F values did not vary significantly. This question was answered by the work of Sillau et al. (11) who found that the increased capillarity in guinea pigs exposed to 5°C is absolute, i.e., is independent of the changes in FCSA. These data are shown in Figures 3 and 4. Not only was the muscle fiber of the cold acclimated guinea pigs more richly vascularized, it was also more elliptical than the fiber of the control animals, reducing the relative diffusion distance to the center of the fiber for any particular level of CD and C/F. It is important to realize that, in high altitude environments, the effects of cold could modify the response of the animal to hypoxia alone. There is no reason to suspect that cold exposure could have played a role in the work of Valdivia (12) and Cassin et al. (3). However, it was important to ascertain to what extent these two factors combined could affect skeletal muscle capillarity.

CAPILLARY SUPPLY IN SKELETAL MUSCLE OF ANIMALS IN COLD AND HYPOXIA

In contrast with observations in chronically hypoxic animals, data obtained in guinea pigs chronically exposed to low temperatures and hypoxia indicate that skeletal muscle capillarity increases when the two environmental stresses are combined. Figure 4 shows the variations in CD in the gastrocnemius and soleus muscles of three groups of guinea pigs, which grew up in three different environments: normoxia at 22°C, normoxia at 5°C, and hypoxia at 6°C. Values of CD were higher in the groups of animals exposed to cold, regardless of the presence or absence of hypoxia. It is of interest to note that the effects of cold plus hypoxia on the gastrocnemius, a muscle with mixed fiber population, were more marked than in cold alone, whereas in the soleus, a muscle composed



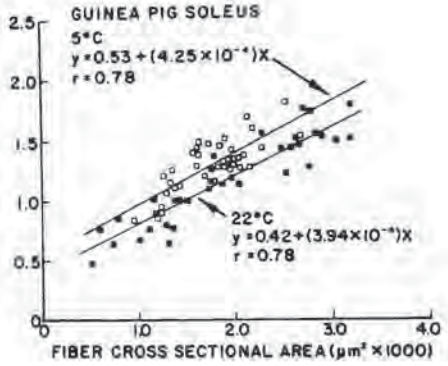
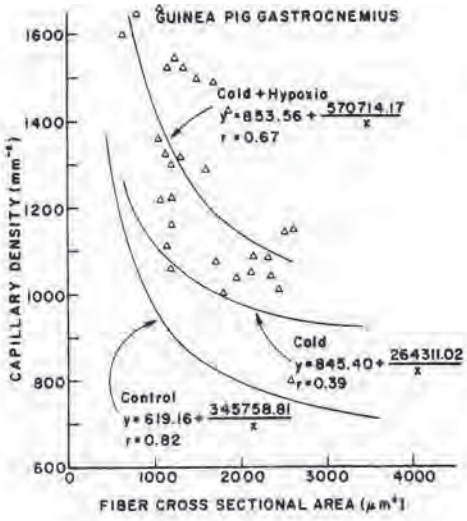


Fig. 3. Relationships between C/F and FCSA in the gastrocnemius and soleus muscles of growing guinea pigs at 22°C and at 5°C. In both muscles, C/F's were significantly higher in the cold acclimated animals (from reference 11).



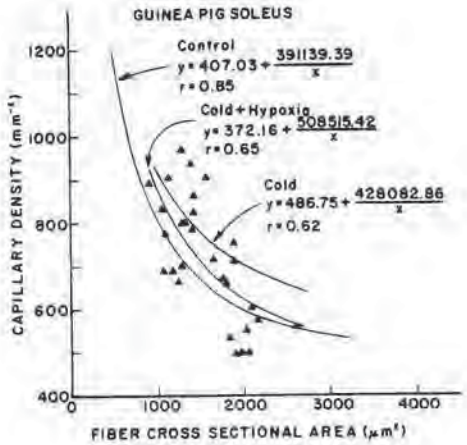


Fig. 4. Relationships between CD and FCSA in guinea pigs acclimated to cold and hypoxia (individual animals are represented by symbols). Also shown are regression lines and equations for a group of growing guinea pigs at 22°C (control) and at 5°C (cold). The CD in guinea pigs acclimated to cold and hypoxia was significantly higher than in controls only in the gastrocnemius muscle. In both muscles CD was significantly higher at 5°C than at 22°C.

