THE PHYSIOLOGIST

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34th Annual Fall Meeting

1983 Refresher Course—Syllabus

Symposium

Society News

Announcements

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Cover: See Physiology of Water Immersion Symposium. The photograph taken by J. David George, British Museum (Natural History), Cromwell Road, London SW7 5BD, UK, is gratefully acknowledged.

The 34th Annual Fall Meeting of the American Physiological Society and the International Conference on Hydrogen Ion Transport in Epithelia

Sheraton Waikiki
Honolulu, Hawaii

August 20-24, 1983

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

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SPECIAL EVENTS

BOWDITCH LECTURE

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Maui Room

Functional Mapping of Cardiovascular Reflexes and the Heart Using C^{14}-2-deoxyglucose

Speaker: David Kostreva, Medical College of Wisconsin and The VA Medical Center, Milwaukee

APS PAST PRESIDENT'S ADDRESS AND BUSINESS MEETING

Monday, 4:30 PM
Maui Room

Crises in Physiological Research

Speaker: Walter C. Randall

APS COMPARATIVE PHYSIOLOGY SECTION BUSINESS MEETING

Tuesday, 4:30 PM
Honolulu Room

EVENING SOCIAL EVENTS

Opening Reception
Saturday, 6:00 PM
Diamond Head Lawn

Luau
Tuesday, 6:30 PM
Diamond Head Lawn

OPEN HOUSE AT THE UNIVERSITY OF HAWAII

The Local Arrangements Committee plans an open house at the University of Hawaii on Thursday, August 25.

INTERNATIONAL CONFERENCE ON HYDROGEN ION TRANSPORT IN EPITHELIA

A series of sessions devoted exclusively to invited papers. These sessions are as follows:

Sunday AM
Symposium—Processes of passive and active H^+ transport in isolated gastric and renal membrane vesicles

Sunday PM
Poster Discussion—Transport in isolated gastric and renal membrane vesicles

Monday AM
Symposium—Epithelial H^+ transport processes and electrophysiology

Monday Evening
Poster Discussion—Regulation of H^+/HCO_3^- transport in gastrointestinal epithelia

Tuesday AM
Poster Discussion—Regulation of H^+/HCO_3^- transport in kidney epithelium

APS TUTORIAL, SYMPOSIA AND REFRESHER COURSE SESSIONS

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Symposium—Autonomic control of coronary tone: Facts, interpretations and consequences.

Sunday PM
Symposium—Neurohumoral control of the circulation

Tutorials
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Calcium exchange in the heart
Physiology of bile

Monday AM
Symposium—Physiology of water immersion

Monday PM
Symposium—Factors influencing vassopressin in body fluids

Tutorials
Structural basis of visual cortical function
Neuronal basis of plastic adaptation in gaze control
Nutrition as a modulator of the aging process

Tuesday AM
Symposium—Prostoglandins, lukotrienes and lung fluid balance

Tutorials
Comparative physiology of the renin angiotensin system
Hypothalamic control of body temperature
Long term reflex regulation of the cardiovascular system

Tuesday PM
Symposium—Sea-bird energetics, Session I

Tutorials
Regulation of blood flow and oxygen transport in skeletal muscle
The aging lung
Contractile properties of vascular smooth muscle

Wednesday AM
Symposium—Sea-bird energetics, Session II

Wednesday AM and PM
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SESSIONS WITH ASSOCIATED ABSTRACTS BY DAY

(APS Tutorials, Symposia and Refresher Course sessions are not listed since abstracts are not required.)

SUNDAY AM

SUNDAY PM

MONDAY AM

MONDAY PM

MONDAY EVENING

TUESDAY AM

TUESDAY PM

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2.1

PROTON/HYDROXYL AND ACID/BASE TRANSPORT THROUGH LIPID BILAYER MEMBRANES. John Gutknecht and Anne Walter, Dept. of Physiology, Duke University, and Duke Marine Laboratory, Beaufort, NC 28516 USA.

Recent studies show enormous discrepancies (2-6 orders of magnitude) in apparent H^+/OH^- and weak acid/base permeabilities of phospholipid bilayers, which are used as models of biological membranes. We used a combination of electrical conductance, pH electrode and tracer techniques to measure H/OH and acid/base permeabilities of planar lipid bilayer membranes. The permeabilities to H^+ and OH^- are about 10^{-9} cm/sec, several orders of magnitude lower than H^+/OH^- permeabilities of biological membranes. Diffusion of molecular HCl produces large H^+ fluxes at low pH, but HCl diffusion is not an important mechanism of H^+ or Cl^- transport at neutral pH. The permeabilities of small acids and bases obey Overton's rule, i.e., permeability is proportional to the hydrocarbon/water partition coefficient. All our results are consistent with the solubility-diffusion model of nonelectrolyte permeation. We will also describe a new electrical method of measuring weak acid/base permeability which utilizes uncouplers (protonophores). For example, the transmembrane diffusion of NH_3 produces a pH gradient in the unstirred layer which causes a transmembrane voltage in the presence of uncoupler. This voltage can be used to calculate the NH_3 permeability if the buffer concentration and unstirred layer thickness are known. (Supported by NIH grants GM 28844 and ES 02289.)

2.2

PROTON-HYDROXIDE PERMEABILITY OF LIPID BILAYERS AND BIOLOGICAL MEMBRANES. D.W. Deamer* and G.L. Barchfeld* (SPON: J. Forte). Dept. Zoology, U.C. Davis CA 95616

Values for proton-hydroxide permeability (P_H) are now available for a variety of lipid bilayer and biological membranes. Typical results for liposomes are in the range of 10^{-4} to 10^{-5} cm/s, orders of magnitude higher than values for other monovalent ions (10^{-11} to 10^{-14} cm/s). At neutral pH ranges, there is evidence for relatively high P_H in planar lipid membranes as well, although at extreme pH ranges values of 10^{-9} cm/s have been reported. P_H values for biological membranes are in the range of 10^{-3} to 10^{-5} cm/s, again much higher than for other monovalent ions. These observations suggest a unique proton-hydroxide flux mechanism, and we are presently investigating the possibility that hydrated defects permit proton-hydroxide to cross membranes by Grotthus conductance, in which proton equivalents move along hydrogen-bonded water structures. We will report results from studies in which melittin was used to produce defects in liposome membranes, with proton-hydroxide flux being monitored by decay of pH gradients. We found that melittin in mole ratios of 10^5 lipid/melittin dramatically increases proton-hydroxide flux. Other evidence suggests that the melittin defect is sufficiently specific to permit proton diffusion potentials as pH gradients decay. This result is consistent with the hypothesis that a hydrated defect can act as a specific proton channel.

2.3

pH-DEPENDENCE OF PROTON TRANSPORT IN YEAST AND NEUROSPORA. C.L. SLAYMAN, A. BALLARIN-DENTI*, M. BLATT*, AND D. SANDERS*. Department of Physiology, Yale University, New Haven, Conn.

Transport processes in plasma membranes of microorganisms are dominated by electrogenic active extrusion of protons, and by electrophoretic H^+-coupled uptake of many substances: e.g. sugars, amino acids, and inorganic ions. A remarkable property of these transport systems is their asymmetric kinetic sensitivity to pH. When secondary involvement of membrane potential is minimized either by voltage clamping (Neurospora) or by de-energization (Saccharomyces), changes of external pH (in the range pH 5 to pH 8) have small effects on transport velocity: usually less than 2-fold per pH unit. By contrast, internal pH (clamped with weak acids; measured with microelectrodes or ^{31}P-NMR) modulates transport much more steeply. For example, saturating current through the proton pump in Neurospora doubles for a 2.5-fold increase of $[H^+]_i$; and amino acid/H^+ cotransport (influx) in Saccharomyces rises 7-fold for a 3-fold elevation of $[H^+]_i$. Kinetic modelling by means of simple carrier reaction diagrams indicates that internal proton binding sites must have pK_a's near the normal pH_i (~pH 7); but external proton binding sites have pK_a's far displaced from neutrality. This conclusion in turn suggests that proton binding or release at the external site is associated primarily with energy conversion, whereas proton reaction at the internal site is associated more with transport control. Supported by NIH Grant GM-15858, NSF Grant PCM-7913412, and NIH Fogarty Fellowship TW-03062.

2.4

PERMEABILITY CHANGES IN ATPase MEMBRANES ASSOCIATED WITH GASTRIC SECRETORY STATE. J.M. Wolosin* and J.G. Forte, Dept. of Physiology-Anatomy, U. Calif., Berkeley, CA 94720.

Depending upon the secretory state, resting or stimulated, the (H^++K^+)-ATPase membrane is recovered from oxyntic cell homogenates in two distinct vesicular types, the microsome and the stimulation-associated vesicle (s.a.v.), respectively (JBC 256:3149). Both systems are capable of H^+-uptake through the action of ATP-driven H^+/K^+ exchange. Microsomes, though, are deficient in K^+ (and Cl^-) permeability needed to provide $[K^+]_{in}$ for the H^+-uptake, e.g., permeabilization by valinomycin is required. In s.a.v., in contrast, KCl transport is fast, exceeding the rate of the H^+/K^+ pump (FEBS Lett. 125:208). Using H^+-gradient dissipation under conditions limiting the compensating flux to either K^+ counter-flow or Cl^- co-flow we have now compared ionic conductances. In s.a.v., the protonophore TCS elicited fast dissipation of H^+-gradients. The H^+ flux increased linearly with [TCS], producing a 20-fold increase at 5 M. In K^+-free medium, H^+ fluxes follow the Goldman flux equation if is assumed to be a sole function of the Cl^- gradient. Thus, large, unlimiting K^+ and Cl^- conductances seem to exist in the s.a.v. membrane. In microsomes, TCS elicits only a 3-fold increase in spontaneous H^+ flux with rate saturation above 1 M indicating limitation of the H^+ flux by the compensating K^+ or Cl^- fluxes. Thus, stimulation of the oxyntic cell induces the appearance of large ionic permeabilities in the (H^++K^+)-ATPase membrane, electrophysiological implications will be discussed.

2.5

PROTON AND BICARBONATE PERMEABILITY OF PLASMA MEMBRANE VESICLES. Ernest M. Wright, Richard E. Schell* and Robert D. Günther*. Department of Physiology, School of Medicine, UCLA, Los Angeles, CA 90024

We have estimated ion permeabilities of brush border membrane vesicles from bi-ionic diffusion potentials and the constant field equation. Vesicles were prepared from rabbit renal cortex, rabbit jejunum and bovine choroid plexus by the Ca^{++} differential precipitation method and diffusion potentials were measured using a voltage sensitive cyanine dye (diS-C_3-(5)). Vesicles were prepared and incubated in solutions containing salts of choline and gluconate buffered between pH 5.5 and 8.5 with 50 mM Tris/HEPES or Tris/MES. Diffusion potentials were generated by replacing choline and/or gluconate in the extravesicular solution with permeable ions or by changing the pH. The dye was calibrated with K-valinomycin and Na-ETH 1097 diffusion potentials. H diffusion potentials were essentially Nernstian in the absence of permeable ions. P_H/P_K and P_{HCO_3}/P_K permeability ratios were estimated from the magnitude of the H/K and HCO_3/K bi-ionic potentials. In all three membranes P_H/P_K ranged between 1 x 10^{-7} and 1 x 10^{-10} whereas P_{HCO_3}/P_K was 0.06 for choroid plexus, 0.3 for jejunum and 2.3 for kidney. We conclude that there are substantial electrodiffusive fluxes of H^+ and HCO_3^- across these plasma membranes.

3.1

INCREASED PULMONARY VASCULAR PERMEABILITY TO PROTEIN FOLLOWING PULMONARY MICROEMBOLI INDUCED BY ECHIS CARINATUS (E.C.) VENOM IN DOGS. R.C. Schaeffer, Jr., S-M. Chilton*, T.J. Hadden*, and R.W. Carlson. Depts. of Med., Wayne State Univ. & Mt. Carmel Mercy Hosp. Detroit, MI 48235.

Pulmonary lymph flow (Q_{lym}, µl/min) was measured from an afferent to the left tracheobronchial lymph node in anesthetized open-chest mongrel dogs (n=7, 12.3-21.5kg). Following 2 hr (baseline) and 2 hr of elevated left atrial pressure ($\uparrow P_{la}$ = 20cm H_2O), the effects of E.C. venom (30 min, 50µg/kg) were studied for 2 hr, plus an additional 2 hr with $\uparrow P_{la}$. This venom activates prothrombin and induces microembolization (Physiologist 24:55, 1981). Steady-state data ($\bar{x} \pm$ SEM; *p < .05) were: lymph/plasma protein ratio (L/P), mean pulmonary artery pressure (P_{pa}, cm H_2O) calculated pulmonary capillary pressure (P_c, cm H_2O; Gaar, et al., Am. J. Physiol. 213:910, 1967) and pulmonary vascular resistance (PVR, cm H_2O/l/min).

	Q_{lym}	L/P	Pc	PVR	P_{pa}
Baseline	25.6±6.8	0.66±.04	12.7±1.0	7.1±1.1	21.7±1.3
$\uparrow P_{la}$	63.4±15.8*	0.54±.16*	24.6±0.7*	7.9±1.3	31.0±4.0*
E.C.venom	62.3±8.4*	0.56±.04*	19.8±0.9*	22.8±3.7*	38.5±2.2*
$\uparrow P_{la}$	117.4±23.5*	0.56±.03*	28.4±1.0*	21.7±5.3*	40.0±2.3*

These were associated with an increased protein clearance (µl/min; 33.8 ± 10.9 at $\uparrow P_{la}$ before E.C., to 62.6 ± 10.5* at $\uparrow P_{la}$ after E.C.). These data suggest that microembolization produced by E.C. venom is associated with an increase in pulmonary vascular permeability to protein. (Supported by MCREC Grant-In-Aid #223-281).

3.2

EFFECTS OF FIBRINOGEN DEGRADATION PRODUCTS (FDP) ON PULMONARY VASCULAR PERMEABILITY TO MACROMOLECULES. Taylor, A.E., D. Martin, J.C. Parker, J. Ryan and W. Curreri. Depts of Physiology and Surgery, University of South Alabama College of Medicine, Mobile, AL 36688.

Prenodal lymphatics were cannulated in open-chested dogs which had been anesthetized with Na+-pentobarbital (30mg/kg). Lymph flows, lymph protein concentrations, plasma protein concentrations, hematocrits, cardiac outputs, systemic and pulmonary vascular pressures and in some instances capillary pressures were measured for control conditions, following administration of a fibrinogen degradation factor isolated from purified human fibrinogen (fragment D, 7mg/kg) or fragment D plus benadryl (2 mg/kg), an H_1-histamine receptor blocker. Two hours following the fragment D challenge, left atrial pressures were elevated in successive steps of 10-15 cmH_2O until the concentrations in lymph did not change even when lymph flow increased. In addition, lung interstitial water was measured using biopsies obtained at control, and at all experimental states. Lymph flows increased after fragment D administration and lymph-to-plasma protein concentration ratios (L/P) decreased. With fragment D and benadryl, the lymph flow increase was smaller, but L/P approached the same values. Lung water was also less with the benadryl treated group and pulmonary capillary pressure was reduced from the 18 mmHg observed with only fragment D to 8 mmHg. Therefore, it appears that fragment D caused a release of histamine, which did not alter pulmonary vascular permeability, but primarily increased capillary pressure and consequently elevated lung water.

3.3

EFFECTS OF REDUCED PLASMA PROTEINS ON CAPILLARY FILTRATION COEFFICIENTS AND ISOGRAVIMETRIC CAPILLARY PRESSURES IN ISOLATED DOG LUNGS. (B. Rippe,* J. Parker, A.E. Taylor).

Using gravimetric techniques, the capillary filtration coefficients ($K_{F,C}$) and isogravimetric capillary pressures (P_{Ci}) were measured in the isolated papavarinized left lower lobe of the dog perfused with: 1) autologous blood, 2) plasma or 3) solutions with very low red cell and plasma protein content. Shifting from blood to a mixture of 4-8g% dextran (Mw~62000) containing 0.2-0.4g% bovine serum albumin increased $K_{F,C}$ slightly (30-60%). P_{Ci} also decreased significantly for dextran concentrations which were iso-oncotic with plasma. Shifting from blood to Tyrode's solution, induced a two to three fold increase in $K_{F,C}$ and bovine serum albumin in low concentrations (0.1-0.5g%) did not totally reverse these changes. Pure plasma perfusion caused only minor changes in $K_{F,C}$ (increased slightly) while P_{Ci} remained essentially the same. In summary, cell-free perfusates, other than plasma appeared to increase capillary filtration coefficients in the dog lung even when small concentration of proteins were still present in the perfusate. Changes in perfusate viscosity and/or in capillary surface area could only account for a small portion of changes. It is concluded that the presence of dextran, lack of perfusate proteins or lack of some other component of plasma or cells somehow decreases capillary permselectivity in dog lungs.

*Parker B. Francis Fellow.

3.4

OXYGEN DELIVERY AND UPTAKE IN DOGS DURING REDUCTION OF CARDIAC OUTPUT BY PLASMAPHERESIS. G.R. Long*, P.T. Schumacker*, and L.D.H. Wood*, (SPON: A. Leff). Critical Care Medicine, University of Chicago, Chicago, IL 60637.

Pulmonary capillary leak is reduced when circulating volume is reduced by plasmapheresis. Yet associated reduction in cardiac output (Qt) may adversely affect oxygen delivery (Qo_2), defined as cardiac output x arterial oxygen content (Cao_2). When Qo_2 was reduced by lowering Cao_2 (decreasing hemoglobin concentration or saturation) in healthy anesthetized dogs, Cain found the critical Qo_2 (Qo_2C), below which oxygen consumption (Vo_2) becomes dependent on Qo_2, to be 9.8 ml/kg/min (JAP 1977, 42:228). It is unknown whether Qo_2C remains the same when reductions in Qt lower Qo_2 at constant Cao_2. In anesthetized, paralyzed healthy dogs ventilated with 100% O_2 we independently measured Vo_2 while plasmapheresis progressively lowered Qt. Qt was measured by thermodilution technique. Catheters in the pulmonary and femoral arteries allowed measurement of vascular pressures, and arterial and mixed venous gases and O_2 contents. Throughout the experiments arterial blood was fully saturated, and measured Cao_2 remained constant. We found the mean value of QO_2C to be 10.9 ± 3.8 ml/kg/min. This value was dependent upon the steady state Vo_2. Measurements at the point where Vo_2 became dependent on O_2 delivery were (mean ± SD):

Cvo_2	Svo_2	Pvo_2	Cao_2-Cvo_2	BP	Qt
8.7±3.5	44±15	32.5±7.1	13.9±3.0	122±34	1.41±.66

As Qt was lowered beyond this point, Pvo_2 and BP continued to fall. We conclude that Qo_2C in dogs remains the same regardless of how Qo_2 is lowered.

3.5

ROLE OF GLYCOSAMINOGLYCAN DEPOLYMERIZATION IN THE PATHOGENESIS OF PERMEABILITY PULMONARY EDEMA DUE TO GRANULOCYTE OXIDANTS. R. B. Fox,* R. H. Demling, C. C. Wong,* B. R. Sumner.* Depts. of Ped. and Surg., Harvard Medical School, Boston, MA 02115

Toxic oxygen radicals, especially the hydroxyl radical (·OH), released from blood granulocytes have been implicated in the pathogenesis of the permeability pulmonary edema (PPE) in Adult Respiratory Distress Syndrome (ARDS). Since glycosaminoglycans (GAG's) are depolymerized by ·OH, and since GAG depolymerization increases glomerular permeability to protein, we hypothesized that breakdown of lung GAG's by granulocyte-derived ·OH contributes to PPE. To examine this hypothesis, PPE was induced in sheep previously fitted with hemodynamic monitoring catheters and lung lymph fistulas by treatment with the granulocyte activator, phorbol myristate acetate (PMA). PMA-treated sheep developed early, transient pulmonary artery hypertension and a subsequent PPE, with increased lung weights (+23%) and flows of protein-rich lymph (+150% baseline). We found depolymerization of lung GAG's as evidenced by decreases in ratios of uronic acid contents (carbazole method) of high:medium molecular weight fractions (PMA: 0.82 ± 0.17 vs. controls: 2.51 ± 0.43) of lung tissue extracts (4M guanidinium, 48 hours) after gel filtration (Sepharose 2B). Decreased tissue contents were accompanied by increases in uronic acid contents of lung lymph, peaking at 1 hour after PMA. PMA-stimulated granulocytes also depolymerized lung GAG's in vitro. Depolymerization and depletion of lung GAG's by granulocyte-derived oxidants may contribute to PPE in ARDS.

3.6

EFFECT OF ENDOTOXIN ON LYMPH FLOW AND COMPOSITION FROM CAUDAL MEDIASTINAL (CMN) AND PREFEMORAL LYMPH (PN) NODES IN UNANESTHETIZED SHEEP. Robert A. Gunther*, Dept. of Surgery, Univ. of California, Davis, CA 95616. (SPON: E.M. Renkin)

Our purpose was to compare the effect of E. coli endotoxin on efferent lymph from the CMN (lung) and PN (skin and skeletal muscle). Seven sheep were prepared with both chronic lung and peripheral lymph fistulae. E. coli endotoxin, 1.5 µg/kg, was given by intravenous bolus injection. Results are given for lymph flow (ml/30 min) and lymph-to-plasma total protein ratios (L/P). Data are mean ± SE.

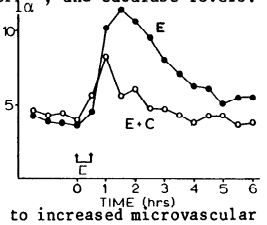
	Lung		Peripheral	
	lymph flow	L/P	lymph flow	L/P
baseline	3.4±1.1	.61±.04	2.7±.45	.51±.02
30 min	4.4±1.1	.51±.06	4.6±.9	.47±.03
1 hr	13.0±3.0	.46±.03	3.8±.5	.41±.04
2 hr	13.0±3.3	.49±.04	3.1±.4	.42±.03
6 hr	9.2±2.6	.60±.03	3.4±.6	.41±.02

Mean pulmonary artery pressure was elevated 23 mm Hg for the first 2 hrs post injection but returned to within 8 mm Hg by 6 hrs. Peripheral lymph protein transport was equal to baseline at 6 hr while CMN lymph protein transport was 3X baseline. These results show that endotoxin at a concentration which profoundly affect the lung lymph has little effect on peripheral lymph. (Supported by NIH HL-07013)

3.7

IMPORTANCE OF H_2O_2 IN THE PULMONARY RESPONSE TO ENDOTOXIN. R.J. Maunder*, R.K. Winn, J.M. Harlan*, J.M. Gleisner*, B.T. Ashleman*, B. Nadir*, J. Hildebrandt. Virginia Mason Research Center and University of Washington, Seattle, WA 98101.

Neutrophils play a key role in septic lung injury. We hypothesized that neutrophil-derived H_2O_2 is an important factor in the microvascular permeability response to endotoxin. In paired experiments using 5 goats with chronic lung lymph fistulae we compared the response to endotoxin (E) with and without catalase (C), a specific H_2O_2 scavenger. C was linked to Ficoll-70 (to prolong $T_{\frac{1}{2}}$) and given 12 hours before E 0.75 μg/kg. We measured hemodynamics, lymph flow ($\dot{Q}_L$), lymph-plasma protein ratio (L/P), TxB_2, 6-keto-$PGF_{1\alpha}$, and catalase levels. The rise in $\dot{Q}_L$ and L/P after E, combined graphically here as protein clearance ($\dot{Q}_L$ x L/P), was attenuated in C-treated animals. C also prevented the drop in cardiac output and arterial pressure after E. Accompanying these changes was a dramatic reduction in the stable metabolites of TxA_2 and PGI_2. We therefore conclude: (1) H_2O_2 contributes to increased microvascular permeability after E; (2) H_2O_2 is also important in the systemic response to sepsis; (3) these effects of H_2O_2 are mediated in part by cyclooxygenase products of arachidonic acid. Supported by: GM 29853, GM 24990, HL 24163, and ALA Fellowship (RJM).



3.8

A MECHANISM FOR ENDOTOXIN PROTECTION FROM OXYGEN TOXICITY. John T. Berg* and Richard M. Smith* (SPON: G.C. Whittow). Dept. of Physiology, Univ. of Hawaii, Honolulu, HI 96822.

Breathing pure oxygen causes pulmonary edema in rats and other mammals. A single intra-peritoneal injection of endotoxin prolongs survival in oxygen and greatly reduces edema formation. Phagocyte derived free radicals have been shown to contribute to lung edema. The present study was designed to determine the effect of In vivo endotoxin on free radical release by lung phagocytes. Rats were injected with endotoxin immediatcly prior to oxygen exposure. Cells were then collected by bronchoalveolar lavage after rats had breathed pure oxygen for 1, 2 or 3 days. The potential of phagocytes to release free radicals was quantitated by zymosan stimulated-luminol dependent chemiluminescence (Cl).

Days in pure O_2	Saline	Endotoxin
1	47 ± 18.8	45.7 ± 20.7
2	60.2 ± 8.6	204 ± 51.7
3	91.3 ± 8	1,903.8 ± 302.4

Control in air = 116.9 ± 28 (± SEM x 10^3)

All saline values, and Day 1 endotoxin values, were lower than Cl for control rats. This may indicate substrate or cofactor depletion in cells which had been overactive in free radical production prior to lavage. Day 2 and Day 3 cells from endotoxin injected rats were able to generate very high Cl (when compared to control levels). This may reflect substrate availability and a lack of free radical release In vivo. NAD-(P)H levels are presently being measured.

3.9

EFFECT OF APROTININ ON LUNG FLUID BALANCE FOLLOWING ENDOTOXIN. J.M. Gleisner*, B.T. Ashleman*, R.K. Winn, J.M. Harlan*, B. Nadir*, R.J. Maunder*, J. Hildebrandt. Virginia Mason Research Center and University of Washington, Seattle, WA 98101.

Increased proteolytic activity has been reported in shock caused by endotoxin, hemorrhage, anaphylaxis and burns. The administration of the broad-spectrum protease inhibitor aprotinin in a number of in vivo shock models, both clinical and experimental, have produced beneficial effects. To assess the role of proteases in the increase in lung vascular permeability following endotoxin administration, we compared the response of goats with chronic lung lymph fistulae to endotoxin with and without aprotinin. We measured hemodynamics, lymph flow ($\dot{Q}_L$) and lymph-plasma protein ratio (L/P) in three paired experiments. Aprotinin reduced the rise in $\dot{Q}_L$ (see table) but little effect was observed on pulmonary arterial pressure, pulmonary wedge pressure, L/P ratios and cardiac output. The data indicate that a protease(s) is involved in lung vascular response to endotoxin. This non-specific protease inhibitor appears to reduce the pulmonary injury even at concentrations of the order 2% of endogenous plasma protease inhibitors. The mechanism of this protection is unknown. Supported by: GM 29853, GM 24990, HL 24163, HL 25706 and ALA Fellowship (RJM).

	Baseline	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	6.69	25.68	23.80	19.92	17.00	16.24	12.12
Aprotinin	5.16	11.00	9.84	9.48	8.24	7.56	7.12

3.10

PULMONARY FLUID BALANCE: DOSE RESPONSE CURVES OF EPINEPHRINE AND NOREPINEPHRINE. Joseph C. Stothert Jr., Robert Winn, Jack Hildebrandt, Brad Nadir*, and Roy Abernathy*. St. Louis University, Dept. Surgery, St. Louis, MO., 63104, and Virginia Mason Research Center, Seattle, WA.

Different concentrations of epinephrine (EPI) and norepinephrine (NOREPI) were infused into monitored, awake goats with chronic lung lymph fistula. Previous work has demonstrated that awake goats respond differently to these catecholamines than anesthetized sheep at higher concentrations (Stothert, J. Surg. Res. 34:367, 1983). Lymph flow ($\dot{Q}L$) and lymph to plasma total protein level (L/P) were monitored in these goats along with cardiac output (C.O.), pulmonary vascular pressures and systemic vascular pressures.

	EPI μg/Kg/min		NOREPI μg/Kg/min		
	1	3	1	3	5
$\dot{Q}L$ (times baseline)	1.4	2.9	1.1	1.6	1.9
Δ L/P	-.01	-.21	-.03	-.03	-.11

EPI in the higher dose range promotes an increase in protein poor lymph which is associated with an increasing pulmonary vascular resistance (P.V.R.) and decreasing C.O. This suggests a downstream pulomary vasoconstriction. NOREPI infusion at the higher dose range is also accompanied by an increasing P.V.R. and decreasing C.O.; however, the increasing lymph flow is not as protein poor suggesting a greater upstream pulmonary vasoconstriction.

3.11

SIMPLIFIED NONINVASIVE DETERMINATION OF THE TRANSPORT COEFFICIENT FOR PULMONARY TRANSVASCULAR PROTEIN FLUX. A. van Grondelle*, I.M. Dauber*, W.T. Pluss* and J.V. Weil* (SPON: J.C. Newell). Dept. of Biomed. Eng., RPI, Troy, NY 12181 and CVP Lab., Univ. of Colorado Health Sciences Ctr., Denver, CO 80262.

To assess the role of increased permeability in the development of pulmonary edema, an accurate method for the determination of permeability is needed. Gorin et al (JCI, 66, 869-877, 1980) determined the pulmonary transvascular protein flux from counts generated by labeled protein and red blood cells in the lung and in blood samples. From this flux, they calculated a transport coefficient α', which reflects permeability to protein. Their method, however, requires extensive calculations. We expressed the counts as a ratio F = (DL/DB)/(RL/RB), in which D = labeled protein, R = labeled red blood cells, L = lung and B = blood sample. F was divided by its value at time 0, resulting in F*. It can be shown theoretically that the slope of F*, α', is approximately equal to α' for sufficiently small time. Using ^{131}I-albumin and ^{99m}Tc-red blood cells, α' and α* were determined from counts obtained between 30 and 150 minutes for 16 lungs with various degrees of lung injury. α* was determined from a single linear regression on F*. The relation between α* and α' was $\alpha^* = 0.08\ 10^{-4} + 0.988\ \alpha'$ ($r = 0.994$) for α' between 4.15 and 35.67 10^{-4}. The excellent agreement between α* and α' shows that the mathematical analysis for Gorin's technique can be reduced to a single linear regression. An important implication of our finding is that, if the blood counts can be obtained from the blood pool in the heart, our method enables on-line determination of α*.

3.12

THE EFFECT OF ELEVATED ALVEOLAR SURFACE TENSION ON MICROVASCULAR PERMEABILITY AND FLUID TRANSFER. CE Bredenberg*, GF Nieman, AM Paskanik*, Upstate Med Ctn, Syracuse NY 13210

The left hilar afferent lymphatic was cannulated in 5 anesthestized dogs. Alveolar surface tension was increased by displacing alveolar surfactant using an aerosol of the detergent dioctyl sodium sulfosuccinate (OT) in a vehicle of 50% ethanol and 50% saline. The aerosol was administered by an ultrasonic nebulizer attached to the ventilator circuit. Lymph (C_L) and plasma (C_P) protein concentration were determined by refractometry. Following a 1 hour control, left atrial pressure (LAP) was elevated to a mean of 20 cm H_2O and held until a steady-state lymph flow ($\dot{Q}L$) and C_L were obtained. A 1 hour rest period (normal LAP) was followed by OT inhalation. Steady-state C_L and $\dot{Q}L$ were again obtained with normal and increased LAP.

Control			OT		
$\dot{Q}L$ (ml/min)	C_L/C_P	LAP (cmH_2O)	$\dot{Q}L$ (ml/min)	C_L/C_P	LAP (cmH_2O)
31	0.67	5.6	67	0.62	6.4
69	0.52	20.1	184	0.46	20.1

The ratio C_L/C_P following detergent inhalation is the same as control, both with normal and elevated LAP indicating no change in pulmonary microvascular permeability. The increase in $\dot{Q}L$ following OT inhalation indicates an increased rate of fluid transfer from the pulmonary microvasculature into the interstitium which causes pulmonary edema.

4.1

CARDIOVASCULAR AND VASOPRESSIN (VP) RESPONSES TO INTRAVENOUS (iv) AND INTRACAROTID (ic) INFUSIONS OF LYSINE VASOPRESSIN (LVP). David P. Brooks*, Leonard Share, Joan T. Crofton and Robin W. Rockhold*. Univ. Tenn. Ctr. Hlth. Sci., Dept. Physiology & Biophysics, Memphis TN 38163.

Previously we have shown that intravertebral infusions of LVP caused similar changes in cardiac output (CO) as iv infusions, but intravertebral infusions caused a significantly greater decrease in heart rate (HR). Plasma VP (P_{AVP}) was unchanged. In the present study we have infused LVP (150 μU/kg·min) ic and iv into morphine/chloralose/urethane anesthetized dogs for 30 min. In 6 vehicle-infused dogs there were no consistent changes in any of the parameters measured. Infusion of LVP iv and ic also had no effect on mean arterial blood pressure (MABP), however, CO decreased 22.2 ± 1.9 and 15.2 + 6.6 ml/min•Kg respectively and HR decreased 6.2 ± 3.3 ($p<0.05$) and 12.3 ± 2.0 beats/min ($p<0.01$) respectively. There were no significant differences between iv and ic LVP infusions. Similarly, there were no differences in P_{AVP} measurements between iv and ic LVP infusions, although there were small decreases in P_{AVP} within the vehicle and LVP iv infusion groups. The results further indicate that peripheral VP can alter HR and CO, but does not feedback to inhibit its own release. (Supported by USPHS grants HL-12990 and HL-19209).

4.2

ANALYSIS OF THE CARDIOVASCULAR EFFECTS OF ARGININE VASOPRESSIN (AVP) IN CONSCIOUS DOGS. U. Tipayamontri* and D. B. Young. University of Mississippi Medical Center, Jackson, MS 39216

AVP infusion has been shown by others to reduce cardiac output (CO), although the mechanism of the reduction is unclear. We have analyzed the effects of infusion of physiological levels of AVP on CO regulation in 6 conscious, chronically instrumented dogs. Several weeks prior to the experiments catheters were placed in the jugular vein, right atrium, and infrarenal abdominal aorta, and an electromagnetic flow probe was placed around the ascending aorta. The following variables were measured 30 minutes after the start of infusion of either normal saline or AVP (1.0 mU/kg/min): MAP, CO, HR, RAP, BV and mean circulatory filling pressure (MCFP). CO function curves were determined on the following day with the same infusion. Compared to normal saline infusion, AVP infusion caused a significant ($p<.05$) reduction in CO from 2.59±0.245 to 1.908±0.214 l/min, and increases in TPR and resistance to venous return from 39.8±3.5 to 58.8±7.6 and 2.57±0.60 to 3.95±0.74 mmHg/l/min, respectively. There were no significant changes in MAP, RAP, HR, SV, MCFP, pressure gradient for venous return or BV. The reduction in CO was due apparently to a direct cardiac effect since neither preload (RAP) nor afterload (MAP) were changed. This hypothesis was tested by determining cardiac function curves during saline and AVP infusion in two dogs. AVP decreased the slope of the curve and the plateau by 35%. These results suggest that AVP has a direct inhibitory effect on the heart. (Supported by HL11678)

4.3

HEMODYNAMIC IMPORTANCE OF VASOPRESSIN DURING HEMORRHAGE. Walter A. Boyle, III* and Guy Valiquette*(SPON: W.H. Sawyer). Columbia University, New York, NY 10032

A number of investigators have demonstrated that vasopressin (VP) is important to blood pressure (BP) maintenance during acute hemorrhage. To determine how VP affects other hemodynamic variables, we examined the hemodynamic response to acute hemorrhage in dogs with and without VP blockade. 15 dogs were anesthetized with halothane and intubated; anesthetic depth and pCO_2 were controlled by end-tidal gas analysis. Aortic and pulmonary artery catheters were inserted; after stabilization, hemodynamic measurements and blood for VP RIA were obtained. Dogs then underwent hemorrhage to 30 ml/kg with frequent measurements. "Pretreatment" (Pt) dogs received VP "pressor" antagonist (BL III-12, gift of M. Manning) prior to hemorrhage (n=5), "Acute-treatment" (At) dogs were given the antagonist immediately after the hemorrhage (n=5), and the remaining five dogs received no treatment (controls). In the Pt dogs there was a significant decrease in BP after minor hemorrhage (10 ml/kg) which was not present in the other dogs. Further hemorrhage was accompanied by significant decreases in BP in all dogs, greatest in the Pt dogs, but the differences between the groups were not significant. Cardiac output (CO) decreased significantly in all dogs during hemorrhage but was significantly less depressed in the Pt dogs. In the At dogs, there was a significant fall in BP and increase in CO with antagonist administration. The greatest differences among the groups were found when systemic vascular resistance (SVR) was considered. SVR was significantly ($p<.01$) depressed throughout hemorrhage in the Pt dogs, compared to the other dogs, and SVR changed dramatically (-64%) in the At dogs with antagonist administration. Our findings confirm that BP is less well maintained during hemorrhage when the "pressor" effects of VP are blocked but, more significantly, suggest that the primary hemodynamic effect of VP involves changes in SVR.

4.4

ANGIOTENSIN II ALTERS THE RELATIONSHIP BETWEEN BLOOD PRESSURE AND PLASMA VASOPRESSIN CONCENTRATION IN CONSCIOUS DOGS. V.L. Brooks, L.C. Keil and I.A. Reid. Department of Physiology, University of California, San Francisco, CA 94143

There is evidence that angiotensin II (AII) increases plasma vasopressin concentration (P_{AVP}); however, this effect has not been observed consistently. A possible explanation for the inconsistency is that the pressor effect of AII counteracts a stimulatory action of AII on vasopressin secretion. This hypothesis was tested by examining the effect of AII on P_{AVP} while the pressor effect of AII was reduced with an infusion of four doses of the vasodilator nitroprusside. When nitroprusside alone was infused, P_{AVP} increased as blood pressure (BP) fell ($r^2 = 0.62$, $p < 0.001$). A 15 min infusion of AII (10 $ng \cdot kg^{-1} \cdot min^{-1}$) alone increased BP but did not alter P_{AVP}. When nitroprusside was infused in the presence of AII, P_{AVP} increased, and the P_{AVP} increase was again related to the BP fall ($r^2 = 0.60$, $p < 0.001$). However, AII shifted this relationship to the right. With total prevention of the BP rise, AII increased P_{AVP} by 13.0±1.6 pg/ml ($p < 0.001$). These data suggest that the pressor effect of AII counteracts a stimulatory action of AII on vasopressin secretion and that when endogenous AII levels are elevated without an increase in BP, as occurs during hypovolemia, AII may stimulate vasopressin release. Supported by USPHS Grant AM06704 and California Heart Association Grant 82-N8.

4.5

EFFECT OF GABA ON VASOPRESSIN RELEASE. Celia D. Sladek* and William E. Armstrong* (SPON: R. Connett), Univ. of Rochester, Rochester, N.Y. 14642.

The role of γ-aminobutyric acid (GABA) in the control of vasopressin (VP) release was examined by exposing organ-cultured explants of the rat hypothalamo-neurohypophyseal system (HNS) to varying concentrations of GABA or to the GABA antagonists, bicuculline (10^{-4}M) and picrotoxin (10^{-5}M) during a one hour test period. VP release was uniformly decreased to approximately half of the control rate by GABA at concentrations ranging from 10^{-3} - 10^{-5}M. In these experiments VP release during the control hour was 103 ± 12 pg/explant. In the presence of GABA it was reduced to 43 ± 6 pg/explant ($p .001$). The addition of GABA antagonists to the culture medium without exogenous GABA resulted in an increase in VP release. In the presence of bicuculline (10^{-4}M), VP release increased during the test hour to 181 ± 17% of the rate observed during the control hour ($p .01$, n=6). Picrotoxin (10^{-5}M) caused a comparable elevation of VP release. The inhibition of VP release by GABA is consistent with the observation that application of GABA decreases electrical activity of supraoptic nucleus neurons. The stimulation of VP release by GABA antagonists is consistent with endogenous GABA activity in the explant. This could represent the influence of GABAergic neurons which border the supraoptic nucleus (Tappaz, et al., Prog. Br. Res. 55:77, 1982). The neural lobe may also be an important site for GABAergic modulation of VP release (Tappaz, ibid). Our experiments do not differentiate between a hypothalamic and pituitary site of action. Supported by NIH grant AM-19761.

4.6

SODIUM LOADING IN RABBITS WITH PRE-OPTIC PERIVENTRICULAR HYPOTHALAMIC LESIONS. Gregory D. Fink*, Mark E. Mann* and Cathy A. Bruner* (SPON: G.L. Gebber). Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Previous studies in the rat have reported that lesions in the anterior third cerebral ventricle (AV3V) will attenuate the excretion of sodium and water after an acute i.v. isotonic saline (0.9% NaCl) infusion. We sought to identify the mechanism of this phenomenon in AV3V-lesioned (AV3V-X) rabbits, and also examined sodium and water handling in AV3V-X rabbits during chronic alterations in sodium intake. Acute saline loading (10% b.wt./hr) caused no significant change in arterial pressure (MAP), but significant increases in central venous pressure (CVP), interstitial fluid pressure (IFP), extracellular fluid volume (ECFV) and interstitial fluid volume (IFV) in sham-lesioned rabbits (n=10). In AV3V-X rabbits (n=10), only IFP increased significantly during the 1 hr infusion. A separate group of 7 rabbits were given food containing 1, 11 and 170 mEq sodium/100 g on 3 consecutive weeks, then received AV3V lesions and the dietary protocol was repeated 2 weeks later. In the pre-lesion period, increasing sodium intake caused a significant increase in MAP and water intake, but no other significant changes in body fluid parameters. After AV3V-X, similar changes were obtained during dietary sodium loading. Furthermore, after AV3V-X the rabbits adjusted urinary sodium excretion normally during step changes in sodium intake. These results suggest that blunted excretory responses to acute saline loading in AV3V-X rabbits may involve an inability to "sense" the load, and that this defect is unimportant in chronic sodium and water homeostasis. (Supported by USPHS grant HL24111.)

4.7

CHRONIC SODIUM DEPLETION DOES NOT ALTER PLASMA CATECHOLAMINE CONCENTRATION. Robert G. Carroll, Thomas E. Lohmeier and Alison J. Brown*. Univ. Miss. Med. Ctr., Jackson, MS. 39216.

To assess the effect of chronic sodium depletion on the activity of the sympathetic nervous system, we measured arterial (N=5) and renal venous (N=3) norepinephrine (NE) and epinephrine (EPI) concentrations in chronically instrumented dogs maintained for 9 days on a low sodium diet; additionally, furosemide (10 mg IM) was administered on days 0 and 4. Renal NE overflow (V_{NE}-A_{NE} effective renal plasma flow) was used as an index of renal sympathetic nerve activity. The following observations were made:

	Control	Day 3	Day 9
Mean Arterial Pressure (mmHg)	93±3	91±4	82±3*
Arterial Plasma NE (pg/ml)	103±8	134±40	90±13
Renal Venous Plasma NE (pg/ml)	172±32	160±26	167±40
Arterial Plasma EPI (pg/ml)	69±13	60±27	101±16
Plasma Renin Activity (ng AI/ml/hr)	.29±.04	.94±.22*	1.18±.27*
Urinary Sodium Excretion (mEq/day)	41±3	5±2*	3±1*

* $p < 0.05$

Although sodium depletion produced a four fold increase in plasma renin activity and a reduction in mean arterial pressure of 11 mmHg, there were no chronic changes in peripheral NE or EPI concentration. Additionally, renal NE overflow was unchanged during sodium depletion. These results do not support the contention that there is sustained activation of the sympathetic nervous system during chronic sodium depletion. (Supported in part by NIH Grants HL 11678 and HL 06341).

4.8

Brain-Renin Angiotensin in the Control of Adrenal Catecholamine Release During Hemorrhage. E.J. Corwin, J.F. Seaton*, M. Hamaji, and T.S. Harrison. Penn State Medical, Hershey, PA 17033.

Angiotensin II (AII) contributes to blood pressure control during hemorrhage (H) by reinforcing adrenal release of catecholamines (CAT). Evidence for this is the fall of adrenal CAT release with H, in nephrectomized (NEPH) animals, which is reversed when the animals are given i.v. AII[1]. We tested the hypothesis that the AII stimulation of CAT release during H is due to a central effect. Dogs were anesthetized, and mean pressure and adrenal secretions of epinephrine (E) and norepinephrine (NE) measured. Bilateral or sham NEPH was performed, and needles were placed ventriculo-cisternally (IVT) to allow drug perfusion ventricularly, before and during H. NEPH dogs receiving only artificial CSF IVT at 1 ml/min showed a small CAT response with H (E= .53 ± .14 µg/min, NE= .1 ± .03 µg/min, $p > .05$) when compared to pre-H levels (E= .16 ± .1, NE= .03 ± .02: E & NE levels with H were comparable to those reported before in NEPH animals hemorrhaged without AII replacement. In contrast, IVT AII at 10 pg/kg/min enhanced CAT release (E= 1.7 ± .34, NE= 1.05 ± .38, $p < .05$) when compared to CSF alone; i.v. AII at this dose had no effect. IVT infusion of .06 ng/kg/min saralasin, a competitive antagonist of AII, prior to H, reduced CAT release during H in intact dogs (E= .97 ± .43, NE= .11 ± .06). The results suggest AII and renal reinforcement of centrally mediated adrenal CAT release during H. [1]Harrison, et al, AJP 224:21-34, 1973-Supported by NIH Grant HL18995

4.9

PHYSIOLOGIC RESPONSE OF WATER IMMERSION IN THE RHESUS MONKEY. B.A. Benjamin*, L.C. Keil, M.S. Shapiro*, M.A. Kirschenbaum, N.S. Bricker, and H. Sandler. NASA Ames Research Center, Moffett Field, CA 94035 and UCLA School of Medicine, Los Angeles, CA 90024

Previous immersion (Im) studies in nonhuman primates have been conducted using anesthetized (A) animals. The present study was designed to compare such changes under tranquilized (T) and conscious (Co) states. Seven experiments were carried out in Co chair-conditioned M. Mullata. Four monkeys were studied A (Nembutal 25 mg/kg) and five were studied T (Ketamine 10 mg/kg). All animals were on a constant Na^+ diet (15 meq/kg/day) with water Ad Lib. The protocol consisted of a 90-min control (c) period, 2 hours Im to the neck in a thermoneutral bath and one hour post-Im. Immersion caused a diuresis and natriuresis in all three groups. Average urine flow during Im was greatest in the Co group and changed from .5±.15 (c) to 1.7±.3 ml/min (x±SE). Urine flow increased from .3±.02 (c) to .99±.2 ml/min with T and from .22±.09 (c) to .7±.03 ml/min with A monkeys. Sodium excretion increased from 8.9±2.5 (c) to 42.4±5.7 µeq/min in Co, from 7.2±2.3 (c) to 40.2±8 µeq/min in A, and from 3±1.7 (c) to 14.8±3 µeq/min in T group. ADH decreased from 56±11 to 24±5 pg/ml ($p<.05$) in A group. T monkeys started at 14±3 and decreased to 6±1.5 pg/ml ($p<.05$) while Co animals decreased from 1.8±.9 to 1.6±.9 pg/ml (N.S.). These studies show that Nembutal and Ketamine significantly alter the physiologic response to Im compared to Co state. Of note, is the marked elevation of basal ADH values in the T and A animals.

4.10

CHANGES IN PLASMA VASOPRESSIN (AVP) CONCENTRATION IN RESPONSE TO DISTENSION OF THE PULMONARY VEIN-LEFT ATRIAL JUNCTIONS. J.R. Ledsome and N. Wilson*. University of B.C., Vancouver, British Columbia, Canada V6T 1W5

Inflation of small balloons placed in the left pulmonary vein-left atrial (PV) junctions with the left lung tied off causes stimulation of left atrial receptors without interfering with blood flow through the left atrium. In chloralose anesthetized dogs this stimulus causes an increase in heart rate, a decrease in renal vascular resistance and a diuresis and natriuresis. In 11 dogs measurements were made of plasma AVP concentration before, 10 min after distension of the PV and 10 min after removal of the distension. Plasma AVP decreased from 5.6 ± 1.3 (mean, ± SE) pg/ml in the control periods to 4.2 ± 1.0 pg/ml during PV distension ($P<0.005$); heart rate (HR) increased from 160 ± 6.2 to 174 ± 8.9 beats/min ($P<0.005$). In 5 dogs propranolol (0.5 mg/kg) decreased HR but did not change plasma AVP. PV distension then caused no change in HR but plasma AVP decreased (7.8 ± 1.8 to 4.8 ± 1.5 pg/ml, $P<0.05$). Cooling both vagi to 10°C increased HR and plasma AVP. PV distension then did not change HR or plasma AVP. The results are consistent with the reduction in plasma AVP with PV distension being caused by increased activity in myelinated afferents from atrial receptors.

(Supported by B.C. Heart Foundation and M.R.C.)

4.11

VASOPRESSIN, ALDOSTERONE, AND URINARY RESPONSES TO HEAD OUT IMMERSION AND SUPINE POSTURE IN MAN. J.R. Claybaugh, D.R. Pendergast, and S.K. Hong. Tripler Army Medical Center, Honolulu, Hi. 96859 and SUNY at Buffalo, Buffalo, N.Y. 14214

Five male volunteers participated in 3 protocols. In each, they ingested water (2.5% body weight) at time 0 and sat for 1 h. No further water was taken. The subjects either remained seated for 5 h (SIT), were supine for 6 h followed by 1 h seated (SUP), or were immersed to the neck for 6 h followed by 1 h seated (IMM). One h after water load the urine flow (V) for SIT, SUP, and IMM groups were 5.9±0.6, 6.1±1.1, and 4.9±1.1 ml/min respectively (NS). In the SIT and SUP groups V steadily decreased and was significantly reduced ($P<0.05$) after 1 h in the SIT group and 3 h in SUP group. In contrast, V increased 73% ($P<0.01$) after 1 h, remained elevated ($P<0.05$) at 2 h and was not significantly lower than pre-immersion until 1 h post immersion. This disparent pattern in V between the SUP and IMM groups cannot be explained by differences in vasopressin or aldosterone. Urinary vasopressin excretion rate decreased 63% ($P<0.05$) during the 2nd h of SUP and decreased 58% ($P<0.05$) during the 2nd h of IMM. Plasma aldosterone concentrations also showed similarity between SUP (decreasing 32% ($P<0.01$) at 4 h) and IMM (decreasing 36% ($P<0.01$) at 4 h. Thus, the aldosterone and vasopressin responses to immersion were mimicked by a supine posture, but the urinary responses were not. (Supported by U.S. Army Healthy Services Command and HL-28542)

4.12

PLASMA VASOPRESSIN, PLASMA RENIN ACTIVITY, AND HEMODYNAMICS DURING HEMORRHAGE IN CONSCIOUS DOGS WITH CHRONIC SINOAORTIC DENERVATION. K. L. Goetz, B. C. Wang, and W. D. Sundet*. St. Luke's Hospital and Foundation, Kansas City, MO 64111

We have reported (Physiologist 25: 288, 1982) that the increase in plasma vasopressin that occurs in normal conscious dogs during hemorrhage is markedly attenuated in dogs with surgically denervated hearts (afferent pathways from arterial baroreceptors intact). We concluded that cardiac receptors play a dominant role in modulating the secretion of vasopressin during hemorrhage. It is possible, however, that both cardiac volume receptors and sinoaortic baroreceptors are necessary for the full expression of the vasopressin response to hemorrhage and that removal of either set of receptors attenuates the response. To test this possibility, we bled 6 conscious dogs with chronic sinoaortic denervation until 30 ml of blood per kg body weight had been removed. Control levels of plasma vasopressin and renin activity were normal. The absolute increases in plasma vasopressin during hemorrhage were decreased (not significantly) when compared with results obtained from sham-operated dogs. The increase in plasma renin activity in response to hemorrhage was indistinguishable from that obtained earlier with sham-operated or cardiac-denervated dogs. These data suggest that arterial baroreceptors play a relatively small role in increasing plasma vasopressin levels during hemorrhage. Our experiments also illustrate that neither arterial baroreceptors nor cardiac receptors are required to produce the renin response to hemorrhage. (Supported by NIH grant HL13623)

5.1

INTERVESSEL DIFFERENCES IN SMOOTH MUSCLE MYOSIN HEAVY CHAINS. C.L. Seidel, V. White* and L. Schildmeyer*. Dept. Med., Sect. CV Sci., Baylor College of Medicine, Houston, TX 77030.

In striated muscle, differences in myosin heavy chain (MHC) mobility have been correlated with differences in muscle shortening velocity. Because vascular smooth muscle (VSM) has different contractile properties than striated muscle and demonstrates intervessel contractile heterogeneity, the existence of differences in MHC molecular weight between striated and vascular muscles was investigated. Rat left ventricle (V), aorta (A) and jugular vein (J) were extracted in 25 mM Na^+ phosphate, 1% βME, 1% SDS, 0.01% proteinase inhibitor. Aliquots (50-100 µg protein) were placed on 5% polyacrylamide-1% SDS gels, electrophoresed and stained with coomassie blue. The mobility (Rf) of the MHC was expressed as a percentage of the mobility of the tracking dye. V had one band, whereas, J and A each exhibited three bands (M_1,M_2,M_3) of similar Rf but of different relative staining intensities (A: M_1 18%, M_2 28%, $M_3$54%; J: M_1 43%, M_2 45%, M_3 12%). The dominant band of J (M_2) had a mobility of 40 ± 1%, A (M_3) 42.6 ± 0.2% and V 44.4 ± 0.7%. All mobilities were significantly different from each other ($p < 0.05$, $n = 5$). These data suggest that the dominant MHC in VSM has a lower mobility than that in V, that VSM may have more than one form of MHC and that the dominant form may differ between VSM. These differences may be related to reported intermuscle differences in contractile properties. (Support by HL 23815, HL 25349 and HL 07282)

5.2

Calmodulin-mediated stimulation of calcium uptake in isolated microsomes from bovine carotid artery. R.C. Bhalla, R.V. Sharma*, and D.W. Doerrfeld*, University of Iowa, Iowa City, IA 52242.

The role of calmodulin and other factors involved in the regulation of calcium uptake in vascular smooth muscle was examined in a microsomal fraction prepared from bovine carotid artery. Calcium uptake required both ATP and Mg^{2+} and was not inhibited by sodium azide. Energy-dependent calcium uptake was greatly enhanced by oxalate in a dose-dependent manner. Calcium ionophore A23187, inhibited calcium uptake to 10-20% of control values in the presence of ATP and oxalate. Calcium uptake was dependent on the free Ca^{2+} concentration in the medium and increased in a dose-dependent manner. The threshhold maximum concentration was approximately 10^{-7} M and maximum uptake was obtained at approximately 2×10^{-6} M. Calmodulin stimulated calcium uptake in EGTA-washed microsomes in a dose-dependent manner and the maximum stimulation was 50-60%. Half-maximal stimulation occurred at approximately 0.2 µM calmodulin. The stimulatory effect of calmodulin was observed over the entire range of calcium concentration. Calmodulin also stimulated phosphorylation of microsomal proteins. The major microsomal protein phosphorylated by calmodulin-dependent kinase showed an apparent molecular weight of 45,000 daltons, estimated by SDS-polyacrylamide gradient gel electrophoresis. These results indicate that calmodulin may play a role in the control of free cytoplasmic calcium concentration in vascular smooth muscle. Supported by NIH grant #HL19027.

5.3

Phorsphorylation of endogenous microsomal proteins by cAMP- and calmodulin-dependent kinases in bovine carotid artery microsomes. R.V. Sharma*, D.W. Doerrfeld* and R.C. Bhalla, University of Iowa, Iowa City, IA 52242.

Bovine carotid artery microsomes were phosphorylated by both cAMP and calmodulin dependent kinases. The catalytic subunit of cAMP dependent protein kinase in the presence of EGTA catalyzed the incorporation of ^{32}P-phosphate in two major bands corresponding to proteins of Mr ≥ 200,000 and 45,000. This phosphorylation was independent of Ca^{2+} and calmodulin, a membrane-bound kinase catalyzed the incorporation of ^{32}P-phosphate into one major band of Mr 45,000 daltons. Several other protein bands of Mr 65,000 and 20,000 were labeled to a lesser extent by calmodulin-dependent kinase. Half maximal phosphorylation of the 45,000 dalton band was achieved at 0.16 µM calmodulin and maximal phosphorylation was observed at approximately 1 µM calmodulin. Ca^{2+} increased calmodulin-dependent phosphorylation of 45,000 dalton protein in a dose-dependent manner and maximum phosphorylation was achieved at approximately 5 µM Ca^{2+} in the presence of 1 µM calmodulin. Calmodulin-dependent microsomal phosphorylation was inhibited by trifluoperazine with an apparent K_i of 2×10^{-6} M. Similarly, 10 mM EGTA inhibited calmodulin-dependent phosphorylation of the 45,000 dalton protein. These data suggest that both cAMP and calmodulin phosphorylate a 45,000 dalton protein which may be involved in the regulation of intracellular calcium concentration.

5.4

CORRELATION OF VASCULAR SMOOTH MUSCLE RELAXATION AND NA^+ PUMP STIMULATION BY FORSKOLIN. J.C. Allen, S.S. Navran*, C.L. Seidel and R.A. Strong*. Department of Medicine, Section of Cardiovascular Sci., Baylor College of Medicine, Houston, TX 77030.

Forskolin (F) has been shown to stimulate adenyl cyclase activity by direct action on the catalytic subunit, increasing tissue c-AMP levels. We have used this affect to study arterial smooth muscle contractile regulation. F relaxes both femoral (FA) and renal (RA) arteries when they are contracted with ED_{50} concentrations of either phenylephrine (PE) or KCl. F (10^{-6} M) is more effective in relaxing RA than FA: PE, RA = 64 ± 13%, FA = 29 ± 6%; KCl, RA = 37 ± 8%, FA = 7 ± 4%. F(10^{-7} - 10^{-5} M) stimulates ouabain sensitive ^{86}Rb uptake, a measure of the Na^+ pump, in relaxed Na^+ loaded RA, but not Na^+ loaded FA. At normal cellular Na^+ levels, when the pump is functioning at lower rates, F also stimulates ^{86}Rb uptake in both arteries. In muscles contracted with ED_{50}'s of either PE or KCl, F stimulation of ^{86}Rb uptake is still maintained in both arteries. These data suggest that F stimulation of the Na^+ pump may be the mechanism of F induced relaxation, and that c-AMP may stimulate the Na^+ pump and thereby exert control over contractility of vascular smooth muscle. (Support by HL 07282, HL 25349, HL 24585 and HL 23815)

5.5

INFLUENCE OF PROSTAGLANDIN ON ARTERIAL MICROSOMAL CALCIUM TRANSPORT. M. E. Soulsby and B. H. Perlmutter*, Univ. Ark. Medical Sciences, Little Rock, AR 72205.

Microsomes isolated from the medial muscularis of bovine aorta were used in this investigation of the effect of 10^{-4} to 10^{-9} M prostacyclin (PGI_2) on passive (no ATP) Ca^{++} binding, ATP-dependent Ca^{++} uptake and Ca^{++} dependent ATP hydrolysis. Incubations were carried out in a medium containing 0.25 mg/ml membrane protein, 104 mM KCl, 50 mM histidine (pH 7.0), 10 mM K-oxalate, 8 mM Na-azide, 5 mM $MgCl_2$, 5.0×10^{-5} $CaCl_2$, 0.1 µCi $^{45}CaCl_2$, and 5 mM Na_2ATP. Aliquots for sampling were taken at 0, 1, 3, 3.6, 5 and 8 min, and PGI_2 was added at 3 min. Control binding was 7.7±0.321 µMol Ca^{++}/gm protein (n=19), whereas after 1.0×10^{-6} PGI_2, binding increased to 9.89±0.385 µMol Ca^{++}/g protein ($p<0.01$). Control ATP-dependent Ca^{++} uptake (total minus binding (no ATP)) was 1.0 µM/g protein/min (n=14). PGI_2 (1.0×10^{-6} M) caused an immediate 85% reduction ($p<0.01$) in uptake, proceeding to 91% after 1.5 min, followed by resumption of normal uptake rate. Control Ca^{++}-dependent ATPase activity was immediately depressed 38% by PGI_2 ($p<0.01$) proceeding to 60% after 1.5 min, followed by resumption of normal ATPase activity. We conclude that PGI_2 vasodilation is the result of increased microsomal passive Ca^{++} binding, as well as depressed active Ca^{++} uptake, secondary to depressed Ca^{++}-dependent ATPase activity. (Supported in part by American Heart Association/Arkansas Affiliate.)

5.6

PRODUCTION OF 6-Keto $PGF_{1\alpha}$ BY AORTA FROM B/W MICE WITH AUTOIMMUNE DISEASE (MURINE LUPUS). Joseph V. Levy (SPON: J.R. Neville). Kuzell Inst. Arthritis Res., MRI, Pacific Med. Ctr., San Francisco, CA.

The B/W mouse derived from NZW X NZB is characterized by many symptoms resulting from spontaneous autoimmune disease (murine lupus-like syndrome). Among the pathophysiological features of this disease is the presence of vasculitis and necrotizing arteritis. It has been speculated that a deficiency in vascular prostacyclin in lupus patients may account for the higher incidence of thrombotic and other vascular disorders. Therefore, experiments were done to determine whether there was a change in prostacyclin production in the B/W lupus model vs. non-lupus control mice (NZB and NZW). Aortae from 27-32 wk. old male (m) and female (f) B/W mice and age matched controls were incubated in physiological buffer for 30 min. at 37 C. The amount of prostacyclin released into the incubate was determined by measuring the stable metabolite of prostacyclin, 6-keto $PGF_{1\alpha}$ (6KPG) by RIA. B/W(f) tissue produced 2.8 times more 6KPG vs. B/W(m). NZW(f) aortae produced 3.47 times more 6KPG than NZW(m). However, no sig. difference was noted between NZB(f) and (m). No sig. difference was seen between B/W (m) and (f) and their matching NZW controls. These data suggest that the vascular changes in this murine lupus model are not associated with a decrement in prostacyclin production under the assay conditions used. Support: Oxnard and Shoong Foundations and Browning Trust.

5.7

INFLUENCE OF TRANSMURAL NERVE STIMULATION (TNS) ON THE EFFECT OF EXOGENOUS NORADRENALINE (NA) IN THE CANINE SAPHENOUS VEIN. M.P.J. Senaratne* and C.T. Kappagoda. Division of Cardiology Department of Medicine, Univ. of Alta. Edmonton T6G 2G3

During recent years the existence of two types of post-junctional alpha-adrenergic receptors mediating the effects of exgenous and endogenous NA in smooth muscle has been documented. It has been shown that TNS inhibits the contraction produced by exogenous NA (The Physiologist 25:209, 1982). The present study was undertaken to further elucidate this inhibitory effect. Response to a single dose of NA was determined as a control. After washing, TNS was applied to produce a contraction 20-70% of the control value. Once this contraction reached a plateau the same dose of NA was added while maintaining TNS. Finally a second control response with NA was repeated. The protocol was repeated with a histamine (n=10) or tyramine (n=16) induced contraction substituted for TNS. The contraction produced by exogenous NA against a background of histamine or tyramine was significantly more than that produced against a background of TNS ($p < .01$). The protocol was also repeated in presence of propranolol (10^{-6} mol/l, n=10) and in the presence of indomethacin ($5x10^{-6}$ mol/l, n=11). The inhibitory phenomenon was still evident under these circumstances suggesting that it is unlikely to be mediated by either beta receptors or by a prostaglandin. These results indicate that the inhibition of exogenous NA mediated contractions observed is specifically associated with a contraction caused by exocytotically released NA.

5.8

NE INDUCED CONTRACTION IN THE RABBIT SAPHENOUS ARTERY: DISMANTLING BIPHASIC TENSION DEVELOPMENT. K. Keef* and A.J. Brady Dept. of Physiology, UCLA, LA, Ca. 90024.

Tension development in response to norepinephrine (NE) in the rabbit saphenous artery consists of two distinct phases. The initial phase (A) rises to maximum in 8 to 20 sec and then falls toward zero. The second phase (B) reaches maximum in 60 to 90 sec and then slowly declines. In 2.5 mM Ca Krebs solution at 37°C these two phases combine such that total tension appears monophasic. When Ca is reduced by buffering with EGTA to obtain free Ca in the μM range total tension is biphasic. When extracellular Ca is completely removed by exposing the vessel to a 0 Ca 1 mM EGTA solution only phase A tension is obtained. We have developed a diffusion dependent model to predict tension development in these two phases. The Ca delivery process for phase A is assumed to be release from a limited store with first order kinetics. The Ca delivery process for phase B is assumed to be an influx from an external source through a Ca channel with first order "on" and "off" kinetics. Removal of Ca from the cytoplasm is assumed to follow first order kinetics. Specific mathematical relationships were defined for these assumptions (including the time required for diffusion of NE into the tissue). Rate constants were obtained by fitting the model to real tension responses. The model was found to be sufficient to describe phasic, biphasic and monophasic plateau type responses. Supported by grant # HL 11351.

5.9

OXYGEN SENSING BY FEMORAL AND RENAL ARTERIES FROM NORMOTENSIVE AND HYPERTENSIVE DOGS. R.N. Pittman and B.A. Graham.* Dept. of Physiol. Biophys., Medical College of VA, Richmond, VA 23298.

We investigated the role of the endothelium in O_2-linked contractile responses of in vitro strips of femoral and renal arteries from normotensive (NT) and one-kidney Goldblatt hypertensive (HT) dogs. The intimal surface of half the strips was rubbed to remove functional endothelium. Isometric force was recorded for strips stimulated with either 20 mM potassium chloride (KCl) or 0.5 μM norepinephrine (NE). Solution PO_2 (P_S) was initially set above the critical P_S (P_S^c) determined for these strips and was lowered in steps to zero Torr. P_S^c depended on the stimulating agent (~123 Torr for KCl and ~69 Torr for NE), but otherwise was similar for femoral and renal arteries whether they were rubbed or unrubbed and NT or HT. Two different patterns of responses were observed: (1) monotonic decrease in force for P_S from P_S^c to 0 and (2) monotonic decrease in force for P_S from P_S^c to P_{min} and subsequent increase in force for P_S from P_{min} to 0. P_{min} was the P_S at which force was minimum and it ranged from 5-10 Torr. Response (1) was observed primarily for renal arteries and response (2) primarily for femoral arteries, although about half the strips stimulated with NE from either site for HT dogs showed either response (1) or (2). Response (2) occurred with or without intact endothelium and we conclude that the site of this O_2-linked contractile behavior is not the endothelium. Response (2) is qualitatively similar to PO_2 dependent prostaglandin-mediated contractile behavior observed by others. (HL25383).

NEUROENDOCRINES, PITUITARY

6.1

CYCLOSPORIN A INHIBITS ORNITHINE DECARBOXYLASE INDUCTION IN KIDNEY IN RESPONSE TO PROLACTIN, GROWTH HORMONE AND INSULIN. Diane Haddock Russell, Douglas F. Larson*, J.R. Womble*, and Jack G. Copeland*, Departments of Pharmacology and Surgery, University of Arizona College of Medicine, Tucson, AZ 85724

Others have shown that prolactin administration results in a marked, early induction of ornithine decarboxylase (ODC) activity in rat kidney (Richards, BBRC 63: 292-299, 1975). Internalization of "coated-pit" hormones, such as prolactin, insulin and possibly growth hormone, may be important in the physiological response to these polypeptide hormones. We found that cyclosporin A, a compound used investigationally as an immunosuppressive agent in organ transplant patients, blocked the induction of ODC in kidney in response to all 3 of these hormones. Cyclosporin A (10^{-6} M) inhibited the ODC response to prolactin by 70%, to growth hormone by 80%, and to insulin by 50%. It did not affect ODC induction in response to hormones whose actions are mediated by cyclic AMP. The structure of cyclosporin A suggests it may be a membrane antagonist of binding sites for these hormones. The administration of cyclosporin A alone did not alter the high basal level of ODC activity present in the kidney. Cyclosporin A alone also did not alter the serum level of prolactin. These data provide the first evidence that cyclosporin A may be a specific inhibitor of binding sites for coated-pit hormones. (Supported by USPHS Research Grant CA-14783 to D.H.R.)

6.2

INFLUENCE OF CORTICOSTERONE ON THE PROLACTIN RESPONSE TO PHYSICAL AND PSYCHOLOGICAL STRESS. Albert Ratner, David B. Yelvington*, and Gerald K. Weiss. Univ. New Mexico School of Medicine, Albuquerque, NM 87131

Both corticosterone and prolactin levels increase in response to stress. In these studies we examined the effect of corticosterone on the prolactin response to both physical (intermittant footshock) and psychological (novel environment) stress. Three groups of rats were used: sham-adrenalectomized (SHAM); adrenalectomized (ADX); and adrenalectomized with corticosterone treatment (ADX+CORT). Blood samples were drawn via an indwelling atrial cannula and prolactin values determined using RIA. ADX rats showed a consistently greater prolactin response to being placed on a platform above water (novel environment) or when receiving intermittant footshock (1 h or 3½ h) than did ADX+CORT rats. The prolactin response of the ADX+CORT rats was similar to that of the SHAM or control animals. Sick rats, with presumably high levels of corticosterone, showed a greatly attenuated prolactin response to footshock when compared to healthy rats. These findings indicate that corticosterone levels of an animal can significantly affect the magnitude and time course of the prolactin response to both physical and psychological stress. These findings further emphasize that the prolactin response to stress is dependent upon more than just the immediate action of the stressor.

6.3

EFFECTS OF TREATMENT OF NEONATES WITH MONOSODIUM GLUTAMATE ON GONADOTROPES IN THE PREPUBERTAL MALE RAT. M.O. Dada* and C.A. Blake. Univ. of Nebraska Medical Center, Omaha, NE 68105

It is established that treatment of neonatal rats with monosodium glutamate (MSG) causes destruction of the hypothalamic arcuate nuclei, and results in a small anterior pituitary gland (APG), often normal serum LH and FSH levels, and reduced gonadal weight. We investigated the effects of MSG on the APG gonadotropes. Male rats were injected with saline or MSG (4 mg/g body weight) on Days 1,3,5,7 and 9 of life (Day 0 = day of birth) and killed by decapitation on Day 40 of life. Trunk blood was collected for assay of serum LH and the pituitary gland was processed for immunocytochemical staining with anti-rat LH-S4 and anti-rat FSHβ. Serum LH levels were similar and virtually all FSH cells contained LH in the 2 groups of rats. Treatment with MSG reduced APG size ($P<0.01$), reduced the average size of both LH and FSH cells ($P<0.01$), was without significant effect on the number of LH cells per unit area, reduced the number of FSH cells per unit area ($P<0.01$), and increased the ratio of LH to FSH cells ($P<0.05$). The results indicate that neonatal treatment of male rats with MSG has marked effects on APG gonadotropes. Supported by a grant from the NIH (HD11011) and the College of Medicine, University of Lagos, Lagos, Nigeria.

6.4

Ovarian effects on mechanisms controlling LH secretion in the androgenized female and feminized male rat. Robert J. Handa* and Roger A.Gorski. Lab. of Neuroendocrinology and Brain Res. Inst., Dept. of Anatomy, UCLA, Los Angeles, CA 90024

Ovarian steroids are involved postpuberally in the cessation of vaginal cyclicity and loss of facilitatory feedback of these steroids on LH secretion in the lightly androgenized female rat. In this study we examined the effects of ovarian transplants on the maintenance of facilitatory feedback mechanisms in the feminized male (gonadectomized within 18 hrs of birth) as compared to those in the lightly androgenized female (1o ug testosterone propionate on day 5). An LH facilitation test (10 ug estradiol benzoate (EB) followed by 2 mg progesterone (P), 72 hrs later) was administered at 45, 120 and 200 days of age. At 45 days of age, 90% of feminized males and 65% of lightly androgenized females showed elevated LH levels in the afternoon following P treatment. These percentages were similar regardless of ovarian presence prepuberally. Transplantation of an immature ovary to the kidney capsule at 50 or 125 days of age in these same animals resulted in the abolishment of the afternoon LH response in 30% of feminized males when tested at 120 or 200 days of age respectively. At these same ages, 89% of all lightly androgenized females ovariectomized prepuberally and 59% of those which remained intact prepuberally lost the positive feedback response to EB and P. These data suggest that ovarian factors play an active role in the loss of positivie feedback seen in the androgenized female and also work to a lesser extent in the feminized male. Supported by HD01182, CM07191.

6.5

HYPOTHALAMIC SEROTONIN (5HT) AND SERUM LUTEINIZING HORMONE (LH) INTERACTIONS IN AGING FEMALE RATS. R.F. Walker* (SPON: D.R. Wekstein) Univ. of Kentucky Medical Center, Dept. of Anatomy & Sanders-Brown Research Center on Aging, Univ. of Ky. Med. Center, Lexington, KY 40536

In this study, patterns of hypothalamic 5HT metabolism and steroid-induced LH surges were compared in young (Y; 3-4 mos. old) and middle-aged (MA; 8-10 mos.) ovariectomized rats. Animals from both groups produced LH surges in response to sequential injections of estrogen (25ug) and progesterone (P; 0.5mg). However, peak levels of serum LH accumulating at 1800h on the day of P administration were significantly higher ($P<0.01$) in Y (2888±260 ng/ml) vs. MA (1458±196 ng/ml) rats. Lower serum LH content in MA rats correlated with smaller mean diurnal changes in hypothalamic 5HT content, which dropped 672 pg/mg between 1500h and 2000h in Y as compared with 251 pg/mg in MA rats. Furthermore, when 5-hydroxytryptophan (5HTP), a precursor of 5HT was administered in addition to the steroids, then LH surges were attenuated in Y (2036±315 ng/ml; $p<0.05$) as well as in MA (822±99 ng/ml; $p<0.02$) rats. 5HTP reduced daily differances in hypothalamic 5HT content in both groups, (Y+344 pg/mg; MA=121 pg/mg), but did not alter hypothalamic catecholamines for the same time interval. These findings suggest that attenuation of steroid-induced LH surges in MA rats results in part, from spontaneous neurochemical changes during aging which blunt the circadian rhythm in hypothalamic 5HT metabolism.
Supported by AG 02867 from the National Institute on Aging

6.6

TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS CONCENTRATE 3H ESTRADIOL.M.Sar,Dept.of Anat.,Univ.of N.C.,Chapel Hill,NC 27514.

Estrogen treatment has been shown to increase dopamine turnover and tyrosine hydroxylase(TH)activity in the hypothalamus. In order to find out anatomical relationships between dopaminergic and estradiol target neurons,localization of ^{3}H estradiol and TH(an enzyme for the synthesis of dopamine)antibodies was investigated by a combined technique of autoradiography and immunocytochemistry(Sar,M.and Stumpf,W.E.J.Histochem.Cytochem.29: 1A,161-166.1981) 2month-old cyclic female rats ovarectomized for 48hrs injected i.v.with .5ug/100b.w.of ^{3}H estradiol spec. act. 90ci/mM.The rats were sacrificed after 1hr,brains were dissected,frozen and processed for thaw-mount autoradiography. After autoradiographic exposure the slides were photographically developed and stained immunocytochemically with antibodies to TH(suppl.by N.Weiner,Dept.of Pharmac.,Univ.of Colorado). TH-positive cells in the arcuate and periventricular nucleus (group A12)show nuclear concentration of radioactivity.In subthalamic region,TH-positive cells that belong to A13group are weakly labeled.Further,a few cells in group A11 are also labeled with ^{3}H estradiol.TH-positive cells of substantia nigra and ventral tegmental area(group A9)as well as scattered cells in thalamic and subthalamic region did not show nuclear uptake of radioactivity.The results demonstrate that certain TH containing neurons in the brain are estradiol target cells and further suggest direct effect of estradiol on tuberoinfundibular dopaminergic neurons.Supported by PHS grant NS17479 and NS00914.

6.7

VALIDATION OF HETEROLOGOUS RADIOIMMUNOASSAYS FOR PROLACTIN, LUTEINIZING HORMONE, AND FOLLICLE STIMULATING HORMONE IN BOTTLENOSED DOLPHIN SERUM. A. Schneyer*, D. Odell* and A. Castro* (SPON: P. Lutz). School of Marine and Atmospheric Science, Univ. of Miami, Miami FL 33149.

Radioimmunoassays (RIA) for prolactin (PRL), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were developed to facilitate the study of reproductive endocrinology of captive and wild bottlenosed dolphins. Antisera to human hormones, tracer and standards (Immuno Nuclear Corp.) were used to measure dolphin PRL, LH, and FSH in sequential saturation RIAs. Either anti-PRL (1:710,000), anti-LH (1:450,000), or anti-FSH (1:275,000) was incubated with serum samples (100ul) for 48 hours at 4°C. Appropriate tracer was added (0.04-0.05ng) and further incubated for 24-36 hours at 4°C. In all 3 assays, standard curves using either human hormone standards, or dolphin standards derived from concentrated serum, produced parallel curves. Another set of standards was produced by adding human hormone to dolphin serum that had previously been stripped of all hormone. The resulting standard curve was parallel to the previous two. The intraassay and interassay CV is about 4% and 10% respectively for all 3 assays. Over 175 samples from captive and wild dolphins have been assayed so far in order to obtain estimates of baseline levels of these hormones. These assays can now be applied to the study of the dolphin reproductive cycle and possible effects of captivity. Partial support provided by Roosevelt Memorial Fund (to AS).

6.8

IMMUNOCYTOCHEMICAL STUDIES OF THYROID GLAND INNERVATION IN THE BRATTLEBORO RAT: George A. Hedge, Linda J. Huffman* and Frank Sundler*, University of Lund, S-223 62 Lund, Sweden.

Brattleboro (DI) rats, which have diabetes insipidus due to a lack of vasopressin (VP), also have high plasma thyrotropin (TSH) levels with impaired thyroid (THY) responsiveness to TSH, and the latter is not reserved by VP treatment. Thus, we investigated the possibility that this reduced responsiveness is due to abnormal THY innervation in DI. Cryostat sections of THY were processed for immunocytochemical demonstration of various neuropeptides using indirect immunofluorescence. Two types of fibers known to exist in rat THY were examined in DI rats and compared to normal Long-Evans (LE) rats: those containing vasoactive intestinal peptide (VIP), or substance P (SP). We also sought to demonstrate fibers containing neuropeptide Y (NPY) or neurophysin (NPH). Urine flows and plasma TSH levels were greater ($p<0.05$) in DI than in LE. VIP fibers were found in both LE and DI and appeared to be more densely distributed in DI. SP innervation in DI was not distinguishable from that of LE. NPY innervation, mainly on blood vessels, was at least as abundant in DI as in LE. Finally, no THY NPH (and presumably VP)-containing fibers were found in any rat. We conclude that the reduced THY responsiveness of DI is not due to an inadequate supply of any of the putative neurotransmitters in this study. Since VIP enhances THY secretion, we suggest that the apparent proliferation of VIP fibers in DI is an attempt to stimulate the THY analogous to the elevation of TSH. (Swed. MRC 4499 & USPHS AM 21348).

6.9

ENKEPHALIN INHIBITION OF ANGIOTENSIN-STIMULATED RELEASE OF OXYTOCIN AND VASOPRESSIN. L. C. Keil, L. M. Rosella-Dampman* S. Emmert* and J. Y. Summy-Long*. NASA-Ames Research Center, Moffett Field, CA 94035

The effect of leucine^5-enkephalin (ENK) on angiotensin II (AII) stimulated release of oxytocin(OT) and vasopressin(VP) was investigated in the conscious male rat. Changes in the plasma levels of both OT and VP were measured in animals after intracerebroventricular (IVT) administration of either artificial cerebrospinal fluid (CSF) with or without AII(10, 50, 100 ng/5 µl) and 60 sec after AII (50 ng/5 µl) or CSF in animals pretreated with ENK (100 ng/5 µl; IVT) or CSF (5 µl). Oxytocin and VP were measured by radioimmunoassay. Angiotensin II at doses ranging from 10-100ng, IVT, increased ($p<0.05$) the plasma level of both OT and VP. Plasma levels of both neurohypophysial hormones remained significantly elevated 300 sec after IVT AII administration. Although the plasma concentration of OT was always greater ($p<0.05$) than VP in AII-stimulated animals the OT/VP ratio did not differ from controls. Leucine^5-enkephalin reduced the rise in OT from 83.6 ± 4.7 (Mean ± S.E.) to 25.2 ± 2.1 pg/ml and VP from 25.2 ± 1.4 to 6.8 ± 1.1 pg/ml stimulated by IVT AII (50 ng/5µ 1). IVT administration of CSF containing only ENK did not alter either OT or VP levels of these neuropeptides. It is concluded that an acute elevation of AII in the CSF stimulated release of both OT and VP from the neural lobe. Leucine^5-enkephalin administered IVT inhibits AII-stimulated OT and VP release.

6.10

AN ANTISERUM TO (ILE^8)-OXYTOCIN (MESOTOCIN). T. I. Koike, H. L. Neldon*, D. E. McKay* and P. L. Rayford. Dept. Physiology and Biophysics, Univ Ark Med Sci, Little Rock, AR 72205

Female New Zealand white rabbits were inoculated with unconjugated mesotocin (MT) or MT covalently linked to bovine thyroglobulin (Tg) or human IgG using carbodiimide (morpho-CDI). MT was iodinated by the lactoperoxidase-glucose oxidase method and monoiodo(^{125}I)-MT (MT*) was purified by chromatography (Dowex 2X-8, Sephadex G-25). Increasing titers were observed in rabbits immunized with the conjugates and a serum obtained after the 5th injection of MT-Tg in one rabbit was selected for additional study (serum K1-7). A radioimmunoassay was developed using a sequential saturation method, 8000-10,000 cpm MT*, and final K1-7 dilutions of 1:10,000 or 1:15,000. Scatchard analysis indicates that K1-7 contains at least 2 populations of antibody binding sites with apparent affinity constants of ~0.5 and 0.01 x 10^{11} L/M. Arginine vasotocin and arginine vasopressin (>10,000 pg/tube) did not cross-react with the antiserum. Cross-reactivity of oxytocin was 1.2%. Fifty % of MT* was displaced by 18-20 pg MT (ED_{50}) and the limit of detection was less than 1 pg (~0.5µU). The slopes of the regression lines relating logit (B/Bo) to log dose and the immunoreactive MT profiles of chromatograms were similar for standard MT and fowl neural lobe extracts. The data indicate that K1-7 may have the requisite sensitivity and specificity to estimate blood and tissue levels of MT in non-mammalian tetrapods.
Supported in part by AM 30415-01

6.11

GROWTH HORMONE-RELEASING FACTOR AND SOMATOSTATIN IN THE GENERATION OF RHYTHMIC GROWTH HORMONE SECRETION. L. Cass Terry, Nancy Petersen*, and Marianne Zorza*. Univ. of Michigan and VA Medical Center, Ann Arbor, MI 48105.

Growth hormone (GH) secretion in the male rat is characterized by secretory episodes that occur rhythmically every 3-4h. The peaks and ebbs of GH are believed to be regulated by two hypothalamic peptides, somatostatin (SRIF), which inhibits GH secretion, and growth hormone-releasing factor (GRF). The purpose of the present experiments was to assess the role of SRIF and GRF in the generation of this rhythm. In the first experiment, thermal lesions were placed in the hypothalamic ventromedial-arcuate nuclei bilaterally to destroy GRF-producing neurons. Animals were then implanted with chronic indwelling intra-atrial cannulae. Two weeks later, blood samples were removed serially every 15 min for 5h, frozen, and later assayed for immunoreactive GH. In the second experiment, the same group of animals was administered cysteamine (150mg/kg sc), an agent known to deplete central and peripheral SRIF. Ventromedial-arcuate lesions obliterated or markedly reduced GH secretory episodes, but did not significantly alter basal trough (ebb) levels. Cysteamine caused a significant rise in basal GH levels, but did not enhance or reinstitute episodic release. These results indicate that 1) the rhythmic pattern of GH secretion is the result of episodic GRF release from hypothalamic neurons, and 2) SRIF appears to have a minor role in the generation of this rhythm. (Supported by AM 28443 and VA Merit Review Grant)

6.12

EFFECT OF GROWTH HORMONE ON THE HEPATIC PORTAL PLASMA CONCENTRATIONS OF SEROTONIN AND CATECHOLAMINES IN DIABETIC DOGS. O.V. Sirek, A. Sirek* and M.N. Hussain*. Department of Physiology, University of Toronto, Toronto, Canada M5S 1A8.

It is known that a number of growth hormone (GH) effects are dependent on insulin and/or insulin-like growth factors. In the present experiments we have attempted to determine whether or not insulin is involved in the alterations which take place in the portal levels of biogenic amines in response to a surge of GH. We have examined the effects of a pulse of GH in unanesthetized, trained, alloxanized and pancreatectomized dogs, fitted with an indwelling portal catheter. A single injection of ovine GH (100 µg/kg, NIH-GH-S9) into a cephalic vein produced in the hepatic portal circulation a statistically significant rise in the concentration of free serotonin and a significant reduction in the concentration of catecholamines in alloxan-diabetic dogs with residual endogenous insulin and in pancreatectomized dogs with exogenous insulin supplement. No change took place in pancreatectomized dogs totally devoid of insulin. The data suggest that a spike concentration of GH requires the presence of insulin to produce the characteristic alterations in the splanchnic concentration of biogenic amines related to hepatic glucoregulation. Supported by the Medical Research Council of Canada, Grant # MA7778.

EXERCISE PHYSIOLOGY I

7.1

THE EFFECTS OF EXERCISE AND CONDITIONING ON WHOLE BLOOD VISCOSITY IN WOMEN. Dale Martin*, Eric Schoomaker*, Susan Wigutoff*, and Earl Ferguson. Department of Physiology, Uniformed Services University, Bethesda, MD 20814

In order to assess whether aerobic conditioning is associated with enhancement of oxygen transport through a reduction in blood viscosity, we studied whole blood viscosity in 44 healthy women. Fifteen sedentary, 15 joggers, and 14 long distance runners had significantly different maximal aerobic capacities of 34.1±5.5, 43.6±5.9 and 51.4±4.4 ml/kg/min respectively as determined by the Bruce protocol. Blood samples were obtained before and immediately after the treadmill test and evaluated for whole blood viscosity (WBV), plasma viscosity (PV) and hematocrit (HCT). WBV and PV were measured with a Wells-Brookfield viscometer and recorded in centipoise (cP).

	Sedentary	Jogger	Long Distance
Resting HCT (%)	41.5 ±2.8	41.1 ±2.9	40.7 ±2.0
Exercise HCT (%)	44.5 ±2.6*	44.9 ±2.4*	44.9 ±2.4*
Resting WBV (cP)	3.75±0.30	3.50±0.31	3.40±0.29
Exercise WBV (cP)	3.92±0.32*	3.99±0.39*	3.86±0.33*
Resting PV (cP)	1.35±0.06	1.33±0.07	1.34±0.08
Exercise PV (cP)	1.42±0.06*	1.41±0.08*	1.43±0.07*

*$p<0.01$ from resting values

Resting HCT, WBV, and PV were not different among the three conditioning groups. All groups showed increases in HCT, WBV, and PV post exercise which were of similar magnitude. In this study conditioning levels and aerobic capacities of women did not correlate with resting or post exercise HCT, WBV, or PV.

7.2

EFFECTS OF VOLUNTARY EXERCISE TRAINING ON INTRACEREBROVENTRICULAR (ICV) INJECTIONS OF ANGIOTENSIN II (AII) IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). J.M. Overton*, J.A. Wegner*, C.V. Gisolfi, and C.M. Tipton. Exercise Science Program, Univ. of Iowa, Iowa City, IA 52242.

Eighteen female SHR were randomly assigned to voluntary activity cage exercise (AC) or control (CC) groups at 4 wk of age. After 8 wk of AC, the AC group had significantly ($P<0.05$) higher values ($\bar{X}\pm SE$) for maximal oxygen uptake (AC=96±2, CC=78±3 $ml \cdot min^{-1} \cdot kg^{-1}$) run time to exhaustion (AC=286±15, CC=162±9 min) and significantly lower resting caudal artery systolic blood pressures (AC=187±4, CC=201±3 mmHg). Subsequently, a stainless steel guide tube was stereotaxically implanted above the lateral ventricle and the femoral artery was catheterized. Microinjections of AII (50 and 500 ng) and artificial CSF were made on the same day separated by 30-60 min. Injections were made in 5-µl volumes over 1 min using a Harvard infusion pump. The latency and magnitude of the pressor responses to AII and AII-induced drinking were recorded. The initial pressor response to the 5-ng dose of AII was significantly lower in the AC vs. CC groups (11±1 vs. 22±1 mmHg). Other measures were not significantly different. These results suggest that the lower resting systolic blood pressure associated with AC by SHR may be due, in part, to central changes in the responsiveness of the renin-angiotensin system to AII. (Supported in part by funds from NIH grants HL-29099-01 and GM-07045-05.)

7.3

EFFECT OF VOLUNTARY EXERCISE BY STROKE-PRONE SHR GROUPS ON SELECT PHYSIOLOGICAL RESPONSES. C.M. Tipton, R.D. Matthes*, J.R. Leininger*, and M. Sturek*. Exercise Science Program, University of Iowa, Iowa City, IA 52242 and College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

Previous studies with SHR populations have indicated that exercise training initiated after weaning and performed in activity cages (AC) will lower caudal artery systolic blood pressure (SBP) by 10-20 mmHg when compared to control groups (CC). To determine whether similar trends would occur with 35-38 d-old SP-SHR animals, a commercial diet (Funabashi) high in Na (3.9 mg/g) and a 1% NaCl drinking solution were provided to 10 F and 5 M assigned to AC and to 8 F and 6 M assigned to CC groups. While M died sooner than F (32±4 vs. 42±2 d), AC had no consistent effect on this fatal event. AC was generally associated with lower SBP in F (224±6 to 204±9 mmHg at 28 d) and higher values in M (204±10 to 193±16 mmHg at 28 d) when compared to CC results. Additionally, AC was also associated with higher $\dot{V}O_{2max}$, reduced daily food intakes, higher daily fluid consumptions, lower body weights, and marked reduction in daily running activity prior to death. Although the histological assessment is incomplete at this time, it was apparent from the experimental conditions of this study that early exposure to strenuous and intermittent voluntary exercise had minimal protective value against the sequence of events associated with strokes. (Supported in part by funds from NIH grant HL-29099-01 and GM-07045-05.)