almost exclusively of intermediate fibers, the values for CD at any given FCSA were less than in exposure to cold alone. It is not clear what mechanisms are involved in eliciting the growth of new capillaries in muscle. One plausible explanation is that capillary sprouting results from a combination of increased muscle blood flow due to the increased oxygen consumption and moderate tissue hypoxia acting either constantly or intermittently. This situation would resemble continuous exposure to bouts of heavy endurance exercise, which is also known to induce increases in capillarity. However, solid experimental evidence in support of this contention is not yet available.

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NOTES

Chapter 6

PATHOLOGICAL EXPRESSION IN THE MICROCIRCULATION: HYPERTENSION AND DIABETES

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The two most common forms of microvascular disease in man are the vascular manifestations of diabetes mellitus and essential hypertension. Assuming all persons with diabetes and hypertension have, or will develop, some form of microvascular pathology, then approximately thirty to forty percent of the population is at risk. The purpose of this review is to describe, from a majority opinion of the data available, the physical and physiological microvascular characteristics one would expect during either hypertension or diabetes. Much of the information to be presented is based on animal models of the disease with correlation to man made as current knowledge will allow.

Hypertension

In the human population, approximately eighty percent of hypertensive individuals have a form of hypertension, often called essential hypertension, whose cause cannot be traced to renal or hormonal origins. The majority of the remaining twenty percent of hypertensives have some form of hypertension related to renal impairment. The person with essential hypertension experiences: no pain, skeletal muscle weakness or tissue degeneration related to hypoperfusion, or tissue edema. Very little seems to be wrong. However, the final result of hypertension can be expressed as accelerated atherosclerosis, stroke, cardiac hypertrophy, renal vascular dysfunction and cardiac failure.

Once essential hypertension is well established in man or the Okamoto-Oaki (27) spontaneously hypertensive rat (SHR), a common animal model of essential hypertension, the cardiac index is essentially normal but total peripheral vascular resistance is chronically elevated. Furthermore, Tobia et al (34, 35) have shown that vascular resistance in all organs and tissues is increased by the same proportion in the early phases of hypertension and possibly throughout the life of the SHR. In other words, sustained hypertension is a disease whose major effector organ is the entire microcirculation of the body.

If increased microvascular resistance is the primary cause of elevated arterial pressure, the fundamental question is how is vascular resistance increased? The possible choices are actually very few; resistance can be increased by vasoconstriction, lengthening the vasculature, decreasing the number of vessels, hyperviscosity, or some combination of these factors. A mild form of hyperviscosity does exist in hypertensive man (32) and SHR (11) but whether it is of sufficient magnitude to explain more than a small fraction of the increased resistance is not known. Likewise, Hutchins and Darnell (21) have shown that the length of individual arterioles is normal during hypertension in SHR. Perhaps the best known hypothesis for increased vascular resistance is vasoconstriction secondary to vessel wall hypertrophy, as originally proposed by Folkow (15). The physiological experimental evidence for this hypothesis is the increased vascular resistance of hypertensive man and rats for any given flow rate in both functionally intact and passive vascular beds. Data of this type presented by Folkow and others (15) certainly indicate a decreased compliance or increased stiffness of the vasculature, which is compatible with hypertrophy of the vessel wall. In vitro histological analysis of the microvasculature of SHR and humans with essential hypertension has demonstrated vessel wall hypertrophy in the arteries which precedes hypertrophy in the microvasculature (16, 25). Data presented by Short (32) and Furuyama (16) from in vitro histological studies do not confirm the presence of vessel wall hypertrophy for microvessels with diameters of less than approximately 100 m in humans with essential hypertension. Direct in vitro observation of the microcirculation in anesthetized spontaneously hypertensive rats and hamsters with renal hypertension (1, 4, 9, 19, 30, 36, 37) has yielded data such as those shown in Figure 1 which, in the main, indicate that the arterioles are neither constricted nor are their vessel walls hypertrophied when compared to normal vessels at similar locations within the vascular branching pattern. The majority of the in vitro data were obtained in the skeletal muscle vasculature and may or may not represent vascular characteristics of other organs. However, data obtained in the SHR during microcirculatory studies do confirm that the typical microvessel has a greater wall tension or stiffness as Folkow (15) had predicted. This follows from the fact that arteriolar diameters are near normal or increased when innervated, yet blood pressure within the arterioles is substantially elevated above normal (1, 2, 37), as is demonstrated in Figure 1.

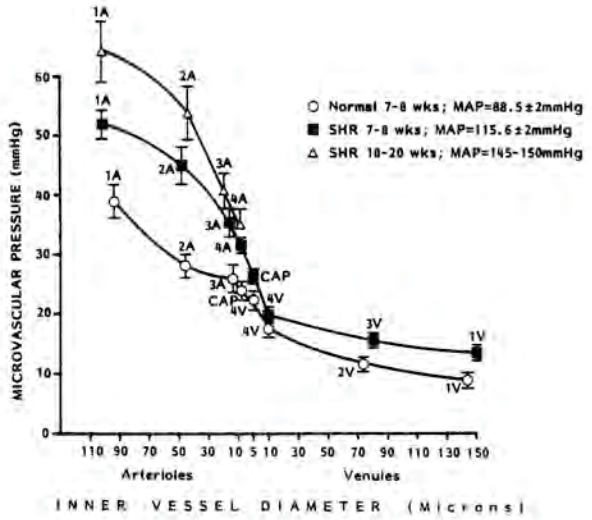

Figure 1. Microvascular Pressures and Internal Diameters in Skeletal Muscle of Normal 7-8 week, SHR 7-8 week and SHR 18-20 week. The mean arterial and microvascular pressures for normal rats at 18-20 weeks is essentially equal to that of 7-8 week animals. Data from refs. 1 and 2.

The most promising data currently available to explain the mechanical origin of the increased vascular resistance during essential hypertension suggest that there is a decreased number of arterioles open to flow in the hypertensive animal. This concept was originally developed from data presented by Hutchins and Darnell (21) for the SHR and has been subsequently confirmed by many investigators (1, 18, 19, 21, 30). Furthermore, Harper, in conjunction with Hutchins and coworkers (18), have shown microvascular rarefaction for the conjunctival circulation of humans with essential hypertension. The rarefaction of arterioles is apparently present in two forms which may occur together or separately. First, a larger than normal percentage of the available small arterioles are closed to blood flow in the skeletal muscle vasculature of the SHR and the majority of the closed vessels tend to open to blood flow upon denervation or disruption of vascular control (1, 19, 30). Secondly, it is possible that the SHR simply has fewer total vessels than do normal rats (21, 30). Whether a given arteriole is closed most of the time or the small vessels cycle and, in effect, take turns being closed is not precisely known.

A partial cause of the active closure of small arterioles may be the hyper-responsiveness of vascular smooth muscle to norepinephrine which has been demonstrated by the majority of studies on this issue (1, 20). Furthermore, Bohlen (1) has demonstrated that the hyper-responsiveness to norepinephrine of small arterioles in skeletal muscle was expressed in vivo as a strong tendency to constrict to closure rather than exhibit a dose dependent partial constricton as occurs in normal arterioles. In view of the hyper-responsiveness to norepinephrine expressed in vivo as vessel closure, the well known sympathetic nervous system activity associated with essential hypertension (8), and frank loss of microvessels, these factors acting in concert may be of very significant importance for the elevated microvascular resistance in hypertension in man and SHR. In addition, the greater mechanical stiffness of small arteries and arterioles, as judged by microcirculatory and isolated vessel studies, and the loss of arterioles may also explain the increased passive resistance of hypertension originally described by Folkow.