7.4

ACUTE REGIONAL BLOOD FLOW RESPONSES DURING DOBUTAMINE INFUSION, DURING COMBINED SYMPATHETIC STIMULATION AND PARASYMPATHETIC WITHDRAWAL, AND DURING EXERCISE IN DOGS. C.C. Haidet,* T.I. Musch,* G.A. Ordway and J.H. Mitchell. Univ. of Texas Health Science Center, Dallas, TX 75235

Previous studies have suggested that intravenously infusing either Dobutamine (DOB) or a combination of atropine, norepinephrine, and epinephrine (ANEE) evokes cardiovascular responses similar to those observed during dynamic exercise (EXER). The purpose of this study was to investigate, at similar levels of estimated myocardial oxygen demand, the acute regional blood flow responses to DOB, ANEE, and EXER. The radioactive microsphere technique was used to compare on separate days the following interventions in dogs: 1) DOB (40µg/kg/min); 2) combined A (0.15mg/kg), NE (0.19µg/kg/min) and E (0.05µg/kg/min); and 3) submaximal treadmill EXER. Blood flow (ml/100g/min) to the brain, intestine, liver, adrenal glands, and kidneys was not changed by any of these interventions. The following differences were noted (*p<0.05 vs rest; n=8):

	Mean % Change From Rest						
	Heart	Lung	Diaphragm	Skel Muscle	Spleen	Tongue	Pancreas
EXER	72*	257*	215*	238*	13	81*	-53*
DOB	67*	68	70	3	37*	-30	-63*
ANEE	69*	51	185*	41	91*	-12	-30*

Thus, at similar levels of estimated myocardial oxygen demand induced by these 3 different interventions, acute regional blood flow responses resulting from EXER, DOB, and ANEE are quite different.

7.5

OXYGEN CONSUMPTION AND HEMODYNAMIC RESPONSES TO DYNAMIC EXERCISE IN DOGS BEFORE AND AFTER SPLENECTOMY. G.A. Ordway, J.C. Longhurst, T.I. Musch,* G.C. Haidet* and J.H. Mitchell. Univ. of Texas Health Science Center, Dallas, TX 75235

We measured the oxygen consumption ($\dot{V}O_2$) and hemodynamic responses of 8 dogs to severe dynamic exercise before and after splenectomy. Absolute work loads were similar for spleen intact and splenectomized dogs, with the result that ($\dot{V}O_2$) did not differ significantly from pre- to post-splenectomy (105.4±10.2 vs 97.3±3.1 ml/min/kg, mean±SEM). Cardiac outputs during exercise were similar before and after splenectomy (17.9±1.3 vs 18.4±1.3 L/min); however, heart rate during exercise was significantly ($p<0.05$) higher following splenectomy than it was before (283±8 vs 264±9 beats/min). Hematocrit during exercise was decreased significantly following splenectomy (46±2 vs 37±3%) which, in turn, reduced the oxygen content of arterial blood (19.1±0.5 vs 15.4±0.8 vol%). The oxygen content of mixed venous blood during exercise was also reduced significantly following splenectomy (4.9±0.6 vs 2.8±0.1 vol%). Additionally, mean arterial pressure during exercise was reduced following splenectomy (145±5 vs 132±4 mmHg), with the result that calculated mean conductance was increased significantly (120±10 vs 140±10 ml/mmHg·min). Thus, the inability of splenectomized dogs to increase their oxygen carrying capacity during exercise by contracting the spleen and increasing hematocrit appears to be offset in part by: (1) a lower oxygen content in mixed venous blood; and (2) an increased conductance of blood to metabolically active tissue.

7.6

THE EFFECT OF PERICARDIECTOMY ON STROKE VOLUME DURING MAXIMAL EXERCISE IN THE UNTRAINED DOG. J. Stray-Gundersen,* G.C. Haidet,* T.I. Musch,* G.A. Ordway and J.H. Mitchell. Univ. of Texas Health Science Center, Dallas, TX 75235

To test the hypothesis that the pericardium limits stroke volume during maximal exercise, we performed pericardiectomies on 4 mongrel dogs (24.9±2.2 kg, mean±SEM) chronically instrumented with aortic (A) and pulmonary artery (PA) catheters. A sham thoracotomy was performed on two similarly instrumented mongrel dogs (22.1±0.8 kg). Dogs were tested by running on a treadmill, and maximal oxygen uptake ($\dot{V}O_2$) was defined as a plateau in cardiac output (CO) and heart rate (HR) with an increase in workload. The dogs were tested 2-4 times both before and after pericardiectomy or sham thoracotomy. We measured cardiac output (dye dilution), HR, and arteriovenous oxygen difference between PA and A blood samples. Oxygen consumption and stroke volume (SV) were calculated from these data. The results are tabulated below:

	Pericardiectomy (n=4)		Sham Thoracotomy (n=2)	
	before	after	before	after
$\dot{V}O_2$(ml/min/kg)	117±8	133±8	118±19	95±23
CO (L/min)	20.8±1.2	25.7±2.7	19.4±1.5	18.0±1.7
HR (beats/min)	273±9	279±7	285±6	288±2
SV (ml)	76±3	92±8	67±3	63±6

These results support the hypothesis that the pericardium limits stroke volume at maximal exercise in untrained dogs.

7.7

PRE- AND POST-TRAINING RESPONSES TO MAXIMAL DYNAMIC EXERCISE DURING α-ADRENERGIC BLOCKADE IN THE FOXHOUND. T.I. Musch,* G.C. Haidet,* G.A. Ordway, J.C. Longhurst and J.H. Mitchell. Univ. of Texas Health Science Center, Dallas, TX 75235

Studies have shown that α-adrenergic blockade (α-B) in untrained dogs reduces oxygen uptake (VO_2) at a given level of exercise by causing a shift toward anaerobic metabolism. To investigate the effect of dynamic exercise training on this response, we studied 8 foxhounds during maximal exercise, with and without α-B (phentolamine, 1 mg/kg IV), before and after 8-12 weeks of dynamic exercise training. Pre-training, α-B significantly ($p<0.05$) reduced VO_2 (123 vs 100 ml/kg/min) and increased venous lactate concentrations (25.6 vs 48.0 mg%) during maximal exercise. Decreases in both arterial oxygen content (CaO_2) and maximal cardiac output contributed to the reduction in oxygen delivery. Post-training, α-B again reduced maximal VO_2 (153 vs 135 ml/kg/min) and increased venous lactate concentrations (22.8 vs 32.0 mg%). Comparing post-training to pre-training, the reduction in maximal VO_2 was similar (12 vs 19%); however, the increase in venous lactate concentration was significantly reduced (44 vs 88%). Additionally, the post-training reduction in maximal VO_2 during α-B was due primarily to a decrease in CaO_2 since maximal cardiac output was maintained. Thus, in the foxhound, α-B during maximal exercise significantly lowers maximal VO_2. After dynamic exercise training, however, the blood lactic acid response to maximal exercise during α-B is attenuated significantly.

7.8

EFFECT OF HEAD-OUT WATER IMMERSION ON THE CARDIORESPIRATORY RESPONSE TO DYNAMIC EXERCISE. L.M. Sheldahl*, F.E. Tristani* and P.S. Clifford* (SPON: J.J. Smith). VA Medical Center and Medical College of Wisconsin, Milwaukee, WI 53193

Head-out water immersion (HOWI) is known to produce several cardiorespiratory adjustments at rest due to a shift in blood volume from the periphery to the thorax. The purpose of this study was to determine the effect of HOWI on the cardiorespiratory response to dynamic exercise. Sixteen healthy middle-aged men performed upright cycling exercise at 40, 60 and 80% maximal oxygen consumption ($\dot{V}O_2max$) on land and in water (30.5±0.5°C) to the shoulders. Cardiac output (measured by the carbon dioxide rebreathing technique) and stroke volume (SV) were significantly greater ($p<0.05$) during exercise in water compared to land. Heart rate (HR) did not differ at 40 and 60% $\dot{V}O_2max$ but was significantly lower ($p<0.05$) in water at 80% $\dot{V}O_2max$. Ventilation, breathing frequency and tidal volume did not differ significantly in water and on land. The data suggests that the central redistribution of blood volume with HOWI results in an increase in SV during exercise via the Frank-Starling mechanism. Since there is not a proportional decrease in HR with the increase in SV, cardiac output is elevated during exercise in water compared to land. The lack of a compensatory decrease in HR may be due to activation of cardiac mechanoreceptors with central hypervolemia. We conclude that the increase in central blood volume with HOWI alters cardiac performance during exercise and results in a change in the cardiac output and oxygen consumption relationship compared to exercise on land.

7.9

EFFECTS OF HINDLIMB POSITION ON THE RHEOLOGY OF THE TERMINAL AORTA IN DOGS. D.R. Gross, G. van Oort* and K.T. Dodd*, Dept Vet Phys & Pharm, College Vet Med, Texas A&M University, College Station, TX, 77843.

We measured pressure-diameter relationships in 6 anesthetized dogs following prior surgical placement of ultrasonic dimension gauges on the terminal aorta, just proximal to the iliac trifurcation. Following recovery from the initial surgery, and time for healing to occur (7-10 days), the dogs were anesthetized with a combination of fentanyl-droperidol and pentobarbital. Using fluoroscopic visualization, a high-fidelity catheter-tip manometer was placed in the distal aorta, via the carotid artery, with the tip placed at the level of the dimension gauges. Measurements were made with the hindlimbs positioned 90° from the body, with both legs fully extended cranially and then caudally,with the right leg extended cranially and the left caudally and vice versa. The pressure elastic modulus (Ep) was calculated for each of the static leg positions. Data collected with both hindlimbs extended caudally were compared to both hindlimbs at 90° from the longitudinal axis of the body. Ep decreased approximately 25%, i.e. the vessel became more compliant in the radial direction. No significant differences were found with the hindlimbs in the other 3 positions.

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7.10

EXERCISE BLOOD FLOW IN RAT MUSCLES AFTER TRAINING. R.B. Armstrong, C. Mitchell*, R. Phelps*, C. Vandenakker*, J. White* and M.H. Laughlin. Oral Roberts U., Tulsa, OK 74171

One group (T) of male Sprague-Dawley rats trained for 1 hr/day for 13-17 wks at 30 m/min on a treadmill at a 5° incline. A second group (UT) of rats was conditioned for 10 min/day for 4 wks at the same speed and incline. BFs in 32 hindlimb muscles were measured with labeled microspheres during preexercise (PE) and while the rats ran for 30 s (E0.5), 5 min (E5), or 15 min (E15) at 30 m/min.

Representative	PE		E0.5		E5		E15	
Muscles	UT	T	UT	T	UT	T	UT	T
Total Hindlimb	30	40	88	104	104	99	89	86
	± 6	± 3	±10	± 4	± 8	± 6	± 7	± 5
Red Gastrocnemius	60	102*	238	280	340	381	273	377*
	±14	±10	±33	±20	±36	±39	±44	±26
White Gastrocnemius	12	16	14	20	24	19	33	17*
	± 2	± 2	± 2	± 4	± 3	± 4	± 5	± 2

Mean ± SE. *T different than UT ($P < 0.05$).

There were no differences in total hindlimb muscle BF between UT and T rats, but T rats had higher PE BFs in the deep red extensor muscles, suggesting a greater anticipatory response. Also, T rats had higher BFs in red extensor muscles during exercise, whereas UT rats had higher BFs in white muscles. These findings demonstrate that exercise training causes changes in the distribution of BF within and among muscles both before and during exercise. Supported by NIH Grants AM25472 and HL26963 and AHA Tulsa Affiliate.

7.11

FITNESS RELATED CHANGES IN THE HEMODYNAMIC RESPONSE TO AN ALPHA-AGONIST CHALLENGE Peter B. Raven, Debbie Rohm-Young*, Michael L. Smith*, and Theodore S. Varas*. Texas College of Osteopathic Medicine, Fort Worth, Tx. 76107.

We have previously shown hemodynamic differences in high fit and average fit individuals when challenged with lower body negative pressure. An α-agonist challenge using phenylephrine HCl (PE) was administered to a similar group of subjects to further delineate these responses. Six high fit (F; $\dot{V}O_2max$ = 62.1 $ml \cdot kg^{-1} \cdot min^{-1}$) and six low fit (UF; $\dot{V}O_2max$ = 33.2 $ml \cdot kg^{-1}$) males ($P<0.01$) were challenged with PE using IV infusion rates of 6,12,20,40, and 60 μg/min. At rest and during the period of stable hemodynamics for each infusion rate, heart rate (HR) using 10 sec averages of R-R intervals, blood pressures, cardiac output ($\dot{Q}$) using the CO_2 rebreathing technique, and forearm blood flow (BF) measured by strain gauge plethysmography were determined. Only HR was significantly different at rest (F=50 beats/min; UF=68 beats/min, $P<0.01$). Mean data at PE of 60 μg/min are summarized below:

	HR (beats/min)	SBP (torr)	DBP (torr)	ΔHR / ΔSBP	$\dot{Q}$ (l/min)	PVR (units)	BF ml/100ml/min
F	41.4	139	96	0.45	6.07	18.71	2.97
UF	56.6	128	82	1.22	7.15	14.31	3.77
P	<0.01	<0.05	<0.01	<0.05	NS	<0.05	NS

The F had a total BF decrease of 42.4% whereas the UF decrease in BF was 14.7%. These data indicate a different vasoconstrictive and hypertensive response between F and UF when challenged by PE.

7.12

CHANGES IN PLASMA CONCENTRATION OF ADRENOCORTICOTROPHIN (ACTH) AND CORTISOL IN MEN IN RESPONSE TO DAILY 2-HOUR RUNS FOR 7 DAYS. C.E. Wade, P. Christ*, M.M. Hunt*, J.R. Claybaugh, C. Hadick*, S.A. Cucinell, and R.H. Dressendorfer*. Letterman Army Medical Center, San Francisco, CA 94129; Tripler Army Medical Center, HI 96859; and William Beaumont Hopital, Royal Oak, MI 48072.

To assess the effects of daily running on the pituitary-adrenal axis, we measured plasma concentrations of ACTH and cortisol in 10 healthy, untrained males before and after 2 hours of daily running for 7 days. The men, age 21 to 37 years, underwent 1 day of control measurements, 7 consecutive days of running for 2 hours, 2 days of rest, and a final day of running for 2 hours. Running (or an equivalent period of rest) was conducted between 0800 and 1030 hours, with fasting blood samples collected before and within 5 min after running. During these 2 hours each man ran an average distance of 18.5 km, which remained constant throughout the study. In response to running, concentrations of plasma ACTH and cortisol levels increased significantly ($P<0.05$). The mean change in levels of plasma cortisol after running progressively decreased from 16.7 ± 3.8 μg/dl on day 1 to 4.3 ± 3.8 μg/dl on day 7. The mean change in levels of ACTH after running was not significantly altered, ranging from 30 ± 10 pg/ml on day 1 to 21 ± 5 pg/ml on day 7. A relationship between plasma cortisol and ACTH was not demonstrated on day 1 ($r = 0.24$, $p>0.20$), while on day 7 a relationship was noted ($r = 0.67$, $p<0.001$). Thus, plasma concentration of ACTH and cortisol levels increased with running, and cortisol response progressively decreased as a result of factors other than ACTH.

8.1

CARDIOVASCULAR ADAPTATIONS TO PREGNANCY PRECEDE INCREASED UTERINE BLOOD FLOW IN THE GUINEA PIG. M.V. Hart*, J.D. Hosenpud*, J.R. Rowles*, M.J. Morton* and A.R. Hohimer. Heart Research Laboratory, Oregon Health Sciences Univ., Portland, OR 97201.

The cardiovascular adaptations to pregnancy were studied cross-sectionally in the guinea pig (GP). Pressures, cardiac output (CO) and regional blood flows, blood volume (BV), estrogen levels, and *in vitro* left ventricular (LV) pressure-volume relations were measured in 14 control and 39 pregnant GPs during early, mid, and late gestation. All animals were prospectively age- and weight-matched prior to conception. The cardiovascular changes during pregnancy are shown in the following table as percentage increase from control GPs.

	CO (%)	SV (%)	LV vol (%)	UBF* (%)	BV (%)	BW+ (%)
Day 20	↑24	↑21	↑16	0	↑30	↑14
Day 40	↑13	↑17	↑25	↑860	↑38	↑36
Day 60	↑12	↑27	↑31	↑1050	↑51	↑70

*UBF = uterine blood flow; +BW = body weight

Aortic, LV end-diastolic and right atrial pressures, heart rate and LV weight remained unchanged during pregnancy. The greatest change in circulating estrogen concentration occurred by Day 20 and was double the control level. Significant cardiovascular changes are seen during early pregnancy and occur prior to a major increase in uterine blood flow. These cardiovascular alterations may be mediated by changing hormone levels.
(Supported in part by NIH HD-10034, Medical Research Foundation of Oregon, & the American Heart Association, Oregon Affiliate.)

8.2

ROLE OF THE SPLEEN IN ERYTHROCYTE VOLUME REGULATION OF THE CONSCIOUS IMMATURE PIG. Carol A. Bossone* and John P. Hannon. Letterman Army Institute of Research, Presidio of San Francisco, CA 94129

When estimated by the dilution of ^{51}Cr-tagged erythrocytes under near-basal conditions, chronically catheterized splenectomized pigs (n=20) had a circulating erythrocyte volume of 17.8±1.64 (S.D.) ml/kg, a calculated plasma volume of 42.8±2.69 ml/kg and blood volume of 60.6 ml/kg. Sham-operated control pigs (n=20) had circulating erythrocyte, plasma and blood volumes of 16.2±1.39, 44.4±2.96, and 60.5 ml/kg, respectively. In the latter animals, kinetic analysis of splenic erythrocyte sequestration showed a splenic erythrocyte volume of 4.51±0.89 ml/kg and a t-1/2 of 9.76±1.93 min. Epinephrine injection (0.5 ml of 1 mg/1000 ml dil) caused rapid mobilization of stored splenic erythrocytes in sham-operated pigs (n=6) with a hematocrit rise from 0.27±0.01 to 0.35±0.02. Similar results were obtained in sham-operated pigs (n=9) subjected to 1 min of physical restraint. In splenectomized pigs (n=7), blood volume estimates based on ^{51}Cr-tagged erythrocyte and ^{125}I-tagged albumin dilutions gave circulating erythrocyte, plasma and blood volumes of 18.4±0.93, 60.7±1.53, and 79.0±2.51 ml/kg, respectively, and a body to large vessel hematocrit ratio of 0.756±0.011. It was concluded that the porcine spleen contains substantial stores of readily-mobilized erythrocytes and that RISA dilution may overestimate porcine plasma volume.

8.3

THE SPLENIC HEMOGRAM OF THE DOG VARIES WITH SPLENIC SIZE. Mortimer Lorber. Georgetown University Schools of Medicine and Dentistry, Washington, D. C. 20007

The canine spleen is known to be a large and muscular blood reservoir, particularly sequestering erythrocytes at higher concentration than in the peripheral circulation. Its volume can be greatly altered by emotion, exercise, and anesthesia. This study wished to learn whether the contracting spleen expels its blood cells randomly or selectively. Ten dogs were anesthetized with sodium pentobarbital which markedly dilates the spleen. From each, one ml of splenic blood was collected in an anticoagulant tube. The spleen was then gently handled to induce moderate contraction and the blood sampling repeated. Blood counts were done manually. The dilated (D) and contracted (C) spleens' blood values were compared by paired Student "t"-tests; $P < 0.05$ being considered significant. The findings were: The contracting spleen does not expel all formed elements randomly. Preferential erythrocyte discharge occurs, thus lowering the splenic microhematocrit by 9% from 69.8 ± 6.6% (D) to 63.3 ± 8.5% (C), ($P < 0.05$). Reticulocytes are not expelled to the same extent as mature RBC; their residual splenic concentration being 77% higher than when dilated, 1.3 ± 0.9% (D) vs 2.3 ± 1.8% (C), ($P < 0.05$). Total and differential leukocyte and platelet counts were not significantly changed. A corollary of this selective expulsion is that the erythroid composition and, thus, oxygenation and plasmacrit of the portal part of the splanchnic circulation changes with splenic tone.

8.4

ADENOSINE AS A PUTATIVE INTRINSIC CONTROLLER OF THE HEPATIC ARTERIAL BLOOD FLOW. W. Wayne Lautt, College of Medicine, University of Saskatchewan, Saskatoon, Sask. Canada S7N OWO

The hepatic arterial buffer response (HABR) is the phenomenon of arterial blood flow changing in the opposite direction to portal blood flow changes. The hypothesis is tested that the mechanism of the HABR involves production of adenosine near the hepatic arterial (HA) resistance vessels. Reduced portal flow would result in reduced washout of adenosine in this region and would thus result in more adenosine in contact with the HA. Several criteria must be met. First, adenosine must dilate the HA. Adenosine is the most potent dilator yet tested, elevating arterial conductance by 200-400%. Second, portal blood flow must have access to HA resistance vessels since changes in portal flow must be able to alter adenosine concentration at the site of the HA resistance vessels. Adenosine infused into the portal vein produces dramatic dilator effects on the HA, albeit at about 1/2 the effectiveness of an equal dose into the HA. Third, potentiators of exogenous adenosine should potentiate the HABR. Dipyridamole, an adenosine uptake blocker, significantly increases the HABR. Fourth, blockers of adenosine must also attenuate the HABR. Isobutyl methyl xanthine (MIX) produced significant reductions in the dilator response to adenosine and also markedly reduced the HABR. When the MIX infusion was discontinued, the responses both returned. The data support the hypothesis that adenosine is the dilator involved with control of the HABR.

Supported by Canadian Heart Foundation and MRC of Canada.

8.5

MEASUREMENT OF HEPATIC BLOOD FLOW BY LASER DOPPLER VELOCIMETRY. G.L. Riedel*, W.F. Ward and A.P. Shepherd. Dept. of Physiol., Univ. Texas Health Science Ctr., San Antonio, Texas 78284

Laser Doppler velocimetry (LDV) could potentially monitor hepatic tissue perfusion directly, if an optical probe were placed against the surface of the liver. LDV measures blood flow continuously in a small volume of laser-illuminated tissue by determining the Doppler shift that light experiences when scattered by moving red blood cells. Several different algorithms have been proposed to extract a flow-dependent signal from the Doppler spectrum. Therefore, we compared a commercially available LDV blood flowmeter (Perimed, Stockholm) with our prototype (Am. J. Physiol. 242:G668-672, 1982) that uses a different flow algorithm. In isolated rat livers pump-perfused via the portal vein, the laser signals from the hepatic surface and total blood flow were linearly related in each experiment, but flow readings at the center of each lobe were higher than at the edges. Both instruments were sufficiently sensitive to measure flow throughout a wide range. A lack of reproducibility in the flow signal from a particular spot on the liver's surface was apparently not due to changing sensitivity of the instruments but to variability in the perfusion of the tissue. We conclude that both instruments provide a linear measure of flow in hepatic tissue but that the blood flow variability in the small measuring volume makes it difficult to calibrate the LDV measurement in absolute units. (Supported by a grant from the American Heart Association and its Texas Affiliate.)

8.6

REGIONAL DIFFERENCES IN INTESTINAL REACTIVE HYPEREMIA STUDIED BY LASER DOPPLER VELOCIMETRY. A.P. Shepherd and G.L. Riedel*. Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284

In a previous study (Am. J. Physiol. 242:G668-G672, 1982) of regional intestinal blood flow by laser Doppler velocimetry (LDV), we noted that the mucosa consistently displayed reactive hyperemia following arterial occlusion but the muscularis did not. Therefore, in this study we measured total blood flow to isolated loops of canine small bowel with an electromagnetic flowprobe on the supply artery. Blood flow in either the mucosa or in the muscularis was measured by LDV. Total and mucosal blood flow consistently showed reactive hyperemia in response to a 60 sec. occlusion, but the muscularis did not. To determine whether metabolic rate influenced reactive hyperemia, we increased enteric oxygen uptake by placing 5% bile and transportable solutes in the lumen. Following a 60 sec. occlusion, the durations of both total and mucosal reactive hyperemia were significantly prolonged by increased metabolic rate. Similarly, the blood flow payback-to-debt ratios in both flows were significantly increased at elevated metabolic rate. These data support the conclusions (1) that reactive hyperemia is more frequent and has a greater magnitude in the mucosa compared with the muscularis and (2) that both total and mucosal reactive hyperemia are profoundly influenced by metabolic conditions. (Supported from a grant from the American Heart Association and its Texas Affiliate.)

8.7

LASER-DOPPLER SKIN BLOOD FLOW: CORRELATION WITH PLETHYSMOGRAPHY. J.M. Johnson, W.F. Taylor*, A.P. Shepherd and M.K. Park*. Dept. of Physiol., Univ. of Texas Health Science Ctr., San Antonio, Texas 78284

Plethysmographic determinations of skin blood flow (SkBF) are the source of most of our understanding of that circulation in man. However, this method is limited to the limbs, is discontinuous, and does not uniquely measure SkBF. Recently, laser-Doppler velocimetry (LDV) has been introduced as an alternate method for measuring SkBF. LDV avoids the above problems. LDV is based on the frequency shift of coherent light scattered by moving red cells within surface tissue. We designed this study to find the relationship between these two measurements of SkBF. Subjects were warmed with water-perfused suits which covered the body except for the arm from which SkBF was measured. Esophageal temperature rose 0.95°C while forearm blood flow (FBF) rose 14 ml/100ml·min. In each of 5 studies, LDV correlated well with FBF (r=0.94-0.98). However, the slope of the LDV-FBF relationship varied between subjects as well as on repeat studies with an individual (40-122 mV per ml/100ml·min). Occlusive zero for the LDV (160-300mV) and the extrapolated intercept of the LDV-FBF relationship (246-599mV) were greater than the instrument zero. We conclude that the two methods are well correlated within a study. However, the relationship of LDV to FBF varied among subjects and between studies in a given subject. (Supported by Grant HL20663)

8.8

STATIC AND DYNAMIC PROPERTIES OF WIRE LOOP PROBES USED IN INDUCTION ANGIOMETRY. J. Krueger* and H. Stinnett, Dept. Elect. Eng. and Physiol., Univ. N. Dak., Grand Forks, ND 58202.

Kolin (Blood Vessels 17: 61-77, 1980) described use of wire loop probes to measure vessel diameter by induction angiometry; commercial systems are available. To determine if probes can follow rapid (cardiac) events without significant vessel wall distortion, data are needed concerning their elastic compression force and rate of loop diameter change. Data for probes with unrestrained loop diameters (UD) of 4.0, 9.4, and 22.5 mm were recorded during loop compression from 95% to 65% of UD. Static compression for all probes yielded a linear (all $F_s > F_{.05(1,4)}$ $P < 0.05$) regression coefficient of 0.213 (4.0 mm), 0.0525 (9.4 mm) and 0.0138 (22.5 mm) grams/% dia. For dynamic measurements a mechanical device was constructed to pulse (p) a loop at varied rates (20 to 250 p/m) and compression/expansion ratios (30/70, 50/50, and 70/30). Probe electronic signals were compared to simultaneous measurements from a linear variable differential transformer (LVDT, Schavitz Engineering). Comparison, of transparancy overlays and electronic differential analysis, of probe and LVDT results indicated the 4.0 and 9.4 mm probes had no hysteresis or lag. The 22.5 mm probe began to lag at rates over 140 p/m. In relation to reports on vessel muscle content and contractile force (Fed. Proc. 42: 2601, 1983) these results indicate that the 4.0 mm probe may be used in arteries but may be too stiff for veins, the 9.4 mm probe is useful in both, while the 22.5 mm probe may not follow events in an artery. Part support AHA Dak. Aff. DA-G-04 and 510.

8.9

OSCILLATIONS IN ENDOCRINE AND CIRCULATORY VARIABLES IN NORMAL CONSCIOUS DOGS. L. A. Benton*, L. B. Loring* and F. E. Yates. Crump Institute for Med. Engr., UCLA, Los Angeles, CA 90024

Homeokinetic physics predicts that the stability of living systems is based on networks of loosely-coupled, nonlinear thermodynamic oscillators. To test this theory we undertook a spectroscopic analysis of the adrenal glucocorticoid system. We examined adrenal blood flow, cortisol secretion rate, concentrations of cortisol and corticosterone, arterial blood pressure and heart rate in conscious, unrestrained dogs. We anticipated that we would discover previously unreported oscillations among these variables. We collected samples at 15 sec, 5 min, or 10 min intervals for 30 min to 8 hr. From the resulting time series we were able to detect three oscillatory epochs: 2 to 4 min, 4+ to 8 min, and 1 to 2 hr. We found significant oscillations in adrenal variables only in the 2-4 min and 1-2 hr ranges. In blood pressure and heart rate we found significant oscillations in both the 2-4 min and the 4+-8 min range; and a strong oscillation in the 1-2 hr domain has been reported in the literature. Thus we saw that heart rate and blood pressure share three oscillations; two of these are present in adrenal blood variables, and one (the middle frequency) is not. Circulatory oscillations are not causal to adrenal oscillations. (Supported by NIH grant GM 23732.)

8.10

IN VIVO DISTRIBUTION OF I-125 HEAT, AN ADRENOCEPTOR, IN MICE. Mozafar K. Karimeddini*, Sheldon M. Robbins*, Richard P. Spencer, Univ. Connecticut Health Center, Farmington, CT 06032

HEAT, 2(B-(4-hydroxyphenyl)ethyl-aminomethyl)tetralone, is available with an I-125 radiolabel, and is an alpha-adrenergic ligand in vitro. We studied the distribution of I-125 HEAT in 25 gm male CD-1 mice after tail vein injection. Animals were sacrificed at 15 minutes, the blood and tissues assayed for radioactivity, and results given as percent dose/gm tissue (PD/G). Studies were repeated at 3 hours after gastric introduction of 0.6 mg of prazosin (an α-adrenergic blocker). Both 24 hour fasted mice and those eating ad libitum were utilized. The lungs are the first major organ reached by the injected I-125 HEAT, after the heart. The lungs had the highest specific activity (27 PD/G). However, radiolabel was widely distributed; pancreas, kidneys and gallbladder had high concentrations. Pretreatment with prazosin had only slight effect on distribution of radiolabel. Urine was collected and chromatographed on silica gel plates, using methanol/chloroform (30/50,v/v). The I-125 HEAT had an Rf of 0.82-0.85. Only about 12% of urinary radioactivity corresponded to the original Rf. There was major degradation and/or metabolism of the I-125 HEAT. Wide distribution of radiolabel, lack of response to prazosin preloading, and chromatographic evidence of alteration, make it problematical whether I-125 HEAT can be used as an in vivo adrenergic label. (Supported by USPHS CA 17802, NCI).

8.11

EFFECT OF TEMPERATURE ON RABBIT EAR ARTERY RECEPTOR AFFINITY AND CONTRACTILITY. Michael Roberts, Susan Maben*, and Clayton Turner*. Dept. of Biology, Linfield College, McMinnville, OR 97128

In isolated ear arteries studied by challenge with exogenous norepinephrine (NE), maximal contractile strength occurs at 25°C, and contraction is reduced at temperatures below or above 25°. To determine the mechanism of this effect, we studied the influence of temperature on two aspects of smooth muscle contraction, receptor affinity for NE and contractility. Rings of artery were mounted for isometric tension recording and stimulated to contract in one of two ways: 1) increasing concentrations of NE from 10^{-8} to 10^{-4} M at 9°,16°,25°,30°,37°, and 42°; 2)direct electrical stimulation over the same range of temperatures. In the first set of experiments receptor affinity was measured at each temperature by comparing dose/response curves before and after the addition of the irreversible alpha-adrenoceptor blocker phenoxybenzamine. Affinity was low at 9° and 16°, increased at 25°, and reduced again at temperatures above 25°. In the second set, response to direct electrical stimulation was low at cool temperatures, and high at temperatures above 25°. Data from both sets suggest that cooling below 25° reduces contractile responses by two means: low affinity and low contractility. Warming above 25° reduces responses by a reduction in affinity even though contractility is high. Supported by NIH Grant HL-29551 and by Yale University BRSG funds.

8.12

MAGNESIUM-FREE SOLUTION DIMINISHES RELAXATION OF ISOLATED ARTERIAL SMOOTH MUSCLE BY ADENOSINE AND SODIUM NITROPRUSSIDE. D.H. Foley. College of Osteopathic Medicine and Dept. of Zoological and Biomedical Sciences, Ohio Univ., Athens, OH 45701

This study was conducted to determine whether relaxation of artery strips by adenosine (Ado) and sodium nitroprusside (NP) would be diminished by the absence of Mg^{2+}. Helical strips of left coronary and femoral arteries from rabbits were suspended in baths with physiological salt solution (PSS) at 37°C and bubbled with 95% O_2 + 5%CO_2. Isometric contractions were induced with acetylcholine and norepinephrine in coronary and femoral artery strips, respectively. Ado and NP produced dose-dependant relaxations which did not change significantly during two consecutive control trials. However, Mg^{2+}-free PSS increased Ado ED_{50} values from 1.9×10^{-7} to 3.7×10^{-7} M* in coronary, and from 4.2×10^{-7} to 8.8×10^{-7} M* in femoral artery strips. In Mg^{2+}-free PSS, the ED_{50} values for NP increased from 2.3×10^{-8} to 6.4×10^{-8} M* in coronary, and from 6.4×10^{-8} to 3.2×10^{-7} M* in femoral artery strips. These effects were not attributable to greater initial tension in Mg^{2+}-free PSS. These results indicate that the capacity for arterial smooth muscle relaxation in response to physiological and pharmacological dilators may be diminished by a decrease in the ambient $[Mg^{2+}]$. This may contribute to increased vascular resistance associated with low plasma $[Mg^{2+}]$ in vivo.
Supported by the Central Ohio Heart Chapter, Inc.
*($P<0.05$)

8.13

LOW SALT DIET DOES NOT ALTER HEAT STRESS-INDUCED RENAL VASOCONSTRICTION IN BABOONS. Duane W. Proppe. Univ. of Texas Health Science Center, San Antonio, Texas 78284.

Based on the reported predominance of the renin-angiotensin system (RAS) in mediating renal vasoconstriction in the baboon during acute environmental heating (EH), this study sought to determine whether this heat stress-induced renal vasoconstriction would be enhanced during elevated RAS activity produced by a low salt diet (LSD). Three baboons were chronically instrumented to measure renal blood flow, arterial blood pressure and arterial blood temperature (T_b). Individual experiments consisted of subjecting the baboon to high ambient temperature (40-45°C) until T_b reached 39.5-40.0°C. These EH studies were performed while the baboons were on a normal-to-high salt diet (N-HSD) and 9-18 days after placement on a LSD. The LSD caused baseline plasma renin activity (PRA) to increase from 2.31±0.84 (mean±SE) to 10.36±0.93 ng/ml/hr. Also, the magnitude of the PRA increase during EH was enhanced while on LSD--for example, at T_b=39.5°C during EH, PRA=10.71±4.30 ng/ml/hr for N-HSD vs. PRA=24.26±4.05 ng/ml/hr for LSD. However, the magnitude of the fall in renal blood flow and increase in renal vascular resistance (RVR) during EH were unaltered in the LSD state. For example, at T_b=39.5°C during EH, RVR had increased by 83.9±14.4% in N-HSD state vs. 87.9±19.0% in LSD state. Thus, the enhanced activity of the RAS in the LSD state is not accompanied by an alteration of the heat stress-induced renal vascular responses in the baboon. (Supported by NIH Grant HL 27504.)

8.14

EFFECT OF RENAL DENERVATION ON THE RENAL RESPONSES OF THE NONHUMAN PRIMATE TO HYPERVOLEMIA. Thomas V. Peterson, Nancy L. Chase* and Debbi K. Gray*. Dept. of Medical Physiology, Texas A&M University, College of Medicine, College Station, TX 77843

In a previous study (Fed. Proc. 42:998, 1983) we reported that chronic renal denervation does not affect the antinatriuretic response to head-up tilt in the monkey. The present experiments were performed in order to determine if the renal nerves are necessary for eliciting the renal responses to an intervention which increases sodium excretion. Male Macaca fascicularis monkeys underwent chronic bilateral renal denervation or sham surgery and were allowed at least a one week recovery period. After that time, the animals were anesthetized with sodium pentobarbital and volume-expanded 20% of estimated blood volume. Two types of volume expansion were used: one with isooncotic dextran in isotonic saline and the other with blood which had previously been withdrawn in exchange for dextran. Both types of expansion caused similar increases in urine flow and sodium excretion. Furthermore, renal denervation did not affect the magnitude or time course of the diuretic and natriuretic responses to either type of expansion. These results demonstrate that, in the nonhuman primate, the decrease in efferent renal nerve activity which occurs with volume expansion is not necessary for eliciting the renal excretory effects. (Supported by NIH Grant No. AM 27689 and a Grant-in-Aid from the American Heart Association in Texas).

8.15

EFFECTS OF FELODOPINE ON SYMPATHETIC TRANSMITTER RELEASE AND VASOCONSTRICTOR RESPONSES TO EXOGENOUS NOREPINEPHRINE IN THE ISOLATED PERFUSED RAT KIDNEY. M. L. Steenberg*, E. Peter*, D. C. Eikenburg*, B. S. Jandhyala* and M. F. Lokhandwala. Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77004.

Felodopine is a dihydropyridine derivative, antihypertensive agent and considered to be an inhibitor of calcium fluxes. In the present study, effects of felodopine on [^{3}H]-norepinephrine (NE) release elicited during sympathetic nerve stimulation and vasoconstrictor responses to exogenous NE were investigated in the isolated perfused rat kidney. Felodopine ($1x10^{-9}$ to $1x10^{-5}$M) did not inhibit stimulus-induced release of [^{3}H]-NE at 0.5 Hz and 2.0 Hz. However, felodopine ($1x10^{-5}$M) significantly enhanced [^{3}H]-NE release at both frequencies. Vasoconstrictor responses to exogenous NE (16 and 32 ng) were progressively reduced with increasing concentrations of the drug and these responses were virtually abolished at the concentration of $1x10^{-5}$M. These data are consistent with the observations that felodopine is more effective in reducing pressor responses of intravenous NE than that of spinal stimulation in pithed rats. These studies collectively suggest that the ability of the drug to reduce vasoconstrictor effects of NE contributes to its vasodilator activity and to its efficacy as an antihypertensive agent. (Felodopine was supplied by AB Haessle, Mölndal, Sweden).

8.16

EFFECTS OF NICOTINE ON RENAL AND SPLANCHNIC BLOOD FLOWS IN CONSCIOUS DOGS. Maria T. B. Bedran de Castro*, H. Fred Downey, George J. Crystal, and Fouad A. Bashour. Univ. Texas Health Science Center, Dallas, Texas 75235

Nicotine (24 µg/kg/min i.v.) was administered to six conscious, instrumented dogs. Left atrial injections of 15 µ radioactive microspheres were made to measure regional blood flows before (control) and at 3 min and 10 min of nicotine infusion. Aortic pressure was elevated at 3 min (+46%) and 10 min (+28%). Flows (ml/min/g) were:

	Kidney Cortex	Pancreas	Duodenum	Liver	Spleen
Control	4.83±.47	1.71±.09	1.33±.19	.46±.10	1.58±.23
3 min	4.79±.47	.77±.12*	1.03±.16	.54±.14	2.58±.65
10 min	5.31±.59	.54±.24*	.79±.14*	.46±.14	1.89±.37

Mean ± SE. * Different from control, $P < .05$.

Nicotine caused significant decreases in pancreatic and duodenal blood flows and vascular conductances. Conductance was also decreased significantly in kidney cortex. These results demonstrate heterogeneous effects of nicotine on renal and splanchnic vasculature of conscious dogs. Supported by the Cardiology Fund.

8.17

EFFECTS OF CHLORDIMEFORM ON CARDIOVASCULAR FUNCTIONAL PARAMETERS IN YOUNG, ADULT, AND GERIATRIC RATS. William P. Watkinson*, Eric H. Hoke* and Kathy S. Robinson* (SPON: M.J. Wiester), U.S. EPA, HERL, Research Triangle Park, NC 27711.

Chlordimeform (CLD), a formamidine pesticide, was administered to four groups of pentobarbital-anesthetized Sprague-Dawley rats in three age brackets. Group A (21-30 d.), Group B (55-65 d.), and Group C (725-737 d.) animals each received sequential intraveneous (IV) injections of 5, 10, 30, 60 and 120 mg/kg CLD. Control animals in each group received multiple injections of normal saline over the same time period. Heart rate (HR), arterial blood pressure (BP), and electrocardiogram (ECG) were monitored for all animals in these groups. Group D animals (21-30 d.) received a single intraperitoneal (IP) injection of either normal saline, or 10, 30, or 60 mg/kg CLD. HR and ECG were monitored on these animals. CLD produced both direct and indirect effects on cardiovascular parameters. Acute changes in HR and BP as well as multiple ECG irregularities occurred with doses as low as 5 mg/kg in Groups A, B, and C. In addition, steady state values of HR and BP were significantly lower following CLD injection when compared to both initial control values and to vehicle-injected controls. Comparable decreases in HR were obtained in Groups A and D at one hour post CLD injection. Age-related differences in arrhythmia production and lethality were observed, with the older animals demonstrating increased vulnerability.

8.18

ELASTIN, COLLAGEN AND THE BIOMECHANICS OF ARTERIAL ANEURYSMS. Philip B. Dobrin. Dept. of Physiology, Loyola University Medical Center, Maywood, IL 60153

Pressure-diameter relations of dog carotid artery and human iliac artery were studied as cylindrical segments in vitro. The vessels were degraded with Worthington ESFF elastase for 16 H or Worthington CLSPA collagenase for 2 H. Treatment of 18 dog vessels with 8µ elastase produced 131% dilation at 100 mmHg but did not cause vessel rupture. Treatment of 18 dog vessels with 64µ collagenase produced only 109% dilation at 100 mmHg, but caused rupture in all vessels tested. Sequential treatment with both enzymes (N=18) produced dilation to 141% at 100 mmHg and rupture of all vessels. Thus, intact elastin is necessary for maintenance of normal dimensions, but intact collagen is required for wall integrity. In 32 human external iliac arteries, treatment with 40µ elastase of 320µ collagenase caused only slight dilation (105% at 100 mmHg), but only treatment with collagenase caused vessel rupture. This suggests that the compliance of elastin in these aged vessels is restricted by collagen. Clinically, human internal iliac arteries become aneurysmal more frequently than do external iliacs. Therefore 9 internal iliac arteries also were studied. Treatment with 40µ elastase or 320µ collagenase produced 105% dilation, but collagenase elicited a marked decline in stiffness and then rupture. Conclusion: arterial integrity requires intact collagen rather than elastin, and human internal iliac artery is especially dependent upon intact collagen. (Supported by NIH HL26056 and a VA Clinical Investigatorship)

8.19

PHYSOSTIGMINE ENHANCEMENT OF THE CEREBROVASCULAR RESPONSE TO HYPOXIA. Oscar Scremin, Dennis M. Hudson* and Ralph R. Sonnenschein. UCLA School of Medicine, Los Angeles, CA 90024

Physostigmine (P), a cholinesterase inhibitor, prolongs survival under hypoxic hypoxia (Stroke 10:142, 1979; 11:548, 1980). The present investigation evaluates the possible contribution of cerebrovascular adjustments to this phenomenon. Internal carotid blood flow (ICBF) was measured with an electromagnetic flowmeter in rabbits (70% N_2O). Under moderate hypoxia (FIO_2=10%), ICBF increased to 118%±9 of control (X ± SE, n=5). Following 0.3mg/kg P (i.v. bolus), this response of ICBF was considerably enhanced (215%±43). The increase in ICBF under severe hypoxia (FIO_2=7%), (212%±36), was only slightly augmented by P (284%±51). The enhancement by P of the ICBF response to moderate hypoxia could not be explained by the 20% increase in arterial pressure. To evaluate the possible contribution of changes in cerebral O_2 uptake ($CMRO_2$), sagittal sinus blood flow was measured (H_2 clearance) and blood was sampled from this same vessel and from the abdominal aorta. Under moderate hypoxia, P induced a decrease in $CMRO_2$ (hypoxia=8±1.3; hypoxia+P=4.8±1.8ml/100g·min; n=3) and an increase in cerebral venous O_2 saturation (hypoxia=8.2±1.4; hypoxia+P=13±1.5%). Conclusion: the increase in blood flow and venous O_2 saturation and the decrease in $CMRO_2$ caused by physostigmine might play a role in the prolonged survival under hypoxia induced by this drug. (Supported by AHA-GLAA 437IG).

8.20

LIVER BLOOD FLOW BY THERMODILUTION TECHNIQUES. Samuel A. Cucinell,* Gordon H. Bryant,* and Peter J. Barcia.* (SPON: J.R.Claybaugh). Tripler Army Medical Center, Honolulu, Hi 96859.

The anatomic position of the hepatic veins lend themselves to the determination of hepatic venous blood flow by the algebraic difference of inferior vena caval (IVC) blood flow at a point cephalad to renal veins but caudal to the hepatic veins, and at a point cephalad to the hepatic veins but below the atrium. Initial studies in dog using electromagnetic flow probes encircling the IVC and thermodilution catheters, placed percutaneously at the same points in the IVC, gave comparable estimations of the blood flow. However, inaccurately high estimation of HVBF was obtained comparing portal venous blood flow (hepatic artery ligated) to percutaneously placed thermodilution catheters in the IVC in the pig. The reason is the loss of the cold thermodiluent in the IVC in the pig. By a comparing of the cardiac output, after atrial and femoral vein injection of the thermodiluent, the number of calories exchanged with the IVC wall can be calculated. From zero to 50% of the thermodiluent calories may be lost depending upon cardiac output, the absolute amount of calories injected, the temperature difference between the injectate and the pig, the speed of blood flow, and the absolute temperature of the animal.

8.21

ACTIVATION OF RENAL LIPOXYGENASE PATHWAYS IN DOGS BY WATER DEPRIVATION. Larry P. Feigen, Tulane Medical Center, New Orleans, LA 70112.

Arachidonic acid (AA) is the metabolic substrate for cyclooxygenase and lipoxygenase enzymes. The former leads to prostaglandins and the latter to lipoxygenase products (LP) including leukotrienes. In anesthetized dogs, renal blood flow was measured electromagnetically and 4 mg AA was injected into the renal artery. In 2/3 of the dogs that had free access to water, AA injection lead to renal vasodilation and in 1/3 it led to initial vasoconstriction followed by vasodilation. In 80% of dogs that were water deprived for 15-18 hours prior to experiments, AA injection led to a biphasic response. The dilator portion of responses to AA was blocked by ibuprofen (a cyclooxygenase inhibitor). The constrictor portion was not affected by ibuprofen but was blocked by lipoxygenase inhibitors. Direct injection of leukotrienes C_4, D_4 and E_4 into the renal artery produced only vasodilation and these agents were not very potent at doses up to 10 µg. These data show that dog kidneys possess the capacity to convert AA to LP. The pathway appears to be normally inactive but can be activated by water deprivation. The LP formed are probably not leukotrienes. (Supported by NHLBI Grant #28356).

MECHANICS OF BREATHING I

9.1

THE ACUTE EFFECTS OF REST ON VENTILATORY MUSCLE (VM) FUNCTION IN PATIENTS WITH SEVERE CHRONIC AIRFLOW LIMITATION (CAL).

B. Rabinovitch*, R.L. Pardy*, S.N.A. Hussain*, P.T. Macklem. Montreal Chest Hospital and Meakins-Christie Labs., McGill University Clinic, Montreal, Quebec.

We studied 7 patients with severe CAL ($FEV_1/VC < 40\%$) by imposing VM rest for 1 hour with an Emerson chest respirator. Control measurements of maximum voluntary ventilation (MVV), Max inspiratory (PI_{max}) and expiratory (PE_{max}) mouth pressures, max. transdiaphragmatic (Pdi_{max}) pressure and minute oxygen consumption ($\dot{V}O_2$) were made. Diaphragm electromyogram (EMG) was recorded with an esophageal electrode. The power in high (H:138-238 Hz) and low (L: 20-40 Hz) frequency bands, the ratio of the two (H/L ratio) and the relationship of the rectified integrated EMG (Edi) to Pdi (diaphragm contractility) were measured before the VM were rested. After 1 hour rest there were increases ($p < .05$) in Pdi_{max}, diaphragm contractility and H/L ratio. We conclude that the VM account for up to 20% of resting $\dot{V}O_2$. Even short periods of VM rest improve diaphragm function in severe CAL. We suggest that VM rest as well as VM training is a reasonable approach to therapy of such patients.

Supported by Royal Edward Laurentian and Parker B Francis Foundations, MRC (Canada), Iraqi Ministry of Higher Education.

9.2

CHEST WALL(CW) RESTRICTION IN EXERCISE. S.N.A. Hussain*, P.T. Macklem and R.L. Pardy*. (SPON: J.Milic-Emili). Meakins-Christie Labs, McGill Univ., and Montreal Chest Hospital.

We studied the effects of rib cage (Rc Res) and Abdominal (Ab Res) restriction on breathing pattern and pressure generation in 5 subjects exercising to exhaustion (tlim) at 80% $\dot{V}max$. During exercise, transdiaphragmatic (Pdi), pleural(Ppl), and gastric (Pga) pressures, and rib cage (Vrc) and abdominal (Vab) volume contributions to tidal volume(V_T) were measured. Without CW restriction mean tlim (± SE)was 6.7±0.8 min. Ventilation($\dot{V}_E$)increased progressively to 110 ± 9 $1min^{-1}$. Vrc $\%V_T$ was 66%. △Pdi insp.increased(due predominantly to greater △ Ppl) to a plateau in the first 3 min. of exercise. △Pga exp. increased progressively due to abdominal muscle recruitment. CW restriction was produced by an inelastic corset. AB Res reduced VC 13% and FRC 37%, and increased Vrc $\%V_T$ to 85%. Ab Res produced no change in tlim, $\dot{V}_E$ or V_T in exercise but increased both △Pdi insp.(due to greater △Pga/△Pdi) and △Pga exp. Rc Res reduced VC 31%, FRC 19% and Vrc $\%V_T$ to 23%. Rc Res decreased tlim by 37%. Although $\dot{V}_E$ and △Pdi insp. in exercise up to tlim were unchanged, V_T was less with Rc Res and △Pga exp. was increased. We conclude that either Ab Res or Rc Res alter the pattern of ventilatory muscle recruitment in exercise. Preventing intercostal-accessory muscle contribution to the increased V_T by Rc Res diminished exercise performance at 80% $\dot{V}max$ (Supported by: Iraqi Ministry of Higher Education, MRC of Canada, and the Parker B. Francis Foundation).

9.3

ORO-NASAL BREATHING AND THE SOFT PALATE. D.C. Stănescu* and D.O. Rodenstein* (Spon. J.A. Will). Cliniques Universitaires St. Luc, Cardiopulmonary Laboratory, Brussels, Belgium.

We investigated in 20 normal naive subjects the ability of the soft palate to direct airflow during breathing. Subjects were connected to a spirometer, without nose clip. No instructions were given on the breathing route. During quiet respiration 15 subjects breathed solely through the nose, despite an open mouth. During forced vital capacity (FVC) maneuvers, 19 subjects expired exclusively through the mouth. When specifically asked to breath quietly through the mouth, pure nasal breathing was no more observed. Tidal volume (Vt) (or FVC) were comparable when subjects were asked to breath through the mouth, without and with a nose clip : 0.67 $\pm$ 0.46 L vs 0.60 $\pm$ 0.21 L for Vt; 4.05 $\pm$ 0.65 L vs 4.18 $\pm$ 0.70 L for FVC. In 8 separate volunteers, the soft palate was shown by fluoroscopy to close the oropharynx during quiet breathing (resulting in pure nasal breathing), and to close the nasopharynx during FVC efforts (resulting in mouth breathing). These data suggest that when both mouth and nose are open, the soft palate makes the choice of the breathing route.

9.4

HEART WEIGHT AFFECTS THE DISTRIBUTION OF LUNG SURFACE PRESSURES. E. Bar-Yishay*, R.E. Hyatt, J.R. Rodarte, and T.A. Wilson. Mayo Clinic and Fdn., Rochester, MN 55905 & Univ. of Minn., Aerospace Eng. & Mech., Minneapolis, MN 55455.

In upright dogs the vertical transpulmonary pressure gradient (VTPG) disappears after pneumothorax (PNX) (JAP 34:590, 1973). We confirmed this finding and observed the heart to move downward and posteriorly with PNX, thus raising the question to what extent is the heart supported by the lungs? We studied a theoretical linear elasticity model using finite element analysis. The lung (L) and heart (H) were assumed to be symmetric along the vertical axis with dimensions obtained from chest radiographs. Reported values of elastic properties of L at FRC and H were assigned. The model was generated first without the heart (L). The heart was then added (L+H). Finally, the effect of doubling heart weight (L+2H) was investigated. The results for L supported by its own weight were similar to those of West and Matthews (JAP 32:332, 1972) although the use of more realistic elastic properties caused a parallel shift in surface pressure values. Adding the heart caused VTPG to increase from 0.2 to 0.32 cmH_2O/cm height, and to 0.52 for L+2H. When the lungs were inverted, surface pressures were less negative than in upright position for L, L+H and L+2H. For L VTPG's were similar in both positions. However, for L+H and L+2H, VTPG's inverted were lower than upright. Heart weight may thus explain why VTPG in the upright dog is greater than lung specific density and why VTPG diminishes with inversion. Supp. by HL21584 and P.B. Francis Fdn.

9.5

REGIONAL VOLUMES (Vr) IN EXCISED RIGHT CAUDAL DOG LUNG LOBES: AIR FILLED-SALINE SUBMERGED. L.E. Olson, T.A. Wilson, D. Stamenovic* and J.R. Rodarte. Mayo Clinic & Fnd., Rochester, MN 55905 and U. of Minn., Dept. of Aerospace Eng. & Mechanics, MN 55455.

Gillett et al (JAP 51:1457, 1981) found a vertical gradient in Vr in lobes submerged in chlorothene less than that predicted from the pressure-volume (PV) curve. We suspended 3 lobes containing lead markers by strings glued to the cephalic surface as if in an upright dog: cephalocaudal, z, was vertical and dorsal ventral, x, and transverse, y, were horizontal. At constant lobe volume, orthogonal videoroentgenographs were recorded before and after submersion in saline. Ptp unsubmerged was 6,9, and 12 cmH_2O. During submersion, bouyancy was overcome by a clip attached to the lobe tip. Vr and large deformation strains in the x, Exx, y, Eyy, and z, Ezz directions were computed. During submersion, Ptp decreased at the bottom and increased at the top which produced vertical gradients in x and y strain and thus volume. Exx and Eyy were < 0 at the bottom > 0 at the top and similar to the strain which would occur if Vr were uniquely determined by local Ptp. The volume gradient was less than that predicted from the uniform PV curve because bouyancy forces produced a positive vertical strain (Ezz) which was greatest at the bottom and nearly equal to Exx and Eyy at the top. This is consistent with a simple model based on linear elasticity and explains the reduced Vr gradient. Supported by HL 21584, 4664, and 07222.

9.6

EFFECTIVE IMPEDANCE OF HUMAN RESPIRATORY SYSTEM. Arthur B. Otis and Cobern V. Peterson, Jr. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

A human subject with lung volume at FRC makes a gradually increasing inspiratory effort against a closed shutter. When mouth pressure (P_{ao}) reaches a chosen preset value (P_o), the shutter abruptly opens. Flow ($\dot{V}$) and P_{ao} are recorded continuously. P_o is the pressure developed by the inspiratory muscles (P_{mus}) at the time of shutter opening. We assume that the "effort" or degree of activation of the muscles remains constant for up to 0.1 sec after the shutter opens. Thus the pressure gradient from muscles to mouth is $P_o - P_{ao}$ and the ratio, $(P_o - P_{ao})/\dot{V}$ is the effective impedance of the respiratory system. Our data show that $\dot{V}$ peaks within 0.015 sec after shutter opening and P_{ao} rises rapidly during the same period. During the next 0.1 sec $\dot{V}$ tends to fall gradually and $P_o - P_{ao}$ slowly rises, while effective impedance increases. This increasing impedance may reflect: 1) an increasing elastic load resulting from changes in shape and volume of the chest; 2) less favorable positions on length-tension curves of inspiratory muscles; and 3) increasing airways resistance. (Supported in part by NIH Grant HL 28263)

9.7

INTERREGIONAL BREATH SOUND COMPARISONS CAN QUANTTATE LOBAR AIRWAY NARROWING AND AIRWAY DISTENSIBILITY. P. E. Krumpe* and J. P. Smith* (SPON: H. Boushey). VA Medical Center, Martinez, Ca 94553.

Inorder to determine if breath sound intensity (I_B) reflects regional airway dynamics, we compared inspiratory I_B and time delays (TD) between peak I_B in 16 pairs of homologous lobes at various transpulmonary pressures (P_L, cm H_2O) before and after lobar airway narrowing (LAN) produced by proximal airway rigid stints or distensible elastic bands in 6 excised dog lungs. Airway diameters (D) were measured from bronchograms at static P_L, and I_B and TD at identical inspiratory flows. Significant differences ($P < .05$ by ANOVAR) were as follows: Before LAN, I_B and D $\uparrow$ as $P_L \uparrow$; TD was always < 20 msecs. With rigid LAN, D and I_B did not change and TD remained > 500 msecs at all levels of P_L. With elastic LAN, D of LAN was < 50% of control lobe D at P_L = 0 and 2, but increased > 80% of control lobe D at P_L = 10 and 15. I_B and TD were similar to rigid LAN at low P_L but similar to baseline data at higher P_L. We conclude that proximal LAN can be identified by TD of peak I_B over the narrowed region when D-LAN/ D-homologous lobe is between 50% and 80%. Furthermore, the distensibility of LAN can be infered from changes in TD as P_L increases from 0 to 15 cm H_2O.

9.8

TRACHEAL LUNG SOUNDS WHILE RECORDING QUASI-STATIC PRESSURE-VOLUME CURVES OF EXCISED RAT LUNGS. D.G. Frazer, L.D. Smith,* C.E. Turick* and N.F. Nehrig*. NIOSH, Dept. of Physiol. W.V.U., Morgantown, W.V. 26505 & F.S.C., Fairmont, W.V. 26554

Excised lungs from Long Evans Hooded male rats were inflated-deflated in a saline-filled plethysmograph housed in an anechoic chamber. The lungs were continuously ventilated for 10 cycles at two different rates (1 and 4 minutes to inflate). Lung sounds were detected as pressure variations (P_s) with a microphone acoustically coupled to the tracheal cannula. The following measurements were made as the lung was ventilated: a) the pressure-volume (P_L-V_L) curve, b) P_s versus P_L, c) the instantaneous and average spectral content of the sound, and d) values of $K\int(P_s^2)\,dt$, (IP_s), during lung inflation and deflation. Results showed that coarse and fine crackles were produced in the excised lung during inflation. The crackles were most intense near the "knee" of the curve but continued to occur until maximum volume (TLC) was reached. The lung was silent during deflation until airway closure occurred. It was found that nearly all crackles were composed of the same spectral content having significant frequency components between 1 KHz and 12 KHz. Gas trapping occurred in the slowly ventilated lung (Frazer and Weber, JAP, 1976) and reached 75% TLC after 10 inflation-deflation cycles. Since crackles occurred during all 10 cycles, alveolar atelectasis prior to lung inflation was not required for sound generation. Lastly, it was found that during inflation IP_s was essentially the same at slow and fast rates.

9.9

Magnitude of the interaction between the bronchomotor effects of sulfur dioxide and those of dry(cold)air. D Sheppard,* WL Eschenbacher,* HA Boushey, RA Bethel*, San Francisco General Hospital and Cardiovascular Research Institute, UC San Francisco. We studied the interaction between airway drying(cooling)and inhalation of sulfur dioxide(SO2)in 8 subjects with mild asthma. On three days we measured specific airway resistance(SRaw) before and after the subject performed voluntary eucapnic hyperpnea at a constant ventilation(30-40l/min)for successive 3 min periods with doubling concentrations of SO2 in dry cold air, in dry warm air, and in partially humidified warm air, and calculated the concentration that would increase SRaw by 100% over baseline(Pc100). The Pc100 for dry cold air with SO2 (0.51±0.20ppm;mean±SD)and for dry warm air with SO2(0.59±0.41) did not differ and were both significantly lower than the Pc100 for humid warm air(0.87±0.41). Repeated hyperpnea with dry cold air without SO2 at the same ventilation had no effect on SRaw. We then had the same subjects perform hyperpnea at increasing ventilation with dry air alone, dry air with 0.1ppm SO2, or dry air with 0.25ppm SO2 and calculated the ventilation that would cause an 80% increase in SRaw under each condition (Pv80). The Pv80 for hyperpnea with 0.25ppm SO2 was significantly lower than that for dry air without SO2. We conclude that sulfur dioxide causes bronchoconstriction at lower concentrations when it is inhaled in dry air than when it is inhaled in partially humidified warm air, and that concentrations of SO2 as low as 0.25ppm can potentiate the bronchoconstriction caused by hyperpnea with dry air itself.

9.10

A CANINE MODEL OF CHRONIC SMALL AIRWAY INJURY INDUCED BY NITRIC ACID. S.G. Peters* and R.E. Hyatt, Mayo Clinic and Foundation, Rochester, MN 55905.

We sought to create a model of chronic small airway disease in dogs which avoids long term toxic exposure. Modifying a proposed model of bronchiolitis obliterans (Whitley et al., ARRD 125:189, 1982) 5 anesthetized mongrel dogs were exposed on alternating days for 4 weeks to 1% nitric acid (HNO_3) nebulized through an endotracheal tube, or through a catheter in the mainstem bronchus of each lung. Controls were treated with saline. Studies of lung mechanics prior to treatment and after 2 and 4 weeks of exposure included volumes, static and dynamic compliance (C_{st}, Cdyn), airway resistance (R_L), flow-volume curves on air and 80% He-20% O_2 for flow at 50% VC ($\dot{V}_{50}$), density dependence ($\Delta\dot{V}max_{50}$) and volume of isoflow ($Viso\dot{V}$), and single breath O_2 for slope of phase III ($\Delta N_2/L$), closing volume (CV) and closing capacity (CC). Significant changes are shown below (mean ± S.D. $p<.05$). There were also increases in $Viso\dot{V}$ and CC, and decreased Cdyn. C_{st}, $\Delta\dot{V}max_{50}$ and CV were not significantly changed. Control dogs showed no significant changes. Histologically, HNO_3 exposure resulted in acute and chronic changes of small airway inflammation. This provides a model for further studies of peripheral airway injury. Supported by Grant HL 21584.

	Baseline	2 4 Weeks
VC(L)	1.76 ±.29	1.23 ±.23
$\dot{V}50_{air}$ (L/s)	9.5 ±.91	4.4 ±2.2
R_L (cmH_2O/L/s)	1.2 ±.4	4.0 ±.8
ΔN_2/L (%)	2.3 ±.6	8.3 ±3.0

9.11

Repetitive Exercise and the Refractory Period in Asthma. B.M. Pichurko,* E.R. McFadden, Jr., B.P. Sullivan,* and P.H. Ingram, Jr. Shipley Institute of Medicine, Brigham and Women's Hospital, Boston, MA 02115

To characterize the events associated with repetitive exercise in asthma, we had 8 subjects undergo exhausting leg exercise under controlled inspired air conditions. Prior to, during and after the challenge, we measured peak expiratory flow rates (PEFR). Six minutes after completion of the work load, while the subjects were experiencing acute bronchospasm, they were re-exercised and the above measurements repeated. The initial exercise produced an increase in PEFR of 16.2 ± 2.5 (SEM) % followed by bronchoconstriction when exercise ceased (ΔPEFR -21.8 ± 5%). During the second challenge the obstruction resolved and PEFR rose 43.3 ± 6.1%. When the subjects stopped work the obstruction returned. Assessment of the size of the response by comparison of pre and post challenge data makes the subjects appear refractory (Ex 1 change = 21.8 ± 5% vs Ex 2 = 2.6 ± 6.0%), however, when mechanical changes are determined from the final minute of exercise to the point of maximal response, no refractoriness is seen (ΔPEFR Ex 1 = 32.7 ± 3.7% vs Ex 2 = 30.8 ± 4.3%). These data are incompatible with the concept of mediator depletion playing a major role in the development of the refractory period. Possibly catecholamine release associated with physical exertion might be a major determinant of this phenomenon.

9.12

METABOLISM OF NOREPINEPHRINE DURING NERVE STIMULATION IN DOG TRACHEA. James A. Russell and Katharyn W. Kircher.* SUNY at Buffalo, Buffalo, N.Y. 14214.

We determined the relative importance of neuronal and extraneuronal uptake in the metabolism of norepinephrine (NE) released during electrical stimulation (ES) of isolated canine tracheal smooth muscle (TSM). Strips of TSM were labelled with L-[^{3}H] NE (2 x 10^{-7}M) and mounted for superfusion. Superfusate was collected continuously before, during, and after ES (15 V, 0.5 ms., 5 H_z). Measurements were made of [^{3}H] NE and its metabolites in superfusate and in tissue. Neuronal uptake followed by metabolism was estimated by measuring the amount of 3,4-dihydroxyphenylglycol (DOPEG). Extraneuronal uptake was estimated by measuring O-methylated metabolites (OMM). ES caused large increases in the efflux of NE, DOPEC and OMM from TSM. However, the overflow of OMM was 5 times greater than that of DOPEG. Cocaine (2 x 10^{-5}M) abolished the increased efflux of DOPEG during ES and enhanced the overflow of NE and OMM. We conclude that extraneuronal uptake constitutes the primary metabolic pathway for NE released from adrenergic nerves innervating TSM.
(Supported in part by grants HL 01074 and HL 27672 from NHLBI.)

9.13

CLOSED VS OPEN LOOP METHODS FOR DIRECT DETERMINATION OF SPECIFIC AIRWAY CONDUCTANCE. W.S. Krell*, K.P. Agrawal, and R.E. Hyatt. Mayo Clinic/Foundation, Rochester, MN 55905.

Specific airway conductance (SGaw) can be derived directly from the relationship between airflow changes at the mouth and flow related pressure changes measured while a subject breathes in a body plethysmograph (Resp. Physiol. 210:65, 1980). The ratio of airflow to flow related box pressure changes is conventionally determined during panting (closed loop method) but can also be measured during tidal breathing, using an open loop method (Resp. Physiol. 43:23, 1981). We compared the value for SGaw obtained during inspiration by each method in 21 normal volunteers and 10 patients with FEV_1 < 75% predicted normal. Results obtained by the two methods correlated well (R=0.87, p < .001). The variance of the closed loop method was 5.8% and of the open loop 5.3%. Two observers' measurements were compared; the median of the coefficient of variation was 5.5% (mean 6.4%). We conclude that values for SGaw by the open loop method are not significantly different than those of the closed loop method. The variability in each is similar. 17/21 normal subjects and 7/10 patients found the tidal breathing easier to perform, and the technicians judged it simpler to teach in 28/31 cases. The open loop method provides an accurate and simple way to obtain SGaw. It may be especially useful in cases where repeated measurements are desired. (Supported by USPHS grant HL21584 and the Parker B. Francis Foundation).

9.14

HINDLIMB MUSCULAR CONTRACTION REFLEXLY RELAXES TRACHEAL SMOOTH MUSCLE IN DOGS. M.P. Kaufman* and K.J. Rybicki* (SPON: J.H. Mitchell). Univ. of Texas Health Science Center, Dallas, TX 75235

Although contraction of hindlimb skeletal muscle is well known to reflexly increase ventilation, heart rate and arterial pressure, little is known about the reflex effect of this maneuver on airway smooth muscle tone. Therefore, in chloralose-anesthetized dogs, we recorded transverse tension from the trachealis muscle while we contracted both gracilis muscles by electrically stimulating the gracilis nerves at 5 and 40 Hz. In 4 of the 5 dogs studied, static (40 Hz) contraction decreased tracheal tension by 22±4 grams. In the remaining dog, static contraction increased tension by 6 grams. The contraction-induced changes in tension were abolished by paralyzing the dogs and were restored after the paralysis had worn off. In addition, contracting the gracilis muscles by stimulating the cut peripheral ends of the gracilis nerves when the dogs were not paralyzed had no effect on tracheal tension. Rhythmic contraction (5 Hz) decreased tension in those dogs in which static contraction decreased tension and increased tension in the one dog in which static contraction increased tension. The changes in tension evoked by rhythmic contraction were prevented by paralysis and were not present when the peripheral cut ends of the nerves were stimulated. In addition, the changes in tension were not secondary to changes in arterial pressure. We conclude that muscular contraction reflexly relaxes tracheal smooth muscle in most dogs.

9.15

MORPHOLOGICAL AND PHARMACOLOGICAL STUDY OF CULTURED AIRWAY SMOOTH MUSCLE CELLS. Richard J. Adams,* May Tom-Moy,* and James K. Brown* (SPON: J.A. Nadel). Veterans Administration Medical Center and Cardiovascular Research Institute, University of California, San Francisco, CA 94121.

Our objective was to culture airway smooth muscle cells, characterize them morphologically, and develop techniques for testing their responses to pharmacological stimuli. Strips of canine trachealis muscle were treated with purified collagenase and elastase, mechanically dispersed, and incubated on plates for 4-7 days. Yield of viable cells was $1.7\pm0.5 \times 10^7$ (mean±SD, n=11) per gram muscle. By electron microscopic analysis, incubated cells showed basal lamina on the outer surface of the plasma membrane, caveolae on the inner surface, and myofilaments in the cytoplasm. When cells were incubated on rigid polystyrene and exposed to acetylcholine (10^{-4}M), no morphologic changes were detected by phase contrast microscopy. When cells were grown on pliable silicone substrata, formed by flaming the surface of dimethylpolysiloxane (60,000 cP), exposure to acetylcholine (10^{-6}M) induced the appearance of extensive wrinkles on the rubber sheets within 1 min; wrinkling was rapidly reversed by atropine (10^{-6}M). When human skin fibroblasts were grown on the silicone substrata, acetylcholine (10^{-4}M) did not produce morphological changes. We concluded that these techniques were suitable for the pharmacological study of monolayers of cultured airway smooth muscle cells. (Supported by USPHS grant HL-27669 and grants from the Veterans Administration and California Lung Association.)

9.16

MECHANISM OF SUBSTANCE P INDUCED CONTRACTION OF THE ISOLATED RABBIT AIRWAY. David T. Tanaka* and Michael M. Grunstein. Natl. Jewish Hosp/Nat.Asthma Center and Univ. of Colo. School of Med., Denver, CO. 80206.

Substance P (SP) contracts airway smooth muscle, however, the mechanism(s) underlying this response has not been systematically evaluated. To elucidate the mechanism of SP-induced airway constriction, isometric tension developed by rabbit tracheal ring segments placed in modified Krebs-Ringer solution was separately tested to methacholine, SP, and its agonist analog ($pGlu^5$,Sar^9)-SP(SP_a). Methacholine (M), SP, and SP_a produced dose-dependent increases in tracheal smooth muscle (TSM) tension in all segments. The peak tension with SP_a averaged 29.1% (range: 9 to 59%) of the maximal tension developed with M. Blockade of both parasympathetic ganglia with hexamethonium and neural transmission with tetrodotoxin had no significant effect on the TSM response to SP_a. On the other hand, TSM contraction with SP_a was: (1) markedly augmented (mean % increase = 440%) by pre-treatment with the cholinesterase inhibitor, neostigmine; and (2) partially inhibited (mean % decrease = 35%) by the cholinergic antagonist, atropine sulfate (10^{-6}M). The TSM response to SP_a was completely blocked by the SP antagonist ($D\text{-}Pro^2$,$D\text{-}Trp^{7,9}$)-SP. These data indicate that: (1) SP produces dose-dependent contraction of TSM; (2) the latter is due both to a direct action (i.e. SP receptor binding) on TSM as well as the release of acetylcholine (Ach); and (3) since the response to SP is unaffected by blockade of neural transmission, it is likely that the Ach release occurs at the neuromuscular junction.

9.17

DYNAMIC COMPLIANCE (Cdyn) AS A MEASUREMENT OF TRAPPED GAS IN BRONCHOCHALLENGED AWAKE GUINEA PIGS. M.J. Wiester, J.L. Tepper*, C.J. Setzer* and J.A. Graham*. U.S. EPA, RTP, NC 27711. Northrop Services Inc. RTP, NC 27709.

The relationship between the plethysmographic measurement of Cdyn and total gas volume (TGV) was evaluated during histamine aerosol exposure in 650g. guinea pigs. One day prior to challenge, intrapleural and right heart cathers were implanted. Tracheal cannulas with a port to measure pressure and a rubber tip to occlude the airway were placed in some animals on the day of the challenge. Tidal volume, intrapleural and tracheal pressure were recorded. Cdyn was computed during tidal breathing at zero flow and TGV, was computed using Boyles law. Additionally, TGV's were obtained from animals instantly killed in the plethysmograph with I.V. $MgSO_4$ at predetermined Cdyn values. Their lungs were removed and TGV measuared by water displacement. Results showed that histamine challenge is accompanied by a reversible hyperinflation. Tracheal pressure during occlusion was identical to the intrapleural pressure regardless of the TGV. Control levels of TGV were found to be 4-5 ml with both measurement procedures and histamine produced increases as high as 22-25 ml. Decreases in Cdyn were functionally related to increases in TGV up to 13-15 ml. Blood gas values, tidal volume, and breathing rate were unreliable predictors of TGV. We conclude that in guinea pigs plethysmographic values of Cdyn reflect TGV and can be used to estimate the amount of trapped air in the lung during histamine challenge.

COMPARATIVE PHYSIOLOGY: RESPIRATION AND CIRCULATION I

10.1

RESPIRATORY COMPENSATION TO METABOLIC ALKALOSIS IN THE YUCATAN MINIATURE BOAR FOLLOWING IMPLANTATION OF DESOXYCORTICOSTERONE-ACETATE (DOCA). J.M. Terris, Department of Physiology, Uniformed Services University, Bethesda, Maryland 20814

With infrequent exceptions, metabolic alkalosis, unlike other acid-base disturbances, has been reported not to result in a compensatory increase in arterial PCO_2 ($PaCO_2$). The respiratory response to metabolic alkalosis resulting from excess mineralocorticoid has not been documented. The present study was performed to evaluate the respiratory response to metabolic alkalosis which results following implantation of DOCA in Yucatan miniature swine. Animals received tap water ad libitum and a pre-determined quantity of pig chow meal supplemented with NaCl (sodium intake 4.5 mEq/Kg body wt/day). Blood samples (indwelling carotid catheters) were obtained daily. After one week of stable baseline measurements, implants (silicone, N=8 animals, or silicone + 100 mg DOCA/Kg body wt, N=9 animals) were placed subcutaneously under light Surital anesthesia. Observations were continued for 3 weeks. Five days post-DOCA, $PaCO_2$, serum pH, bicarbonate, carbonic acid, CO_2 capacity, and CO_2 content were significantly elevated. $PaCO_2$ remained relatively constant during the remainder of the study. Serum pH and bicarbonate continued to rise. CO_2 content exceeded CO_2 capacity. Although compensated pH was consistently less than uncompensated pH, it did not return to control. It is concluded from these studies that respiratory compensation to metabolic alkalosis in these animals is minimal, and is virtually complete within 5 days following DOCA administration.

10.2

RESPIRATORY ADAPTATIONS FOR DIVING IN THE MUSKRAT. Gregory K. Snyder and Edward Binkley*. Department of EPO Biology, Box 334, University of Colorado, Boulder, Colorado 80309

Selected components of the total oxygen stores, blood respiratory properties and capacity for anerobic energy production were compared in the muskrat, Ondatra zibethica, and the laboratory rat. Of the potential oxygen stores, lung volume and blood hemoglobin concentrations were comparable for the two species while skeletal muscle myoglobin, 13.3 ± 0.5 mg/g, was over four-fold higher in the muskrat. The whole blood P_{50}, 24.4 ± 1.4, and Hill coefficient, 2.4, were significantly lower in the muskrat while the Bohr effect, -0.64 ± 0.07, was significantly higher for the diving species. Blood buffering capacity and red cell 2,3-DPG were comparable. Differences in the capacity for anerobic energy production were not apparent. Concentrations of glycogen in the heart, gastrocnemius and brain were comparable and activity levels of pyruvate kinase in these tissues actually lower in the muskrat. Normally, the voluntary dives for the muskrat are of short duration and aerobic in nature. The primary differences in oxygen stores, over those found in the rat, are in a higher blood volume, not reported here, and tissue myoglobin concentrations. The affinity of the whole blood for oxygen may be important in utilizing the oxygen stores in the lung, especially since an increase in lung volume is prevented by buoyancy problems. The need for oxygen stores in the diving muskrat is best understood when equated to the high mass-specific metabolic rate which is a function of the small body size of this diver.

10.3

SIMILARITY ANALYSIS OF MAMMALIAN CARDIAC ENERGETICS.
John K-J. Li. Rutgers University, New Brunswick, NJ 08854.

Considerable interest has in recent decades been centered on the relation between energy requirement of the heart and its pumping performance. Whether this relation holds for all mammalian species has not been examined. To investigate this, pertinent physiological parameters such as mean arterial blood pressure ($\bar{p}$), stroke volume (V_S), heart rate (f_h; sec^{-1}); metabolic rate (MR; J/sec), and heart and body weights (W;Kg) were selected for the analysis. A new similarity principle was established by the use of allometric equations and the applications of dimensional analysis and Buckingham's pi-theorem. The relation is

$$I = \frac{EW \cdot f_h}{MR} = .013\ W^{.06}$$

where EW ($=\bar{p} \cdot V_S$) is the left ventricular external work (J). I, the invariant number, is dimensionless, and is practically independent of mammalian body weights. It thus qualifies as a similarity principle. It can be stated that, in mammals, the external left ventricular work performed per cardiac cycle normalized by their respective metabolic rates generated is a constant. The significance of the present finding is that mammalian resting heart rate governs the relation between energy generation and cardiac pumping ability.
(Supported in part by the AHA-NJ Affiliate 82-21)

10.4

Distribution of Blood Flow in the Turtle Pseudemys scripta during Progressive Anoxia.
Timothy B. Bentley,* Peter Lutz, Myron Rosenthal and Tom J. Sick.* University of Miami, Miami, Florida 33149

The primary response to anoxia in air breathing divers involves the cardiovascular system. This response is well demonstrated in marine mammals where blood flow is confined primarily to the heart and brain during extended dives. Although turtles have a much greater diving capacity nothing is known of the pattern of blood flow during diving in these animals. We hypothesize that turtles show a two stage response to progressive anoxia, the first stage being the maintenance of blood flow to the lungs which function as the primary oxygen store. The second stage occurs when lung oxygen is depleted and the blood flow alters to increase the transfer of substrates necessary for anaerobic energy production. Experiments are being conducted on Pseudemys scripta using three radioactively labelled microspheres which allow determination of control, short term and long term anoxia blood flows. We have found that the GI tract, kidneys and bladder experience reduced blood flow during anoxia while the lungs and brain showed increases in flow. The liver showed an initial decline in flow and then a subsequent rise.

Support for this work was provided by NIH grant No. NS16655.

10.5

INTRAFILAMENTAL FLOW DISTRIBUTION AND PLASMA SKIMMING IN THE PERFUSED GILL OF THREE TELEOSTS. Kenneth R. Olson. Indiana University School of Medicine, South Bend Center, Notre Dame IN. 46556

Distribution of flow and red cells between the efferent (epibranchial) and venous pathways was examined with an isolated perfused gill adapted to separately collect the two effluents. Gills from two species (Ictalurids) with abundant prelamellar arteriovenous anastomoses (AVAs) were compared to those of the trout which contain few AVAs. The gills were perfused with Ringer or ^{125}I albuminated Ringer containing ^{51}Cr tagged red cells (blood).

In Ringer perfused gills efferent outflow decreased as efferent pressure increased. Epinephrine prevented the decrease in efferent flow at elevated efferent pressures. In all species around one third of the control blood perfusing the gill drained via the venous pathway. At constant efferent pressure epinephrine increased and acetylcholine decreased efferent outflow. These results suggest that tonic adrenergic stimulation is necessary for normal branchial perfusion.

The hematocrit of efferent effluent was greater than venous effluent in all species. No consistent effects of epinephrine or acetylcholine on plasma skimming were observed. Comparison of measured microhematocrit and hematocrit calculated from ^{51}Cr red cell space and ^{125}I-albumin plasma space show that the red cells in the venous effluent are larger than those from the efferent pathway and support the concept of a nutritive function for the venous pathway. (Supported in part by NSF Grant No. PCM 79-23073.

11.1

ADENYLATE CYCLASE AND CYCLIC AMP MEDIATION OF OCTOPAMINE-STIMULATED ION REDUCTIONS IN LIMULUS LEG MUSCLE EXPOSED TO HYPOOSMOTIC STRESS. S.K. Pierce* and S.C. Edwards* (SPON: H. Levitan). Univ. of Maryland, College Park, MD 20742

We have previously shown that the biogenic amine, octopamine (OCT), may play a neurohormonal role in cell volume regulation in Limulus exposed to hypoosmotic stress. OCT potentiates the hypoosmotically induced reduction of two major intracellular osmotic solutes, Na^+ and Cl^-, in isolated walking leg muscle. Now we report that this action of OCT appears to be mediated via adenylate cyclase and intracellular cyclic AMP. Application of 10^{-5}M forskolin, a nonspecific adenylate cyclase stimulator, to isolated Limulus muscle mimics the OCT-induced ion reduction. In addition, 10^{-7}, 10^{-6}, and 10^{-5}M OCT elevates cyclic AMP in isolated muscles exposed to isoosmotic and hypoosmotic media from the basal level of 2 pmoles/mg protein to approximately 4, 9, and 15 pmoles/mg protein respectively. Finally, treatment of the muscle with the OCT receptor blocker, phentolamine, 10 min prior to and during exposure to OCT results in competitive inhibition of both the cyclic AMP elevation and the OCT-stimulated ion reductions. (Supported by NIH GM-23731 and TS&GCMBA, Inc.)

11.2

MUSCLE FIBER LENGTHS AND TENDON ARRANGEMENTS IN DORSAL AND VENTRAL MUSCLES OF THE DOLPHIN (TURSIOPS truncatus). R.R.Roy, M.A. Bello,* I. Oxman* and V.R. Edgerton. Brain Research Institute and Dept. Kinesiology, UCLA, L.A.,CA 90024.

The architecture of the dolphin axial musculature may be an important factor in this mammal's ability to swim. To examine this question, the architecture of dorsal and ventral axial muscles was studied. A frozen specimen (∿5 years old and 180 kg body weight) was thawed and fixed in 10% formalin. The muscles were removed and digested in acid (Sacks and Roy, J. Morphol., 173:185, 1982). Fiber lengths in the dorsal muscles ranged from 160-226 mm ($\bar{X}$ = 190 mm). Fiber lengths in the ventral muscles had a larger range (37-185 mm),were shorter ($\bar{X}$ = 90) and appeared to vary considerably in different regions of the same compartment. The fibers toward the caudal end were longer than those in the more cranial end in both the ventral and dorsal muscles. Angle of pinnation with respect to the tendon was ∿ 15° in most muscles. The dorsal fibers were attached to thin, long tendons in a "net-like" arrangement which eventually converged with the larger tendons near the fluke. In the ventral muscles, some fibers appeared to be arranged in series. These differences may have functional implications with respect to proposed differences in the upward and downward strokes during swimming.

(Supported by Naval Ocean System Center N-66001)

11.3

FIBER TYPE AND FIBER SIZE DISTRIBUTIONS IN THE AXIAL MUSCULATURE OF THE DOLPHIN (TURSIOPS truncatus). M.A.Bello,* R.R.Roy, I. Oxman,* T. Martin,* and V.R. Edgerton. Brain Research Institute and Dept. Kinesiology, UCLA, L.A., CA 90024.

To begin to understand the bioenergetic efficiency of the swimming dolphin, muscle fiber size and type of the axial musculature was investigated. All tissue samples were taken from a single specimen (∿5 years old and 180 kg body weight). "Fast" (F) and "slow" (S) fiber types were identified from frozen sections stained for myosin ATPase. Fiber area and type was determined using an automated image processing system. Generally, both the dorsal and ventral muscles consisted of 50% S and 50% F fibers. One dorsal muscle (extensor caudae medialis) (Strickler, Am. J. Anat. 157:49, 1980) had one region that consisted of about 70% S. The caudal end of the dorsal and ventral muscles had about 70% F fibers. Mean cross-sectional area (CSA) of the fibers in the ventral muscles was ∿65% greater than in the dorsal muscles (1750 vs 1072 μm^2). The F fibers were 40% (2200 vs 1317 μm^2) and 30% (1213 vs 879 μm^2) larger than the S fibers in the ventral and dorsal muscles, respectively. These fiber sizes are smaller than for most terrestrial mammalian muscles. The observation that the ventral muscles had larger and shorter fibers (Roy, et al., Physiologist, 1983) suggests that they are specifically designed for force production. In contrast, the dorsal muscles are designed to optimize velocity and displacement. (Supported by Naval Ocean System Center N-66001)

11.4

DOLPHIN TENDON MATRIX COMPONENTS. M. Russell*, A.C. Vailas*, V.R. Edgerton, C. Sasson*, A. Nekooi* and J. Durivage*. Univ. of California, Los Angeles 90024.

Virtually nothing is known about the important tendonous structures that are involved in the transmittance of muscular forces to the fluke of a Dolphin. Therefore the primary purpose of this study is to characterize essential matrix components in tendons of the Dolphin tail region and make comparisons to similar components in tendons from lower extremities in terrestrial mammals. Essential matrix components, collagen and proteoglycans, were evaluated from papin digests of tendons obtained from the tail region of the Dolphin and compared to Achilles tendons of rats. Also, cellularity and non-collagenous protein content were determined from the same papain digests. In contrast to rat Achilles tendons, Dolphin tail tendons were similar with respect to cellularity, and had greater concentrations of proteoglycan (31%) and non-collagenous protein (26%), but lower collagen (29%). Also, there were equal amounts of glucosamine and galactosamine containing proteoglycans. However, the ratio of glucosamine: galactosamine for rat Achilles tendons was <1, suggesting that the rat tendon contains a greater amount of galactosamine proteoglycans. We know that the amino sugars are representative of the proteoglycans in tendon and not from other sources of hexosamines. These data suggest that the lower collagen content and greater glucosamine containing proteoglycans parallel the composition of elastic tendons such as the ligamentum nuchae of terrestrial mammals. (Navy-N66001).

11.5

ANTIHYPERTROPHIC ACTIONS OF PROPRANOLOL, VERAPAMIL AND SARALASIN ON EXERCISE AND DRUG INDUCED CARDIAC ENLARGEMENT. L. Norris, K. Rouse, M.S. Holder and L.N. Cothran*, College of Pharmacy, Florida A&M University, Tallahassee, Florida 32307.

Hypertrophy was induced in male adult rats by either exercise (E) or angiotensin II (A-II). The present study was done to determine whether or not chronic pretreatment with Verapamil (V), Saralasin (S) or Propranolol (P) would prevent the development of A-II and E induced hypertrophy. Eight groups of animals were continuously infused with S, V and P for one week. Beginning the second week, one-half of the animals were infused with A-II while the others were exercised twice a day for one-half hour. At the end of the second week, animals were sacrificed, hearts were removed and body weights (BW) and ventricle weights (VW) were obtained. VW/BW ratios and their percent difference (%) from control were calculated. In the acute studies A-II was simultaneously infused with V, P or S from the onset of the experiment. Results show that acute and chronic A-II induced hypertrophy was 12.4 and 22.5% respectively. Both were blocked by S (acute = 1%, chronic = −2.7%); V reduced both chronic and acute responses down to 0% change. VW/BW after P was 5.9% for the acute and −2.7% for the chronic. Exercise induced hypertrophy was generally higher than A-II induced hypertrophy (avg. 27%). Acute exercise hypertrophy was significantly reduced by P and V but not S. However, there was still a large hypertrophy present after the blockade with either one alone. On the contrary, only V blocked chronic E, reducing it to 2%. The results suggest that E may be contractility related and is independent of changes in A-II. Furthermore, chronic A-II induced hypertrophy seems to have a significant sympathetic component. (Supported by DRR-MBRS-NHLBI 08111)

15.1

Ca^{++} AS MODULATOR OF GASTRIC SECRETION AND VESICULAR H^+ TRANSPORT. Fabián Michelangeli and Marie Christine Ruiz*
IVIC, Apdo. 1827, Caracas 1010A, Venezuela.

The involvement of Ca^{++} as second messenger in stimulus-secretion coupling has been studied in isolated amphibian mucosa (IAM) and membrane vesicles from mammalian stomachs (mv). a) Identification of Ca^{++} as messenger: Artificial increase in $[Ca^{++}]_i$ using A23187 mimic response to secretagogues (stimulation of H^+ secretion and histamine release). A23187 acts directly on oxyntic cells, but parallel action of histamine is required for full effect; b) Mechanism of $[Ca^{++}]_i$ increase: Although secretagogues release Ca_i^{++} as evidenced by chlortetracycline fluorescence and disappearance of mitochondrial deposits (EM), ACh and A23187 also require Ca^{++}_o for their action. It appears that Ca^{++} entry is required in cholinergic and A23187 stimulation. Ca_i^{++} release may be important in histamine and cAMP stimulation; c) Action of Ca^{++} at H^+ pump complex: In mv's, Ca^{++} (1-10 µM) increases KCl permeability thereby stimulating H^+/K^+ ATPase and H^+ transport. Using ANS^- as potential probe we can show that ATP increases Cl^- permeability and Ca^{++} + ATP increase K^+ permeability, suggesting two separate channels for K^+ and Cl^-. These effects may be mediated through membrane phosphorylation; d) Interaction of Ca^{++} with cAMP: Full stimulation by increases in $[Ca^{++}]_i$ require elevation of $[cAMP]_i$. In a number of instances we can show Ca^{++}-cAMP potentiation during ACh and A23187 stimulation. Intracellular interactions between Ca^{++} and cAMP during S-S coupling are responsible for triggering the HCl secretory process.