The elevation of microvascular pressures in the SHR, as presented in Figure 1 and documented in other studies (1, 2, 37), indicates that the elevated mean arterial pressure of the hypertensive does invade the microvasculature. Zweifach et al (37) suggest that capillary pressures in the spinotrapezius muscle are within a normal range based on the near normal pressures within the smallest arterioles and venules. Bohlen and coworkers (1, 2) report that capillary pressures in the cremasteric muscle are slightly elevated. Both groups (1, 2, 37) agree that as the mean arterial pressure is elevated with age, pressures within the arteriolar vasculature are increased. This phenomenon is demonstrated by the data in Figure 1 for SHR for age 7-8 and 18-20 weeks. The point to be made is that although the capillary pressure in skeletal muscle may be preserved, or nearly so, during hypertension, the majority of arterioles are forced to endure a substantially elevated internal pressure which continues to rise as hypertension becomes more severe. The increased internal pressure may, as Folkow (15) originally speculated, be a stimulus for vascular smooth muscle hypertrophy during chronic hypertension. Whatever the fate of the arteriolar wall may be, there is no question that some form of anatomical or physiological vessel wall compensation occurs to protect the vessels from over-distention because the arterioles typically have a near normal internal diameter or are constricted.

Diabetic Vascular Disturbances

The microvascular characteristics of chronic diabetes mellitus which are also common to essential hypertension include a loss of microvessels (3, 5, 5, 7, 13, 14, 24), increased capillary permeability (29), and increased blood viscosity (33). In contrast to essential hypertension as modeled by the SHR, the arterioles of chronically diabetic rodents tend to be constricted at rest (5, 6, 7) and vascular resistance in both diabetic man and animals fails to decrease as much as normal under conditions which should cause vasodilation (5, 6, 7, 10, 17). These latter two characteristics do appear to be manifestations of renal and mineral corticoid forms of hypertension in rats (36) but not in hamsters (23). Perhaps the most important distinctions between the

hypertensive and diabetic microvasculature is that the vascular pathology of hypertension appears to be a hyperactive process which does not harm the majority of host tissues, whereas diabetic vascular pathology may be a degenerative vascular process which does limit or impair the function of the host tissue.

The degenerative process which occurs in the diabetic microvasculature may be related to the thickening of the capillary basement membrane and the generalized atrophy of the wall structure of arterioles. Even though the capillary basement membrane is thickened during diabetes, apparently the integrity of the capillary as a semipermeable barrier is impaired (23, 24, 28, 29). Based on studies of vascular permeability in both diabetic man and animals, there is no question that capillary permeability to both small and large molecules is increased very early in the disease process and may even contribute to tissue and vascular damage (29). The loss of capillaries and arterioles in both man (13, 14, 24) and animals (3, 5, 6, 7, 28) during diabetes may be related to the vessel wall degeneration. For arterioles in diabetic rats and mice, Bohlen and coworkers (3, 5, 6, 7) have shown that the arteriolar wall cross-sectional area, which is primarily smooth muscle, decreases by one-third to one-half. In addition, platelet and red blood cell thrombi spontaneously and repeatedly form in arterioles, which is highly unusual in normal animals, and may reflect the overall degeneration of the vessel wall as well as the increased fragility of platelets (26) and red cell aggregation abnormality (31) which accompanies diabetes.

Many possible causes of diabetic vascular pathology in both man and laboratory animals are suspected but evidence in favor of any given hypothesis is as yet circumstantial. Dietzel (12) has proposed that intermittent hypoxia caused by the impaired oxygen release from glycolysated hemoglobin is a major factor in the development of microangiopathy. In rodent models of diabetes mellitus, severe microvascular disease occurs even though usually five percent or even less of the total hemoglobin is glycolysated and a normal percentage of glycolysated hemoglobin is two to three percent (3, 5, 6, 7). Even in diabetic man, the P50 for hemoglobin is within a few mmHg of the normal P50 (12). Therefore, one would not expect more than a very mild state of hypoxia to exist due to impaired hemoglobin; furthermore, the elevated concentration on 2,3-DPG in diabetes may assist oxygen exchange at the tissue (12). The loss of arterioles and capillaries during chronic hyperglycemia may contribute to a lower than normal tissue oxygenation even though the loss of vessels may or may not be specifically related to hypoxia.

Assuming the loss of microvessels is related to vessel wall defects previously mentioned, the excessive availability of glucose directly or indirectly may be closely related to the occurrence of vessel wall damage. Papachristodoulou et al (28) have shown that a diet rich in rapidly absorbed simple sugars will cause normal rodents to develop capillary disturbances similar to those in diabetics. In addition, Bohlen and Niggl (5) have shown that hyper- and hypoinsulemic diabetic mice have equivalent microvascular problems even though their only common characteristic is hyperglycemia. Recently, Bohlen and Hankins (3) have shown that twice daily intraperitoneal injections over a 4-5 week period of an isotonic saline solution which contained 300 mg% glucose did not cause systemic hyperglycemia at any time. However, the intestinal microvasculature in contact with the hyperglycemic saline demonstrated the characteristic vasodilation of short term diabetes, and the cross-sectional area of the arteriolar wall was decreased by one third to one half. These vascular anomalies were qualitatively and quantitatively similar to those in the intestine of age matched animals which had been made diabetic with streptozotocin for four to five weeks. The distribution distance of capillaries, as shown in Figure 2 for passive vascular conditions, is virtually identical for diabetic and glucose treated animals in terms of the actual distribution, mean and median of the population. Furthermore, the complete right-shift of the distribution for diabetic and glucose treated animals indicates that the animals were not only simply losing capillaries but the pattern of capillarization is very different from normal. In effect, the intestine of otherwise healthy animals developed what appears to be diabetic microangiopathy possibly because of the local presence of excess glucose.

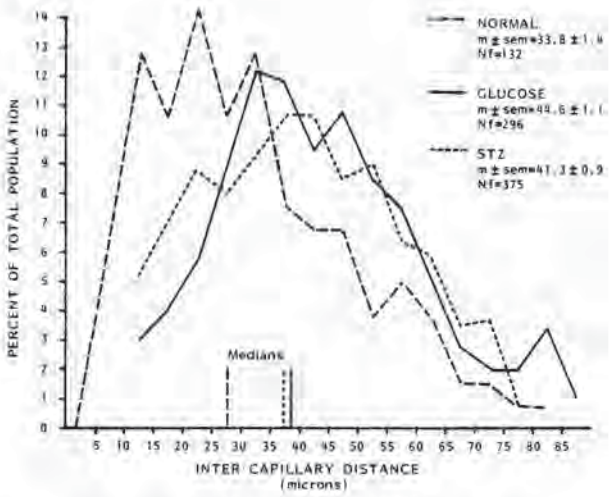

Figure 2. Distribution of Intestinal Muscle Inter-Capillary Distances During Passive Conditions in Normal, STZ-Diabetic and Intraperitoneal Glucose Treated Rats. Diabetes and intraperitoneal glucose injection uniformly increase capillary separation distances relative to normal. Additional details in text: From H. G. Bohlen and K. D. Hankins, Diabetologia, in press.