15.2

CONFORMATIONAL CHANGES IN $(K^+ + H^+)$-ATPase

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Mg^{2+} and K^+ have several effects on $(K^+ + H^+)$-ATPase. Previously we have shown that the rate of inactivation by butanedione and DTNB, agents which modify arginine and sulfhydryl groups respectively, is affected by these ions. The level of binding of the ATP analog is also affected by Mg^{2+} and K^+. The ATP/ADP exchange activity of the enzyme can be stimulated by K^+. This stimulatory effect can be antagonized by Mg^{2+}. The stimulatory effect of K^+ on this reaction increases with the pH, which can be interpreted as a K^+/H^+ antagonism. The dye eosin, which is a competitive inhibitor of $(K^+ + H^+)$-ATPase (K_I= 5 µM), binds to the enzyme. This leads to an enhancement of eosin fluorescence. The fluorescence can be further enhanced by Mg^{2+}, while K^+ and ATP lower the fluorescence level. At low pH, more Mg^{2+} is needed to increase fluorescence.

These findings suggest a Mg^{2+}/K^+ and a Mg^{2+}/H^+ antagonism. This indicates that ions which are transported by the enzyme and also Mg^{2+} induce specific conformational states of the enzyme.

15.3

INHIBITION OF GASTRIC AND SECRETION BY OMEPRAZOLE, STUDIES ON ITS MECHANISM OF ACTION. Björn Wallmark*, Britt-Marie Jaresten*, Pia Lorentzon* and Arne Brändström*. (SPON: John G. Forte).
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The inhibitory action of the benzimidazole, omeprazole (OME) was investigated in two in vitro models: isolated rabbit gastric glands and purified hog gastric H^+,K^+-ATPase. In the glandular system OME inhibited basal and stimulated acid formation. Inclusion of mercapto compounds, such as ß-mercaptoethanol (ß), to the glandular incubates, prevented inhibition by OME. This protective effect appeared specific for benzimidazoles, since no protection of the inhibitory action of SCN^- or cimetidine was observed. Also when inhibition by OME was induced before addition of ß, the inhibition was reversed. The H^+,K^+-ATPase was found to be inhibited in a pH-dependent manner by OME, the inhibition being accelerated at pH below 7.4. Inclusion of ß prevented inhibition of the ATPase activity. Binding studies showed a saturable incorporation of the radiolabel into the H^+,K^+-ATPase. The binding of ^{14}C-OME was prevented by the addition of ß. When ß was added to the preformed enzyme-inhibitor complex the ATPase activity was restered and the ^{14}C-radiolabel was released. Titration of sulfhydryl groups (SH) in the preparation showed modification of one (SH) for each inhibitor molecule bound. In the two preparations used, ß could both protect and reverse the inhibition indicating similar inhibitory mechanism for OME. Thus, (SH) in the H^+,K^+-ATPase appears essential for its ATPase activity, since modification of (SH) by OME leads to deactivation.

15.4

LIGHT DEPENDENT LABELLING OF THE ACTIVE SITE OF GASTRIC ATPase WITH 8-AZIDO ATP. G. Saccomani*, L. Cole* and E. Mukidjam*. (Sponsor J.G. Forte). Univ. Alabama in Birmingham, B'ham, AL

Photoaffinity labelling of purified hog gastric $(H^+ + K^+)$ATPase with (^{3}H) or (α-^{32}P) 8-azido ATP resulted in 1.4-1.6 nmol/mg prot. of specifically bound reagent. Half-maximal incorporation of the label (promoted by Mg^{++}) at pH 7.4 and at 24°C was obtained at a concentration of 30 µM. The amount of 8-azido ATP bound was decreased about 80% when excess of ATP (or ADP) was added to the sample prior photolysis, but an equal excess of AMP caused no decrease. Photoinduced binding of 8-azido ATP irreversibly inhibited 40-50% of the K^+ATPase activity as well as the steady-state level of E-P formed by 5 µM (γ-^{32}P)ATP. In the absence of photolysis, 8-azido ATP could be utilized as substrate for the gastric enzyme with an E-P level formed by 80µM (γ-^{32}P) 8-azido ATP, approaching 3.0 nmol/mg prot. A combination of IEF and 2-D gels of the enzyme phosphorylated by (γ-^{32}P) 8-azido ATP showed that 80% of the protein applied was separated into 5 major subunits of pI ranging between 6.3 and 5.5, all with the same M.W. of 100 Kd and containing ^{32}P label as detected by autoradiography. When the enzyme was photolyzed in the presence of (α-^{32}P) 8-azido ATP the label was found only in the peptides region of pI 5.9-5.5 while phosphorylation of the enzyme following 8-azido ATP binding showed labelling to occur in the region of pI 6.3-5.9. These results suggest that there many be 2 classes of ATP-binding sites on subunits differing in pI and that catalytically, one site on the enzyme can phosphorylate while the other binds ATP. (NIH,NATO support).

15.5

DISTRIBUTION OF MICROFILAMENT-RELATED PROTEIN IN THE GASTRIC EPITHELIUM AND ISOLATED MEMBRANE FRACTIONS. T.M. Forte*, J.M. Wolosin* and J.G. Forte, Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Microfilaments have been implicated in membrane transformations that accompany the secretory cycle of the oxyntic cell. Whole mucosal scrapings (rabbit, pig) contained 29-36 µg actin/mg prot., and isolated gastric glands (rabbit) contained 42 µg/mg prot. This actin was predominantly (60%) in the f-actin form. Cytochemistry of f-actin with fluorescent-labeled phallicidin showed: filamentous staining in resting oxyntic cells, consistent with the tortuous canaliculi; broadly stained tracts in stimulated cells, consistent with apical microvillar elaboration; minimal staining in chief cells. The $(H^+ + K^+)$-ATPase of resting tissue, associated with the microsomes, was devoid of actin. In secreting tissue, much of the $(H^+ + K^+)$-ATPase redistributes to heavier membrane fractions, called stimulation-associated membranes (s.a.m.). Actin content and $(H^+ + K^+)$-ATPase activity were correlated in the purification of s.a.m., with values of about 90 µg actin/mg prot. Partial delipidation of s.a.m. produced ghost structures revealing filamentous actin. SDS gels of residual "ghost" protein showed abundant actin and several other bands that may belong to class of actin-binding/regulating proteins. We conclude: oxyntic cells are rich in actin; association of f-actin with s.a.m. supports our view of their origin from the apical membrane of the stimulated cell; and membrane redistribution a la the recycling hypothesis. (Support by USPHS #AM10141.)

15.6

MICROTUBULAR FUNCTION IN SECRETION OF H^+ AND PEPSINOGEN BY GASTRIC MUCOSA. Dinkar K. Kasbekar, Dept. of Physiology, Georgetown Univ., Washington, D.C. 20007.

Previous studies have shown that colchicine (10 mM) and vinblastine (1 mM) abolish H^+ and pepsinogen secretion in the in vitro frog gastric mucosal preparations. Because of the possible nonspecific effects of these agents at relatively high concentrations, we have attempted to characterize the distribution of microtubules in oxyntic and chief cells of the rabbit gastric fundic glands in their resting and stimulated states. The dispersed glands were incubated under appropriate conditions with a) no additions, b) 100 µM histamine to stimulate H^+ secretion, c) 10 nM cholecystokinin octapeptide (CCK-8) to stimulate pepsinogen secretion, and d) 1 mM burimamide, an H-2 antagonist and 1 mM dibutyryl cyclic GMP, a CCK antagonist, to obtain the resting states of oxyntic and chief cells respectively. Transmission electron microscopy on the triton-solubilized glands indicates an abundance of microtubules in close association with the mitochondria in histamine-stimulated gland oxyntic cells relative to those in the burimamide treated resting glands. The microtubule content of the chief cells in the resting and stimulated glands is virtually undetectable. These findings can be confirmed by indirect immunofluorescence studies with FITC conjugated antitubulin antibody treated gastric glands. The implications of these observations vis a vis the ultrastructural changes associated with secretory events will be discussed. NSF support.

15.7

DISTRIBUTION AND REGULATION OF Na^+/H^+ ANTIPORTERS IN RENAL MEMBRANE POPULATIONS. David G. Warnock, Harlan E. Ives*, Victoria J. Yee*, and Austen K. Mircheff. Dept. of Med., SFVAMC and UCSF, San Francisco, CA 94143 and Dept. Physiol. and Biophys., USC, Los Angeles, CA 90033

Na^+/H^+ exchange appears to play a central role in the vectorial transport of H^+ in the mammalian proximal tubule. We examined the subcellular distribution of Na^+/H^+ antiporters using the acridine orange assay in fractions obtained from sucrose density gradient fractionation of membranes from the rabbit renal cortex. Na^+/H^+ antiporter activity was confined to membranes co-migrating with brush border membranes and was absent from basolateral membranes. Further fractionation of brush border membranes by counter current distribution with polyethylene glycol:dextran revealed 2 distinct peaks of Na^+/H^+ antiporter activity - one associated with maltase activity; the other associated with acid phosphatase activity. Kinetic studies of Na^+/H^+ antiporter activity in brush border membranes revealed non-competitive inhibition by amiloride and Li^+ at an external modifier site distinct from the transport site.
Conclusions: 1) Vectorial H^+ transport in the proximal tubule results from the polar distribution of Na^+/H^+ antiporters in the cell. 2) Na^+/H^+ antiporter activity appears to be associated with intracellular membranes, in addition to brush border membranes, raising the possibility that total antiporter activity could be regulated by membrane recycling. 3) The Na^+/H^+ antiporter has at least one modifier site, which could be a site for physiological regulation. (Supported by NIH grants AM 07219, AM 28408, AM 19407, AM 0068).

15.8

Na^+-H^+ EXCHANGE IN ISOLATED RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV): REGULATION BY METABOLIC ACIDOSIS AND GLUCOCORTICOIDS. J. L. Kinsella* and B. Sacktor, NIA/NIH, Gerontology Research Center, Baltimore, MD 21224.

Previously, we reported that glucocorticoids increase amiloride sensitive Na^+-H^+ exchange activity in BBMV (PNAS 79, 4932, 1982). The present study examines the effect of metabolic acidosis and the role of glucocorticoids on Na^+-H^+ exchange activity. The Na^+ uptakes (5 s) in the presence of a pH gradient (pH_i=5.5, pH_o=7.5) were significantly greater (3.28 ± 0.20 vs 2.36 ± 0.21 nmol/mg) into BBMV isolated from acidotic compared to control animals. Amiloride sensitive Na^+ uptakes (2 s) were determined at different $[Na^+]$. Acidosis increased V_{max} from 11.3 ± 0.9 to 15.3 ± 0.7 nmol/mg/ 2 s without affecting the app K_{Na} (10.2 ± 0.5 vs 10.2 ± 0.6 mM). The rates of pH gradient generation were measured when Na^+ loaded vesicles (pH=7.5) were diluted into Na^+ free medium (pH=7.5) by monitoring acridine orange absorbance at 492 relative to 600 nm. BBMV from acidotic animals generated pH gradients more rapidly than BBMV from control animals. Adrenalectomy of acidotic rats reduced Na^+ uptakes (5 s) to that found in control rats (2.40 ± 0.32 nmol/mg). If the adrenalectomized acidotic rats were given dexamethasone (30 µg/100 g b.w.) 16 and 24 hr prior to BBMV isolation, Na^+ uptakes (5 s) were increased to levels found in the acidotic rats (3.58 ± 0.19 nmol/mg). We conclude: 1) metabolic acidosis increases Na^+-H^+ exchange activity by increasing V_{max} and 2) the increase is dependent upon an intact adrenal gland or glucocorticoid supplements.

15.9

RENAL FAILURE, METABOLIC ACIDOSIS AND PARATHYROIDECTOMY INCREASE NA^+-H^+ EXCHANGE IN ISOLATED RENAL BRUSH BORDER MEMBRANE VESICLES. M.R. Hammerman, S. Klahr, and D.E. Cohn* Washington Univ. School of Medicine, St. Louis, MO 63110

We have recently identified an amiloride inhibitable Na^+-H^+ exchanger in canine renal brush border membrane vesicles (BBMV). The activity of this exchanger was found to be enhanced in BBMV prepared from the remnant kidneys of dogs with chronic renal failure (CRF). Na^+-H^+ exchange in BBMV increased progressively as plasma creatinine increased. In order to ascertain whether changes in H^+ excretion by the kidney observed in chronic metabolic acidosis and in states of altered parathyroid function might result from altered Na^+-H^+ exchange across the renal cortical cellular brush border membrane, we measured Na^+-H^+ exchange in BBMV from kidneys of dogs with chronic metabolic acidosis and from kidneys of thyroparathyroidectomized dogs. Increased initial rates (20s) of amiloride sensitive 1mM $^{22}Na^+$ uptake measured under H^+ gradient conditions (intravesicular pH < extravesicular pH) were demonstrated in BBMV from kidneys of both acidotic (1.54 ± .18 nmol/mg protein) and hypoparathyroid (1.78 ± .20 nmol/mg protein) compared to normal (0.96 ± .03 nmol/mg protein) dogs ($p < 0.05$, both experimental groups > normal). These findings suggested that adaptations in H^+ excretion in chronic metabolic acidosis and hypoparathyroidism might be explained by increased activity of a renal brush border membrane Na^+-H^+ exchanger. The adaptation in CRF may result from the need to excrete more H^+ per nephron. (Supported by NIH R01AM27600).

15.10

PROTON TRANSLOCATING ATPASES FROM BOVINE CLATHRIN-COATED VESICLE AND RENAL MEDULLA. Dennis K. Stone*, Xiao-Song Xie*, and Efraim Racker*. (Spon: Juha P. Kokko) Cornell University, Ithaca, NY 14853.

Clathrin-coated vesicles harvested from bovine brain catalyzed ATP-driven proton translocation, as measured by acridine orange (A.O.) quenching and $^{32}P_i$-ATP exchange. Both activities were insensitive to oligomycin (5 µg/mg protein) and were inhibited by N-ethylmaleimide (NEM) 1 mM. The preparation was devoid of the lysosomal marker 5' nucleotidase (pH 5.0), and ATP-driven A.O. quenching was precipitated by monoclonal anti-clathrin antibody. Chloride and bromide, but not fluoride, sulfate, phosphate, or gluconate, were effective in counterbalancing electrogenic proton pumping. ^{36}Cl uptake, driven by a K gradient $[K]_{out}/[K]_{in}$ = [40 mM]/[0 mM] in the presence of valinomycin, was completely inhibited by DIDS (1x 10^{-5} M) and duramycin (1 µg/10 µg protein). Kidney vesicles, prepared by differential centrifugation and passage through a sucrose gradient, were enriched in oligomycin-insensitive ATP-generated A.O. quenching and were devoid of 5' nucleotidase activity (pH 5.0). The kidney proton pump is not distinguishable from the brain-coated vesicle proton pump with respect to inhibitor sensitivity, substrate dependency, cofactor requirement, or dependence upon chloride or bromide as effective counterions. Moreover, the kidney vesicles catalyze $^{32}P_i$-ATP exchange and contain a DIDS and duramycin sensitive ^{36}Cl transporter, which is responsible for counter-ion movement.

15.11

A BIOCHEMICAL EVALUATION OF RABBIT RENAL DCCD-SENSITIVE ATPase ACTIVITIES. Diana Marver . UTHSCD, Dallas, Tx. 75235.

Microperfusion studies with isolated medullary collecting ducts have shown that aldosterone enhances tubule HCO_3 reabs.-H^+ secr. To evaluate steroid effects on absolute levels of a putative plasma membrane H^+ ATPase along the nephron, an ultramicro assay has been established, using ± DCCD+oligomycin+ouabain+tubule in the initial reaction. However since DCCD titrates several H^+-translocating enzymes with varying physiological functions, initial studies will attempt to correlate the apparent act. with function in a given segment. Thus the putative H^+-ATPase activities will be compared with resident NaK ATPase, carbonic anhydrase, acid phosphatase, G6PDH and NEM-sens. ATPase, as well as monitored for steroid-dependence. To initiate these experiments, assays were performed on plasma-/lysosomal membrane fractions from cortex, outer and inner medulla (C,OM,IM) from 6 normal rabbits. DCCD-sens. ATPase act.(pH 6.8, 37°C) was 0.135±0.018, 0.496± 0.096 and 0.770± 0.288 U/mg prot. in C, OM and IM, resp., or 46, 62 and 34% of the total oligomycin+ouabain (+ vanadate)-insens. act. In contrast, under the conditions of the assay, NEM inhibited 29, 9 and 24 % of the total ATPase act. Thus a major discrepancy appeared in the relative NEM:DCCD sens. in OM, suggesting some variation in either the aff. of the probe or the number of sites titrated in each case. Of note was that NEM-sens. act. in OM was equivalent at both 100 and 500 µM conc. Studies are currently underway to evaluate these differences at the isolated nephron level (Supported by AM 14677).

16.1

RENAL AND LOWER INTESTINAL CONTRIBUTIONS TO WATER ECONOMY IN WILD DESERT-DWELLING PARTRIDGES. Berry Pinshow, A. Allan Degen* & David H. Thomas*. Blaustein Inst. Desert Res., Ben-Gurion Univ., Sede Boqer Campus 84990, Israel.

Osmotic and ionic concentrations were measured in plasma (P), ureteral urine (U), and rectal luminal (R) and voided (V) fluids of Sand Partridges and Chukars to determine contributions of their kidneys and lower intestines to water economy. In summer, after 2-3.5 days water deprivation of birds held outdoors, P, U, R and V osmolalities increased, as did Na^+ and Cl^-. K^+ remained unchanged, while body masses decreased. Wild birds had most fluid compositions between those of watered and water-deprived captive birds. Exceptions were Na^+ and Cl^- of U and V which were usually much higher in wild birds. These measurements indicated that in the wild, neither species was short of water and that their diet was high in NaCl. Osmolalities of P and U were higher in wild Sand Partridges than in wild Chukars, suggesting that they may endure longer periods of water deprivation between drinks than Chukars. P and U concentrations in wild Sand Partridges were similar to captive water-deprived ones, whereas in wild Chukars, these concentrations were similar to captive watered ones. Highest U/P osmolality ratios (1.8-1.9) were found in water-deprived Sand Partridges. Results indicated that the lower intestine modifies U slightly when the birds are concenrating their urine and have a diet containing more than adequate salt, as in wild birds. However, there was modification of U in captive watered birds; osmolality, Na^+ and Cl^- were lower in V than in U.

16.2

SEASONAL WATER AND ELECTROLYTE BALANCE IN FREE LIVING FAT SAND RATS. A.A. Degen*, B. Pinshow & M. Ilan*. Blaustein Institute for Desert Research, Ben-Gurion University, 84990 Sede Boqer Campus, Israel.

Fat sand rats (Psammomys obesus) are large (200 g), diurnal, desert rodents that feed entirely on halophytic plants (family Chenopodiaceae). We measured tritiated water fluxes, and plasma and urine osmolalities (P_{osm}, U_{osm}) and concentrations of Na^+, Cl^- and K^+ (P_{Na}, P_{Cl}, P_K, U_{Na}, U_{Cl}, U_K) in free-living sand rats to determine their water and electrolyte balances. Osmolalities and ion concentrations of food and feces were also measured. Mean adult water fluxes were significantly different among seasons (0.27, 0.43 and 0.18 ml H_2O/g day in winter, spring and summer, respectively). Mean P_{osm} and U_{osm} were 344 and 2119 mOsm/kg, respectively, and were not different between winter and spring, as were not P_{Na}, U_{Na}, P_{Cl} and U_{Cl} (145, 750, 118 and 775 mmol/l, respectively). However, U_K increased in spring (207mmol/l vs 163 in winter). Maximum urine osmolality in winter and spring was over 3000 mOsm/kg and fecal water content was 43%; no summer measurements were made. Using mean values for osmolalities and ion concentrations, sand rats require about 0.2 ml H_2O/g day for ion excretion which exceeds mean summer water flux. Assuming maximal U_{osm} and 43% fecal water content in summer, sand rats would require about 0.15 ml H_2O/g day for ion excretion. Supported by a Bat-Sheva de Rothschild grant to B.P.

16.3

REDUCED CALCIUM AND INCREASED CHLORINE INTAKE RAISES PLASMA CHOLESTEROL. B. H. Douglas, N. W. Revis*, L. J. Tillman*, P. T. McCauley, Jr.* and R. J. Bull*. Univ. Miss. Med. Ctr., Jackson, MS 39216 and Oak Ridge Nat. Lab., Oak Ridge, TN 37830

Five groups of rats (10 each group) and three groups of rabbits (5 each group) were used to study the interaction of Ca and Cl on plasma cholesterol levels. The rats were given standard lab chow and received 0, 1, or 10 ppm Cl in the drinking water for a period of 7 weeks. Half of the rats received water which was chlorinated with NaOCl. The others received chlorinated water obtained from the Jackson, Mississippi Water Treatment Plant. Plasma cholesterol levels were 63 ± 3 mg% in the control group. After 7 weeks of Cl ingestion, the plasma cholesterol level was higher ($P<0.05$) in the animals which received 10 ppm Cl. There was a 14% increase in the plasma cholesterol level in the group which received NaOCl and a 12% increase in the group which received chlorinated water from the water treatment plant. The rabbits received a diet containing 80% of the minimum daily requirement of Ca. Groups I, II and III received 0, 0.1 and 15 ppm Cl respectively. After 2 months treatment plasma cholesterol levels were 48 ± 18 mg% (Group I), 61 ± 34 mg% (Group II) and 87 ± 29 mg% (Group III). The studies demonstrate that reducing the calcium intake increases the hypercholesterolemic effect of chlorinated drinking water.

16.4

CIRCULATING ALDOSTERONE TITERS IN ELECTROLYTE-DEPLETED AND NaCl-LOADED AMBYSTOMA TIGRINUM LARVAE.
Daniel F. Stiffler and Peter B. Hanson*. California State Polytechnic Univ., Pomona, CA 91768.

Larval A. tigrinum were maintained in either distilled water (changed daily) or 150 mM NaCl for two weeks. At the end of this period, plasma samples were analysed for $[Na^+]$, $[K^+]$, [Inulin] (injected the previous day) and aldosterone titer (RIA). Urine was analysed for $[Na^+]$, $[K^+]$ and [Inulin]. Fractional tubular reabsorption was estimated from electrolyte and inulin urine: plasma ratios. Plasma $[Na^+]$ decreased from 104 in control larvae (CL) to 76 mM in depleted larvae (DL) and increased to 127 mM in salt-loaded larvae (SL). Plasma $[K^+]$ was constant at 5 mM in all groups. Urine $[Na^+]$ was 8 mM in CL, 2 mM in DL, and 133 mM in SL. Urine $[K^+]$ was 1.3 mM in CL, 1.6 mM in SL and 0.4 mM in DL. Fractional Na^+ reabsorption was 94% in CL, 99% in DL and 75% in SL. Fractional K^+ reabsorption was 74% in CL, 95% in SL and 96% in DL. Plasma aldosterone titers increased from 161 pg/ml in CL to 1,009 pg/ml in DL and decreased to 101 pg/ml in SL. These data are consistent with the possibility of a physiological role for aldosterone in Na^+ and perhaps K^+ homeostasis in this species. This project was supported in part by NSF grant SPI 8026274.

16.5

SEA URCHIN LARVAE LACK APPARENT REGULATORY MECHANISMS FOR AMINO ACID TRANSPORT.
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Bacteria-free larvae of Strongylocentrotus purpuratus remove neutral free amino acids (FAA) form very dilute solution. At submicromolar concentrations, influx of radio-labeled FAA and net removal of substrate, determined by high performance liquid chromatography (HPLC), occur at the same rate. In the present study, larvae were exposed to unnaturally high concentrations of serine (50-1000 μM) to obtain further evidence of net entry by following changes in internal serine concentration. It was also of interest to determine the response of the FAA transport system to experimental manipulation of the internal FAA pool. Exposure of larvae to 50 or 250 μM serine for four hours produces a 14-22 fold increase in internal serine concentration. More prolonged exposure leads to leakage of neutral FAA to the medium. Although the entry rate of labeled serine appears to decrease, correcton of the data to take account of the increasing ambient concentration produced by this leakage indicates there is no compensatory change in influx kinetics. No leakage of acidic or basic amino acids is observed. Supported in part by NSF grant PMC 82-08185 and DOC grant NOAA 04-8-M01-89.

16.6

BRAIN TEMPERATURE REGULATION IN HEAT STRESSED, WATER DEPRIVED DESERT PHASIANIDS. S. Kleinhaus*, B. Pinshow & M.H. Bernstein, Blaustein Institute for Desert Research, Ben-Gurion Univ., Sede Boqer Campus 84990, Israel.

Brain temperature regulation in two desert phasianids, the chukar (wide spread in mesic and desert areas) and the sand partridge (endemic to the desert) was compared by measuring core and hypothalamic temperatures (T_c, T_h), and breathing and panting frequencies (f_b, f_p) in watered (W) and water-deprived (WD) birds exposed to ambient temperatures (T_a) from 28 to 45°C. T_h and T_c increased with increasing T_a in W and WD birds of both species. No differences were found between their abilities to maintain constant T_c - T_h in the above range of T_a. At T_a > 38°C, the slope values of the regression lines relating T_h to T_b were lower for WD than for W birds of both species. In general, f_b and f_p were lower for WD than for W birds in both species. Onset of panting occurs at higher T_h and T_b in WD than in W birds. There is apparently no difference in the brain temperature regulation ability of the two species. Results suggest changes in cephalic circulation leading to increased brain cooling efficiency by the Rete Mirabile Ophthalmicum in WD birds. Supported by NSF grant PCM 79-21856 and U.S.-Israel BSF grant 2496/81.

16.7

ENERGETICS OF THE HIGH ARCTIC SPITZBERGEN PTARMIGAN
A. Mortensen * and A.S. Blix. Dept. of Arctic Biology,Univ. of Tromsø, 9000 Tromsø, Norway.

The winter at the high arctic archipelago of Svalbard (79 - 81°NL) is characterized by low ambient temperatures, three months of darkness and poor food quality and availability. The Spitzbergen ptarmigan, a native galliform at this location, accumulates large amounts of fat during late summer and fall. The fat stores enables the bird to survive periods of acute starvation during midwinter, and also give a minor contribution to the daily energy consumption untill March, when the fat stores are exhausted. In the period of fat combustion the food intake is voluntarely reduced, but this partial anorexia is accompanied by a reduction in daily energy expenditure, which for a period approaches the resting metabolic rate. During winter resting metabolic rate is reduced by about 10% and insulation increased by about 30% compared to summer, leaving the lower critical temperature unchanged (-5°C). Fasting of birds in the fall leads to a small (7%) decrease in resting metabolic rate whereas no effect is seen in mid-winter. In contrast to the normal response daily energy expenditure is not increased by fasting. Thus, the low energy requirement, combined with the emergency depots of fat makes this bird especially well prepared to cope with reduced food availability in midwinter. This adaptation is certainly of great survival value for a bird living in an area where episodes of starvation is a common experience during the prolonged winter night.

16.8

BRAIN COOLING IN DEHYDRATED HEAT EXPOSED FOWLS. **Z. Arad* and U. Midtgård*. (SPON: M. H. Bernstein). Univ. of Copenhagen, Denmark.**

Body (T_b) and hypothalamic (T_h) temperatures were measured in heat exposed fowls during normal hydration (NH) and dehydration (D). The body-to-brain temperature difference ($\Delta T = T_b - T_h$) decreased from 0.68 ± 0.38°C (mean ± SD) during NH to 0.35 ± 0.27°C during D (T_a = 26-42°C, $p < 0.001$). The slope of the regression line relating T_h to T_b during NH (0.79) was significantly lower than unity ($p < 0.001$) indicating increased brain cooling with increasing T_b. The slope during D (0.96) was significantly higher ($p < 0.02$) and did not differ from unity. The dehydrated fowls were characterized by significantly higher plasma osmolality and sodium and chloride concentrations ($p < 0.001$). The ΔT was significantly correlated ($p < 0.02$) with the heat exchange area of the rete ophthalmicum. It is suggested that a decreased heat loss during dehydration resulted in a decreased heat exchange in the retia ophthalmica and consequently in higher brain temperatures and lower ΔT. The thermoregulatory and osmoregulatory responses during dehydration are probably interrelated to maintain water and thermal homeostasis. **(Supported by a Danish-Israeli cultural exchange grant and by NSF grant PCM-8118956).**

16.9

METABOLIC ADAPTATION TO LONG TERM FASTING IN MALE EMPEROR PENGUINS AND IN KING PENGUIN CHICKS. Jean-Patrice Robin, Yves Cherel, René Groscolas and Yvon Le Maho. Laboratoire de Physiologie Respiratoire, Centre National de la Recherche Scientifique, 67087 Strasbourg & Laboratoire de Physiologie Animale et de la Nutrition, 21004 Dijon, France.

Male emperor penguins, which assume the incubation of eggs, starve for 4 months during the antarctic winter. Three periods have been characterized from the changes in their rate of decrease in body mass per unit of body mass (dm/m) during the course of this fast. dm/m decreases rapidly in period I (4-5 days), tends to remain constant during period II (80-100 days) and increases dramatically in period III. Emperors usually leave their colony at the beginning of period III to feed at sea. Analysis of body composition indicated that period II is a period of protein sparing and preferential utilization of lipids. Period III is critical in that proteins are no longer spared, lipids being depleted. Changes in the plasma level of uric acid (a good index of protein catabolism in birds) were found to remarkably parallel the changes in dm/m. Thus, period I is an adaptation period marked by a decrease in protein utilization and changes in dm/m may be considered as good indicators of protein mobilization. We found that king penguin chicks, which may be temporarily abandoned by their parents during the subantarctic winter, may still be vigourous after 4-6 months of starvation. Three periods were also characterized from their changes in dm/m, suggesting that their adaptation to starvation is similar to that observed in emperors.

REPRODUCTION

17.1

ALTERED FOLLICULAR STEROIDOGENESIS IN THE PREGNANT HAMSTER AND RAT. G. S. Greenwald. Univ Kansas Med Ctr, Kansas City, KS

Five Graafian follicles were dissected at 0900h from proestrous and pregnant hamsters (Day 1 = sperm positive). The follicles were incubated for 1h in medium 199 (h1) the medium then replaced with 100 ng ovine LH and the follicles incubated for another h (h2). The medium was assayed for: progesterone (P_4), 17 hydroxyprogesterone (17OHP), androstenedione (A) and estradiol (E_2). For the proestrous follicle, during h1, E_2 accumulated at about 2X the level as follicles from pregnant animals except on Day 8 when the values were about the same as for proestrus & on Days 12 & 16 when E_2 was usually undetectable. Addition of 100 ng LH showed that the proestrous follicle was considerably more active in E_2 production than any of the follicles of pregnancy with maximal stimulation on Day 8 and minimal responsiveness on Day 16. Androstenedione secretion by the proestrous and pregnant follicle closely paralleled the profile of E_2. During h1 the proestrous follicle accumulated much less P_4 than E_2 in comparison to the situation during pregnancy. When stimulated by LH, maximal follicular production of P_4 was on Day 16 of pregnancy when conversion to E_2 and A was minimal. Just the reverse relationship exisfed on Day 8. Although the pathway from C-21 to C-18 steroids was "open" at all times for the hamster follicle, the rate at which P_4 was converted to E_2 varied at different stages of gestation. In contrast, the 8 Day rat follicle after exposure to LH synthesized 8 ng P_4 but the pathway was blocked beyond the production of C-21 steroids.

17.2

INVESTIGATION OF GONADOTROPIN RECEPTORS IN HUMAN OVARIAN CANCERS. R.L. Stouffer, E.A. Surwit*, and M.S. Grodin*. University of Arizona, Tucson, AZ 85724

The ovary is a target organ for gonadotropins and contains receptors for follicle stimulating hormone (FSH) and for luteinizing hormone (LH)/chorionic gonadotropin (CG). To establish whether ovarian cancers are targets for gonadotropins, we analyzed ovarian tumor biopsies from 18 women for the presence of specific FSH and LH/CG binding sites. Various concentrations of 20000g particulates (2.5-10 mg tissue equiv.) were incubated for 20hr at 25C with 10ng ^{125}I-human (h)FSH and -hCG; selected tumors were also incubated with increasing amounts (0.25-50 ng) ^{125}I-hFSH and -hCG. Ovarian tumors of epithelial origin (n=17) displayed low levels of apparent specific FSH and CG binding, which were not dependent on tissue concentration and were not saturable. In contrast, specific FSH binding to a granulosa cell-theca cell (GC-TC) tumor was directly proportional to tissue concentration and demonstrated saturation in the presence of >10 ng ^{125}I-FSH. Scatchard analysis of equilibrium binding data resulted in a linear plot. The binding capacity (23.3 fmol/mg) of the tumor exceeded that of ovaries from estrogen-primed rats (5.2 fmol/mg). The dissociation constant (Kd; 1.36 x 10-9M) was similar to that for FSH binding to rat ovary (0.63 x 10-9M). Specific hCG binding sites were not detected in the GC-TC tumor. We conclude that the GC-TC tumor contained receptors for FSH, but not for LH/CG. However, more common ovarian tumors of epithelial origin are not gonadotropin target tissues. NIH CA33336

17.3

ETHANOL MODULATES THE GONADOTROPIN (LH/CG) RECEPTOR-ADENYLATE CYCLASE SYSTEM IN RAT CORPORA LUTEA (CL). D. Danforth*, K. Eyster*, and R. Stouffer. Univ. of Arizona, Tucson, AZ 85724

In vitro exposure to ethanol (EtOH) unmasks LH binding sites in luteal tissue of the monkey (Endo. 110: 1451, 1982). To examine the effect of EtOH on the gonadotropin receptor-adenylate cyclase system in corpora lutea of psuedopregnant rats, 20000g ovarian particulates were incubated with ^{125}I-hLH or α^{32}P-ATP in the presence of 0-20% EtOH. Specific LH binding and cAMP production were examined. Conditions which enhanced LH binding to monkey luteal particulates (1-12% EtOH, 25C for 20h; 4-8% EtOH, 37C for 2h) failed to increase LH binding to rat luteal particulates. In kinetic studies, EtOH did not increase LH uptake above steady-state levels at any time during incubation. Indeed, exposure to 8% EtOH at 37C for >30 min reduced LH uptake relative to control levels. In contrast, EtOH increased basal adenylate cyclase (AC) activity in a dose-dependent manner; 8% EtOH increased cAMP production 3-fold (14.8±3.7 vs 5.3±2.1 pmol cAMP/10 min at 37C, $\bar{x}$±SE, $p<0.05$, n=3). Whereas gonadotropin (hCG) alone stimulated AC activity 5-fold, addition of EtOH decreased relative CG stimulation in a dose-dependent fashion. The presence of 8% EtOH abolished the stimulation of AC by CG. In conclusion, EtOH does not unmask LH binding sites in corpora lutea of psuedopregnant rats. However, EtOH stimulates AC activity and uncouples gonadotropin activation of AC in rat luteal membranes. These findings suggest that the gonadotropin receptor system and/or membrane characteristics differ between the primate and rodent CL.

17.4

THE ADENYLATE CYCLASE (AC) SYSTEM OF THE PRIMATE CORPUS LUTEUM: RESPONSIVE TO GONADOTROPINS BUT NOT TO CATECHOLAMINES. K. M. Eyster* and R. L. Stouffer. Univ. of Arizona, Tucson, AZ 85724

AC activity in corpora lutea (CL) of rats and rabbits is stimulated by the catecholamines, epinephrine (EPI) and isoproterenol (ISO), as well as by the gonadotropins, luteinizing hormone (LH) and chorionic gonadotropin (CG). To determine whether the AC system of primate CL exhibits similar responsiveness, the conversion of α32P-ATP to 32P-cAMP by homogenates of luteal tissue from rhesus monkeys was assessed in the presence of various hormones. CL (n=8) were obtained at mid-luteal phase of the menstrual cycle. Human (h)CG and hLH, but not deglycosylated hCG (dCG; 70% of carbohydrate removed), stimulated cAMP production in a dose-dependent manner; maximal stimulation (2.3x control) occurred at 3.75 µg LH or CG (per ml). Whereas 10 µg CG increased cAMP production compared to control (3.8±0.2 vs 1.4±0.1 pmol cAMP/10 min/mg tissue), the presence of 10 µg dCG blocked CG stimulation of AC (1.2±0.1 pmol; $p<.05$). Although EPI and ISO enhanced cAMP production in a dose-dependent manner in rat luteal homogenates (14x control), the catecholamines did not alter AC activity in macaque CL. EPI (50µg) did not stimulate cAMP production (1.7±0.1 vs 1.5±0.1) nor alter CG stimulation of AC (3.3±0.2 vs 3.6±0.2, $p>.05$). In conclusion, (1) the AC system of the primate CL is responsive to LH-like gonadotropins, but, unlike the rodent, is insensitive to catecholamines, and (2) hCG devoid of normal amounts of carbohydrate is a gonadotropin antagonist in the primate CL. NIH HD12333

17.5

AVAILABLE RECEPTORS FOR CHORIONIC GONADOTROPIN (CG) IN THE PRIMATE CORPUS LUTEUM (CL) DURING SIMULATED EARLY PREGNANCY. J.S. Ottobre*, A.C. Ottobre* and R.L. Stouffer. Univ. of Arizona, Tucson, AZ 85724

Stimulation of the primate CL by endogenous CG in early pregnancy, or by exogenous human (h)CG (APL, Ayerst Labs) in simulated early pregnancy, is *transient*, despite continued exposure to rising concentrations of CG. To determine if this ephemeral response is associated with changes in available CG receptors, CL were removed from rhesus monkeys during simulated early pregnancy, and the number and affinity of ^{125}I-hCG binding sites were estimated by Scatchard analyses. Whereas peripheral venous concentrations of progesterone increased within 9hr of CG treatment and remained elevated for 3 days, the binding capacity of CG during this interval (9.8±1.2 fmol/mg tissue, $\bar{x}$±SE, n=14) was comparable to that just prior to treatment (9.4±1.1, n=5). However, CG binding capacity declined ($p<0.05$) after 6 days (2.4±0.5, n=3) and 10 days (1.7±0.8, n=3) of treatment, concomitant with declines in circulating progesterone and CL weight. The dissociation constant (Kd) for CG-binding was greater after 6 days of treatment (4.7±0.9 M x 10^{-10} n=3, $p<0.05$) than prior to treatment (1.1±0.2, n=5), reflecting a decrease in receptor affinity. In conclusion, the macaque CL maintains a constant population of available CG receptors amidst dramatic stimulation of luteal function during early CG exposure. The subsequent diminution of number and affinity of available CG receptors during prolonged exposure to CG may compromise CL function. (HD 12333).

17.6

ENDOMETRIAL PROSTAGLANDIN $F_{2\alpha}$ SYNTHESIZING CAPABILITY IN MARES WITH SPONTANEOUSLY PROLONGED CORPUS LUTEUM SYNDROME. Sheryl S. King* and J. Warren Evans. University of California, Davis, CA 95616

The spontaneous prolonged corpus luteum (SPCL) syndrome is a common reproductive problem in mares contributing significantly to lowered rates of conception. The cause of this syndrome is as yet unknown. The objective of this study was to compare the $PGF_{2\alpha}$ synthetic capability of endometrial tissue from mares experiencing the SPCL syndrome with endometrial tissue obtained during different stages of the normal estrous cycle. Uterine biopsies were obtained after 30 days of postovulatory diestrus behavior from 10 mares experiencing the SPCL syndrome. Prolonged luteal activity was verified by daily plasma progesterone concentrations. Uterine biopsies were obtained from mares during normal estrous cycles on days 5, 10, 12, 14, 16 and 20 postovulation. $PGF_{2\alpha}$ synthetic capability was low during the early stages of diestrus (3.37 ± .91, 4.44 ± 1.12 and 9.30 ± 2.55 ng $PGF_{2\alpha}$/mg dry wt on days 5, 10 and 12 postovulation, respectively), rose to a peak on day 14 (13.37 ± 1.89 ng $PGF_{2\alpha}$/mg dry wt) and returned to minimal concentrations by day 20 (2.56 ± 0.28 ng $PGF_{2\alpha}$/mg dry wt). Endometrial $PGF_{2\alpha}$ synthesizing capabilities during SPCL syndrome were the same as those observed during early diestrus and estrus (3.95 ± 0.85 ng $PGF_{2\alpha}$/mg dry wt). It is concluded that a causative factor to the SPCL in the mare is the failure of the synthesis and release of $PGF_{2\alpha}$ from the endometrium normally occurring during late diestrus.

17.7

PURIFICATION OF EQUINE RELAXIN. Dennis R. Stewart* and George H. Stabenfeldt. VM:Reproduction, U. C. Davis, Davis, CA 95616

Relaxin has previously been shown to be produced in the placenta of the mare during pregnancy (Stewart, Stabenfeldt, Hughes, Meagher, 1981). Equine placentas were collected at term and stored frozen. Placental material was extracted following the procedures of Sherwood and O'Byrne for porcine relaxin. Purification through acid-acetone extraction, gel filtration, and ion exchange chromatographies was monitored by a porcine relaxin radioimmunoassay and biological assay using the mouse interpubic ligament assay. Three peaks eluted from the ion exchange chromatography that contained high amounts of immunoactivity. Each of these three peaks was demonstrated to contain biological activity by the mouse interpubic ligament assay and was confirmed by Dr. Bernard Steinetz (CIBA-GEIGY) utilizing the guinea pig palpation assay. Only one peak was obtained in sufficient quantitiy to allow a dose response comparison with porcine relaxin. Equine relaxins showed parallelism with porcine relaxin but a potency of only 28 units/mg. The content of equine placentas is low, such that only about 1 mg is obtained from 5 km starting material. Sufficient material has been isolated, however, to raise antibodies against equine relaxin for the development of a homologous equine relaxin radioimmunoassay.
Supported by a grant from the Grayson Foundation.

17.8

THYROID-GONAD RELATIONSHIP IN BRONCHIAL ASTHMA. O. Parshad*, M. Kumar* and G.N. Melville*, University of the West Indies. (SPON: L.H. Hamilton, Medical College of Wisconsin, Milwaukee WI 53226).

Thirty men of African origin aged 35 to 50 years, with bronchial asthma, were studied to elucidate the relationship between thyroid functions and level of serum testosterone. Serum thyroxine (T_4), triiodothyronine (T_3), triiodothyronine uptake (T_3U), free thyroxine index (FTI), thyrotropin (TSH) and testosterone (T), were measured by RIA and compared with those from 30 healthy male subjects matched for age. Results as mean ± S.E. (*$P<0.001$):

SUBJECT	T_4 (µg/100 ml)	T_3 (ng/100 ml)	T_3U (%)	FTI	TSH (µIU/ml)	T (ng/100 ml)
Control	8.59 ±0.27	126.77 ±2.87	30.26 ±0.43	2.58 ±0.07	1.19 ±0.08	692.67 ±36.27
Asthmatics	7.78* ±0.42	79.27* ±5.66	35.77* ±0.92	2.70 ±0.10	1.44 ±0.12	369.40* ±34.12

In bronchial asthma: (i) there were decreases in both serum T_3 and T levels which were positively correlated (r=0.5539); (ii) significant increases in T_3U were not associated with changes in FTI, indicating an abnormality in the thyroid binding proteins, and (iii) in spite of decreases in serum T_3, the TSH levels did not differ significantly. It was concluded that the hypothalamic-hypophyseal-thyroid-gonadal axis is apparently set at a lower level in asthmatic patients.

17.9

The Effect Of ContraSperm™ On Sperm Count And Motility Of Primates And Humans.

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Rational Alternative Corporation, P.O.Box 2547, Mission Viejo, California,92690. U.S.A.

Ecbelatericin™, the active ingredient of ContraSperm™, is a unique, sophisticated preparation extract of the plant Ecballium elaterium, Linn. Its preparation and use are disclosed in United States Patent 4,148,892 and the European Patent O 005 367. It is revealed as a simple, effective and non toxic Male Oral Contraceptive, free of steroids and hormones, and capable of reducing sperm count and motility from pretreatment levels, to infertility and nil values, within thirty minutes to one hour after oral ingestion in both Rhesus macaques and Humans, for a period up to twelve hours. No side or after effects have been indicated, after short or long term usage.All histopathological, teratology and blood studies did not show any changes at the cellular and tissue levels before and after treatment.

17.10

INTERRELATIONSHIPS OF TESTES WEIGHT, TESTICULAR SPERM NUMBER, DUCTUS (D.) DEFERENS SPERM NUMBER AND EJACULATE VOLUME AND SPERM NUMBER OF MATURE TURKEYS. Helene C. Cecil and Murray R. Bakst*. USDA, ARS, Avian Physiology Laboratory, Beltsville, MD 20705.

In a flock of breeder turkeys ejaculate volumes between individuals can vary from 0.01 to 1 ml. Little is known of the mechanisms which determine this variation. Regression analyses were used to determine the interrelationships between testes weight, testicular sperm number, d. deferens sperm number and ejaculate volume and sperm number in a flock of mature turkeys between 38 and 52 weeks of age. Testes weight was positively correlated with the total number of testicular sperm ($P < .0001$) and with the total number of sperm in the d. deferens ($P < .009$). The total number of testicular sperm was not correlated with the total number of sperm in the d. deferens ($P=.07$). The total number of sperm in the ejaculate was not correlated with testes weight or the total number of testicular sperm. From 38 to 52 weeks of age the total number of sperm in the d. deferens decreased ($P < .02$), but testes weight and sperm content did not decrease significantly nor did ejaculate volume change. These data suggest that the ejaculate volume and sperm number is not related to the testes weight or testicular sperm number. However, the d. deferens sperm number is related to testes weight and possibly to the rate of sperm production.

17.11

GLUTAMATE DEHYDROGENASE ACTIVITY IN RAT VENTRAL PROSTATE. Renty B. Franklin and Leslie C. Costello. University of Maryland, Baltimore, MD 21201

We have proposed that aspartate transamination is an important source of oxalacetate (OAA) in prostate. However, transamination would require an adequate continual source of keto-acid. Consequently, we proposed that in prostate glutamate dehydrogenase (GDH) recycles α-ketoglutarate (αKG) by oxidative deamination of glutamate. In order to measure enzyme activity with glutamate as substrate at pH 7.0-8.0, we developed an assay system utilizing the tetrazolium salt 2,2'-di-(p-nitrophenyl)-5,5' diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene) detetrazolium chloride (NBT) as the final electron acceptor. GDH activity was determined in mitochondrial preparations from rat ventral prostate and rat kidney. Maximum catalytic activity (forward direction) for the prostate and kidney preparations was 3.1 and 3.6 nmoles/mg pr/min respectively. However, when αKG was substrate (reverse reaction) the kidney preparation was more than 3 times more active than prostate. We also incubated prostate mitochondria in a reaction mixture which was essentially the same as the assay system with the addition of acetyl CoA and aspartate. Results demonstrated that prostate mitochondria could synthesize citrate from aspartate and glutamate at pH 7.4 at a rate of 6 nmoles/mg pr/min. These results demonstrate the presence of GDH activity in prostate mitochondria. Furthermore, they demonstrate that prostate mitochondria can accumulate citrate from amino acids, in the presence of acetyl CoA. Supported by NIH grants AM28015 & HD16193.

17.12

TESTOSTERONE EFFECTS ON CITRATE OXIDATION BY RAT VENTRAL PROSTATE. Myong W. Kahng*, V. Akuffo*, R. B. Franklin and L. C. Costello. Univ. of Maryland, Baltimore, MD 21201

The metabolic mechanism(s) which results in the accumulation and secretion of extraordinarily high levels of citrate by the prostate gland has not been elucidated. In addition the mechanism by which testosterone regulates prostate citrate secretion needs to be established. In previous studies we demonstrated that citrate oxidation by mitochondria isolated from rat ventral prostate was very low due to a limited aconitase activity. The present report is an extension of the earlier studies and also demonstrate the effect of testosterone on citrate oxidation. Citrate oxidation was determined by $C\text{-}14O_2$ production from 6-C14 citrate, and citrate utilization was determined as the total disappearance of citrate. Castration (72 hour) resulted in a decrease (30%-45%) citrate oxidation and utilization by rat ventral prostate mitochondria as compared to sham animals. Similarly citrate oxidation by ventral prostate fragments was decreased by castration. The administration of testosterone to castrated rats (1-5 mg per rat for 24 hours) stimulated citrate oxidation and utilization back to normal levels. This effect of testosterone would have a tendency to decrease citrate levels by increasing citrate oxidation which is contrary to the action of testosterone which increases prostate citrate levels and secretion. These results indicate that the regulation of citrate production by testosterone is not mediated via an effect on citrate oxidation by prostate. Supported by NIH grants AM28015 & HD16193.

ENVIRONMENTAL PHYSIOLOGY

18.1

CIRCADIAN RESPONSES OF MAMMALS TO THE HYPERDYNAMIC ENVIRONMENT. Charles A Fuller, David W. Griffin*+ and John M. Horowitz+. Div. Biomedical Sci., Univ. of Calif., Riverside, Ca 92521 and +Dept. Animal Physiol., Univ. of Calif., Davis, CA 95616.

Mammals demonstrate depression of deep body temperature in hyperdynamic environments. However, the influence of circadian body temperature rhythms on this response have not previously been investigated. The present study examined such time of day influences on acute exposures of animals to $2G_z$. Colonic temperatures were measured in eight monkeys and eight rats exposed to 70 min of $2G_z$ via centrifugation at two times during the day, with a minimum of four recovery days between exposures. The order of exposure was reversed for half of each group of animals. During the 70 min control periods prior to centrifugation, all groups demonstrated stable body temperatures. At $2G_z$ during the day, the diurnal monkeys showed a 1.4°C fall in colonic temperature to about 37.3°C. During the night, body temperature did not change and was regulated at about 36.9°C. The nocturnal rats showed a reversed response. At $2G_z$, during the day, colonic temperature was depressed 1.6°C, to about 36.2°C. At night, these animals showed an average temperature depression of 2.3°C to about 35.5°C. Thus, there are clear circadian differences in response to the hyperdynamic environment, with the greatest fall in temperature during the animals' active phase. Further, the animals clearly have some ability to regulate temperature as demonstrated by the capacity to minmize the changes in body temperature during their rest phase. (Supported by NASA Grants NAGW-309, NSG-2234 and PHS Grant BRS RR-05816).

18.2

ACID-BASE STATUS DURING SHORT-TERM IMMOBILIZATION IN MONKEYS (M. NEMESTRINA). D. R. Young and R. S. Swenson.* Ames Research Center, Moffett Field, CA 94035

In an earlier study of the effect of chronic immobilization with monkeys, we observed an increased urine net acid excretion (NAE) largely due to a sustained rise in ammonium production. Within 2-3 weeks, arterial pH and bicarbonate increased approximately 10%, and the stable alkalemia persisted throughout several months of continuous immobilization. Potential causes of immobilization-associated alkalosis in primates incude mineralocorticoid excess, potassium depletion, and reduction of blood volume. The early responses to immobilization were studied in order to elaborate mechanisms which can stimulate aldosterone production. Adult male animals were restrained on a soft couch for 9 days and measurements were made on venous blood and urine samples. Body weight declined 320g and blood volume decreased 90 ml. During the first 4 days, plasma aldosterone rose significantly and urine aldosterone was elevated throughout the test. There was a hypokalemia and hyponatremia. Urine titratable acidity (TA) increased significantly along with endogenous phosphate excretion. NAE was significantly elevated. Venous pH was relatively unaffected. We conclude that hypovolemia and hyponatremia promote the rise in aldosterone production. Renal ammoniagenesis occurs as a result of mineralocorticoid excess and hypokalema however there may be a delay of 2-3 weeks prior to the expression of the response in immobilized animals.

18.3

SPACELAB-4:THE FIRST SHUTTLE MISSION DEDICATED TO LIFE SCIENCES RESEARCH. C. Dant*, C. Schatte, L. F. Cipriano, NASA-Ames Research Center, Moffett Field, CA 94035

In early 1986, NASA will launch Spacelab Mission 4, the first mission dedicated to the Life Sciences. This mission will carry 14 nonhuman and 10 human investigations proposed from an international group of investigators. The Life Sciences Flight Experiments Project (LSFEP) at NASA-Ames Research Center manages, develops, and implements spacelab nonhuman Life Sciences investigations. The Spacelab-4 nonhuman studies, designed to investigate physiological responses to spaceflight in rodents, squirrel monkeys, amphibians, and plants include: vestibular physiology; musculoskeletal metabolism; macro and microcirculatory physiology; fluid and electrolyte balance; erythropoiesis and blood volume regulation; thermoregulation; amphibian embryogenesis; and plant nutation. The Research Animal Holding Facility and General Purpose Work Station are among many common reusable Life Science laboratory equipment items developed for such Life Science investigations. Spacelab-4 will carry non-career astronaut Payload Specialists, professionals chosen from the Life Sciences community to carry out the investigations directly in space. Spacelab-4 is the first of many flight opportunities available to Life Scientists; future investigations will include more dedicated flights, minilabs, and locker-sized carry-on experiments. Research capabilities using a space platform/space station for long-term Life Sciences research is now under study. The presentation will focus on the nonhuman Spacelab-4 investigations and future opportunities.

18.4

FACTORS AFFECTING ATROPHY OF LOAD BEARING MUSCLES OF RATS IN SIMULATED WEIGHTLESSNESS. Herbert S. Ginoza* and Emily Morey-Holton* (SPON: J. Oyama). NASA Ames Research Center, Moffett Field, CA 94035

We have previously shown that unloading of hind limbs of rats by suspension leads to selective atrophy and decrease in the rate of protein synthesis of the soleus muscle. The present study was initiated to determine: (1) whether alterations in the respiratory capacity of mitochondria in the atrophying muscle elicit changes in the muscle mass and (2) the role of high circulating glucocorticoids in weightlessness induced muscle atrophy. The hind limbs of Sprague-Dawley rats, 150 - 200 grams were made non-weight bearing with a modified Morey rat model (BioScience 29:168, 1979). Mitochondria from soleus, EDL and gastrocnemius muscles were isolated from rats suspended for 7 days and respiration was measured using glutamate as a substrate. The state 3 respiration of mitochondria from pooled atrophied soleus muscles was 26.65 nano/atoms oxygen utilized/mg protein/min compared to 61.89 for the non-atrophied control muscles. No significant differences were found in the respiratory activity of the EDL and gastrocnemius muscles. When non-suspended rats were injected intraperitoneally with triamcinolone acetonide, 5 mg/kg body weight, daily, for 4 days, the size of the soleus muscle was not significantly different from untreated control. However, when steroid treated and non-treated rats were suspended for 4 days, the size of the soleus muscle decreased more rapidly in the hormone treated rats.

18.5

EFFECT OF HIGH ALTITUDE (HA) AND ACETAZOLAMIDE (AZ) ON PLASMA LACTATE (LAC) AND ENDURANCE TIME TO EXHAUSTION AT 90% VO_2 MAX. R.L. Burse, R.C. Feccia*, A. Cymerman, W.L. Daniels, P.B. Rock* and J.T. Maher. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

An experimental treatment (Tr) of either 500 mg AZ (E group, n=7 young men) or placebo (C group, n=5) was given b.i.d. for 2 days before and for 3 days after ascent to HA (4300 m) to assess the effect of AZ and HA on resting arterialized venous pH, endurance time to exhaustion at 90% of cycling VO_2 max at that altitude-Tr combination (ET90), and venous LAC 3 min after exhaustion. Subjects performed at sea level without Tr, at HA with Tr 3 days after ascent and at HA 3 days after Tr was stopped. At HA, pH was lower in E than C with AZ, but reverted after cessation. Although LAC at exhaustion was reduced acutely with AZ and progressively over 6 days HA exposure, ET90 was not significantly affected. Respiratory alkalosis of HA thus qualitatively differs from a metabolic alkalosis at sea level which enhances both LAC accumulation and endurance time in exhausting heavy exercise.

		n	SL, no Tr	HA, Tr	HA, no Tr
pH	C	5	7.42 ± .01	7.46 ± .01	7.46 ± .01*
	E	6	7.42 ± .01	7.39 ± .01§#	7.45 ± .01*
ET90	C	5	397 ± 59	399 ± 30	401 ± 51
(sec)	E	7	548 ± 64	488 ± 74	538 ± 65
LAC	C	4	11.6 ± 1.4	8.0 ± 1.4	6.6 ± 0.5*
(mmol/l)	E	6	11.0 ± 1.3	4.3 ± 0.6§#	6.4 ± 0.6*

$P<.05$: # E differs from C; * differs from corresponding SL value; § differs from no-Tr value at HA; Table shows $\bar{X} \pm SE$.

18.6

HYPOCAPNIA AND SUSTAINED HYPOXIA BLUNT VENTILATION ON ARRIVAL AT HIGH ALTITUDE. J.T. Reeves, S.Y. Huang*, J.K. Alexander, R.F. Grover, J.T. Maher, R.E. McCullough*, R.G. McCullough*, L.C. Moore, J.B. Sampson*, J.V. Weil*. Univ. of Colorado Health Sciences Cntr., Denver, CO 80262

Hypoxia at high altitude stimulates ventilation but inhibitory influences limit the ventilatory response. Possible inhibitory influences include hypocapnia and non-sustained ventilation during continued hypoxia. Our approach was to compare hypoxic ventilatory responses at low altitude with ventilation at high altitude. In 12 subjects we compared responses to acute (<10 min) isocapnic hypoxia, acute poikilocapnic (no CO_2 added) hypoxia and prolonged (30 min) hypoxia in Denver, 1600 M, with ventilations measured on each of 5 days on Pikes Peak, 4300 M. On Pikes Peak day 1 ventilation (V_E=10.2 l/min, SaO_2=82%) was less than predicted by either acute isocapnic or poikilocapnic tests. However prolonged poikilocapnic hypoxia (SaO_2 =82%) in Denver yielded ventilation similar to that on Pikes Peak day 1. By Pikes Peak days 4 and 5, end-tidal PCO_2, pHa, and arterial oxygen saturation approached plateaus, and ventilation 12.4 l/min was as predicted by the acute isocapnic test. Thus the combination of hypocapnia plus nonsustained ventilation may have blunted the ventilatory increase on Pikes Peak day 1, but apparently not after 4 or 5 days of acclimatization. (Supported by NIH Grant HL 14985 and US Army Rsch. Institute Contract DAMD-81-C-1057)

18.7

FACTORS INFLUENCING RIGHT VENTRICULAR HYPERTROPHY AND SURVIVAL OF GUINEA PIGS IN HYPOXIA. N. Banchero, S.R. Kayar and C.I. Blake*. Univ. of Colorado Medical School, Denver, CO 80262.

The pulmonary arterial hypertension of mammals in chronic hypoxia is due to increased pulmonary vascular resistance. Hypoxia produces vasoconstriction first and anatomical reduction of the pulmonary vascular lumen later. This increases the work of the right ventricle and causes hypertrophy (RVH). Hematological and heart weight data obtained in growing guinea pigs (GP) have been analyzed to investigate factors affecting RVH. GP were exposed to dilution hypoxia (FO_2=0.126, ambient PO_2=80 torr) equivalent to 5100 m, and to hypobaric hypoxia (PB=435 torr, ambient PO_2=90 torr) equivalent to 4600 m, for up to 16 weeks. A control group was studied in Denver (PB=635 torr, ambient PO_2=133 torr, 1610 m). Body growth rates were the same for all groups. The weight of the right ventricle (RVW) increased linearly with body weight (BW), but in the hypoxic GP the RVW were significantly higher than in the controls at any BW. The degree of RVH, calculated as the percent increase in RVW over the control group in Denver, was exponentially related to Hct ($RVH=0.97e^{0.07\ Hct}$, R=0.77). High Hcts (>65%) caused severe RVH (>100%); 20% of the GP exposed to hypoxia developed right heart failure and peripheral edema, resembling chronic mountain sickness. In these GP the average Hct was 68%. The increased blood viscosity of severe polycythemia aggravates the pulmonary arterial hypertension causing severe RVH and in some cases RH failure. Supported by NIH HL28849 and HL06527.

18.8

INCOMPATIBILITY BETWEEN ALVEOLAR GASES AND PREDICTED PERIPHERAL CHEMOSENSORY DRIVE FOR VENTILATION ON THE EVEREST SUMMIT. S. Lahiri. University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104, USA.

Resting alveolar PCO_2 on the Mt. Everest summit (8848 m) in man was predicted to be 10-12 Torr assuming a barometric pressure between 247 and 250 Torr (Dejours, 1980; West and Wagner, 1980). The average value found in a single subject was 7.5 Torr at an alveolar PO_2 of 35 Torr (West et al., 1983). The calculated arterial pH was 7.7. The extreme hyperventilation was assumed to be driven by the peripheral chemoreceptors due to hypoxia. However, these blood gases and $\{H^+\}$ values are close to the stimulus threshold for arterial chemoreceptors in the normal sea level cats (e.g. Lahiri et al., 1978). Therefore, acclimatization to chronic hypoxia and subsequent exposure to extreme hypoxia must have caused some differences which require investigation. We investigated the arterial chemical stimulus threshold for carotid body chemoreceptors in the cats which were exposed to PIO_2 of 70 Torr for 3-4 weeks. We found that arterial PO_2 of 30 Torr and pH 7.7 are still very close to the stimulus threshold for carotid body chemoreceptors in these cats as well. The conclusion that the peripheral chemosensory drive alone could not account for the observed alveolar gases on the Everest summit is inescapable, assuming that the experimentally observed chemosensory data in the cat are applicable to man.

18.9

VENTILATION AND O_2 CONSUMPTION IN HYPOXIA AND COLD-ACCLIMATED GUINEA PIGS. C.I. Blake*, S.R. Kayar and N. Banchero. Univ. of Colorado School of Medicine, Denver, CO 80262

Cold-acclimated mammals require greater amounts of O_2 to meet the increased metabolic demands for maintenance of body temperature. The O_2 requirements of hypoxia-acclimated mammals with similar metabolic needs to those at low altitude must be met under conditions of reduced O_2 availability. To determine the effects of acclimation, we measured O_2 consumption ($\dot{V}_{O_2}$), tidal volume (V_T), and breathing frequency (f) in cold- and hypoxia-acclimated guinea pigs (GP). Growing males maintained in cold (5°C, ambient P_{O_2}=133 torr) for 8 weeks, hypobaric hypoxia (26°C, ambient P_{O_2}=90 torr) for 11 weeks,and in Denver(22°C, ambient P_{O_2}=133 torr) were placed awake and unrestrained in a transparent chamber. Gas flow rate through this chamber was 1.2 L/min. After 30 min, resting $\dot{V}_{O_2}$ was determined by measuring the fall in O_2 concentration between inlet and outlet. The chamber was then closed to serve as a plethysmograph and oscillations in pressure were recorded from which f and V_T were obtained. In cold GP, V_T was 28% greater and f was 46% greater than in controls. In hypoxic GP, V_T was 40% greater and f was 15% greater than in controls. Thus, minute ventilation ($\dot{V}_E$) was 100% greater in the cold-acclimated animals and 60% greater in hypoxia-acclimated animals. The relation between $\dot{V}_E$ and $\dot{V}_{O_2}$ was parabolic, with $\dot{V}_{O_2}$ increasing rapidly at $\dot{V}_E$ values above 50 ml/g·hr. Some GP showed increased $\dot{V}_E$ and a concomitant increase in $\dot{V}_{O_2}$ which appeared due to restlessness and not enviromental stress. NIH HL28849

18.10

SIGNIFICANCE OF LUNG STRUCTURE IN BIRDS AND MAN FOR PERFORMANCE AT HIGH ALTITUDE. P. Scheid and J. Piiper. Inst. f. Physiol., Ruhr-Univ., Bochum, and Dept. Physiol., Max-Planck-Inst. f. exp. Med., Göttingen, FRG.

Only few people have succeeded to climb Mt. Everest (altitude, 8848 m) breathing ambient air (inspired PO_2 about 42 Torr), and maximum exercise is restricted to low levels at this altitude. In contrast, birds have been reported to fly or soar well above 10,000 m. We have estimated the significance of lung structure for the apparent higher altitude tolerance of birds compared with man, as the avian parabronchial lung, due to its cross-current arrangement, is known to have a higher gas exchange efficiency than the alveolar lung. For this, we have calculated the change in the inspired-to-arterial PO_2 difference that would occur in man upon replacement of his alveolar lung by a cross-current parabronchial lung, keeping all other pulmonary parameters (e.g., O_2 uptake, ventilation, blood flow, diffusing capacity) unchanged. The results show that, for unaltered arterial PO_2, inspired PO_2 with the avian lung can be 5 Torr lower than with the mammalian lung, which corresponds to a gain in altitude of about 800 m. Lung structure thus plays an important role in the high altitude tolerance of birds. However, since birds appear to tolerate an even higher altitude, other factors that enable birds to endure the extreme altitude are expected to be involved as well.

18.11

EFFECTS OF ISOPROTERENOL INFUSION ON THE KINETICS OF NITROGEN WASHOUT IN RATS. Gary W. Mack* and Y.C. Lin. Department of Physiology, University of Hawaii, Honolulu, HI 96822.

In a perfusion limited model for inert gas exchange, extracted rate constants depend only on the rate of tissue perfusion and the partition coefficient of the gas. Unanesthetized male rats previously prepared for determination of cardiac output ($\dot{Q}$) by thermodilution, femoral artery blood and venous infusion were given saline (day 1) or isoproterenol (1.17 μg/kg·min, day 2) at a rate of .0035 ml/min during a two hour isobaric washout with 100% O_2 at 1 ATA. Whole body nitrogen washout kinetics were determined and three rate constants were calculated and labeled by order of extraction as: K1=slow, K2=medium and K3=fast. $\dot{Q}$ increased from 390 ml/min·kg during saline infusion to 550 ml/min·kg with isoproterenol. When K2 is plotted against $\dot{Q}$ one obtains a straight line with a slope of zero. K1 vs $\dot{Q}$ shows an initial linear increase with $\dot{Q}$ as predicted by the equation $K=\lambda \cdot \dot{Q}$ but at higher flow rates the plot shows a steep exponential rise in K1. These results suggest that perfusion limitations to nitrogen washout reside primarily in the slower tissues but at higher flow states other factors begin to influence significantly the half-times for desaturation. (Supported in part by Hawaii Heart Assoc.).

18.12

AUXILIARY COOLING: A COMPARISON OF VARIOUS METHODS IN HOT/DRY CLIMATE. *Y. Epstein, Y. Shapiro*, S. Brill, D. Zakai. Heller Institute of Medical Research, Chaim Sheba Medical Center, Israel.*

The physiological hazard involved in elevated body temperature and the reduction in performance urged the seeking of a proper solution for an efficient external cooling system. Since total air-conditioning is usually unfeasible, mainly because of power considerations, individual cooling is the most practical solution to alleviate heat stress problems. Individual cooling includes gas, liquid, or ice cooled systems, covering the entire body or a limited segment, usually head or torso. Seven different cooling devices were compared under the same hot/dry climatic conditions (50°c,30% rh). Using the latin-square routine, 8 male subjects tested water/air cooled garments (vests and hoods), ice-bags vest, zone cooling and a fan for their beneficial effect on physiological parameters. The strain index (SI) of Craig (SI=HR/100+ΔT_r+SR) was used in order to evaluate the physiological status of the subjects. Cooling the torso was found to be more effective than cooling the head. Systems based on cooled air resulted in similar physiological impact as systems based on circulated water, in spite of their lower cooling capacity. Subjective sensation of comfort was found to be highly correlated to the physiological strain index ($r=0.61, p < 0.001$). It is suggested that the SI might serve as a useful tool to compare different physiological stressful situations.

19.1

NON-INVASIVE ESTIMATION OF WORK OF BREATHING AND COMPLIANCE WITH SURFACE INDUCTIVE PLETHYSMOGRAPHY. N.E. Moavero*, D.S. Lipton*, G.A. Jenouri*, J. Pine*, and M.A. Sackner. Mt. Sinai Med. Ctr., Miami Beach, FL 33140

Movement of suprasternal fossa monitored with surface inductive plethysmography (SIP) reflects changes of intrapleural pressure (P_{pl}). Compliance (C_L) may be calculated indirectly using Mead & Whittenberger approach (JAP 5:779, 1953) if value of oscillatory resistance during tidal breathing is substituted into inspiratory pulmonary resistance (R_L) loop. SIP gain is sensitive to changes in neck position. Should this occur, the procedure obviates recalibration of SIP which would be necessary by a time-consuming Null Procedure (Tobin, JAP, in press). We studied 4 seated COPD patients and 1 normal with simultaneous SIP and intraesophageal balloon methods. Values at tidal breathing (17 br/min) and 20 br/min were compared to line of identity: 1) work of breathing (W_{Br}) all values ± 30%, R_L all values ± 35%, 3) C_L 66% were ± 40% and 100% were ± 60%. In 6 COPD patients, tidal breathing ($\dot{V}_I$=12.4 L/min) comparisons with SIP method seated and supine revealed no significant differences.

Mean (SD)	R_L $cmH_2O/L/sec$	C_L L/cmH_2O	W_{Br} K_gM/Br	Elastic Work %
Seated	7.8(2.6)	.18(.11)	.067(.021)	43(18)
Supine	7.1(2.4)	.20(.08)	.063(.042)	44(18)

This new method which provides semi-quantitative data on work of breathing and lung compliance, suggests that C_L is not altered by changing from upright to supine posture in COPD.

19.2

WIDE-BAND VERSUS DISCRETE EXCITATION DURING HIGH-FREQUENCY OSCILLATION. J.R. Clarke, D. Kerem, E.T. Flynn, M.E. Bradley and L.D. Homer. Naval Medical Research Institute, Bethesda, Md. 20814

High-frequency oscillations (HFO) used as a ventilatory aid are generally applied at a single discrete frequency, which is chosen empirically. We sought to compare the relative efficiency of discrete versus multiple frequencies as forcing functions during HFO. Oscillations were applied to anesthetized, paralyzed dogs either directly to the trachea via a loudspeaker or to the chest-wall by a shaker. The driving input was either sinusoidal (15 Hz) or band-pass filtered (5-50 Hz) noise reflecting the admittance characteristics of the canine respiratory system. Acoustical power delivered to the lower trachea was measured by a Millar catheter pressure transducer, and was related to the area under a modified 3-min nitrogen washout curve (Clarke et al., Fed Proc 42:1351, 1983). For both internally and externally applied oscillations, matched tracheal RMS powers resulted in similar washouts regardless of the nature of the exciting function, even though the power at 15 Hz was 250 times lower during noise application than during 15 Hz sinusoidal oscillation.

(Supported by NMRDC Work Unit M0099PN.01B.0009.)

19.3

ENDOGENOUS AND EXOGENOUS INFLAMMATORY MEDIATORS CAUSE SECRETION OF $^{35}SO_4$-LABELED MACROMOLECULES FROM FERRET TRACHEA. A.D. Mackay,* D.B. Borson, E.M. Jensen,* and J.A. Nadel, CVRI, UCSF, San Francisco, Calif. 94143.

To determine whether endogenous and exogenous inflammatory mediators cause mucus secretion, we studied the release of $^{35}SO_4$-labeled macromolecules from ferret tracheas in the presence or absence of indomethacin, an inhibitor of prostaglandin synthesis or in the presence of histamine or prostaglandins E_2, $F_{2\alpha}$, and D_2. We excised tracheas from ferrets anesthetized with pentobarbital sodium (45-60 mg/kg i.p.). Half the trachea was placed in medium M199/Earle's salt solution and half in medium containing indomethacin (10^{-5}M) for the duration of the experiment. We mounted pieces of the anterior portion of the trachea in Ussing chambers filled with 5 ml medium equilibrated with 95% O_2/5% CO_2. We exposed the submucosal side of each tissue to 0.167 mCi $Na_2{}^{35}SO_4$ throughout the experiment. We measured nondialyzable $^{35}SO_4$ released into the luminal halves of the chambers by draining and refilling them at 15 min intervals over 4 h. Indomethacin-treated tissues secreted less $^{35}SO_4$ than untreated ones; mean inhibition 23% ($p<0.05$, $n=8$). This inhibition was not seen in tissues stripped of epithelium ($n=4$). In 5 other animals, exogenous mediators caused secretion with an order of potency (at 10^{-5}M): $PGE_2 > F_{2\alpha} = D_2$ = histamine. These results suggest that endogenous mediators, possibly from the epithelium, may modulate baseline secretion of macromolecules. (Supported by USPHS Grant HL-24136 and a grant from Vicks Division Res. & Develop.)

19.4

ENHANCED PERIPHERAL MUCUS CLEARANCE WITH HIGH FREQUENCY CHEST WALL COMPRESSION. D.Gross*,C.O'Brien*, D.Wight*,L.Rosenthall* A. Zudulka*,and M. King. School of Physical and Occupational Therapy and Meakins Christie Lab, McGill University and Montreal General Hospital, Montreal, Quebec, Canada H3G 1Y5

The effects of high frequency chest wall compression (HFCWC) on mucociliary clearance (MCC) in the small airways was studied in 7 anesthetized nonparalyzed dogs. HFCWC was achieved by a piston pump oscillating the pressure in a modified double blood pressure cuff wrapped around the lower thorax. $A^{99m}Tc$ sulfur colloid aerosol was introduced into the lungs via a Bird neubilizer connected to an endotracheal tube. Aerosolization continued until total lung radioactive count reached 100,000-150,000 per min. and the lungs appeared well outlined. Lung retention was quantified in various lung zones using a gamma camera and subsequent computer analysis. HFCWC at 13 Hz, with a peak cuff pressure of 100 cm H_2O, applied for 30 min, significantly enhanced clearance from the lower peripheral zones when compared with spontaneous ventilation (mean value of 20.1%±13% SD, $p<.01$). In the upper zones MCC was not enhanced. HFCWC at 13 Hz with a peak cuff pressure of 50 cm H_2O also enhanced clearance from the lower peripheral zones in the 3 dogs tested. The enhancement of clearance may be brought about by mechanical effects, changes in mucus viscosity or neural or chemical factors. These results suggest that, provided safety can be demonstrated, HFCWC might be of potential benefit as a mode of chest physiotherapy in patients with mucus hypersecretion. (Supported Can.CF Fdn, & Can.Lung Ass'n).

19.5

NEUTROPHIL DEPLETION INHIBITS AIRWAY HYPERRESPONSIVENESS INDUCED BY OZONE. P. O'Byrne,* E. Walters,* B. Gold,* H. Aizawa,* L. Fabbri,* S. Alpert,* J. Nadel, M. Holtzman.* UCSF, CVRI, San Francisco, Calif. 94143.

We studied whether ozone-induced hyperresponsiveness could be inhibited by neutrophil depletion in dogs. Responsiveness was assessed with dose-response curves of acetylcholine aerosol versus pulmonary resistance; depletion was assessed by counting neutrophils in venous blood and in biopsies of the airway epithelium. Each was assessed 5 d and 1 d before ozone and 1 h after ozone (3.0 ppm, 2 h) in 6 untreated dogs and in 6 dogs treated with hydroxyurea (200 mg/kg daily for 5 d starting 5 d before ozone). In untreated dogs, responsiveness and neutrophil numbers 5 d and 1 d before ozone did not change, but responsiveness and epithelial neutrophils increased markedly after ozone. In treated dogs, circulating neutrophils decreased from 8.9 ± 2.2 to 0.6 ± 0.01 x 10^3 per mm^3 (mean ± SEM), and responsiveness before ozone did not change. Furthermore, increases in responsiveness and epithelial neutrophils did not occur after ozone. Six wk after stopping hydroxyurea, responsiveness and epithelial neutrophils again increased markedly after ozone. The results suggest that ozone-induced hyperresponsiveness may depend on the mobilization of neutrophils into the airways. (Supported by NIH Grant Nos. HL-24136, HL-00797 and by grants from the Council for Tobacco Research and California Air Resources Board.)

19.6

BW755c INHIBITS AIRWAY HYPRERESPONSIVENESS INDUCED BY OZONE IN DOGS. L.M. Fabbri,* H. Aizawa,* P.M. O'Byrne,* E.H. Walters,* M.J. Holtzman,* J.A. Nadel, CVRI, UCSF, San Francisco, Calif.

To follow up our previous observations that airway hyperresponsiveness induced by ozone (O_3) is linked to airway inflammation (neutrophil infiltration and ciliated cell desquamation), we investigated the effect of BW755c, a nonsteroidal anti-inflammatory drug, on the responses to O_3 in 5 dogs. We determined the concentration of acetylcholine (%ACh) that increased baseline pulmonary resistance by 5 $cmH_2O \cdot L^{-1} \cdot s$, and we counted the number of each cell type in bronchoalveolar lavage 1 wk before and 1 h after exposure to O_3 (3 ppm; 2 h). Airway responsiveness and number of epithelial cells in lavage increased significantly after O_3 in the placebo studies but not after BW755c (10 mg/kg, iv; 15 min before exposure). The number of neutrophils in lavage increased in placebo studies and after drug intervention (see table).

	Placebo		BW755c	
	Pre O_3	Post O_3	Pre O_3	Post O_3
%ACh	0.16	0.01	0.13	0.07
Epithelial cells*	1.20	33.00	7.90	17.60
Neutrophils*	6.40	36.40	6.90	41.20

*values = number of cells x 10^3 per ml of lavage fluid.

We conclude that BW755c markedly inhibits ozone-induced airway hyperresponsiveness in dogs, probably by inhibiting arachidonic acid metabolism. (Supported in part by USPHS HL-24136, a grant from the Fisons Corp, Council for Tobacco Research-USA, and California Air Resources Board)

19.7

RAT LUNG POLYAMINE METABOLISM AND SURVIVAL IN HYPEROXIA; AGE RELATED DIFFERENCES. Allen D. Hacker*, and Donald F. Tierney. Department of Medicine, U.C.L.A., Los Angeles, Ca. 90024

Polyamine metabolism is closely related to cell growth including proliferation. Thirty day old rats survive continuous exposure to 1.0 atm O_2 for several weeks; 60 day old rats die within 72 hrs. We exposed 30 day (125 gm) and 60 day (250-300 gm) rats to 1.0 atm O_2 for 24,48,and 56 hrs. Only 30 day old rats survived for 72 hrs. In the 60 day rat, ornithine decarboxylase (ODC) activity increased 360% by 24 hrs and continued to increase until it was 1100% greater by 56 hrs, whereas in the 30 day rat it reached a peak at 48 hrs and then declined by 56 and 72 hrs. The absolute value of ODC per mg DNA was greater in the 30 day rat under all circumstances. By 48 hours putrescine doubled in both age groups but S-adenosylmethionine decarboxylase (SAMDC) activity,spermidine, spermine, and ^{3}H thymidine incorporation into DNA did not increase. In the 30 day rat, but not the 60 day rat, SAMDC activity increased 44% at 56 hrs and 230% by 72 hrs and spermidine content increased 26% by 72 hrs. Spermine content did not change in either age group. ^{3}H thymidine incorporation into DNA was below control through 56 hrs but increased 196% above control by 72 hrs in the 30 day rat. Presumbly lung repair after hyperoxia requires cell proliferation to replace damaged or destroyed cells. The delayed increases of SAMDC activity and spermidine content may be related to the suppression of DNA synthesis that occurs for several days with hyperoxia. (Supported in part by USPHS-NHLBI Grant HL 27309)

19.8

REGIONAL DIFFERENCES IN POLYMORPHONUCLEAR LEUKOCYTE (PMN) TRANSIT THROUGH THE DOG LUNG. B. A. Martin,* S. Lee,* M. Quiroga*and J.C. Hogg. UBC Pulmonary Research Laboratory, St. Paul's Hospital, Vancouver, B.C., Canada.

Previous studies have shown that there is a large marginating pool of PMN in the lung and that the release of cells from this pool is dependent on blood flow (1). The purpose of this study was to examine the movement of these cells through different lung regions and determine their fate when they leave the lung. A bolus of ^{51}Cr-labelled PMN and ^{99m}Tc red blood cells was injected into the right atrium, followed by a bolus of I^{125}-labelled macroaggregates to mark regional pulmonary blood flow. The animals were sacrificed at 10 or 30 min after the injection and the number of PMN in the lung, liver, spleen and total blood volume was calculated. The % of the injected PMN found in the lung, liver, spleen and blood was (mean ± SE) 35±3, 37±2, 9±2 and 12±1 at 10 min (n=5), and this changed to 18±2, 43±3, 7±2 and 8±1 at 30 min (n=5). At 10 min the number of labelled PMN/gm lung was least in the upper region ($p < .05$). At 30 min it was least in the lower region ($p < .05$). We conclude that the transit of PMN through the marginating pool in the lung takes longer in upper regions with low flow and that the liver appears to be the site of accumulation of the labelled PMN released from the lung.

1. J. Clin. Invest. 69: 1279-1285, 1982.

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19.9

PULMONARY TISSUE VOLUME IN PIGS USING THE PIGS' OWN TISSUE SOLUBILITY. M.F. Petrini, M.S. Phillips*, W.C. Pinkston*, K.R. Tullos* and J.R. Norman*. Univ of Miss. Med. Ctr., Jackson, MS. 39216.

We measured pulmonary tissue volume (V_t) in 4 pigs with average body weight of 17.3 ± 1.7 Kg; V_t was estimated by rebreathing acetylene using an inspired volume of 1 L at a frequency of 20-30 breaths/minute. The rebreathing started above residual volume but below functional residual capacity; data was collected and analyzed on line The mean coefficient of variation was 6%. The tissue volume consistently underestimated the wet weight of the lung by nearly 10% when the Bunsen solubility coefficient (alpha) of human lung tissue (0.768 ml gas/ml of tissue) was used. We then measured alpha in the lung tissue of the 4 pigs. After removing the passively drained lungs from the chest we obtained the solubility coefficient in small amounts of tissue ((0.4 gm) by double extraction. The alpha = 0.645 ± 0.017 ml gas/ ml tissue. The mean recalculated V_t was 275 ml, which is an 8% overestimation of wet lung weight This agrees with values found in dogs. Thus we conclude that the solubility coefficient is crucial in V_t determinations. (Supp. by NIH Grant HL26051 and a Mississippi Lung Assoc. Grant-in-Aid of Research).

19.10

A mathematical model of the pulmonary circulation: effect of Histamine (H), serotonin (5HT), norepinephrine (NE) and increased vascular pressure (IP). J.C. Parker, B. Rippe* and A.E. Taylor, Dept. of Physiology, University of South Alabama, College of Medicine, Mobile, AL 36688.

A mathematical model which represents the pulmonary circulation as four resistance (R) segments and three capacitance (C) segments was programmed on a digital computer. The R and C values were adjusted to simulate dynamic and steady-state values of filtration mid-point (P_{CI}), arterial occlusion (P_{AC}), venous occlusion (P_{VC}) and double occlusion pressure (P_D) previously obtained under constant pressure (CP) and constant flow (CF) conditions in isolated, blood-perfused lung lobes during IP and drug infusions. These simulations indicate that: (1) Total R decreased to 40% of control as P_{CI} increased for 10 to 30cm H_2O, and this decrease was mostly attributed to distention or recruitment of small arteries and veins; (2) H increased R and decreased C mainly in the large veins; (3) 5HT increased R in all vascular segments but markedly decreased arterial C; (4) NE increased R primarily in large arteries and veins but decreased venous C; (5) increased R effects of drugs on the small vessels was largely reversed at high P_{CI} due to distention or recruitment; (6) P_D deviated from P_{CI} when arterial and venous C became unbalanced; and (7) constriction of capacitance vessels during drug infusions at low P_{CI} produced greater vascular volume losses than predicted by the passive model. Supported by HL-24571 and HL-22549.

19.11

GRANULOCYTES MEDIATE HYPOXEMIA IN SHEEP INFUSED WITH ACTIVATED COMPLEMENT. T.M. Egan*, N.R. Saunders*, S.C. Luk*, and J.D. Cooper* (SPON: E.A. Phillipson) Univ. Toronto, Canada M5S 1A8

Infusion of "zymosan-activated plasma" ZAP into the superior vena cava in sheep results in leukopenia, pulmonary leukostasis and pulmonary hypertension associated with generation of thromboxane, and hypoxemia;these changes commence rapidly and resolve over 20 to 30 mins. following cessation of infusion. This study was undertaken to elucidate the role of polymorphonuclear leukocyte (PMN) in pulmonary dysfunction induced by infusion of ZAP. 9 sheep were rendered severely granulocytopenic by pretreatment with hydroxyurea (PMN < 100/cu.mm). Their response to ZAP infusion was compared to a group of 11 normal animals. The neutropenic animals elaborated equivalent amounts of thromboxane B2 (measured by RIA) and demonstrated the same degree of pulmonary hypertension as normal sheep. However, granulocytopenic sheep were significantly less hypoxic during and after infusion ($p < .01$). Serial lung biopsies were undertaken in 3 normal and 3 neutropenic sheep infused with ZAP. Pulmonary leukostasis with margination was observed in normal sheep. Electron microscopy demonstrated interstitial edema in normal animals. Edema was absent or markedly attenuated in granulocytopenic animals. Both normal and PMN-depleted animals demonstrated equivalent decrements in pulmonary dynamic compliance. This study provides evidence that the thromboxane generated in-vivo in response to infusion of ZAP is independent of neutrophils. The hypoxemia and associated interstitial edema are granulocyte mediated.

19.12

INFLUENCE OF PROSTAGLANDIN INHIBITION ON A CANINE MODEL OF HYPOXEMIC RESPIRATORY FAILURE. I. Mayers*, J. Burgett*, S. Gottlieb*, P.H. Breen*, L.D.H. Wood*, and G.R. Long*. (SPON: A. Leff). Critical Care Medicine, University of Chicago, Chicago, IL 60637.

Inhibition of endogenous prostaglandins may alter hypoxic vasoconstriction and its associated venous admixture (Qva/Qt) in pulmonary edema. We tested this in anesthetized, artificially ventilated dogs using a model of acid aspiration (1 ml/kg of .1 N HCl intratracheal). Pulmonary capillary wedge pressure (Pcwp) was matched at 12 mm Hg in a control group (C) with a treatment group (I) given indomethacin (5 mg/kg) 1 hour post aspiration. Over the next 4 hours we measured cardiac output (Qt), Qva/Qt and, by thermal green dye (TGD), extravascular lung liquid in vivo (EVLL). The animals were exsanguinated, and gravimetric estimates were expressed as wet lung weight/body weight (W/B). Mean measurements at 5 hours post HCl are shown in the table:

	W/B (ml/kg)	EVLL (ml)	Qva/Qt (%)	Qt (l/min)
C	27.0	575	27.0	6.8
I	25.2	323	15.2	5.3

At a similar Qt and Pcwp, indomethacin tended to decrease W/B, and reduced EVLL and Qva/Qt. Conceivably, indomethacin decreased perfusion in edematous regions by augmentation of hypoxic vasoconstriction resulting in decreased edema formation. Alternatively, redistribution of Qt away from edematous units caused TGD to underestimate the extent of the edema.

20.1

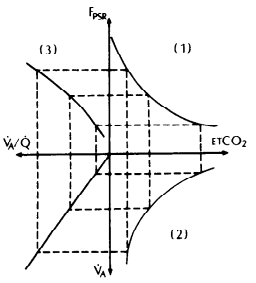
A THEORETICAL DESCRIPTION OF CO_2 TRANSPORT IN MEDULLARY CHEMORECEPTOR MICROVASCULATURE. J. M. Adams, A.C. Roth* and J.B. Ferguson*. Univ. of Virginia, Charlottesville VA 22908

Lipscomb and Boyarsky (Resp. Physiol. 16:362, 1972) suggested that "... substances which stimulate or depress respiration when introduced into the CSF produce their effects more directly at deep neurons after diffusion into the arterial supply to the medulla." To better understand the possible exchange of CO_2 across the medullary microvasculature, we constructed a mathematical description of CO_2 transport between CSF perfusate, arterioles, tissue, capillaries and venules. The salient features of the steady state model include: 6 coupled, linear, differential equations, parameters derived from vascular length and radius, diffusivity and solubility of CO_2, and blood flow rates from pial arteriole velocities and hydrogen washout in chemoreceptor tissue. We found that tissue PCO_2 (P_T) is nearly equal to blood PCO_2 (P_B) half-way along the capilary and that P_B in small ($< 22\mu$diameter) arterioles in the tissue is equal to P_T. When CSF perfusion rate is high, small arterioles in CSF have P_B equal to PCO_2 in CSF but hardly any exchange occurs in larger ($> 63\mu$) arterioles. Assuming that smaller arterioles in CSF perfuse only tissue near the surface and that larger arterioles penetrate more deeply to the respiratory centers, we conclude that CSF perfusion will affect arterial supply to surface layers but not to deeper tissue. (supported by NIH HL 25606)

20.2

A MECHANISM FOR SENSING $\dot{V}_A/\dot{Q}$ DISTRIBUTION? A. Huszczuk*, B.J Whipp and K.Wasserman. Harbor-UCLA Med. Ctr.,Torrance, CA 90509.

Based upon the experimentally-determined relationship between end-tidal PCO_2 and the rate of pulmonary stretch receptor (PSR) discharge [(1) in Fig], it is commonly assumed that these receptors have little regulatory power within the physiological range of PCO_2. However, we and others have presented evidence (Fed. Proc. 41: 1102, 1982 & 42: 1013, 1983) suggesting an an appreciable role for PSR-mediated CO_2 feedback control. We have therefore developed a scheme which accounts for the apparently greater sensitivity of this mechanism, based upon the interrelationships among the essential variables involved in regional pulmonary gas exchange (Fig.) This demonstrates that the projection of the alveolar CO_2 clearance relationship [(2)] through its PSR transduction function [(1)] yields a prominent dependence of integrated PSR input on the distribution of $\dot{V}_A/\dot{Q}$ [(3)]. This suggests, therefore, that $\dot{V}_A/\dot{Q}$ distribution may elicit a component of feedback control sensitive throughout the physiological range and which operates through integration of PSR activity in a manner characteristic of Hering-Breuer reflex mechanisms.



20.3

PHRENIC MOTONEURON ACTIVITY DURING CONSTANT FLOW VENTILATION (CFV). G.C.W. Man*, S.F.P. Man, C.T. Kappagoda, Dept. of Medicine, Univ. of Alberta, Edmonton, Alta, Canada.