Summary

The diversity of microvascular changes which accompany hypertension and diabetes mellitus indicates microvascular pathology is not a simple process. For example, the arteriolar vasculature of a normal animal need only constrict approximately ten to fifteen percent to support the typical range of advanced hypertensive arterial pressures. However, in vivo observation of the microvasculature in SHR indicates little or no constriction of vessels open to flow. Instead, the hypertensive vasculature experiences temporary closure of some vessels and either fails to grow or permanently closes other vessels. The hypertensive vasculature is not following the expected behavioral pattern which cardiovascular physiologists associate with the normal vasculature. In the case of vascular pathology during diabetes mellitus, the atrophy of the arteriolar wall, loss of microvessels and diminished vascular tone does not appear to be a form of compensation but actual degeneration of the microvasculature. If these changes do represent some form of compensation, the logic of the process eludes our understanding based on normal vascular behavior. These inconsistencies of vascular behavior during normal and pathological conditions do emphasize the continued need for the study of pathological expression in the microvasculature.

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SOCIETY NEWS

Centennial Book Series Inaugurated

The Publications Committee and the Centennial Celebration Committee of the American Physiological Society are pleased to initiate the book-publishing aspect of the celebration of the Society's Centennial with the reprinting of *Circulation of the Blood: Men and Ideas*. This book, edited by Alfred P. Fishman and Dickinson W. Richards, received wide acclaim when originally published by Oxford University Press in 1964.

In Fishman's preface to the 1982 edition of *Circulation of the Blood: Men and Ideas* he states three reasons for launching the celebration of the American Physiological Society's Centennial with the reprinting of this volume. First, the centennial of a learned Society is a time to reflect on the advancement of knowledge by the use of great ideas as landmarks. Second, participants in science are more likely to perceive the personal element in scientific discovery than are its observers. Finally, scientist-authors who were leaders of a famous laboratory or school of physiology are well suited to place the roles of imagination, discipline, tradition, and the environment in nurturing the creative process. The authors of the chapters in this book were such participants and leaders.

Circulation of the Blood: Men and Ideas covers the development of ideas on the function of the cardiovascular system by scientists from ancient to modern times. The contributions of William Harvey are emphasized, which is appropriate as it was he who perceived order in the nature of blood circulation where others had been unable to do so. Further he translated these ideas into reality by systematic observation and experimentation. His work marks the beginning of modern physiology.

What a fitting way to celebrate the centennial of the American Physiological Society by honoring Harvey, for a centennial is not only a time to reflect but also is a time to add new vigor to the field that the Society serves. In this vein the Publications Committee is pleased to announce plans to continue the approach used in this book with a "People and Ideas" series. Samuel M. McCann has agreed to organize and edit the first new book in this series, *Endocrinology: People and Ideas*. Other books are being considered.

This edition of *Circulation of the Blood: Men and Ideas* has been sturdily and attractively casebound to ensure its long life. Society members may order copies of this book, with 879 pages and 248 figures, from the APS Business Office, 9650 Rockville Pike, Bethesda, MD 20814. The price to APS members is $45.00 ($56.00 to nonmembers).

H. E. Morgan, Chairman
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Publications Committee

Contributions and Matching Gifts to APS

It is gratifying that many Society members are now adding voluntary contributions to their annual dues. These contributions are most welcome. They have the effect of reducing inflationary pressure to raise dues in order to maintain valued Society programs.

One category of members does not automatically have the opportunity to make such contributions. These are members whose dues are paid by the institutions for which they work. We would like to ask all members in this category to consider sending a voluntary personal contribution to the Society each year. Such contributions are, of course, tax deductible and help the Society do a better job serving all its members.

It is also worth noting that many industrial concerns now have Matching Gift Programs through which they will match an employee's gift to a tax-exempt organization such as APS, sometimes at a rate of 2 to 1. Companies with matching programs include Abbott Laboratories, Allied Chemical Corp., Burroughs Wellcome Co., Hoffmann-LaRoche, Inc., Merck and Co., Inc., Pfizer, Inc., Scherring-Plough Corp., Smith Kline Foundation, and Squibb Corp. Members who work for companies with matching programs are urged to contact their companies in order to explore the possibility of a joint contribution.

William F. Ganong, Chairman
Financial Development Committee

APS Committees, Their Principal Functions and Membership (1982-1983)

Publications Committee

Manages all Society publications including the appointment of editors and editorial boards. A subcommittee of this committee is responsible for developing an annual symposium as the basis for an APS publication in the basic and clinical sciences.

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Reviews the proposed annual budget and fiscal plan for all Society activities and recommends a final budget and implementation plan to Council. Supervises the investment of the Society's financial resources subject to approval by Council.

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Detailed functional statements for each committee are included in The APS Operational Guide distributed annually to officers and committee chairmen.

Future Meetings

1982	
APS Fall Meeting	Oct 10-15, San Diego
1982	
FASEB Annual Meeting	Apr 10-15, Chicago
APS "Fall" Meeting	Aug 20-24, Honolulu
IUPS Congress	Aug 28-Sep 3, Sydney
1984	
FASEB Annual Meeting	Apr 1-6, St Louis
*APS "Fall" Meeting	Jul 29-Aug 7, Lexington
1985	
FASEB Annual Meeting	Apr 21-26, Anaheim
*APS "Fall" Meeting	Aug 4-9, Buffalo

*Campus meeting

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Reviews and evaluates applications received from candidates for membership and recommends to Council the nominees for election to Regular, Associate, Corresponding and Student membership.

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Consists of one member from each state who is responsible for reporting to the Public Affairs Executive Committee any legislation or regulation pertaining to biomedical research being considered or proposed by the state or any political subdivision thereof.

Members of this committee may develop a network of Society members within their states to assist in identifying pertinent proposed legislation or regulation and help to provide information to proper agencies regarding their effects on biomedical research, if enacted.

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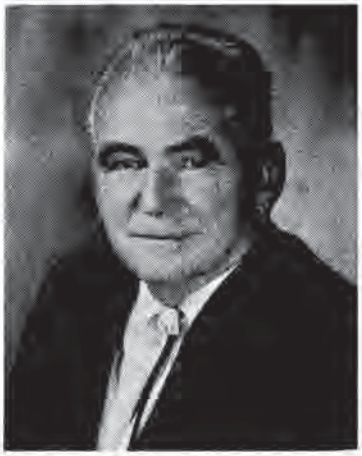

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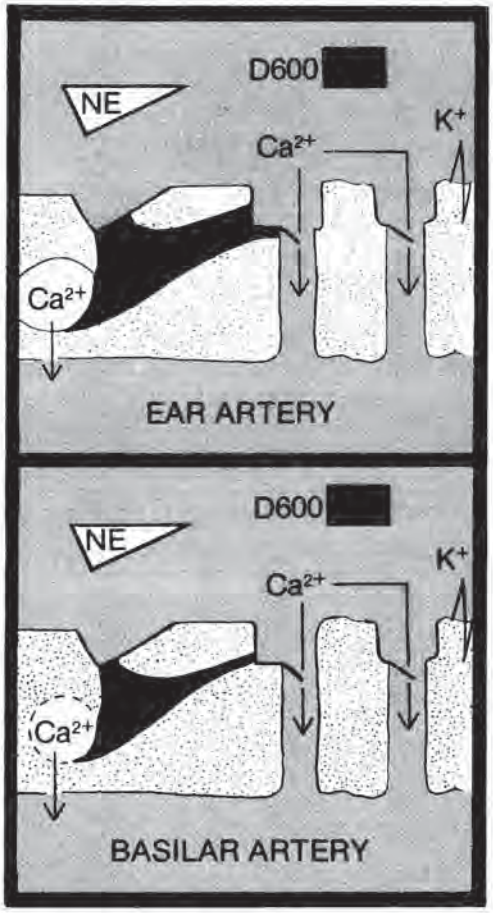

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