We recorded action potentials from 10 single phrenic fibers in 4 pentobarbital-anesthetized dogs ventilated by CFV, provided through two 1.5mm I.D. tubes introduced to the level of the carina. A gas flow of 8L/min was delivered through each tube continuously. Mean airway pressure (Paw) measured at the mid-tracheal level was steady and was manipulated by restricting the exhalation passage. Arterial PCO_2 was monitored continuously by a Clarke electrode incorporated into a vascular loop of the femoral artery. By adjusting the gas mixture (O_2:CO_2) the arterial PCO_2 could be maintained at different levels between 35-75mmHg. At each level of PCO_2, the phrenic activity was recorded while the Paw was increased in steps from 2 to 8mmHg (two minutes for each step). The burst frequency (f), impulses per burst (n), and inspiratory time (T_I) decreased while the expiratory time (T_E) increased when Paw was increased. These changes were less in the higher CO_2 range.

PCO_2	Paw	f(%, mean ±SE)	n	T_I	T_E
35-45	2	100	100	100	100
	8	15.5±10.1	69.5±4.5	41.5±21.0	419±53
>65	2	100	100	100	100
	8	48.7±14.3	88±10	68.1±12.2	271±62

We conclude that during CFV the effect of increasing airway pressure on phrenic motoneuron activity was intact but attentuated at higher CO_2 levels.

20.4

IN VIVO RECORDING FROM CAT TRACHEAL PARASYMPATHETIC GANGLIA R.A. Mitchell, D.G. Baker, C.B. Basbaum* and D.A. Herbert* Cardiovascular Research Institute and Depts. of Anatomy, Physiology and Anesthesia, University of California, San Francisco, SF, CA 94143

We previously reported that tracheal smooth muscle contracts during inspiration (Physiologist, 25:225, 1982). To further investigate this phenomenon, we recorded intracellular activity from 24 spontaneously active tracheal parasympathetic ganglion cells in anesthetized, paralyzed and artifically ventilated cats. Ganglion cells on the dorsal surface of the trachealis muscle were impaled with micropipettes containing 4 M K acetate or 5% Lucifer Yellow. We identified 3 types of neurons based on firing pattern. Eleven cells had an inspiratory rhythm and were inhibited by lung inflation sufficient to abolish phrenic nerve activity. Lucifer Yellow, injected into 5 inspiratory cells, revealed axonal projections to the trachealis muscle but terminal arborizations could not be visualized. Seven cells fired during expiration and fired continuously when phrenic activity was abolished by lung inflation. Four cells with a random firing pattern were unaffected by lung inflation. These findings are consistent with our hypothesis that preganglionic motor fibers that synapse on ganglion cells innervating airway smooth muscle are driven by the same central pattern generator as that driving phrenic motor nerves. (Supported by NIH Grants #HL27319, #HL24136 & #HL28364).

20.5

CONTRIBUTION OF THE INSPIRATORY INTERCOSTAL MUSCLES AND THE DIAPHRAGM TO AUGMENTED BREATHS IN ADULT AND NEWBORN DOGS. F.B. Sant'Ambrogio, G. Sant'Ambrogio, J.T. Fisher and O.P. Mathew. Dept. of Physiology and Dept. of Pediatrics, U.T.M.B. Galveston, TX 77550

We recorded the electromyograms (e.m.g.) of the diaphragm and of an inspiratory intercostal muscle in 3 adult dogs and in 11 puppies (1-30 days old). The animals were anesthetized with chloralose and urethane and were spontaneously breathing through a tracheal cannula. The amount of activity present in the diaphragm and the intercostal muscle was measured as the peak value of the integrated e.m.g. In adult animals during spontaneous augmented breaths the increment in intercostal activity always exceeded that of the diaphragm. In the majority of newborn puppies (5 out of 7 of the 1 to 2 day olds) the increment in diaphragmatic activity during spontaneous augmented breaths exceeded that of the inspiratory intercostal muscle. Older puppies showed a progressive change toward the adult behavior: i.e. a progressive increment in the intercostal contribution. The weaker activation of the inspiratory intercostal muscles during the first days of life could be attributed to an immature proprioceptive control that constitutes an important component in the activation of these muscles in the adult. Such a condition can be considered as a factor of mechanical instability in the respiratory function of the newborn.
Supported by N.I.H. Grants HL-20122, 29169, MRC and A.L.A.

20.6

VENTILATORY RESPONSES TO INSPIRATORY FLOW RESISTIVE LOADING DURING STEADY STATE EXERCISE. Peter H. Abbrecht. USUHS, Bethesda, Md. 20814

Six men, 25-45 years old with normal pulmonary function, rode a bicycle ergometer while breathing through an inspiratory resistance of 36 cm H_2O/l/sec. Each subject did one-hr runs at 20% and 30% of maximum oxygen consumption ($\dot{V}O_2$ max). End-tidal PCO_2, tidal volume, breathing frequency, and minute ventilation were measured throughout the experiments. In all subjects, PCO_2 and minute ventilation reached steady state values within five minutes after starting exercise. At 20% $\dot{V}O_2$ max, tidal volume rose quickly to a new steady state value. At 30% $\dot{V}O_2$ max, tidal volume rose initially and then decreased to below pre-exercise values in association with an increase in breathing rate. In all runs, mean inspiratory flow rate (V_t/T_I) did not change after steady state CO_2 was achieved. Maximum inspiratory pressures were the same before and after exercise, indicating that respiratory muscle fatigue did not occur. We conclude that in steady state exercise with inspiratory flow resistive loading, end-tidal CO_2 is controlled by manipulation of mean inspiratory flow rate. (supported by USUHS Protocols RO7648 and RO7659)

20.7

EFFECT OF CO_2, HYPOXIA AND VAGOTOMY ON DECAY OF POST-INSPIRATORY MUSCLE PRESSURE. S.B. Gottfried*, A. Rossi*, L. Zocchi*, P.M.A. Calverley*, W.A. Zin*, and J. Milic-Emili.
Meakins-Christie Labs., McGill Univ., Montreal, Quebec, Canada

Experiments were performed on 8 pentobarbital sodium anesthetized adult cats spontaneously breathing through a tracheal cannula (a) room air both before and after vagotomy, (b) 60% O_2, (c) 2-6% CO_2, and (d) 10-12% O_2. The pressure developed by inspiratory muscles during expiration (PmusI) was quantified as previously described (J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54:408, 1983). In all instances, the rate of decay of PmusI was an exponential function of expiratory time (Te) which could be quantified in terms of a time constant (τ). Correlation coefficients ranged between 0.95 and 0.99. During CO_2, hypoxia and after vagotomy, tidal volume (Vt) and initial PmusI (Te=0) increased. However, the decay of PmusI changed proportionally so that the relative rate of decay was constant (τ unchanged). With CO_2 and hypoxia, inspiratory (Ti) and expiratory time decreased while Vt/Ti increased. In contrast, after vagotomy both Ti and Te increased while VT/Ti was unchanged. In conclusion, the relative rate of decay of PmusI is a constant exponential function, independent of the absolute level of PmusI. The decay of PmusI is essentially unaffected by changes in respiratory timing occurring with chemical stimulation and vagotomy.
(Supported by the MRC of Canada and NIH grant HL-27617).

20.8

INHIBITION OF BREATHING ARISING FROM THE GALLBLADDER (GB) IN DOGS, G.T. Ford*, K.S. Rideout*, L.K. Bozdech*, W.A. Whitelaw* and J.S. Davison* (SPON: C.A. Guenter).
University of Calgary, Calgary, Alberta. T2N IN4.

Decreased diaphragm activity is seen after cholecystectomy in man and dog. We studied the pulmonary reflexes which could be evoked when the GB was stimulated in anesthetized dogs by mechanical traction or squeezing. The electrical activity of the diaphragm and parasternal intercostals was measured with six bipolar percutaneous needle electrodes and time averaged ($\int$Edi, $\int$Eic). Inspiratory time (T_I),total breath time (T_{TOT}), breath frequency (F), and tidal volume (V_T) were measured. Traction on the gallbladder caused an immediate apnea up to 22 seconds with a gradual resumption in breathing. T_I/T_{TOT}, F and V_T decreased and there was an accompanying proportional fall in $\int$Edi when compared to V_T, with a gradual increase in $\int$Eic. Release of the traction caused an immediate "overshoot" in T_I/T_{TOT}, F and V_T with a gradual return to pretraction levels. Squeezing the GB caused an initial decrease in T_I/T_{TOT}, F and V_T with marked fluctuation of T_I/T_{TOT} and F during the squeeze. V_T decreased 50% and $\int$Edi increased during expiration and showed a paradoxical fall during inspiration. There was a gradual return towards baseline over the next 20-30 minutes. We conclude that (1) stimulation of GB afferent nerves by traction causes reflex inhibition of breathing (2) squeezing the GB reflexly stimulates paradoxical breathing (3) the changes in diaphgram activity seen after cholecystectomy in man or dog may be mediated by this same mechanism.

20.9

STIMULATION OF PULMONARY AND BRONCHIAL C-FIBERS BY INTERSTITIAL LUNG EDEMA IN DOGS. A.M. Roberts, J. Bhattacharya, H.D. Schultz, H.M. Coleridge, and J.C.G. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Pulmonary C-fibers (J or juxta-pulmonary capillary receptors) are known to be stimulated when pulmonary capillary pressure increases. We have attempted to determine whether the increased lung C-fiber activity associated with pulmonary congestion is maintained by interstitial lung edema after pulmonary vascular pressures are returned to normal. We recorded impulses from afferent vagal fibers arising from the lung in anesthetized open-chest dogs during acute extracellular volume expansion produced by I.V. infusion of Krebs-Henseleit solution (10-20% of body weight). After the infusion, pulmonary arterial and left atrial pressures were returned to control by withdrawal of blood. Measurements of extravascular lung water (4.4 $\pm$ 0.4 g/g dry) and histological examination (which revealed perivascular cuffing) provided evidence of interstitial lung edema but not of alveolar edema. During infusion when pulmonary vascular pressures were elevated many pulmonary and bronchial C-fibers were stimulated, and C-fiber activity remained high after intravascular pressures returned to control levels. We conclude that lung C-fibers are stimulated by interstitial pulmonary edema.
(Supported by HL-24136, HL-07192 and HL-25548 from NHLBI.)

20.10

REFLEX EFFECTS OF PULMONARY EDEMA. W.B. Wead and S.S. Cassidy, University of Texas Health Science Center, Dallas, Tex. 75235

We have previously shown that lung inflation and injection of either capsaicin or the toxic substance, alloxan initiate the pulmonary depressor reflex (PDR) responses of decreased heart rate (HR), blood pressure (BP), and frequency of diaphragmatic contraction (DCf). Our objective was to determine if hydrostatic edema would elicit the PDR. In six anesthetized, ventilated, open chest dogs, the left pulmonary artery (LPA) and veins (LPV) were ligated and cannulated, isolating the left lung from the systemic circulation. The entire tidal volume and cardiac output were directed to the right lung. The left lung was suspended and continuously weighed (LLWt). Pulmonary edema was produced by a 5 min. infusion of saline into the LPA and increasing the pulmonary capillary pressure to 30 mmHg. Since edema formation is gradual, these results were compared to those produced by a 5 min. ramp left lung inflation (LLI) to 25 cmH2O left airway pressure (LAWP). Edema produced no significant changes in HR, BP, or DCf as LLWt increased 72%. LLI produced a progressive reduction in HR, reaching -19% at 25 cmH2O LAWP. BP did not change. Amplitude of DC progressively decreased until cessation of DC occurred at an average LAWP of 19 cm H_2O. Left lung wet/dry weight was 9.86±0.47 while the right lung was 4.08±0.22. These data indicate that pulmonary edema induced with increased hydrostatic and decreased oncotic pressure does not elicit PDR responses.

20.11

P_{CO_2} DIFFERENCE BETWEEN ARTERIAL BLOOD AND VENTRAL MEDULLARY SURFACE CHEMORECEPTORS Mary J. Stafford, Paul J. Feustel and John W. Severinghaus. Univ. Calif.,San Francisco, CA 94143.

Isocapnic hypoxic ventilatory depression might be caused by decreased central chemoreceptor tissue P_{CO_2} accompanying hypoxic vasodilation. To measure the tissue to arterial ΔP_{CO_2}, a fiber optic P_{CO_2} probe 1.2mm long, 0.6mm dia. was sealed under mylar on the ventrolateral medullary chemosensory surface in 4 cats, anesthetized with pentobarbital (30 mg/kg), artificially ventilated at constant P_{CO_2} and subjected to normal, low and high P_{O_2}. At $P^a_{O_2}$ = 120±14, ΔP_{CO_2} = 3.46±2.1 (n=12, $P^a_{CO_2}$ = 33.1±1.2). At $P^a_{O_2}$ = 530±51, ΔP_{CO_2} = 4.89±2.1 (n=11, $P^a_{CO_2}$ = 35.0±1.9). At $P^a_{O_2}$ = 39.6±5.0, ΔP_{CO_2} = 3.12±2.0 (n=10, $P^a_{CO_2}$ = 33.6±1.9). ΔP_{CO_2} was significantly greater in hyperoxia than in normoxia ($p<0.05$) or hypoxia ($p<0.01$). This increased ΔP_{CO_2} may be accounted for by the decreased Haldane effect in hyperoxia and possible vasoconstriction by cerebral vessels. Although hypoxia caused an initial fall, ΔP_{CO_2} in the steady state was not significantly less than in normoxia. In hypoxia, ΔP_{CO_2} may have been kept from falling by CO_2 produced by lactacidotic titration of tissue HCO_3^-, despite vasodilation. These data suggest that chemoreceptor tissue flow/metabolism ratio is about 3x that of brain cortex where ΔP_{CO_2} averages about 9 mmHg. Thus, at most a 3 mmHg rise of arterial P_{CO_2} in hypoxia could be attributed to chemoreceptor vasodilation.
(Supported by NIH Grant HL-26167).

20.12

EFFECTS OF NASAL BREATHING AT DIFFERENT TEMPERATURES ON CO_2 SENSITIVITY, DYSPNEA AND ALAE NASI EMG ACTIVITY. K.R. Burgess*, and W.A. Whitelaw. Faculty of Medicine, University of Calgary, Calgary, Alberta. T2N IN4

Steady state nasal CO_2 response was measured in five normal volunteers at "cold" (-0.8±2.1°C) and "warm" humidified (32.1±3.8°C) temperatures. Volume was measured by pneumotachygraph and electrically integrated. Minute ventilation ($\dot{V}_E$) was computed from five breaths at each temperature (T) and end tidal CO_2 (ET_{CO_2}). ET_{CO_2} was sampled at the nares and measured by a mass spectophotometer. Temperature of inspired gas was measured by transistorized probe. "Breathlessness" was scored (BS) by a modified closed Borg scale at each T and ET_{CO_2}. Alae nasi EMG was measured with surface electrodes and time average ($\int$Ea.n.). The slope of the mean CO_2 response was increased from 1.15±0.3 l/min/mmHg "cold" to 1.51±0.8 l/min/mmHg "warm". The mean intercept at 8 l/min ($\hat{x}$) was reduced from 33.6±5.4 mmHg "cold" to 26.4±21.1 "warm". At 10 l/min mean "warm" $\int$Ea.n. was 19.7±16.6 mm compared to 1.86±6.9 mm for the "cold" ($P<0.005$, t test, n=3) indicating a significant left shift of the $\int$Ea.n./$\dot{V}_E$ curve. At 20 l/min. mean BS was 3.2 units "warm" compared to 0.86 units "cold" ($P<0.5$ 1 tailed t test). The mean BS was approximately 2 units higher during the "warm" runs compared to the "cold". (scale 0 to 10). We conclude that breathing warm humidified air by nose increases CO_2 sensitivity. It also significantly increases Alae nasi EMG activity and dyspnea compared to cold air at the same minute ventilation.
Supported by Alberta Heritage Foundation for Medical Research and Alberta Lung Association.

20.13

STIMULATION OF GALLBLADDER AND STOMACH THIN-FIBER AFFERENTS CAUSES AN INCREASE IN RESPIRATORY OUTPUT. T.G. Waldrop,* K.J. Rybicki,* T.I. Musch* and M.P. Kaufman* (SPON: J.H. Mitchell). Univ. of Texas Health Science Center, Dallas, TX 75235

Stimulation of thin-fiber afferents (groups III and IV) from the gallbladder and stomach is known to cause reflex increases in arterial pressure (AP), heart rate (HR) and ventricular contractility. However the ventilatory effects of activating gallbladder and stomach thin-fiber afferents has not been determined. Therefore, we applied capsaicin, a known stimulant of groups III and IV afferents, to the serosal surfaces of the gallbladder and stomach while measuring AP, HR and phrenic nerve activity (processed as a neural equivalent for respiratory output) in cats anesthetized with α-chloralose and urethane. Stimulation of gallbladder and stomach receptors with capsaicin caused increases in phrenic nerve activity, AP and HR in paralyzed, ventilated cats as well as in spontaneously breathing cats. Cervical vagotomy did not alter the responses to stimulation of either visceral organ. However, the respiratory and cardiovascular responses to capsaicin were greatly attenuated after transection of the sympathetic trunks at a level just above the diaphragm. We conclude that: 1) in addition to the cardiovascular effects, stimulation of gallbladder and stomach thin-fiber afferents causes an increase in respiratory output, and 2) the afferent limb of this reflex is via the sympathetic trunks and not the vagus nerves.

20.14

EVIDENCE THAT PULMONARY C-FIBERS TRIGGER BOTH THE APNEA AND RAPID SHALLOW BREATHING OF THE PULMONARY CHEMOREFLEX. J.F. Green, N.D. Schmidt,* H.D. Schultz, A.M. Roberts, H.M. Coleridge, and J.C.G. Coleridge. University of California, Davis, CA 95616 and San Francisco, CA 94143.

There is good evidence that pulmonary afferent C-fibers initiate the apnea evoked by pulmonary arterial injections of capsaicin in dogs, but their role in the subsequent rapid shallow breathing (RSB) is disputed. We attempted to determine if RSB could be triggered from the lungs without influence from a site downstream. In open-chest dogs anesthetized with halothane, pulmonary and systemic circulations were independently pump-perfused at constant PCO_2, PO_2 and blood flow. The lungs were statically inflated at constant pressure (3 cm H_2O). Ventilatory drive was assessed from a phrenic neurogram. Pulmonary arterial injections of capsaicin evoked an immediate decrease in systemic arterial blood pressure and cessation of phrenic firing ("apnea"); when firing resumed, the frequency of phrenic bursts was higher than before and amplitude less ("RSB"). Effects were abolished by vagotomy. Apneic duration was dose-dependent up to 4 μg/kg; phrenic burst frequency increased linearly with dose. "RSB" was prevented or abolished by ventilating or hyperinflating (5-10 cm H_2O) the lung. Results suggest that both the apnea and rapid shallow breathing of the pulmonary chemoreflex are due to stimulation of pulmonary C-fibers, since in these experiments capsaicin could not reach the systemic circulation.

(Supported by NHLBI grants 20371, 24136 and 07192)

20.15

LARYNGEAL MECHANORECEPTORS IN PUPPIES. J. T. Fisher, O. P. Mathew, F. B. Sant'Ambrogio and G. Sant'Ambrogio. Dept. of Physiology and Biophysics and Dept. of Pediatrics, U.T.M.B, Galveston, TX 77550

We studied 26 laryngeal mechanoreceptors responding to collapsing and/or distending pressure applied to an isolated upper airway in 6 anesthetized puppies (3-35 days old). During negative pressure application 20 receptors increased their discharge, 4 were unaffected and 2 had an "on" response. Positive pressure stimulated 7 receptors, inhibited 3, elicited an "on" response in 9 and had no effect on 7. Another 34 receptors were classified as pressure, flow and "drive" on the basis of their behavior during: tracheostomy breathing, upper airway breathing, tracheal occlusion and upper airway occlusion. Pressure receptors, the most frequent type (n=19), responded to negative pressure most often. Flow receptors, the least common type (n=2), responded to inspiratory flow. The remaining endings (n=13) were "drive" receptors (i.e. stimulated by respiratory activity of upper airway muscles, mostly inspiratory). During upper airway breathing an inspiratory modulation was present in 62% of the receptors, expiratory modulation in 6% and 32% were unmodulated. Predominance of an inspiratory activity and the presence of the 3 sensory modalities (i.e. pressure, flow and "drive") are in agreement with our previous observations in adult dogs.

Supported by N.I.H. grants HL-20122, 29169, MRC, A.L.A.

20.16

EFFECTS OF HYPOXIA AND HYPEROXIA ON VENTILATION AND THE PATTERN OF BREATHING IN RABBIT PUPS. T. Trippenbach, R. Affleck* , and G. Kelly*. Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6.

External intercostal EMG (INT), 'integrated' phrenic activity (PHR), tidal volume (Vt), and esophageal pressure (Pes) were recorded in anaesthetized 9-12 day old rabbits for 3-5 min during room-air breathing and 30s after breathing with 100% O_2 or 12% O_2. Arterial blood gases were measured. During hypoxia, there was an increase in minute ventilation ($\dot{V}_E$), Vt, rate of breathing (f), PHR, INT, and Pes. During hyperoxia increase in $\dot{V}_E$ was less but still significant. Changes in f were comparable to those under hypoxia; Vt, INT, and Pes were unaffected and PHR decreased. At both O_2 levels increase in f was due to a shortening in Ti. Linear relationships: Vt vs Ti, PHR vs Ti, expiratory time (Te) vs Ti, were present only during hyperoxia. Hypoxic response was independent of vagal feedback. Hyperoxia caused a decrease in Vt, PHR and Pes but f was unaffected. At both O_2 levels there was a negative Vt vs Ti relationship. Neither Te vs Ti nor PHR vs Ti relationships were present. It is suggested that vagal input and increased P_aO_2 work in favour to maintain the instantaneous ventilation constant in young rabbits. The results also showed that two mechanisms are responsible for an increase in $\dot{V}_E$ as effect of changes in O_2 levels. During hypoxia, the central mechanisms controlling Vt and Ti are involved. During hyperoxia, an increase in $\dot{V}_E$ was exclusively related to vagal input that exerts some control over Ti duration. (Supported by the MRC of Canada).

20.17

SHORT-TERM EXPOSURE TO SEVERE HYPOCAPNIA CAUSES A PROLONGED INHIBITION OF RESPIRATION. D.E. Millhorn. Dept of Physiology University of North Carolina, Chapel Hill, N.C. -27514-

Exposure to hypoxia (F_{IO2}=10) in cats whose peripheral chemoreceptors had been denervated caused a marked depression of respiratory output(RO). Upon return to control conditions (F_{IO2}=100), RO increased but remained significantly depressed for more than one hour (Millhorn et al, Proc IUPS, 1983). Furthermore, we showed that this post-hypoxic inhibition of RO was prevented by pretreating animals with theophylline, an antagonist of adensoine. Because severe hypocapnia causes a decrease in brain blood flow (Am J Physiol 206: 25, 1964) and tissue hypoxia, I wondered if a brief exposure to hypocapnia might also activate the mechanism responsible for the prolonged depression of RO. Anesthetized, paralyzed cats whose vagi and carotid sinus nerves had been cut were studied. RO was quantified from phrenic nerve activity. During control and recovery the animals were ventilated with air. To induce hypocapnia (PCO_2=15 torr) the animals were hyperventilated for 10 min. This always led to apnea. Upon return to normocapnia, RO increased but remained significantly below the control level for more than 1 hr. If cats were pretreated with theophylline (13.6 mg/kg iv), RO returned to or exceeded the control level within 10 min after return to normocapnia. It appears therefore that brief exposure to severe hypocapnia activates a central adenosine mechanism that inhibits RO for a long time after return to normocapnic conditions.

(Supported by AHA 82-640, HL-17689 and NS-11132).

20.18

COMPUTER PROCESSING OF PHRENIC NEUROGRAMS DURING STEP CHANGES IN END-TIDAL PCO_2. Howard J. Bryant and Peter H. Abbrecht. Dept. of Physiology, USUHS, Bethesda, MD 20814.

On physiological grounds the integrated or averaged phrenic neurogram should consist of 1. a constant baseline firing rate during expiration, 2. an initial step increase from baseline, 3. a slower ramp-like increase in firing rate reflecting increased central inspiratory excitation, 4. a peak value representing the off threshold or the end of inspiration, and 5. a fairly rapid decrease in firing rate progressing back to the baseline. Values for these waveform parameters may be obtained by careful manual analysis of strip-chart recordings but this procedure is extremely time consuming and if the waveform is noisy, subject to significant errors in reading. Thus, computer algorithms were developed to estimate these model parameters during step changes in end-tidal PCO_2. The smoothed first derivative of the averaged neurogram was used to establish the positions at which parameters were calculated from the smoothed neurogram. Over 2000 breaths were processed using these techniques. For 2 complete data sets of over 100 records each, repeated manual processing or processing by different individuals produced a variability of ± 10% in parameter estimates. Computer produced parameter estimates fell outside of this range less than 11% of the time. The computer processing algorithms allow rapid analysis of large amounts of data with accuracy consistent with, and reproducibility far exceeding that obtained by manual processing. Supported by USUHS Grants RO7648 and RO7659.

20.19

MEDULLARY BLOOD FLOW DURING HYPOCAPNIC HYPOXIA. D. G. Davies, W. E. Nolan and J. Sexton. Dept. of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The increase in cerebral blood flow (CBF) during hypoxia is attenuated by hypocapnia. If this occurred to a significant degree in the medullary chemoreceptor area, central regulation of ventilation could be affected. We measured dorsal and ventral medullary blood flows during 60 min of isocapnic-, mild hypocapnic- and severe hypocapnic-hypoxia (inhalation of 10% O2) in 22 chloralose-urethane-anesthetized cats (Pco2 values: isocapnia-25, mild-22, severe hypocapnia-17 mmHg). During mild hypocapnic-hypoxia the increase in blood flow to both areas was markedly reduced, while in severe hypocapnia the response was completely abolished. The absence of an increase in medullary blood flow during hypoxemia accompanied by severe hypocapnia might be expected to cause a marked decrease in ECF pH and could result in increased ventilatory drive. (HL 25984).

20.20

RESPIRATORY RHYTHM IS NOT GENERATED BY MEDULLARY INSPIRATORY PREMOTOR NEURONS IN THE CAT. D.F. SPECK, D.R. McCRIMMON* AND J.L. FELDMAN. Departments of Physiology and Anesthesia, Northwestern University, Chicago, IL. 60611.

We examined the effects on respiratory neural outflow elicited by synchronous electrical stimulation (25-100 uA, 100 us, 1-300 Hz) of bulbospinal inspiratory neurons. Experiments were conducted in chloralose-urethane anesthetized, paralyzed, vagotomized and artificially ventilated cats. Ventolateral C2 spinal cord stimulation at intensities of 100 uA antidromically activated approximately half of the inspiratory premotor neurons in the ventrolateral nucleus of the tractus solitarius and the nucleus retroambigualis. Stimulation pulses elicited a 2-6 msec orthodromic excitation (2-4 msec onset latency) of the ipsilateral phrenic nerve, followed by a 4-30 msec period of inhibition of phrenic nerve discharge. However, continuous stimulation had little effect on the duration of inspiration or expiration. Similarly, brief trains of bilateral spinal cord stimulation had only a transient effect (< 60 msec) on the burst pattern of phrenic nerve discharge. Since synchronous activation of a portion of a rhythm generator would be expected to produce a phase shift, we conclude that the bulbospinal respiratory neurons do not themselves generate respiratory rhythmicity and have limited (or no) collateral projections to the neurons that do. (Supported by NIH grants NS-17489, HL-00554, and HL-06331 and the Parker B. Francis Foundation.)

SKELETAL AND SMOOTH MUSCLE PHYSIOLOGY

21.1

EFFECT OF ATP AND ATP ANALOGS ON Ca^{2+} BINDING TO GLYCEROL-EXTRACTED RABBIT PSOAS MUSCLE FIBERS. Franklin Fuchs, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

A double isotope procedure was used to measure the binding of Ca^{2+} to (detergent + glycerol)-extracted psoas fiber bundles, a) in rigor, b) in the presence of nucleotides which bind to myosin but do not energize contraction (ADP and AMPPNP), and c) in the presence of nucleotides which energize contraction (ATP and ITP). Rigor fibers bound a maximum of four moles Ca^{2+}/mole troponin-C, with half-maximal binding at pCa 6.7-6.9. The presence of ADP or AMPPNP had no influence on binding affinity or number of binding sites. In the presence of ATP there was less binding at all pCa values (maximum ~ three mole Ca^{2+}/mole troponin-C), both in unloaded fibers and fibers generating isometric force. Binding in the presence of ITP was identical to that in the presence of ATP. However, Ca^{2+} regulation was severely impaired with ITP, so that at pCa 7.0 ITP-energized fibers generated 80-90% maximal force as compared to <10% maximal force generated by ATP-energized fibers. These results suggest that, 1) cycling of force-generating cross-bridges causes a reduction in Ca^{2+} binding, and 2) the amount of Ca^{2+} bound by the working filament lattice depends on the free [Ca^{2+}] but not on the steady-state force developed. (Supported by NIH AM10551).

21.2

THE EFFECT OF ACID pH ON THE ACTIVATION AND CALCIUM BINDING OF RABBIT SKELETAL MYOFILAMENTS. R. John Solaro, Bo-Sheng Pan* and Edward M. Blanchard. University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

The effects of acid pH on Ca activation of rabbit skeletal muscle myofilaments predict a decrease in the affinity of regulatory sites on troponin (Tn) for Ca. Yet reports in the literature on pure Tn or Tn in rigor myofilaments show no effects of acid pH on bound Ca. We compared directly the effect of acid pH on ATPase activity of rabbit psoas myofilaments and bound Ca under the same incubation conditions--2 mM MgATP, 2 mM free Mg, 0.12 ionic strength. The ATPase activity of the myofilaments was half maximal at pCa 5.83 at pH 7.0, 5.54 at pH 6.5 and 5.43 at pH 6.2. These shifts in sensitivity were the same whether or not EGTA was used to buffer pCa. The amounts of Ca bound to myofibrils or chemically skinned fibers as a function of pCa decreased as pH was lowered from 7.0 to 6.5 to 6.2 in a manner predicted from the shifts in the activity associated with these decreases in pH. At saturating pCa's myofilament Tn bound 4 mol Ca/ mol regardless of the pH. These results indicate that the effect of acid pH on skeletal myofilaments is due to a change in Tn Ca binding properties and that to show this effect may require that Tn be in the myofilament lattice.

21.3

EFFECT OF INCLINE RUNNING ON CONTRACTILE AND BIOCHEMICAL PROPERTIES OF SKELETAL MUSCLE. K.M. Baldwin, W.M. Mullin*, D.B. Thomason*, and R.E. Herrick*. Physiology Dept., Univ. Calif., Irvine, CA 92717.

This study examined the effects of 12 wks of incline running (15 meters/min, 30% grade, 90 min/day) on selected in situ derived contractile properties and Ca^{++} regulated myofibril (Myo) ATPase of rodent medial gastrocnemius (MG) muscle. Four groups were studied: normal-sedentary (NS), normal-runners (NR), overload-sedentary (OS), and overload-runners (OR). MG overload was done by surgical removal of synergists. Twitch contraction and one-half relaxation times were not different among the groups, suggesting that sarcoplasmic reticulum function was not altered. Compared to the NS group, all experimental groups demonstrated a decrease in relative isometric force output for a given stimulation frequency (5-75 Hz; $p < 0.05$). Maximal tetanic force normalized for muscle mass was similar among the groups. All three experimental groups demonstrated an increased resistance to fatigue while contracting isometrically under tetanic stimulation conditions covering a 20 min period relative to the NS group ($p < 0.05$). Consistent with the above functional data, the three experimental groups demonstrated a decrease in Myo ATPase in the oxidative regions of the muscle relative to the NS group ($p < 0.05$). The relative magnitude of the adaptive change among groups was as follows: OR>OS>NR>NS. These data suggest that dynamic locomotion against an incline requiring increased force production in fast-twitch skeletal muscle induces a reduction in contractile protein energy turnover, which could increase contractile effectiveness. Supported by NIH 30346-01A1.

21.4

DOSE DEPENDENT LENGTH-TENSION CURVES OF VASCULAR SMOOTH MUSCLE. Joel M. Price and D.L. Davis. Univ. of South Florida, Department of Physiology, College of Medicine, Tampa, Florida 33612.

Previous studies have found the dose-response relationship depends on length.[1] These studies suggest that the shape of the length-tension (L-T) relationship may depend on the concentration of agonist.[2] The objective of this study is to test this hypothesis directly using ring segments of the dog anterior tibial artery. Media thickness and internal circumference (or muscle length) of the rings were measured on-line with a video caliper. The active force at each length was normalized to the maximum active force in a L-T experiment. Length was normalized to the initial length for resting force. The normalized L-T curve with 10^{-6}M norepinephrine (NE) was significantly lower than the normalized curve with 10^{-5}M NE. The length for maximum active force and the initial length for an active response were significantly longer with 10^{-6}M NE than with 10^{-5}M NE. Repeated L-T curves using the same concentration of NE were obtained from the same vessel in a separate series of experiments. There was no significant difference between the repeated curves when 10^{-5}M NE was used or when 10^{-6}M NE was used. We conclude that the L-T relationship of the dog anterior tibial artery depends on the concentration of NE.

1. Price, J.M., et al., Am. J. Physiol. 241: H557-H563, 1981.
2. Price, J.M., et al., Am. J. Physiol., In Press, 1983.
Supported by NIH Grant #HL 21103 and Am. Heart Assoc. Florida Affiliate.

21.5

COMPARISON OF MEMBRANE POTENTIALS IN SMOOTH MUSCLE CELL CULTURES EXPOSED TO OUABAIN, LOW TEMPERATURE AND LOW POTASSIUM F.A. Kutyna*, H.J. Bryant, M.B. Pamnani and F.J. Haddy. Dept. of Physiology, Uniformed Services Univ., Bethesda, MD 20814.

The magnitude of the resting transmembrane potential (E_m) in arterial smooth muscle depends to a considerable extent on the activity of the Na^+-K^+ electrogenic pump. The present study compares the effects of some Na^+-K^+ pump inhibiting conditions on the E_m of arterial smooth muscle in cell culture. Smooth muscle from Wistar rat tail arteries was grown in primary cell culture. The plated cells were allowed to grow in a modified GIBCO 199 medium for 7 to 10 days before recording. The cell monolayers were superfused with modified Krebs-Henseleit (KH) solution at 37°C which contained from 0.1 to 100 mM potassium or 0 to 10^{-2}M ouabain (at K^+=5.9 mM). The effects of these solutions on transmembrane potential were compared to that produced by 0.1 mM K^+ (KH) superfused at 15°C. Cells maintained an average E_m of 67.3±1.0 mV over the range of 0 to 10^{-5} M ouabain whereupon they increasingly depolarized at higher concentrations, reaching 14 mV at 10^{-2} ouabain. Superfusion of 0.1 mM K^+ (KH) at 37°C depolarized the cells to 34.9±1.9 mV. 3 mM K^+ (KH) slightly hyperpolarized them to 72.5±1.7 mV and at normal 5.9 mM K^+ (KH) the Em was 65.9±1.02 mV. Cells cooled to 15°C in 0.1 mM K^+ (KH) depolarized to 12.6±1.0 mV. Ouabain at a concentration of 10^{-2} M appears to be as effective a depolarizing agent as the combination of low temperature and low potassium in this cell model. (Supported by NIH Grant HL21525-05, USUHS C07605 and C07607)

21.6

LIPID SOLUBLE TOXINS FROM A DINOFLAGELLATE, *GAMBIERDISCUS TOXICUS*, ISOLATED FROM A CARIBBEAN REGION SUPPORTING CIGUATERIC FISH. Donald M. Miller, Robert W. Dickey* and Donald R. Tindall*, Depts. of Physiology and Botany, SIU-C, Carbondale, IL, 62901.

A crude (GT) and three purified (GT-1, 2, and 3) ether-soluble, acetone precipitated, extracts were isolated from mass cultures of a dinoflagellate, *Gambierdiscus toxicus*. The crude extract (GT) when tested on 20 g mice resulted in an LD-50 of 99 µg within 48 hours. All four extracts at a concentration of 4 mg/ml were effective against; the synapse, nerve and striated muscle in the frog sciatic nerve-muscle preparation; and acetylcholine and histamine receptors on smooth muscle in the guinea pig ileum. The three purified extracts (GT-1, 2 and 3) from silicic acid chromatography were effective at 5 ng/ml on the guinea pig ileum preparations. Tetrodotoxin was utilized to suppress nervous elements in the ileum preparation in order to establish the effect of GT-1, GT-2, and GT-3 on the ileal muscle and the results followed Michaelis-Menten kinetics for a competitive inhibition of both histamine and acetylcholine receptors. Dose ratio determinations of a further purification of GT-2, allowed us to estimate an apparent affinity constant for that component. This study has established the presence of multiple toxins in the dinoflagellate, *G. toxicus*, outlined a method for their assay in small quantities, and identified at least one of the major effects of these toxins in animals. (Supported by: Dept. of Botany, Sch. of Med. SIU-C, and FDA Contract #223-79-2287)

21.7

TIME DEPENDENT POTENTIATION OF THE RATE OF FORCE REGENERATION FOLLOWING QUICK RELEASE IN CANINE TRACHEAL SMOOTH MUSCLE. Susan J. Gunst. Mayo Clinic & Fdn., Rochester, MN 55905.

Trachealis strips were mounted in a tissue bath between a force transducer and a rod which could be moved at constant rate (20 mm/sec) to alter muscle length over a preset distance. Muscle length, tension (P), and dP/dt were continuously recorded. Lmax was first determined as the length at which a standard electrical stimulus produced maximal active tension. The muscle was then stimulated at Lmax using a supramaximal stimulus (60 cps, 15 v) and released to .90 Lmax at various time intervals following the onset of force development (FD). Releases were performed prior to the onset of FD (0 sec), at peak dP/dt ($\sim$ 0.5 sec), during the decline in dP/dt (1,2,5 and 10 secs) and after a plateau in force had been achieved (15 sec). Force dropped to zero during the release. Stimulation was continued for 15 sec following release and dP/dt recorded. Instantaneous dP/dt was plotted against P for force redevelopment (FR) at each release time. Releases performed between 0 and 0.5 sec had little effect on dP/dt during FR. Releases performed after peak dP/dt resulted in a marked initial potentiation of the rate of FR which diminished as force increased but did not alter maximal force achieved. Potentiation was greatest at 2 sec, where peak dP/dt increased to 2.45 ± 0.6 (SD)(n=6) of peak dP/dt at 0 sec. After 5 sec, depression of dP/dt during FR was present at all forces, and a lower maximal force was achieved. Supported by HL29289, HL21584 and the Parker B. Francis Fdn.

21.8

COMPARISON OF CELL MEMBRANE CONFIGURATION IN RELAXED AND CONTRACTED SMOOTH MUSCLE. L.J. McGuffee, E.S. Wheeler-Clark* and S.A. Little*. Univ. of New Mexico, Albuquerque, N.M. 87131

As a smooth muscle contracts it can shorten markedly. This decrease in length has previously been suggested to be associated with an increase in folding and wrinkling of the plasma membrane. In the present study, we have attempted to relate the extent of wrinkling of the plasma membrane with the contractile state of the tissue. Cells of the rabbit renal artery were exposed to norepinephrine (NE) to induce contraction or to nitroprusside (NP) to induce relaxation. Tissues were prepared for microscopic examination as previously described (McGuffee et al., J. Cell Biol. 90:201-210, 1981). Thick, 0.5 µ sections were cut and were photographed at 400 X magnification. All photography and analysis of the micrographs were carried out in a blind manner. Cells were classified as either wrinkled or smooth depending on the extent of folding of the plasma membrane. The results indicate that the degree of wrinkling of the plasma membrane could not be related to the contractile state of the tissue. Thus, under the conditions of this study, no consistent difference in the configuration of the cell surface in contracted vs. relaxed cells was observed. (Supported by NSF Grant PCM 79-11230)

21.9

MEASUREMENT OF INTRACELLULAR IONIC ACTIVITY WITH ION-SELECTIVE MICROELECTRODES IN GASTROINTESTINAL SMOOTH MUSCLE. N.L. Shearin. Univ. of Utah Medical Center, Salt Lake City, Utah 84132

The development of liquid ion selective microelectrodes (ISE) has made possible direct intracellular measurements of ionic activity within small cells. However, the use of these microelectrodes in smooth muscle has been limited and few measurements of intracellular ionic activity have been reported in smooth muscle from the gastrointestinal tract. The methodology of ISE construction, calibration, and use will be outlined and different types of ISE will be discussed. The difficulties, limitations, and advantages of using ISE will also be discussed. Some applications of the ISE in research problems encountered in studying the physiology of gastrointestinal smooth muscle will be analyzed.

23.1

STATUS OF ELECTROGENIC PROTON PUMP IN GASTRIC MUCOSA IN LIGHT OF RESULTS OF VESICLE STUDIES. W. S. Rehm, M. Schwartz, and G. Carrasquer. Univ. of Louisville, Louisville, KY 40292.

In previous work, it has been found for the in-vitro frog gastric mucosa that with Cl-free media, the PD is inverted (secretory side becomes positive). A number of explanations, other than the postulate of an electrogenic proton pump, are possible for the inverted PD. However, it was found with Cl-free media that during inhibition of the H^+ rate, there is a precise linear relationship between the PD and the H^+ rate which cannot be easily explained except by an electrogenic proton pump. However, work on gastric vesicles show that there is an ATP-driven neutral pump in which K exchanges with H^+ and the possibility that the inverted PD is due to a K gradient from the cell to the secretory fluid has been considered. However, this latter postulate is untenable since we will show that a) in Cl-free media, the K partial conductances of the secretory and nutrient membranes are equal and b) with 80 K (K for Na) in both bathing fluids, the magnitude of the PD is the same as with regular (K = 4 mM) solutions. The latter finding would demand, with the K gradient hypothesis, absurdly high levels of cellular K. We will also present evidence that makes untenable the possibility of other ion gradients producing the inverted PD. Electrogenic proton pump models incorporating the neutral H^+-K^+ mechanism will be presented. (NSF support)

23.2

ELECTROPHYSIOLOGICAL STUDIES ON INDIVIDUAL CELLS OF ISOLATED GLANDS OF RABBIT GASTRIC MUCOSA. Trifone Schettino+, Martin R. Köhler+ and Eberhard Frömter+(SPON: J.G. Forte). Max-Planck-Institut für Biophysik, 6000 Frankfurt, Fed. Rep. Germany

Rabbit gastric tubules were isolated either by collagenase or by microdissection. The former technique yielded gland fragments which were fixed in a perfusion chamber to a layer of agarose, whereas the latter yielded whole glands which were held in suction pipettes. Mitochondria-rich parietal cells and mitochondria-poor chief cells were distinguished by an autofluorescence which presumably arises from mitochondrial flavines, or by staining of the mitochondria with rhodamine 6 GO. This proved more reliable than staining of presumably acid regions in the cells by acridine-orange. Cells of collagenase-treated glands were easy to impale with microelectrodes but showed spontaneous sinusoidal potential fluctuations of ~3 mHz frequency and up to 20 mV amplitude. Cells of microdissected glands were more difficult to impale and the distinction between membrane potentials and microelectrode tip artifacts was critical. Nevertheless constant membrane potentials of the order of -30 mV (and occasionally -60 mV) were recorded, both in parietal cells and in chief cells, which responded as expected to elevation of bath K^+ concentration and to harmaline (1 mmol/l) which is thought to reversibly inhibit the Na^+/K^+ pump. Instead of a transient depolarization, replacement of Cl^- by SO_4^{--} hyperpolarized the parietal cells by up to -40 mV which cannot be explained as tip potential artifact.

23.3

GASTRIC EPITHELIAL DESQUAMATION AND RAPID REPAIR. S. Ito,* E.R. Lacy, M.J. Rutten and W. Silen. Harvard Medical School and Beth Israel Hospital, Boston, MA 02115

Destruction of gastric epithelial cells by hypertonic solutions, ethanol, or aspirin has been acknowledged but the subsequent rapid repair process or its fine structural details has not been elucidated or fully recognized. In recent studies we have used in vivo rat and in vitro guinea pig and frog stomachs to study the damage and repair process. Rat stomachs briefly exposed to absolute ethanol loose their surface epithelium but about half of the exposed basal lamina is covered within 7 min by migrating mucous cells. Within 15 min there is about 85% coverage and virtually complete reconstitution within an hour. The Ussing chambered guinea pig stomach preparation, exposed to hypertonic NaCl shows extensive cell destruction followed by morphological reconstitution of the epithelium in about 20 min and recovery of its electrical properties within 90 min. The chambered bullfrog mucosa is severely damaged by 1M NaCl but reconstitutes its epithelial lining in 1-2 hrs with electrical recovery in 4-6 hrs. These features and other parameters which inhibit or promote the repair process will be presented.
Supported in part by NIH grants Am-30303, AM-31158, AM-06345.

23.4

TRANSPORT OF H^+ AND HCO_3^- IN NECTURUS GALLBLADDER EPITHELIUM. Luis Reuss and Steven A. Weinman* Washington University School of Medicine, St. Louis, MO. 63110.

Intracellular and extracellular electrophysiologic techniques and tracer unidirectional Na uptake measurements were employed to study the mechanism of NaCl uptake across the apical membrane of Necturus gallbladder epithelium. Amiloride-inhibitable Na/H exchange occurs at this membrane in the presence and nominal absence of HCO_3, as shown by measurements of luminal acidification, effect of luminal Na and amiloride on intracellular pH, and effect of luminal pH on intracellular Na. The increase in intracellular Na produced by sudden mucosal Na addition to ouabain-treated, Na-depleted tissues, and the unidirectional tracer Na uptake into gallbladders incubated under control conditions were both inhibited by about 50% by amiloride (1 mM), suggesting that at least 50% of Na entry is through this pathway. The rate of luminal acidification was doubled immediately after reducing luminal [Cl] (from 114 to 4.5 mM, cyclamate substitution), and decreased slowly during prolonged exposure to the low-Cl medium. Immediately after replacing the full luminal [Cl], alkalinization was observed. These effects can all be explained by Cl-gradient driven HCO_3 (or OH) fluxes. We conclude that Na/H and Cl/HCO_3 (OH), exchanges coexist at the luminal membrane of this epithelium and account for a major portion of NaCl entry. Supported by NIH Grant AM 19580.

23.5

BUFFER BASE TRANSPORT BY RENAL DISTAL TUBULES. Maurice B. Burg. NHLBI, NIH, Bethesda, MD 20205

Previously, rabbit cortical collecting ducts (CCD) perfused in vitro were observed to either absorb or secrete bicarbonate depending on whether the animals from which they were obtained were acidotic or alkalotic. Recent studies with rat CCD give virtually identical results. In contrast rabbit outer medullary collecting ducts (OMCD) from both acidotic and alkalotic rabbits absorbed bicarbonate, but the rate was paradoxically higher in OMCD from alkalotic animals. Recent studies with rat OMCD yield the same result, except the absolute rates of transport are three times as high. Rabbit thick ascending limbs previously were not found to transport bicarbonate. In contrast, significant bicarbonate absorption is now found in rat TAL. Rat TAL now are also found to absorb considerable amounts of ammonia with equal ammonia concentrations in the perfusate and bath. The ammonia transport is inhibited by furosemide and cannot be by nonionic diffusion since the lumen fluid is acidic. Ammonia absorption by TAL may represent a single effect in counter current multiplication of ammonia, contributing to the high ammonia concentrations observed in renal medulla and urine. The mechanisms of the buffer base transport in these segments, and their role in urinary acidification will be discussed.

24.1

TRANSMURAL UNIFORMITY OF CORONARY BLOOD FLOW IN HYPOXIC, NON-ISCHEMIC, LEFT VENTRICULAR MYOCARDIUM. H. Fred Downey, George J. Crystal, Arthur G. Williams*, and Fouad A. Bashour. Univ. Texas Health Science Center, Dallas, Texas 75235

The left anterior descending coronary artery (LAD) of eleven anesthetized dogs was cannulated and perfused with blood deoxygenated in an extracorporeal lung. Thus, the transmural distribution of coronary blood flow was determined in the absence of hemodynamic and reflex changes that accompany whole body hypoxia. Regional myocardial blood flow was measured with 15 micron radioactive microspheres administered into the LAD perfusion line during normoxic perfusion (control) and after 3 min of hypoxic perfusion. LAD perfusion pressure (control, 100 ± 2 mmHg) equaled mean aortic pressure. Hypoxia had no effect on aortic and left atrial pressures or heart rate. Mean ± SE flows (ml/min/g):

Control			Hypoxia		
Epi	Mid	Endo	Epi	Mid	Endo
0.81±0.08	0.82±0.09	0.81±0.08	4.4±0.4	4.9±0.4	4.7±0.4

Mean coronary flow increased by 576%, but remained uniform transmurally (P> 0.4). These results are similar to our findings in myocardium whose coronary vasculature was dilated by ischemia or by pharmacologic agents (Circ. Res. 37: 111-117, 1975) and are consistent with a transmural gradient of vascularity favoring the subendocardium. Supported by HL-21657 and the Cardiology Fund.

24.2

AN α_1-ADRENOCEPTOR CORONARY CONSTRICTION DURING PARTIAL CORONARY STENOSIS. Carl E. Jones, Isabella Y.S. Liang, Tim A. Farrell*, and Robert Ator*. Texas College of Osteopathic Medicine, Fort Worth, TX, 76107.

Studies using general α-adrenoceptor blockers have suggested an α-constriction in the coronary circulation under certain conditions. However, the increase in coronary flow with general α-blockade in these conditions may be due to blockade of postsynaptic α_1- or presynaptic α_2-receptors with a consequent increase in NE release and metabolic vasodilation. In the present experiments, the left coronary artery of dogs was perfused at constant pressure. Partial stenosis was simulated by reducing pressure to 50 mmHg. Effects of the selective α_1-blocker prazosin (2 mg i.c. over 20 min, N=6)and the general α-blocker phenoxybenzamine (0.25 mg/kg i.c. over 30 min, N=5) on ventricular Contractile Force, Flow, $(a\text{-}v)O_2$, and $M\dot{V}O_2$ were measured. Prazosin increased Flow, $M\dot{V}O_2$, and Contractile Force by 21% (P<0.05), 12% (P>0.05), and 18% (<0.05), respectively. Phenoxybenzamine increased these variables by 40% (P<0.05), 37% (P<0.05), and 48% (P<0.05), respectively. All increases were greater with phenoxybenzamine (P<0.05), but the increase in Flow more paralleled the increase in $M\dot{V}O_2$. These results demonstrate an α_1-adrenoceptor constrictor tone during coronary stenosis. However, the increased coronary flow caused by a general α-blocker may be attributed to blockade of both α_1- and α_2-receptors. (Supported by the AHA with funds contributed in part by Texas Affiliate).

24.3

EFFECTS OF ALPHA ADRENERGIC TONE ON CORONARY TRANSCAPILLARY EXCHANGE. M.H. Laughlin, K. Rouk* and S. Mohrman*. Oral Roberts U., Tulsa, OK 74171

We were interested in the effects of neurohumeral influences on coronary capillary exchange. We have observed that coronary infusions of phenylepherine (1-20 µg/min) produce no significant effects on capillary permeability-surface area products (PS). The purpose of this study was to determine the effects of α_1 adrenergic blockade (Prazosin, 2-5 mg i.a.) on coronary PS with blood flow autoregulation intact and during maximal vasodilation with adenosine (Ado)(3-10 µmoles/min i.a.). ^{51}Cr-EDTA extractions and PSs were determined with the single injection indicator diffusion method (^{125}I-albumin reference) in intact working hearts of anesthetized dogs (Nembutal 30 mg/kg. The LAD branch of the left coronary artery was cannulated and pump-perfused under constant pressure conditions (≈ 100 torr) while aortic, central venous and coronary perfusion pressures, heart rate, ECG and coronary flow were monitored.

	Baseline	α_1 Block	Adenosine	α_1 Block & Ado
PS	21 ± 2	28 ± 3	48 ± 5	54 ± 7
F	62 ± 6	106 ± 12	291 ± 33	328 ± 38
n	12	12	16	16

($\bar{x}$ ± SE). F = plasma flow. PS and F units of ml/min/100g.

α block caused significant ($p < 0.05$) increases in F and PS under both conditions. However, with autoregulation intact α block caused a greater increase in F than PS (71% vs 31%) while during Ado plus α block the relative increases in PS and F were similar. Supported by NIH #HL 26963.

24.4

EVIDENCE THAT AN ALPHA-ADRENERGIC VASOCONSTRICTION LIMITS CORONARY FLOW AND CARDIAC FUNCTION DURING EXERCISE IN DOGS. Patricia A. Gwirtz, Dean Franklin, and Howard J. Mass*, Texas College of Osteopathic Medicine, Fort Worth, TX 76107, and University of Missouri, Columbia, MO 65201.

Alpha-adrenergic receptor modulation of coronary blood flow and cardiac function was examined in exercising dogs. Dogs were chronically instrumented to measure left circumflex blood flow velocity (CBFV, pulsed Doppler), heart rate (HR), regional left ventricular function (systolic shortening, %ΔL and velocity of shortening, dL/dt using sonomicrometers) and global left ventricular function (left ventricular pressure, LVP, and dP/dt). The specific postsynaptic alpha$_1$-receptor antagonist prazosin (0.5 mg) and nonselective alpha-blocker phentolamine (1.0 mg) were injected through an indwelling coronary artery catheter to produce local adrenergic blockade during exercise. Exercise significantly increased HR (103-250 bpm), LVP (119-150 mmHg), peak +dP/dt (2430-6100 mmHg/sec), %ΔL (15-24%) and dL/dt (1.18-2.15 mm/sec/EDL), and CBFV (21-42 cm/sec). Neither antagonist caused changes in HR, LVP, or %ΔL, but with both antagonists, increases in dP/dt (+1291 mmHg/sec) and dL/dt (+0.64 mm/sec/EDL) were associated with increases in CBFV (+10 cm/sec). It is suggested that an alpha-vasoconstriction limits both coronary vasodilation and cardiac function, during exercise. Supported by NIH Grant #RO1-HL31144 and RO1-HL26436.

24.5

INHIBITION OF β-ADRENERGIC RELAXATION BY PROSTAGLANDIN SYNTHESIS IN ISOLATED CORONARY ARTERIES: MECHANISM OF HYPOXIC VASOSPASM? Gabor Rubanyi* and Richard J. Paul. Dept. of Physiology, Univ. Cincinnati, College of Medicine, Ohio, 45267, U.S.A.

In a recent report (Fed. Proc. 42:1347), we showed that a decrease in bath pO2 from 95% to 40%, 20% or 10% (hypoxia) elicited a contraction in isolated porcine coronary arteries. This hypoxic vasoconstriction was abolished by either indomethacin or aspirin. Isoproterenol, which relaxed these coronary vessels, potentiated the PG-mediated hypoxic vasoconstriction indicating that hypoxia may inhibit β-adrenergic relaxation. These results suggested that β-adrenergic relaxation may be modified by intrinsic PG's. We tested this hypothesis by measuring isoproterenol dose-relaxation curves for isolated porcine coronary artery rings under conditions in which PG synthesis was stimulated (decreasing bath pO_2 from 95% to 40%) or inhibited (5.5µM indomethacin). Although the absolute sensitivity of the coronary arteries to isoproterenol varied with the vasoconstrictor (histamine>ouabain>KCl), stimulation of PG synthesis by decreasing pO_2 inhibited, while indomethacin significantly potentiated β-adrenergic relaxation. To our knowledge, this is the first evidence that intrinsic vascular PG synthesis modulates β-adrenergic relaxation. This PG-mediated, pO_2 sensitivity of β-adrenergic relaxation may underlie the phenomenon of hypoxic vasoconstriction in the large coronary arteries. Supported in part by NIH 1RO1 23240 and AHA Established Investigatorship #81-148 (RJP).

24.6

EFFECT OF PICROTOXIN ON REGIONAL CORONARY VASCULAR TONE IN CATS. S.A. Segal and J.E. McKenzie. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814

We have previously shown that the GABA antagonist drug, picrotoxin (PT), causes sympathetically-mediated coronary vasoconstriction in cats. To assess the effect of this vasoconstriction on the distribution of coronary blood flow (CBF) within the myocardium, regional CBF was measured in 4 regions of the left ventricle in 11 chloralose-anesthetized, vagotomized cats. Fifteen µm radiolabeled microspheres were injected immediately prior to and at various times following the administration of 2 mg/kg PT i.v. Results obtained in the whole left ventricle reconfirmed those of previous studies: coronary vascular resistance [CVR (mm Hg·ml^{-1}·min·100g)] increased significantly after PT administration ($\bar{x}$= +.12±.01, P<0.05). On a regional basis, control CVR was significantly higher in the epicardium than in the endocardium ($\bar{x}$= .40±.02 vs .34±.02, P<0.05). The PT-induced increase in CVR was significantly higher in the anterior epicardium than in the posterior epicardium, anterior and posterior endocardium ($\bar{x}$= +.16±.02 vs. +.12±.03, +.10±.03 and +.09±.02, P<0.05). CBF decreased following PT administration in all regions, with the anterior epicardium showing the greatest decrease, despite a tendency for arterial pressure to increase. A controversy exists regarding the differential effects of sympathetic innervation on epi- vs. endocardial coronary arteries. These data support the concept that there may be regional differences in sympathetic innervation to the coronary arteries. (USUHS Grant RO7682)

24.7

EFFECTS OF PYRUVATE (Pyr) AND LACTATE (Lac) ON MYOCARDIAL ADENOSINE (AR) RELEASE. R. Bünger, R.R. Curnish, R.M. Berne. Departments of Physiology, Uniformed Services University, Bethesda, MD, and University of Virginia, Charlottesville, VA

In isolated working guinea pig heart (preload: 12 cm H_2O, afterload: 90 cm H_2O) the influence of exogenously supplied Pyr (0-2.0 mM) and Lac (0-2.0 mM) on coronary effluent AR was studied. Pyr and Lac were determined enzymatically, AR was measured with standard HPLC techniques. Since 15 mM glucose plus 10 mM acetate (in presence of 5 U/l insulin) were the main energy-providing substrates, the concentration of coronary venous Pyr + Lac was similar to the concentration of the infused arterial Pyr + Lac (Pyr + Lac = 1.0 or 2.0 mM). 2.0 mM Pyr caused a 14.2±6.3% decrease ($p<0.05$) in AR release (control: 932±102 pmol/min x g dry wt, n=12) but 2.0 mM Lac did not significantly change AR release. Similarly, low arterial Lac/Pyr ratios (0.1 to 1.0) resulted in small decreases in AR release whereas with high arterial Lac/Pyr (>10-100) AR release was increased slightly in 5 out of 6 hearts. Myocardial oxygen uptake ($M\dot{V}O_2$) did not appreciably change under the same conditions. In contrast, 0.2 µM norepinephrine (NE) strongly stimulated $M\dot{V}O_2$ resulting in an approx. 3-fold increase in AR release in the absence of exogenous Pyr and Lac. Coronary resistance decreased approx. 24%. Obviously, changes in the cytosolic redox state produced by exogenous Pyr plus Lac can be associated with small, but significant, changes in cardiac AR metabolism. However, the influence of NE enhancement of myocardial energy demand is of comparatively major importance.

24.8

ADENOSINE IS UNIMPORTANT FOR UNSTRESSED CORONARY REGULATION. Keith Kroll* & Eric O. Feigl, Dept. of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

If adenosine (Ado) is important in the regulation of resting coronary artery blood flow, then an imposed decrease in interstitial Ado should decrease coronary flow. This was tested in closed-chest dogs where the left main coronary artery was cannulated and perfused at a constant pressure. Histamine was infused into the entire perfused bed through the main cannula in order to increase vascular permeability to macromolecules, while 1600 U of adenosine deaminase (ADA) was infused selectively into one region of the coronary vascular bed. After the cessation of both infusions, regional coronary flow was measured with radioactive microspheres. Flow in the region selectively treated with ADA was not different from flow in the untreated control region of the same heart, indicating that Ado is unimportant in flow regulation. Functional enzymatic activity in the treated region was assessed in two ways. 1) During an infusion of exogenous Ado into both regions, the flow increase in the treated region was about 40% less than the flow increase in the control region, demonstrating the action of ADA. 2) When EHNA (an ADA inhibitor) and Ado were infused simultaneously, flow in both regions was the same, indicating the specificity of the enzyme's action.

It is concluded that adenosine is not important in the regulation of coronary blood flow under unstressed conditions.

(Supported by NIH grants HL 16910 & HL 07090.)

24.9

HEART RATE (HR) AND CORONARY FLOW (CF) EFFECTS OF ADENOSINE (ADO) AND ADO ANALOGUES IN GUINEA PIG HEART. R. P. Steffen, S. J. Haleen*, and D. B. Evans*. Warner-Lambert/Parke-Davis Pharm. Res. Ann Arbor, MI 48105

The purpose of this study was to determine the HR and CF effects of ADO and the ADO analogues; R-N^6-phenylisopropyladenosine (R-PIA) and 5'-N-(ethylcarboxamide) adenosine (NECA) in the isolated spontaneously beating non-working heart of mature guinea pigs. A microprocessor driven system was used to maintain drug concentration and coronary perfusion pressure constant independent of changes in CF. Values are threshold molar concentrations producing significant changes. Through biochemical and binding studies ADO receptors have been classified as A_1 or A_2.

	ADO	R-PIA*	NECA*
HR	10^{-6}	3×10^{-8}	10^{-7}
CF	10^{-7}	3×10^{-8}	10^{-9}

*Asystole at 10^{-7}

Studies from this laboratory, using rabbits, have reported that ADO's effect on HR and CF are through A_1 and A_2 receptors respectively (Fed. Proc. 41:1736, 1982). We report here that in the guinea pig, ADO receptor selectivity is the same as in the rabbit with an A_1 potency of R-PIA>NECA>ADO and A_2 potency of NECA>R-PIA ADO. Both species are equally sensitive to these agents for A_1 (HR) effects yet the guinea pig is 30-100 times more sensitive to the agents for A_2 (CF) effects. Whether this species difference is anatomic or physiologic in origin and what the physiologic significance is for a species difference in A_2 receptor activity awaits further study.

24.10

MYOCARDIAL CAPILLARY AND ARTERIOLAR PERFUSION RESERVE WITH VASODILATION. H.R. Weiss, G.J. Grover*, J. Kedem*, and M. Rosolowsky*. U.M.D.N.J.- Rutgers Medical School, Dept. of Physiology and Biophysics, Piscataway, N.J. 08854.

The purpose of this study was to compare the effects of vasodilation caused by hypoxia (8-12% O_2) and adenosine (0.4 mg/kg/min) on various morphometric indices of the arteriolar and capillary bed reserve of the rabbit heart. FITC-dextran was injected into rabbits under control, adenosine infusion and hypoxic conditions to label the perfused vessels for fluorescent microscopy. The tissue was then stained for alkaline phosphatase for detection of the total capillary and arteriolar bed. Standard morphometric techniques were used to find total and perfused capillary/arteriolar number/mm^2 and volume and surface area/mm^3. Under control conditions, 52±6% and 57±5% (Mean ± SE) of the arteriolar and capillary bed volume/mm^3 were perfused. With hypoxia, both arteriolar and capillary bed volume/mm^3 increased greatly, 81±7% and 96±1%. Adenosine infusion increased arteriolar values close to maximum, 91±8%, but increased capillary values significantly less, 71±11%. No significant subepicardial vs. subendocardial differences were found under any condition. These results show that control hearts have a high degree of arteriolar/capillary reserve that is mobilized equally by hypoxia but adenosine mobilizes arteriolar reserve to a greater extent than capillary.

24.11

VISCOELASTIC BEHAVIOR OF IN VIVO CORONARY ARTERY CAPACITANCE. J. M. Canty, Jr.*, R. E. Mates and F. J. Klocke. SUNY at Buffalo, N.Y. 14215

Although excised arteries exhibit viscoelastic effects, it is uncertain whether the magnitude of coronary capacitance is influenced by the rate of pressure change applied. Accordingly, the cannulated circumflex artery of seven open-chest heart-blocked dogs was perfused with a programmable pressure control system during adenosine vasodilation. During long diastoles coronary pressure was made to decline and subsequently rise at a constant rate (dP_{LC}/dt) and pressure-flow curves were constructed over a pressure range of 25-75 mm Hg. For a given dP_{LC}/dt, capacitance was calculated from the flow differences between the two curves, using an RC model.

dP_{LC}/dt (mm Hg/sec)	Circumflex Pressure 30 mm Hg	50 mm Hg	70 mm Hg
30	20.0 ± 0.95	14.7 ± 0.97	8.93 ± 0.82
50	18.1 ± 0.85*	12.9 ± 0.54*	9.44 ± 0.79
70	17.1 ± 0.69	11.5 ± 0.58*	8.28 ± 0.86
90	16.0 ± 0.71	11.0 ± 0.72	7.53 ± 0.83

Mean ± 1 SEM *$p<0.05$ compared to next lower dP_{LC}/dt

Thus, at any given distending pressure, coronary capacitance decreases as dP_{LC}/dt increases. These data indicate that viscoelastic properties do influence coronary capacitance and capacitive flow in vivo, but to a lesser degree than distending pressure and/or vasomotor tone. Supported by NHLBI #15194.

24.12

DOES RETROGRADE FLOW REPRESENT TOTAL COLLATERAL FLOW? Konrad W. Scheel and Barbara Eisenstein*. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

In an isolated heart system in which coronary flows to the anterior descending, A, circumflex C, right and septal arteries were simultaneously measured at maximal dilation with adenosine, retrograde flows from A were determined before and after embolization of A with 20 and 25 micron spheres in 8 dogs. We found a consistent increase of 100 ± 4.6% in retrograde flow from A with the cannula tip pressure maintained at zero pressure. Embolization of A also increased retrograde flow measured on C by 65 ± 6.4%. There was a linear relationship between graded embolization of A and increase in retrograde flow. Even with optimal embolization of A, a residual flow of 10.3 ± 2.8% of max flow was recorded. Flows to other coronary vessels were not affected by A embolization. The collateral perfusion pressure measured on A after embolization was 89% of C perfusion pressure prior to adenosine infusion and decreased to 62% with vasodilation of C. After embolization of A, the collateral pressure flow relationship was linear with a pressure intercept of zero. We conclude that intramyocardial collaterals may be responsible for the increase in retrograde flow after embolization of A. This work was supported by NIH grant HL 28948.

25.1

REFLEX AUGMENTATION OF ACTIVITY OF UPPER AIRWAY DILATING MUSCLES (UADM) BY ESOPHAGEAL AFFERENTS. Musa A. Haxhiu,* Erik van Lunteren,* Jyoti Mitra* and Neil S. Cherniack. Department of Medicine, Case Western Reserve Univ., Cleveland, OH 44106.

Distention of the esophagus (Eos) inhibits the inspiratory activity of the diaphragm but its effects on other respiratory muscles have not been examined. We recorded the electrical activity of UADM, alae nasi, genioglossus, and posterior cricoarytenoid, as well as that of the diaphragm (D) and inspiratory parasternal (IPS) muscles, in nine anesthetized dogs, before and during esophageal distention. During O_2 breathing, a graded inflation of a balloon positioned in the middle third of the Eos induced a graded inhibition of D with a concomitant progressive augmentation of IPS and UADM activities in all dogs. Distention of Eos with 100 ml of air caused an immediate statistically significant decrease in D activity to 80% of control, and an increase in IPS and UADM activities exceeding 250% in all muscles. There was an insignificant decrease in tidal volume while breathing frequency increased primarily due to shortening of expiratory time. No significant change in minute ventilation, end-tidal PCO_2, mean systemic blood pressure and heart rate was found. Bilateral mid-cervical vagotomy abolished the respiratory effects of Eos distention. These results indicate that visceral afferent inputs can differentially affect motoneurons which control D activity and regulate inspiratory intercostal muscles and UADM activities. (Support: NIH HL-25830, HL-30012, HL-07288, and V.A. Merit Review.)

25.2

REFLEX RESPONSES CAUSED BY PULMONARY C-FIBERS. S.S. Cassidy, W.B. Wead, M.P. Kaufman, J.H. Ashton and Y. Monsereenusorn. Univ. of Texas Health Science Center, Dallas, Texas 75235.

Our purpose was to quantitate the pulmonary depressor chemoreflex (PDC) caused by stimulation of pulmonary C-fiber receptors (PC-fbr) with capsaicin (CAP). In 25 dogs this was made possible using an open-chest preparation in which the left pulmonary artery (LPA) and veins and left airway were cannulated enabling us to inject CAP into the LPA without its entering the systemic circulation. Frequency (f) of diaphragmatic contractions (DC) were monitored with a strain gauge on the diaphragm and systemic blood pressure (BP), heart rate (HR), cardiac output (C.O.), left ventricular contractility (LV contr.), and hindlimb vascular resistance (HVR) were monitored using standard techniques. Gas exchange was maintained by mechanically ventilating the right lung which received the entire C.O. Single fiber afferent nerve impulses were recorded from the left cervical vagus nerve. The CAP dose giving maximum reflex responses (< 10 μg/kg) could be repeated at 10-min. intervals without diminution of reflex responses or PC-fbr discharge. BP, HVR, HR and LV contr. and C.O., fell by 30-40% when CAP was injected through the LPA. CAP caused a 30-60s cessation of DC followed by a return to the pre-CAP f-DC. Left pulmonary vagotomy abolished all responses to LPA injection of CAP. We conclude that stimulation of lung receptors with CAP inhibits brainstem centers via ipsilateral afferent vagal C-fibers to cause apnea without subsequent tachypnea and a substantial depression of all cardiovascular functions.

25.3

RESPIRATORY MECHANORECEPTORS IN THE LARYNX. G. Sant'Ambrogio, O.P. Mathew, J.T. Fisher and F.B. Sant'Ambrogio. Departments of Physiology and Pediatrics, UTMB, Galveston, TX 77550.

The larynx has a rich sensory supply which is the main source of several respiratory reflexes. These reflexes, that influence both the patency of the upper airway and the pattern of breathing, are related to transmural pressure and/or airflow in the upper airway. Yet hardly any information is available on the response of laryngeal mechanoreceptors to transmural pressure and airflow. We recorded action potentials from single fibers separated from the superior laryngeal nerve of anesthetized dogs spontaneously breathing either through a tracheostomy or the upper airway. The airway could be occluded above or below the larynx. On the basis of their behavior during tracheostomy breathing, upper airway breathing, tracheal occlusion and upper airway occlusion, laryngeal mechanoreceptors were classifed as pressure receptors, flow receptors and "drive" receptors (stimulated by the respiratory activity of upper airway muscles). Pressure receptors were encountered most frequently, representing 55% of our sampling (77 receptors), "drive" receptors constituted 26% and flow receptors the remaining 19%. Our findings indicate that, even though the three types of receptors differ in sensory modality, they concur in exhibiting a predominant activity during inspiration. In fact, 90% of all receptors are activated by conditions present during inspiration. Moreover, their activity increases markedly during upper airway obstruction. Supported by NIH Grants HL-20122, 29169, MRC Canada and A.L.A.

25.4

DEVELOPMENT OF SLOWLY ADAPTING AIRWAY RECEPTOR (SAR) ACTIVITY IN THE OPOSSUM. **J.P. Farber, J.T. Fisher, G. Sant'Ambrogio. UTMB, Galveston, TX; OU Health Sciences Ctr., Oklahoma City, OK.**

Characteristics of SAR discharge were evaluated in pentobarbital-anesthesized, gallamine-paralyzed, artificially ventilated, open chested opossums (Didelphis marsupialis). Animals were tested at 20, 30, 55, and 100 days of age; results were compared with data from adults. The percentage of SARs active at a transpulmonary pressure (P_{tp}) of 0 cmH_2O did not vary systematically as a function of age (ranging from 27% to 49% of receptors tested among the different age groups). Firing threshold as a function of P_{tp} was variable for SARs in the 20 and 30 day old groups. At high static levels of P_{tp} (15 and 20 cmH_2O), SAR discharge rate was progressively reduced as a function of decreasing age; but at P_{tp} = 5 cmH_2O only SARs of 20 day old animals had a significantly reduced firing frequency. When the lungs were rapidly inflated from a P_{tp} of 2 cmH_2O to a P_{tp} of 10 cmH_2O, the adaptation index (Respir. Physiol. 37:303, 1979) was similar at 20 days (28%), 30 days (24%) and in adults (27%). About 80% of tested SARs were inhibited by CO_2 at 20 and 55 days as well as in adults. The opossum, a marsupial, at 55 days is generally developed to the stage of a placental newborn such as the dog or cat; but, in contrast to those newborns, the 55 day (or even 30 day) old opossum has a well maintained static discharge at low P_{tp} levels. Possibly there is a stimulus for receptor development which occurs because of early utilization of the opossum lungs. **(Supported in part by HL-20122, 29169, and 00619 from NIH).**

25.5

APNEUSTIC-LIKE BREATHING PRODUCED BY INTRAVENOUS ADMINISTRATION OF BACLOFEN IN THE CAT. A.M. Taveira Da Silva, J.A. Quest*, P. Hamosh and R.A. Gillis*. Depts. of Pharmacology, Physiology and Medicine, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007 and National Toxicology Program, NIEHS, NIH, Bethesda, MD 20205

Injection of GABA into the cisterna magna (CM) and local application of GABA to Schlaefke's area (S area) of the ventral medulla produces a decrease in tidal volume but little change in respiratory rate (f) (Brain Res. 248: 71, 1982). This study was initiated to determine whether baclofen, a drug known to activate GABA B receptors, would produce a similar response. Since baclofen crosses the blood-brain barrier, we administered cumulative doses of 0.5, 1, 2 and 4 mg/kg i.v. to 5 chloralose-anesthetized cats while monitoring pulmonary ventilation (V_E), arterial pressure (BP) and heart rate. After a total dose of 4 mg/kg, apneustic-like breathing occurred (i.e., inspiratory duration increased from 1.0 + 0.1 to 16.1 + 4.2 sec; $P < 0.05$). This was associated with a decrease in V_E from 615 + 59 to 323 + 77 ml/min ($P < 0.05$) which was due to a decrease in f. BP also decreased. Baclofen injected into the lateral ventricle also produced apneustic-like breathing, whereas injection into the CM or application to S area did not. These results indicate that the respiratory depressant effect of baclofen differs from that of GABA in terms of both type of breathing pattern and CNS site of action (HE 29562).

25.6

NEURAL ELEMENTS RESPONSIBLE FOR FACILITATION OF PHRENIC NERVE ACTIVITY BY MODERATE LUNG INFLATION. D.B. Averill*, A.J. Berger, and W.E. Cameron*. Department of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Slowly adapting pulmonary stretch receptors (PSRs) may exert a facilitatory action on the discharge of phrenic motoneurons during inspiration (DiMarco et al., '81) which may be mediated through a disynaptic pathway (Iscoe et al., '79). We have recorded simultaneously from the neural elements that may subserve this facilitatory reflex. Experiments were carried out on anesthetized, paralyzed and artificially ventilated cats. Extracellular activity from individual PSRs was recorded in the right nodose ganglion. Central respiratory outflow was recorded from the left C5 phrenic root. Single unit activity of dorsal respiratory group (DRG) neurons was recorded in the right medulla. Cross-correlation analysis of simultaneous spike activity from PSRs and Iβ neurons demonstrated monosynaptic excitatory connections between PSRs and DRG Iβ neurons in 6 of 21 neuron pairs. The same Iβ neurons provided the triggering event for spike-triggered averaging of left phrenic activity; 5 of these 6 Iβ neurons also had monosynaptic projections to the contralateral C5 phrenic motoneuron pool. These results demonstrate that PSRs project monosynaptically to Iβ which, in turn, project monosynaptically to phrenic motoneurons. This is the first demonstration of the components of the neural pathway responsible for facilitation of phrenic motoneurons by lung stretch receptors. This work was supported by USPHS Grant NS 14857 and NRSAs HL 06233 and 06474.

25.7

MORPHOLOGY OF DORSAL MEDULLARY INSPIRATORY NEURONS AS REVEALED BY INTRACELLULAR INJECTION OF HRP. A.J. Berger, D.B. Averill* and W.E. Cameron*. Department of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Inspiratory neurons in the cat are concentrated in the dorsal medulla associated with the ventrolateral nucleus of the tractus solitarius (vl-NTS). An understanding of the somal, axonal and dendritic morphology is important to understand the integration of sensory inputs by these neurons. The detailed morphology of 10 well-stained inspiratory neurons has been investigated utilizing the technique of intracellular injection of HRP. After fixation, the medulla was sectioned at 100 µm, preincubated in $CoCl_2$ and reacted with DAB. All labeled cells were within the vl-NTS. Six cells sectioned in transverse plane had a mean somal diameter of 30.4 µm, while 4 others sectioned in horizontal plane had a mean of 37.3 µm. Many of the axons were observed to run ventrally from the soma and then turn medially to cross the midline of the medulla. Stained neurons had from 4 to 10 primary dendrites. The main dendritic arborizations ran parallel and ventrolateral to the tractus solitarius (TS) for up to 1.0 mm from the cell bodies. These dendrites possessed numerous spines and appendages. We suggest that this orientation of the dendritic arbors of vl-NTS inspiratory neurons optimizes the surface area available to receive synaptic contacts from sensory afferents emerging from the TS. Supported by USPHS NS 14857 and NRSAs HL 06233 & 06474.

25.8

POST-SYNAPTIC RESPONSES OF POST-INSPIRATORY NEURONS TO ROSTRAL PONTINE STIMULATION. J.P. Baker, Jr., J.E. Remmers, R. Takeda*, K.P. Madden*, D.W. Richter* and J. Farber. Departments of Physiology and Medicine, University of Texas Medical Branch, Galveston, TX 77550.

Glass micropipettes filled with 3M KCl were used to record membrane potential of post-inspiratory (PI) neurons (Richter, J. Exp. Biol. 100:93-107, 1982) in pentobarbital-anesthetized, paralyzed cats. The animals were pneumothoraxed and held in a stereotaxic frame, with the spine clamped. A phrenic-driven servorespirator was used to ventilate the animals. Antidromic stimulation of the cervical spinal cord and vagus demonstrated that PI neurons had neither vagal nor spinal axons. PI neurons were hyperpolarized during inspiration and neural expiration, but were sharply depolarized just after the end of inspiration. Measurements of input resistance and membrane potential after Cl^- reversal revealed the hyperpolarization to be due to inhibitory post-synaptic potentials (ipsp). Ipsilateral pontine stimulation (in the region producing global phrenic inhibition) produced excitatory post-synaptic potentials (epsp) in the PI neurons with latency about 4 msec. The gating of the action potentials during inspiration appeared to be due to ipsp-mediated shunting and hyperpolarization. Some PI neurons also had weak excitatory responses to contralateral pontine stimulation and some received epsp from vagal stimulation. (Supported by NIH grant HL-27190).

25.9

INFLUENCE OF CERVICAL AND THORACIC DORSAL ROOT AFFERENT INFORMATION ON MEDULLARY INSPIRATORY NEURON ACTIVITY DURING MECHANICAL LOADING. R. Shannon, W.T. Shear*, A.R. Mercak*, D.C. Bolser* and B.G. Lindsey. Dept. Physiology, Col. Med., Univ. South Florida, Tampa, Fl. 33612

Studies were conducted to determine the first-breath (neural reflex) response of dorsal and ventral respiratory group inspiratory (I) neurons to the mechanical loading (tracheal occlusion, TO) of inspiration in unanesthetized (decerebrate), vagotomized cats.

Tracheal occlusion produced an increase in activity in 52% of the I-neurons (9 cats), which was characterized primarily by a prolongation of the firing duration. There was a decrease in activity in 21% of the I-neurons, which was most apparent in late firing neurons. The increase in I-neuron activity following loading has not been reported in previous studies using anesthetized vagotomized cats. These changes in I-neuron activity with TO were still present in cats with their cervical dorsal roots (C3-C7) or thoracic dorsal roots (TI-T9) cut. The changes in neuron activity with TO were absent when both cervical and thoracic dorsal roots were cut.

The most probable sources of the cervical and thoracic afferent information altering medullary I-neuron activity during loading is the diaphragm and external intercostal muscle proprioceptors (i.e., muscle spindles and/or tendon organs). (Supported by NIH Grant HL-17715)

25.10

THE RESPIRATORY PARAMETER OF THE SCHIFF-SHERRINGTON PHENOMENON Robert E. Schuhmann, Sharon K. Coles*, and Hebbel E. Hoff Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030

Fifteen cats and five dogs were decerebrated at the midcollicular level and then cordotomized at the twelfth thoracic level. Minute volumes were measured after decerebration, before and after cordotomy. All animals developed extensor rigidity after decerebration, and exhibited significant increase in forelimb rigidity following cordotomy (Schiff-Sherrington phenomenon). Increase in forelimb rigidity was accompanied by a simultaneous step-wise increase in tidal volume and respiratory rate, averaging a 46% increase in minute volume. This increase appeared after a 5-15 second period of total inhibition immediately following the cordotomy and continued throughout periods of survival of four to thirty hours. The results are interpreted as pointing to the presence of a neuromechanism linking the increased ventilation with the Schiff-Sherrington effect; the release of postural reflexes in the Schiff-Sherrington phenomenon seems to be accompanied by the release of respiratory activity as well. The presence of this integrated response in the midcollicular decerebrate preparation establishes that the center for postural-respiratory integration is situated caudal to the hypothalamus and thalamus.

25.11

ELECTRICAL STIMULATION OF THE DIAPHRAGM IN CATS - A PHYSIOLOGICAL MODEL FOR PACING IN THE HUMAN INFANT. David G. Fleming, Dennis Shelby* and Fred Montague*. Case Western Reserve Univ. Cleveland, Ohio 44106

Disorders of respiratory control in infants include central alveolar hypoventilation and cervical cord injuries. In as much as respiratory stimulants are usually ineffective, mechanical ventilation and/or electrical stimulation of the phrenic nerves bilaterally are the current treatment methods of choice. A modified form of electical stimulation, directly to the diaphragm, is used by the authors, to minimize the possible hazzard of nerve damage in a chronic implant. Four stainless steel electrodes are implanted in the diaphragm, two per side, and the electrode leads externalized to a connector on the back of the neck. Tidal volumes in the range of 8-10 ml per kilo of body weight are obtained in anesthetized animal with bilateral stimulation. Electrodes have remained functional in a 3.0 kilo cat for more than 8 months after implant with the only lead breakage in the external cable.A study of the pattern of chest wall to abdominal movement comparing spontaneous to paced respiration demonstrated that the cat functions as a model for direct pacing in the human infant in that both species the amplitude of ventilation is limited by paradoxical rib cage movement. There is also an alteration in the ventilation perfusion relationship during diaphragmatic pacing in that most of the volume exchange is limited to the lower lobes.
Supported by NIH grant HL 27065

25.12

EFFECT OF A SINGLE BREATH OF 100% OXYGEN ON RESPIRATION IN NEONATES DURING SLEEP. Tazeem Aizad*, Jaya Bodani*, Don Cates*, Leanne Horvath*, and Henrique Rigatto. Dept. of Pediatrics, Univ. of Manitoba, Winnipeg, Canada.

To determine the effect of a single breath of 100% O_2 on ventilation, we studied 10 term (BW 3360±110 gm (SE); GA 39.3 ±.36 wk, postnatal age 2.6±.6 days) and 10 preterm neonates (BW 2020±60 gm, GA 34.1±1.8 wk, PNA 8.7±1.6 days) during sleep. The single breath method measures the peripheral chemoreceptor response. To standardize the control period for all infants we adjusted FiO_2 to 16±0.6% to obtain a control O_2 saturation of 83±1%. After 1 minute of control in each sleep state, they were given a single breath of O_2 followed by 21% O_2. We measured $\dot{V}_E$, V_T, f, PAO_2, P_ACO_2, O_2 saturation (ear oximeter) and $tcPO_2$. $\dot{V}_E$ always decreased with inhalation of O_2 ($p<0.01$). In non-REM sleep, the decrease in $\dot{V}_E$ was less in term (14%) than in preterm (40%) infants ($p<.001$). This decrease in $\dot{V}_E$ was primarily due to a fall in V_T in term infants as opposed to a fall in f and V_T in preterm infants ($p<0.05$). Apnea as part of the response was more prevalent in preterm than in term infants. In REM sleep the decrease in $\dot{V}_E$ was similar in term (19%) and preterm (21%) infants ($p>0.5$). These results suggest a greater peripheral chemoreceptor response in preterm than in term infants as reflected by a more pronounced decrease in $\dot{V}_E$ with O_2. The results are compatible with a more powerful peripheral chemoreceptor contribution to breathing in preterm than in term infants.

26.1

MODIFICATION OF BLOOD PRESSURE BY BIOFEEDBACK ASSISTED RELAXATION. A.V. McGrady*;G.A.A. Bernal*;T. Fine*;J Higgins*;J. Turner*;S. Utz*;M. Woerner*(SPON: L. Nelson). Medical College of Ohio, Toledo, Ohio, 43699.

EMG (electromyograph) biofeedback assisted relaxation training has been shown to induce lowering of blood pressure in patients with essential hypertension. However, little is known about the mechanisms by which hypertensives affect the decrease in pressure. This study explored the relationship between blood pressure cortisol and aldosterone in 7 unmedicated hypertensives. They participated in a 6 week baseline, 10 weeks of treatment, and a 3 month follow-up. Blood pressure was measured at home and in the clinic and averaged 142/90 during baseline and 133/85 post-treatment. Muscle tension levels decreased by 42% during treatment. Plasma cortisol decreased from 14.5 to 11.2 ugm%; urinary cortisol decreased from 71 to 63 ugm/gm creatinine. Plasma aldosterone decreased from 9.5 to 9.2 mg%. The decreases in mean blood pressure, muscle tension and urinary cortisol were significant ($p < .05$, $p < .01$, $p < .02$, respectively). Changes in urinary cortisol were correlated with changes in home mean blood pressure: $r = 0.87$. Changes in plasma cortisol and in plasma aldosterone were correlated ($r = 0.74, 0.75$) with changes in clinic mean blood pressure only for patients who decreased their cortisol and aldosterone levels. These results indicate that the adrenal cortex plays a role in the relaxation response of persons with essential hypertension. Biomed Res 5 S01 RR 05700 12

26.2

AN ANALYSIS OF ANGIOTENSIN II and III IN THE BRAIN WITH HIGH PRESSURE LIQUID CHROMATOGRAPHY. M. Ian Phillips and Birgitta Stenstrom*. Department of Physiology, University of Florida, Gainesville, Florida 32610.

To answer the question of whether angiotensin II exists in the brain independently of peripheral angiotensin, a sensitive radioimmunoassay with high recovery rates was used to measure fractions eluted from high pressure liquid chromotography (HPLC) assay of male adult rat brains. Rats were nephrectomized bilaterally and 24 hrs. later the brains were extracted after boiling in acetic acid. A Sep Pac-18 was used to purify the angiotensin followed by high pressure liquid chromatography (HPLC). The fractions were collected and analyzed by an Ang II radioimmunoassay with a 93% recovery. HPLC revealed two peaks which comigrated with authentic (Ile) angiotensin II and (Ile) angiotensin III. Both angiotensins were found in the hypothalamus blocks which included parts of the septum thalamus and mid-brain. Angiotensin II concentration was 63 to 873 pg/g tissue. Angiotensin II, but not angiotensin III, was also found in cortex but in lower quantities (14-179 pg/g tissue). The results demonstrate that the antibody which was previously used in the immunocytochemical localization of angiotensin in the hypothalamus detects authentic angiotensins. The present data provide critical evidence for endogenous angiotensin in the brain. Physiological implications of brain angiotensin can be explored with this procedure. [Supported by NIH Grant No. 1-R01-HL27334 to MIP.]

26.3

CENTRAL NERVOUS SYSTEM SARALASIN INJECTION REDUCES BLOOD PRESSURE DURING INDUCED PREECLAMPSIA IN RATS. Douglas J. Eder* and Mecca T. McDonald* (SPON: James W. DeClue). Southern Illinois Univ. at Edwardsville, IL. 62026

We report that angiotensin II acting in the brain causes at least 50% of the rise in blood pressure observed during experimental preeclampsia (pregnancy-induced hypertension) in the laboratory rat. We surgically induce preeclampsia at day 14-17 of pregnancy in Sprague-Dawley rats by means of an aortic coarctation. Under Equithesin anesthesia we place a silk ligature around the aorta caudal to the renal arteries and tighten it sufficiently to reduce mean blood pressure below the coarctation by about 35% (Abitbol method). At the same time we cannulate the left carotid artery and introduce a permanent cannula into a lateral ventricle of the brain. Typically carotid blood pressure rises during 3-5 days postoperatively in the conscious pregnant rat from a normal mean of 98 to 118 mm Hg. Urinary protein content (Lowry method) in a 24-hour sample rises from about 16 to 80 mg/100 ml in hypertensive animals only. Intracerebroventricular injections of the angiotensin II antagonist, saralasin (100 ng/µl), reduce hypertension by at least 50%. This reduction has not occurred in normally pregnant or non-pregnant controls nor during vehicle injections. We conclude that angiotensin II activates blood pressure control centers in the brain as part of the pathophysiology of preeclampsia.

Grant support: American Heart Association/Illinois Affiliate

26.4

HYPOTHALAMIC DISCONNEXION AND RENAL HYPERTENSION. A DUAL RESPONSE. O.U.Lopes*, A.L.Castro*, E.F.Alemida* and R.Vadenal* (SPON: A.C.Guyton). Departamento de Fisiologia, Universidade de São Paulo, 05508, São Paulo, SP, Brasil.

The role of the central nervous system in general, and of the hypothalamus in particular, in the genesis of various forms of experimental hypertension has been the object of increased investigation. In the present experiments by means of a stereotaxically placed curved knife (2 mm radius) the anterior hypothalamus was disconnected from caudal neuroaxis at the level of the arcuate nucleus. This lesion by itself induces polydpsia, increased urinary sodium excretion and reduction of pressor effects of i.v. angiotensin II. If lesioned rats are offered the choice, they consume large amounts of 0.9% saline in preference to tap water. The interactions of simultaneously performed hypothalamic disconnexion (HD) and Goldblatt one-kidney, one clip (HG_1) or two-kidney, one clip (HG_2) hypertensions were studied. It was found that HD retards and attenuates the development of HG_1 hypertension but does not materially affect the evolution of the HG_2 model. Rats with established HG_1 or HG_2 hypertensions were not affected by HD, whereas rats with chronic HD (4 weeks) showed slight and slow developing hypertension in response to HG_1. These results suggest that the anterior region of the hypothalamus contains separate neural mechanisms, involved in renin and nonrenin-dependent renal hypertension.

26.5

HEMODYNAMIC PATTERNS IN 2 KIDNEY, 1 CLIP (2K1C) GOLDBLATT HYPERTENSIVE CONSCIOUS RATS. Karen A. Stanek*, William R. Murphy*, and Thomas G. Coleman. Univ. Miss. Med. Ctr., Jackson, MS 39216

Renovascular hypertension was produced in male Sprague-Dawley rats by applying a silver clip (.2 mm ID) to the left renal artery. Cardiac index (CI), mean arterial blood pressure (MABP), total peripheral resistance (TPR) and organ flows and resistances were measured at 3 and 6 weeks after clipping and compared to Sprague-Dawley controls (C). MABP and TPR for C(N=11), 3-week 2K1C (N=7), and 6-week 2K1C (N=8) respectively increased with time:115 vs 166† vs 170† mmHg; 3.5 vs 3.8 vs 4.4† mmHg/ml/min/100g. CI was increased at 3 weeks and remained elevated (33 vs 44† vs 40† ml/min/100g). Heart weight and blood flow per unit weight increased significantly in both hypertensive groups. The clipped kidney in both hypertensive groups maintained its weight while absolute blood flow was decreased slightly 2.7 vs 2.5 vs 2.5 ml/min/100g body weight. The right kidney was hypertrophied in both groups compared to C, where C was .38 vs 3-week 2K1C at .57† vs 6-week 2K1C at .54† g/100g body weight. Absolute flow to the right kidney was maintained; however, expressed in ml/min/g it was reduced; C was 7.4 vs 3-week 2K1C at 4.7† vs 6-week 2K1C at 4.8†ml/min/g. Limb skeletal muscle showed vasoconstriction, which was significant at 6 weeks. Hence, hemodynamics in 2K1C hypertension appears to involve both increased vascular resistance and alterations in blood flow distribution. († Significant at $P < .05$ compared to C.) (Supported by grant HL26412).

26.6

REVERSAL OF ONE-KIDNEY, ONE CLIP (1-K, 1C) HYPERTENSION (HT) IN RATS. EFFECT ON CARDIOVASCULAR MUSCLE NA^+,K^+ PUMP ACTIVITY AND CIRCULATING OUABAIN-LIKE FACTOR (OLHF). M. Pamnani, H. Bryant, K. Knoble* and F. Haddy. Department of Physiology, Uniformed Services University, Bethesda, MD 20814

We have reported that 1-K, 1C HT (E) rats have increased OLHF and decreased vascular Na^+,K^+ pump activity, myocardial Na,K-ATPase, and vascular smooth muscle cell (VSMC) resting membrane potential (Em). In this study, the effects of unclipping on these parameters were examined in relation to blood pressure. After 4 weeks of hypertension in E rats and a similar time period in paired one-kidney, normotensive control (C) rats, the clips on the renal arteries were removed. Systolic blood pressure (SBP) was monitored at 3 and 6 hrs and then daily for 3 or 7 days. The rats were then anesthetized, tail arteries removed for measurement of ouabain-sensitive (OS) and ouabain-insensitive (OI) ^{86}Rb uptakes and Ems, hearts excised for measurement of Na,K-ATPase, and blood collected to assay for OLHF. Following unclipping, SBP of E rats decreased from 190±3 to 120±2 mmHg, N=16, $P<0.001$ in 3 h. Both at 3 and 7 days there were no significant decreases in ^{86}Rb uptakes and Na,K-ATPase or increase of OLHF in E compared to C. Em's also were not decreased at 3 days but at 7 days E was slightly depolarized relative to C. These data suggest that unclipping 1-K, 1C HT rats not only reverses HT but also abolishes increase in OLHF and decrease in Na^+-K^+ pump activity of cardiovascular muscle cells, suggesting a causal relationship between HT and OLHF.

26.7

AMELIORATION OF RENIN-DEPENDENT HYPERTENSION BY AMILORIDE. Kenji Shimoda* and Thomas C. Lee. Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, California 90048.

We reported that amiloride, a potassium-sparing diuretic with kallikrein-inhibiting activity, was as effective in preventing furosemide-induced rises in plasma renin activity (PRA) as aprotinin, an inhibitor of serine-proteases which include renal kallikrein. As a logical sequel, the potential efficacy of amiloride in ameliorating renin-dependent hypertension was tested in rats with severe unilateral renal artery constriction. Amiloride was added to the drinking fluid to provide an approximate dose of 1 mg/kg/day. Treated rats (n=14) maintained body weight better ($p<0.01$) than non-treated rats (n=14) and their systolic blood pressure measured in the conscious state was remarkably lower (166±6 vs 198±8 mm Hg, $p<0.005$) after an 8-day regimen. Renin-dependency of their hypertension as assessed by the hypotensive response to saralasin-induced angiotensin II blockade was also significantly reduced in the treated rats than that of non-treated rats (-23±6 vs -40±7, $p<0.05$). All rats exhibited contralateral compensatory polyuria and amiloride exerted no discernible natriuretic or antikaliuretic effect. Plasma potassium concentration was similar in both groups. Thus, the antihypertensive effect of amiloride was due not to a greater diuresis nor hyperkalemia, but to suppression of hyperreninemia. (This study was supported by NHLBI Grant #HL-22069, BRSG #RR-05468 and Award #415-IG-15 from the American Heart Association - Greater L.A. Affiliate).

26.8

NITRENDIPINE CONTROL OF BOTH SPONTANEOUS HYPERTENSION AND DOC-SALT HYPERTENSION AND THE ACCOMPANYING DIABETES INSIPIDUS-LIKE SYNDROME. Charles E. Hall and Shirley Hungerford Univ. of Texas Medical Branch, Galveston, TX 77550.

Prehypertensive Aoki-Okamoto (SHR) or mononephrectomized female Sprague-Dawley (SD) rats injected with 5 mg/kg/wk of deoxycorticosterone pivalate (DOCP) and given 1% NaCl solution to drink, had a 25mg pellet of nitrendipine (NTR) implanted s.c. on day 0 and then weekly. NTR prevented both spontaneous and DOC hypertension. Cessation of implantation at 40 days in SHR and after 20 days of DOC treatment led to hypertension in the former, and to rising although still normotensive pressures in the latter, after about ten days. Reimplantation of pellets reduced arterial pressures in both, although not to the levels prevailing before discontinuance of implantation. The drug had no effect on the weight of hearts, kidneys or adrenals of SHR: it prevented heart but not kidney enlargement from DOC treatment. Urinary studies conducted 24 hr. after implantation of an NTR pellet (51st day), or 1 hr following a 30 mg/kg subcutaneous injection, (61st day), revealed that the administration of 3 ml/100g body wt of 0.45% NaCl and 0.25% KCl caused rats given only DOC to excrete a large volume of low osmolality and more total Na^+ than controls: on the 61st day they also excreted more K^+ and exhibited hypernatremia, hypokalemia and an elevated Na^+/K^+. NTR treatment prevented the urinary abnormalities, but not the serum changes. (Supported by a grant from Miles Laboratories, Inc. and HL 09911 from the NIH)

26.9

THYMUS IMPLANTS FROM NORMOTENSIVE RATS ATTENUATE HYPERTENSION IN OKAMOTO SPONTANEOUSLY HYPERTENSIVE RATS (SHR). R.A. Norman, Jr., D.J. Dzielak*, K.L. Bost*, M.A. Cuchens, and A.A. Khraibi*. Univ. Miss. Sch. Med., Jackson, MS 39216

We have demonstrated that chronic immunosuppressive therapy with cyclophosphamide will attenuate the hypertension in SHR, suggesting that this form of spontaneous hypertension may be due in part to an autoimmune mechanism (Physiologist 25:337, 1982). It has been postulated that autoimmunity in SHR may be the result of a thymic defect. To test this hypothesis 3-week-old SHR were given thymus implants subcutaneously at weekly intervals for 3 weeks from Wistar-Kyoto donor rats (WKY). Sham-implanted SHR developed hypertension between 8 to 12 weeks of age. These thymus implants did not interfere with development of hypertension. However, additional thymus implants at weeks 12, 13 and 14 attenuated the hypertension in SHR by week 14. At week 16 the tail-cuff pressure averaged 171±2.5 (mean±SE) mmHg in 7 sham-implanted SHR and 152±2.8 mmHg in 14 thymus-implanted SHR. This reduction of blood pressure in thymus-implanted SHR was not associated with weight loss, which was possibly a confounding factor with chronic immunosuppressive therapy. Preliminary data indicate that thymus implants from Wistar donor rats may be more effective than implants from WKY (tail-cuff pressure reduction of 31 mmHg after 2 weeks treatment). These results support the hypothesis that hypertension in SHR may be due in part to an autoimmune mechanism. Supported by NIH Grant HL 11678

29.10

LOW-DOSE INTRAPERITONEAL CADMIUM EXACERBATES HYPERTENSION IN SH RATS. Gurdarshan S. Thind, M.D. Hypertension Unit, Univ. of Louisville, School of Medicine, Louisville, Kentucky 40202.

Twenty-five, 60 day-old SH rats were kept in stainless steel cages, and controlled environment, fed ad libitum low cadmium (Cd) diet and de-ionized distilled water containing essential vitamins (IP) 2 mg/Kg Cd acetate (10 rats) or equal volume of normal saline (NSS)(15 rats). Indirect systolic blood pressure (BP) from the tail-artery was done one- to 30-minutes post-injection under light (up to 25 mg/Kg) pentobarbital. Total body weights (TBW) and BP's were monitored chronically for 10-weeks. The BP significantly increased ($P < 0.05 - 0.001$) post-Cd (158 ± 6, 1-min.; 168 ± 5, 5-min; 179 ± 5, 15-min. and 193 ± 5, 30-min.) compared to pre-Cd BP of 137 ± 5 mm Hg. The Cd rats showed no significant BP elevation at week 1-3, and week 6, but significant ($P < 0.025 - 0.001$) post-Cd BP elevations were found at week 4, 5, and 7-10 (range of 155 ± 6 to 176 ± 9 mm Hg). The NSS rats showed no significant BP elevations acutely or from week 1-10. The TBW of Cd & NSS rats did not show significant changes. It is concluded that IP 2 mg/Kg Cd produced acute and chronic worsening of pre-existing genetic hypertension in SH rats, an ideal model for studying the role of environmental factors in the genetically hypertensive rats.

26.11

EFFECTS OF BARIUM ON ANIMALS SUSCEPTIBLE TO AND RESISTANT TO HYPERTENSION. L. J. Tillman*, B. H. Douglas, V. L. Hill*, P. T. McCauley* and R. J. Bull*. Univ. Miss. Med. Ctr., Jackson, MS 39216 and Health Effects Research Lab., Cincinnati, OH 45268

Some trace elements affect blood pressure in humans and experimental animals. Because of its presence in drinking water in some areas, the effects of Ba ingestion on the experimental animals was examined. Twenty-two groups of animals received Ba for 16 weeks. Blood pressures were monitored weekly. Kidney tissue was taken for ultrastructural evaluation at the end of 16 weeks. Five groups received 0, 3, 10 or 100 ppm Ba in the drinking water. Five additional groups received similar amounts of Ba in 0.9% NaCl. Four groups of uninephrectomized animals received 1, 10, 100 or 1000 ppm Ba in either drinking water or 0.9% NaCl. Dahl sodium sensitive and sodium resistant rats (4 groups each) received 1, 10, 100 or 1000 ppm Ba in 0.9% NaCl. Ba alone did not exert a hypertensive effect. Ba did not modulate the hypertensive effect of NaCl nor alter the sensitivity or resistance to the effects of NaCl. Histopathological changes were not observed in the kidneys of animals receiving low doses of Ba. Animals which received larger doses exhibited ultrastructural changes in the glomeruli which included basement membrane thickening, epithelial foot process fusion and the presence of myelin figures. Ba ingestion for longer periods might affect blood pressure if the kidney is further damaged. (This is an abstract of a presentation and does not necessarily reflect EPA policy.)

26.12

ALTERED VASCULAR RESPONSES TO NOREPINEPHRINE AFTER UNCLIPPING RENAL ARTERY IN GOLDBLATT HYPERTENSIVE RATS. K.A. Smith, A.W. Dow,* B. Brooks,* E.E. Muirhead, Univ. of Tennessee Cen. for the Health Sciences, Memphis, TN 38163

Phospholipids isolated from renal papilla and renal vein blood following removal of the renal artery clip in one-kidney Goldblatt hypertensive rats (GHR) have been shown to have adrenergic antagonist activity in some systems. To test whether this procedure alters vascular sensitivity to norepinephrine (NE), responses were assessed in GHR (N = 9). Renal artery clip removal significantly lowered mean arterial pressure (MAP) from 188 to 123 mm Hg at 3 to 4 hours, and to 114 mm Hg at 3 to 5 days. NE responses (area beneath curve) were reduced significantly for 0.05, 0.1, and 0.5 ug/kg NE at 3 to 4 hours and 3 to 5 days. Thus, the vascular responses to low doses of NE are decreased significantly after removal of the renal artery clip in GHR. A paradoxical response was seen at 3 to 4 hours to 5 or 10 ug/kg NE. Following the pressor responses to NE, a prolonged (26 minutes) decrease in MAP occurred with an average maximum reduction of 38 mm Hg. Since a specific phospholipid, acetylated lysolecithin, can be isolated from renal vein blood and has adrenergic antagonist activity, in some systems, it may be involved partly in the lowering of MAP and the altered vascular responses to NE observed following removal of the renal artery clip. (Supported by an NIH Grant USPHS Gr H1 19287-07)

27.1

FUNCTION OF THE PERICARDIAL-PERITONEAL CANAL IN ELASMOBRANCH FISHES. Daniel C. Abel*, Ralph Shabetai*, and Jeffrey B.Graham* (SPON: Fred N. White). Physiol. Res. Lab., Scripps Instit. of Oceanography, La Jolla, CA 92093.

The rigid pericardium of elasmobranchs connects to the peritoneum by the pericardial-peritoneal canal. We investigated dynamic relationships of the canal, pericardium, and heart to assess the canal's function. Intrapericardial pressures of six species,recorded from anesthetized fish lying supine out of water, were negative (-10 to -1 cm H_2O). Infusion of elasmobranch saline into the pericardium gradually increased pressure until the threshold for canal opening was reached (1 to 8 cm H_2O). Further infusion did not raise pressure but resulted in steady flow to the peritoneum. Infusion after canal ligation increased pressure exponentially to values above threshold. Technetium antimony sulfide, injected into the pericardium of a thornback ray,became concentrated in the heart, blood, liver and peritoneal fluid following 4 h of swimming. This indicates uptake by the lymphatic and/or circulatory system(s) and suggests that the canal function is not soley to compensate for poorly developed mechanisms of pericardial drainage. Thus, the canal may ensure heart function by relieving high intrapericardial pressures resulting from body tension occurring during swimming, passage of food through the esophagus, or increased venous return during activity as the heart expands in accordance with Starling's law. (Partially supported by NSF Predoctoral Fellowship, the Veterans Administration, NIH S07 RR07011, and the Aquarium Dept. at Sea World, San Diego).

27.2

HEMOGLOBIN FUNCTION IN TARICHA GRANULOSA: A NEWT APPROACH TO THERMAL ACCLIMATION. S.C. Wood. University of New Mexico, School of Medicine, Albuquerque, NM 87131

Acclimation to temperature is manifested in many poikilothermic animals as a change in metabolic rate (long term Q10 < short term) and a change in hemoglobin-oxygen affinity (long term dP50/dT < short term). This study tested the hypothesis that these patterns of acclimation would occur in Taricha granulosa, a newt that is aquatic much of the year with water temperatures ranging from 3°C (Jan) to 25°C (July). Newts were kept in the lab at 5°C or 22°C for 1 month. Subsequently, the O2 uptake and respiratory properties of blood and Hb solutions were determined. There was a significant increase in O2 capacity of blood in warm acclimated newts. There were no differences between cold and warm acclimated animals with respect to: Hb electrophoretic pattern, O2 uptake, O2 affinity of blood or Hb solutions (at pH 7.7, 20°C), Hill's "n", dlogP50/dT, or red cell [NTP]. The lack of change in blood P50 contrasts with previous data for other species. However, an unexpected finding complicates the interpretation of this; e.g., cold acclimation resulted in a reversal of the normal Bohr effect (dlogP50/dpH = -0.12 in warm acclimated newts and +0.13 in cold acclimated newts). Supported by NSF Grant PCM 77-24246.

27.3

THE EFFECT OF PHASIC AND MEAN CHANGES OF PULMONARY CO_2 (LCO_2) ON VENTILATION IN THE TECU LIZARD. G.O. Ballam, and J.W. Hicks*. Lovelace Medical Foundation, Albuquerque, NM 87108.

The posterior portion of each lung of the lizard Tupinambus nigropunctatus was cannulated with a silastic exit tube (ID =5mm) which passed through the body wall to the exterior. An endotracheal tube was inserted and warmed, humidified gas blown unidirectionally into the lungs. The gas passed through the lungs and out the exit tubes. Ventilatory movements caused changes in lung volume but did not alter LCO_2 which was dependent on the amount of CO_2 added to the gas stream entering the endotracheal tube. Increasing LCO_2 within the range of 3 to 5% caused an increase in tidal volume and ventilatory frequency. Setting background LCO_2 between 4 and 5% while elevating or reducing LCO_2 by 1.5% for approximately 200msec with each breath increased the ventilatory frequency compared to maintaining the background LCO_2 constant. Increasing the 200msec LCO_2 reduction greater than 1.5% further elevated the ventilatory frequency. These results indicate that increasing CO_2 delivery to the lungs by an increase in metabolism would probably stimulate ventilation both by increasing the mean LCO_2 and increasing the amplitude of phasic LCO_2 changes. These results also suggest that elevating inspired levels of CO_2 would increase mean LCO_2 and tend to increase ventilation but would decrease the amplitude of phasic changes in LCO_2 which would tend to decrease ventilation. (Supported by NIH Grant HL 29342.)

27.4

EFFECTS OF INSPIRED OXYGEN ON PREFERRED BODY TEMPERATURE IN THE IGUANA. J.W. Hicks* and S.C. Wood. University of New Mexico, School of Medicine, Albuquerque, NM 87131

Animals exposed to hypoxia, either external (lowered lung PO2) or internal (due to cardiovascular shunts) must make physiological and/or behavioral adjustments to improve oxygen conductance. A previously reported computer model describing O2 transport in vertebrates with cardiovascular shunts (Wood and Hicks, The Physiologist, 25:214, 1982) predicted that arterial PO2 would increase as temperature increase until a "breaking point" occurred and PaO2 then would decrease. The temperature at which the "breaking point" occurred would be lowered by external hypoxia. This study tested the hypothesis that a reduction in the preferred body temperature will occur during exposure to hypoxia. We established a temperature gradient of 20 to 40°C within a chamber and determined the preferred body temperature (mini-mitter telemetry) for Iguana iguana, breathing room air and when the inspired O2 fraction was reduced to 0.07. Preferred body temperature changed from 36°C breathing room air to 26°C breathing 7 % O2 and returned to 36°C with the return of normoxia. The physiological significance of this may be to keep the arterial PO2 above the "critical" PO2. Supported by NSF Grant PCM 77-24246.

27.5

BLOOD OXYGEN TRANSPORT IN A PASSERINE BIRD POSSESSING MULTIPLE HEMOGLOBINS. Leigh A. Maginniss and Peter Han*. Division of Biology and Medicine, Brown University, Providence, RI 02912

Adult house sparrows (Passer domesticus) exhibit hemoglobin heterogeneity; isoelectric focusing techniques reveal two structurally distinct isohemoglobins in a three to one molar ratio. We measured isocapnic O_2 equilibrium curves (O_2EC) for Passer whole blood by thin film dual wavelength spectrophotometry (542 - 560 nm) and electrode oximetry. Blood film pH was computed from a buffer line and the P_{CO_2}. At in vivo pH (7.47 ± .02) and 41°C, the half saturation P_{O_2} was 43.4 ± 0.5 torr. The shape of the sparrow O_2EC deviated from the Severinghaus curve for normal human blood. The Hill plot for Passer blood was alinear; the Hill coefficient (n) increased from a value of 2.7 below 40% S to 3.6 above 60% S. These sparrow equilibrium data are accurately described by the equation:

$$S = [(6.5 \times 10^6)/(P^4 + 16P^3 + 340P^2 + 2.4 \times 10^4 P) + 1]^{-1}.$$

This complex O_2EC shape may reflect the presence of multiple hemoglobins. The high P_{50} and steep upper limb of the O_2EC may facilitate blood O_2 transport in small passerine birds at altitude. The fixed acid and CO_2 Bohr effects ($\Delta \log P_{50}/\Delta pH$) were -.39 ± .03 and -.49 ± .01, respectively. These results demonstrated a significant pH-independent effect of CO_2 on Hb-O_2 affinity. The fixed acid and CO_2 Bohr slopes were both independent of saturation. (Supported by NSF PCM82-02702).

27.6

WHAT IS RESPIRATORY DEAD SPACE IN BIRDS? Randolph H. Hastings and Frank L. Powell. Department of Medicine M-023, University of California, San Diego, La Jolla, CA 92093

In four pump ventilated ducks we measured Bohr (VDB), Enghoff (VDE), Fowler (VDF) and physiologic (VDP) dead spaces and compared them with upper airway plus instrument volume (VDA). VDB, VDE and VDP were estimated as $VT \bullet ((Px - P\bar{E})/Px)$ where Px was end-tidal, arterial and ideal-expired PCO_2 respectively and $P\bar{E}CO_2$ was measured at the outlet of a 2.3 L heated mixing box. Ideal $PECO_2$ was found on an O_2-CO_2 diagram at the intersection of a line through the $\bar{E}$ and inspired points and a computer modeled cross-current $\dot{V}/\dot{Q}$ line. PCO_2 and PO_2 were measured with blood-gas electrodes. VDF was determined from a plot of PCO_2, measured with an infrared CO_2 analyzer, vs. expired volume. In one bird with VT=112 ml and VDA= 30 ml, VDB=31.2 ml, VDF=38.4 ml and VDP=43.7 ml. Phase 3 of the Fowler measurement showed a significant slope. VDE is not a good measure of VD in birds because $PaCO_2$ does not always approximate ideal $PECO_2$ in cross-current lungs. In another duck with VDA=55 ml, VDE decreased 4 ml but VDP did not change when VT was increased from 88 to 127 ml. The reason for $VDA \approx VDB < VDF < VDP$ may be differential sensitivity of the measurements to mesobronchial shunt, temporal variations in parabronchial ventilation and asynchronous emptying of lungs and air sacs. Thus, the various VD estimates may be used to distinguish between these mechanisms of ventilatory limitation to gas exchange. (NIH RO1 HL26050, PHS GM07198-08)

27.7

EFFECTS OF HYPOXIA ON VENTILATION-PERFUSION MATCHING IN BIRDS. Frank L. Powell and Randolph H. Hastings. Department of Medicine M-023, Univ. of Calif., San Diego, La Jolla, CA 92093.

We measured the effects of hypoxia on $\dot{V}/\dot{Q}$ inequality in 3 anesthetized Pekin ducks using the inert gas elimination technique modified for cross-current lungs (Resp. Phys. 48: 233, 1982). FIO2 was changed between .21, .09 and .06 and ventilation was adjusted to give PaO2 values as reported for awake unacclimatized ducks at similar levels of PIO2 (Resp. Phys. 39: 217, 1980). Most $\dot{V}/\dot{Q}$ distributions were unimodal and centered near $\dot{V}/\dot{Q}$ = (VE - VD)/cardiac output at all FIO2. Quantifying $\dot{V}/\dot{Q}$ inequality as log-standard deviation of the main $\dot{Q}$ mode (σ) showed no effect of hypoxia. σ = .87 ± .04, .87 ± .10 and .84 ± .08 at FIO2 = .21, .09 and .06. Low $\dot{V}/\dot{Q}$ areas were seen in some ducks but the fraction of cardiac output to areas with $\dot{V}/\dot{Q}$ <.1 was only 1.5 ± .2% and was not affected by hypoxia. We did not observe high $\dot{V}/\dot{Q}$ areas in ducks as we did earlier in geese but using the above protocol in 1 goose, we did find a high $\dot{V}/\dot{Q}$ mode that was not affected by hypoxia. Differences in high $\dot{V}/\dot{Q}$ areas between species should not lead to differences in hypoxic tolerance since a computer model of cross-current gas exchange that considers $\dot{V}/\dot{Q}$ inequality predicts PaO2 and PaCO2 changes <1 torr if a fixed amount of $\dot{V}$ is shifted between a high $\dot{V}/\dot{Q}$ mode and dead-space. Because $\dot{V}/\dot{Q}$ distributions did not change with hypoxia, the improved efficacy of O2 exchange reported for hypoxic birds is probably due to exchange occurring on the steeper part of the O2 dissociation curve. (NIH RO1 HL 26050).

27.8

OXYGEN TRANSPORT IN THE OBESE ZUCKER RAT. Paul M. Nagel and Stephen C. Wood, Dept. Physiology, Univ. New Mexico School of Medicine, Albuquerque, NM 87131

The obese Zucker rat is massively overweight with the majority of total body weight adipose tissue. The presence of lean littermate controls make this animal an excellent model of human obesity. To assess the effects of obesity on oxygen transport, we examined five male littermate pairs at rest and during swimming exercise. Body weight for obese and lean animals was 644.9±23.4 and 407.5±10.8 (X±SEM), respectively. Blood was sampled from right atrial and carotid cannulae with cardiac output (Q) calculated from the Fick principle. Oxygen consumption (VO2) was measured by closed circuit technique in a swimming pool metabolic chamber. At rest, total body VO2 was significantly elevated with no differences in Q or arteriovenous oxygen difference. Fifteen minutes of weighted swimming represented a different metabolic stress to lean and obese rats. Although total body VO2 was similar in both groups, the lean animal raised VO2 2.4 times rest, while the obese increased VO2 1.6 times rest. Both groups increased Q to 1.2 times resting levels while the lean animals almost double oxygen extraction and obese rats increase extraction to 1.3 times rest. These results suggest that the obese animal exhibits minimal oxygen transport impairment at rest but responds to swimming exercise with an inappropriate cardiorespiratory response for the low metabolic demand encountered. (Supported by NSF Grant PCM 77-24246.)

27.9

CHANGES IN RESPIRATORY GAS EXCHANGE ASSOCIATED WITH SLEEP-WAKING CYCLES IN RATS. Marshall E. Yacoe. Scripps Inst. of Oceanography, La Jolla, CA 92093

Three parameters of respiratory gas exchange ($\dot{V}CO_2$, $\dot{V}O_2$, and R.Q.) were measured in unrestrained rats during normal diurnal sleep-waking cycles. An open-circuit respirometer with rapid washout kinetics ($T_{1/2}$ = 1.3 min) was used and values for each parameter were calculated every 20 s by a microcomputer. Sleep-waking cycles were documented by behavioral observation. Eleven cycles in five animals were analyzed. Values for $\dot{V}CO_2$ and $\dot{V}O_2$ are expressed as $ml \cdot g^{-1} \cdot h^{-1}$ at T_a = 25°C. During entry into sleep $\dot{V}CO_2$ (1.084 ± .048) and R.Q. (0.764 ± .007) were significantly lower (P<.01 in both cases) than in the resting awake state (1.318 ± .074; 0.802 ± .006, respectively). During steady-state sleep $\dot{V}CO_2$ (1.014 ± .045) and $\dot{V}O_2$ (1.302 ± .055) were significantly lower (P<.05; P<.01, respectively) than in the resting awake state (1.318 ± .074; 1.644 ± .093, respectively), whereas R.Q. values did not differ significantly from those of awake animals. During arousal R.Q. (0.853 ± .012) was significantly greater than during waking or sleeping states (P<.01 in all cases), while $\dot{V}CO_2$ and $\dot{V}O_2$ did not differ from waking values. These data are consistent with the hypothesis that CO_2 is retained during entry into sleep. The parallel between R.Q. shifts and changes in $\dot{V}O_2$ suggests that CO_2 retention may be related to the supression of metabolism during sleep.

GASTROINTESTINAL HORMONES AND PEPTIDES

28.1

PROSTAGLANDIN E_2 (PGE_2) PRODUCTION IN CANINE GASTRIC CELLS IN RESPONSE TO ACID SECRETION. M.L. Skoglund, M.R. Feller*, A.I. Vinik*. Department of Surgery, University of Michigan, Ann Arbor, MI 48109, and The Procter & Gamble Company, Cincinnati, OH 45247.

The effect of acid secretion on prostaglandin production was investigated by measuring the amount of PGE_2 produced by isolated gastric cells. It has been postulated that endogenous prostaglandins are produced by the gastric mucosa in response to acid secretion. Once produced, these prostaglandins could then act as negative feedback inhibitors of acid secretion. Thus, we have examined the effect of four different acid secretagogues on prostaglandin biosynthesis by canine gastric cells.

Two Ca^{2+} dependent acid secretagogues, carbachol and gastrin, increased PGE_2 produced biosynthesis by gastric cells. However, two cAMP-dependent acid secretagogues, histamine and dibutyryl, cAMP, decreased PGE_2 produced biosynthesis by gastric cells. We conclude that acid secretion does not stimulate PGE_2 production by gastric cells. Instead, prostaglandin synthesis is controlled by some other intracellular signal such as Ca^{2+}, similar to control of prostaglandin synthesis by most other cell populations.

28.2

POTENT CENTRAL NERVOUS SYSTEM ACTION OF A STABILIZED TRH ANALOG, RX77368, TO STIMULATE GASTRIC SECRETION IN RATS. Y.TACHE, Y.GOTO*, AND M. LAUFFENBURGER*. CENTER FOR ULCER RESEARCH AND EDUCATION, VA WADSWORTH, UCLA SCHOOL OF MEDICINE, LOS ANGELES,CA 90073.

A TRH analog, RX-77368 (Pyr-His-dimethylProNH_2) more resistant to enzymatic degradation was tested for its influence on gastric secretion. Studies were performed in urethane-anesthetized rats in which the esophagus and pylorus were ligated and double lumen cannula inserted into the stomach, the lumen was flushed every 10 min with 5ml boli of 0.15M NaCl and 5ml of air. Acid output was determined by titration of the flushed perfusate with 0.1NaOH. Intracisternal (i.c.) injection of RX 77368 (3-100ng) induced a dose-dependent increase in gastric acid output. The maximal secretory response to 10 or 100ng dose occured within 20 min and is long lasting. Based on dose-response curve, the analog appears 10 fold more potent than TRH to stimulate gastric secretion. Intravenous infusion of RX-77368 ($100ngkg^{-1},h^{-1}$) did not modify gastric secretion whereas a $1\mu gkg^{-1}h^{-1}$ dose slightly stimulated acid secretion. RX-77368 action (100 ng, i.c.) is partly blocked by acute subdiaphragmatic vagotomy but not by bicuculine injected at adose (1mg/kg i.p.-10min) which reversed the vagally mediated stimulatory effect of i.c. pcpGABA. These data demonstrate that RX77368 is more potent than TRH to act within the rat brain to increase gastric acid secretion. Its action is mediated in part by vagal dependent mechanisms unrelated to gabaergic system.

28.3

LOCALIZATION OF GASTRIN RECEPTORS ON CANINE FUNDIC MUCOSAL CELLS. Lovick P. Thomas,VI*, Andrew H. Soll, and Deborah A. Amirian*. CURE, VA Wadsworth and UCLA Medical Centers, Los Angeles, CA 90073.

While there is little controversy concerning the presence of histamine H_2 receptors and muscarinic receptors on parietal cells, the locus of receptors for gastrin within the fundic mucosa remains subject to controversy. Using enzyme dispersed canine fundic mucosal cells, we have studied the localization of gastrin receptors using radioligands. Biologically active ^{125}I-(15-leucine)-gastrin-17 binds specifically and reversibly to dispersed canine fundic mucosal cells. When fundic mucosal cells were separated by size in a Beckman Elutriator rotor, ^{125}I-Leu-gastrin binding correlated to the large cell fractions which contained parietal cells. However, elutriation separation enriched parietal cells only to about 55%. To further enparietal and chief cells, sequential step density gradients were performed using bovine serum albumin and Ficoll. In the lighter and denser fractions respectively, parietal and chief cells were enriched to greater than 90%. Specific ^{125}I-Leu-gastrin binding correlated positively with parietal cells (r = 0.98) and negatively to chief cells (r = -0.96). To confirm the localization of gastrin receptors to the parietal cell, autoradiography was performed using cytocentrifuge slides coated with Kodak NTB2. Discrete localization of exposed silver grains was found to plasma membranes of parietal cells, but not of chief cells. With these techniques, we detect gastrin receptors on canine parietal cells, but not on chief cells.

28.4

QUANTITATION OF DUODENAL CHOLECYSTOKININ IN THE RAT, HAMSTER, AND DOG. G. Greeley, J. Burdett,* F. Hill,* A. Spannagel,* J. Trowbridge,* and J.C. Thompson. The University of Texas Medical Branch, Galveston, Texas 77550.

The purpose of this study was to quantitate, by radioimmunoassay, the molecular forms of cholecystokinin (CCK) in the rat, hamster, and dog duodenum, using a carboxyl-terminal CCK antibody (CT-Ab) or an antibody which detected only the entire CCK molecule (E-Ab).

Species	Acid Extracted Duodenum	
	CT-Ab	E-Ab
Rat	21.7 ± 1.2 ng/g	115 ± 8.3 ng/g
Hamster	14.1 ± 1.3 ng/g	39.4 ± 3.9 ng/g
Dog	40.5 ± 6.9 ng/g	458.5 ± 146.5 ng/g
	Aqueous Extracted Duodenum	
Rat	34.5 ± 3.4 ng/g	74.0 ± 15.2 ng/g
Hamster	45.8 ± 7.1 ng/g	21.5 ± 2.6 ng/g
Dog	13.4 ± 1.7 ng/g	116.9 ± 20.5 ng/g

Conclusions: 1) In the rat, hamster, and dog duodenum, both small and large molecular forms of CCK are present. 2) As determined with the CT-Ab, there appears to be a greater proportion of smaller molecular forms in the rat and hamster duodenum. In the dog duodenum, however, the larger molecular forms predominate. 3) According to the data from the E-Ab, the larger molecular forms predominate in all three species.

28.5

COMPARISON OF BIOLOGICAL ACTIVITIES OF CHOLECYSTOKININ$_{58}$ ISOLATED FROM CANINE INTESTINE AND BRAIN. H. Sankaran,A. Wong,L. Way,V. Eysselein,J. Reeve Jr.,J.H. Walsh and C.W. Deveney. CURE,Los Angeles,CA and Surgical Service,VA Hospital,San Francisco,CA 94121.

The biological activity of 58 amino acid cholecystokinin isolated from the canine intestine and brain was compared. CCK$_{58}$ purified by affinity chromatography and High Pressure Liquid Chromatography possessed identical immunoreactivities to antisera specific to the amino terminus of CCK. Both the brain and intestinal CCK's were tested for their biological potencies in in vitro isolated mouse pancreatic acini and mouse brain particulate preparation. The results are as follows

CCK	MAX. AMYLASE RELEASE FROM ACINI	CONC. OF PEPTIDE REQUIRED FOR: 50% INHIBITION OF CCK BINDING: ACINI	PARTICULATE
CCK$_8$	100 pM	300 pM	3 nM
brain	100-200 pM	600 pM	1-3 nM
intestine	300 pM	300-600 pM	3 nM

Furthermore, amylase release stimulated by both brain and intestinal CCK$_{58}$ could be blocked by 10^{-4} M dibutyryl cyclic GMP. Conclusion: Whether CCK$_{58}$ is only an intracellular precursor of other smaller forms, or an important circulating hormone, it appears to be the major molecular precursor of all smaller forms in brain and gut. Our results, however, indicate that the two CCK's isolated have identical potencies, compared to CCK$_8$, in eliciting amylase release from acini and in the inhibition of CCK binding to membrane receptors.

28.6

SOMATOSTATIN INHIBITION OF CAERULEIN INDUCED PANCREATIC GROWTH. J. Morisset. Centre de recherche sur les mécanismes de sécrétion, Sherbrooke University, Sherbrooke, Qué., Canada, J1K 2R1.

These studies were undertaken to evaluate the antitrophic potency of somatostatin on caerulein induced pancreatic growth. Sprague-Dawley rats (220-225g) were divided in 5 groups: control(c), caerulein(cae), caerulein + antisomatostatin(cae+ASS), caerulein + somatostatin 300 μg, kg^{-1} (cae+SS300) and caerulein + somatostatin 600 μg kg^{-1} (cae+SS600). Rats were given the following S.C. in gelatin: saline (c), caerulein 1 μg kg^{-1}, somatostatin 300 or 600 μg kg^{-1} thrice a day for 2 days and anti SS, 0.5 ml I.P. once daily. They were fasted overnight before sacrifice. Cae induced significant increases in pancreatic weight (32%), total contents of protein (57%), amylase (55%), ChTg (186%), RNA (33%), DNA (11%) and thymidine incorporation into DNA (464%). The addition of somatostatin antibody to caerulein significantly increased pancreatic weight (10%), total DNA (10%) and thymidine incorporation (32%). SS at the 300 or 600 μg kg^{-1} dose reduced significantly the following increases initiated by caerulein: pancreatic weight (9%), total protein (17%) and ChTg (37%), and thymidine incorporation (51%). Total RNA and DNA were respectively reduced by 7 and 5%. In conclusion, inhibition of endogenous somatostatin by a specific antibody had an additive effect on the trophic effect of caerulein. These data indicate that somatostatin can reduce pancreatic growth induced by caerulein. Supported by grants A6369 from NSERC of Canada and 733 from ME Quebec.

28.7

ISOLATION OF TWO STIMULANTS OF GASTRIC ACID SECRETION FROM NON-ANTRAL CANINE STOMACHS. M.S. Orloff*, N.W. Bunnett*, H.J. Corbet*, J. Reeve*, J.H. Walsh.

Three stimulants of gastric acid secretion, histamine, gastrin and bombesin are present in the stomach. An activity was identified in acidic extracts of canine non-antral gastric tissues that stimulated acid secretion in the conscious and anesthetized rat. After high pressure liquid chromatography the activity was separated into two components, neither containing gastrin, bombesin-like immunoreactivities nor histamine. The fraction eluting earlier on the HPLC was provisionally named oxyntin 1 and the later eluting fraction, oxyntin 2. Both oxyntins stimulated acid secretion when infused intravenously into urethane anesthetized rats prepared with gastric fistulas. Both activities were purified to apparent homogeneity by sequential HPLC steps. Preliminary analysis of acid hydrolysates revealed no identifiable amino acids in oxyntin 1 while oxyntin 2 had a composition consistent with a peptide of 10 to 15 amino acids. The composition is Lys 1, Asx 2, Ser 2, Glx 2, Pro 2, Gly 4, Ala 1. Non integral amounts of Lys 0.2, Ser 0.4 and Leu 0.3 were also found. Oxyntin 2 could not be sequenced presumably due to a blocked amino terminus. The presence of Glx in the composition could be consistent with a pyroglutamic residue at the amino terminus of this peptide. Ongoing work is aimed at purification of stimulants in larger amounts. Currently a chronic gastric fistula preparation in the conscious rat is being used as the bioassay, as it appears to be more sensitive to the two activities.

28.8

CATABOLISM OF A BOMBESIN-LIKE PEPTIDE IN THE INTERSTITIAL FLUID OF THE STOMACH. N.W. Bunnett*, M.S. Orloff*, J. Reeve*, J.H. Walsh. Center for Ulcer Research and Education, VA Wadsworth Medical Center, Los Angeles, CA 90073

Bombesin-like neuropeptides in nerve fibers of the stomach wall are putative neurotransmitters and may be inactivated locally after secretion. To examine the local pathways of catabolism we have developed a new technique called interstitial fluid dialysis. Under general anesthesia 2 bundles of cellulose dialysis fibers were implanted into the region of the submucosal or myenteric nerve plexuses in the gastric corpus of dogs and rats. Experiments were performed on conscious animals after recovery from surgery. A synthetic bombesin analog, Tyr^4-bombesin, was radioiodinated and perfused through 1 set of dialysis tubes. The adjacent bundle of tubes was filled with 5% BSA in 0.9% NaCl to collect the catabolized label. Catabolic products were separated by HPLC. In dogs after 1 hour there was a diminution of the intact peptide and appearance of 3 additional labeled compounds that eluted earlier. Almost 50% of the radioactive material remaining after 1 hour appeared to be undegraded peptide. In rats the pattern of products was similar, but these were generated much more rapidly than in the dog. These results suggest that neuropeptides released into the extracellular fluid of the stomach are degraded by specific mechanisms that may not require passage into cells or into the vascular space.

28.9

STIMULATORY EFFECT OF THE MONOAMINE OXIDASE (MAO) INHIBITOR, NIALAMIDE, ON AMINO ACID-INDUCED GASTRIN (G) SECRETION. L.M. Lichtenberger, L.A. Graziani* and R. Delansorne*. Univ. of Texas Medical School, Houston, TX 77025

We have reported that amines, either present in the diet or produced intracellularly by the decarboxylation of amino acids, are potent in vivo and in vitro stimulants of G release (Am. J. Physiol. 243:G429, 1982). If amines are intracellular stimulants of G release, it would follow that the activity of the degradative enzyme, MAO, in the antral mucosa should play an important role in the regulation of the local conc of amines and their efficacy to stimulate G release. In order to investigate this possibility, the effect of secretory stimulants on G release from both in vivo and in vitro systems was studied in the presence of the MAO inhibitor, nialamide. In the in vivo studies it was demonstrated that serum G levels 1 hr after ingestion of an amino acid rich-Vivonex meal was significantly ($P<0.02$) increased 3.1 fold if rats were pretreated with nialamide (200 mg/kg,i.p.). i.p.). Similarly, G release from isolated G cells in response to phenylalanine (10mM) was significantly ($P<0.05$) increased 1.5 and 1.8 fold by the addition of 10^{-7}M and 10^{-6}M nialamide respectively to the incubation medium. Conclusion: Inhibition of MAO activity results in an enhancement in amino acid-induced G release. Thus, the intracellular amine concentration plays an important role in the regulation of G cell secretory activity. (Supported by NIH Grants AM20686 and AM00842)

28.10

GASTRIC SECRETION AND BLOOD LEVELS OF GASTRIN PEPSINOGEN I IN CHILDREN WITH PRIMARY DUODENAL ULCER. G.L. de Angelis*,S.Scuto*,G.Banchini*,G.Gregori* and E.Molina. Hospital and University of Parma. Italy.

We studied 6 cases of duodenal ulcer (D.U.) in male children (mean 8.8 yrs., range 6-12 yrs., mean weight 29.8 Kg.). Ten healthy children, matched by weight, age and sex were used as controls. All the 16 children were studied by: A) a protein meal for gastrin and pepsinogen I response; B) pentagastrin 6 ug/Kg i.m. for acid secretion.

RESULTS	NORMAL (n=10)	DUODENAL ULCER (n=6)
A) - GASTRIN pg/ml		
basal	67.5 ± 7.3	68.3 ± 9.1
peak after food	115.0 ± 22.2	121.7 ± 14.7
PEPSINOGEN I ng/ml		
basal	48.7 ± 2.73	73.5 ± 12.5**
peak after food	54.3 ± 2.65	88.2 ± 15.2**
B) - BAO mmol H^+/hr/Kg b.w.	0.023 ± 0.02	0.102 ± 0.03*
MAO " " "	0.223 ± 0.04	0.408 ± 0.06*
PAO " " "	0.274 ± 0.04	0.599 ± 0.06**

The results are expressed as MEAN + S.E.M. (*$p < 0.05$; **$p < 0.01$).

Conclusions: The elevated gastric acid response associated with D.U.is already present in children, and seems to be cause rather than consequence.Finally a strong family history and elevated pepsinogen I further strongly support an inherited basis of D.U. in children. (Supported by a grant from C.N.R.-Rome).

28.11

POTENTIATION OF HISTAMINE BY GASTRIN IN THE GASTRIC FISTULA DOG.
Tobias O. Yellin* and Richard A. Macia*, (Spon: G. A. Feigen). Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897.

The maximal acid response to histamine (0.5 μmol/kg/hr) was halved by atropine (100 μg/kg bolus plus 20 μg/kg/hr), whereas the secretagogue action of pentagastrin (2 μg/kg/hr) was abolished by the same treatment. The characteristic fade observed with control histamine infusions was absent in atropinized dogs. Atropine's effects could not be overcome by doubling or quadrupling the dose of histamine nor by vagal stimulation with 2-deoxy-D-glucose (200 mg/kg, i.v.). Nevertheless, the response to histamine (0.5 μmol/kg/hr), with a rate of fade not different from control, was fully restored in the presence of atropine (100 μg/kg bolus plus 20 μg/kg/hr) by the addition of pentagastrin infusion (2 μg/kg/hr). It follows that gastrin potentiates the action of histamine on acid secretion in vivo, independently of cholinergic excitation, just as it does in canine oxyntic cell suspensions as reported by Soll (J. Clin. Invest. 61, 381, 1978). Our conclusion is in agreement with Johnson and Grossman (Gastroenterology 56, 687, 1969), but contradicts Hirschowitz, et al. (Amer. J. Physiol. 244, 509, 1973).

FETAL AND NEONATAL BIOLOGY

29.1

THE STIMULATORY EFFECT OF REPRODUCTIVE AMINO ACIDS ON DIMETHYLBENZANTHRACENE (DMBA) PRIMED MAMMARY GLAND NUCLEIC ACID CONTENT DURING LACTATION. Howard S. Pitkow, Michael Goldman* and Bill Urbas*. 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. We also found that some amino acids had gonadotropic-like and/or estrogenic-like activity. In order to determine the effects of reproductive amino acids on DMBA primed mammary gland tissue, female adult virgin Long Evans rats (12 animals/group) were intraperitoneally injected daily with 1 mg DMBA in 0.2 ml sesame oil on days 8 through 12 of pregnancy. On days 8 through 17 of gestation these rats were subcutaneously injected with 150 mg/kg body weight of either L-tryptophan, L-arginine, or L-glycine in 1 ml saline solution. On day 1 of lactation, we observed that L-tryptophan and L-glycine significantly increased the depressed DMBA control values for total RNA (mg), RNA (mg/100g B.W.), RNA (mg/100mg M.G.), and RNA/DNA to that of sesame oil controls. L-glycine significantly increased both the total DNA and DNA (mg/100g B.W.) to that of sesame oil control levels whereas L-tryptophan had a positive effect only on the latter parameter. However, L-arginine had no stimulatory effect on the RNA or DNA values. Our data suggests that L-glycine, which increases growth hormone release, and L-tryptophan, which enhances and mimicks prolactin secretion, negate the harmful effects of DMBA by stimulating mammary gland growth (i.e., DNA) and secretion (i.e., RNA).

29.2

THE EFFECTS OF THYROID HORMONES ON DEVELOPMENT AND MYELINATION OF THE NEONATAL RAT SCIATIC NERVE. D.M. Van Wynsberghe and Joan M. Macdonald*. Univ. of Wisconsin, Milwaukee, WI 53201

Thyroidectomized neonatal rats were given daily subcutaneous injections of 1 ug/10 gm body weight of triidothronine (T_3), thyroxine (T_4), or triiodothyroacetic acid (T_3AC) from the fifth day of age. Left and right sciatic nerves were removed from the T_3, T_4 and T_3AC treated rats as well as control and thyroidectomized (T_X) animals at 10, 15 or 20 days of age to observe the effects of altered thyroid states on the ultrastructure and myelination of this peripheral nerve. Axon density, myelinated fiber size distribution, axon area, myelin area, and myelin area/axon area ratio were determined. Thyroidectomized and T_3 and T_3AC treated neonates demonstrated no significant changes in axon size, density or myelination, in contrast to reported changes in size, density and myelination in CNS fibers. T_4 significantly increased axon density and decreased axon size, thus decreasing the area available for myelination and total myelin content.

29.3

PITUITARY-ADRENAL RESPONSES TO ETHANOL AND MORPHINE IN INFANT RATS EXPOSED TO ETHANOL PRENATALLY. A.N. Taylor, B.J. Branch*, L.A. Lane*, L.R. Nelson* and R.E. Poland*. UCLA Sch. of Med. & Brentwood VA Med. Ctr., Los Angeles, CA 90024 & Harbor-UCLA Med. Ctr., Torrance, CA.90509.

Fetal ethanol-exposed (FEE) rats show enhanced corticosterone responses to ethanol (E) and morphine (M) as adults. These same challenges were given to FEE neonates to determine if comparable effects occur in infants. Subjects were the offspring of Sprague-Dawley dams fed either a 5% w/v E-containing casein-supplemented liquid diet (BioServ) ad lib, pair-fed an isocaloric liquid diet without E, or lab chow and water ad lib from gestation day-8 to birth. At birth, all pups were cross fostered to normal dams with food and water available ad lib. At 7 days of age, pups were injected s.c. with E (1.5 g/kg), M (3.0 mg/kg) or saline and sacrificed 1 hr later. Serum corticosterone was measured by radioimmunoassay. In contrast to the adult findings, FEE pups showed a blunted response to both M and E, only the latter being significant ($P<0.01$). Responses to saline were: 17.6±1.1 (SEM) ng/ml in normal pups, 14.7±1.9 in pair-feds and 18.3±2.4 in E-derived; to M: 65.2±5.6, 55.2±6.3 and 50.5±4.6, respectively; and to E: 50.5±5.9, 43.5±3.1 and 27.3±2.2, respectively. These results suggest that tolerance to the effects of E and M exists for at least the first postnatal week and that any possible enhanced responses may be masked by the persistent effects of the fetal E exposure. Developmental patterns for these effects are now being determined. (Supported by VA Medical Research Service.)

29.4

ADRENAL EPINEPHRINE (E), NOREPINEPHRINE (N) AND DOPAMINE (D) IN THE FASTING NEWBORN RAT. J. J. Hannigan*, L. Witek-Janusek, and C. L. Anderson*. Depts. of Pediatrics, Physiology and Nursing, Loyola University, Maywood, IL 60153

Adrenal catecholamines (CA) are important in neonatal adaptation to extrauterine life. Most studies of the neonatal adrenal report total CA content. This study used a sensitive assay, high performance liquid chromatography with electrochemical detection, to measure E, N, and D in newborn and day old rat pups. On day 21, pregnant dams were decapitated and pups were delivered by C-section. Adrenals were obtained from pups randomly decapitated at birth and 1, 2, 4, and 6 hrs postnatal. Pups kept beyond birth were maintained in an incubator (39°C). Adrenals were also obtained from 12 vaginally delivered, day old, nursing pups. Individual adrenal CA did not differ from birth to 6 hrs; thus, these values were combined for the newborn. CA values (μg/gm wet wt) are below.

	E	N	D	E/N
Newborn (N=48)	2.4±0.4*	1.9±0.2*	0.23±0.03*	1.3*
% of Total CA	53.0%	42.0%	5.0%	
Day Old (N=12)	64.4±5.6	15.6±1.3	2.6±0.2	4.2
% of Total CA	78%	18.9%	3.1%	

E, N, D and E/N were significantly (*$P<.05$) less in the fasting newborn from birth to 6 hrs compared to the fed day old rat. Depletion of adrenal CA in the newborn is most likely due to multiple stressors of birth, maternal deprivation and/or lack of CA precursors (dietary tyrosine).
(Supported in part by USPH 5 S07 RR05368BRSG)

29.5

THE EFFECT OF PROSTACYCLIN ON ANGIOTENSIN II INDUCED PLACENTAL VASOCONSTRICTION. V.M. Parisi* and J.H.G. Rankin. Depts. Ob-Gyn & Physiology, University of Wisconsin, Madison, WI 53706.

Significant alterations in vascular responsiveness to angiotensin II (AII) have been documented during pregnancy. We have observed that PGI_2, a potent vasodilator, does not dilate the placental vasculature of sheep. However, we thought that PGI_2 might modulate the placental vasoconstriction induced by AII. We measured regional blood flows and resistances (R) by the radioactive microsphere technique in 6 near-term sheep. Blood flows were measured in the control condition (C), and 15 min after beginning an infusion of AII at 5 ug/min (T1). Additional measurements were made 15 min after the addition of 50 ug/min of PGI_2 to the AII infusate (T2), and again 15 min after removing PGI_2 while continuing AII (T3). Results are given below:

	C	T1	T2	T3
Blood Pressure (mmHg)	84± 6	103± 6	66± 4	96± 5
Renal R (PRU)	0.14±.02	0.30±.04	0.15±.03	0.31±.03
Uterine R (PRU)	0.60±.09	1.35±.31	0.62±.10	1.77±.28
Placental R (PRU·kg)	0.33±.04	0.42±.06	0.62±.10	0.45±.09

The renal and uterine vasculatures showed the expected vasoconstriction to AII, which was then reversed to control levels by PGI_2. Unexpectedly, PGI_2 did not reverse the AII vasoconstriction in the placenta, but further increased R ($P<.03$). We conclude that PGI_2 does not modulate AII induced placental vasoconstriction. It follows from these data that PGI_2 may not be involved in the regulation of placental blood flow.
Supported by NIH grant HL27358.

29.6

FEEDBACK CONTROL OF FETAL PLACENTAL BLOOD FLOW IN 11 LAMBS. Debra F. Anderson and J. Job Faber, Dept. Physiol., S.M., OHSU, Portland, OR 97201.

Placental blood flow was monitored by means of a sensor on the aorta below the renal arteries and injections of microspheres. An occluder was placed distal to the flow sensor. After a control period of one week, placental flow was restricted for at least another week to an average of 54.5% of its control value by means of the occluder; no increase for presumed fetal growth was allowed. Fetal arterial Po_2 and pH fell after restriction; changes (control vs restricted) in arterial blood pressure proximal to the occluder (50 vs 50 mm Hg) and hematocrit (34 vs 35 %) were not significant. Fetal placental resistance to flow fell by 3.85% per day ($P<0.005$) which is approximately the normal rate of decrease in the last third of gestation in sheep. The fetuses continued to grow at a rate of 2.0 %/day ($P<0.001$), according to measurements of plasma volume with radio iodinated plasma albumin (normal growth is approximately 5%/day). These results were not influenced by the duration of the restriction of flow up to 21 days. It is concluded that fetal placental blood flow is not defended by negative feedback control in the last third of gestation in sheep.
Supported by USPHS ROI HL 27194.

29.7

LEFT VENTRICULAR STROKE VOLUME IN THE VENTILATED FETUS. K.L. Thornburg and M.J. Morton*. Dept. of Physiology and Medicine (Heart Research Laboratory), Oregon Health Sciences University, Portland, OR 97201

Left ventricular stroke volume (LVSV) increases substantially at birth in the lamb despite increased arterial pressure. This increase in LVSV is not explained by elevated filling pressure. To determine the role of ventilation in augmenting LVSV, we prepared 6 near-term fetal lambs with a tracheal tube, aortic electromagnetic flow sensor, carotid, jugular, pericardial and left atrial catheters. After 9 ± 2 (mean ± SD) postoperative days, blocking doses of propranolol (1 mg/kg) and atropine (0.25 mg/kg) were administered. Blocked control values were pH 7.36 ± .02, P_{CO_2} 46.3 ± 2.9 mmHg, P_{O_2} 18.6 ± 3.3 mmHg, arterial O_2 content 8.2 ± 2.6 $ml \cdot dl^{-1}$. LV function curves were generated by rapidly withdrawing and infusing blood before and during *in utero* ventilation (VENT) with 100% O_2 to determine maximum LVSV ($LVSV_{max}$). Two lambs with immature lungs were not oxygenated, but showed a 25% increase in $LVSV_{max}$ during VENT. In 4 lambs, O_2 content doubled (17.1 ± 2.1 $ml \cdot dl^{-1}$) during VENT. These lambs showed a 56% increase in $LVSV_{max}$ during VENT. We conclude that: 1) the fetal lamb can be respired *in utero*, 2) VENT itself augments $LVSV_{max}$, 3) oxygenation further augments $LVSV_{max}$, and 4) LVSV in the ventilated, oxygenated fetus approximates neonatal values.
(Supported by HL 29324.)

29.8

THE PERICARDIUM IMPOPTANTLY AFFECTS FETAL CARDIAC FILLING PRESSURE. Mark J. Morton* and Kent L. Thornburg. Oregon Health Sciences University, Portland, OR. 97201

Pericardial restraint of cardiac filling is well described in adult animals. Fetal cardiovascular pressures are frequently referenced to amniotic fluid pressure (AMN). Accordingly, we investigated the contribution of pericardial (PC) and thoracic pressure (TH) to observed mean right atrial pressure (RA). Eight fetal lambs were instrumented with vascular catheters, a silastic pericardial and polyvinyl pleural catheter, both with multiple side holes. Subsequent autopsies showed no evidence of pericardial or pleural reaction. Fourteen studies were performed 10 days (range 4-21) after surgery. Amniotic, pleural, pericardial and RAP were measured during rapid hemorrhage and re-infusion of blood and crystalloid. During three studies, pericardial fluid was added and measurements repeated. Transmural pressures during control were RA-PC 2.7 ± 1.5, PC-TH 1.2 ± 1.5, TH-AMN -0.2 ± 1.7 mm Hg. During volume infusion, PC-TH remained at control until RA-TH was 3.9 ± 1.4 mm Hg. At higher RA-TH pressures, PC-TH rose linearly with slopes ranging from 0.5-1.0. Addition of fluid to the pericardium shifted the PC-TH vs RA-TH relationship to the left with a slightly steeper slope. We conclude that the pericardium importantly restrains fetal cardiac filling at pressures above control, addition of volume to the pericardium further restrains cardiac filling, lastly that fetal cardiac growth must be precisely matched by increased pericardial volume. (Supported in part by NIH HL-29324.)

29.9

EFFECT OF ISCHEMIA ON FATTY ACID METABOLISM IN THE LUNGS OF RABBIT FETUSES. Anita Neogi* and Dipak K. Das. State University of New York at Stony Brook and Long Island Jewish-Hillside Medical Center, New Hyde Park, New York 11042

The effects of ischemia on in vivo fatty acid metabolism in fetal lung were studied using rabbit fetuses of 25 to 28 gestational age. Ischemia was produced by inflating the aortic balloon thereby reducing the uterine blood flow. Ischemic insult resulted significant increase in lactate/pyruvate and NADH/NAD ratios and decrease in ATP/ADP ratio in the fetal lung. Levels of CoA, acetyl CoA, carnitine and acetyl carnitine decreased while those of long chain acyl CoA and long chain acyl carnitine enhanced. Tissue content of these metabolites returned to normal after 2 hr stabilization following 20 min of ischemic insult. Ischemia also caused small increase in the lipogenesis and the neutral lipid content of the fetal lungs. Our results thus suggest that -oxidation in fetal lung is inhibited and becomes rate-limiting for the fatty acid oxidation during ischemia (supported in part by a grant from Long Island Jewish-Hillside Medical Center).

29.10

GLYCOGEN METABOLISM IN FETAL AND NEONATAL LIVER: EFFECT OF MATERNAL ETHANOL INGESTION. S.P. Singh, A.K. Snyder*, G. Pullen* and S.K. Singh*. VA Medical Center and Chicago Medical School, North Chicago, Il 60064.

We have previously reported perturbations in perinatal blood glucose (BG) levels in the offspring of rats fed ethanol (EtOH) during pregnancy. To provide insight into these effects, liver glycogen (LG) levels were measured in term fetuses and neonates of rats fed liquid diet containing moderate (2.8% w/v, ME) or high (5% w/v, HE) levels of EtOH. Controls were pair-fed (MP and HP) and ad libitum-fed (A) diet in which carbohydrates replaced ethanol isocalorically. Maternal weight gain and term fetal weights were significantly reduced ($p < 0.001$) in groups HE and HP due to reduced maternal food intake. Ethanol intake was 3.3 ± 0.1 and 2.5 ± 0.1 g/day for groups HE and ME. LG was significantly reduced ($p < 0.01$) in cesarean-derived term fetuses from HE rats (42.7 ± 6.0 mg/g liver vs 94.3 ± 3.6 in HP controls and 92.4 ± 5.4 in A controls). Liver glycogen levels were 90.7 ± 7.2 mg/g liver in ME fetuses and 106.9 ± 8.5 in MP control fetuses. LG was depleted more rapidly in both ethanol-fed groups. Four hours after delivery, 15% of HE and 57% of ME term LG stores remained, compared to 84% in A and 81% in MP and HP offspring. Simultaneously, HE offspring were significantly hypoglycemic relative to the other groups. It is concluded that ethanol exposure in utero can alter glycogen levels in the perinatal period. Research supported by the VA.

29.11

THE EFFECT OF CHRONIC ALCOHOL CONSUMPTION IN MATERNAL EFFICIENCY IN PRODUCTION OF FETAL TISSUE. T. Kepic*, A. Snyder*, S. Singh and M. Bennett*. Chicago Medical School and VA Medical Center, North Chicago, Il 60064

We studied the effects of chronic ethanol (EtOH) consumption during pregnancy on maternal nutrient storage and efficiency in production of fetal tissue. Timed-pregnant Sprague-Dawley rats were assigned to three groups: One group (EF) received liquid diet containing EtOH, 5% w/v. Control diet was provided by pair feeding (group PF) or ad libitum (group AF). Weight gain was reduced in both EF and PF dams ($p < 0.001$). Liver (LI), lung (LU) and placenta (PL) from cesarean-derived term fetuses were used for wet weights. There was a significant decrease ($p < 0.001$) in body weight (BW) and LI, LU and PL wet weights in the EF pups as compared to PF and AF groups. Maternal efficiencies were expressed as mean offspring body or organ weight per KCal maternal food consumption. Efficiencies of PF dams were the same or significantly greater than those of AF dams for BW (4.68 ± 0.58 $p < 0.001$), LI (24.28 ± 3.78 $p < 0.001$), LU (11.58 ± 0.81 $p < 0.02$) and PL (43.49 ± 4.01 $p < 0.001$) in agreement with previous reports for undernourished animals. Moreover, it appears that EtOH interferes significantly in the efficiency of production of LI (12.61 ± 2.74), LU (6.74 ± 0.48) and PL (35.67 ± 6.74). Research supported by VA.

29.12

CALCIUM CONTENT AND WEIGHT OF VENTRICULAR MYOCARDIUM DURING EARLY DEVELOPMENT - INFLUENCE OF ANGIOTENSIN II, NOREPINEPHRINE AND NUTRITIONAL INTAKE. Maurice S. Holder, Zelda D. Johnson and LaVal N. Cothran*, College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Physiological demands are thought to induce structural and contractile alterations in the myocardium even in the early stages of development. Such alterations may be modulated by humoral agents and subject to nutritional intake. The involvement of Angiotensin II (Ag II), Norepinephrine (NE) and different diets in the alteration process was investigated in developing rats at 4 and 8 weeks of age. Ventricular weight (VW), body weight (BW), free calcium in myocardium (MCa) and plasma (PCa) were determined after periods of chronic Ag II, NE and Calcium gluconate (Ca^{++}) treatment, and after regulation of type and amount of nutritional intake. Three groups of rats were studied, slow growers (SG), medium growers (MG) and fast growers (FG) depending upon the rate of post partum development. Results show that the pattern of VW/BW change seen in control rats was common in SG, MG and FG although there was a marked inter group difference in absolute size of heart and body with age. The difference was more pronounced at 4 than at 8 weeks. Diets rich in carbohydrate, protein or lipid significantly increased (10, 14, 25%) VW/BW; however, its decreasing pattern from 4 to 8 weeks was still present. Ag II but not NE significantly inhibited it. Diet types did not affect the level of MCa or PCa, however, both Ca^{++} and Ag II increased MCa at 4 and 8 weeks (8, 11%) while PCa was relatively unchanged. The most pronounced change in VW/BW was induced with Ca^{++} and both this and the Ag II effect was inhibited with Verapamil. The results suggest that the observed alterations in myocardial size may be Ca^{++} related and that Ag II plays a positive role in the response. (Supported by DRR MBRS-NHLBI 08111).

30.1

ROLE OF THROMBOXANE (TxA_2) IN THROMBIN-INDUCED INCREASE IN LUNG MICROVASCULAR PERMEABILITY. T. Noonan*, R.R. Garcia-Szabo* A.B. Malik. Albany Medical College, Albany New York 12208.

We examined the role of TxA_2 by using TxA_2 synthetase inhibitor, Dazoxiben (DAZ); and by the ability of TxA_2 mimic, U46619, to reverse any effect of DAZ on the thrombin-induced alterations in lung transcapillary fluid and protein exchange. We used the acute sheep lung lymph fistula model to compare the effects of thrombin (T)(85 U/kg b.wt.) in (i) Control; (ii) DAZ-pretreatment (10 mg/kg i.v. and 4 mg/kg/hr), and (iii) DAZ-pretreatment plus U46619 (1.5-2.0 µg/kg/hr). Results (µ±SE) are given below. (Qlym=pulmonary lymph flow, L/P=lymph/plasma prot. conc.):

	Qlym		L/P		PVR	
	Baseline	3 hr	Baseline	3 hr	Baseline	3 hr
GROUP						
Control T	3.8±1.0	13.4±3.5	.74±.07	.81±.04	5.5±.6	15.5±4.6
DAZ + T	4.7±0.8	6.1±1.1	.78±.04	.74±.06	6.3±.7	13.1±2.2
DAZ + T + U46619	4.1±1.2	13.9±5.6	.73±.03	.65±.01	5.3±.7	14.7±2.5

DAZ prevented to a great extent the thrombin-induced increases in Qlym and transvascular protein clearance (Qlym x L/P), and infusion of U46619 in DAZ-pretreated sheep restored the increases in Qlym and clearance. Therefore, TxA_2 may be an important humoral mediator contributing to the increase in lung vascular permeability after thrombin-induced intravascular coagulation. (HL 17355 and HL 26551).

30.2

INTRATHORACIC CONTRIBUTIONS TO CAUDAL MEDIASTINAL NODE (CMN) EFFERENT LYMPH IN SHEEP. K. Koike*, K.H. Albertine and N.C. Staub. Cardiovascular Research Institute and Departments of Physiology and Anatomy, University of California, San Francisco, CA 94143

Most extra-thoracic sources of CMN efferent lymph can be removed by resection of the node tail and superficial cauterization across the surface of the diaphragm (PHYSIOLOGIST 25:311, 1982). To determine whether there are any intra-thoracic non-pulmonary contributions, we measured lymph flow and its protein concentration, in 12 anesthetized, ventilated, prone sheep, as we resected various tissues that may send lymph to the CMN. Because the experiment is distructive, we did the resections in various combinations and waited one hour between steps. Baseline CMN lymph flow averaged 0.9±.5 ml/15 min (mean + S.D.). Cutting the pulmonary ligaments decreased flow by an average of 60%. Cauterizing around the lung hila in five sheep reduced flow 16% more. There were no detectible contributions from chest wall in five sheep but about 10% from the esophagus. After nearly complete isolation of the node, except for artery and vein, 14% of baseline flow remained. Lymph protein concentration did not change significantly with any procedure. We estimate that 75% of baseline intrathoracic CMN efferent flow comes from the lung. [Supported in part by HL25816 (Program Project)].

30.3

MEDIATORS OF TRACHEOBRONCHIAL FLUID FORMATION AND PULMONARY DESTRUCTION FOLLOWING SMOKE INHALATION. D.N. Herndon*, D.L. Traber, L.D.Traber*, G.C. Kramer, and J.T. Flynn. Jeff. Univ. Phil. PA, UC, Davis CA, U. TX. Md. Br. and Shriners Burns Inst. Galveston, Texas 77550.

Lung injury was produced by insufflating sheep with cotton smoke. Twelve to 24 hrs post-injury tracheobronchial damage was evidenced by wheezing, mucosal sloughing of casts 5 to 6 cm in size. Arterial PO_2 fell from 106±5 to 62±5 mmHg. Tracheobronchial fluid (TBF) collected from five animals was produced at a rate of 20±2 mℓ/hr. Total protein content of TBF was 4.5±0.6 g/dℓ relative to 6.2±0.2 g/dℓ in the plasma, a ratio of 0.75±0.05. Albumin, Igg and Igm ratios in TBF relative to plasma were determined in one animal and were 0.8, 0.68 and 0.2 respectively. Prostacyclin and Thromboxane (TXA_2) as measured by the stable metabolites in TBF were 0.498±0.305 ng/mℓ and 2.50±0.886 ng/mℓ respectively. Measurements in three animals revealed $3.6x10^6$ neutrophils/mℓ TBF and 29.5±0.4 units/mℓ TBF of the proteolytic enzyme β-glucuronidase. Marked elevation of the potent vasoconstrictor TXA_2 and the presence of leukocytes and proteolytic enzymes in the lung following insufflation of smoke may result in the demonstrated bronchospasm, local tissue destruction, tracheobronchial fluid accumulation and fall in arterial PO_2. The fluid is an ultrafiltrate of plasma producedd by increases in hydrostatic pressure and concomitant elevations in microvascular permeability.

30.4

EFFECTS OF ALLOXAN ON PULMONARY MICROVASCULAR PERMEABILITY. GF Nieman, CE Bredenberg*, AM Paskanik*, Upstate Medical Center, Department of Surgery, Syracuse NY 13210

Mongrel dogs were anesthetized with sodium pentobarbitol (30mg/kg), placed on a piston ventilator and subjected to a left thorocotomy. The following parameters were monitored: femoral, pulmonary and left atrial (foley balloon catheter) pressures, cardiac output and blood gases. A left tracheobronchial afferent lymphatic was cannulated and lymph flow ($\dot{Q}L$) measured. Lymph (C_L) and plasma (C_p) protein concentrations were measured by refractometry. $\dot{Q}L$ and C_L were monitored for one hour with normal left atrial pressure (LAP) to establish base line values. LAP was then increased to elevate capillary pressure from a mean of 5.4 to 22.9 cm H_2O and maintained until a new steady-state was obtained. A 1 hour period with normal LAP was followed by I.V. administration of Alloxan (85 mg/kg). LAP was again elevated (5.8-20.7 cm H_2O) with a second steady-state obtained. Vascular integrity was assessed by looking at C_L/C_p ratio at the high lymph filtration rates caused by increased LAP. Control C_L/C_p demonstrated the fall in C_L/C_p with elevated LAP characteristic of normal microvascular membrane permeability. However, following Alloxan infusion and increased LAP, C_L/C_p increased to greater than control levels (control C_L/C_p 0.56; Alloxan C_L/C_p 0.66) indicating increased microvascular endothelial permeability.

30.5

THE EFFECT OF BUMETANIDE (B) ON CANINE OLEIC ACID (OA) PULMONARY EDEMA. J. Ali* K. Duke* (Spon. N. Anthonisen). University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0Z3.

To determine if B, a potent loop diuretic, like furosemide, improves shunt in OA edema by pulmonary vasoactivity we produced left lower lobe (LLL) edema by injecting .03ml/Kg OA into the LLL pulmonary artery in 20 anesthetized open chested dogs ventilated on 100% O_2. Fractional perfusion to the LLL ($Q_L/Q_{T\%}$) was measured with electromagnetic flow probes and LLL shunt ($Q_{SL}/Q_{T\%}$) from LLL pulmonary venous O_2 content at: (1) Baseline (2) 1 1/2 hrs. after OA (3) 15 min. after either 0.1mgm/Kg B was given I.V. to 10 dogs (Group I) or an equivalent volume of saline to the other 10 dogs (Group II) and (4) 2 1/2 hrs. after OA. Mean (± S.D.) were (*denotes $p<.05$ compared to preceding values):

$Q_L/Q_{T\%}$:	1	2	3	4
Group I	29.9 ± 1.8	14.7 ± 1.1*	14.6 ± 0.7	15.3 ± 1.9
Group II	28.6 ± 2.1	14.2 ± 1.1*	14.2 ± 1.4	9.9 ± 1.1*
$Q_{SL}/Q_{T\%}$:				
Group I	9.3 ± 4.0	54.3 ± 13.6	54.7 ± 13.6	38.6 ± 12.0*
Group II	8.7 ± 1.6	45.1 ± 8.8	48.3 ± 7.8	70.4 ± 6.2*

Wet to body weight ratios were less ($p<.05$) in the right lower lobe (2.5 ± 0.2 vs 2.9 ± 0.3) and the LLL (4.7 ± 0.4 vs 6.0 ± 0.5) after B. Since Q_L/Q_T and Q_{SL}/Q_T did not change at 15 mins. after B, we conclude that B improves shunt by reducing the OA edema following diuresis and not through pulmonary vasoactivity.
(Supported by the Manitoba Lung Association).

30.6

LEUKOCYTES ARE NOT REQUIRED FOR OLEIC ACID INDUCED LUNG INJURY IN SHEEP. M.R. Flick, M. Julien*, and J.M. Hoeffel*. University of California, San Francisco, CA 94143.

We studied the effect of leukocyte depletion on oleic acid induced lung injury in 4 unanesthetized sheep with lung lymph fistulas. We measured pulmonary arterial (Ppa) and left atrial (Pla) pressures, cardiac output ($\dot{Q}b$), and lung lymph fluid ($\dot{Q}lym$) and protein ($\dot{Q}prt$) flows. After baseline periods we infused oleic acid (0.06 ml/kg intravenously) in the same sheep when normal and when made profoundly leukopenic by repeated injections of a nitrogen mustard. Mean data were:

Condition	Ppa (cmH_2O)	Pla (cmH_2O)	$\dot{Q}b$ (l/min)	$\dot{Q}lym$ (ml/h)	$\dot{Q}prt$ (mg/h)
Normal					
baseline	15	1	6.2	10.0	264
oleic acid	28	-1	5.9	31.8	832
Leukopenia					
baseline	15	-2	5.7	6.3	172
oleic acid	27	-5	5.4	27.2	663

Hemodynamic and lung fluid balance variables were the same in the baseline and injury periods in the same sheep when normal and when leukopenic. Leukocytes do not play a central role in oleic acid induced lung injury in sheep.
(Supported by NIH Grants HL-26913 and HL-19155 and the Medical Research Council of Canada)

30.7

OLEIC ACID LUNG INJURY IS NOT AFFECTED BY METHYLPREDNISOLONE NOR IBUPROFEN IN SHEEP. M. Julien*, J.M. Hoeffel*, and M.R. Flick. University of California, San Francisco, CA 94143

We studied the effect of methylprednisolone (MPS) or ibuprofen (IBU) pretreatment on oleic acid induced lung injury in unanesthetized sheep with lung lymph fistulas. We measured pulmonary arterial (Ppa) and left atrial (Pla) pressures, cardiac output ($\dot{Q}b$), and lung lymph fluid ($\dot{Q}lym$) and protein ($\dot{Q}prt$) flows. After baseline periods we infused oleic acid (0.06 ml/kg intravenously) in the same sheep when untreated and when pretreated with MPS (30 mg/kg) or IBU (12.5 mg/kg).

Condition (n)	Ppa (cmH_2O)	Pla (cmH_2O)	$\dot{Q}b$ (l/min)	$\dot{Q}lym$ (ml/h)	$\dot{Q}prt$ (mg/h)
Untreated (6)					
baseline	14	2	5.5	8.8	295
oleic acid	24	0	5.3	30.8	960
MPS (4)					
baseline	15	0	5.7	8.1	255
oleic acid	26	-1	5.0	26.3	710
IBU (2)					
baseline	14	3	5.7	7.2	270
oleic acid	25	0	5.5	31.9	1048

Hemodynamic and lung fluid balance variables were the same in the baseline and oleic acid injury periods in the sheep under all 3 conditions.

(Supported by NIH Grants HL-26913 and HL-19155 and MRC Canada)

30.8

BETA RECEPTORS AND LUNG FLUID BALANCE IN AWAKE SHEEP. P.L. Culver, W.H. Rao, P. Dodek and N.C. Staub. Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, CA 94143.

We studied seven awake sheep with lung lymph fistulas to determine whether beta adrenergic receptors in the pulmonary circulation play a role in normal lung fluid balance. We measured pulmonary arterial (PA) and left atrial (LA) pressures, cardiac output ($\dot{Q}$), lung lymph flow ($\dot{L}$) and lymph/plasma protein concentration ratio (L/P). We calculated pulmonary vascular resistance (PVR). After a 6h baseline we did the following: 1) 2h baseline + 4h of propranolol [2 mg/kg IV, then 0.25-0.5 mg/(kg x h)] (5 sheep) or 2) 2h baseline + 4h of terbutaline [0.6 µg/(kg x h)] (4 sheep). The table summarizes the data (mean ± S.D.) of 2h baseline and 4h drug infusion.

	PA	LA	$\dot{Q}$ l/min	PVR units	$\dot{L}$ (ml/h)	L/P
Baseline	19±4	4±2	5.0±1.1	3.0±1.7	6.4±1.3	.63±.06
Propranolol	18±4	4±4	4.5±1.1	3.6±1.8	6.5±2.3	.61±.05
Baseline	19±5	3±2	6.0±1.1	2.2±0.8	9.2±1.1	.56±.04
Terbutaline	22±5	5±3	6.5±0.9	2.7±0.8	9.2±2.4	.56±.03

Neither beta stimulation nor blockade affected hemodynamics, except terbutaline increased and propranolol decreased cardiac output slightly. Lymph dynamics were unaltered. We conclude that beta receptors are not involved in normal lung fluid balance in sheep. [Supported by HL25816 (Program Project)].

30.9

METHYLPREDNISOLONE PROTECTS THE LUNG'S MICROCIRCULATION FROM ULTRASTRUCTURAL DAMAGE DURING AIR EMBOLIZATION IN AWAKE SHEEP. K.H. Albertine, P.C. Culver*, W-H. Rao*, and N.C. Staub. Cardiovascular Research Institute and Departments of Anatomy & Physiology, University of California, San Francisco, CA 94143.

Steroids protect the lungs from pathophysiologic changes during experimental injury. There ought to be correlated structural protection. We studied the protective effect of methylprednisolone (MPS) in four awake, chronically-instrumented sheep. After a 2h baseline period, we air-embolized the lungs for 4h in 3 sheep; in two we gave 30 mg/kg MPS 1h before infusing air. At 6h the 3 embolized and one control sheep were anesthetized and the left caudal lobe was insufflated with fixative. We examined all vessels in each of five tissue blocks/lobe, except capillaries of which we examined 10. The table shows the total number of vessels and lesions, defined as endothelial cell gaps or basal lamina disruption, in each category.

Condition (# sheep)	Pulmonary arterioles		Pulmonary venules		Alveolar capillaries		Bronchial vessels	
	#	Lesions	#	Lesions	#	Lesions	#	Lesions
Control (1)	10	0	9	0	50	0	5	0
4h air (1)	9	10	8	0	50	0	8	0
MPS/air (2)	18	5	8	0	100	0	3	0

After MPS, there were fewer lesions in the pulmonary arterioles. There were also fewer neutrophils at the blood-embolus interface. We conclude that MPS, partially protects the pulmonary arterial microcirculation from ultrastructural damage [Supported by HL-29377 (NIRA) and HL-25816 (Program Project)].

30.10

INTERSTITIAL FLUID PRESSURE MEASURED BY MICROPUNCTURE OF ISOLATED, EDEMATOUS DOG LUNGS AT DIFFERENT LEVELS OF INFLATION. J. Bhattacharya, M.A. Gropper and A. Eaton. Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, CA, U.S.A. 94143

Previously, we reported an interstitial fluid pressure (Padv) measurement obtained by micropuncturing adventitia surrounding venules (30 µm) in constantly inflated lungs (FED PROC 40:448, 1981). Now we have measured Padv at two inflation levels. We blood perfused 6 isolated left lower lobes of dog lung which we continuously weighed. When lobe weight doubled we equalized vascular pressures to 10 cmH_2O to maintain isogravimetric conditions; then measured Padv by micropuncture at constant alveolar pressure. The data referred to ambient (pleural) pressure are shown (mean S.D. cmH_2O).

Alveolar Pressure	Padv
7	2.4 ± .7
21	3.7 ± 1.0

At the lower inflation Padv was 4.6 cmH_2O less than alveolar pressure. When alveolar pressure tripled Padv increased slightly (P>0.01). We conclude that in edematous lungs, first alveolar pressure was poorly transmitted to the peri-venular interstitium; second, inflation increased Padv, probably by compressing interstitial fluid. (Supported by the California Lung Association.)

30.11

BRONCHIAL BLOOD FLOW INCREASES IN INFLAMMATORY LUNG EDEMA. S. Lakshmi, S.K. Jindal*, W. Kirk* and J. Butler. Department of Medicine, Univ. of Washington, Seattle, WA 98195

The pulmonary vessels in injured lung regions are constricted, destroyed or thrombosed. How then are the inflammatory and repair processes carried on? Our hypothesis is that it is via the anastomotic bronchial blood flow. We compared bronchial blood flow ($\dot{Q}Br$) before and after injuring the left lower lobe (LLL) in anesthetized, open-chested dogs. Systemic blood pressure, cardiac output, PO_2, PCO_2 and acid-base status were kept constant. The test lobe was hung from a strain gauge and weighed. The LLL pulmonary circulation was isolated and perfused with autologous blood. $\dot{Q}Br$ was measured as the sum of the blood overflowing the lobe's closed pulmonary vascular circuit plus its weight change. LLL injury (reflected by LLL weight gain) was caused by airways aspiration of 0.1 N HCl (2 ml/kg) or by pulmonary arterial injection of ANTU (αNaphthyl thiourea) (2 mg/kg).

$\dot{Q}Br$ (ml/min/100 gm dry lobe weight/100 torr $\bar{P}sa$: mean ± SEM)

Type of injury	Before injury(control)	After injury(minutes) 5	60	120	Total weight gain (gms.)
HCl(n=4)	16 ± 4.5	69*±19.0	57*±12.0	47*±13.5	78.8 ± 7.3
ANTU(n=4)	25 ± 5.6	77*±17.0	52*± 7.8	54*± 8.5	93.5 ±36.0

*P < 0.05 compared to control

$\dot{Q}Br$ also increased in 3 dogs after aspiration injury with glucose-glucose oxidase and in 2 with vascular injury due to oleic acid. We conclude that there is an immediate, significant increase in $\dot{Q}Br$ in inflammatory lung edema which is sustained for at least 2 hours.

30.12

METHACHOLINE INCREASES ANASTOMOTIC BRONCHIAL BLOOD FLOW IN DOGS SK Jindal*, S Lakshminarayan, W Kirk* and J Butler. Department of Medicine, Univ. of Washington, Seattle, WA 98195

What happens to the bronchial blood flow ($\dot{Q}Br$) following the inhalation of bronchodilator and bronchoconstrictor aerosols? The peribronchial bronchial vascular plexus sends branches through gaps in the cartilage and muscle fiber bundles into the submucosa where another network is formed. We postulate that these systemic vessels may be dilated by cholinergic agents. Such effects would be blocked by atropine. In anesthetized, open-chested dogs the right lung was ventilated with 100% O_2 to maintain systemic blood gases and the left lower lobe (LLL) with 5% CO_2 in air. The pulmonary artery and vein of the LLL were isolated and perfused with autologous, heparinized blood. $\dot{Q}Br$ was measured as the sum of the blood overflowing out of the closed pulmonary vascular circuit plus any lobe weight changes. After nebulizing 1 mg of methacholine in 10 cc saline in the LLL airway bronchoconstriction was confirmed from a rise in peak airway pressure at constant rate, tidal volume and static pressures. $\dot{Q}Br$ (ml/min/100 gm dry lobe weight/100 torr $\bar{P}sa$; mean ± SEM) =

Before (control)	After methacholine
20 ± 8.4	43* ± 10.8

*P < 0.05

Nebulized atropine (2 mgm in 10 cc saline), 30 minutes after the methacholine, lowered $\dot{Q}Br$ (and the airway pressure) towards control. We conclude that methacholine aerosol increases $\dot{Q}Br$ and that this effect is partly reversed by atropine.

30.13

EFFECT OF BLOOD pH ON LUNG HYPOXIC MICROVASCULAR PRESSURE PROFILE IN CATS. Y. Nagasaka, J. Bhattacharya, M.A. Gropper and N.C. Staub. Cardiovascular Research Institute and Dept. of Physiol., University of California San Francisco, CA 94143.

In our first micropuncture determination of the site of hypoxic vasoconstriction in cats (FED PROC 42:595, 1983) the blood was alkaline (pH=7.6). We have made more measurements at lower pH (increased Pco2). We perfused six isolated cat lungs with blood at constant flow [80±20 ml/(kg x min)] and left atrial pressure = 9 cmH_2O. By micropuncture (J APPL PHYSIOL 52:634, 1982), we measured pressure in 30-50 μm diameter arterioles and venules at alveolar pressure=8 cmH_2O; first at FiO_2=.3, then .02. Blood Pco2 (33±5 mmHg) and pH (7.40±.07) were nearly constant in each experiment. The table summarizes the pressures in cmH_2O(mean ± S.D.)

Condition	Artery	Arteriole	Venule	Left Atrium
30% O_2	18.2 ± 0.5	15.2 ± 0.7	12.4 ± 0.9	9.0
2% O_2	28.3 ± 2.6	19.0 ± 3.7	14.5 ± 3.9	9.0

In normoxia the pressure drop was distributed relative uniformly .33 arterioles, .30 alveolar wall microvessels, .37 veins. In hypoxia driving pressure was increased in all segments, as previously reported, but the arterial vasoconstriction was accentuated. Reducing blood pH (increased Pco2) changes the normoxic pressure profile slightly and amplies hypoxia vasoconstriction, mainly in the arteries. [Supported by HL25548].

30.14

EFFECT OF FORWARD AND RETROGRADE PERFUSION ON THE DISTRIBUTION OF PULMONARY VASCULAR RESISTANCE. P. Rock*, A. Patterson*, S. Permutt, J. Sylvester. Depts. of Anesthesiology-Critical Care Med., and Medicine, Johns Hopkins Med. Inst. at Balto. City Hosp. Baltimore, Maryland 21224.

The arterial and venous occlusion technique allows measurement of pressure gradients across the arterial (ΔPa), venous (ΔPv), and middle (ΔPm) segments of the pulmonary vasculature. ΔPm could exist, however, without a resistance in the middle compliant segment, if there were a Starling resistor with a high critical pressure in the non-compliant arterial segment. We assessed this possibility during normoxia (N) and hypoxia (H) in 14 isolated pig lungs perfused forward (F) and retrograde (R) at 50 ml/kg/min. Alveolar pressure was 3 mmHg and outflow pressure, 5 mmHg. During N, the total pressure gradient (ΔP_T) was 20.9 and ΔPm was 10.9 mmHg during F and 27.3 and 17.0 mmHg during R. During H, ΔP_T was 33.2 and ΔPm 17.7 mmHg during F and 35.7 and 21.8 mmHg during R. Since ΔPm was present during R as well as F, we conclude it was not caused by a Starling resistor in the arterial segment but by a resistance in the middle compliant segment, which we have previously found to act like a Starling resistor. The increase in ΔPm during R may have resulted from an increase in the critical pressure of this Starling resistor or because retrograde perfusion unmasked an ohmic resistance on the venous side of the Starling resistor. Supported by NIH Grant #HL-26752.

30.15

CYCLOSPORIN A (CyA) AND DEXAMETHASONE (DX) PROTECT RATS FROM THE CARDIOPULMONARY EFFECTS OF MONOCROTALINE PYRROLE (MCTP). Leon H. Bruner*, Katherine S. Hilliker* and Robert A. Roth* (SPON: G.L. Gebber). Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

Monocrotaline is a plant toxin that is metabolized to MCTP which causes lung injury and right ventricular hypertrophy (RVH). The onset of major pulmonary and hemodynamic changes is delayed four to seven days after a single dose of MCTP suggesting that an immune mechanism is involved in the pathogenic process. Rats were treated with a single dose of chemically synthesized MCTP (4 mg/kg, iv). Daily injections of the immunosuppressant, CyA (10 mg/kg, sc) were started two days prior to MCTP. Other groups received either MCTP and olive oil vehicle (OI), dimethyl formamide (DMF) and CyA, or DMF and OI. Rats were killed 14 days after MCTP. Administration of CyA with MCTP resulted in protection from increased lung weight to body weight ratio. There was significant RVH in the rats which received MCTP/OI whereas right ventricle weight in MCTP/CyA treated rats was not significantly different from rats which received DMF/CyA or DMF/OI. In a second experiment, MCTP-treated rats were given daily injections of DX (27 μg/kg, ip). Other groups received either MCTP and saline (S), DMF and DX, or DMF and S. Administration of DX with MCTP resulted in protection from increased lung weight and RVH. These results suggest that the immune system is involved in the mechanism by which MCTP causes cardiopulmonary injury. (Supported by USPHS grant ES02581; LHB supported by HL07404; KSH by the Pharmaceutical Mfrs. Assn. Fdn.)

30.16

EFFECT OF COLD AIR HYPERVENTILATION (hv) ON BRONCHIAL BLOOD FLOW IN THE DOG. E.M. Baile,* R.W. Dahlby* and P.D. Paré. U.B.C. Pulm. Res. Lab., St. Paul's Hospital, Vancouver, Canada.

Bronchial artery vasoconstriction has been postulated as a mechanism in the induction of the bronchoconstriction produced by cold air hv. We measured bronchial blood flow ($\dot{Q}br$) to central airways and lung parenchyma in dogs before, during and after isocapnic hv of cold air. 5 anesthetized, supine, open-chest dogs were hyperventilated (f = 40; V_T = 30-35 ml/kg) for 25 min. with (1) warm, humidified room air (RA); (2) cold (-20°C), dry air and (3) RA. End tidal CO_2 was kept constant by adding CO_2 to the inspired line. During each period of hv we recorded vascular pressures, cardiac output and tracheal, inspired and expired temperatures. Using a modification of the reference flow technique, ^{113}Sn, ^{153}Gd and ^{103}Ru-labelled microspheres were injected into the left atrium to make separate measurements of $\dot{Q}br$ at each intervention. After the final injection dogs were killed and the lungs excised. Knowing the radioactivity in the tissue and reference flow blood sample, Qbr to airways and lung parenchyma was calculated (ml/min/g dry). Results:

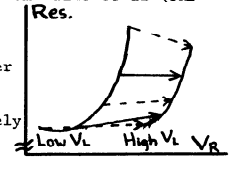
	RA	COLD	RA	
Airways	.63 ± .10	1.25 ± .20*	.69 ± .11	$\bar{x}$ ± SE
Parenchyma	.36 ± .04	.28 ± .03	.22 ± .03	*p < .01

We conclude that cold air hv increases $\dot{Q}br$ to central airways and speculate that this may play a role in the induction of exercise induced asthma.

30.17

PULMONARY VASCULAR RESISTANCE (R) IS NOT STRONGLY DEPENDENT ON LUNG VOLUME (Vl). K.C. Beck and S.J. Lai-Fook, Mayo Clinic, Rochester, MN.

R in isolated lungs is dependent on Vl, though the variation in R over the range of Vl becomes less at higher vascular pressures (Beck & Lai-Fook, Fed. Proc. 41 '82). To determine the implications of these findings for distributions of R in intact lungs in which Vl increases and vascular pressures decrease with height above the lung base, we derived blood flow ($\dot{Q}$) distributions using data from isolated perfused rabbit lung studies in zones 2 and 3 conditions. To quantify $\dot{Q}$, we derived separate equations for zones 2 and 3 using multiple linear regression that included linear, squared and cross product terms in the relevant variables. In zone 2, $\dot{Q}$ depends on pulmonary artery relative to alveolar pressure (Ppa') and Vl. In zone 3 when Ppa-Ppv is constant $\dot{Q}$ depends on pulmonary venous relative to alveolar pressure (Ppv') and Vl. Data for regional Vl in 2 cm slices at various lung heights in supine dogs at normal and high Vl were taken from Baile et al (JAP 52: p.914, '82). Derived regional R versus Vl curves were shifted nearly horizontally, as found by Baile et al. The shape of R vs Vl and the shift after increasing Vl are due to the relative effects of Vl, Ppa' and Ppv' as determinants of $\dot{Q}$. Vl per se has a relatively minor influence. Supported by NIH #HL06244 and Am. Lung Assoc. grants.



30.18

REVERSED PULSUS PARADOXUS. S.V. Lichtenstein*, T.W. Rice*, A. Panos*, K. Teoh* and M. Stilwell*. (SPON: C.E. Bayliss). Univ. of Toronto, Toronto, Ontario M5S 1A1

The transient increase in arterial pressure associated with positive pressure ventilation is referred to as "reversed pulsus paradoxus". This is in contrast to the normal decrease in arterial pressure with spontaneous inspiration, "pulsus paradoxus". Classically, it is believed that the differences observed are due to the pulmonary venous return to the left heart. No serious consideration has been given to the possible significance of the effects of pleural pressure on left ventricular (LV) function. We evaluated the hemodynamic effects of positive pressure ventilation in man and compared these to spontaneous respiration. This complex phenomenon was broken into its component parts by looking at lung inflation at constant pleural pressure and lung inflation at constant aortic blood flow. In addition, we evaluated LV blood volume at end inspiration and end expiration. Our results suggest that "reversed pulsus paradoxus" results from an increase in left ventricular stroke volume because of a primary decrease in left ventricular afterload and not a primary increase in left ventricular preload as previously thought.

30.19

BREATHING AT BIRTH STIMULATES PULMONARY PROSTACYCLIN SYNTHESIS. C.W. Leffler, J.R. Hessler, and R.S. Green*. U. TN Ctr. Hlth. Sci., Memphis, Tennessee 38163

Previously, we showed that ventilation of pump perfused fetal lungs stimulates pulmonary prostacyclin (PGI_2) synthesis; and indomethacin attenuates ventilation-induced pulmonary vasodilation by these fetuses. The present study investigated pulmonary PGI_2 synthesis by unanesthetized fetuses during spontaneous onset of breathing at birth. Fetal lambs and goats were instrumented at 0.93 term and delivered by C-section at 0.95 term. They began breathing spontaneously. PGI_2 was determined by RIA of 6-Keto $PGF_{1\alpha}$ using methods validated by comparison to gas chromatography with electron capture detection. Fetal left pulmonary PGI_2 production ([pulmonary venous concentration minus pulmonary arterial concentration] x pulmonary blood flow) was undetectable (-1.7 ± 1.0 (SEM) ng $PGI_2 \cdot kg^{-1} \cdot min^{-1}$) and fetal pulmonary vascular resistance (PVR) high (5.1 ± 0.9 mmHg·kg·min^{-1}). PGI_2 production increased to 30.1 ± 12.3 ng $PGI_2 \cdot kg \cdot min^{-1}$ and PVR declined to 0.5 ± 0.1 mmHg·kg·min^{-1} 15 min after birth. PVR remained low even though PGI_2 production fell 2-5 hrs after birth. These results suggest that pulmonary PGI_2 synthesis participates in the decline of PVR that accompanies the onset of ventilation at birth, but may be less important in maintenance of low PVR once established. (Supported by Am. Heart Assoc., NIH, TN Lung Assoc., Biomed Res. Support Grant from UT Col. of Med.)

BODY FLUID REGULATION

31.1

PREDICTION OF BODY COMPOSITION BASED ON WHOLE BODY ELECTRICAL IMPEDANCE. Lyle H. Hamilton, Steven M. Horvath, Michael G. Maksud, Ernest D. Michael, Gerald B. Spurr, Ronald L. Jackson* Wood VA Med. Ctr. and Med. Coll. of Wisconsin, Milwaukee, WI 53193; Univ. of Calif., Santa Barbara, CA 93106; Oregon State Univ., Corvallis, OR 97331.

Measurements of whole body impedance (Z) or the ratio of Z at 2 frequencies have been proposed as an index of body composition. Anthropometry, body density (BD) by underwater weighing, skin-fold thickness (SF) at 3 sites (in most subjects) and Z at 10 KHz (ZL) and at 100 KHz (ZH) were measured in 45 males (M) and 93 females (F) ranging in age from 18 to 71 years. Most were college students in good to excellent physical condition. Correlations between % body fat calculated by density and by skin-fold thickness based on the method of Jackson and Pollack were r=0.721 for M and 0.788 for F. Correlations between body density and ZL, ZH and the ratio ZL/ZH (RZ) were, respectively -0.91, -0.157 and 0.417 for M and -.203, -.262 and 0.354 for F. Multiple regression analyses were necessary to provide prediction equations useful for body density. Equations were:

M: BD = 0.813119 + 0.158143RZ + (1.122E-3)Ht - (1.189E-3)Wt - (1.77E-4)ZH

F: BD = 0.995104 + 0.088820RZ + (3.52E-4)Ht - (7.69E-4)Wt - (1.26E-4)ZH - (7.33E-4)Thigh SF

SFaffected predictions for F, but not for M. Multiple correlation coefficients were 0.817 for M and 0.779 for F.

31.2

NASAL SALT GLAND SECRETION INHIBITED BY ANGIOTENSIN II. H. T. Hammel and J. E. Maggert* Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, CA 92093.

Adult Pekin ducks received an intravenous control infusion of 1000 mosm NaCl/Kg H_2O at 0.4 ml/min for 90 min. The amount of salt and water secreted by the nasal salt glands was 100±5% of the amount infused. When the infusate included Angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), Sigma A4390, at the rate of $8x10^{-9}$ gm min^{-1} Kg^{-1}, 70% of the salt solution was secreted during the infusion and 30% subsequently. When angiotensin II accompanied the infusate at the rate of $80x10^{-9}$ gm min^{-1} Kg^{-1}, only 3% of the salt solution was secreted during infusion and more than 90% was secreted during the subsequent 60 min. Since the rate of secretion by the nasal salt gland can not be fully explained by variations in plasma osmolality, NaCl concentration, extracellular and intracellular volumes, we postulate that variations in the circulating level of Angiotensin II are affecting the secretory rate, NSF Grant PCM 78-23460.

31.3

ALTERING PLASMA CONCENTRATION OF ARGININE VASOTOCIN CHANGES GLOMERULAR FILTRATION RATE IN SALINE-ADAPTED PEKIN DUCKS. R. Kaul*, R. Gerstberger*, D.A. Gray* and E. Simon. Max-Planck-/W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, FRG.

Reabsorption of nearly isotonic renally excreted salt and water, combined with hypertonic extra-renal salt excretion, is believed to produce free water for nitrogen excretion in birds with salt glands. Increased glomerular filtration rate (GFR) in response to salt loading has been reported for gulls and geese, but salt loads also elevate plasma concentration of arginine vasotocin (AVT) and intravenous AVT reduces GFR in birds lacking salt glands. We have therefore measured GFR (C-14 inulin clearance) in birds with functional salt glands while manipulating plasma [AVT] (radioimmune assayed). In 8 Pekin ducks (2.3 ± 0.2 kg, $\bar{x} \pm$ SE) adapted for at least 30 days to 600 milliosmolal (mO) NaCl, GFR during constant iv infusion of 200 mO NaCl at 1 ml/min was 2.9 ± 0.1 ml/min·kg (n = 19) at a plasma [AVT] of 19 ± 1 pg/ml (n = 9). Doubling plasma [AVT] by rapid injection of synthetic AVT (10 ng/kg) invariably reduced GFR by 40 to 90%, and reduction of plasma [AVT] by iv injection of antibodies to AVT always increased GFR. Endogenous AVT release, when stimulated by continuous push-pull perfusion of the third ventricle with hypertonic artificial CSF or inhibited by hypotonic third ventricular perfusion, was also invariably associated with respectively decreased and increased GFR. Thus, in a bird with functional salt glands, GFR is inversely correlated with even reflexly regulated endogenous AVT.

31.4

EFFECTS OF MORPHINE AND 2-DEOXY-D-GLUCOSE AND WATER INTAKE IN RATS. G.K.W. Yim,* T. Mack,* J.D. Roache,* A. Malave,* and J.E. Zabik.* (SPON: P.V. Malven). Dept. of Pharmacology and Toxicology, Sch. of Pharmacy and Pharmacal Sci., Purdue University, West Lafayette, IN 47907.

The reduction of immobilization-induced saline drinking, by naloxone, suggests that endogenous opioids may mediate the stress-induced increase in salt intake (Fed. Proc. 42: 1983). The aim of this study was to determine if morphine and 2-deoxy-D-glucose (2-DG) would increase salt intake. Male Sprague Dawley rats were given access to 0.15 M NaCl and tap water for two weeks. The control rats preferred tap water. Morphine sulfate (4.0 and 8.0 mg/kg, s.c. at 1800 hr) resulted in a dose related increase in nocturnal saline intake, without appreciably affecting total fluid intake. Thus, preference was reversed to saline (from 0.4 to 0.6 saline/total fluid intake). Daytime administration of morphine to the non-deprived rats increased fluid consumption, but not saline preference. 2-DG (400 mg/kg, s.c.) did not increase daytime or nocturnal saline intake. Since 2-DG-induced hyperphagia is reduced by naloxone, these results indicate that the opioid mechanisms involved in food intake differ markedly from those involved in salt intake. (Supported in part by BMSG 55861; Univ. of Puerto Rico Fellowship to AM. Purdue Research Foundation Fellowship to JR and Mark Summer Science Program to TM).

31.5

HIGH METABOLIC ACTIVITY IN THE SEPTAL TRIANGULAR NUCLEUS IN RAT MODELS OF THIRST. M. Kadekaro*, P.M. Gross, & L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The septal triangular nucleus (STN) and subfornical organ (SFO) have been identified as components of the cerebral circuit of structures subserving fluid balance. Anatomical studies have demonstrated: 1) afferent fibers from STN to SFO; and 2) merging of the ventrocaudal extent of STN with the ventral stalk of SFO, findings that suggest a functional relationship between these two structures. We previously reported high rates of glucose metabolism in the SFO of homozygous Brattleboro rats and in normal rats deprived of water for 18-120 hours. We report here high metabolic activity in the STN of thirsty animals. Rates of glucose utilization were determined by the autoradiographic [^{14}C]deoxyglucose method and computerized image-processing. Values presented are the mean μmol/100 g/min ± SE for STN of conscious animals. *p<0.05 compared to the respective control.

Water-sated Sprague-Dawley	49±2	n=8
120 hour water-deprived Sprague-Dawley	85±6*	n=8
Water-sated Long-Evans	49±4	n=7
18 hour water-deprived Long-Evans	63±5*	n=8
Water-sated homozygous Brattleboro	68±5*	n=8
Vasopressin-treated homozygous Brattleboro	44±2*	n=7
18 hour water-deprived homozygous Brattleboro	107±6*	n=8

The findings indicate that the STN is a locus of high metabolic activity during conditions of thirst.

31.6

BODY COMPOSITION, TISSUE WATER AND ELECTROLYTE, AND PHYSIOLOGICAL BALANCES IN STRAIN 13 GUINEA PIGS (GP) WITH PICHINDE VIRUS INFECTION. C.T. Liu, M.J. Griffin*, G. McNamee*, P.B. Jahrling*, and C.J. Peters*. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD 21701

Arenaviruses produce fatal infections without marked histopathological changes. Weight loss is characteristic of both human and experimental Lassa fever. To study the body wasting in an animal model analogue of Lassa fever, Pichinde virus infection in GP was used. Energy and nitrogen balances were negative by 10 days after virus inoculation and body water, Na^+, and K^+ retention decreased. By day 14, GP lost ~25% of their initial body weights and deaths occurred between days 13-19. Tissues were analyzed on day 14 and after pentobarbital euthanasia in other animals, the intact body was dessicated at 100°C and pulvarized. In the infected GP, body water, Na^+ and K^+ content, and lean body mass decreased significantly as did total body lipids. Relative weights of lung, liver, and kidney increased, but the heart wasted. Lipids accumulated in liver. Total lipids, water, and Na^+ content of cardiac muscle decreased. Skeletal muscle showed significant decreases in total water, Na^+, K^+, and in intracellular Na^+. In contrast to the water losses elsewhere, the lung showed edema. The fatal course of this arenavirus infection is associated with muscle mass loss, dehydration, pulmonary edema, changes in renal function and cardiac muscle composition, and hepatic fat accumulation.

31.7

THE CHRONIC EFFECTS OF HYPERPROTEINEMIA ON ARTERIAL PRESSURE AND RENAL FUNCTION. R.D. Manning, Jr. Univ. Miss. Med. Ctr., Jackson, Miss. 39216

Plasma protein concentration (PPC) was increased in unanesthetized dogs over a 17 day period by daily intravenous infusion of 300 ml of autologous plasma to determine the long term effects of hyperproteinemia on renal function and arterial pressure. The sodium content of the diet (80 mEq/day) was adjusted to compensate for sodium in the infused plasma during the experimental period. Control values of PPC, plasma colloid osmotic pressure (PCOP), mean arterial pressure (MAP), glomerular filtration rate (GFR), and effective renal plasma flow (ERPF) were 6.9 gm/dl, 20.2 mmHg, 92.6 mmHg, 64.7 ml/min, and 166.2 ml/min, respectively. By day 9 of the experimental period, PPC had increased to 9.1 gm/dl; PCOP had increased to 26.7 mmHg; MAP had increased 10 mmHg; GFR had increased to 73.2 ml/min; ERPF was 244.2 ml/min; filtration fraction had decreased from a control value of .393 to .301; iothalamate space had increased 12%; and plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were both decreased. A reduction in sodium intake to 5 mEq/day for 3 days while maintaining the elevated PPC caused little change in GFR or ERPF. However, an increase in sodium intake to 330 mEq/day caused GFR and ERPF to increase to 76.1 ml/min and 261.8 ml/min, respectively, while MAP increased to 118% of control. I conclude that chronic elevations of PPC are associated with increases in MAP, GFR, ERPF, and iothalamate space and decreases in PRA, PAC, and filtration fraction. (Supported by NIH grant HL11678).

31.8

ATRIAL EXTRACT (AE) INHIBITION OF ANGIOTENSIN II (AII)-CONTRACTILE RESPONSE IN THE RABBIT AORTIC RING. H.D. Kleinert*, J.E. Sealey*, J.H. Laragh* , and T. Maack. Cardiovascular Center and Dept. Physiol., Cornell Univ.Med.Coll., NY,NY 10021

We have previously shown that rat AE-induced natriuresis is of renal hemodynamic origin and have proposed that AE may have the functional characteristics of an agonist/antagonist of AII or another vasoconstrictor agent (Physiologist 25:298, 1982; Abstr. 5th Sci. Meeting, Inter-American Soc. Hyper, p. 99, 1983.) To test this hypothesis we examined the effect of boiled supernatant of rat AE (0.3 mg protein/ml.) on the isometric contractile response of rabbit aortic rings to AII. Rings were bathed at 37°C in 10 ml of oxygenated Krebs buffer. Dose-response curves to AII (2.5 to 1000 ng AII) were measured in the presence of saline (C, n=21), 5 μl of ventricular extract (VE, n=5) or AE (0.1 μl, n=4; 1.0 μl, n=7; 5.0 μl, n=5). C, VE or AE alone had no significant effect on baseline contraction. AE, but not VE, shifted the dose response curve of AII to the right in approximately parallel fashion. The following AII doses (mean±SEM) produced a Δ contraction of 0.5 g: C=7.8±0.8 ng; VE=9.5±2.0 ng; 0.1 μl AE=18.3±4.4*ng; 1 μl AE=29.0±6.9*ng, and 5 μl AE=246±68*ng (p*<0.05, compared to C). Lineweaver-Burke plots of the dose response curves to AII show that increasing doses of AE led to a progressive increase in the slope without significantly affecting the intercept. The results are consistent with the hypothesis that atrial extract contains a substance which acts as a competitive inhibitor of angiotensin II.

31.9

PLASMA AND CEREBROSPINAL FLUID VASOPRESSIN DURING DEHYDRATION IN THE STEER. F.R. Bell* and P.A. Doris* Spon. W.R. Adey) Dept. of Physiology & Biochemistry, Univ. of Reading, Reading, England.

Friesian steer calves were implanted with a cannula in the cisterna magna for sampling of cerebrospinal fluid (csf). Levels of vasopressin (AVP) were measured in csf and concurrently obtained plasma samples by radioimmunoassay. During 4 days of dehydration, plasma osmolarity (pOsm) rose progressively from control hydrated levels of 301.3 mOsm/l±0.62 mOsm/l (mean SE, n=6) to 338.5 mOsm/l±3.00 mOsm/l (p<0.001). Hematocrit increased from 39.9%±0.64% to 44.7%±1.24% (p<0.001). Plasma AVP level in hydrated animals was 1.3 uU/ml±0.19 uU/ml and rose during dehydration to 16.9 uU/ml±1.88 uU/ml (p<0.001) after 4 days. Csf AVP level in hydrated animals was 5.4 uU/ml±0.97 uU/ml and rose significantly above hydrated level after 3 days without water, reaching 15.2 uU/ml±1.83 uU/ml (p<0.001) after 4 days. Both plasma and csf AVP could be related to pOsm by an equation of the form logY = a + bX, where Y = p or csf AVP and X = pOsm. Concurrent levels of plasma and csf AVP were linearly correlated. During dehydration, neurons secreting AVP to sites accessible to csf appear to respond to similar stimuli as neurons secreting AVP into blood. (Supported by The Wellcome Trust and Medical Research Council, GB.)

31.10

HALF-LIFE($t_{\frac{1}{2}}$),VOLUME OF DISTRIBUTION(VD)AND METABOLIC CLEARANCE RATE(MCR) OF ARGININE VASOPRESSIN(AVP)IN CIRRHOSIS OF LIVER(CL) USING TWO-POOL MODEL.M.H.JAWADI*AND L.S. HO*(SPON:D.C.JOHNSON). UNIV. KANSAS SCH. MED.-W. & VAMC, WICHITA, KS 67214

Increased AVP levels have been reported in cirrhosis of liver with ascites and been implicated in the water retention in this disease. Whether the ↑ AVP levels are due to ↑ secretion or impaired liver metabolism are not clear. We studied 3 controls (CON) and 6 patients with biopsy-proven CL with ascites and edema. Two-pool model was used to calculate $t_{\frac{1}{2}}$, VD, and MCR: $\%dose/\ell = Ae^{-\alpha t} + Be^{-\beta t}$, $VD = 1/A+B$ at t=0, and $MCR = \alpha\beta/A\beta+B\alpha$. The subjects were given water p.o. until endogenous AVP was suppressed. A bolus of 2 U. of Pitressin was injected i.v. Blood was drawn at baseline, every 2 min. for 20 min., every 10 min. for next 40 min., and every 20 min. for another 60 min. Plasma AVP was determined by RIA.

	Fast Comp $t_{\frac{1}{2}}$min	Slow Comp $t_{\frac{1}{2}}$min	VD %Body Weight	MCR ml/min/Kg
CON(3)	3.03±0.09	56.67±19.23	17.90±3.76	18.70±3.10
CL (6)	4.60±0.39*	75.02±13.61,NS	29.75±9.43,NS	17.68±3.67,NS

*p<0.05, Wilcoxon Rank Sum Test

We conclude that 1)Fast Comp $t_{\frac{1}{2}}$ is longer in CL suggesting a delayed hemodynamic effect or receptor saturation. 2) ↑ VD could be due to ↑ distribution of AVP in expanded third space. 3)Contrary to common consensus that liver disease retards AVP and other hormone metabolism, we found no differences in MCR of AVP in CL. 4)Data point to ↑ secretion or release as a likely cause of ↑ AVP in CL.

31.11

ROLE OF CARDIAC VOLUME RECEPTORS IN THE CONTROL OF ANTIDIURETIC HORMONE (ADH) RELEASE IN MAN. V.A.Convertino, B.A.Benjamin*, L.C.Keil, W.M.Savin*, E.Gordon*, W.L.Haskell*, J.S.Schroeder*, and H.Sandler. NASA-Ames Res. Cntr., Moffett Field, CA 94035, and School of Medicine, Stanford Univ., Palo Alto, CA 94304.

Hemodynamic responses and ADH were measured during body position changes designed to induce central blood volume shifts in 10 cardiac transplant subjects to assess the contribution of cardiac volume receptors in the control of ADH release. Each subject underwent 15 min of a sitting control period (C) followed by 30 min of -6° headdown tilt (T) and 30 min of resumed sitting (S). Venous blood samples and cardiac dimensions (echocardiography) were taken at 0 and 15 min of C, 5, 15, and 30 min of T, and 5, 15, and 30 min of S. Blood samples were analyzed for hematocrit and ADH. Plasma volume (PV) was measured by T-1824. Heart rate (HR) and mean arterial pressure (MAP) were recorded every 2 min. Mean (±SE) HR decreased ($P<.05$) from 101 ± 4 bpm in C to 94 ± 4 bpm in T and returned to 101 ± 4 bpm with S. MAP was not significantly altered during body position changes. PV was increased by 6.3% ($P<.05$) in T but returned to C levels following S. Heart volume was increased ($P<.05$) with T and reduced ($P<.05$) with S. These responses were similar in control subjects. Preliminary data demonstrated that ADH was reduced with T and increased with S in cardiac transplant subjects as well as controls. These data may suggest that cardiac volume receptors are not significant in the control of ADH release in man.

METABOLISM AND ENDOCRINES

32.1

RESTORATION OF NORMAL DYNAMIC INSULIN SECRETION IN AGING RATS. Francis H. Premdas*, Joseph M. Molina* and Loren G. Lipson. U.S.C. School of Medicine, Los Angeles, California 90033.

Glucose-stimulated insulin secretion is decreased from islets of older rats. Mechanisms postulated for this have been both decreased activity of adenylate cyclase and decreased glucose oxidation. In attempts to define this age-related defect, we showed that insulin secretion to D-glyceraldehyde is not diminished in aging. To gain further insight into the effects of D-glyceraldehyde versus D-glucose in aging and to ascertain if normal insulin release could be restored in islets of older rats, dynamic insulin secretion from isolated islets from 2.5 and 13 month old rats was studied by perifusion to 2.8 mM and 16.7 mM D-glucose or 2.8 mM D-glucose with 5, 10 or 14 mM D-glyceraldehyde. The resulting perifusates were assayed for insulin by RIA. Total insulin release was reduced by 36% from islets of older rats in the presence of 16.7 mM D-glucose,but total release was similar from both islets of older and young rats at any of the glyceraldehyde concentrations. In the presence of 16.7 mM D-glucose, first phase secretion was blunted and the second phase reduced. When D-glyceraldehyde was used in islets from young and old rats, the biphasic nature of the release process was present and comparable in both groups.Thus glyceraldehyde overcomes the age-related diminished insulin secretion to glucose, suggesting that the decreased insulin release from islets of older rats involves changes in a rate-limiting step in stimulus-secretion coupling between glucose and the trioses.

32.2

EFFECT OF GROWTH HORMONE ON THE HEPATIC PORTAL PLASMA CONCENTRATIONS OF BIOGENIC AMINES IN NORMAL DOGS. A. Sirek*, M.N. Hussain* and O.V. Sirek. Department of Physiology, University of Toronto, Toronto, Canada M5S 1A8.

The present investigation is based on the results of previous experiments in which acute surges of plasma growth hormone (GH) concentrations stimulated insulin, glucagon and somatostatin secretion. The effects of a spike concentration of GH on portal and peripheral levels of free serotonin and catecholamines were studied by radioenzymatic methods. Experiments were conducted in trained, conscious, normal adult dogs fitted with an indwelling portal catheter. An injection of ovine GH (100 µg/kg, NIH-GH-S9) into a cephalic vein produced in the hepatic portal circulation, but not in the peripheral circulation, a transient but statistically significant rise of serotonin and a concomitant significant reduction in the concentrations of epinephrine, norepinephrine and dopamine. These findings indicate that sudden peak concentrations of GH, and by inference endogenously occurring pulses also, affect the carbohydrate metabolism of the liver by varying the levels of a number of glucoregulatory agents. This was further substantiated by measuring glucose turnover which, in spite of the absence of appreciable differences in plasma glucose levels, was found to be increased more than sevenfold. Supported by the Medical Research Council of Canada, Grant # MA7778.

32.3

EFFECTS OF INSULIN UPON FOOD INTAKE AND BODY WEIGHT IN NORMAL AND DIABETIC RATS. Dennis A. VanderWeele and Alan D. Gorang* Occidental College, Los Angeles, CA 90041.

When insulin is dramatically raised by acute injection, increased food intake often occurs. Levels of insulin used probably induce hypoglycemia and would never occur physiologically. When insulin levels were more modestly elevated for prolonged periods by an osmotic Minipump, decreased food intake occurred. We implanted pumps intraperitoneally infusing 1.2 or 2.4 U regular insulin per day into growing rats of both sexes (n=48) and found reliable decreases in eating accomplished by reductions in the size of spontaneous meals. Subdiaphragmatic vagotomy reduced this decrease in food intake.

Additionally, rats treated with streptozotocin (diabetogen) at subdiabetogenic doses (20-22 mg per 400-500 g rat), became glucose intolerant and significantly hyperphagic. Correcting the glucose intolerance by Minipumps administering 2.4-4.0 U regular insulin per 24 hours eliminated the hyperphagia. Rats which received 60 mg/kg of streptozotocin developed frank diabetes and marked hyperphagia. The hyperphagia of diabetic rats was attributable to an increase in size of spontaneous meals; only after significant weight loss occurred was an increase in the frequency of spontaneous meals recorded.

We interpret these data to indicate that insulin may play a physiological role in terminating spontaneous meals, either by facilitating nutrient uptake into the liver (glycogenesis) or by acting on an "insulin receptor" which adjusts feeding.

Supported by faculty development funds, Occidental College.

32.4

SPONTANEOUS DIABETIC WISTAR RAT: EFFECT OF KETOACIDOSIS ON GLUCOSE UPTAKE AND INSULIN REMOVAL BY LIVER AND MUSCLE. C. E. Mondon, G. M. Reaven*, and R. Rabkin*. V.A. Medical Center, Palo Alto, CA 94304

Liver and skeletal muscle are major sites of insulin removal and are responsive to insulin-induced glucose uptake. To ascertain the effect of diabetes on these processes, glucose uptake and insulin removal was assessed in liver and muscle from ketoacidotic diabetic (KD), nonketotic diabetic (D) and normal (N) rats. Before study, blood pH averaged 6.93, 7.34 and 7.33 for KD, D and N rats and serum glucose averaged 580, 406 and 144 mg/dl, respectively. Livers and hindlimbs were isolated and perfused with blood-buffer media with added insulin (∿28 ng/ml). Livers from N rats exhibited an uptake of 18.7 ±4.1 µmol glucose/g liver whereas livers from KD and D rats showed an outflow of 9.0 ±2.6 and 10.7 ±3.4 umol glucose/g each. Hepatic clearance of insulin by D rats, was significantly greater ($p<.05$) than N rats (5.9 ±.2 vs 4.9 ±.4 ml/min), but less ($p<.05$) than N in KD rats (3.4 ±.5). Glucose uptake by muscle, calculated as a clearance, was significantly less in KD rats than in D and N rats (8.9 ±.8 vs 12.7 ±1.3 and 17.1 ±1.6 µl/min/g muscle, respectively). In contrast, muscle clearance of insulin was similar in all groups (1.1 ±.1 ml/min). These findings indicate that insulin removal by muscle is unaffected by diabetes whereas insulin removal by liver is increased by diabetes but is decreased with ketoacidosis. However, glucose uptake by liver and muscle are both impaired by diabetes with ketoacidosis further impairing muscle uptake. Thus, diabetes has different effects on insulin removal and glucose uptake in liver and muscle.

32.5

LACK OF AN EFFECT OF SOMATOSTATIN ON EPINEPHRINE STIMULATED HEPATIC GLUCOSE PRODUCTION IN VIVO. P.E. Williams*, K.E. Steiner*, R.W. Stevenson* and A.D. Cherrington, Vanderbilt University School of Medicine, Nashville, TN, 37232.

The present study was undertaken to examine the effects of somatostatin on epinephrine stimulated hepatic glucose production (GP). GP was assessed using a primed constant infusion of 3-^{3}H-glucose. Two experiments consisting of 3 periods, (tracer equilibration (80 min), control (40 min) and test (60 min)) were performed on each of four overnight fasted conscious dogs. In the first experiment (EXP. I) epinephrine (E) was infused at 0.08 μg/kg-min for 1h. Two weeks later (EXP II) E was again infused but with somatostatin (0.8 μg/kg-min), and intraportal replacement amounts of insulin (I) and glucagon (G). The latter were given in such a way as to mimick the I and G levels observed during E infusion in EXP. I. In EXP. I epinephrine caused Δ's in I and G of 9±3 and 4±2 μU/ml and -1±3 and -22±12 pg/ml at 5 and 60 min respectively. GP peaked at 15 min (Δ 0.83±0.24 mg/kg-min) and increased by an av of 0.38±0.12 mg/kg-min. In EXP. II intraportal replacement of I and G during E infusion resulted in Δ's in I and G of 8±6 and 2±3μU/ml and 2±2 and -21±7 pg/ml at 5 and 60 min respectively. GP peaked at 15 min (Δ 0.63±0.13 mg/kg-min) and increased by an av of 0.40±0.11 mg/kg-min. In conclusion somatostatin at the dose employed had no significant effect on epinephrine stimulated glucose production in the overnight fasted conscious dog.

Support from N.I.H. grant AM18243.

32.6

EVIDENCE FOR A PHYSIOLOGIC ROLE FOR PANCREATIC SOMATOSTATIN IN VIVO. Gerald J. Taborsky, Jr. Univ. of Washington and VA Medical Center, Seattle, WA 98108

Somatostatin in islet D-cells has long been hypothesized to inhibit the secretion of insulin and glucagon from the neighboring B- and A-cells, despite lack of direct evidence in vivo. To provide such evidence, we inhibited somatostatin (SS) secretion in anesthetized dogs by infusing a non-immunoreactive SS analog and observed the effects of that suppression upon insulin and glucagon output from the right lobe of the in situ pancreas (see Table and Summary below).

	Dose of Analog (μg/min IV)					
Response (%Δ basal)	0.55	1.1	1.7	5.5	11	17
SS	-7±9*	-16±5	-31±10	-45±6	-75±12	-84±3
Insulin	-10±7*	+22±9	+35±17	+62±27	+6±18*	-61±9
Glucagon	+36±29*	+86±26	+179±39	+250±60	+174±15	+95±35

* = nonsignificant; all others = significant ($p < 0.05$)

Summary: 1) Increasing doses of the analog produce a progressive suppression of pancreatic SS secretion. 2) Low to moderate doses produce a modest stimulation of insulin and a marked stimulation of glucagon. 3) High doses produce net inhibition of insulin and less stimulation of glucagon. **Conclusions:** 1) At low to moderate doses the analog stimulates insulin and glucagon secretion indirectly by decreasing the inhibitory effect of pancreatic somatostatin. 2) At high doses, this indirect stimulatory effect is blunted (for glucagon) or overwhelmed (for insulin) by a somatostatin-agonist effect of this analog directly upon the A- and B-cells. 3) Pancreatic somatostatin tonically restrains basal insulin and glucagon release in vivo.

32.7

THE QUANTITATIVE SEPARATION OF CHYLOMICRONS AND CHYLOMICRON REMNANTS BY COLUMN CHROMATOGRAPHY. J.M. Felts, M.C. Gould*, R.A. Gorman* and A. Frank*. VA Medical Center, San Francisco, CA 94121 and the Department of Physiology, UCSF.

Chylomicrons (CM), upon entering the circulation, are acted on by endothelial lipoprotein lipase to produce smaller chylomicron remnants (RM) which are removed by the liver. The metabolism of RM are difficult to study in vivo, since the spectrum of these lipoproteins overlap the CM spectrum with respect to size and density. Thus, RM are still defined by an operational definition as lipoprotein particles that are recognized and taken up by the liver. We have determined that RM, during their formation, acquire new constituents which distinguish them from CM. RM acquire a lipoprotein lipase component probably derived from the endothelium. Compared to CM, RM have a large increase in free fatty acid content and have aquired a 10 fold increase in heparan sulfate per particle. These chemical changes markedly alter RM surface characteristics. We have taken advantage of these properties to devise two techniques for the complete separation of CM and RM which we produced in vivo. Chromatography of a mixture of CM and RM on DEAE Sephacel (Pharmacia) effects a complete separation of the particles. Likewise, chromatography of a mixture of CM and RM on a protamine-Affigel-10 (Bio-Rad) affinity column effects a complete separation of the particles. These techniques are useful in studies of the metabolism of CM and RM in vivo.

32.8

ROLE OF HYPERLIPIDEMIA, GLOMERULAR INJURY AND GLOMERULOSCLEROSIS IN CHRONIC RENAL FAILURE. K.D.G. Edwards*(SPON:J.B.Coelho). Cornell/Memorial Sloan-Kettering Cancer Center, New York, NY.

In the "response to injury" hypothesis of the pathogenesis of atherosclerosis (R. Ross et al., 1976-7), endothelial injury allows access of plasma LDL-cholesterol and platelet- and monocyte-derived growth factors to medial smooth muscle cells, with intimal migration and proliferation, new connective tissue formation and lipid accumulation. In the glomerulus, mesangial cells are known to be related in form and function to smooth muscle cells, so that the mesangium represents a modified arteriolar wall, and glomerulosclerosis (GS) may be considered to be a form of atherosclerosis (M.S.Schilthuis & J.D.Elema, 1978-9). Prospective studies have demonstrated the ability of dietary hyperlipidemias to induce focal GS due to impairment of healing of aminonucleoside-induced glomerular injury in normotensive and spontaneously hypertensive rats (SHR), leading to progressive chronic renal failure and enhanced 1-y mortality (K.D.G. Edwards, Controv. Nephrol. III, Masson Pub. USA, New York 1982, pp. 3-19); GS occurred in rats with hyperlipidemia caused by sucrose-lard-casein-cellulose diets. Addition of halofenate or hydralazine or both treatments in SHR or a change of diet to cereal-based laboratory chow controlled dietary hyperlipidemia and protected the rats against impaired healing (chronic renal failure, proteinuria, focal GS and arteriolar thickening or necrosis) after aminonucleoside. It is concluded that the control of hyperlipidemias by diet and drugs protects against progression of renal failure in response to injury.

32.9

SOCIAL STRESS ON PLASMA LIPIDS AND ATHEROSCLEROSIS OF ATHEROGENIC-FED ROOSTERS. H.Y.C. Wong, S. Patel*, H.A. I. Newman. Dept. of Physiol., Howard Univ. Coll. of Med., Washington, DC 20059 and Dept. of Path., Ohio State Univ., Columbus, OH.

Emotional stress has been implicated in the pathogenesis of atherosclerosis. We have studied the effects of social stress (S) on plasma lipids and aortic atherogenesis of roosters fed an atherogenic diet (AD), consisting of 2% cholesterol + 5% cottonseed oil added to mash. A total of 34 15 month-old Hyline roosters were divided into 4 groups as follows: I. Controls, plain mash (PM); II. PM + S; III. AD; and IV. AD + S. Birds were stressed by moving them daily from one cage to another. After 18 weeks these data were obtained: 1. total plasma cholesterol (PC) of grp III compared to grp IV showed no statistical difference; however, PC of these grps were significantly elevated when compared to grps I and II. TG values of grp II were significantly increased compared to grp I ($p < 0.05$) and even higher in grps III and IV than grp I or II ($p < 0.02$); and 2. gross grading (based on 0-4) for atheromatosis of abdominal aorta was as follows: in grp I, almost all birds had lesions avg. 1.2; II. 1.67; and III 3.0; and IV 4.0. Stressed birds had more severe lesions than control, but they were not significantly different. Thoracic atheroma was observed in grp II only; 0.5. Conclusions: Stressed birds, on PM or AD, had no effect on PC, but S or AD increased TG levels. Aortic lesions of stressed grps were more severe than the non-stressed. (Supported by grants from USPHS MBRS 2 S06 RR0816 and Hoffman LaRoche Inc.)

32.10

GCMS ANALYSIS OF MULTI-LABELLED GLUCOSE. Marta H. Wolfe*, Gerald Shulman*, and Robert R. Wolfe. Harvard Medical School, Massachusetts General Hospital, and Shriners Burns Institute, Boston, MA 02114

Hydrogen atoms are lost from glucose at specific points in glycolysis. By means of the primed-constant infusion of appropriately labelled glucose using stable isotopes, it is therefore possible to quantify the rates of hepatic gluconeogenic/glycolytic "futile" cycling in humans. This requires the simultaneous administration of [2-d_1]-, [3-d_1]-, and 6,6-d_2-glucose, and the measurement of the subsequent enrichment of the plasma glucose at those positions. We have accomplished this by using a combination of chemical (CI)- and electron impact (EI)-ionization mass spectrometry. The enrichment of the sum of 2-d_1 and 3-d_1 glucose was measured at m/e 116 in the EI spectrum. The 6,6-d_2- and 3-d_1-glucose enrichments were measured at ions m/e 171 and 170, respectively, in the CI spectrum. The 2-d_1-enrichment was then calculated by subtraction. Using this technique we found that in normal volunteers the rate of cycling between glucose ⟶ g6P ⟶ glucose was 0.59 ± 0.37 mg/kg·min, and the rate of cycling between F6P ⟶ FDP ⟶ F6P was 0.63 ± 0.28 mg/kg·min. Thus, 37.5% of the total glucose output (3.25 ± 0.29 mg/kg·min) was accounted for by "futile" cycling of glucose.

32.11

SUBSTRATE AVAILABILITY AND MUSCLE FATIGUE IN McARDLE'S DISEASE STUDIED BY ^{31}PHOSPHORUS NUCLEAR MAGNETIC RESONANCE. S.F. Lewis,* R.G. Haller,* R. Negro-Vilar,* J.D. Cook* and R.L. Nunnally.* (SPON: R.L. Johnson, Jr.). University of Texas Health Science Center, Dallas, Texas 75235.

The mechanism of easy fatigue in myophosphorylase deficiency (McArdle's disease) is poorly defined. Using ^{31}phosphorus nuclear magnetic resonance (^{31}P NMR), inorganic phosphate (Pi), phosphocreatine (PCr), and adenosinetriphosphate (ATP) were measured in the forearm muscles of a McArdle patient (MP) and two healthy subjects (HS) at rest and during handgrip exercise (HGE; repetition of a 5 sec maximal contraction and 5 sec rest) during control conditions (C) and with the same arm 10-15 min later during I.V. infusion of 60 ml of 50% glucose (G). MP stopped HGE (123 kg/min) after 130 sec due to an impending contracture of the forearm muscles during C, but exercised easily for >7 min at a higher load (145 kg/min) during G. For MP, PCr fell from 100 (rest; arbitrary units) to 38 and from 117 (rest) to 67 by 2 min HGE during C and G, respectively, and during G PCr was 59 at 7 min HGE. Pi showed directionally opposite changes of similar magnitude while ATP declined only slightly and was not affected by G. For HS, PCr and Pi changed modestly and ATP not at all and G had no effect on the PCr, Pi, ATP responses or HGE performance. The data firmly link the rapid fatigue in McArdle's disease to a limited substrate availability and to marked changes in muscle PCr and Pi, but not ATP.

32.12

TISSUE INTERACTIONS OF [^{14}C]RETINOIC ACID IN THE MOUSE. Carolyn Marlowe* and William J. Waddell. Dept. of Pharmacol. and Toxicol., Univ. of Louisville, Louisville, KY 40292.

Retinoic acid has been demonstrated to be effective in animals in preventing the action of some carcinogens. However, the disposition of retinoic acid differs in some respects to that of other retinoids. In an effort to define more precisely the disposition of retinoic acid, [^{14}C]retinoic acid was studied by whole-body autoradiography. Adult male C57BL/6J mice were given, IV, 6.8 mg/kg of [carboxyl-^{14}C]vitamin A acid (0.3 µCi/g). At 0.1, 0.33, 1, 3, 9, and 24 hr, the mice were anesthesized with ether and frozen by immersion in dry ice/hexane. Whole-body sagittal sections were processed for whole-body autoradiography. The sites of heavy accumulation of radioactivity at the earlier time intervals were kidney, liver, choroid plexus, Harderian gland, brown fat, adrenal cortex, myocardium, bile and urine. At the later time intervals, heavy retention was seen primarily in intestinal contents, liver, kidney, Harderian gland, brown fat, esophageal epithelium and spleen. It is unknown whether the radioactivity was in unchanged retinoic acid or a metabolic fragment. Nevertheless, there was minimal correlation of radioactivity with sites where this molecule prevents the action of carcinogens. The studies do not clarify whether the chemoprevention is by a direct action on tissue sites or by an indirect effect. (Supported by Pharmacon Research Foundation, Inc. and Tobacco and Health Research Institute).

32.13

A COMPARATIVE KINETIC STUDY OF HUMAN ERYTHROCYTE UROPORPHYRINOGEN DECARBOXYLASE ACTIVITY IN SPORADIC AND FAMILIAL PORPHYRIA CUTANEA TARDA. S.K. Mukerji*, M. Burns* and N.R. Pimstone*, (SPON: J. Green), Dept. of Internal Medicine, University of California, Davis, CA 95616

All patients with porphyria cutanea tarda (PCT) have defective hepatic uroporphyrinogen decarboxylase (UROD) activity. However, RBC UROD activity is normal in sporadic but reduced in familial PCT. We have compared for the first time the kinetic properties of RBC UROD in sporadic PCT with that in a familial PCT patient, the latter with bone marrow expression of the enzymatic defect (Blood 59, 725,1982). Using pentacarboxylic porphyrinogen I as substrate, the activity in RBC hemolysate/mg Hb was normal in sporadic but 75% diminished in the familial PCT patient. The enzyme was partially purified by freeze-thawing the RBC thrice, separating the Hb by column chromatography on DEAE-cellulose, precipitating the enzyme to 80% saturation with ammonium sulfate and dialysing prior to assay. Results: (1) The V_{max} (nmoles coproporphyrinogen formed/hr/mg protein) was the same in normal and sporadic PCT but 34% of normal in familial PCT. (2) The Km values were 1.4 µM, 2.5 µM and 4.55 µM for the normal, sporadic, and familial PCT respectively. The results suggest (i) a structural alteration of the UROD enzyme which differs in the two forms of PCT, and (ii) a high Km and low or normal V_{max} could perturb heme biosynthesis in vivo thereby predisposing to clinical disease.

32.14

ALTERED PROTEIN METABOLISM IN DIAPHRAGMS FROM THERMALLY INJURED RATS. James J. Newman,* Cleon W. Goodwin, Arthur D. Mason, Jr.* and Basil A. Pruitt, Jr.* US Army Institute of Surgical Research, Fort Sam Houston, Texas 78234

Large surface area burns to the skin are known to cause increased protein catabolism in uninjured muscle during the postburn period, but the mechanisms involved in this process are not clear. Protein metabolism in diaphragms isolated from burned rats (50% total body surface, full-thickness wounds) 3 days after injury was assessed by measuring the rates of [^{14}C]tyrosine uptake and release as an indicator of protein synthesis and degradation, respectively. Incorporation and degradation rates (nmoles/mg/3 hr) are:

	Incorporation	Release
Control		
- Insulin	0.338±0.027	0.891±0.190
+ Insulin (0.1 U/ml)	0.470±0.035**	0.760±0.185
Burned		
- Insulin	0.250±0.030*	1.61±0.211*
+ Insulin (0.1 U/ml)	0.302±0.031	1.25±0.202

* Significantly (P<0.05) different from control group.
** Significantly (P<0.05) larger increase than burned group.

The diaphragm shows a depressed rate of protein synthesis and increased rate of degradation after thermal injury. Insulin supplementation does not stimulate synthesis significantly in the burned group.

32.15

ANABOLIC HORMONES AND PROTEIN SYNTHESIS IN REGENERATING RAT SKELETAL MUSCLE. Jessica Schwartz, B. Carlson*, Margot Moore* Lili Yamasaki*. Univ. of Michigan, Ann Arbor, MI 48109

To determine whether the growth of regenerating skeletal muscle, like developing skeletal muscle, is regulated by anabolic hormones, we examined the influence of insulin and pituitary hormones on protein synthesis in regenerating rat extensor digitorum longus (EDL). Earlier work indicated that protein synthesis was elevated 4-6 fold for 10 days following autograft with nerve intact, and then subsided. The effect of hormone withdrawal on the elevated incorporation of ^{3}H-Phe into protein was measured 10 d after surgery in rats with grafted or sham-operated (op) EDL. After insulin withdrawal, produced by streptozotocin (100 mg/kg ip), protein synthesis in sham-op and control EDL was comparable to that in normal rats. However, ^{3}H-Phe incorporation into protein of grafted EDL was 4-10 fold greater than in its contralateral control. Thus, insulin deficiency did not impair, and in some cases enhanced, protein synthesis in regenerating EDL. Since the pituitary is the source of a number of anabolic hormones, the effect of their withdrawal on regenerating EDL was examined in hypophysectomized rats. In grafted EDL, protein synthesis was 5-7 fold above that in the contralateral control, which was again comparable to normal or sham-op. These data indicate that withdrawal of insulin or pituitary hormones does not impair the elevation in protein synthesis in regenerating rat skeletal muscle. Other anabolic hormones/factors may be involved. (Supported by NIH grant NS17017).

32.16

EFFECT OF CHRONIC HYPERCAPNIA ON THE ß ADRENERGIC RECEPTOR AND ADENYLATE CYCLASE OF RAT HEART. Martin C. Müller*, J. Hedley-Whyte, and M.L. Steer. Departments of Anaesthesia and Surgery, Harvard Medical School and the Charles A. Dana Research Institute at Beth Israel Hospital, Boston, MA 02215

Acute hypercapnia increases circulating levels of catecholamines and may induce arrhythmias. We have studied the effects of chronic, up to 9 days, hypercapnia (81 ± 1 mm Hg) on Sprague-Dawley rat heart ß-adrenergic receptors and adenylate cyclase. Concentration-dependent binding of [^{125}Iodo]cyanopindolol to membranes was studied. A modest, but significant (P<0.05) reduction in ß-receptor density was observed which is consistent with so-called "down-regulation" induced by elevated circulating hormone levels. No change in ß-receptor affinity for antagonist was noted. The values for K_d and B_{max} were 27.8 pM and 39.3 fmol/mg protein respectively for control samples and 25.9 pM and 32.8 fmol/mg for samples from rats, hypercapnic for 2 days. Adenylate cyclase activity was measured under basal conditions as well as in the presence of isoproterenol (30 µM) and NaF (10mM). During hypercapnia, cardiac ß-receptor stimulated adenylate cyclase activity was not altered. This suggests that during hypercapnia receptor "down-regulation" is not associated with a reduction in the ability of the heart to be stimulated by ß-agents and may, in part, explain the observation that hypercapnia does not diminish cardiac sensitivity to catecholamine-induced arrhythmias.

32.17

CHOLESTEROL MODULATES BETA-ADRENERGIC RECEPTOR NUMBER BY MECHANISMS OTHER THAN CHANGES IN MEMBRANE FLUIDITY. Philip J. Scarpace*, Stephen W. O'Connor*, Elsa I. Fernandez*, and Itamar B. Abrass*. (SPON: M. Sambhi.) GRECC, Sepulveda VA Medical Center, Sepulveda, CA 91343.

The accessibility of cell surface receptors to ligand binding appears to be modified by changes in membrane lipid composition. Beta-adrenergic receptor number is increased or decreased by modulation of membrane composition by phospholipids or cholesterol depending on the tissue investigated. A prevailing hypothesis suggests that phospholipids or cholesterol alter membrane fluidity and thus both beta-adrenergic receptor number and diffusion controlled coupling to adenylate cyclase. In the present study the effects of both cholesterol and temperature were assessed on lung membrane beta-adrenergic receptor number in female CDF (F-344) rats. Cholesterol (0.5 mg/mg membrane protein in a total volume of 1 ml) decreases membrane fluidity as determined by electron paramagnetic spectra while increasing beta-adrenergic receptor density from 136±16 fmoles/mg protein to 216±13, p<.001. In the same membrane preparations temperature (37°C, 25°C, 10°C, 0°C) which greatly decreased membrane fluidity over this range, did not alter beta-adrenergic receptor number (138±10 fmoles/mg protein; 144±13; 97±6.5; 132±14, respectively). These data suggest that cholesterol modulates upward the number of rat lung beta-adrenergic receptors, but by a mechanism other other than changes in membrane fluidity. (This work was supported by the Medical Research Service of the Veterans Administration.)

32.18

INFLUENCE OF DIETARY LEAD ON FRACTURE HEALING IN CHICKS Milton A. Lessler and David A. Ray* Dept.of Physiology, Ohio State Univ. Columbus, Ohio 43210

We found that fracture healing of the radii of 2 week old White Leghorn cockerels was markedly affected by the presence of 5000 ppm of lead in the diet (Pb-fed). Three day post-hatch chicks were maintained on Purina Startina Grower Mash for 11 days; then 5000 ppm of lead (as Pb-acetate) was added to the diet of 30 of 60 chicks. At 14 days post-hatch the right radius of each chick was fractured by gentle digital pressure and the ulna left intact to act as a natural splint. The rate of healing was followed through callus formation and bony union (at 15 to 18 days post-fracture). Addition of Pb to the diet depressed chick growth about 50% as blood-Pb levels rose above 600 µg/dl within 12 hours after exposure to the Pb-diet. Although there was little observable difference in rate of callus formation and calcification between controls and Pb-fed chicks (as evidenced by wet to dry weight ratios of callus samples), epiphysis ratios were significantly lower than the controls; suggesting that Pb-poisoning tends to increase mineral deposition in endochondrial bone formation. The Pb-fed chicks had larger calluses, as compared to controls, and showed a significant delay in the rate of callus reabsorption. These data suggest interference of the healing process by dietary lead.

32.19

ELECTRICAL AND SECRETORY PROPERTIES OF NORMAL HUMAN PARATHYROID CELLS (NHPC) IN VITRO. J.T. Posillico*, G.S. Leight, Jr.*, N. C. Anderson, Jr., Duke University Med. Ctr., Durham, NC, 27710.

Both the secretion of biologically active parathyroid hormone (BioPTH) and the transmembrane potential (TMP) of NHPC were measured concomitantly as a function of the extracellular calcium concentration in a rapid flow superfusion chamber (1ml/min). The resting TMP of NHPC in 2.5 mM calcium (Ca) Krebs-Henseleit (KHB) was -19.2 mV ± 1.3 mV (SEM) (N=81). Exposure to low Ca (0.5mM) initiated a rapid hyperpolarization (HYP) of the cell membrane to -56.0mV ± 4.9 mV. In synchrony with the HYP response BioPTH secretion increased. Basal secretion in 2.5mM Ca KHB was 0.16 pg/mg/min ± .07 which increased to 44.0 pg/mg/min ± 3.7 in 0.5mM Ca (p 0.001). The low Ca-induced HYP state was rapidly reversed (i.e., depolarized) by the addition of 0.5mM LaCl, 0.1mM D-600 or increased $[K^+]_o$ (12 to 71mM). LaCl produced a 40.8% inhibition of BioPTH release (p 0.01) whereas D-600 had no significant effect on PTH secretion. Increased $[K^+]_o$ stimulated hormone secretion up to 228% in an additive fashion over that of 0.5mM Ca alone (p 0.001). These data suggest that the low Ca-induced HYP in NHPC: 1) is highly correlated with BioPTH secretion over the normal physiological range of Ca, 2) provides a direct measure of the secretory stimulus strength, 3) arises from a Ca-activated potassium conductance, and 4) may involve voltage-dependent Ca channels.

32.20

ENHANCEMENT BY CAFFEINE OF THE DEVELOPMENT OF CARCINOGEN INDUCED RAT MAMMARY GLAND CARCINOMAS. Clifford w. Welsch, Karen Scieszka* and Jane V. DeHoog*. Department of Anatomy, Michigan State University, East Lansing, Michigan, 48824.

158 female Sprague-Dawley rats were intubated i.g. with 7,12-dimethylbenzanthracene (DMBA) (5mg/rat) at 50-53 days of age. 5 months after DMBA treatment, the animals were divided into 2 groups, those with (Exp. #1) and without (Exp. #2) palpable mammary tumors. These groups were subdivided into 3 groups: controls, caffeine treated (low dose) and caffeine treated (high dose). Caffeine was added to the drinking water at 250 mg/L (low dose) and 500 mg/L (high dose) for a period of 6 weeks. In Exp. #3, 90 female Sprague-Dawley rats were intubated with DMBA at 53 days of age (5 mg/rat) and 3 days later divided into the same 3 groups and treated for 21 weeks. Mammary carcinoma (MC) incidence in Exps. 1, 2 and 3 was as follows.

	Exp. #1		Exp. #2		Exp. #3	
	No. of rats	No. MC	No. of rats	No. MC	No. of rats	No. MC
Controls	24	78	29	22	30	45
Caffeine-low dose	24	86	29	32	30	62
Caffeine-high dose	24	118	28	45	30	66

In all 3 experiments, rats which were treated with caffeine had a higher incidence of MC than did control rats ($P<0.05$). Combining the 3 studies and both dose levels of caffeine, caffeine consumption increased MC incidence by ≈50%. (NCI-NO1-CP-05717)

32.21

GLUCOCORTICOID REGULATION OF HUMAN MILK SODIUM, POTASSIUM CONCENTRATIONS AND CIRCADIAN VARIATION. B.S. Keenan*, S.W. Buzek*, A.E. Boyd* and C. Garza* (SPON: H-P Sheng). Baylor College of Medicine, Houston, TX 77030

Circadian variation (CV) of milk sodium $[Na^+]$ and potassium $[K^+]$ was studied in 29 lactating women obtaining samples every 4h for 3d. Mean 24h concentrations of Na and K ($\bar{X}_{Na}$ and $\bar{X}_K$) were calculated for day 1 and day 3. A reproducible pattern of CV of $[Na^+]$ was seen with peak at 330h and nadir 1412 h. The CV of $[K^+]$ was reciprocal to $[Na^+]$. In 8 control subjects no change was seen in $\bar{X}_{Na}$, $\bar{X}_K$, or CV over 3d. Administration of dexamethasone (dex), decreased $\bar{X}_{Na}$ from 6.9 ± 1.2 to 4.2 ± 0.5 (s.e.) mEq/d ($p < 0.01$, n=9) and $\bar{X}_K$ increased from 14.2 ± 0.06 to 14.9 ± 0.09. Δ Na correlated with Δ K: $r = -0.81$, $p < 0.01$. The CV pattern was disrupted by dex: peak $[Na^+]$ shifted to 715h, and amplitude of milk Na variation decreased in all subjects. Serum prolactin (prl) did not change on dex. Metoclopramide (mcl), increased basal prl in 7 subjects from 65 ± 17 to 163 ± 17 to 163 ± 23 ($p < .01$) ng/ml and stimulated levels increased from 127 ± 13 to 176 ± 24. $\bar{X}_{Na}$, $\bar{X}_K$ and CV did not change with mcl. A 10 mEq Na diet in 5 subjects produced a 5-fold increase in urinary aldosterone and Na, but $\bar{X}_{Na}$, $\bar{X}_K$ and CV pattern in milk did not change. These data indicate that milk $[Na^+]$ and $[K^+]$ are regulated by glucocorticoids and are unaffected by changes in aldosterone or prl within the physiological range. Work supported by Children's Nutrition Research Center, U.S.D.A./ARS, Texas Children's Hospital, Houston, TX.

32.22

MODULATION OF GLUCOCORTICOID RECEPTOR BY AURINTRICARBOXYLIC ACID. Virinder K. Moudgil and Virginia M. Caradonna*. Oakland University, Rochester, MI 48063

Effects of aurintricarboxylic acid (ATA, a triphenylmethane dye with known inhibitory effects on activities of nucleotidyl transferases) were examined on the DNA binding of rat liver glucocorticoid receptor. DNA-cellulase binding capacity of glucocorticoid-receptor complex was completely abolished by a pretreatment of receptor preparation with 0.1-0.5mM ATA at 4°C. The half-maximal inhibition (I.D. 50) in the DNA binding of [^{3}H]triamcinolone acetonide [^{3}H-TA]-receptor complex was observed at 40- and 130µM ATA depending upon whether the inhibitor was added prior to or following the receptor activation. The entire [^{3}H]-TA-receptor complexes adsorbed on DNA-cellulose in the control experiments could be extracted in a concentration-dependent manner by incubation of resin-bound receptor with 20-100µM ATA. The amount of available [^{3}H]-TA-receptor complexes remained unchanged under these conditions. The inhibition in the DNA binding of ATA-treated glucocorticoid receptor was not due to complexing of ATA to the affinity resin. The DNA-binding capacity of ATA-treated receptor preparations could be recovered upon exhaustive dialysis. ATA appeared to be interacting with the DNA-binding site(s) of the receptor rather than reversing the transformed form of receptor into a non-DNA binding cytosolic form. The use of ATA should offer a good chemical probe for analysis of different domains of glucocorticoid receptor.
Supported by NIH Grant AM-20893.

32.23

INESCAPABLE BUT NOT ESCAPABLE STRESS ALTERS IMMUNE FUNCTION. Y. Shavit*, S.M. Ryan*, J.W. Lewis*, M.L. Laudenslager*, G.W. Terman*, S.F. Maier*, R.P. Gale*, and J.C. Liebeskind. Psychology Depts.: UCLA, Los Angeles, CA 90024; Univ. of Denver, CO 80208 and Univ. of Colorado, Boulder, CO 80309.

Exposure to inescapable shock suppresses activity of natural killer (NK) cells, and this effect seems to be mediated by opioids (Shavit et.al., 1983). Inescapable but not escapable stress causes learned helplessness and release of opioids. Inescapable but not escapable stress suppresses mitogenic proliferation of lymphocytes (Laudenslager et.al., 1983). Here we compared effects of inescapable and escapable stress on NK activity. Rats received either escapable (ES), inescapable (IS) or no (NS) shock. The ES group received 80 tail shocks that they could terminate. The IS (yoked) group received the same shocks, but had no control over their termination. 24 hr later ES and IS groups received 5 "mild" inescapable shocks, a procedure known to produce opioid analgesia in the IS group only. Splenic NK cytotoxicity against labelled YAC-1 target cells was assessed by a 4 hr chromium release assay. NK activity was suppressed in the IS but not ES group compared to controls. Thus, inescapable but not escapable shock suppresses NK activity, an effect that may be associated both with learned helplessness and opioid release. (Supported by a gift from the Brotman Foundation, NIH grant NS07628, and NSF grant BNS-8200944).

32.24

CHEMICAL PEPTIDE SYNTHESIS OF ANTIGENIC DETERMINANTS OF THE PLASMODIUM KNOWLESI CIRCUMSPOROZOITE SURFACE PROTEIN. David H. Schlesinger, Richard Melton,* Nigel Godson* and Victor Nussenzweig*. N.Y.U. Med. Ctr., N.Y., N.Y. 10016.

In the mosquito salivary glands the sporozoite stages of the Plasmodium protozoan parasites that cause malaria are covered with a highly immunogenic surface protein which seems to participate in the invasion of the vertebrate host liver cells. Elucidation of the structure of this protein could lead to the chemical synthesis of antigenic determinants (peptide epitopes) that may be used as a malaria vaccine.

cDNA fragments of the monkey malaria parasite, P. knowlesi, sporozoite surface antigen gene that contains the epitopes of the protein has been cloned, (Ellis, J. et al., Nature, 302:536, 1983). The deduced amino acid sequence of the immunogenic region of the surface antigen consists of 12 amino acid unit tandemly repeated 12 times and constitutes at least 1/3 of the total size of the protein. This dodecapeptide (Q-A-Q-G-D-G-A-N-A-G-Q-P) has been synthesized and has the properties of the surface antigen epitope, as judged by its ability to bind monoclonal antibodies and to specifically compete in a radioimmune assay with the authentic in vivo synthesized circumsporozoite the surface protein. Synthetic analogs of this epitope demonstrate that the essential amino acids required for antigenic activity comprise heptapeptide epitope D-G-A-N-A-G-Q. (Supported by AID, WHO, NIH, NYU).

35.1

CONTRACTION IN "MARGINALLY ISCHEMIC" MYOCARDIUM DURING BEHAVIORALLY & PHARMACOLOGICALLY INDUCED PRESSOR EVENTS IN BABOON. D. Randall, K. Ogilvy*, R. Wilson* & S. Vallance*. Univ. Kentucky Dept. Physiol. & Biophys., Lexington 40536

Increases in BP and HR during acute myocardial ischemia augment metabolic demand and may thus expand the zone of contractile dysfunction. To test this we used ultra-sound to measure muscle shortening in 3 regions of the baboon heart (n=3). One crystal pair was located at the immediate level of an inflatable occluder around the anterior descending coronary artery. Trials consisted of 1 min. pre-occlusion (PO) followed by 5 min. occlusion. BP was increased during the 3rd and 4th min. of occlusion by behavioral conditioning (BC) or phenylephrine (PE) infusion; a control (C) behavioral manipulation induced no pressor response. Mean data are given for PO and the 4th min.; shortening (△L) and end-diastolic length (EDL) are given as % of PO:

Condition	BP (mmHg)		HR (/min)		△L (%)	EDL (%)
	PO	4th	PO	4th	4th	4th
C	77	80	110	119	67	101
BC	76	100	116	145	61	101
PE	73	88	112	112	61	102

Muscle shortening recorded from a crystal pair above the occluder was not decreased; a segment below the occluder exhibited markedly impaired shortening or expansion. Increased metabolic demand causes only modest additional impairment of function in marginally ischemic tissue. (Supported by NIH grant HL 19343)

35.2

FLUOROCARBONS IN CORONARY OCCLUSION FOLLOWED BY REPERFUSION. Patrick K.C. Chun, William F. LaPenna, J. Judson McNamara. Tripler Army Medical Center and Cardiovascular Research Laboratory, Queen's Medical Center, Honolulu, Hawaii 96859

Perfluorochemicals have a higher oxygen solubility, are smaller in size, and have a lower viscosity than red cells. We examined the effect of fluorocarbon (oxypherol [FC-43], and fluosol-DA [FA-DA]) on infarct size produced by 2 hours of occlusion followed by reperfusion. In 28 baboons (Papio anubis), the left anterior descending artery was ligated and the ligature released 2 hours later. There were 4 groups: 1. (n=8), nonexchanged control; 2. (n=6), Ringer's lactate exchange control; 3. (n=8), FC-43 treated; and 4. (n=6), F-DA treated. Those animals exchanged were bled to a mean Hct of 20%. Twenty-four hours after ligation, the left ventricle was sectioned at 5mm intervals, and examined as to infarction (I) and perfusion bed (PB) size. Planimetry yielded the areas of I and PB. The sections summed yielded the volume of I and PB. The infarct volume and the perfusion bed volume were compared by regression analysis. R values for the nonexchanged and exchanged controls had an excellent fit: .89 and .91, respectively. R values for the FC-43 and F-DA baboons were poor: .16 and .38, respectively. In the primate model, 2 hours of occlusion followed by reperfusion, the I size increases proportionately with PB (without treatment). This relationship is lost with fluorocarbon treatment. Thus fluorocarbons alters the relationship of infarct size to perfusion bed.

35.3

UNIFORMITY OF TRANSMURAL WALL THICKENING WITH GRADED CORONARY STENOSES. C.J. Hartley, L.H. Michael, K. Allen*, M.L. Entman, and R.M. Lewis*, Baylor College of Medicine, Houston, TX 77030

To determine the alterations in the transmural distribution of left ventricular (LV) ejection phase thickening (LVT) with reduced perfusion, 4 acute dogs were instrumented with left circumflex flow probes and occluders and 4 mm ultrasonic transducers sutured to the epicardium. A 10 MHz pulsed Doppler was used to digitally integrate the velocity of myocardial layers passing through each of 8 sample volumes (SV) distributed across the LV wall. LVT was plotted vs SV depth in each animal by averaging 10 beats at each depth and at each level of flow. The slope of the curve at each depth represents local thickening fraction (TF). Linear regression analysis was used to assess the degree of uniformity (r value) and average TF (slope) at control (C) and at each level of reduced flow as summarized in the table. Labelled microspheres were used to estimate perfusion in 3 layers underlying the transducer at 3 levels of flow. The high r values indicate that thickening was evenly distributed across the LV wall and remained so with ischemia even though microspheres showed a redistribution of flow toward the epicardium. The poor correlation between local TF and perfusion each measured as %C (r=.222) suggests strong tethering of myocardial layers and indicates that measured transmural function is a poor indicator of the transmural extent of ischemia.

FLOW %C	%TF	"r"
100%	9.3%	.987
60-90%	10.0%	.974
25-50%	5.9%	.987
occluded	1.6%	.944

35.4

USE OF AN INTRAVENTRICULAR BALLOON TO INDUCE ACUTE MYOCARDIAL DEPRESSION IN THE INTACT DOG. P. Heerdt* and R. Caldwell* (SPON: S. Bealer). UTCHS, Memphis, TN 38163.

Evaluation of inotropic agents in normal myocardium probably does not accurately reflect the activity of a drug in a compromised heart. The purpose of this study was to develop a stable model of depressed left ventricular (LV) function in the intact dog. Eight animals (8.6-14.0kg) were anesthetized with Na pentobarbital (30mg/kg), mechanically ventilated and a sternotomy performed. LV pressure (LVP) was recorded and its first derivative (dP/dt) regarded as an index of LV contractile force. Through a small incision in the apex a balloon-tipped catheter (45cc capacity) was introduced into the LV and slowly filled with saline. All animals showed a decrease in dP/dt in response to balloon filling. In 3 dogs rapid emptying of the balloon resulted in ventricular fibrillation (VF). In 5 animals the balloon volume was slowly removed and VF did not occur. LV dP/dt remained depressed in these animals (44.8±7.8%) and none showed recovery 40 minutes later. Decreases in arterial pressure and cardiac output, as well as increases in pulmonary artery pressure and heart rate were also noted. IV injection of 30μg/kg ouabain (O) moderately increased dP/dt but also induced arrhythmias. Histologic examination of the LV revealed separation of myofibrils and capillary disruption. Thus, this method can produce a compromised, stable cardiac preparation moderately responsive to the inotropic effects of O but sensitive to O-induced arrhythmias.

35.5

EFFECT OF CHRONIC SUBHYPERTENSIVE NOREPINEPHRINE INFUSION ON THE RESPONSE OF THE HYPERTROPHIED HEART TO SYMPATHETIC STIMULATION. Francis M. Siri* (SPON: Martin D. Rayner) Univ. of Hawaii, Honolulu, HI 96822

Depletion of myocardial norepinephrine (NE) stores in cases of severe afterload-induced cardiac hypertrophy has been well documented. Diminished responsiveness of such hearts to sympathetic stimulation has also been reported. A study was designed to ascertain the ability of a chronic subhypertensive infusion of NE to prevent these effects. Adult male Wistar rats underwent an initial operation, consisting of implantation of an Alzet osmotic minipump for intravenous infusion of either NE (0.05 mcg/kg/min) or vehicle (sham) followed by constriction of the abdominal aorta (AC) or placement of a loose aortic ligature (sham). This resulted in 4 groups, which were compared 7 days postoperatively. The hearts of AC, NE-infused rats showed normal NE content compared to the double-sham group. In contrast, the NE content of afterloaded hearts exposed to sham infusion was significantly less than that of the double-sham group ($P < 0.01$). Electrical stimulation of the cervical sympathetic trunk produced significantly lesser increases in heart rate and left ventricular dP/dt max in AC rats, compared to sham-constricted rats, and this deficit was not ameliorated by NE treatment. There was evidence ($P < 0.05$) that NE infusion was associated with even further reduction in these responses. The results indicate that maintenance of the heart's NE stores, in this model, is insufficient to restore myocardial adrenergic function.

35.6

CORONARY VASCULAR GROWTH IN THYROXINE-INDUCED CARDIAC HYPERTROPHY. W.M. Chilian, R.J. Tomanek, M.L. Marcus. CV Center & Depts of Physiol, Anat, & Med., U of I, Iowa City, IA 52242

Pressure-induced left ventricular hypertrophy (LVH) is associated with decreased coronary reserve, increased minimal coronary resistance, and decreased capillary density. To examine these parameters in a different type of hypertrophy, we measured coronary reserve, minimal coronary resistance, and capillary density in age-matched (7-month) Wistar Kyoto (WKY) and thyroxine treated (Th) (0.25 mg/kg s.c./day x 2 mo) WKY rats. The degree of hypertrophy was assessed by the LV/BW ratio (mg/g). Coronary reserve was assessed by measuring peak blood velocity to resting blood velocity ratio (PV/RV) during maximal coronary reactive hyperemia. Minimal coronary resistance (MCR) (mmHg·min·100g/ml) was measured as the quotient of mean arterial pressure and myocardial perfusion (microspheres) during maximal coronary dilation with dipyridamole (1 mg/kg/min). Capillary density (D) (capillaries/mm^2) was determined by microscopic analysis in perfused-fixed hearts.

Results ($\bar{x}$ ± SEM); *p < 0.05 vs WKY

	PV/RV	MCR	D	LV/BW
WKY	2.1±0.2	0.13±0.02	4029±143	2.3±0.2
TH-WKY	2.2±0.3	0.08±0.02	4645±201*	3.2±0.3*

Despite, LV hypertrophy, the Th-WKY rats had normal coronary resistance, normal reactive hyperemic responses, and increased capillary density which is in contrast to pressure-induced LVH. Thus, the response of the coronary bed is dependent on the stimulus which produces LVH.

35.7

THE BIAXIAL STRESS-STRAIN RELATIONSHIP OF CANINE PERICARDIUM. P. H. Chew*, R.K. Strumpf*, F.C.P. Yin. Johns Hopkins Med. Inst., Baltimore, Md. 21205

Pericardial constitutive properties may demonstrate anisotropy and may be dependent upon the exact loading pattern. Assessment of these properties cannot be made from uniaxial tests but can be made from planar biaxial tests. On a specially designed, servo-controlled testing apparatus we performed biaxial stretching tests on sheets of canine pericardium. Three specimens of anterior canine parietal pericardium, 4 x 4 cm, were mounted in the apparatus and floated in oxygenated Krebs solution at 37°C. Specimens were subject to cyclic stretching over a 50 sec period to 500 gm in each axis. Protocols included simultaneous biaxial loading as well as loading in which one axis was fixed while the orthogonal axis was stretched and vice versa. From the measured forces and deformations in the center of the specimen we calculated the Green's strains and Kirchhoff stresses. Using a modified Marquardt nonlinear regression technique, we estimated the generalized elastic coefficients of the following pseudo-strain energy function

$$W = 0.5(\alpha_1 E_1 + \alpha_2 E_2 + 2\alpha_4 E_1 E_2) + 0.5C\,(\exp[A_1 E_1^2 + A_2 E_2^2 + 2A_4 E_1 E_2])$$

A representative listing of coefficients from three protocols from one specimen follows (* denotes axis that is stretched):

	A_1	A_2	A_4	α_1	α_2	α_4	C
X^*/Y^*	10.1	11.6	-5.53	.187	.740	.070	.00025
X^*/Y	5.7	6.1	0.66	.065	.595	.086	.00003
X/Y^*	5.3	5.6	0.28	.457	.983	.372	.00016

Two of three specimens had near equality of corresponding coefficients whereas one specimen had differing coefficients over these same protocols. Equal coefficients for different protocols suggests a unique strain energy function, i.e., material properties are independent of loading pattern. Differences between A_1 and A_2 indicate anisotropy. This technique allows us to assess anisotropy and load dependence of pericardial material properties.

35.8

EFFECTS OF ARTERIAL INPUT IMPEDANCE ON THE MEAN LEFT VENTRICULAR PRESSURE-FLOW RELATIONSHIP. Kiichi Sagawa, Kenji Sunagawa, W. Lowell Maughan. Johns Hopkins Medical Institution, Baltimore, MD 21205

The mean left ventricular pressure-flow relationship (LVPFR), determined under a constant preload and variable peripheral resistance (R), has been proposed as an index of left ventricular pump function (Elzinga and Westerhof, 1979). To test whether the LVPFR is independent of arterial compliance (C) and characteristic impedance (R_c), we used 7 isolated cross-perfused canine hearts in which end diastolic volume (EDV) and inotropic state (I) could be precisely controlled. The LVPFR was determined always by varying R of a simulated arterial load in 5 steps from 6.0 to 0.375 mmHg-sec/ml while keeping EDV, I, C and R_c at different constant values. All of 27 LVPFRs thus determined were moderately nonlinear. With 3 levels of EDV (35.8 ± 7.4, 31.8 ± 7.1 & 27.1 ± 7.4 ml) there was an approximately parallel shift of LVPFR ($p<.0001$). At 3 levels of I (mean LVP of isovolumic contractions at constant EDV 34.3 ± 8.2, 48.0 ± 6.3 & 59.2 ± 9.6 mmHg) the LVPFR shifted with mostly slope change ($p<.0001$). Changing C at 3 levels (0.2, 0.4 & 0.8 ml/mmHg) caused a statistically significant but quantitatively minimal cross over of the LVPFR curves ($p<.0001$). Changing R_c to 0.1, 0.2 & 0.4 mmHg-sec/ml caused a highly significant ($p<.0001$) divergence of LVPFR over the high flow range. We conclude that this sensitivity of LVPFR to C and R_c make its use as contractility index difficult. Supported by PHS Grant HL 14903.

35.9

ASSESSMENT OF MYOCARDIAL CONTRACTILITY USING PULSED DOPPLER ULTRASOUND. D. Alverson*, W. Berman, Jr.*, T. Blomquist*, M. Eldridge*, C. Intress*, D. Christensen*. (SPON: D. Priola). University of New Mexico, Dept. of Pediatrics, School of Medicine, Albuq. NM 87131

We studied the relationship of aortic blood flow velocity variables to left ventricular (LV) pressure events under a variety of inotropic and afterload conditions in nine anesthetized adult mongrel dogs. The peak first derivative of left ventricular pressure with respect to time (dP/dt max) was used as an index of the myocardial contractile state. Peak dP/dt was derived from LV pressure waveforms using a transducer tipped Millar catheter. Blood flow velocity waveforms were derived from a transducer tipped 20 MHz pulsed Doppler catheter in the ascending aorta. Inotropic state was changed using dobutamine or propranolol. Afterload was altered with a balloon tipped catheter in the descending aorta. Strong correlation existed between dP/dt max and time to peak blood flow velocity (Tvmax) in all inotropic conditions, independent of afterload ($r = -0.92$, $p<0.001$). Linear regression slopes and y - intercepts comparing these two variables were similar in all dogs studied. The peak first derivative of velocity with respect to time (dV/dt max) and peak velocity (V max) were very afterload dependent as reported previously. Tvmax approximates closely LV dP/dt max over a range of afterload and inotropy and can be obtained noninvasively with currently available instrumentation.

35.10

RELATIONSHIP OF ß-ADRENERGIC RECEPTOR NUMBER TO TISSUE AND CIRCULATING CATECHOLAMINES IN PRESSURE OVERLOAD LEFT VENTRICULAR HYPERTROPHY. D.E. Vatner*, C.J. Homcy, S.P. Sit & S.F. Vatner. Dept. of Med., Harvard Med. Sch., MGH, & Brig. & Womens Hosp. & N.E. Reg. Prim. Res. Ctr., Southboro, MA.

Depletion of myocardial catecholamines is characteristic of heart failure, but less is known about catecholamines in hypertrophy (H) without heart failure. To determine if left ventricular (LV) H alters ß-adrenergic receptor regulation, LV tissue and plasma catecholamines, and ß-adrenergic receptor numbers were compared in 6 dogs with chronic LVH (LV/body wt=6.0±0.5), and in 6 control dogs (LV/body wt 3.3±0.2). Hemodynamics were measured in conscious dogs 1 month after instrumentation with LV pressure and diameter gauges. In dogs with LVH, LV systolic pressure was twice normal but LV end diastolic pressure (10.5±0.9mmHg) and diameter (30±2.2mm) were not significantly different from values in normal dogs of 8.8±1.1mmHg and 35±2.5 mm, respectively. Catecholamine levels were determined by the method of DaPrada and Zurcher and ß-adrenergic receptors were quantitated by receptor ligand binding. Plasma norepinephrine and epinephrine were not different in LVH (223±26 and 81±13 pg/ml) from normal dogs (251±23 and 122±17 pg/ml), but LV norepinephrine was markedly depleted in LVH (163±48 pg/mg), compared to normals (835±166 pg/mg). ß-adrenergic receptor number increased ($p<0.01$) in LVH (108±7) compared to normal dogs (58±6 fmol/mg protein). Thus, upregulation of ß-adrenergic receptor number in LVH occurs in the presence of normal circulating, but depressed myocardial, catecholamine levels.

35.11

REDISTRIBUTION OF BLOOD FLOW: A MAJOR DETERMINANT OF CARDIAC OUTPUT. T.W. Rice*, S.V. Lichtenstein* and A. Panos* (SPON: C.E. Bayliss). Univ. of Toronto, Toronto, Ont. M5S 1A1

A two compartment model of the peripheral circulation is proposed. Nine anesthetized, normothermic patients were studied before and during epinephrine infusion while on total cardiopulmonary bypass, after the aortic root was cross clamped and the heart isolated from the peripheral circulation. Under these conditions, peripheral vascular compliance, arteriolar, and venous resistance and the systemic time constant were determined directly. Epinephrine infusion increased blood pressure and decreased blood volume by an average of 360 ml at constant blood flow and right atrial pressure. Systemic vascular compliance decreased because of veno-constriction and the resistance to venous return changed significantly, surprisingly, it decreased. Analysis of transient blood volume changes after a step change in right atrial pressure at constant blood flow, showed that epinephrine redistributed blood flow away from the compartment with the longest time constant by constricting the arterioles leading to it. This accounts for the major increase in venous return and is a significant mechanism of increased cardiac output in the normal individual after epinephrine, independent of its effects on the heart! Our results suggest that an increase in total peripheral resistance may indeed increase cardiac output, rather than decrease it, as is generally believed.

35.12

EFFECT OF ATRIAL FIBRILLATION ON REGIONAL BLOOD FLOW. H.S. Friedman, J.O'Connor*, S. Kottmeier*, R. McGuinn* Downstate Medical Center, (SUNY), Brooklyn, New York, 11203.

The effect of atrial fibrillation(AF)on regional blood flow has not been studied. Accordingly,in 10 awake dogs,AF was induced and regional blood flow was measured. With AF,ventricular rate increased from 118±8 to 196±17 min^{-1} ($P<0.01$)and cardiac output declined from 3.7±0.3 to 2.9±0.2 L/min($P<0.05$),but aortic and left atrial pressures did not change.

		REGIONAL BLOOD FLOW (ml/min/100g)		
Heart:	Left ventricle	114±12	115±11	NS
	Right ventricle	68±8	74±8	NS
	Left atrium	73±14	109±16	< 0.05
	Right atrium	39±7	84±14	< 0.05
Brain:	Cortex	70±3	59±3	< 0.01
	White matter	42±3	36±4	< 0.05
	Cerebellum	63±3	53±3	< 0.01
	Brain stem	43±4	36±4	< 0.01
Splanchnic:	Liver	52±12	31±6	< 0.05
	Intestine	88±9	68±11	< 0.01
	Pancreas	149±30	86±15	< 0.01
Kidney:	Outer cortex	701±49	552±41	< 0.01
	Inner cortex	402±59	344±42	NS
	Medulla	36±7	31±7	NS

Thus,in awake dogs atrial fibrillation produces marked changes in cardiac output and regional blood flow.

MICROVASCULAR TRANSPORT

36.1

EFFECT OF HYALURONIDASE ON BLOOD FLOW AND TRANSCAPILLARY FLUID AND SOLUTE EXCHANGE IN THE DOG HEART FOLLOWING ACUTE CORONARY ARTERY OCCLUSION. John N. Diana and Samuel A. Maxey, Depts. of Physiology and Pathology, LSU School of Medicine in Shreveport, Shreveport, LA 71130.

Measurements were made of left ventricular (LV), aortic (Ao) and left coronary artery (LAD) pressures, heart rate (HR), EKG, coronary vascular resistance, regional myocardial blood flow (7 to 10μ microspheres), microvascular extraction (E) and permeability-surface area (PS-product) of lipid insoluble molecules in the control state and following acute LAD occlusion in the working dog heart. The above measurements were also made following an i.v. injection of hyaluronidase (100 to 600 units/Kg). Hyaluronidase and the coronary occlusive procedure had no significant effect on LVP, dP/dt/P, Aop, HR and EKG. Regional myocardial blood flow of the left ventricular free wall and septum tended to increase in both control and LAD occluded hearts following hyaluronidase but the increase was not statistically significant. The endocardial/epicardial ratio, E and PS-product were unchanged with hyaluronidase in both control and LAD occluded hearts. It is concluded that hyaluronidase does not significantly change blood flow or diffusion of low molecular weight substances in the dog heart. The myocardial extravascular fluid content is reduced following hyaluronidase resulting in a smaller volume of distribution for solutes. The mechanism of the volume decrease appears to be a reduction in capillary hydrostatic pressure following direct venodilation. Supported by Grant HL23196 from NHLBI.

36.2

EFFECT OF HISTAMINE ON CAPILLARY PERMEABILITY TO SUCROSE IN THE RABBIT HEART. Jack T. Saari. Physiology Department, University of North Dakota, Grand Forks, ND 58202.

Much evidence exists in the literature supporting the concept that histamine (HA) increases capillary membrane permeability in a variety of tissues. Little work has been done on the effect of HA on capillary permeability in heart muscle. In this study the effect of HA on the capillary membrane permeability coefficient (P) to sucrose was studied in the isolated Langendorff-perfused rabbit heart. The osmotic transient method (Vargas and Johnson, Am. J. Physiol. 213: 87-93, 1967) was used to measure possible changes in P. In this method the rate constant k for decay of volume flow across the capillary membrane during an osmotic weight transient is estimated to be PA/V, where, A is exchange area and V is distribution volume for permeant (in this case sucrose). Experimentally determined values of k were corrected for variation in A (based upon initial flow resistance) and for V (based upon initial heart rate). This yielded a value for k of .057 ± .014 $(SD)_1$ for six control hearts and a value for k of .079 ± .020 sec^{-1} for six test hearts (HA = 20 mg/liter) ($P < .05$, t-test). Since these k values are corrected for variations in A and V, their difference indicates a significant increase in P with histamine in rabbit heart capillaries. Supported by NIH Grant HL 28217.

36.3

HISTAMINE INCREASES PERMEABILITY OF THE CANINE CORONARY MICROVASCULATURE. Charles F. Pilati* and Michael B. Maron. Program in Physiology, N.E. Ohio Univ. Col. Med. Rootstown, Ohio 44272.

Coronary vascular permeability was assessed under elevated venous pressure conditions in the isolated, blood perfused, canine heart after histamine treatment. The ratio (V_F,pr/V_F, Hct) of the apparent volumes filtered into the interstitium, calculated from the increases in plasma protein concentration (V_F,pr) and hematocrit (V_F,Hct) was used to evaluate changes in permeability. In the absence of histamine, the transudate was essentially protein-free (V_F,pr/V_F,Hct = 1.05 ± 0.11). On exposure to histamine (3.8 ± 0.3 μg base/ml blood), coronary vascular permeability increased and V_F,pr/V_F,Hct decreased to 0.37 ± 0.06 ($p<0.001$). This value remained constant throughout the 60 min of histamine exposure. Aortic perfusion pressure decreased significantly ($p<0.05$) during the first min of histamine treatment and rose slowly thereafter. In 4 of the 7 hearts, concomittant increases in left ventricular isovolumic pressure development (13-31 mmHg) and heart rate (4-29 b/min) were observed. In the remaining hearts, neither variable was affected by histamine. We conclude that histamine causes an increase in the permeability of the canine coronary microvasculature but failed to consistently increase heart rate or left ventricular performance. (Supported by the American Heart Association, Akron District Chapter.)

36.4

SOLUTE TRANSPORT AND PLASMA VOLUME IN THE PITUITARY GLAND OF CONSCIOUS RATS. P.M. Gross, R.G. Blasberg*, H. Nakagawa*,M.H. Yen*, K. Shima*, J.D. Fenstermacher and C.S. Patlak. National Cancer Institute and National Institute of Mental Health, Bethesda, Maryland 20205.

The three lobes of the pituitary gland have different endocrine functions and contiguous, but individually unique, vascular beds. Some basic physiological mechanisms of pituitary blood vessels are likely to vary, therefore, from lobe to lobe and in different endocrinological states. We determined capillary solute transport (K) with ^{14}C-α-aminoisobutyric acid (AIB, MW=103) and vascular plasma volume (V_p) with ^{125}I-albumin in the pituitary lobes of conscious albino rats. The effects of dehydration (5 days of water deprivation), which stimulates vasopressin secretion from the pituitary neural lobe, were also evaluated for K and V_p. We made regional tissue measurements of K and V_p from intact horizontal sections of whole pituitary glands by quantitative autoradiography. V_p for the neural, intermediate and anterior lobes was 34, 11 and 76 μl/g, respectively. V_p increased in each of the lobes in dehydrated rats. The rank order of K for AIB in the lobes of normal animals was neural>>anterior>intermediate. K was increased in each of the lobes in dehydration, most markedly in the neural lobe where a 4 to 5-fold increase in uptake occurred. Our results indicate that these vascular measurements are unique to the individual pituitary lobes. Inter-lobe differences likely reflect both the vascular anatomy and the physiological response to dehydration in each lobe.

36.5

LACK OF SELECTIVITY TO SMALL IONS IN HYDROPHILIC PORES IN MUSCLE AND BRAIN CAPILLARIES. Christian Crone. The Panum Institute, University of Copenhagen, 2200 Copenhagen, Denmark

Salt gradients across a membrane create diffusion potentials that depend on relative ion mobilities (or transport numbers) in the membrane.

Mobilities of K^+ and Cl^- relative to Na^+ were determined in single capillaries in frog brain and muscle from diffusion potentials across the capillary wall in superfusion (S) or perfusion (P) experiments, or in combinations of S and P. The potentials were created by salt gradients (2:1 and 10:1) or bi-ionic substitutions (KCl:NaCl). The potentials were symmetrical across the wall. Analysis of sign and magnitude based on Planck-Henderson equations showed ion mobilities to be proportional to free solution mobilities reflecting absence of charge effects in the hydrophilic pathways. The 'pores' in continuous capillaries are highly hydrated, neutral or weakly charged channels that are wide compared to small ion size.

	P_{Cl}/P_{Na}	P_K/P_{Na}
Brain capillary	1.42	1.74
Muscle capillary	1.35	*
Free Solution	1.52	1.46

(*high K^+-solutions cause twitching)

36.6

O-β-HYDROXY RUTOSIDE FAILS TO BLOCK BRADYKININ-INDUCED EDEMA FORMATION IN THE CANINE FORELIMB. David E. Dobbins, Connie Y. Soika* and Joe M. Dabney. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

O-β-hydroxy rutoside (Venoruton®) has been reported to alleviate edema formation in chronic venous insufficiency. In an attempt to elucidate Venoruton®'s potential as an antiinflammatory agent, we infused Venoruton® (20 mg/min) intraarterially into the canine forelimb perfused at constant flow (N=7) during a simultaneous local intraarterial infusion of bradykinin (2 µg/min). The infusion of Venoruton® alone did not significantly affect forelimb vascular pressures, systemic pressure or skin lymph flow, total protein concentration or total protein transport. Subsequent infusion of bradykinin caused a significant decrease in forelimb perfusion pressure and skin small artery pressure which then returned to control values, a small but significant and sustained decrease in systemic pressure and a sustained increase in skin small vein pressure. These results were similar to those seen in a separate series of animals (N=7) during the infusion of bradykinin alone. Infusion of bradykinin during the simultaneous infusion of Venoruton® resulted in a marked increase in skin lymph flow, protein concentration and protein transport which tended to be greater than that seen with the infusion of bradykinin alone. These data indicate that the intraarterial infusion of Venoruton® at this dosage does not inhibit the ability of simultaneously infused bradykinin to increase transvascular fluid and macromolecular efflux in the canine forelimb.

36.7

SELECTIVE PERMEABILITY OF KIDNEY CAPILLARIES TO GLUCOSYLATED ALBUMIN. Stuart K. Williams and Gabriel G. Pinter. Jefferson Medical College, Philadelphia, PA 19107 and University of Maryland School of Medicine, Baltimore, MD

The transendothelial transport of normal and nonenzymatically glucosylated albumin was studied in the kidneys of normal and diabetic rats. Rats were injected with a mixture of fluorescein isothiocyanate labelled-glucosylated albumin (FITC-GSA) and tetramethylrhodamine isothiocyanate labelled-normal albumin (TRITC-SA). Plasma, kidney lymph and urine were sampled at intervals for 120 minutes, analyzed for FITC and TRITC specific fluorecence, and the ratio of FITC/TRITC (F/T) calculated for each sample. We found no significant change in the F/T ratio in samples of plasma and lymph from normal rats. However, the urine F/T ratio more than doubled indicating the preferential appearance of glucosylated albumin in the urine of normal rats. Diabetic rats exhibited a significant decrease in the plasma F/T ratio and a significant increase in the lymph and urine F/T ratio. Decreased plasma F/T ratio indicates the preferential escape of glucosylated albumin from the vascular space. Increased F/T ratio in the urine and lymph samples suggest the preferential transendothelial transport of glucosylated albumin across glomerular and peritubular capillaries. Supported by NIH HL-29152 and NIH AM-17093.

36.8

JEJUNAL CAPILLARY PERMEABILITY TO ENDOGENOUS PLASMA PROTEINS. N.A. Mortillaro, Dept. Physiology, Univ. South Alabama, Mobile, AL 36688.

In autoperfused segments of cat jejunum, steady-state lymph flows, lymph protein concentration (C_L) and plasma protein concentrations (C_p) were measured at jejunal venous outflow pressures of 0, 10, 20 and 30 mmHg. In addition to determining total protein concentrations, samples of lymph and plasma were subjected to polyacrylamide gradient gel electrophoresis to establish lymph protein concentrations of albumin and nine protein fractions. The osmotic reflection coefficient σ_d for total proteins and the various fractions was estimated from $\sigma_d = 1 - C_L/C_p$ when C_L/C_p became filtration-rate independent, i.e., at high lymph flow induced by the elevation of venous outflow pressure. In 15 animals the estimated $\sigma_d = 0.83$ for total proteins. In addition to determining σ_d, a graphic analysis was employed and an estimate of equivalent pore radii were obtained. Results suggest that the characteristics of jejunal capillary permeability are similar to those of the stomach. (Supported by NHLBI Grants 22392 and 29455.)

36.9

A LOGNORMAL DISTRIBUTION OF PORE RADII IN CAPILLARY MEMBRANES G. Bloom and J. A. Johnson Dept of Physiology, 6-255 Millard Hall, 435 Delaware St. SE , University of Minnesota, Minneapolis , Mn. 55455

A model of a membrane that is heterogeneous in the ultra-microscopic domain is proposed. The influence of the solute size on passive transport via diffusion and osmosis is derived and compared to experimental observations. A lognormal distribution of pore radii is postulated. The shape of the pores is assumed to a right circular cylinder. Hydrodynamic interaction of an uncharged spherical solute and water with the pore is given by expressions generated by Paine and Scherr. The lognormal distribution was chosen because it occurs in many systems, it does not extend to negative pore sizes, and the because ofd the mathematical economy implicit in the ability to specify the distibution by only two parameters, the mean and the standard deviation. This may be compared to a model which employs two distinct pore sizes which must be specified along with the ratio of their occurence. A graphical approach which plots the diffusive solute vs solute radius, or 1/solute radius vs osmotic water transport allows a simple assessment of the fit of the data to the proposed model. In addition, numerical integration of these equations generates a set of results that include the transport to be expected from a population of pores of a single size. A better fit of the data from several experiments is obtained with a distribution of pore sizes.

37.1

SHELL CONDUCTANCE, METABOLISM, AND GAS TENSIONS IN INCUBATING HEN'S EGGS: AN *IN VIVO* METHOD FOR MEASURING DIFFUSION COEFFICIENTS. C. V. Paganelli, P. R. Sotherland, and H. Rahn. SUNY at Buffalo, Buffalo, NY 14214

The normal metabolic gas exchange of incubating hen's eggs occurs by diffusion through the shell. We have developed a method which makes use of this property to obtain diffusion coefficients in the gas phase. Simultaneous measurement of O2 consumption ($\dot{M}O2$) and CO2 production ($\dot{M}CO2$) during incubation, together with $\Delta PO2$ and $\Delta PCO2$ across the shell from electrodes chronically implanted in the air cell, allow us to calculate shell conductance (GO2 and GCO2) as the ratio $\dot{M}/\Delta P$ for each gas. For eggs incubated in air at 38°C, GO2 and GCO2 in 9 eggs were 16.0 ± 3.1 and 12.0 ± 1.6 ml (STPD) day^{-1} $torr^{-1}$, (mean ± s.d.), respectively. The average GCO2/GO2 was 0.76, which agrees well with a value of 0.78 computed from the ratio of the binary diffusivities of CO2 and O2 in air at 38°C. The proportionality between G and diffusivity permits calculation of unknown diffusivities from measured G values, provided one diffusivity is known.

(Supported in part by USPHS Grant #HL-28542.)

37.2

PULMONARY DMO_2 IN THE MONITOR LIZARD. (V. salvator). B. Burns MIEMSS, Critical Care/Anesthesia Research, University of Maryland, Baltimore, Maryland 21201.

Rebreathing measurements of pulmonary membrane diffusing capacity (DMO_2 - ml/min/torr STPD) were made using the Na dithionite technique in 4 Varanids weighing from 2.2-3.2 kg (120-150 cm length). Following ketamine anesthesia (30 mg/kg, i.m.) the animals were placed supine, tracheostomized and mechanically ventilated. A small ventral incision was made, the pericardium exposed and the pulmonary outflow tract cannulated through the right ventricle. The lungs were pump perfused by artificial plasma at 25 °C(albumin 4 g%) containing 80 mM Na dithionite (used to maintain capillary PO_2=0 during gas exchange measurements) and the left atrium incised to allow removal of perfusate. Results: In 16 measurments the mean values (±s.e.) were DMO_2=.64 (±.11); V_L = 170 (±11.7) ml; the ratio DMO_2/V_L=.00375 (±.00046); and the ratio DMO_2/kg body wt =.25 (±.047). Argon mixing during rebreathing at a rate of 1-2/sec and a tidal volume of 50 ml was a double exponential process with half-times of approximately 1.4 and 4 sec, reflecting ventilation/volume heterogenieties and relatively inefficient mixing in the multicameral lung of V. salvator. The O_2 disappearance half-time during rebreathing was approximately 10 times longer than the longest Argon mixing half-time, indicating that mixing was not grossly rate-limiting for DMO_2 measurments. The ratio DM/V_L in this species is approximately 1/40th of the mammalian lung.

37.3

OXYGENATION AND DEOXYGENATION KINETICS OF HUMAN RED BLOOD CELLS STUDIED BY A STOPPED-FLOW TECHNIQUE. J. Piiper, K. Yamaguchi*, D. Nguyen-Phu* and P. Scheid. Dept. Physiol., Max-Planck-Inst. f. Exp. Med., Göttingen, FRG.

The kinetics of O_2 uptake into, and release from, human red blood cells was measured by a double-beam spectrophotometer (560 and 577 nm) at 37°C using a stopped-flow technique. A simple model, with a discrete O_2 diffusion resistive layer between hemoglobin and medium, was used for calculation of diffusive conductances from the rate of change of O_2 saturation (SO_2) and the calculated corresponding effective intra-extracellular O_2 pressure difference. For the same SO_2 range, O_2 uptake and release (without dithionite) yielded identical conductance values. Addition of albumin in varied concentrations and variation of dithionite concentration in the medium suggested an important diffusion limitation exerted by an extracellular stagnant layer. Desaturation measurements (with sufficiently high dithionite concentration) starting at varied SO_2 values yielded identical conductance values, independent of SO_2. The corrected O_2 transfer conductance of human red cells (i.e. excluding extracellular diffusion) was estimated at 8.7 ml O_2/(min·Torr·ml red cells), corresponding to θ = 3.9 ml O_2/(min·Torr·ml blood) for blood with a hematocrit of 45%. There was no indication for limitation of O_2 transfer by kinetics of O_2-hemoglobin reactions in the SO_2 range of 10 to 75%.

37.4

OXYGEN AFFINITY OF FETAL BLOOD CONTAINING CARBOXYHEMOGLOBIN; A TEST OF HALDANE'S LAWS. Robert Blake Reeves and Hae Kun Park. SUNY, Buffalo, N.Y. 14214

The utility of Haldane's simple formulation for the competitive equilibrium between O_2 and CO for Hb binding in blood rests on the experimental data of Roughton & Darling (1944). This formulation may be expressed as: $S_{O2}T + S_{CO} = f[P_{O2}(1 + S_{CO}/S_{O2}T)]$ where saturation (S) is expressed as fraction of total (T) Hb. We measured the oxygen equilibrium curve (O_2EC) of **human term fetal blood** over a range of S_{CO} values from 0-.5 and compared our results with Haldane's law computed values. Ligand saturation was measured using dual wavelength spectrophotometry (430-453 nm) on thin blood films one red cell thick held between 6 μm Teflon membranes. Oxygen tension was measured with an O_2-cathode. Continuous O_2EC sampled at 500 points were run in about 10'. HbCO was pre-loaded and S_{CO} did not change during the measurement of the O_2EC. Our CO-free std. fetal curve had a P_{50} of 22.4 torr and was isomorphic with the adult std. curve. The dependence of P_{50} on S_{CO} (P_{50} = 22.4 - 0.219*S_{CO}) followed Haldane's laws acceptably. Error plot of P(obs)-P(calc) vs. $S_{O2}A$, however, deviated progressively and significantly in the range .6 < $S_{O2}A$ < .95 (deviation <1 to >14 torr). Data of Roughton & Darling, limited in this saturation range, also tended to deviate from predicted in this region. We conclude that Haldane's laws are of limited utility for calculating the O_2EC of fetal blood in the presence of significant quantities of HbCO. (Supported by NIH HD13999.)

37.5

DECREASE OF DIFFUSING CAPACITY (D_L/V_A) WITH INCREASED HEIGHT IN HEALTHY NON-SMOKERS. Edith Rosenberg, Dept. of Physiology and Biophysics, College of Medicine, Howard University, Washington, D.C. 20059.

The use of the single breath diffusing capacity of the lungs for CO (D_L) and (D_L/V_A) as an index of interstitial lung disease requires prediction formulas for healthy lungs. Most recently published regression equations of D_L and D_L/V_A for normal people showed that both indeces decreased with the age of the subjects but Ayers et al (West. J.Med. 123: 255, 1975) reported a strong negative correlation between D_L/V_A and height (H). We have examined the measurements made on 67 non-smoking normal men who participated in an epidemiological study (Bernice H. Cohen et al, Johns Hopkins Med.J. 137: 95, 1975) and found that the regression equation of D_L/V_A vs age and -H was statistically significant while the regression of the same data on age alone was not significant. All of the eight regressions of D_L/V_A vs age and H that have been reported have negative coefficients for H. The probability of this occurring by chance is 1/1000 and indicates that the decrease of D_L/V_A with increased height is not a statistical artifact. It suggests that tall people have a larger proportion of low D_L/V_A regions than shorter ones. The low D_L/V_A regions may be in the apices of the lungs or in the central portion of the alveolar region. If the observed negative correlation of D_L/V_A with height is due to low D_L/V_A in lung apices it should be eliminated when D_L/V_A is measured in the supine or prone position.

37.6

INTERACTION OF SOLUBLE GASES WITH TRACHEAL AIRWAY MUCOSA. David D. Ralph* and Michael P. Hlastala. Univ. of Washington, Seattle, WA 98195

In models of pulmonary gas exchange, the assumption is usually made that there is no interaction of flowing gas with the airway mucosa. We quantitated such interaction by comparing movement during tracheal flow of two gases of similar molecular weight (MW) but different blood-gas partition coefficients (λ). Tracheostomies were performed in anesthetized dogs. The expiratory flow was controlled at a constant 100 ml/min by a piston ventilator. A 1.0 ml gas bolus containing traces of krypton (MW 84, λ 0.6) and diethyl ether (MW 74, λ 9.3) was injected at the start of exhalation into the trachea along injection sites at the tip and 15 cm distal to the tip of the tracheostomy tube. Appearance of each gas at the proximal tracheostomy tube was monitored by a Balzers quadrupole mass spectrometer. After distal injection the elimination of the more tissue soluble ether was delayed relative to krypton. Such delayed elimination did not occur when the gases were injected into airflow through dry lucite tubes. The depth of penetration of ether into the tracheal mucosa, calculated from the tracheal surface area and distribution volume, averaged 42 μ. We conclude that interaction of soluble gases with the tracheal mucosa delays elimination of soluble gases during unidirectional ventilation.

(Supported by NHLBI Grants HL12174, HL24163 and HL00891)

37.7

INFLUENCE OF THE CHEST WALL (CW) ON GAS EXCHANGE DURING MECHANICAL VENTILATION (MV). H. I. Modell, Virginia Mason Research Center, Seattle, WA 98101

Possible CW states during MV ranges from a coordinated inspiratory effort by CW muscles during assisted ventilation (AV) to a passive CW during controlled ventilation with paralysis (CV). To determine the extent to which CW mechanics may affect gas exchange during MV, 4 pentobarbital anesthetized, adult mongrel dogs were ventilated in AV and CV modes. Arterial and mixed venous blood gas status, cardiac output, and minute ventilation were measured during AV (airway pressure <0 during inspiration) and during CV after paralysis with succinylcholine. Tidal volume (15 ml/kg) and inspiratory flow rate were fixed. Frequency during CV was set at the animal's rate during AV. Results (mean ± SD) are shown below. No significant difference in computed venous admixture was detected, but Bohr dead space was higher with CV than with AV ($P<.05$). Analysis with a 3-compartment model shows that 82% of the Po_2 fall from AV to CV can be explained by the lower alveolar ventilation in CV. Whether this change is due primarily to absence of CW muscle activity directing ventilation or secondarily to redistribution of perfusion from increased intrapulmonary pressure remains to be determined. (Sponsored by AFOSR Contract F49620-78-C-0058)

	Po_2 (Torr)	Pco_2 (Torr)	$\dot{Q}$ (L/min)	$\dot{V}_E$ (L/min)
AV	68.7 ±15.5	50.6 ±11.7	3.99 ±0.90	2.44 ±0.71
CV	60.5 ±14.2	58.8 ±16.5	3.03 ±0.80	2.35 ±0.56
(Paired-t) P<	0.005	0.005	0.005	N. S.

37.8

EFFECT OF BODY POSITION ON REGIONAL LUNG EXPANSION: A COMPUTER TOMOGRAPHIC APPROACH. E. A. Hoffman, L. J. Sinak,* and E. L. Ritman. Mayo Med Sch, Rochester, MN 55905

The Dynamic Spatial Reconstructor (DSR) (Science 210:273-280) was used to estimate total and regional lung air content (LAC). Anesthetized dogs were scanned prone and supine (2 dogs) or in right and left lateral decubitus (1 dog) positions. Roentgen attenuation values for each 3D picture element were normalized so that air = 0% and water = 100% thus providing an index of % LAC. Each dog was scanned at 3 volume steps between FRC and TLC in each of 2 body positions. Change (Δ) in total LAC as calculated from DSR volume imaging (y) matched the known inflation step (x) (tidal volume range) to within 6% (range was 1-6% with a mean of 3% ± .5 SEM, N = 8; $y = 1.005x-.768$; $r = .997$). A gradient of decreasing % LAC was measured in nondependent to dependent direction at FRC in the supine, right and left lateral body positions ranging from 84 to 53; 69 to 38; and 69 to 49%, respectively. Regional fractional Δ LAC with lung inflation in these body positions were greater in the dependent than in nondependent lung regions. In contrast, regional LAC prone (FRC) was approximately uniform along the nondependent to dependent direction and was 68% ± .6 SEM. No linear right to left oriented gradients were observed prone or supine. However, ventral-dorsal FRC gradients in the nondependent lung with the dog in the right and left lateral positions were 69-57% and 86-53%, respectively. (HL-04664, RR-00007, and HL-29886 from NIH)

37.9

GAS TRANSPORT AND VENTILATION DISTRIBUTION AS A FUNCTION OF TIDAL VOLUME AND BREATHING FREQUENCY IN A MECHANICAL LUNG MODEL. Joel Deitz, M.D.*, Neil MacIntyre, M.D.*, Nelson Leatherman, Ph.D.* (SPON: Herbert Saltzman, M.D.). Duke University Medical Center, Durham, NC 27710

To study gas transport and ventilation distribution as a function of ventilatory frequency, we measured both total and regional ventilation in a mechanical lung model with two parallel compartments. This model allowed us to alter the compliance (C_L) or the resistance (R_{aw}) of each compartment individually. A wide range of frequency (f = 10-1000 BPM) and tidal volume (V_T = 10-2500 cc) combinations were used to produce three fixed ventilator flowrates (10, 25, 50 liters/minute). Effective ventilation ($\dot{V}_{Eff}$) to each compartment was determined by inert gas washout ($\dot{V}_{Eff}$ = compartment volume/washout time constant). Total $\dot{V}_{Eff}$, as well as a ratio of the $\dot{V}_{Eff}$'s in the two compartments, were determined. We found that $\dot{V}_{Eff}$ was a function of f and V_T when V_T > dead space (V_D), but that $\dot{V}_{Eff}$ appeared to be a function of f and V_T^2 when $V_T < V_D$. In addition, we found that with a unilateral increase in airway resistance, ventilation became less uniform as frequency increased; however, with a unilateral decrease in compliance, ventilation became more uniform as frequency increased.

(Sponsored by AHA 82-1037, NIH F32HL06648-01, and NC Lung Association.)

37.10

THE EFFECTS OF MANIPULATION OF PULMONARY BLOOD FLOW ON V/Q DISTRIBUTION IN UNILATERALLY UNVENTILATED LUNG T.S. Lee, M.D.*, B.D. Wright, M.D.*, and S.O. Jakobson, M.D.*. (SPON: Donald Frazier) University of Kentucky Medical Center., Lexington, Kentucky. 40536

The Wagner-West method with gas chromatogram technique was used to study V/Q distribution. Seven mongrel dogs were included. Minute ventilation was mechanically maintained constant at FIO_2 of 0.5. The experimental sequence consisted of 4 stages: Stage I: Ligation of the left main bronchus only. Stage II: Adding 50% occlusion of the left main pulmonary artery, Stage III: De-clamping of the left main pulmonary artery, Stage IV: 100% occlusion of the left main pulmonary artery. Stage I produced a large shunt (QS/QT 0.55) and hypoxemia (PaO_2 64.2) torr. At Stage II, QS/QT decreased significantly ($P<0.02$) from 0.55 to 0.30. Stage III again caused significant V/Q mismatch with shunt back to 43% ($P<0.001$) and PaO_2 decreased to 63.5 torr ($P<0.05$). At the Stage IV, the QS/QT reduced to 0.23 ($P<0.001$) and PaO_2 improved significantly ($P<0.01$). The proportion of cardiac output distributed to V/Q ratio between 0.1-10.0 were 45% (Stage I), 68% (Stage II), 54% (Stage III) and 76% (Stage IV) respectively ($P<0.05$). Since cardiac output and PCWP do not change significantly the improvement of arterial oxygenation can be attributed to the favorable redistribution of blood flow and the decrease in true shunt.

CELL PHYSIOLOGY I

38.1

COUMADIN INDUCED COAGULOPOIETIN PLASMA ENHANCES CARBOXYLATION OF LIVER MICROSOMES. Simon Karpatkin and Margaret Karpatkin*. NYU Medical School, New York, N.Y. 10016

Previous work in this laboratory has demonstrated that when plasma from Coumadin treated rabbits is injected into normal animals, biologic activity of vitamin K dependent clotting factors increases in plasma of the recipients. This indicates the presence of a humoral substance(s) or coagulopoietin(s) which regulates these clotting factors. In the present study 5 normal rabbits received plasma from Coumadin treated animals. Plasma levels of factors II, V, VII and X were measured biologically in the recipients; factors II and X were also measured by immuno-assay. Biologic activity of II, VII and X rose to 149±13% (SEM), 160±6%, and 160±24% respectively compared to base line levels. Factor V did not rise. Factors II and X measured immunologically also increased (115±7.2% and 134±5.4%) but less than did the biologic activity. This indicated increased specific activity of these two factors. The recipient animals were sacrificed and carboxylase activity of the liver microsomes compared to that of control animals. Microsomal carboxylase activity was greater in the animals who had received Coumadin plasma than in controls. The difference was 2.4 fold employing endogenous microsomal precursor for carboxylation and 6.2 fold employing the synthetic substrate, phe-leu-glu-glu-val. We conclude that a Coumadin induced coagulopoietin enhances biological activity of Vitamin K dependent coagulation factors in recipient animals and carboxylase activity of their liver microsomes.

38.2

THE RATE OF PLASMA CLEARANCE OF CROSSLINKED AND PYRIDOXYLATED HEMOGLOBINS IN THE RAT. L. Triner, R. Benesch*, R. E. Benesch*, S. Kwong* and M. Verosky*. Departments of Anesthesiology and Biochemistry, Columbia University, New York, N. Y. 10032

Hemoglobin crosslinked with 2-nor-2-formylpyridoxal phosphate (HbXL) as well as hemoglobin pyridoxylated at either the α chain or the β chain N termini, or at both chains were compared to unmodified hemoglobin (Hb). Hemoglobin solutions were injected i.v., plasma and urine hemoglobin concentrations were measured as cyanmethemoglobin. Volume loss due to sampling and diuresis was replaced by saline. At a dose of 0.4 mg/g, the initial plasma concentration was 0.7-1 g% and the rate of clearance was exponential with a $t_{1/2}$ of ≈ 1 hr for Hb and pyridoxylated hemoglobins and about 2 hrs for HbXL. As shown previously (Fed. Proc. 41: 652, 1982), HbXL was not excreted in the urine, in contrast to Hb and all uncrosslinked pyridoxylated hemoglobins (20-30% of the given dose was excreted in the urine in the first 3 hrs). At a dose of 2 mg/g, the initial plasma level was about 3 g%. The unmodified Hb plasma concentration decreased at a rate of $t_{1/2}$ ~ 90 min, while the clearance of HbXL was biphasic: ~ 1 g% was cleared from the plasma with a $t_{1/2}$ of ≈ 50 min, followed by a slower, linear decline at a rate of 1 g% in 8 hrs. Supported by N.I.H. grant HL-05791 (to R.E.B.) and N.S.F. grant PCM-7911610 (to R.B.).

38.3

LEUKOCYTE ACCUMULATION IN OCULAR INFLAMMATION: ANOMALOUS EFFECT OF ANTI-INFLAMMATORY STEROIDS. Christopher A. Paterson and Richard N. Williams*. University of Colorado Medical School, Denver, CO 80262

The effect of anti-inflammatory steroids upon bacterial endotoxin induced inflammation of the rabbit eye was examined. Polymorphonuclear leukocyte (PMN) infiltration into ocular tissues was quantified by measuring myeloperoxidase (MPO) activity. Intravitreal injection of endotoxin (1 µg) caused dilation of conjunctival and iridial blood vessels, leakage of protein into the aqueous humor, and appearance of PMN's in iris-ciliary body and aqueous humor. Topical or systemic administration of prednisolone sodium phosphate, dexamethasone sodium phosphate or hydrocortisone acetate immediately after the intravitreal injection of endotoxin inhibited PMN accumulation in the aqueous humor but increased MPO activity in iris-ciliary body. However, pretreatment with these steroids, either topically or systemically, inhibited endotoxin induced PMN accumulation in both aqueous humor and iris-ciliary body. These findings could be qualitatively confirmed by histopathology. It was observed that both pre- and post-treatment with steroids reduced the clinical signs of endotoxin-induced inflammation. We suggest that, in the eye, the initial therapeutic effect of anti-inflammatory steroids is not directly related to inhibition of PMN infiltration and accumulation.

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38.4

CORRELATION BETWEEN THE DOSE-RELATED EFFECTS OF GENERAL ANESTHETICS ON PHOSPHOLIPID SURFACE TENSION AND BRAIN OXYGEN CONSUMPTION. Edwin M. Nemoto, Marc Uram,* and Peter M. Winter.*

General anesthetics differ in their dose-related effects on cerebral metabolic rate for O_2 ($CMRO_2$). Thiopental decreases $CMRO_2$ a maximal 30% of normal even at doses 6 times higher than for anesthesia. Halothane decreases $CMRO_2$ linearly with increasing dose. Because the primary effects of anesthetics are believed to be exerted on membranes, we compared the effects of thiopental, halothane, enflurane and isoflurane on the surface tension of monolayers of brain phospholipid (PL), namely, phosphotidylinositol (PI) to determine whether they correlated with their effects on $CMRO_2$. A Cahn 2000 electrobalance/surface tension unit was used to measure surface tension (γ) by the Wilhemy plate method. Monolayers of PI were prepared over bicarbonate buffer. Thiopental was added to the monolayer or the gaseous anesthetic in air, was perfused through the surface tension chamber. Changes in surface pressure ($\Delta\pi$) were calculated by the formula, $\Delta\pi = \gamma_o - \gamma'$, where γ_o = surface tension of the PL monolayer alone and γ' = surface tension of the PL monolayer plus drug. Whereas the inhalation anesthetics increased $\Delta\pi$ linearly up to 10 dynes/cm, thiopental had a maximal plateau effect with a 3 dynes/cm change in π. There is an apparent correlation between the effects of general anesthetics on $CMRO_2$ and their effects on PL surface tension. The differential effect on $CMRO_2$ may be due to differential effects on membrane ion transport and membrane-bound enzyme activities.

38.5

Amiloride Inhibition of H^+ Extrusion from B-Cells Triggers Electrical and Secretory Activity. Caroline S. Pace. University of Alabama in Birmingham, Birmingham, AL 35294

Regulation of intracellular pH may occur via the activity of Na:H and HCO_3:Cl antiporters in the B-cell plasma membrane since the inhibitory influence of amiloride or DIDS (4,4' diisothiocyano-2,2'stilbene disulfonic acid) induces an increase in the duration of the active phase of glucose-induced (11.1 mM) oscillatory electrical activity similar to that obtained by use of a permeable weak acid to decrease pH_i (Pace, C.S. and J.T. Tarvin 73: 39, 1983). We used amiloride to determine whether a decrease in pH_i is a sufficient stimulus to trigger electrical activity and insulin release in the presence of subthreshold glucose concentrations. The electrical response of mouse islet B-cells was compared to the secretory response obtained from rat islets placed in a perifusion chamber. At 0.1 mM amiloride, electrical activity was induced at 4.8 ± 0.3 mM (M ±SE) glucose and constant spike activity at 10.6 ± 0.9 mM, whereas the respective values for glucose alone were 7.0 ± 0.4 and 24.0 ± 1.5 mM. Amiloride did not influence basal insulin release at 2.8 mM glucose, but elicited a secretory response at 4.2 mM glucose of 101 µU/100 islets/min above basal release determined during a 45 min period; and 124 and 133 µU/100 islets/min at 5.6 and 7.0 mM glucose, respectively. These results indicate that inhibition of Na:H exchange at subthreshold glucose is sufficient to initiate electrical events which may play an important role in information transfer to the secretory complex.

38.6

QUINACRINE COMPETITIVELY INHIBITS NA/CA EXCHANGE IN CARDIAC SARCOLEMMAL VESICLES. J.L. Sutko*, P.K. Williams*, P. de la Peña* and J.P. Reeves*(SPON: G. Templeton). Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235 and Roche Res. Ctr., Roche Inst. Mol. Biol., Nutley, NJ 07110

Na/Ca exchange diffusion is thought to play a key role in controlling the level of free calcium in cardiac muscle cells; but definition of its actual contributions awaits the identification of an inhibitor of this process. Quinacrine and other acridines block sodium channels in nerve (Oxford & Hudson, Biochem. Biophys. Res. Comm. 104:1579,1982), therefore, we investigated its ability to antagonize another sodium-dependent process, Na/Ca exchange, in plasma membrane vesicles isolated from bovine and canine myocardium. In the vesicle system, quinacrine (1-200 µM) competitively and reversibly inhibits both Na_i-dependent Ca_o uptake and Na_o-dependent Ca_i efflux with a K_i of 10-25 µM. Quinacrine also inhibits the passive efflux of calcium from vesicles previously loaded with calcium via Na/Ca exchange. Moreover, quinacrine antagonizes both Na/Na and Ca/Ca exchange modes of operation of this transport system. In the latter case, this effect was observed in both the presence and absence of a stimulating monovalent cation. At the concentrations tested, quinacrine did not inhibit ATP-dependent calcium uptake. In conclusion, quinacrine is an effective antagonist of the Na/Ca exchange process in cardiac plasma membrane vesicles. Supported by NIH grant #HL26810.

38.7

A CORRELATION OF I- ANTIGEN EXPRESSION AND FETAL HEMOGLOBIN CONTENT IN ERYTHROCYTES OF INDIVIDUALS WITH SICKLE CELL ANEMIA. M. K. Basu*, P. Goldie*, T. Boussios*, M.R. Condon*, W.G.Ramey* and J.F. Bertles. St. Luke's-Roosevelt Hospital Center, Columbia University, New York, N.Y. 10025.

Expression of the I(and i) antigen on the membrane surface of erythrocytes from individuals homozygous for the sickle-cell gene (SS) is greater than on normal (AA) erythrocytes,but with considerable variability from cell to cell in both genotypes. In attempts to separate strongly and weakly I-expressing SS cells, we took advantage of the well-known marked heterogeneity in cell density within SS erythrocyte populations. Venous blood samples were obtained with informed consent. Each AA and SS erythrocyte population was fractionated through a discontinuous density gradient (Dextran T40). Proportions of fetal hemoglobin (Hb F) in each fraction were measured. Cells of each fraction were assayed by autoanalyzer for agglutination by anti-I antibody, and for expression of I antigen by single cell immunofluorescence and by flow cytofluorometry in a fluorescence-activated cell sorting (FACS) analyzer. The expression of I antigen on AA erythrocytes was constant throughout the density range by all three techniques. In contrast, the expression of I antigen on SS erythrocytes peaked by all three techniques in the same density fraction that demonstrated the greatest proportion of Hb F. Refinement of separation of maximally and minimally fluorescing SS cells by FACS may provide explanations of these findings. (Supported by NIH grants HL-28033,AM 30942, and the Clark Foundation)

38.8

INSULIN EFFECTS ON pH_i AND V_m OF FROG MUSCLE. R.W. Putnam* and A. Roos. Dept. of Physiology and Biophysics, Washington Univ. School of Medicine, St. Louis, Missouri 63110.

Membrane potential (V_m) and intracellular pH (pH_i) (measured with glass microelectrodes) were followed for up to 2 hours in frog muscle (semitendinosus) in Ringer with 1 mU/ml insulin and 0.1% BSA (22 C). In 11 fibers, the membrane hyperpolarized by 5.4±1.9 mV from -90±2 mV. After a delay of 19±5 min, the initial pH_i, 7.24±0.04, rose during 30 min by 0.08±0.01 (range 0.03-0.16). In 3 fibers, pH_i did not change, but the membrane hyperpolarized by 3 mV. The pH_i in 27 other fibers, pre-soaked in insulin for more than 1 hour, was 7.38±0.02, whereas in 21 control fibers it was 7.29±0.02. A similar insulin-induced pH_i increase from 7.31±0.02 (21) to 7.40±0.02 (22) was seen in fibers depolarized in 15 K, but not in 50 K. Insulin (400 mU/ml, no BSA) did not affect, in 2.5 K, the very slow pH_i recovery (0.03±0.02 ΔpH_i/h) from 5% CO_2-induced acidification (pH_o constant at 7.35), nor the brisk recovery (0.28±0.03 ΔpH_i/h, n=9) in fibers depolarized in 50 K (Abercrombie, Putnam and Roos, J. Physiol.(Lond.), in press). However, the slow recovery in 15 K, 0.05±0.02 ΔpH_i/h (24), was nearly tripled to 0.13±0.01 ΔpH_i/h (24) by insulin. This recovery could be inhibited by 1 mM amiloride. The results suggest that insulin can activate Na:H exchange in frog skeletal muscle.

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39.1

EFFECTS OF CIGARETTE SMOKING AND ALCOHOL ON BLOOD LIPIDS AND LIPOPROTEINS IN PHYSICALLY ACTIVE AND INACTIVE MIDDLE-AGED FEMALES. B.A. Stamford, S. Matter*, R.D. Fell and S. Sady*. University of Louisville, Kentucky 40292.

Exercise and alcohol are believed to increase serum HDL-C whereas cigarette smoking may act in a contrary manner. The purpose was to examine separate and combined effects of these variables. More than 300 middle-aged females were screened for exercise and smoking habits. Body fat (via hydrostatic weighing), cardiovascular responses to treadmill exercise, age, height, weight, serum total cholesterol, HDL-C, LDL-C, HDL-C/total ratio and triglycerides were determined. Estimates of partial regression coefficients revealed that HDL-C of chronic exercisers was 6.6 mg/dl higher than nonexercisers. Each ounce of alcohol contributed to an increase in HDL-C of 3.8 mg/dl. HDL-C of nonsmokers was 7.6 mg/dl higher than smokers. Analysis of covariance with adjustments for body fatness, age, physical activity, alcohol, and smoking revealed: (1) smokers who exercise and/or consume alcohol demonstrated lower HDL-C levels than nonsmokers with similar habits; and (2) HDL-C was higher in subjects who both exercise and consume alcohol when compared with subjects who practice one or the other separately. It was concluded that: (1) smoking attenuated the beneficial effects of exercise and/or alcohol to raise HDL-C; and (2) chronic exercise combined with alcohol exerted an additive effect to raise HDL-C. (Supported by AHA, Kentucky Affiliate and the Graduate Research Council, Univ of Louisville)

39.2

EFFECT OF EXERCISE ON METABOLISM, HEART RATE AND TEMPERATURE IN PIGS. H. H. Erickson, F. M. Faraci*, and S. C. Olsen*. Kansas State University, Manhattan, KS 66506

Domestic pigs (47.1±7.9 Kg) were exercised for 5 minutes at five different treadmill speeds, 1.0 - 1.8 $m \cdot sec^{-1}$, on a 3° incline while oxygen consumption ($\dot{M}O_2$), carbon dioxide production ($\dot{M}CO_2$), the electrocardiogram (EKG), and body temperature (T_b) were continuously recorded. Data were taken at rest, during exercise, and at 2, 5, 15 and 30 minutes after exercise. $\dot{M}O_2$, $\dot{M}CO_2$, and heart rate (HR) showed progressive increases with increasing treadmill speed. The respiratory quotient (R) increased during exercise and approached 1.0, but peak values were seen shortly after exercise. HR, $\dot{M}O_2$, $\dot{M}CO_2$, and R reached steady-values after 2 minutes of exercise that were maintained for the duration of exercise. In most cases, these variables had returned to control levels 15 minutes after exercise. In contrast, T_b continued to increase after exercise, and at higher running speeds was still elevated at 30 minutes post-exercise. A high correlation (R = 0.98) between HR and $\dot{M}O_2$ was found in these animals. Prominent increases in T-wave amplitude of the EKG were associated with exercise and early recovery. Metabolic and cardiac changes associated with exercise in these animals were qualitatively similar to responses observed in exercising man, supporting the use of the pig as a good model for studying the cardiopulmonary responses to exercise in man. (Supported in part by USDA 55604-0337).

39.3

TRAINING INDUCED REPRODUCTIVE HORMONE CHANGES IN REGULARLY MENSTRUATING VS. OLIGO-AMENORRHEIC RUNNERS. S.J. Pepper, R.W. Hale*, and D.A. Lally*. Dept. of Physiology and Dept. of Ob/Gyn., University of Hawaii, Honolulu, HI 96822

Reproductive hormone levels of 35 females were measured. Seven were nonathletes (NA) and 28 were athletes, of which 12 had normal menstrual cycles (RA), 10 were oligomenorrheic (OA), and 6 were amenorrheic (AA). There were no differences in age, hours exercised, or body composition among the athletes however significantly more of the RA (83%) compared with the AA (17%) had been pregnant. The RA also had significantly higher pre-exercise follicular phase estradiol levels compared with both the AA and the NA. Reproductive hormone responses immediately after and 30 minutes after standardized treadmill runs during the follicular and luteal phases of the menstrual cycle were compared for each group. Estradiol, progesterone, and prolactin levels increased following exercise and the RA hormone levels were generally 2 to 3 times higher than for the AA, both prior to and following exercise. The fact that NA and AA had similar reproductive hormone levels but different menstrual histories clearly demonstrates the need for further study. The consistently higher steroid hormone levels of the RA, both before and following exercise, suggests that for athletes to continue regular menstrual cycles they may need to maintain elevated reproductive hormone levels.

39.4

ADULT FITNESS PROGRAMS INVOLVING SEDENTARY FACULTY MEMBERS. Roger G. Soule. Biola U., La Mirada, CA 90639

15 adult faculty members: 7 men, 8 women (avg age men, 43.5; women, 30.0) participated in a 10 wk fitness program consisting of: jogging, swimming, or volleyball, 3 times a wk, 45 min/session. Each session consisted of: 5 min calesthenics, 30 min selected activity, 5-10 min cool down exercises; stretching, walking etc. Measurements taken pre/post program were: Resting HR, BP, % body fat-underwater weighing, & $\dot{V}O_2$ max. The groups were not matched--5 joggers (3 men 2 women) 4 swimmers (2 men 2 women) & 6 volleyball players (2 men 4 women). Over the 10 wk program there were no changes in body composition, HR or BP. However, all groups increased significantly in $\dot{V}O_2$ max; joggers 30%, swimmers 20%, & volleyball 12%. It is concluded that low intensity activity such as volleyball is stressful enough to bring about significant $\dot{V}O_2$ max improvement in low fit adults.

Supported by Biola Faculty Research Grant

39.5

EFFECTS OF OVARIECTOMY ON BONE STRUCTURE AND CALCIUM IN EXERCISED RATS. R.L. Pohlman*, L.A. Darby*, and A.J. Lechner. Dept. Physiology, St.Louis Univ. Sch. Med., St.Louis,MO 63104.

The rate of bone calcium loss and other osteoporotic changes associated with menopause were studied in exercising ovariectomized rats. Female Sprague-Dawley rats were divided into three groups: 1)ovariectomized-young(120 day old)OY, $\overline{BW}$= 344.6g; 2)ovariectomized-retired breeders, OR, $\overline{BW}$=351.5g; 3)nonovariectomized-control/young, CY, $\overline{BW}$=292.2g. All rats were run on a treadmill 1 hr/day, 5 days/wk for 4 weeks at a speed of 19.2 m/min and 0% grade. Rats were anesthetized with sodium pentobarbital (30mg/kg, i.p.) and sacrificed. Training effects were verified by muscle enzyme levels and fiber typing. Bones were measured for length and diameter with calipers (±.02mm) and volumes and densities by fluid displacement (±.01ml). Calcium concentrations and contents of femurs and 7th ribs were assayed after acid hydrolysis in 5N HCl. Although the OY group showed greater femur length and volume associated with their greater $\overline{BW}$, bone densities and Ca contents and concentrations were significantly lower in the OY and OR groups than in the CY group: OY [Ca]=132.50 mg/g fresh wt., Ca content=124.86g; OR [Ca]=136.00, Ca content=124.37; CY [Ca]=165.24, Ca content=150.39. No differences among groups were found for 7th ribs. Thus, significant skeletal Ca losses occurred within 4 weeks of ovariectomy in both young and old rats. Whether such Ca losses would be more pronounced in a sedentary population remains to be determined. (Supported by NIH Grants HL29640 and HL07050)

39.6

GLUCOSE KINETICS IN EXERCISE: THE ROLE OF CHANGES IN INSULIN AND GLUCAGON. Robert R. Wolfe, Ethan R. Nadel, James H.F. Shaw*. Harvard Med Schl, Shriners Burns Inst, Boston, MA and John B. Pierce Fnd, Yale Med Schl, New Haven, CT 06519.

To examine the role of insulin and glucagon in maintaining glucose homeostasis during mild exercise (40% $\dot{V}O_2$ max) and recovery, we have studied human subjects in hormonal clamp conditions. We clamped insulin and glucagon by inhibiting their secretion with somatostatin, simultaneously infusing insulin and glucagon at constant rates throughout the experiment. Results were compared to the responses in the situation when the hormones were unclamped. Glucose kinetics were determined using a stable isotopic tracer of glucose (6,6-d_2 glucose or 1-^{13}C-glucose).

With no hormonal clamp there was a balance between an increased rate of glucose clearance and glucose production in exercise such that plasma glucose homeostasis was maintained. In contrast, hypoglycemia developed in all subjects during exercise. The mean glucose concentration fell from 95.4±17.8 at rest to 50.6±6.1 mg/dl at the end of exercise, and did not rebound in recovery. Glucose clearance increased markedly, and despite the hypoglycemia and 10-fold increase in plasma catecholamine concentrations, glucose production remained at the resting level.

Thus, increased sensitivity to insulin in even light exercise necessitates that a small drop in insulin and/or increase in glucagon occur or glucose homeostasis will be lost.

39.7

SHORT TERM RECOVERY EXERCISE: BLOOD LACTATE DISAPPEARANCE AND EXCESS POSTEXERCISE OXYGEN CONSUMPTION. R.J. Moffatt*, B.A. Stamford and B. Golden*. Western Washington University Bellingham, Washington 98225.

The purpose was to determine effects of a brief bout (15 min) of exercise at 40% $\dot{V}O_2$max on recovery from exhaustive exercise. Comparisons were made with passive (P) recovery over 45 min. Arterialized venous blood (PO_2=77.8, PCO_2=41.2) was sampled regularly via catheter from a dorsal hand vein in five male volunteers and $\dot{V}O_2$ was determined throughout. During 40% exercise recovery at min 15, blood lactate concentration was 7mM, pH 7.340, and HCO_3 21 mM as compared with 13.3 mM, 7.260, and 16.5 mM, respectively for P. Recovery was essentially complete at 30 min for 40% exercise whereas 45 min were required for P. These results agree with our previous data in which exercise was performed throughout recovery. Excess postexercise oxygen consumption (EPOC) fast components did not differ significantly between protocols (P=3.16L; 40%=3.54L) whereas slow components did (P=7.29L; 40%=5.39L). There was no relationship between EPOC and lactate disappearance and the overall correlation coefficient (r) was 0.04. Individual r values ranged from 0.06 to 0.39. It was concluded that a brief bout of aerobic exercise is very effective in promoting recovery from exhaustive exercise and may be as helpful as exercise throughout. Also, lactate disappearance from the blood was not related to EPOC. (Supported by the Bureau of Faculty Research, Western Washington University).

39.8

PHYSIOLOGICAL RESPONSES OF PARAPLEGIC AND QUADRIPLEGIC SUBJECTS TO ELECTRICALLY INDUCED EXERCISE AND WALKING. Jerrold S. Petrofsky, Chandler A. Phillips and Enrique Pantoja * Wright State University, Dayton, Ohio 45435

Physiological stress including cardiovascular and bone density measurements were examined in quadriplegics' and paraplegics' measurements during electrically induced isokinetic and dynamic exercise on the bicycle ergometer and, in some subjects, walking. Blood pressure in quadriplegic and paraplegic subjects was similar to exercise in nonparalyzed individuals. Except that both systolic and dyastolic blood pressure increased in paraplegic and quadriplegic subjects during the exercise. The increases in blood pressure appeared to be linked to reflex activation from muscle since occlusion of the circulation at the end of the contraction could sustain the increase in hypertension following the termination of the exercise. Cardiovascular responses improved with training. Also, bone density increased in typical subjects by greater than 15% bone mineral content with several months of training. Following exercise training, paralyzed subjects were able to tolerate walking with small increase in blood pressure and heart rate. This work was supported in part by the Spinal Cord Society and the American Paralysis Association.

39.9

PROTEIN TURNOVER IN TRAINED AND UNTRAINED RATS DURING REST AND EXERCISE. Scott A. Henderson*, Arthur L. Black*, and George A. Brooks. Exercise Physiology Laboratory, Univ. of California, Berkeley, CA 94720.

The effects of training on protein flux were studied in sedentary and endurance-trained female rats during rest and exercise. Leucine turnover and oxidation were assessed in vivo by continuous infusion of [1-^{14}C]leucine. The infusions were maintained for 60 min of rest, 40 min of easy exercise (17 m/min, 1% grade), and, in the case of trained rats, 20 min of moderate exercise (25 m/min, 1% grade). Arterial blood was assayed for leucine specific activity and expired air monitored for $\dot{V}O_2$, $\dot{V}CO_2$, and the specific activity of $^{14}CO_2$. The leucine turnover rate was verified independently for the same two groups of animals, by determining the accumulation of [1-^{14}C]leucine in the hemoglobin pool and applying the occupancy principle. Based on leucine turnover and oxidation, it was estimated that protein degradation and protein synthesis were higher in trained rats during rest and exercise. In addition, exercise resulted in a net catabolism of protein proportional to the metabolic rate and due to a decreased rate of protein synthesis. It was estimated that protein supplied 4-6% of the energy required for submaximal exercise. The results suggest that protein metabolism is affected by training and exercise, and that chronic endurance exercise may increase the dietary requirement for protein.

Supported by a grant from the American Heart Association-California Affiliate.

39.10

ELECTRICALLY INDUCED EXERCISE OF PARALYZED LEG MUSCLES: METABOLIC AND CARDIOPULMONARY RESPONSES. S.R. Collins*, R.M. Glaser, S.D. Feinberg*. Wright State Univ. Sch. of Med., Dayton, OH 45435 and Rehabilitation Inst. of Ohio, Miami Valley Hosp., Dayton, OH 45409

The purpose of this study was to determine aerobic metabolism and cardiopulmonary responses of 4 spinal cord injured subjects during electrical stimulation induced exercise of their paralyzed legs. For this, a computer controlled closed loop electrical stimulation system, using surface electrodes over motor points of the quadriceps muscle groups, automatically regulated 4 min bouts of 70° leg extension exercise (alternating 7 sec contractions; 2 per min for each leg). Exercise was discontinuous (10 min rest between bouts) and progressive with respect to the load weight attached to the lower leg (5,10,15,20 lb). During the final min of each exercise bout steady state oxygen uptake ($\dot{V}O_2$), pulmonary ventilation ($\dot{V}E$), heart rate (HR) and arterial blood pressure (BP) were determined. $\dot{V}O_2$ and $\dot{V}E$ were linear with exercise load and were 2X rest at 20 lb. In contrast, HR did not rise above rest. BP increased several mm Hg above rest during exercise, but was similar during each exercise level. These data suggest that the commonly monitored cardiovascular variables of HR and BP are not valid indicators of the stressfulness of peripherally stimulated exercise in spinal cord patients. In contrast, $\dot{V}O_2$ and $\dot{V}E$ appear to be well regulated with respect to exercise intensity. (Supported in part by the Veterans Administration)

39.11

USE OF ELECTRICALLY STIMULATED LEG MUSCLES FOR LOCOMOTION. R. M. Glaser, S.R. Collins*, S.D. Feinberg* and J.A. Gruner*. Wright State Univ. Sch. of Med., Dayton, OH 45435 and Rehabilitation Inst. of Ohio, Miami Valley Hosp., Dayton, OH 45409

The purpose of this project was to design and construct a vehicle to permit locomotion via electrically stimulated paralyzed leg muscles. For this, a conventional wheelchair was modified by the addition of two moveable footplates which were coupled to the drive wheels via ratchet-type transmissions. Thus, reciprocating movements of the legs result in forward propulsion of the vehicle. Steering can be accomplished by consecutive movements of a single leg. A two-channel battery powered electrical stimulator (square wave impulses of 10-1000 microseconds wide, 0-135 V, 0-150 ma, 20-125 Hz) using surface electrodes over motor points of the quadriceps muscle groups controls contractions and locomotive characteristics. Multiple electrode sequential stimulation is used to obtain smooth contractions at a relatively low impulse frequency and less fatigue of the muscles. With the onset of fatigue, higher stimulation levels are required to recruit more muscle fibers. This leg propelled vehicle (LPV) has been successfully operated by paraplegic and quadriplegic subjects. Chronic use of an LPV may contribute to the rehabilitation of non-ambulatory individuals by improving their: locomotive capability; circulation in the lower extremities; cardiovascular and respiratory fitness; strength and size of the exercised muscles and bones; and, self-image. (Supported in part by the Veterans Administration)

39.12

SKELETAL MUSCLE ADAPTATION TO DYNAMIC EXERCISE TRAINING IN THE FOXHOUND. D. Parsons,* R.L. Moore,* T.I. Musch,* G.C. Haidet* and G.A. Ordway. Univ. of Texas Health Science Center, Dallas, TX 75235

Eight to 12 weeks of dynamic exercise training increases maximal oxygen consumption approximately 30% in foxhounds. This increase in maximal oxygen consumption results primarily from an increase in maximal stroke volume and cardiac output, with no change in maximal arterial-venous oxygen extraction (Fed Proc 42:735). The purpose of this study was to determine if dynamic exercise training produces biochemical or histochemical adaptations in skeletal muscle in foxhounds. Analyses were performed on samples removed from medial gastrocnemius muscles of 7 foxhounds before and after a treadmill running program. Biochemical analysis showed that training produced no alteration in the activities of phosphofructokinase, β-OH acyl CoA dehydrogenase, succinate dehydrogenase or total phosphorylase. Histochemical analysis of myofibrillar actomyosin ATPase demonstrated 2 distinct fiber types. Oxidative and glycolytic potentials, as indicated by NADH-TR or α-glycerophosphate dehydrogenase staining intensity, were unaltered by training. Additionally, there was no positive correlation between oxidative capacity and fiber type. Thus, unlike humans and other mammals (i.e., rat), foxhounds do not demonstrate biochemical or histochemical adaptations in skeletal muscle as the result of exercise training. Further, these results are consistent with the finding that exercise training does not alter maximal arterial-venous oxygen extraction in foxhounds.

40.1

CHARACTERISTICS OF MECHANORECEPTORS IN THE AIR BREATHING ORGAN OF THE HOLOSTEAN FISH, AMIA CALVA. William K. Milsom and David R. Jones, Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 2A9.

Mechanoreceptors associated with the air breathing organ were identified and characterized, in vivo, in double-pithed specimens of the bowfin (Amia calva) using standard techniques for single fibre nerve recording. These receptors were innervated by the vagus nerve and located within the lung, apparently along the ventral wall where the lung and gut retain a connective tissue attachment. All receptors increased tonic discharge with step increases in lung volume above a threshold level and were slowly adapting. Most were not active at lung volumes below 25 $ml \cdot Kg^{-1}$. There was a dynamic, rate sensitive burst of activity also associated with lung inflation and a dynamic, rate sensitive inhibition of discharge associated with deflation. In a few fibres, rapid step inflation of the lung produced a burst of activity followed by a rebound inhibition of activity with discharge then returning to a new tonic level which was dependent on lung volume. All receptors were insensitive to changes in intrapulmonary, carbon dioxide partial pressure. These observations suggest that receptors capable of transducing both rate and degree of inflation and deflation are associated with even the most primitive air breathing organs. Furthermore, these receptors bear characteristics in common with both gut receptors and pulmonary mechanoreceptors of other vertebrates. (Supported by the NSERC of Canada)

40.2

LIMITATION OF LACTATE EFFLUX FROM FISH MUSCLE TISSUES AFTER EXHAUSTING EXERCISE. N. Heisler*, G.F. Holeton* and P. Neumann* (SPON: J. Piiper). Dept. of Physiology, Max Planck Inst. for Experimental Medicine, Göttingen, FRG

The slow efflux of lactate from fish muscle tissues after anaerobic activity has often been attributed to partial or even complete shut-down of blood perfusion. In order to test this hypothesis the blood flow to tissues of rainbow trout was studied before and 5, 30 and 120 min after exercise by application of the microsphere method. Radioactively labelled microspheres (MS) were injected via indwelling catheters into dorsal aorta and caudal vein, and the distribution of the MS lodged in systemic and gill tissues was taken as representative for the respective blood flow. The distribution of blood flow in the gills remained unchanged, whereas blood flow in the systemic tissues was considerably redistributed. White and red muscle received up to +230 or +490% of the pre-exercise blood flow, much more than attributable to the maximal increase in cardiac output (+60%). Model calculations based on the tissue flow rates and data on the body compartment volumes indicated that maximal lactate concentration values in the blood would be expected 2.5 min after the end of exercise, if the process of lactate efflux from muscle tissues was perfusion-limited. Comparison with the time required for this process in vivo (about 2h) suggests that the efflux of lactate from fish muscle tissues is largely diffusion-limited.

40.3

COMPENSATORY PLASMA BICARBONATE MODULATION DURING ENVIRONMENTAL HYPERCAPNIA IN CARP. J.B. Claiborne* and N. Heisler* (SPON: J. Piiper). Dept. Physiology, Max Planck Institute for Experimental Medicine, Göttingen, FRG.

When fish are exposed to elevated levels of ambient CO_2, the resulting respiratory acidosis is compensated by an increase in plasma $[HCO_3^-]$. Some species achieve full recovery of pH to pre-hypercapnic values in less than 24 hours while others may require several days and do not achieve complete compensation. In order to test the regulatory mechanisms responsible for plasma $[HCO_3^-]$ adjustments, carp (Cyprinus carpio) were exposed to hypercapnic ambient water gassed with 1 or 5% CO_2 in air while plasma pH, P_{CO2}, and $[HCO_3^-]$ were periodically monitored. During exposure to 1% hypercapnia, plasma $[HCO_3^-]$ increased from 13.8 to 21.6 mM over 48 hours, thus compensating about 50% of the expected pH depression at constant $[HCO_3^-]$. 5% ambient CO_2 induced a larger plasma pH depression (from 7.86 to 7.09 in the first hour) which was finally compensated by about 45% over 96 hours when plasma $[HCO_3^-]$ had reached a steady-state level of 28 mM. In a seperate series of experiments, fish, which after 48 hours of hypercapnia (1% CO_2) had increased plasma $[HCO_3^-]$ to 20 mM, were infused with $NaHCO_3$ (5 mM/kg). Following the infusion, serosal pH reached control levels concurrent to an increase in plasma $[HCO_3^-]$ to 40 mM but quickly returned to pre-infusion values as $[HCO_3^-]$ decreased to about 24 mM. It is concluded that carp can retain a maximal $[HCO_3^-]$ of 25 to 28 mM. Thus, the extent of compensatory pH regulation by the augmentation of plasma HCO_3^- during hypercapnia is delimited by this maximal threshold.

40.4

THE PHYSIOLOGICAL RESPONSES OF THE TURTLE, CHRYSEMYS PICTA BELLII, TO APNEA AS A FUNCTION OF TEMPERATURE. Christine V. Herbert* and Donald C. Jackson. Brown Univ., Providence, R.I.

Freshwater turtles were submerged in low O_2 water at 3°, 10° 15°, and 20°C until the blood pH was reduced to 7.0 or the plasma HCO_3^- to 10 mM. The duration of anoxia at the 4 temperatures was 91 days, 10 days, 3 days and 0.5 days respectively. During submergence and subsequent recovery blood pH, PCO_2, PO_2 and the plasma concentrations of HCO_3^-, Na^+, K^+, Cl^-, Ca^{++}(tot) Mg^{++}(tot) and lactate were periodically measured. Peak lactate values decreased with temperature and mean values ranged from 96.0 at 3°C to 19.7 mM at 20°C. Peak blood PCO_2 values increased with temperature and ranged from 13.3 T at 3°C to 64.4 T at 20°C. The type of acidosis, therefore, changed from being primarily metabolic at low temperature to being primarily respiratory at high temperatures. Associated with the diving were significant increases in plasma K^+,Ca^{++}, and Mg^{++} and decreases in HCO_3^- and Cl^-. These changes each correlated with plasma lactate independent of temperature, but did not correlate with either the fall in pH or the rise in PCO_2. The ionic changes served to minimize the drop in pH by maintaining a positive strong ion difference. These changes reversed during recovery. With submergence metabolic rate was shown to decrease by 90% at 3°C and 80% at 20°C. The greatly prolonged submergence at 3°C can thus be attributed to two factors: 1) the absence of a significant respiratory acidosis, and 2) greatly reduced metabolic rate.

40.5

THE EFFECTS OF DIFFERENT TYPES OF ACIDOSIS AND EXTRACELLULAR CALCIUM ON MECHANICAL ACTIVITY OF TURTLE ATRIA. Hal F. Yee* and Donald C. Jackson. Brown Univ., Providence, R.I. 02912

The effects of acidosis and calcium on the force and frequency of contraction were studied in the isolated spontaneously contracting atria of the freshwater turtle (Chrysemys picta) at 20°C. The atria were subjected to treatments of lactic, hypercapnic, or chloride acidosis in the presence of both normal (2.0 mM) and high (10.0 mM) [Ca]. In all cases of acidosis, pH was reduced to 6.80 from a control pH of 7.80. All three forms of acidosis significantly depressed contractile force. During lactic and chloride acidosis, a progressive decrease was seen, while during hypercapnic acidosis, a spontaneous partial recovery occurred after an initial drop in force. High calcium during lactic and hypercapnic acidoses significantly moderated the negative inotropic effects of acidosis, although force remained below control values. During chloride acidosis with high calcium, force was not different from the control. Each type of acidosis caused a significant decrease in the frequency of contraction; however, the reductions were not affected by increased calcium. Hypercapnic acidosis affected mechanical activity fastest, while chloride acidosis had the slowest effect. In conclusion, the freshwater turtle may have mechanisms to compensate for the negative inotropic effects of acidosis, and may thereby be able to preserve cardiac function during prolonged diving and accompanying acidosis. (Supported by NSF Grant PCM-82-02419)

40.6

VENTILATION AND ACID BASE BALANCE DURING LACTIC ACID INFUSION IN THE LIZARD VARANUS SALVATOR. G.S. Mitchell and T.I. Gleeson*. Univ. of Wisconsin, Madison, WI 53706 and Univ. of Colorado, Boulder, CO 80309.

Although reptiles rely heavily on anaerobic metabolism and lactic acid production during activity, little is known concerning their ventilatory response to the attendant metabolic acidosis. We measured arterial PCO_2, H^+ and lactate (L) concentrations, and the rates of CO_2 ($\dot{M}CO_2$) and O_2 ($\dot{M}O_2$) exchange in V. salvator (n=9) during intravenous infusions of lactic acid (HL) or sodium lactate (NaL; 250 mM) at rest. Two protocols were used: 1) 15 min infusions of 0.42 ml/min at both 25° and 35°C with measurements every 5 min; 2) 4.5 min infusions of 1.73 ml/min at 35°C with measurements at 4.5 min. At 25°C, the results of HL infusion were: 1) [L]a increased progressively, reaching values as high as 9mM above control, 2) $\dot{M}CO_2$ increased 3.9 fold, 3) R ($\dot{M}CO_2/\dot{M}O_2$) increased 2.3 fold, 4) pHa decreased 0.11 and 5) $PaCO_2$ decreased 1.3 Torr. At 35°C, control pH decreased from its value at 25°C with a slope of -0.005/°C and $PaCO_2$ increased 7 Torr. During slow infusions at 35°C: 1) [L]a increased 6.5 mM, 2) $\dot{M}CO_2$ increased 2.5 fold, 3) R increased 1.6 fold, 4) pH decreased 0.09 and 5) $PaCO_2$ remained unchanged from control. During fast infusions at 35°C: 1) [L]a increased 18.6 mM, 2) $\dot{M}CO_2$ increased 4 fold, 3) R increased 2.2 fold, 4) pH decreased 0.27 and 5) $PaCO_2$ was unchanged. In NaL infusions, only small changes were observed except in [L]a. The results indicate that: 1) $\Delta pH/\Delta T$ in V. salvator is less than other poikilothermic vertebrates, but consistent with other varanid lizards, 2) respiratory compensation is slight in response to acute metabolic acidosis in this species and 3) ventilation follows changes in $\dot{M}CO_2$ rather closely, accounting for precise regulation of $PaCO_2$ despite 4 fold increases in $\dot{M}CO_2$ elicited by bicarbonate buffering and increased metabolic rate.

40.7

INTRA- AND EXTRACELLULAR pH AND TEMPERATURE IN THE BLUE CRAB, Callinectes sapidus. James N. Cameron* and Chris M. Wood* (SPON: J. L. Larimer). Univ. of Texas, Port Aransas Marine Lab., Port Aransas, TX 78373 and McMaster Univ., Hamilton, Canada L8S 4K1.

The blood acid-base status of Blue Crabs acclimated to 10, 20 and 30 C seawater showed patterns similar to water-breathing vertebrates, with a pH/temp. slope of about -0.015. The adjustment was achieved by only small changes in [HCO_3^-] and Pa_{CO_2}. The values of intracellular pH of various muscle tissues, measured with DMO (5,5-dimethyl-2,4-oxazoladinedione) had slopes ranging from -0.014 to -0.016, but the "mean whole body" estimate derived from the total DMO dose distribution was consistently higher than that of individual tissues, and had a temperature slope of only about -0.006. This anomalous "mean whole body" estimate was explained by the discovery of a large fluid compartment in the carapace which had a mean pH of 8.33 at 20 C, and a flat temperature slope. The consequences of this large alkaline compartment for buffering of acute acid-base disturbances, and the acid-base consequences of formation of the carapace during moulting are being investigated. The carbonate pool of the carapace is approximately 600-fold greater than the combined intra- and extracellular total CO_2 pools.

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40.8

GAS EXCHANGE AND ACID BASE STATUS IN HEAT STRESSED DESERT PHASIANIDS. R. Frumkin*, Y. Weinstein*, B. Pinshow and M.H. Bernstein. Blaustein Institute for Desert Research, Ben-Gurion Univ., Sede Boqer Campus 84990, Israel.

To compare respiratory gas exchange and acid base status of two Negev desert avian species, the Chukar (wide spread in mesic and desert areas) and the Sand Partridge (endemic to the desert), we measured $\dot{V}O_2$, $\dot{V}CO_2$, evaporative water loss (EWL) and acid base variables of birds exposed to T_a = 16 - 45°C. From their thermoneutral zones (TNZ) to the highest T_as ($T_a$max) of exposure, T_b in both species increased from 41 to 43°C. Mean SMR of Chukars and Sand Partridges were 1.1 and 1.3 cm^3 O_2 /(g h), respectively. Mean TNZ EWLs were 1.2 and 4.3 mg H_2O/(g h), respectively. At $T_a$max, EWL of Chukars increased 5-fold while that of Sand Partridges increased only 3-fold. Arterial blood PO_2, PCO_2, pH, [Hb] and Hct of both species did not vary significantly with T_a. Arterial lactate concentration in Chukars increased from 0.7 mmol/l at 32°C to 1.8 at 42.3°C while that of Sand Partridges increased from 0.7 mmol/l at 35°C to 1.5 at 42°C. We conclude that while both species used similar mechanisms to tolerate heat stress, Sand Partridges evaporated more water in their TNZ than did Chukars (probably due to higher breathing rates), whereas at high T_as, Chukars evaporated more water for heat dissipation. Close regulation of acid base status by both species may best explain their ability to function at high T_as. Supported by NSF grant PCM 79-21856 and U.S.-Israel BSF grant 2496/81.

40.9

THE INITIATION OF CARDIOVASCULAR ADJUSTMENTS TO DIVING IN REDHEAD DUCKS. Robert A. Furilla* and David R. Jones. Dept. of Zoology, Univ. of British Columbia, Vancouver, BC V6T2A9.

Redhead ducks (Aythya americana) diving voluntarily or submerged forcibly display an immediate bradycardia. This fall in heart rate is unaffected by pre-breathing gases of various oxygen contents when either diving freely or submerged forcibly. The heart rate during the dive is directly proportional to the pre-dive heart rate. This relationship holds for both types of dive suggesting that the depth of bradycardia seen in forcibly submerged redhead ducks is governed primarily by the physiological state of the animal at the time the dive commences and that restraint per se is not an important factor in the expression of diving bradycardia. To test the role of nasal receptors in the cardiovascular adjustments to submersion, Xylocaine in aerosol form was administered into the nares of 7 redhead ducks that were then forcibly submerged. The mean pre-dive heart rate for untreated animals was 92 $\pm$ 2 (S.E.) beats·min^{-1} (bpm) and for Xylocaine treated animals was 98 $\pm$ 3 bpm; however, 2 seconds after submergence the mean heart rates were 36 $\pm$ 4 bpm (untreated) and 96 $\pm$ 4 bpm (Xylocaine treated), and 10 seconds after submergence were 29 $\pm$ 3 bpm (untreated) and 82 $\pm$ 5 bpm (Xylocaine treated). We conclude, therefore, that nasal receptors in redhead ducks play an important role in initiating cardiovascular adjustments to submergence.

40.10

EFFECT OF CO_2 AND pH ON COLLOID OSMOTIC PRESSURE (COP) OF HUMAN BLOOD AND PLASMA, IN VITRO. Christopher S. Ogilvy*, C. Bruce Wenger, and Arthur B. DuBois. John B. Pierce Foundation Laboratory and Yale Univ., New Haven, CT 06519.

COP is influenced by two factors as the CO_2 content of whole blood is increased. One is an increase in COP as fluid is drawn into the red blood cells. The other factor is a decrease in COP due to a change in pH because the pH affects the charge on the plasma proteins. Using a membrane osmometer at 37°C we measured the COP of whole blood (true plasma), or of separated plasma, tonometered to different concentrations of CO_2. The loss of CO_2 from plasma samples in the oncometer was prevented by use of appropriate concentrations of CO_2 in the sample chamber. COP of true plasma increased from 26.3 mm Hg at a pH of 7.40 to 26.5 mm Hg at a pH of 7.30 while the total CO_2 content decreased by 3.4 mM/ℓ. But for separated plasma, the COP decreased 0.2 mm Hg as the pH fell from 7.40 to 7.30. We also measured the change in total osmotic pressure of true plasma and it was similar (about 0.9 mOsm/mM CO_2) to that found by others. We conclude that the change in COP is much less than that reported previously 'in vitro' (Kakiuchi, et al, J. Appl. Physiol. 44:254-257, 1978) though not unlike that 'in vivo' (Kakiuchi, et al, Am. J. Physiol. 236: F419-422, 1979). We also conclude that if COP is used to calculate the amount of fluid that shifts from plasma to red cells during the addition of CO_2 'in vitro', this amount would be underestimated if the effect of pH on COP were neglected. (NIH grant HL 17407).

41.1

EFFECTS OF CARDIAC DENERVATION ON THE RESPONSE TO NALOXONE IN CANINE HEMORRHAGIC SHOCK. R.B. LECHNER*, N.J. GURLL, D.G. REYNOLDS AND M.J. BRODY, Departments of Surgery and Pharmacology, University of Iowa, Iowa City, IA 52242.

The opiate antagonist naloxone improves cardiovascular performance in canine hemorrhagic shock, however it is not known if this is neurally or humorally mediated. The purpose of this study was to investigate the role of cardiac nerves in mediating this response. One week after sham or actual intrapericardial denervation, dogs were anesthetized, instrumented and placed in one of 3 groups: Group I (n=10) sham denervation, Group II (n=10) denervation, and Group III (n=10) denervation plus ß-adrenergic blockade. The dogs were hemorrhaged and held at 45 mmHg pressure for one hour at which time half received saline and half received naloxone (2 mg/kg + 2 mg/kg hr). The differences between the naloxone and saline responses for: mean arterial pressure (MAP, mmHg), maximum left ventricular dp/dt (LV dp/dt, mmHg·10^3/sec), cardiac output (CO, L/min), total peripheral resistance (TPR, mmHg·min/L) and heart rate (HR, beats/min) 30 minutes after treatment were:

Group	MAP	LV dp/dt	CO	TPR	HR
I	28±6	1.1±0.2	0.2±0.1	16±5	-1±4
II	28±3	1.1±0.1	0.3±0.1	10±3	42±6*
III	19±6	0.4±0.1*	0.2±0.1	10±3	2±3

*significantly different (p<.01) from other groups

Cardiac denervation did not attenuate the MAP, CO or LV dp/dt responses. In denervated dogs there was a non-neurogenic, ß-adrenergically mediated increase in HR and LV dp/dt.

41.2

CHANGES IN BETA-ENDORPHIN, ADRENOCORTICOTROPIN AND CORTISOL LEVELS IN THE CONSCIOUS PIG DURING CONTROLLED NONLETHAL HEMORRHAGE. J.D. O'Benar, J.P. Hannon, J.L. Peterson* and C.A. Bossone.* Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

To explore some of the neuroendocrine changes associated with hemorrhage, beta-endorphin, adrenocorticotropin, and cortisol levels were measured by radioimmunoassay before, during, and after controlled bleeding of conscious splenectomized pigs. The bleeding of the animals was accomplished through a chronic indwelling catheter placed in the ascending aorta through the left common carotid artery. Blood pressure and heart rate were also recorded. After 30 minutes of recumbent rest, 3 sets of control samples were taken at 10-minute intervals. Then an estimated 50% total blood volume was removed over a 1-hour period on an exponential scale during which blood samples were taken at 10% intervals. After hemorrhage, 6 more samples were taken during the recovery period as blood pressure returned towards control values. All animals showed significant increases in the three neuroendocrine substances during hemorrhage. Endorphin values initially ranged from 29 to 78 pg/ml and rose to a peak of 263 to 564 pg/ml at the nadir of blood pressure. ACTH showed a similar pattern, increasing from 49±10 pg/ml (mean ±SE) to a peak of 518±56.2. Cortisol values reached their peak of 18.2±2.5 ug% somewhat later during the recovery phase. High negative correlations existed between endorphin and blood pressure for both hemorrhage period and during recovery.

41.3

THE EFFECTS OF DIFFERENT VASOACTIVE MEDIATOR ANTAGONISTS ON HEMORRHAGIC SHOCK IN DOGS, Phillip D. Toth*, Steven A. Hamburger*, Steven Barefoot*, and William V. Judy, Department of Medical Research, Methodist Hospital of Indiana, Inc., 1604 N Capitol Ave., Indianapolis, Indiana 46202.

Opiate and prostaglandin antagonists have both been separately shown to improve hemodynamics and survival in hemorrhagic shock models, no study has examined both types of antagonists in the same model. The present study examines naloxone(N), an opiate antagonist, and fenoprofen(F), a cyclooxygenase inhibitor in the same hemorrhagic shock model. Impedance cardiography and invasive methods were used to measure various cardiovascular parameters. After pentobarbital anesthesia, all dogs (beagles) were bled to and maintained at a mean arterial pressure (MAP) of 60 mm Hg for 90 minutes and then were given either naloxone (2mg/kg) (n=7), fenoprofen (10mg/kg) (n=6), or saline (n=7). After another 90 minutes all the shed blood was reinfused. Both drugs improved MAP secondary to an increase of total peripheral vascular resistance (TPR) ($p < .05$). F had a greater increase in TPR than N. Representative data observed 60 minutes after drug administration are as follows:

	MAP	TPR
Control	62.0 ± 12.4	43.9 ± 6.1
Naloxone	100.0 ± 7.3	62.1 ± 6.0
Fenoprofen	99.6 ± 5.6	81.8 ± 5.1

There were no significant improvements in the other cardiovascular parameters. These data demonstrate that both mediator antagonists are equally effective in improving MAP in this hemorrhagic shock model secondary to an increase of TPR. Further work is in progress to determine the mechanism(s) by which both drugs are improving vascular resistance. (Supported by Methodist Hospital)

41.4

RESPONSES OF THE SKELETAL MUSCLE MICROCIRCULATION TO HEMORRHAGIC HYPOTENSION. M. Boegehold*, I. Torres*, S. House*, R. Baker, E. Bouskela-Torres* and P.C. Johnson. Dept. Physiol., Univ. Arizona Coll. Med., Tucson, Arizona 85724.

The initial response of the cat sartorius muscle microcirculation to acute blood loss and the subsequent changes occurring over a 4-hour period were examined with intravital microscopy using an exteriorized preparation with intact neural and vascular supply. After a 1-hour control period, blood was withdrawn until mean arterial pressure fell to 60 mm Hg, at which it was maintained throughout the experimental period. Vessel diameter and red cell velocity were measured on-line in arterioles (7-87µm i.d.) and venules (12-187µm i.d.) and capillary red cell velocity was measured off-line. Arteriolar diameter changes following hemorrhage were found to be dependent on control diameter. During the first hour after hemorrhage, arterioles larger than 35µm i.d. constricted to a mean of 78% control diameter. Arterioles smaller than 35µ i.d. dilated to a mean of 146% of control diameter. No significant changes in venular diameter were observed. Volume flow fell sharply in all vessels to a mean of 27% of control with no significant differences between groups of vessels. The proportion of capillaries exhibiting red cell flow fell to 26% of control. The dilation of the smaller arterioles in this preparation may be an autoregulatory response to the reduction in pressure and flow following hemorrhage which is augmented locally by constriction of larger upstream arterioles. (Supported by the Claudia Gips Foundation, Inc. and a grant from NIH (HL07249))

41.5

BLOOD FLOW NOT HYPOXIA PRODUCES THE CATECHOLAMINE RESPONSE TO SHOCK. Kevin Turley*, Yee-Phoung Chang*, Michael F. Roizen* and Paul A. Ebert* (SPON:A.M. Rudolph). U.C.S.F., CA 94143

The effect of flow vs hypoxia as the primary stimulus for catecholamine response during the "shock" state has not been investigated. 30 infant lambs aged 2-12 days mean 5.1 days were subjected to deep hypothermia (<14°C). Each was anesthetized using 1.5% halothane in oxygen and pancuronium. Five animals underwent bilateral adrenalectomy. Each lamb was perfused at 100ml/kg and cooled to <14°C. 5 had high blood flow (H) (100ml/kg); 5 low flow (L) (10ml/kg); 5 total circulatory arrest (TCA); 5 upper body arrest (C); and 5 hypoxemia at high flow (N) for 1 hour. The 5 bilateral adrenalectomy had 100ml/kg (A). Venous samples were obtained pre and postintervention and norepinephrine (NE) and epinephrine (E) levels were determined using a radioenzymatic assay. Results demonstrate significant changes in NE and E levels between H and TCA. Minimal changes occurred with C and A as with L and H. Results demonstrate TCA produces a massive rise in NE and E significantly different than the response produced by hypoxemia alone.

	H	TCA	C	A	L	N
NE	-1,749	+9,558*	+30	+1,070	+1,551	745
	±1,497	±4,384	±165	±310	±767	±883
E	-2,579	+13,636*	+180	+308	+2,072	3,348
	±2,013	±9,829	±242	±258	±375	±1,995

Analysis of variance *P<.001

41.6

SELECTIVE SPLANCHNIC VASOCONSTRICTIVE RESPONSE TO DECREASED CARDIAC OUTPUT (CO). G. B. Bulkley and A. Oshima*. Johns Hopkins Medical Institutions, Baltimore, Md. 21205.

We studied the splanchnic vascular response to decreased systemic perfusion in anesthetized piglets. Following 15% hemorrhage, we progressively decreased CO by inducing graded cardiac tamponade with dextran (n= 14), while continuously monitoring splanchnic and systemic hemodynamic parameters. When superior mesenteric artery resistance was plotted against total peripheral resistance, the slope varied between 1.8 and 4.1, always significantly ($p<.01$) greater than 1.0. This demonstrates a disproportionate splanchnic vasoconstriction in response to decreased systemic perfusion. This was abolished by ablation of the renin-angiotensin axis with: 1) angiotensin converting enzyme blockade with teprotide (n=7), 2) competitive inhibition of angiotensin II with saralasin (n= 7), and 3) bilateral nephrectomy (n=7). Sympathetic ablation by perivascular denervation (n=7), α blockade (phenoxybenzamine) (n=8), β blockade (propanolol) (n=7), and $\alpha + \beta$ blockade (n=7) all failed to abolish this effect. In the absence of hemorrhage and tamponade, the exogenous infusion of angiotensin II (n=5) mimicked the selective splanchnic vasoconstriction seen with decreased CO, whereas norepinephrine infusion did not. The disproportionate reduction in splanchnic blood flow seen in response to hypoperfusion is due to selective mesenteric vasoconstriction that is mediated primarily via the renin-angiotensin axis, not the sympathetic nervous system.

41.7

FETAL ENDOCRINE RESPONSES TO SLOW FETAL HEMORRHAGE. Cecilia Y. Cheung and Robert A Brace. Division of Perinatal Biology, School of Medicine, Loma Linda Univ., Loma Linda, CA 92350.

It has been demonstrated that there are multiple endocrine responses to rapid fetal hemorrhage. The purpose of this study was to determine the fetal endocrine responses to a slow graded fetal hemorrhage. Chronically catheterized fetal sheep averaging 130 days gestation were studied 5 days post surgery (n = 6). For the hemorrhage, 4 to 10 ml of arterial blood was removed at 10 minute intervals over a 2.5 hr period. During recovery, 4 ml samples were obtained at hourly intervals 2 to 5 and 24 to 26 hr post hemorrhage. Basal plasma hormone concentrations (± SE) were dopamine (DA), 272 ± 90 (pg/ml); epinephrine (EPI), 64 ± 21 (pg/ml); norepinephrine (NE), 57 ± 9 (pg/ml), prolactin (PRL), 33 ± 12 (ng/ml); vasopressin (AVP), 3.8 ± 1.0 (pg/ml); and plasma renin activity (PRA), 3.2 ± 0.6 (ng/ml/ hr). There were no changes in DA or EPI during or after hemorrhage of 0 to 25% of the initial blood volume. NE increased (43%) for hemorrhages >20% and remained elevated 2 to 5 (145%) and 24 to 26 (99%) hr post hemorrhage. Both PRL and AVP were elevated (60%, 224%, resp.) 2 to 5 hr post hemorrhage if >20% of the initial blood volume was removed. In 5 of 6 fetuses PRA increased in proportion to the severity of the hemorrhage, and remained elevated at 2 to 5 hr for >20% hemorrhages. In summary, these data suggest 1) PRA is the most sensitive hormone in response to slow fetal hemorrhage, and 2) DA, EPI, NE, AVP, and PRL responses to slow hemorrhages of <20% are surprisingly small.

41.8

FETAL CARDIOVASCULAR RESPONSES TO SLOW FETAL HEMORRHAGE. Robert A. Brace and Cecilia Y. Cheung. Div. of Perinatal Biol., Sch. of Med., Loma Linda Univ., Loma Linda, CA 92350.

The effects of rapid hemorrhage on the fetal cardiovascular system has been frequently studied. The purpose of this study was to determine the changes in the fetal cardiovascular variables in response to a slow fetal hemorrhage. Chronically catheterized fetal sheep averaging 130 days gestation were studied 5 days post surgery (n=6). An average of 28% of the measured blood volume gradually was removed over 2.5 hrs. We found that fetal arterial pressure did not decrease during the hemorrhage, was reduced by an average of 5 to 7 mm Hg 2 to 5 hrs after the hemorrhage, and returned to normal 24 hrs post hemorrhage. Venous pressure did not change significantly. The only changes in heart rate occurred 2 to 5 hrs post hemorrhage and averaged +20 bpm. Fetal blood volume returned to control by 3 hrs post hemorrhage and was elevated by 8% 24 hrs after the hemorrhage. Arterial oxygen tension decreased while carbon dioxide tension increased by an average of 2 to 3 mm Hg but neither returned to normal after 24 hrs. Arterial pH decreased by 0.05 units during and after the hemorrhage but returned to normal the next day. These data suggest that slow fetal hemorrhage may induce a discoupling between fetal arterial pressure, heart rate, and blood volume because 1) at the end of the hemorrhage arterial pressure and heart rate were normal when blood volume was reduced, and 2) 3 hrs later arterial pressure was reduced and heart rate elevated when blood volume had returned to normal.

41.9

EFFECTS OF HIGH ENERGY ELECTRON RADIATION ON VENOUS RETURN IN THE ANESTHETIZED RAT. Robert N. Hawkins. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814

In many animal species whole-body radiation produces a shock-like sequalae with an apparent decrease in venous return. To determine the cardiovascular response in rats, 14 chloralose-anesthetized animals were exposed to 10K rads of 14 MeV electrons. Preradiation cardiovascular values were: mean arterial pressure (MAP) 114 ± 4 mmHg, heart rate (HR) 366 ± 13 beats per minute, cardiac output (CO) 117 ± 7 ml/min, total peripheral resistance (TPR) 1.0 ± 0.1 mmHg · min/ml, mean circulatory filling pressure (MCFP) 5.6 ± 0.3 mmHg, and gradient for venous return (GVR) 5.6 ± 0.2 mmHg. Radiation resulted in an immediate but transient decrease in MAP to 67.9 ± 4.5 mmHg. By 1 min postradiation MAP, HR, CO, and TPR were not significantly different from preradiation values whereas MCFP and GVR were significantly elevated ($p < 0.05$) to 7.0 ± 3.0 mmHg and 6.6 ± 0.3 mgHg, respectively. Throughout the remainder of the 60-min postradiation observation period, only MCFP and GVR remained significantly different ($p < 0.05$) from preradiation values. These data suggest that failure to observe radiogenic shock in rats during this time may result from compensatory adjustments in venous return.

42.1

PLANNING FOR LIFE SCIENCES RESEARCH ON A SPACE STATION. Milton Heinrich* and Roger Arno* (SPON: R. E. Grindeland). NASA, Ames Research Center, Moffett Field, CA 94035

Questions of physiological adjustment to weightlessness become more important with the increase in duration and/or frequency of space flights. Plans for a Space Station in the 1990s provide facilities to study both the altered functions which may lead to crew health problems, and the fundamental changes in organisms when "normal" gravity is removed. Important physiological responses to space flight which require long-term study include vestibular changes leading to Space Adaptation Syndrome, fluid shifts to the upper body, cardiovascular deconditioning, and loss of bone and muscle tissue. Fundamental questions include: how do organisms adapt to microgravity; what are the mechanisms of gravity sensing in animals; will an animal embryo develop normally in the absence of gravity, and how does the resulting animal adapt to 1-g? Present Space Station plans include: a vivarium for animals and plants; a centrifuge to provide 1-g for control organisms; a laboratory for specimen manipulation, dissection, sampling, analysis, and preservation; continuous manning; and crew exchange every 90 days. The life sciences community has provided a cross-section of about 100 representative experiments appropriate to the Station. These are being studied to determine facility requirements. The Space Station will offer an opportunity for long-term studies in weightlessness, with sampling of organisms before reexposure to earth gravity.

42.2

THERMOREGULATION IN RATS DURING WARM OR COLD EXPOSURE AT 3 G. C. B. Monson, Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

Previous investigators reported a fall in core temperature, T_c, of rats during the first hour of exposure to acceleration fields (Am. J. Physiol. 221:271, 1971; J. Appl. Physiol. 42:74, 1977). After this initial fall, T_c stabilized at a low level during the next several hours of hypergravic exposure. To test the proposal that rats then regulate T_c at this low level, in this study 22 Long-Evans hooded male rats (464 ± 3.7 g, mean ± S.E.) were first exposed to an ambient temperature, T_a, of either 34 or 14°C; T_c was then monitored for 2 hours (with T_a at 24°C) to determine if T_c returned toward the low reference level. Throughout the experimental trials rats were in a 3 G field (controls were at 1 G). During the 1 hour period of warm exposure, T_c increased from 33.9 ± 0.32 to 36.7 ± 0.35°C. T_c 2 hours after the end of warm exposure stabilized at a value 1.8°C higher ($p < .01$, paired t-test) than at the beginning of warm exposure. Two hours after the termination of cold exposure, T_c was significantly lower (by 1.7°C, $p < .001$) than at the beginning of cold exposure. In 1 G controls, 2 hours after warm exposure T_c was 1.7°C higher than prior to warm exposure (36.3 ± 0.32°C), and 2 hours after the termination of cold exposure, T_c was not significantly different than 36.3 ± 0.32°C. In conclusion, rats in 3 G fields do not maintain core temperature at the same level before and after thermal stress as well as 1 G controls (supported by NASA grant NS G-2234).

42.3

EFFECTS OF RELATIVE HUMIDITY (RH) ON THERMAL CONDUCTIVITY OF FUR IN MACAQUES. Habsah Arshad*, R.L. Coulson, and W.S. Hunter. SIU-C, Medical School, Carbondale, IL 62901.

Macaques use eccrine sweating as a significant means of evaporative heat loss, but insulation by their fur might be expected to reduce the effectiveness of sweating. Since the effect of increased humidity within the air-fur complex (coat) of these animals was unknown, it was hypothesized that variations in relative humidity could induce variation the insulative capacity of the coat. Two samples from each of the freeze-dried pelts of Macaca mulatta and Macaca Fascicularis were sewn to form 0.65 cm I.D. by 38 cm long sleeves and mounted, fur side out, on a copper tube calorimeter. With air temperature (Ta) controlled at 31°C, thermal conductivity (K) was measured at RH=20, 36, and 97% before and after shaving the fur off the skin. The relationship between thermal resistance (Z) and ambient RH is well represented by the hyperbolic equation Z=Ro (Ho + 200-RH)/(Ho + RH). Ro =horizontal asymptote, Ho = vertical asymptote, and $Z = K^{-1}$. The range of K from 0 - 100% RH is 3.25 x 10^{-4} to 3.79 x 10^{-4} (cal/sec·cm²·deg C/cm) and 3.76 x 10^{-4} to 4.46 x 10^{-4} for M. Mulatta and M. fascicularis, respectively. These results indicate that at constant Ta, increasing water vapor content of the coat can signifcantly ($p<0.01$) increase the thermal conductivity of the coat, facilitating heat loss when RH is high, and skin temperature > Ta.

42.4

DEVELOPMENT OF FIXED, PERFUSED ORGANS FOR STUDIES OF TISSUE HEAT TRANSFER (HT), K.R. Holmes, W. Ryan*, P. Weinstein*, and M.M. Chen*. Dept. of Veterinary Biosciences, Dept. of Mechanical and Industrial Engineering, and Bioengineering Faculty. University of Illinois, Urbana, IL 61801

In the examination and modeling of biological HT mechanisms, investigators have often drawn conclusions from measurements made in tissue phantoms such as gels, water filled sponges, or containers of sand or glass beads and water mixtures. Convective HT is presumed to be adequately represented when water is forced thru the sponge or porous bed and the fluid space is taken to represent the tissue vasculature. However, the vascular architecture of real organs is organized, highly structured, and often complex. We (MMC & KRH) have shown (N.Y. Acad. Sci. 335:137, 1980) that the angioarchitecture may contribute significantly to bio-HT mechanisms. Where perfusion contributes to HT, measurements in phantoms are best made in materials possessing an intrinsic system of perfusion channels closely resembling that of living tissue. We have developed such a phantom using rabbit or cat kidney and spleen. After excision from the anesthetized, heparinized animal, the organ vasculature is purged with 4% Mannitol-saline. The organ is then fixed with 80% alcohol. Organ vascular resistance subsequent to rehydration closely approximates that measured in freshly excised tissues, or values reported in the literature. Cycles of fixation/rehydration have been made over several months without significant change in perfusion characteristics.

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42.5

EFFECTS OF RESTRICTED WATER INTAKE ON PERFORMANCE IN A COLD ENVIRONMENT. D.E. Roberts, J.F. Patton, J.W. Pennycook, M.J. Jacey*, D.V. Tappan*, and E. Heyder*. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760 and Naval Submarine Medical Research Laboratory, Groton, CT 06349.

Eighteen male subjects were housed for 10 days in an environmental chamber maintained at 70°F for the first 3 days and then lowered to -10°F for the next 5 days. Subjects received a standard ration containing 4200 calories and either 1.5L (Group 1) or 3.0L (Group 2) of water per day begining on the first cold day. Their daily routine included a standard workload (16 miles/day walking on treadmills with or without packs), psychological testing, weighing (BW), blood and urine collection and either endurance testing (cycle ergometer at 75% at VO_2 max for 30 min) or cold stress testing, which consisted of sitting in a cold chamber (32°F) dressed in cold weather clothing with the right hand bare for 90 mintues. All subjects were slightly dehydrated (2% BW) prior to cold exposure. The weight change for Group 1 was 3.49 ± .35% BW while Group 2 regained most of their initial loss and was down only .14 ± 35% BW. There was no significant difference in the groups ability to perform endurance tests, but Group 1 showed a significant degree of cooling ($p < .025$). These data indicate that exercise can be performed satisfactorily even when subjects are not well hydrated, but their response to environmental conditions is adversely affected. These data indicate that a person can function and remain hydrated on 3.0L H_2O/day even under these severe conditions.

42.6

SHIVER OF THE ANKLE. P. A. Iaizzo*, L. E. Wittmers*, and R. S. Pozos* (SPON: P. C. Royce). Univ. of Minn., Duluth, Sch. of Med., Duluth, MN 55812.

The frequency of pathological ankle clonus is considered to be between 5-7 Hz, and can be elicited by applying a dorsiflexing force to the ankle. A similar oscillation in normal subjects has readily been initiated by cooling the subject. To determine if this clonic oscillation could be considered "shiver," the metabolic rates of the subjects were determined before and during the cold exposure. Seven normal adults, who passed EKG and stress tests, were seated in a 0°C environmental chamber for a period ranging from 15-60 minutes until intense oscillations occurred. Throughout each experiment several parameters were recorded: acceleration of the leg, electromyograms (EMG's) from four muscles, respiratory gas partial pressures, respiratory rate, and core temperature. The data was then analyzed by a MINC-11 computer at various stages of the experiment. At the onset of large clonic-like oscillations of the ankle (acceleration values > 1,000 g/sec^2), an average increase in metabolic rate of approximately 30% was observed. Such an increase is sufficient to define this large amplitude oscillation as shiver of the ankle. This oscillation has frequency and amplitude characteristics similar to pathological clonus and may prove to be a useful model of this motor disorder. (Supported by Sea Grant; #DOC/NA82AA-D-00039)

42.7

DOES SOCIAL INTERACTION INFLUENCE SLEEP-WAKE ACTIVITY DURING SMALL GROUP CONFINEMENT IN A CONSTANT ENVIRONMENT?
Daniel C. Holley, Charles W. DeRoshia*, Keith Ogawa*, Kevin Winterfield*, and Charles M. Winget. Dept. of Biological Sciences, San Jose State University, San Jose, CA 95192, and NASA-Ames Research Center, Moffett Field, CA 94035.

Two groups (N and S) of three male subjects (S), 20-24 years old were maintained in confined and socially attenuated rooms (size=3.4X5.2m) for 105 days (d). A third group (n=3) served as ambulatory controls and lived at the test center under 16L:8D conditions. Urine volume and electrolytes, rectal temperature (RT), and heart rate were continuously monitored. Performance on a flight simulator and psychiatric interviews were done daily. In addition various objective psychological questionnaires were periodically administered. At the beginning of the last lighting regimen (L:L) on d84 an individual from groups N and S were exchanged. The RT and sleep/wake rhythms (period and phase) of the 3 S (group N) exhibited parallel changes during the first 15d, indicating relative coordination or control by social interaction. Hostility and depression increased and the need for interpersonal relationships decreased during this time. In the last 7d (group N) the exchanged S's rhythms desynchronized from the rhythms of the other two S's who phase delayed, but remained mutually phase locked. After group desynchronization the circadian rhythms of the exchanged S internally desynchronized. These data imply a role for social interaction as a modulator of circadian rhythm stability in confined groups.

42.8

ALTERATIONS IN CIRCADIAN RHYTHMS IN CELL DIVISION OF THE MOUSE GASTROINTESTINAL TRACT AFTER BILATERAL SUPRACHIASMATIC NUCLEAR LESIONS (SCN). J. N. Pasley, E. W. Powell*, T. H. Tsai*, Depts of Physiology and Anatomy, Univ. Ark. Med. Sci., Little Rock, Arkansas 72205.

The effect of SCN ablation (SCNA) was examined on rhythms that characterize the incorporation of (^{3}H)-TdR into DNA in various regions of the mouse gastrointestinal tract. Thirty-five day old female $BD2F_1$ mice were caged in groups of 6-7 mice each. Four groups each were placed in 8 separate isolation chambers maintained at 23° C and were standardized to LD 16:8 and received food and water ad libitum. After one week the animals underwent bilateral stereotaxic lesions of the SCN area or sham-operation. After operation, mice were caged as before with one cage of 7 sham-operated mice in each isolation chamber. After 45 days the animals were killed in groups that represented 7 time points over a single 24 hr span. After fixation of the carcass in 10% buffered formalin a standard size piece of tissue was obtained from tongue, esophagus, stomach (gastric portion), colon and the DNA extracted. DNA labeling in the gastrointestinal tract of SCNA mice with verified lesions was characterized by an acrophase advance in all tissues and an amplitude reduction in the esophagus, tongue with an amplitude increase in stomach and colon compared to controls. The overal mesor was increased also in the stomach and colon in SCNA mice. The results further confirm our postulated role of the SCN area as a phase resetter for circadian rhythms.

42.9

POSTBURN METABOLISM IN THE RAT. D.R.Strome,* L.H.Aulick, A.D.Mason,Jr.* and B.A.Pruitt,Jr.* U.S.Army Institute of Surgical Research. Ft. Sam Houston, TX. 78234.

Burn patients are hypermetabolic in their thermoneutral zone. The burned rat has been used as a model without clear evidence that it is hypermetabolic at thermal neutrality. Male rats (500 gm; n=39) were placed as a group in a respiration chamber and metabolic rate was determined over 3-6 hr at ambient temperatures of 9-36 °C. Rectal temperatures (T_{re}; °C) and body weights were measured after each run. Metabolic rate at thermal neutrality (MR; W/m^2), lower critical temperature (LCT) and thermal conductance at 20 °C (k; W/m^2·°C) were determined on the rats as normals, after shaving and following 50% total body surface scald burns.

	MR	LCT	T_{re}	k
NORMAL	42.6 ± 0.5	27.5	36.6 ± 0.1	3.36
SHAVED	42.0 ± 0.8	30.0	36.6 ± 0.1	4.47
BURNED	47.2 ± 0.6*	32.5	36.9 ± 0.1*	5.33

Both shaving and burning created insulative deficits. Only in the shaved group could this deficit be offset by environmental heating. In burned animals T_{re} and MR remained elevated and constant at temperatures above the LCT. This extra heat production must reflect the basic metabolic cost of injury since thermoregulatory demands on metabolism are eliminated in the thermoneutral zone. Although the responses of burned rats mimic those of patients, the limited increase in MR after burn restricts the utility of the model. (*=p<0.05 burn vs. normal.)

42.10

POSTBURN HYPERMETABOLISM IN THE PIG. Louis H. Aulick and Arthur D. Mason, Jr.* USA Institute of Surgical Research, Fort Sam Houston, TX 78234.

Research in postburn hypermetabolism is hampered by the constraints of patient studies and the limited response of small animal models. To test the utility of a large animal model, metabolic and temperature measurements were performed on three, 40-80 kg pigs before and for three weeks after a 25% total body surface burn. Unrestrained, postabsorptive animals were studied overnight in a respiration chamber at temperatures from 10° to 35°C. Resting metabolic rate at thermal neutrality (25° and 30°C) increased from 69.5 ± 1.0 to 84.8 ± 2.7 W/m^2 (mean ± SE, $p < 0.001$) after injury. Peritoneal temperature was elevated only during the first week postinjury. Core-to-air thermal conductance increased, and lower critical temperature remained unchanged during the next two weeks. Postburn hypermetabolism was not reduced by raising ambient temperature to 35°C. This, plus the absence of fever, suggests that this large animal response, like that in the human, was not temperature dependent. The pig response exceeds that of small animals with the same size injury but is less than half that of humans. The limited metabolic response and absence of a sustained fever reduces the utility of this model.

42.11

EFFECTS OF CONTINUOUS WAVE AND PULSED RADIOFREQUENCY RADIATION UPON THERMAL RESPONSES IN RATS. M.R. Frei*, J.R. Jauchem* and F. Heinmets* (SPON: A.E.U. Edisen). Trinity Univ., San Antonio TX 78284 and Technology Incorporated, San Antonio TX 78216.

The high peak power density of pulsed radiofrequency radiation (RFR) has led to speculation that it may cause greater biological effects than continuous wave (CW) RFR. The present study investigates the effects of these two types of RFR upon thermal responses in rats. Ketmaine-anesthetized female rats were exposed in an alternating fashion to 2.8 GHz pulsed and CW RFR at average power densities of 60 mW/cm^2 as measured by a Narda #8616 EMR monitor and #8623 isotropic probe (specific absorption rate ~16 W/kg). Pulses were 2 μs in duration at a repetition rate of 500 pps. During intermittent exposures which produced 1°C colonic temperature cycles (between 38.5 and 39.5°C), CW irradiation resulted in significantly ($p < 0.001$) less time [7.2 ± 0.2 min (mean ± SEM); N = 6] required to achieve a 1°C increase than pulsed RFR (8.9 ± 0.2 min). No significant difference was noted in the time required to recover to the initial temperature upon cessation of CW or pulsed irradiation; 11.5 ± 1.0 min and 11.5 ± 0.9 min, respectively. The data suggest a possible difference in energy deposition in animals exposed to CW and pulsed RFR of equivalent average power densities or a difference in the average power densities as measured by currently accepted methods. Results of experiments to resolve this issue will be reported. (Performed at USAF School of Aerospace Medicine, Brooks AFB, TX 78235; supported by USAF Contract No. F33615-80-C-0614.)

42.12

TERMINAL EXPOSURE TO RADIOFREQUENCY RADIATION (RFR): INCREASED SUSCEPTIBILITY DUE TO CHLORPROMAZINE. J.R. Jauchem*, M.R. Frei* and F. Heinmets* (SPON: A.E.U. Edisen). Technology Incorporated, San Antonio, TX 78216 and Trinity University, San Antonio, TX 78284.

Our previous studies have shown that chlorpromazine (CPZ) enhances thermoregulatory efficiency during intermittent RFR exposure when colonic temperature (Tc) is not allowed to rise above 39.5°C. The present experiments were performed to assess the effect of acute CPZ on terminal RFR exposure in ketamine-anesthetized female Sprague-Dawley rats. Tc was monitored with a Vitek 101 temperature probe. Animals were exposed in the H-orientation to continuous wave RFR at a frequency of 2.8 GHz, average power 60 mW/cm^2, specific absorption rate ~16 W/kg. Starting at a Tc of 38.5°, exposure was performed until termination (cessation of respiration). The Tc's at which death occurred were similar in both saline- (43.1 ± 0.1°; mean ± SE, N = 6) and CPZ-treated (42.7 ± 0.3°; N = 6) rats. Survival time in animals treated with CPZ (22.6 ± 1.1 min) was significantly ($p < 0.05$) less than in saline-treated rats (32.7 ± 1.2 min). The results indicate that although CPZ enhances thermoregulatory efficiency during intermittent RFR exposure below a Tc of 39.5°, CPZ increases susceptibility to terminal RFR exposure. Similar temperature-dependent effects of CPZ have been observed by other investigators during environmental heat exposure. (Performed at USAF School of Aerospace Medicine, Brooks AFB, TX; USAF Contract #F33615-80-C-0614.)

42.13

SUBMERSION HYPOTHERMIA IN DOGS. F. G. Hempel, L. E. Wittmers, R. Dromeshauser*, D. Howard*, and R. S. Pozos. Office of Naval Research, Arlington, VA 22217 and Department of Physiology, Sch. of Med., Univ. of Minn., Duluth, MN 55812.

The bradycardia and apnea which characterize the "dive reflex" are thought to have survival value during total immersion in cold water. It has not been determined whether the aspiration of cold water into the lungs during immersion may be even more protective by rapidly cooling the brain and heart, and blood in the major vessels. For this reason we investigated the rate at which the aspiration of fresh water at 10°C cooled the shallow brain (sb), deep brain (db), left ventricle (lv), rectum (rec), esophagus (eso), and subcutaneous space (scn) of dogs after whole body submersion.

Male and female dogs were anesthetized with sodium pentobarbital, and the electrocardiogram and arterial blood pressure were recorded. Spontaneously breathing dogs were submerged and allowed to aspirate water for 4 minutes. An average temperature drop observed in all animals was: 4.6°C (db), 4.2°C (sb), 5.9°C (lv), 2.3°C (rec), and 10.0°C (scn).

We have found that the inspiration of cold water rapidly cools the heart and brain, at a rate of 1.2°C/minute, and have concluded that rapid cooling of these organs may explain the exceptional survival of individuals who have been accidentally submerged in cold water for long periods.

Supported in part by Minnesota Sea Grant Number DOC/NA 82AA-D-00039.

42.14

Diet Induced Hyperphagia Decreases Thermic Responses of Hamster Adipocytes In Vitro. Richard J. Schimmel and Linda McCarthy*. Rutgers Medical School, Piscataway, NJ 08854.

Thermogenesis in brown adipose tissue is increased in animals consuming a diet supplemented with palatable food items (cafeteria diet). To study the mechanisms by which thermogenic capacity of brown fat is increased during cafeteria feeding, in vitro respiration of brown adipocytes from hamsters given a cafeteria diet was measured and compared with that of adipocytes from animals eating a chow diet. Oxygen consumption was measured polarigraphically. Basal respiration in adipocytes from cafeteria-fed hamsters was slightly less than that of cells from control animals, and stimulated respiration by adipocytes from hamsters fed a cafeteria diet was only 40-50% of the rate in cells from control animals. This difference in respiration was present when the stimulus was isoproterenol, 3-isobutyl-1-methyl xanthine or forskolin. The reduced thermogenic responses of brown adipocytes prepared from cafeteria-fed hamsters was reversed when the animals were returned to the standard diet. Because, diet induced thermogenesis is associated with increased norepinephrine turnover in brown fat, we suggest that the diminished in vitro responses of brown adipocytes from hyperphagic hamsters results from the prolonged sympathetic stimulation preceeding their isolation. We further suggest that the increased brown fat thermogenesis during cafeteria feeding may be a consequence of adipocyte proliferation.

42.15

REGULATION OF BROWN ADIPOSE TISSUE THERMOGENESIS BY ADENOSINE AND ANTILIPOLYTIC AGENTS. Ludwik J. Bukowiecki and Dieter Szillat*. Laval University, Medical School, Dept. Physiology, Quebec, P.Q., Canada G1K 7P4.

Nanomolar concentrations of adenosine competitively inhibited the stimulatory effects of the beta adrenergic agonist, (-)-isoproterenol, both on lipolysis and respiration in hamster brown adipocytes. The low value of the apparent k_i for respiratory inhibition by adenosine (7 nM) indicated that the nucleoside may control brown adipocyte function under physiological conditions. Significantly, the dose-response curves for isoproterenol -stimulation of lipolysis and respiration were both shifted by adenosine to higher agonist concentrations by the same order of magnitude, providing additional evidence for a tight coupling between lipolysis and respiration (Bukowiecki, L. et al. (1981) J. Biol. Chem., 256 (24): 12840-12848). The inhibitory effects of adenosine were rapidly reversed by (a) transforming extracellular adenosine into inosine with adenosine deaminase, (b) adding agents known to increase intracellular cyclic AMP levels (isoproterenol, iso-butyl-methylxanthine, dibutyryl cyclic AMP), and (c) directly stimulating respiration with palmitic acid, thus bypassing the early metabolic steps associated with activation of adenylate cyclase and lipolysis. These results combined with the fact that adenosine failed to affect respiration evoked either by dibutyryl cyclic AMP or by palmitic acid, stongly indicate that adenosine regulates brown adipose tissue respiration by inhibiting lipolysis via a direct effect on the adenylate cyclase complex. The observation that antilipolytic agents other than adenosine, such as adrenergic blockers (propranolol, alprenolol), insulin, prostaglandins E1 and E2, all inhibited respiration, suggests that lipolysis represents the "flux-generating step" modulating brown adipose tissue thermogenesis. (Supported in part by the Medical Research Council of Canada).

42.16

CIRCADIAN HEMODYNAMICS IN THE UPRIGHT SLEEPING MONKEY: NOCTURNAL SYMPATHETIC NERVOUS COMPENSATION TO MAINTAIN CENTRAL BLOOD VOLUME? Bruce M. Halpryn*, Frank Sulzman, David Murrish, and Harold Sandler. NASA/Ames Research Center, Moffett Field, Ca. 94040 and S.U.N.Y. @ Binghamton, Binghamton, N.Y. 13901

Thirteen male Macaca mulatta monkeys were instrumented to allow chronic monitoring of heart rate, electrocardiogram, arterial blood pressure, cardiac output, and rectal temperature. Total peripheral resistance and stroke volume were calculated. Heart rate and rectal temperature were higher during the day than during the night. Cardiac output showed no statistical difference between day and night values. Blood pressure, total peripheral resistance and stroke volume were higher at night than during the day (the opposite of what has been documentated in other diurnal mammals). This demonstrates an increased sympathetic nervous tone to the vasculature at night. Unlike most other diurnal animals, these monkeys do not assume a horizontal position at night, they remain upright. Gravity is acting on the upright monkey to cause blood pooling in the legs. This pooling is counteracted by the skeletal muscle pump during the day. We believe that nocturnal vasoconstriction is a compensatory mechanism to counteract blood pooling, and maintain nocturnal cerebral blood flow. This research sponsered in part by NASA grant NGT-331-888-00

43.1

BIOLOGICAL ACTIVITY OF COMPONENTS OF CHOLECYSTOKININ(CCK)-OCTAPEPTIDE ISOLATED FROM HUMAN BRAIN. C.W. Deveney,A. Wong,L. Way, V. Eysselein,J. Reeve Jr.,J.H. Walsh*, and H. Sankaran. CURE, Los Angeles,CA and Surgical Service,Veterans Administration Medical Center,San Francisco,CA 94121.

We investigated the bio- and immunoreactivities of two components of CCK_8 isolated from the human brain. Affinity chromatography and High Performance Liquid Chromatography of the brain extracts yielded two distinguishable components (1) a CCK_8 component coeluting with synthetic sulfated CCK_8 and (2) an oxidized form of CCK_8. Both forms reacted identically with antisera specific to carboxy terminal CCK_8. The biological activities of the two forms tested in vitro on isolated mouse pancreatic acini and mouse brain particulate preparation are as follows:

	CONC. OF CCK_8 REQUIRED FOR:		
FORMS OF CCK_8	MAX. AMYLASE RELEASE FROM ACINI	50% INHIBITION OF CCK BINDING: ACINI	PARTICULATE
synthetic	100 pM	300 pM	1-2 nM
nonoxidized	60-100 pM	300 pM	1-3 nM
oxidized	$>$1 nM	$>$3 nM	$>$3 nM

Conclusion: These data demonstrate that the two forms of CCK_8 isolated from the human brain exhibit identical immunoreactivities; however there are significant differences in their biological potencies to elicit appropriate responses from the target tissue. It also is clear that the brain particulate receptors, unlike acinar receptors, do not exhibit a significant difference in their binding characteristics to either the oxidized or nonoxidized form of CCK_8.

43.2

SERUM GASTRIN, PEPSINOGEN I AND ACID OUTPUT IN MALE AND FEMALE CHILDREN. E.Molina, G.Banchini*, G.Gregori*, G.L.de Angelis*, C.Ghinelli*,C.Zanacca*and S.Scuto*. Hospital and University of Parma, Italy.

We studied 20 healthy children, 10 males and 10 females with same age and weight (mean 8.8 yrs.and 30 Kg. respectively): the gastrin release was stimulated by a protein meal (hamburger and a glassful of milk) and the fasting serum gastrin and pespinogen I were measured.

RESULTS

	MALE (n-10)	FEMALE (n-10)
GASTRIN (pg/ml)		
1) Basal	67.5 $\pm$ 7.3	45.3 $\pm$ 6**
2) Peak after food	115.0 $\pm$ 22.2	82.0 $\pm$ 9**
PEPSINOGEN I (ng/ml)		
1) Basal	48.7 $\pm$ 3	37.9 $\pm$ 3**

The results are expressed as MEAN $\pm$ S.E.M. (**$p < 0.01$).

CONCLUSIONS: The fasting serum gastrin and the gastrin after a protein meal levels are statistically higher in male children ($p < 0.01$). The fasting serum pepsinogen I levels are also significantly lower ($p < 0.01$) in females that in male. This critical difference in the endogenous part of the stomach between males and females should be considered in the evaluation of gastric secretory tests (i.e. D.U.'s versus normals).

(Supported in part by a grant from C.N.R. - Rome).

43.3

VARIATIONS IN BASAL, FOOD AND BOMBESIN-STIMULATED LEVELS OF GI PEPTIDES IN DOGS OVER EIGHT HOURS. P. L. Rayford, K. Inoue and D. McKay*. Dept. Physiology and Biophysics, Univ. Ark. Med. Little Rock, AR 72205

This study reports plasma levels of gastrin (G), cholecystokinin (CCK) and pancreatic polypeptide (PP) in dogs over a 8 hr duration. Methods: Dogs were fasted for 18 hr. Four blood samples were collected each 10 min for 30 min (control). Six dogs received only water (basal study), 16 dogs received food (food study) and 6 dogs received a 1 hr infusion of 1µg/kg-hr bombesin (BBS study). G,CCK and PP (pmol/L) were measured by radioimmunoassay. Results: Mean control levels were 10.9±1.4 for G, 10.8±0.7 for CCK; and 39.9±6.4 for PP. In the basal study, G and PP were not altered for 8 hr; CCK decreased at 120,180,210 and 240 min. In the food study, G was 30±3 and PP was 644±101 at 5 min; each remained significantly elevated over control throughout the study, PP showed a biphasic response. CCK increased significantly at 30,90,120,180,210 and 240 min and thereafter was not different from basal. In the BBS study, G was 20±1.2 at 5 min, CCK was 15±1 and PP was 122±5 at 10 min; G and PP remained significantly elevated for 60 min and CCK for 30 min after BBS was stopped. Conclusion: Decreases in levels of CCK in the basal study suggest that an 18 hr fast is not sufficient to lower CCK to basal levels. The prolonged rises in G and PP after food indicate that food is a long acting and potent stimulus of these peptides. The relatively fast decline in peptide levels after BBS suggest that BBS, though potent, is rapidly catabolyzed. Supported by NIH AM30415-01.

43.4

PROSTAGLANDIN PREVENTS DECREASE IN ETHANOL-INDUCED NON-PROTEIN SULFHYDRYL LEVELS IN CANINE GASTRIC MUCOSA. L.L. Shanbour, Y.-J. Kuo*, D. Li* and T.A. Miller. Depts. of Physiology and Cell Biology and Surgery, Univ. of Texas Med. Sch., Houston TX. 77025

Previous studies with the rat stomach have suggested that gastric mucosal nonprotein sulfhydryl compounds may play a role in mediating prostaglandin cytoprotection (Sci. 214: 200-202, 1981). To determine whether this same action exists in canine mucosa, the dog-flap preparation in which the blood supply is maintained intact and the gastric mucosa partitioned into two halves was the experimental model for the studies. The gastric mucosa was exposed to topical ethanol (40%) and/or to 16,16 dimethyl prostaglandin E_2 (1 ug/ml). The tissue nonprotein sulfhydryl levels (mmole/100 gm tissue) when compared with paired control (saline) mucosa were decreased to 35 $\pm$ 6% by ethanol and increased to 132 $\pm$ 5% by PGE_2. Pretreatment with PGE_2 prevented the decrease in nonprotein sulfhydryl levels produced by ethanol (102 $\pm$ 21% as compared with matched control tissue). Prostaglandin E_2 induced maintenance of tissue nonprotein sulfhydryl levels even in the presence of the potent damaging agent ethanol may be one of the mechanisms of its cytoprotective action. (Supported by NIH grants AM 2583804 and AA 00194-11).

43.5

EFFECT OF STIMULATION IN THE MEDULLA OBLONGATA ON GASTRIC ACID SECRETION IN THE CAT. M.S. Armush*, C.F. Nassar, S.K. Agulian* and S.J. Jabbur*. Dept. of Physiology, American University of Beirut, Lebanon.

The effect of localized electrical stimulation within the medulla oblongata on gastric secretion was investigated. A pH-sensitive glass electrode inserted into the gastric antral region through a fistula recorded immediate acid concentration changes. In cats anesthetized with pentobarbital sodium (35-40 mg/kg, i.p.), electrical stimulation with a coaxial electrode (train of pulses at 300-500 Hz for 10-30 sec; individual pulse width 0.1-0.5 msec and amplitude not exceeding 0.5 mA) induced an increase in gastric acid secretion equivalent to 1.34±0.57 units. In decerebrate cats, the same type of stimulation elicited a more intense gastric juice secretion (3.13±1.85 units). The latency of these responses ranged from immediate to a few seconds. Reversible blockage of the vagus nerves eliminated these responses. Our results indicate that electrical stimulation in the posterior region of the medulla oblongata evokes a significant increase in gastric acid secretion which is mediated through the vagus nerve.

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43.6

CORTICOSTERONE (B) DOSE EFFECTS ON NEUROTRANSMITTER ENZYME ACTIVITIES AND HISTOLOGY OF THE RAT GI TRACT. M.M. Heitkemper, J. Shaver* and M.J. Cowan.* Physiological Nsg., Univ. of Washington, Seattle, WA 98195

Previous experiments demonstrated that chronic exposure to a low dose of B, which slightly elevated basal plasma B levels, produced increases in acetylcholine esterase (AChE) and monoamine oxidase (MAO) which synthesize and degrade acetylcholine and norepinephrine, respectively, in the rat GI tract. To determine if this effect is dependent upon the basal level of B, 3 doses of B were administered to male, Sprague-Dawley, 21 d old rats for 14 d. Rats in the B treated (BT) group received 50, 100 or 200 ug/ml of B in their drinking water. Non-treated (NT) and BT groups were sacrificed at 35 d of age and AChE and MAO activities assayed in 4 GI segments (fundus, duodenum, ileum, colon) as well as adrenal and plasma B level determinations. The BT rats exhibited dose-related increases in plasma B levels, decreased adrenal weights and adrenal B content, along with increased AChE and MAO activities in most segments examined. In order to investigate concomitant structural changes, segments of pylorus from 3 NT and 7 BT rats were examined histologically. Selective smooth muscle cell necrosis of muscularis externa was evident in 6 of 7 BT rats. Elevated plasma B levels for two weeks can produce increases in neurotransmitter degradative enzyme activities as well as pyloric structural alterations in the rat GI tract.

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43.7

NERVOUS CONTROL OF THE EXOCRINE PANCREAS IN THE GUINEA-PIG. J.S. Davison* and Val Dickson* (SPON: F. Lorscheider). Univ. of Calgary, Calgary, Alberta, Canada. T2N 1N4

Adult guinea-pigs were anesthetized with urethane or pentobarbital and pancreatic secretion collected from a cannula inserted into the pancreatic duct. Stimulation of the cervical vagus (10Hz.10V.0.5ms.15min) caused a brisk flow of pancreatic juice and an increase in the output of pancreatic amylase. The increased amylase output was associated with a rise in amylase concentration in the pancreatic juice. Electrolyte analysis of the vagally induced secretion showed a rise in [HCO_3^-] and a fall in [Cl^-] comparable to that evoked by secretin (1µg IV). Atropine (1-10µg/g/IV) produced a significant depression of amylase output by about 30% but was without effect on fluid and electrolyte secretion. Fluid and amylase responses were blocked completely by hexamethonium (>7mg/Kg IV). It appears therefore that the secretomotor nerves of the guinea-pig pancreas can be activated by preganglionic vagal fibres via conventional nicotinic synapses. These fibres can activate acinar cells causing enzyme secretion and duct cells causing fluid and HCO_3^- secretion. The duct cells appear to be entirely under the control of noncholinergic nerves whereas acinar cells are controlled by cholinergic and noncholinergic nerves. The degree of reduction of amylase output by atropine correlates well with the proportion of cells receiving a functional cholinergic innervation as revealed by electrophysiological studies, implying that cholinergic and noncholinergic fibres each innervate a different population of acinar cells.

43.8

ACTION OF ETHANOL ON ISOLATED PANCREATIC ACINI. M. Lewin,H. Sankaran,A. Wong,C.W. Deveney,M. Wendland, and M.C. Geokas* Departments of Medicine,Veterans Administration Medical Center, Martinez,CA;University of California,Davis, and Department of Surgery,Veterans Administration Medical Center,San Francisco, CA;University of California,San Francisco,94121.

Ethanol has been shown to affect various biochemical processes in the cell. In the pancreas, the action of ethanol is not conclusive. We employed isolated rat pancreatic acini to study the action of ethanol on basal and cholecystokinin(CCK)-induced enzyme secretion and on binding of CCK to acini. In isolated acini, the CCK8-amylase dose response curve, in the presence of 600 mM ethanol, was identical to that of control, but the amylase response was diminished by over 30%. Amylase release induced by maximal concentration of CCK8(300 pM) was inhibited by ethanol and this inhibition was concentration(0.3-1.3 M ethanol)-dependent. Basal amylase release was increased 50% by ethanol between 0.3-1.3 M; whereas greater than 1.3 M concentrations caused a greater enhancement of amylase release both in the absence and presence of 300 pM CCK8. ^{51}Cr release from prelabeled acini reveal that there was significant cell membrane damage to acini treated with greater than 1.3 M ethanol, thereby suggesting leakage of secretory proteins. Ethanol, also in a dose-dependent manner, inhibited the binding of ^{125}I-CCK to specific membrane receptors on acini. Both of the inhibitory effects of ethanol are reversible on washing and reincubating ethanol-treated acini. These data suggest that 600 mM ethanol inhibitory action is a transient membrane effect.

43.9

ACTION OF ACETALDEHYDE ON ISOLATED PANCREATIC ACINI. WENDLAND, M.,LEWIN,M.,WONG,A.,DEVENEY,C.W.,SANKARAN H.,LEIMGRUBER,R.M., AND GEOKAS,M.C* Departments of Medicine,V.A. Medical Center, Martinez,CA;University of California,Davis, and Department of Surgery,V.A. Medical Center,San Francisco,CA;University of California,San Francisco, 94121.

Acetaldehyde, a metabolite of ethanol, is known to affect various cellular processes. We investigated the effect of acetaldehyde on basal and cholecystokinin(CCK)-induced enzyme release and on the binding of ^{125}I-CCK to receptors on isolated rat pancreatic acini. Basal amylase release was inhibited up to 37% by 6-100 mM acetaldehyde, whereas greater than 100 mM concentrations caused an increase in amylase secretion. In the presence of 45 mM acetaldehyde(half maximal inhibitory dose), the shape of the dose-response curve for CCK8-induced amylase release was the same as that of the control, but the amylase release was inhibited by 50%. Increasing concentrations of acetaldehyde(6-300 mM) inhibited, in a dose-dependent manner, amylase release elicited by maximal concentration of CCK8(300 pM). Acetaldehyde inhibition of ^{125}I-CCK binding was observed between 100 mM and 1 M, with half maximal inhibition at 300 mM. However, no correlation between the two inhibitory actions of acetaldehyde could be established. There was no significant cell membrane damage to acini by acetaldehyde in the concentration range of 10-300 mM; greater than 300 mM caused significant damage to the acinar cell membrane. These results suggest that acetaldehyde inhibition of CCK8-induced enzyme release is not a membrane effect, but may be intracellularly mediated.

43.10

IMPAIRED AMYLASE RELEASE FROM PANCREATIC ACINI DURING ACUTE PANCREATITIS INDUCED BY HIGH DOSE OF CAERULEIN. L. Larose* J. Morisset, J. Wood* and T.E. Solomon. Centre de recherche sur les mécanismes de sécrétion, Sherbrooke Univ., Québec, Canada and Truman VA Hospital, Columbia, MO, 65201.

These studies were performed to evaluate possible damages to the pancreatic secretory function during development of acute pancreatitis in rats. Caerulein, 12 µg kg^{-1} was given S.C. thrice a day for 2 days. Control and treated groups were killed 4 h after the 1st, 2nd and 3rd inj. and 8 h after the 6th inj. Pancreas were removed, acini prepared and amylase release in response to carbachol 10^{-7} -10^{-4}M measured. Acini amylase conc. drop progressively during treatment by 28% after 1 inj., 52% after 2 inj., 58% after 3 inj. and 75% after 6 inj. These data indicate a progressive deterioration of the pancreatic tissue. Secretory studies: Basal amylase release (% total content) was not affected during treatment. Maximal amylase outputs (U/DNA) in response to carbachol were progressively decreased by 45% after 1 inj. to 85% after 6 inj. and paralleled changes in acini amylase conc. Maximal amylase secretion (% total content) was also reduced with a maximum decrease of 40% reached 4 h after 2 inj. Amylase release in response to carbachol shows major shifts to the right in the dose response curves with a maximum effect 4 h after 2 inj. The secretory response to caerulein seems likewise affected. Thus development of acute pancreatitis results in severe modifications in acini secretory responses to secretagogues.
(Supported by MRC of Canada MT-7320)

43.11

EFFECT OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) INFUSION ON CARDIOVASCULAR FUNCTION IN MAN. G.J. Krejs,* L.L. Frase,* F.A. Gaffney,* and C.G. Blomqvist.* (SPON: J.M. Lipton). Univ. of Tex. Hlth. Sci. Ctr., Dallas, Texas 75235

Circulatory disturbances such as hypotension and flushing are occasionally observed in patients with pancreatic cholera syndrome. In this study cardiovascular function was assessed in healthy man during constant intravenous VIP infusion (400 pmol/kg/h for 100 min). VIP infusion caused flushing in all 6 subjects. A marked reduction in total peripheral resistance was associated with increased forearm blood flow (control [C]: 1.7, VIP: 4.4 ml/min/100 g tissue; all numbers are mean values). To maintain systolic pressure heart rate (C: 74, VIP: 94 beats/min) and cardiac output (C: 6.7, VIP 8.6 l/min) increased while stroke volume remained unchanged for 60 min (C: 92, VIP 93 ml). Late in the VIP infusion period cardiac output fell (7.7 l/min) due to a decrease in stroke volume (81 ml). Arterial pressure was maintained by tachycardia. The fall in stroke volume indicates a progressive decrease in venous return. Simultaneous decreases in both arterial and venous tone would explain this finding suggesting that VIP is both an arterial and venous vasodilator. The immediate return of total peripheral resistance to normal after the infusion with a sustained decrease in venous return (stroke volume during 60 min after VIP infusion: 70 ml) suggests that the time course of action of VIP on the arterial and venous capacitance beds is different.

43.12

COMPARATIVE EFFECTS OF WHEAT BRAN DIETS ON NIBBLING (N) AND MEAL-EATING (ME) RATS. Stanley T. Omaye* and Faye I. Chow* (SPON: John P. Hannon). Nutrients Research Unit, USDA, ARS, Western Regional Research Center, Berkeley, CA 94710.

Semipurified diets containing 5% or 20% wheat bran (WB) were fed ad libitum (N) or for restricted 2-hour periods daily (ME) to male Sprague-Dawley rats for 35 days. The basal diet was a modified AIN-76 formulation, substituting Hard Red Spring WB (30 Mesh, Certified by Amer. Assoc. Cereal Chemists) for the nondigestible and up to 15% of the digestible carbohydrates. There was a total of 4 groups with 6 rats per group. Body weights (BW) of ME rats paralleled the BW of N rats after an initial loss of BW. The following results were found at the end of 35 days: Body, total intestinal tract, stomach, and colon weights and BW gain and food intakes were increased ($P < 0.05$) in N rats compared to ME rats, regardless of WB intake. The deposition of epididymal and renal fat was increased ($P < 0.05$) in N rats compared to ME rats fed either 5% or 20% WB. Comparing the effect of WB on ME or N rats showed no influence of WB on BW; however, the proximal colon weights were increased ($P < 0.05$): 28.0 ± 8% (ME) and 39.8 ± 5% (N) in rats fed 20% WB. Distal colon weights were increased 42.5 ± 1% in N rats fed 20% WB ($P < 0.05$). Plasma levels of vitamin E were 27.5 ± 2.8% (ME) and 28.1 ± 3.7% (N) less ($P < 0.05$) in rats fed 20% WB. These results indicate a trend for lower plasma fat-soluble vitamins and larger intestinal tract weights with decreased meal-frequency and increased dietary wheat bran.

43.13

BIOAVAILABILITY OF VITAMIN E IN RATS FED GRADED LEVELS OF PECTIN. E.E. Schaus*, B.O. deLumen*, F.I. Chow*, and S.T. Omaye* (SPON: J.P. Hannon). Dept. of Nutritional Sciences, Univ. of Calif., Berkeley, CA 94720 and Nutrients Res. Unit, Western Regional Res. Ctr., USDA, ARS, Berkeley, CA 94710.

Citrus pectin (CP, 6.7% methoxyl groups by wt) was fed at levels of 0%, 3%, 6%, and 8% (w/w) of the diet for 8 wks to male weanling Sprague-Dawley rats (8 rats per group). A semipurified diet containing .001% vitamin E (VE) was used as a basal diet and fed ad libitum. The purpose of this study was to evaluate whether the bioavailability of VE was affected by CP. There was a nonsignificant (NS, $P > 0.05$) trend for an inverse relationship between food intake and CP levels. In general, rats fed 3% CP were not different in (NS) any parameters from rats fed 0% CP. In rats fed 6% or 8% CP, liver VE levels were reduced ($P < 0.05$) after 8 wks compared to liver VE at the start of the study. By wk 8, both groups had reduced body wt and increased red blood cell hemolysis compared to the 0% CP group. Rats fed 8% CP also had reduced plasma VE ($P < 0.05$). In rats fed 6% CP, heart VE was less ($P < 0.05$) compared to the 0% CP group at 8 wks. Fecal fat excretion was not different between groups but wts of the small and large intestine were increased in rats fed 6% or 8% CP. These results indicate that CP at 3% of the diet does not reduce the bioavailability of VE in the rat, but higher levels do. Based on our data and extrapolating to common Western CP consumption in humans, we would not expect an adverse effect of CP on VE status.

43.14

THE EFFECT OF PROGLUMIDE ON PENTAGASTRIN AND NERVE INDUCED MOTILITY CHANGES IN THE CANINE STOMACH. P.F. Schmalz and J.H. Szurszewski. Mayo Foundation, Rochester, MN 55905

Strips of corporal muscle (0.2 x 1.0 cm) were cut parallel to the circular muscle fibers, suspended between two platinum electrodes in an organ chamber, and bathed in oxygenated Krebs solution at 37°C. Proglumide concentration-response relationships were obtained for excitation and inhibition induced by pentagastrin (PG), carbamylcholine chloride and nerve stimulation. Pentagastrin, acting directly on the muscle, increased the frequency and amplitude of spontaneously occurring phasic contractions. Both effects were significantly reduced by proglumide (10^{-2} M). Carbamylcholine chloride (10^{-8} M to 10^{-5} M), a muscarinic agonist, increased the frequency and amplitude of spontaneous contractions which were not affected by proglumide (10^{-2} M). In normal Krebs solution, transmural electrical nerve stimulation has both excitatory and inhibitory effects on motor activity. The excitatory effect is cholinergic whereas the inhibitory effect is due to release of an unknown inhibitory transmitter. We have previously shown that pentagastrin potentiates the inhibitory effect of nerve stimulation by an action on the inhibitory neuron. The potentiating effect of PG (10^{-9} M) on the inhibitory effect of nerve stimulation was reduced by proglumide (5×10^{-3} M). These data suggest that proglumide, in millimolar concentrations, may be an antagonist for gastric receptors located on inhibitory nerves and smooth muscle. (Supported by A.H. Robins.)

43.15

HISTAMINE RELEASING MECHANISMS IN AMPHIBIAN GASTRIC MUCOSA. M.C. Ruiz* and F. Michelangeli. Centro de Biofísica y Bioquímica, IVIC. Caracas Venezuela

The mechanism by which histamine is released from mucosal stores was studied in isolated gastric mucosae of Rana catesbeiana mounted in Ussing chambers. Histamine in bath was measured by fluorometry. H^+ rate was measured by titration. ACh, tetragastrin (TG), K^+ depolarization ($50K^+$) and Na^+ free nutrient released histamine which peaked and faded even in continuous presence of the agonist. D/R curves for ACh and TG showed sigmoidal relationship. Atropine inhibited release by ACh but not by TG. Hexamethonium did not block release by ACh. Metiamide did not block release by any agent tested. Co^{++} (1.25 mM) blocked release by ACh and Na-free medium. D600 did not have any effect on release by ACh or $50K^+$-free solution inhibited release by ACh and TG. Compound 48/80 had no effect. Histamine appears to be released by a non-mast cell type (Paracrine?). The results are consistent with an action of ACh (muscarinic) and TG on separate receptors on the histamine-releasing cell This interaction would result in an entry of Ca^{++}may be following depolarization. An increase in intracellular Ca^{++} would trigger histamine release which in turn acts on the oxyntic cell to stimulate H^+ secretion. However, interactions between secretagogues and released histamine occur at the oxyntic cell.

44.1

Na EXCRETION INDUCED BY NaCl LOADING IN FROG SKIN. L.W. Frazier and J.C. Vanatta, Baylor Coll. Dent. and Univ. Tx. Southwestern Med. Sch., Dallas, TX 75246;75235

Skin sacs were made from the hind limbs of Rana pipiens to compare Na excretion in normal (I) and NaCl loaded frogs (II). Twelve sacs were in each series, made from six each of I and II. Group II received 0.1 ml/G of 120mM NaCl by injection, 2 x on day 1 and 2 and 1 x on day 3. The sacs were filled with ∿2 ml of Ringer solution. They were immersed in 15 ml of a solution containing 2 mM KCl and 2mM NaCl. After equilibration the sacs were immersed in the solution for 6 hrs. The $[Na^+]$ was determined in the outside solution and the excretion calculated. Excretion for I was 2.09±0.30μM/hr x 50G frog and II was 4.22±0.98 ($P/2<0.025$). Unidirectional ^{22}Na fluxes were determined on paired skins from similarly loaded frogs. Skins were mounted in chambers and the inside and outside solution were as given above. Each chamber was 2 ml in vol and the flux period was for 60 min. Excretion in units of nM/100 mg skin x min for I had a net outside to inside flux of 514±101.2, while II had a net inside to outside flux of 157.2±65.8 units. Electrical studies were made on similar groups of skins. Skins were mounted in chambers with identical Ringer's on both sides. The PD(mV) was measured every 5 min for 30 min. Following each PD reading short-circuit current (SCC) was recorded. The SCC in I averaged 59.2±8.33μA/cm^2 and II (N=10) averaged 30.0±4.15 μA/cm^2($P<0.025$). We conclude that frog skin can excrete Na in response to a Na load. (Supported in part by NIH grant 2-S07-RR RR07175 and by NIH grant AM-18689).

44.2

EFFECT OF ADRIAMYCIN ON SHORT CIRCUIT CURRENT ACROSS TOAD URINARY BLADDER EPITHELIUM. J.S. Chen, Y.H. Kang* and B.L. Chen*, Physiology, USUHS and NIH, Bethesda, MD 20814

Adriamycin is a potent antitumor antibiotic widely used in the chemotherapy. The major side-effect of this drug is its cardiac toxicity. The mechanism of adriamycin toxicity is still unclear. In this study, we investigated the effect of adriamycin (ADR) on the short circuit current (SCC) across the toad bladder in an attempt to detect a possible interaction between the drug molecule and the toad bladder epithelial cell membrane. Our preliminary results show that when the mucosal surface of the epithelium is exposed to 52 μM ADR, a significant increase in SCC occurs within 10 min if the epithelium is responsive to the drug treatment. Our data indicate that the increase in SCC after the treatment with ADR is attributable to an enhancement of Na transport channels at the apical membrane but not to changes in Na pump (Na,K-ATPase) activity in the epithelium. The epithelial cell membrane also hyperpolarizes as a result of the drug treatment. Mucosal ADR treatment also causes significant increases in the cell contents of Na and Ca. Relative to the ultrastructure of the control bladder epithelium, ADR causes a reduction of microvilli and microfilaments as well as the size of cells, and the accumulation of secretory granules in the apical regions of the epithelial cells. This study thus suggests that toad urinary bladder epithelium may be a target tissue for probing the cardiac toxicity of adriamycin. (Supported by USUHS Grant C07662).

44.3

ELECTRICAL RESISTANCES OF CELL MEMBRANES AND PARACELLULAR PATHWAY IN RABBIT PROXIMAL TUBULES. Elsa Bello-Reuss* (SPON: C. Hunt), Washington University School of Medicine, St. Louis, MO 63122.

Transepithelial specific resistance (R_e) was measured by luminal cable analysis in isolated and perfused rabbit proximal convoluted tubules. Intracellular microelectrode techniques were used to calculate the electrical resistances of the cell membranes and of the shunt pathway from (a) Re, (b) the apparent ratio of cell membrane resistances and (c) the effects of peritubular Ba^{2+} addition on basolateral and apical membrane voltages (assuming that Ba^{2+} acts only at the basolateral membrane). The values of R_e (17 ± 2 ohm cm^2) and the space constant (218 ± 39 μm) were found to be higher than previously reported. R_e increased significantly to 31 ± 9 ohm cm^2 when the temperature was lowered from 37 to 10°C and was practically abolished by removal of Ca^{2+} from the bathing solution (R_e in Ca^{2+} free medium: 2.0 ± 0.4 ohm cm^2). The ratio of membrane resistances (luminal/basolateral) was 3.6 ± 0.6. The values of apical and basolateral membrane resistances (R_a and R_b) were 146 and 41 ohm cm^2 (ideal cylindrical tubule). If a correction for a membrane folding factor of 36 is introduced, the values of R_a and R_b can be calculated to be 5250 and 1480 ohm cm^2, respectively. The low paracellular resistance (19 ohm cm^2), explains the low transepithelial resistance. Supported by NIH Grant AM09976.

44.4

THE IMPORTANCE OF BASIC AMINO ACID RESIDUES IN THE RENAL PAH TRANSPORT SYSTEM. S.S. Tse*, C. Bildstein*, D. Liu* and R.D. Mamelok. Stanford University, Stanford, CA 94305

Basal-lateral membranous vesicles prepared from the rabbit renal cortex contain a transport system for p-aminohippurate (PAH) which is irreversibly inactivated by concentrations of trypsin which do not affect either Na^+-dependent uptake of L-glutamate or the "glucose space" of the vesicles. This suggests that the transporter contains a peptide bond(s) near the surface of the membrane, involving arginine or lysine residues, and that this bond(s) is essential for maintaining the functional integrity of the transporter. In order to explore the role of arginine and lysine residues in the function of the PAH transporter we investigated the effects of phenylglyoxal which react with arginine residues and of trinitrobenzenesulfonic acid (TNBS) and citraconic anhydride which react with lysine residues and N-terminal amino groups. Phenylglyoxal inhibited the total transport of PAH into vesicles by 38%. TNBS inhibited uptake by 29%; however, citraconic anhydride was not inhibitory. Clostripain, which cleaves predominantly bonds involving carboxyl groups of arginine, was then tested for its ability to decrease the transport of PAH. After the vesicles were incubated for 15 minutes with clostripain, about 50% of probenecid inhibitable uptake of PAH was abolished. We conclude that a residue of arginine is important to the integrity and function of the transporter. The importance of a residue of lysine and N-terminal amino groups cannot be determined unambiguously from the data.

44.5

RADIATION INACTIVATION ANALYSIS OF TARGET SIZE FOR AN AMINO ACID TRANSPORT CARRIER IN INTESTINE. Robert L. Preston* and J. Clive Ellory* (SPON: W. A. Riddle). Illinois State Univ., Normal, IL 61761 and University of Cambridge, Cambridge, England CB2 3EG.

The apparent molecular weight of a transport carrier for L-serine was measured using radiation inactivation analysis of target size on rabbit intestinal brush border membrane vesicles. Brush border membrane vesicles from rabbit intestine were prepared using the calcium precipitation method, lyophilized, and then irradiated under vacuum with 20 MEV electrons. The vesicles were then restored by gentle homogenization and incubation for 45 minutes in mannitol/Tris buffer at pH 7.6. ^{3}H-serine transport was measured at 20°C using a 5 second flux period, followed by ice-cold mannitol buffer dilution and rapid filtration through 0.45 μm filters. Diffusion corrections were made by subtracting the residual flux in 100 mM serine from the flux measured at 1 mM serine. It was found that under tracer exchange conditions the apparent molecular weight of the serine transport carrier was 165,000 daltons. The molecular weights of three-brush border marker enzymes were also measured in this preparation using radiation inactivation. The molecular weights were γ-glutamyl transpeptidase, 123,000-daltons; L-leucine aminopeptidase, 135,000 daltons; and alkaline phosphatase, 57,400 daltons. It is concluded that this preparation should be useful for target size analysis of other brush border intestinal transport systems. (Supported by USPHS grant 1 F33 AM 06687-01).

44.6

PROPERTIES OF Cl^-/HCO_3^--STIMULATED ATPase IN APLYSIA CALIFORNICA GUT. G.A. Gerencser and S.H. Lee*, Department of Physiology, University of Florida, Gainesville, FL, NIEHS/NIH., C.V. Whitney Laboratory, St. Augustine, FL, USA.

The serosa negative transepithelial potential difference across Aplysia gut is generated by a Na^+-independent, active, electrogenic Cl^- absorptive mechanism. In order to possibly clarify the Cl^- absorptive mechanism, plasma membranes from Aplysia enterocytes were isolated by differential centrifugation and sucrose density gradient techniques and assayed for ATP hydrolyzing capability. Marker enzymes for the plasma membrane fraction included 5' nucleotidase, glucose-6-phosphatase, alkaline phosphatase and Na^+/K^+-stimulated ATPase while succinic dehydrogenase and cytochrome oxidase were used as marker enzymes for the mitochondrial fraction. Both Cl^- and HCO_3^--stimulated ATPase were found in the plasma membrane fractions. Maximal anion-ATPase activity was achieved with either 25 mM Cl^- or 25 mM HCO_3^-. The apparent K_A for Cl^- activation of the ATPase was 10.3 mM while the K_A for HCO_3^- was 9.7 mM. ATP was the most effective nucleotide for both HCO_3^- and Cl^--ATPase activities. These results suggest that the active Cl^- transport mechanism in Aplysia gut could be a Cl^-/HCO_3^--stimulated ATPase found in the enterocyte plasma membrane fractions. Supported by Whitehall Foundation Grant No. 78-156 ck 1.

44.7

Cl^- ABSORPTION BY AMPHIUMA SMALL INTESTINE: DEPENDENCE ON SEROSAL Na^+ RATHER THAN MUCOSAL Na. John F. White, Dorothy Ellingsen* and Kevin Burnup*. Department of Physiology, Emory University, Atlanta, GA 30322.

Tracer techniques and Cl^--sensitive microelectrodes were used to determine the site of the requirement for Na^+ for active electrogenic Cl^- absorption in Amphiuma small intestine. In vitro segments of stripped intestine incubated in a Na^+-free (choline) media exhibited an enhanced short-circuit current consistent with greater Cl^- absorption when Na^+ was added simultaneously to mucosal and serosal media. Simultaneously the absorptive flux of Cl^- ($J^{Cl}_{m\to s}$) was increased 0.46±0.05 μeq/hr·cm^2. The s→m flux was unchanged. In paired tissues addition of Na^+ only to s increased $J^{Cl}_{m\to s}$ by 0.43±0.07 μeq/hr·cm^2 while addition to the m side increased $J^{Cl}_{m\to s}$ significantly less ($P<0.01$). Addition of choline gluconate or Tris gluconate to s was ineffective in stimulating $J^{Cl}_{m\to s}$. In Na^+ containing medium intracellular Cl^- activity (a^i_{Cl}) measured with double-barrelled microelectrodes was 29.4±2.8 mM in villus cells. While the mucosal membrane potential (ψ_m) was -30.3±3.2 mV indicating active Cl^- accumulation. Cl^- accumulation was eliminated and ψ_m greatly depolarized 2 hrs after replacement of serosal Na^+ with choline or after addition of 1 mM ouabain to the serosal bath. In contrast, after replacement of mucosal Na^+, Cl^- accumulation and ψ_m remained at control levels. These results indicate that Na is required at the serosal rather than the mucosal membrane for active electrogenic Cl^- absorption by the small intestine. Supported by USPHS grants AM17361 and AM26870

44.8

CATION TRANSPORT BY HOG GASTRIC VESICLES. P.K. Rangachari, A. Soumarmon and M.J.-M. Lewin. (SPON: M.J. Rutten). I.N.S.E.R.M.U.10 Hopital Bichat. Paris 75018. France

We studied the transport of ^{86}Rb and ^{22}Na by vesicular preparations from hog stomachs. ^{86}Rb uptake was resolved into a fast, osmotically sensitive component (t 1/2 45 secs) and a slower component presumably representing membrane adsorption. Rb uptake was inhibited by cations in the following order: K > Rb > Cs >> Na ≃ Li. The system showed the characteristics of a K K or a K H antiport. ^{22}Na uptake was slower (t 1/2 96 secs), inhibited by cations in the following order: Na ≃ Li >> K ≃ Rb ≃ Cs and behaved like a Na H exchange system. Maximal uptakes of Na and Rb were equivalent. The uptakes were not affected by ouabain; bumetanide and amiloride had inconsistent effects. N-ethyl maleimide inhibited Rb uptake. Replacing chloride with sulphate made no apparent difference to the transport of either cation. Thus chloride insensitive antiports for Na and K exist in hog gastric vesicles. (Supported by INSERM, France).

44.9

ADVANTAGES OF AN ELECTROGENIC OVER A NEUTRAL NaCl SYMPORT. G. Carrasquer, M. Schwartz, and W. S. Rehm. University of Louisville, Louisville, KY 40292.

A neutral NaCl symport has been suggested for a number of epithelial tissues including the gastric mucosa. Our experimental findings on the gastric mucosa are more adequately explained with an electrogenic NaCl symport. A major objection to the neutral NaCl symport arises as follows. With a passive neutral NaCl symport the products of the Na and Cl activities in the cell and in the medium determine the direction and driving force for NaCl. Why is this product so much lower in most epithelial cells (including gastric cells) than in the medium across the membrane where the NaCl symport is located? This could be explained by a sluggish NaCl symport combined with a very active Na-K ATPase, making the NaCl symport trivial. In contrast, with an electrogenic NaCl symport (e.g., more Cl's than Na's transported per cycle), the equilibrium concentrations of both ions across the membrane where the symport is located would be dictated not only by the product of their concentrations but also by the transmembrane PD. Illustration: assume 2 Cl and 1 Na are transported per cycle. Because of the electrogenicity, the following equation is derived (Carrasquer et al. AJP 242:G620-G627, 1982), namely, $PD = 120 \log (Cl_N/Cl_C) + 60 \log(Na_N/Na_C)$. With a ratio of 2 for Cl_N/Cl_C and for Na_N/Na_C, a transmembrane PD of 54 mV is obtained, a reasonable value. The electrogenic rather than the neutral NaCl symport is more appropriate to explain the low intracellular Na and Cl concentrations. (NSF support)

44.10

EQUATIONS AND ELECTRICAL CIRCUITS FOR THE (Na + K)-ATPase PUMP. Manuel Schwartz, Gaspar Carrasquer, and Warren S. Rehm. University of Louisville, Louisville, KY 40292

It is the aim here to show that equations based on linear non-equilibrium thermodynamics can be reduced to an equivalent electrical circuit and, from the latter, conclusions can be drawn readily for varying PD responses due to changes in ion concentration. Such equations for the (Na + K)-ATPase pump are presented by Heinz (Electrical Potentials in Biological Membrane Transport, Springer-Verlag 1981). In one eq. the fluxes $J_{Na} + J_K = 0$ for electroneutrality and in the other, additionally, $J_{Na} = J_K = 0$ for static head. These equations are electrically equivalent to $V = E_L + R_{Na}R_K (R_{Na} + R_K)^{-1} i_p$ where the PD across the leak pathways of Na and K has two terms: E_L, the PD across the pathways due to diffusion potentials of Na and K and the second the PD due to pump current $i_p = i_{Na} - i_K$ flowing into the leak pathways. This eq. can be put in the form $V = (-R_K E_{Na} + rR_{Na}E_K)(R_K + rR_{Na})^{-1}$ where $r = i_{Na}i_K^{-1}$. Emphasis in steady state is on leak pathways. If we consider open-circuit conditions, pump emf is determined from classical equilibrium thermodynamics to which non-equilibrium thermodynamics reduces in this case. We will show that, for $J_{Na} + J_K = 0$, r of pump (P) ≠ r of leak (L) and that, for $J_{Na} = J_K = 0$, r of pump = r of leak. With ΔE_p and ΔE_L representing changes in magnitude of pump and leak emf's, we will further show that $R_P R_L^{-1} < (\Delta E_P)(\Delta E_L)^{-1}$ for an anomalous PD response due to ion concentration changes such as K and $R_P R_L^{-1} > (\Delta E_P)(\Delta E_L)^{-1}$ for a normal PD response. (NSF support)

44.11

APPARENT IONIC PERMEABILITIES OF NON-SENSORY EPITHELIUM OF THE MAMMALIAN INNER EAR. Daniel C. Marcus and Nancy Y. Marcus.* Washington Univ. Med. Schl., St. Louis, MO 63110

Non-sensory cells of the utricle produce a luminal fluid rich in K (ca. 140 mM) and low in Na (ca. 12 mM). The cellular mechanisms responsible are unknown. A non-sensory tubular region (ca. 500 μm dia. by 500 μm long) of gerbil utricle was isolated in vitro from the rest of the vestibular labyrinth by introducing columns of stained castor oil. Transepithelial potential (V_T) was measured by puncturing the tubule wall with a glass microelectrode (1 M KCl). The bath contained stirred, HCO_3-buffered media at 37° C. Under control conditions V_T was 7.2 ± 0.9 SEM mV (N=9), lumen positive. This potential was sensitive to 1 mM ouabain (decline of V_T: 7.2 ± 1.6 mV (N=3)). Reduction of bath K from 5 mM to nominally K-free (Na substitution) also reduced the potential, by 3.7 ± 0.7 mV (N=3). Reduction of bath Cl from 130 mM to nominally Cl-free (SO_4 substitution) increased V_T by 8.9 ± 3.2 mV (N=3) and subsequent replacement of K for Na (also Cl-free) led to a further increase of V_T by 8.2 ± 0.7 mV (N=3). Decreasing bath Na from 150 to 28 mM by substitution with N-methyl-D-glucamine (NMDG) decreased V_T by 4.3 ± 0.5 mV (N=3). Substitution of K for NMDG elevated V_T by 7.4 ± 1.4 mV (N=3). These results suggest that there is a significant conductance for at least K and Cl in the basolateral membranes and/or paracellular pathway. Determination of the exact loci and magnitudes of these conductances require further investigation. Supported by NSF & NIH.

44.12

CHARACTERISTICS OF CALCIUM PERMEABILITY AND TRANSPORT IN THE MAMMALIAN LENS: ANALYSIS OF ^{45}Ca FLUXES. Nicholas A. Delamere & Christopher A. Paterson, University of Colorado Medical School, Denver, CO 80262

The influx and efflux of ^{45}Ca from the crystalline lens of the rabbit was examined. ^{45}Ca accumulation reached a maximum level within 12 hr with a lens/medium ^{45}Ca ratio of 0.08. This value is similar to the lens/medium ratio seen with ^{14}C inulin, suggesting that ^{45}Ca might exchange only with calcium in lens extracellular spaces. ^{45}Ca efflux analysis supported this suggestion. The lens ^{45}Ca efflux pattern resolved into three components. However, the efflux rate of each of the components could be explained on the basis of simple diffusion of ^{45}Ca along extracellular space pathways of varying tortuosity. Neither metabolic inhibition nor freeze-thawing the lens had any influence upon the ^{45}Ca efflux characteristics. These studies indicated that ^{45}Ca exchanges primarily with extracellular lens calcium. This suggests that a very low membrane permeability to calcium restricts calcium entry into lens cells. This suggestion was supported by the finding that ^{45}Ca was accumulated intracellularly only in experiments where lens membrane permeability was increased.

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44.13

EFFECTS OF COTTON BRACTS EXTRACT ON CANINE TRACHEAL EPITHELIUM. M.M. Cloutier,* K. Lesniak,* J.A. Russell and M.S. Rohrbach. SUNY at Buffalo, Buffalo, N.Y. 14214 and Mayo Clinic, Rochester, Minn. 55905.

Aqueous extracts of cotton bracts (CBE) cause airway smooth muscle contraction and 5-hydroxytryptamine release from human platelets. We tested the effects of CBE on the isolated canine tracheal epithelium and on the paracellular fluxes of ^{14}C-mannitol. Mucosal addition of CBE produced decreases in transepithelial potential difference, short-circuit current and tissue resistance and the effects were dose-dependent. Five µl of CBE produced no change in mannitol fluxes even after five hours, while higher concentrations resulted in increases in mannitol fluxes at 90 min. Submucosal CBE (100 µl) produced no change in bioelectric properties or mannitol fluxes. The effects of 5 µl were reversible but were not blocked by mucosal amiloride (10^{-4}M) or indomethacin (10^{-6}M). We conclude that low concentrations of CBE alter active ion transport at the apical cell membrane without affecting the paracellular pathway while higher concentrations alter paracellular fluxes. These effects may produce changes in secondary water transport, allow access of CBE to airway smooth muscle and the interstitium and may contribute to the pathophysiology of byssinosis.

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44.14

RELATIONSHIP BETWEEN PHOSPHATE REABSORPTION AND PLASMA pH. Harald Langberg*, Anders Hartmann* and Fredrik Kiil* (SPON: F. Rector). University of Oslo, Institute for Experimental Medical Research, Ullevaal Hospital, Oslo, Norway

Bicarbonate reabsorption varies with plasma pH. To examine whether the reabsorption of another buffer also varies with plasma pH, phosphate reabsorption was measured in volume expanded dogs. By altering renal arterial perfusion pressure, reabsorption rates could be compared at similar glomerular filtration rates (GFR). Plasma pH was varied between 7.1 and 7.8 by altering P_{CO_2} or plasma bicarbonate concentration (P_{HCO_3}). At a plasma phosphate concentration (P_{P_i}) of 3.4 $\pm$ 0.1 mM, similar relationships were obtained in thyroparathyrodectomized (TPTX) and intact dogs. By raising P_{CO_2} and P_{HCO_3} at constant plasma pH, bicarbonate excretion increased but phosphate reabsorption remained unaltered at similar GFR. Phosphate and bicarbonate reabsorption were reduced by 30-40% by raising pH from 7.4 to 7.8 either by inducing hypocapnia or by bicarbonate loading. By inducing hyperchloremic acidosis, bicarbonate reabsorption fell whereas phosphate reabsorption increased as much as during hypercapnia. Thus, phosphate reabsorption varies with plasma pH under conditions of adequate filtered loads in both TPTX and intact dogs. On average a reduction from pH 7.4 to 7.2 increases phosphate reabsorption by 28% and an increase from 7.4 to 7.8 reduces phosphate reabsorption by 56%.

45.1

AMILORIDE INHIBITS CARBACHOL-INDUCED AMYLASE SECRETION AND ^{45}Ca EFFLUX IN RABBIT PANCREATIC ACINI

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The diuretic drug amiloride is an inhibitor of two different Na^+-transport mechanisms: a conductive Na^+-entry mechanism (high amiloride affinity) and a Na^+-H^+ exchange mechanism (low amiloride affinity). The Na^+-H^+ exchange mechanism plays a role in a number of different physiological processes and is inhibited by relatively high concentrations of amiloride (10^{-5}-10^{-3}M). In the exocrine pancreas, the neurotransmitter acetylcholine and the peptide hormone pancreozymin stimulate amylase secretion. Their mechanism of action is based on an increase in the intracellular Ca concentration, due to the release of bound Ca from intracellular pool(s). In rabbit pancreatic acini, amiloride (10^{-4}-10^{-3}M) inhibits the carbachol-stimulated amylase secretion in an apparently competitive manner. However, it does not inhibit the pancreozymin-stimulated amylase secretion and the ^{45}Ca efflux. This suggests that amiloride in concentrations, in which it also inhibits the Na^+-H^+ exchange process, is either a competitive antagonist of the action of carbachol at the acetylcholine receptor level, or that the Na^+-H^+ exchange mechanism is involved in the mechanism of carbachol-stimulated enzyme secretion.

45.2

BICARBONATE SECRETION BY DUODENAL SURFACE EPITHELIUM AND ITS PROTECTIVE ROLE. G. Flemström*, E. Kivilaakso* and A. Garner* (SPONS: J.G. Forte and F.C. Rector). Uppsala Univ. Biomed. Ctr., S-751 23 Uppsala, Sweden; Helsinki Univ. Ctr. Hosp., SF-00290 Helsinki, Finland and ICI Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, England.

Surface epithelium in duodenum in a variety of mammals in vivo transports HCO_3^- into the lumen at a considerably greater rate than does stomach or more distal small intestine. The effects of luminal acid on this secretion was studied in anesthetized Sprague-Dawley rats. Luminal alkalinization was titrated (pH stat) either at neutral luminal pH after a 5 min exposure of the duodenal lumen to pH 2, or during continuous exposure of the lumen to pH 2 or 5. In the latter experiments, pH at the luminal cell surface was measured with antimony microelectrodes. Values of pH $\geq$ 7 excluded that passive diffusion of H^+ from lumen into tissue contributed to luminal alkalinization. Luminal pH 2 for 5 min caused a sustained (>90 min) rise in HCO_3^- secretion and intravenous aspirin or indomethacin reduced the duration of this response. Continuous exposure to pH 2 caused a ~ 700% rise and exposure to pH 5 a ~100% rise in secretion. Intravenous aspirin inhibited only the response to pH 2 and inhibition resulted in mucosal damage. The lipoxygenase inhibitor BW755C (5-20 mg/kg), which increases mucosal formation of prostaglandins, also increased the HCO_3^- secretion. The results suggest endogenous prostaglandin production as one mediator of the duodenal alkaline response to acid and this response to be important in mucosal protection

45.3

FACTORS REGULATING GASTRODUODENAL EPITHELIAL HCO_3 SECRETION. Andrew Garner*, Gunnar Flemström*, Jon R. Heylings*, and Bryan C. Hurst*. (SPON: F.C. Rector and J.G. Forte). ICI Pharm. Div., Macclesfield, Cheshire SK10 4TG, U.K. and Uppsala Univ. Biomed. Ctr., S-751 23 Uppsala, Sweden.

Gastric and duodenal epithelia transport HCO_3 in a secretory direction. Normally, contents of stomach and proximal duodenum are acidic and HCO_3 is neutralized close to the mucosal surface. In the absence of luminal acid, HCO_3 secretion can be measured as a titratable alkalinity. Gastric HCO_3 secretion amounts to 2-10% of maximal H^+ secretion and is due to a Cl-HCO_3 exchange process. Duodenal HCO_3 rates exceed those in stomach by 2 to 10-fold and the predominant mechanism is transcellular transport of HCO_3. In both regions, HCO_3 output is attenuated by inhibitors of metabolism, carbonic anhydrase and PG-cyclooxygenase, stimulated by glucagon, E and F-type PGs and low luminal pH but unaffected by histamine, gastrin or secretin. Variations in basal rate and in sensitivity to exogenous stimulants and inhibitors occur, not only between stomach and duodenum but also between species and even between tissues. Such variations may in part reflect differences in endogenous mediator levels e.g. as a consequence of acid secretory status or experimental factors. Exposure to luminal acid (or feeding) increases both gastric and duodenal HCO_3 secretion in vivo and in vitro. This response, mediated via local PG production and tissue specific humoral agents, may be important in the local regulation of H^+ disposal and mucosal protection in upper gastrointestinal tract.

45.4

MEASUREMENT OF IN SITU pH IN THE STOMACH AND PROXIMAL DUODENUM WITH SINGLE AND MULTIPLE ELECTRODE SYSTEMS. Simon J. Rune* and Steen Hannibal* (Spon: J.G.Forte). Glostrup Hospital, University of Copenhagen, DK-2600 Denmark.

Luminal pH was measured in the stomach and proximal duodenum in man using a small glass electrode with a built-in Ag/AgCl reference electrode (GK 2801C, Radiometer, Denmark). Duodenal pH recordings at reproducible distance from the pylorus were obtained by strapping 4 electrodes together at 1.5 cm intervals. Electrodes situated in the stomach record steady acid pH while electrodes distal to the pylorus show fluctuating pH, thereby enabling the electrodes to be localized from the pH pattern on an analogue recorder. Digital pH values were sampled and stored at a rate of 2/s from each electrode. For each 10-min period mean hydrogen ion activity, percentage of time pH was < 3.0 and number of spikes were computed.

Log mean hydrogen ion activity in the proximal part of the duodenal bulb within 1.5 cm from the pylorus was (median and interquartile range from 14 normal subjects): fasting pH 2.40 (2.23-3.00); 0-60 min after liquid test meal: pH 5.7 (4.92-5.95); 60-120 min: pH 2.31 (1.95-3.20); 120-180 min: pH 2.20 (1.99-2.44). Repeated studies in 12 subjects showed a linear correlation between 1. and 2. study, r = 0.87.

The pH gradient from the proximal to the distal part of the duodenal bulb was determined by simultaneous measurements from 2 electrodes spaced 1.5 cm apart. The median gradient was in fasting state from pH 2.6 to 3.5, postprandial from pH 2.3 to 3.0.

45.5

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

An SPD and sudden drop in resistance (SR) was regularly observed by Kidder in frog gastric mucosa during anoxia with serosal pH <7.1, in Cl^- bathing solutions only, and with a PD which changes across the region of 10mV (AJP 230:61-66, 1976). We have found that anoxia is not a prerequisite, since SPD and SR can be reliably produced under oxygenated conditions when H^+ is inhibited with metiamide or SCN^- if nutrient pH <7.0. Normal nutrient [Cl^-] and a luminal [Cl^-] of > 40mM are necessary for SPD and SR. Change in luminal [Cl^-] from 90 to 0mM or vice versa produced a sudden ΔPD of 22.6±1.7mV in the post SPD state, a ΔPD of 12.9 ±1.4mV during anoxia with nutrient pH >7.1, and a ΔPD of 8.1+0.6mV for control conditions. In the post SPD state, changes in luminal [HCO_3^-] produced a small increased Δ PD over that in control conditions, but Δ PD was negligible after changes in luminal [Na^+] or [K^+]. Neither DIDS nor 16-16 dm PGE_2 in either bathing solution prevented an SPD. Influx of Cl_{36} into the mucosa from the luminal solution was increased in the post SPD state but transmural J_{Cl} was unaffected. No consistent morphologic changes were associated with the SPD, but severe damage of the oxyntic cells occurs after one hour even though electrophysiological recovery ultimately occurred. We conclude that the SPD is produced by a Cl^- shunt from lumen to cell, and that inhibition of H^+ secretion together with a relatively acidotic nutrient pH are prerequisites.

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45.6

BACK DIFFUSION OF H^+ IN FROG STOMACH. Richard P. Durbin. University of California, San Francisco, CA 94143

Loss of H^+ from the secretory solution, bathing the luminal surface of isolated bullfrog gastric mucosa, was quantitated by direct titration of weighed aliquots of this solution. Secretory to nutrient Cl fluxes were also measured, using ^{36}Cl. Mucosae brought to a resting state with cimetidine were relatively impermeable to H^+ and resistant to ulceration. In experiments in which 50 mM HCl (made isotonic with NaCl) was instilled as secretory solution, H^+ permeability was 0.5×10^{-5} cm sec^{-1} (resting mucosae; n = 6). In the same experiments, the presence of exogenous H^+ increased secretory to nutrient Cl flux to an extent indicating that approximately 75% of H^+ back diffusion occurred as HCl. The rate of H^+ back diffusion was greater in mucosae maximally stimulated to secrete acid, as previously found in canine stomach in vivo (Moody & Davis, Gastroenterology 59:350-357, 1970). A further increase in rate of H^+ back diffusion in stimulated mucosae could be induced by addition of SCN^- to the secretory solution. In the presence of SCN, addition of H^+ reduced secretory to nutrient Cl flux, suggesting competition between HCl and HSCN at a mucosal site. (Supported by grants NSF PCM 78-22520 and NIH AM 25986).

45.7

THE HISTAMINE STIMULATORY PATHWAY IN ISOLATED GASTRIC GLANDS --MODIFICATION BY DMSO. Thomas Berglindh* and Olof Nylander * (SPON: G.Sachs). CURE Membr. Biol. VA Wadsworth, Los Angeles, CA 90073 and Dept. Physiol., Univ. Uppsala, Sweden.

The histamine pathway involved in stimulation of isolated gastric glands can be altered in several ways by dimethylsulfoxide (DMSO). DMSO, which is a dipolar, aprotic solvent, has been shown to interact with biological systems in multifaceted ways due to its ability to substitute for water in membranes, proteins and in the cytoskeleton. The histamine-induced acid secretory response as measured by aminopyrine (AP) accumulation or oxygen consumption was dose-dependently increased by DMSO (1-10%). At low histamine concentrations, however, the DMSO response was below that of control. This effect was reflected in an increased $K_{0.5}$ for histamine + DMSO. The apparent change in histamine receptor affinity was corroborated by a significant decrease in pA_2 for cimetidine from 6.2 to 5.5 at 4% DMSO. In contrast, the basal as well as the responses to db-cAMP, IMX, IMX+gastrin and acetylcholine were all inhibited by DMSO. This unique potentiation of the histamine pathway can be partly explained by an increase in histamine-dependent cAMP formation, but must also reflect DMSO-activated mechanisms closer to the H^+ production site.

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45.8

A somatostatin receptor coupled with phosphoprotein phosphatases in parietal cell. MJM Lewin and F. Reyl-Desmars, INSERM U10, Hop. Bichat, 75018, Paris, France.

Pronase-dissociated and Percoll-purified rat parietal cells were found to contain specific and high affinity binding sites for ^{125}I-Tyr 1- somatostatin 14 (KD = 5×10^{-11}M). These sites were further shown to be reversibly associated with phosphoprotein phosphatase subunits (PPPase; substrate ^{32}P-histones), these being released (i.e. activated) upon somatostatin binding. Both binding and somatostatin-stimulated PPPase activity were enriched in cytosolic (100,000 x g x 1h) fraction. Both were sensitive to temperature and were inhibited by blockers and uncouplers of oxidative phosphorylation (rotenone, DNP), blockers of Na^+ transport (ouabaïne, amiloride) and specific inhibitors of transglutaminases (methylamine, m-dansyl cadaverine). We therefore suggest that somatotatin inhibition of gastric HCl secretion is due to a dephosphorylation process mediated by a specific intracellular or internalized receptor.

45.9

CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASE FROM ISOLATED GASTRIC GLANDS. S.J. Hersey and M. Miller Dept. Physiology Emory Univ. Atlanta, GA 30322.

Gastric glands isolated from rabbit were used to study the biochemical and functional properties of cyclic nucleotide-dependent protein kinase(PK). Gastric glands were found to contain a high activity (5,000U per mg protein) of cAMP activated PK. Half-maximal activity was observed with 100nM cAMP. Activation of PK was observed also with cIMP and cGMP with a potency sequence cAMP>cIMP>cGMP. Nucleotide activation of PK was inhibited by the specific inhibitor of cAMP-dependent PK regardless of the nucleotide employed. The 8-bromo forms of the cyclic nucleotides activated PK with potencies similar to those for the native nucleotides and this activation was inhibited also by the specific inhibitor of cAMP-dependent PK. cGMP-dependent PK activity could not be detected in gastric glands. Examination of whole gastric mucosa or isolated surface epithelial cells also failed to show any cGMP-dependent PK activity. These observations are interpreted to support the hypothesis that stimulation of acid and pepsinogen secretion in gastric glands by 8-BrcGMP reflects activation of cAMP-dependent PK and does not indicate a role for cGMP in these secretory processes.

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45.10

SECOND MESSENGERS AND THEIR SITE OF ACTION IN GASTRIC EPITHELIAL CELLS: Karl J. Öbrink*, Elisabet Bergqvist*, Olle Nylander* and Mona Schenholm*. (SPON: J.G. Forte). Dept. of Physiology and Med. Biophysics, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden.

Isolated gastric glands from rabbit were used to study the liberation and action of histamine. Histamine stimulates the parietal cells in these preparations but pentagastrin seems to be without effect. It has been made probable, however, that other cells in the glands have receptors for pentagastrin and the ability to liberate histamine in the suspending fluid. As no mast cells are present in the preparation, histamine seems to be liberated from one of the endocrine type cells in the gland. Antibodies to histamine-albumin complex have been produced and used for immunofluorescence microscopy. So far the parietal cells seem to be free of these immunoreactive sites. The amount of histamine liberated is not influenced by moderate doses of cimetidine but may possibly be enhanced by very large ones. Somatostatin may have an influence on pentagastrin-liberated histamine but not on the basal rate of histamine formation. The effect of prostaglandins does not show any regular pattern. On the other hand both exogenous and endogenous prostaglandins (the latter indirectly studied by the use of blockers for the prostaglandin synthesis) inhibit the action of histamine on the parietal cell activity measured both with aminopyrine accumulation and oxygen consumption. They have, however, also an effect on the stage beyond the cAMP as they interfere with the action of db-cAMP and 8-Br-cAMP.

45.11

HISTAMINE AND CYCLIC AMP: MAJOR NODES OF STIMULUS PATHWAYS FOR GASTRIC ACID SECRETION. E. B. Margareta Ekblad* (SPON: Richard P. Durbin). University of California, San Francisco CA 94143

Role of both histamine and cyclic AMP in gastric acid secretion is, generally, acknowledged. However, there is a persistent controversy whether these two are the final common mediators of any neural and humoral stimulation. In a series of experiments in which acetylcholine, pentagastrin and histamine were used as stimulators and atropine, metiamide, isobutylmethylxantine and calmodulin inhibitor were intercepting the secretagogues stimulus pathway we attempted to clarify the role of histamine and cyclic AMP. Sustained histamine stimulation gives rise to a transient cyclic AMP tissue changes and sustained acid secretion. A sustained acetylcholine stimulation leads to a transient histamine release and a similar pattern of acid secretion. Atropine, metiamide and calmodulin inhibitor disrupt the sequence of biochemical events such that acid secretion associated with acetylcholine can be understood only in terms of taht due to histamine. By following in details the teporal patterns of acid secretion after histamine a unique relationship between the total acid secreted and exposure to histamine was established. When tissue was stimulated by pentagastrin the acid secreted was that corresponding to the released histamine. From these data we conclude that histamine and cyclic AMP are crucial for secretagogue signal transmission in gastric mucosa. (Supp. NIH AM 21448).

45.12

ROLE OF CALCIUM AND MEMBRANE PHOSPHORYLATION IN REGULATING GASTRIC ACID SECRETION. Linda J. Shlatz*. Medical College of Ohio, Toledo, Ohio 43699

Using parietal cells isolated from the rat gastric mucosa, the calcium ionophore, A23187, was found to stimulate acid secretion as measured by the accumulation of [^{14}C]aminopyrine. The stimulation of acid secretion by the ionophore was time dependent, required the presence of extracellular Ca^{2+}, and varied as a function of ionophore concentration. In the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, the maximal response to the ionophore was approximately 45% of that observed in the presence of dibutyryl cAMP. The ionophore was found to potentiate the histamine dependent response but not carbachol- or gastrin-stimulated secretion. In addition, the membrane component of the parietal cells was shown to be phosphorylated by both dibutyryl cAMP- and Ca^{2+}-dependent mechanisms. The Ca^{2+}-dependent phosphorylation of the parietal cell membrane could be inhibited by trifluoperazine, a phenothiazine known to inactivate calmodulin. These studies suggest that Ca^{2+} does serve as a second messenger in stimulating gastric acid secretion and that a Ca^{2+}-dependent phosphorylation of membrane proteins may play a role in regulating parietal cell function. (Supported by USPHS Grant AM 27348).

48.1

ELECTROGENICITY OF THE ATP-INDUCED ACIDIFICATION IN ISOLATED PLASMA MEMBRANE VESICLES OF TURTLE BLADDER EPITHELIAL CELLS. S.J. Youmans*, W.A. Brodsky, Mt. Sinai Sch. Med., N.Y., N.Y. 10029

Plasma membrane vesicles isolated from turtle bladder epithelial cells contain a primary active mechanism for proton transport. Energized by the extravesicular addition of ATP, the mechanism induces the development of transmembrane gradients of pH (inside, more acidic) and electrical potential (inside, more positive), as indicated respectively by the quenched fluorescence of acridine orange (AO) and oxonol V, respectively. Evidence for these claims is as follows. (i) After ATP addition to vesicles suspended in tris buffered media (containing KCl or NaCl or choline Cl or the NO_3 of these cations), Cl or NO_3 is carried passively into the vesicular interior along with the pumped protons. (ii) After the same ATP addition to vesicles that have been pre-loaded with K gluconate and treated with valinomycin, K is carried passively out of the vesicular interior while protons are again pumped into the vesicular interior. (iii) The same addition to vesicles in buffered media devoid of transportable ions induces the development of a transmembrane electrical potential (inside, more positive) with no net transport of protons. (iv) However less than 20% of the ouabain-resistant ATPase is required for maintenance of 100% of the ATP-driven proton transport in these vesicles as indicated from data on the effects of FCCP, DCCD, Triton X-100, and $HgCl_2$. (NIH-supported)

48.2

THE USE OF ^{31}P NUCLEAR MAGNETIC RESONANCE (NMR) TO STUDY H/HCO_3 TRANSPORT IN EPITHELIA. J. P. Kokko, S. I. Helman, J.S. Stoddard*, and R.L. Nunnally*. Univ. of Texas Health Science Center, Dallas, Texas 75235 and Univ. of Illinois, Urbana, Illinois 61801.

There exists a number of different techniques by which intracellular pH (pHi) can be determined. Recently it has become apparent that nuclear magnetic resonance is another technique by which pHi can be measured noninvasively with a relative degree of accuracy (±0.03 pH units). The theoretical basis for this is the observation that the position of the inorganic phosphate peak is pH dependent. While NMR does offer some unique advantages, it currently has the disadvantage of requiring large quantities (about a gram wet weight) of homogeneous and viable tissue. The purpose of the present studies was to use NMR to measure pHi in epithelial sheets of frog skin in response to acute stepwise changes in pCO_2 (0, 2, 5, and 10% CO_2) in the presence and absence of Cl. All studies were conducted at room temperature using Cl or SO_4 Ringers. The control pHi (0% CO_2) in Cl Ringers was 7.19± 0.07 while in SO_4 Ringers it was 7.42±0.12. With increasing pCO_2 there was a polynominal decrease in pHi with the pHi in the SO_4 Ringers being more alkaline than in Cl Ringers (the polynominal regressions are different at the p = 0.11 level). These results are consistent with the view that the intracellular hydrogen ion concentration is lower than predicted from Nernst potential, and further, the results support the existence of HCO_3/Cl exchange mechanism.

48.3

REGULATION OF ACIDIFICATION IN RABBIT MEDULLARY COLLECTING DUCTS. H.R. Jacobson. UTHSCD. Dallas, Texas 75235

We have recently demonstrated that the rabbit medullary collecting duct (MCD), when perfused and bathed in vitro with symmetrical Ringer's bicarbonate solutions, demonstrates a lumen positive transepithelial voltage (V_T) and reabsorbs HCO_3 at a rate of 10-12 pmoles·mm^{-1}·min^{-1}. Net HCO_3 reabsorption ($JHCO_3$) is matched by net Cl secretion (JCl). Additionally, net bicarbonate reabsorption is mineralocorticoid sensitive, Na independent, dependent on peritubular Cl, and inhibited by peritubular acetazolamide and SITS. The present studies examine the effects of [K], pCO_2, [HCO_3] and 8-Bromo cyclic AMP on MCD perfused in vitro. Increasing peritubular [K] from 5 to 50 mM (Na replacement) does not change $JHCO_3$ (12.1±1.9 vs 12.1±2.0) or V_T. However, increasing both luminal and peritubular [K] to 75 mM (Na replacement) and reducing [Cl] to 50 mM (gluconate replacement) decreases $JHCO_3$ and changes $JHCO_3$/JCl from 1 to 0.5. Acute reduction of pCO_2 (40 to 10 mmHg) reduces V_T and $JHCO_3$ by 50%. Acute reduction of bath HCO_3 to 5 mM (pH 6.8) significantly increases lumen positive V_T and $JHCO_3$. Finally, 10^{-5} M 8-Bromo cyclic AMP significantly increases V_T by 30% and increases $JHCO_3$ from 6.9 to 9.8. Thus, rabbit MCD is a relatively high capacity distal nephron acidifying segment that: 1) is not affected by large changes in peritubular [K]; 2) appears to preferentially shunt H secretion with Cl secretion; 3) is regulated by changes in ambient pH; and 4) is stimulated to secrete H by cyclic nucleotides.

48.4

FLOW DEPENDENCE OF PROXIMAL TUBULE ACIDIFICATION. Robert J. Alpern* (SPON: F.C. Rector, Jr.), Dept. of Med., Univ. of Calif., San Francisco, CA 94143

In order to examine the mechanism of flow dependence of proximal bicarbonate absorption (JHCO3), rat proximal convoluted tubules were microperfused at varying rates. In tubules perfused with 25 mM HCO3, at 15, 33, and 49 nl/min, JHCO3 was 105 ± 4, 176 ± 8, and 209 ± 7 pmol/mm•min, respectively. Only 15% of this stimulation of JHCO3 was attributable to flow dependent changes in the measured axial HCO3 concentration profile of the luminal bulk fluid. In addition, changes in diffusive or convective fluxes could not account for the observed stimulation. In tubules perfused with 58 mM HCO3, (a perfusate previously demonstrated to achieve maximal rates of acidification), increasing luminal flow rate from 15 to 49 nl/min did not stimulate JHCO3. These results demonstrate that increasing luminal flow rates stimulate active proton secretion at low luminal HCO3 concentrations but do not affect the maximal rate. The failure to increase Vmax excludes an increase in the number of Na/H antiporters. This kinetic behavior is most consistent with the presence of a flow dependent luminal diffusion barrier in the proximal tubule. According to this thesis, flow dependent stimulation of JHCO3 is secondary to flow dependent changes in luminal HCO3 concentration occurring by two mechanisms: 1) flow dependent increases in the measured axial luminal HCO3 concentration profile, and 2) flow dependent decreases in radial luminal HCO3 concentration gradients. In order for such radial gradients to exist, HCO3 diffusion must be restricted in the microenvironment of the brush border membrane. (This work was supported by NIH grants AM 27045 and AM 07219.)

48.5

FACTORS AFFECTING TUBULAR ACIDIFICATION: INTERPRETATION BY AN ANALOG MODEL. G. Malnic*, C. Amorena*, D.T. Fernandes*, N.A. Rebouças*, F.Z. Gil* and M. Mello Aires* (SPON.: F.C. Rector). University of São Paulo, 05508 São Paulo, Brasil.

The stopped-flow microperfusion technique for the study of renal tubular acidification kinetics has shown that different experimental conditions affect the pH gradient, acidification half-time (t/2) and/or net H (HCO_3) fluxes. We have devellopeda an electrical analog model in which H movement, electroneutral or not, is described by a circuit consisting of a proton-motive force (E) with a series resistance (Rh), in parallel with a capacitance (C) and a shunt resistance (Rs). Changes in CV, the capacitor charge, follow an exponential course toward steady-state, in the same way as luminal Na_2HPO_4 or $NaHCO_3$. Changes in t/2 are due to modifications in Rh, Rs or C. Experimentally, an increase in capillary pCO_2 from 2 to 10% at pH 7.2 reduces proximal t/2 from 5.58 to 3.71 s. At pCO_2 of 10%, acetazolamide increases t/2 from 3.71 to 6.09 s. Both alterations are attributed to changes in Rh depending on cellular H availability. Situations in which the number of luminal transport sites (e.g. by maleate treatment) or peritubular base transfer are modified also affect Rh. The fall in distal t/2 during amphotericin B treatment is ascribed to a decrease in Rs. The proposed model is able to account for a number of experimental modifications of tubular acidification, incorporating gradient-dependent active H ion secretion, an active pump conductance and transepithelial shunt conductance, the latter including both passive H and HCO_3 movement.

48.6

DETERMINANTS OF RENAL CORTICAL CO_2 TENSION. Thomas D. DuBose, Jr. Departments of Medicine, Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550

In vivo micropuncture studies employing PCO_2 microelectrodes have demonstrated CO_2 tensions in the renal cortex which are significantly greater than systemic arterial PCO_2 (65 vs 40 mmHg). Our findings demonstrate that CO_2 gas is in, or near, diffusion equilibrium in the superficial cortex. PCO_2 in the renal vein is indistinguishable from renal artery PCO_2. A mathematical model of CO_2 generation from our laboratory suggests that metabolic CO_2 production (MCO_2) in combination with vascular -to- vascular gas transfer in the cortex can explain these findings. The purpose of this study was to evaluate the role of MCO_2 and CO_2 removal in the generation and maintenance of renal cortical PCO_2 by examination of changes in renal blood flow (RBF) and the effect of metabolic or transport inhibitors on cortical PCO_2. Six groups of rats were maintained on a respirator and PCO_2 was measured in the early and late proximal tubules and the stellate vessel during a control and experimental period: I. 5% isotonic expansion, II. Hyperoncotic albumin expansion, III. Aortic constriction, IV. Na_3VO_4 (20 $\mu M \cdot kg^{-1} \cdot h^{-1}$), V. Rotenone 0.1 $mM \cdot L^{-1}$ via renal artery, and VI. DNP 50 $mM \cdot L^{-1}$ via renal artery. Arterial blood PCO_2 and [tCO_2] did not differ among the groups. RBF increased in II (6.9 to 18.3 $ml \cdot m^{-1}$) and decreased in groups III, IV, V, and VI. PCO_2 was 65 in I, 71 in II, 54.0 in III, 55 in IV, 57 in V, and 87 mm Hg in VI. Therefore, RBF can be dissociated from changes in renal cortical PCO_2. Metabolic CO_2 production is an important determinant of the elevated PCO_2 in the renal cortex.

48.7

DETERMINANTS OF TUBULAR AND CAPILLARY PCO_2 IN THE RAT RENAL CORTEX. F.J.Gennari*, D.A.Maddox, L.J.Atherton* and W.M.Deen. U.of Vt., Burlington VT 05405 and MIT, Cambridge MA 02139.

CO_2 is produced in the kidney by both cell metabolism and HCO_3 reabsorption. Consistent with this unique CO_2 burden, PCO_2 in surface tubules and peritubular capillaries (PC) is notably higher than in arterial blood (Art). The PC-Art PCO_2 gradient can be altered markedly by a variety of experimental maneuvers, but it shows no correlation with proximal HCO_3 reabsorption ($APRHCO_3$) in our studies. PC-Art PCO_2 varies inversely with renal blood flow in rats with comparable $APRHCO_3$, suggesting that cortical PCO_2 is influenced by the rate of CO_2 removal. In Munich-Wistar rats, we have found Bowman's space (BS) PCO_2 to be 10-15 mmHg higher than Art, indicating that CO_2 must be added to the blood prior to its arrival at the superficial cortex. PCO_2 rises further, by 5 mmHg, between BS and the early proximal tubules and is 2-3 mmHg higher in PC than in BS, reflecting CO_2 production in surface nephrons. These small increments are consistent with the view that the CO_2 produced by HCO_3 reabsorption is consumed in the tubular cell to form new HCO_3, and that metabolic CO_2 is rapidly buffered. Although the PCO_2 increment may be small at a given level in the cortex, we propose that PCO_2 is increased progressively in the interlobular arteries as they traverse the cortex, by a process of exchange with the associated venous return and by diffusion from deep nephrons, resulting in the high cortical-arterial PCO_2 gradients normally observed.

48.8

HYDROGEN ION SECRETION AND BICARBONATE-DEPENDENT REABSORPTION IN DOGS. Fredrik Kiil*, Harald Langberg*, and Anders Hartmann* (SPON: F. Rector). University of Oslo, Institute for Experimental Medical Research, Ullevaal Hospital, Oslo, Norway

Bicarbonate reabsorption and associated water and NaCl reabsorption can be reduced either by reducing P_{CO_2} or by administering a carbonic anhydrase inhibitor such as acetazolamide. To examine the interrelations between these inhibitory effects, experiments were performed on anesthetized dogs at a constant plasma bicarbonate concentration of about 30 mM during continuous infusion of ethacrynic acid. By altering P_{CO_2} between 120 and 20 mmHg, plasma pH varied between 7.2 and 7.8 and relationships with similar slopes were obtained between bicarbonate reabsorption and plasma pH or log P_{CO_2} before and after administration of acetazolamide in doses of 30 and 100 mg/kg body weight. On average bicarbonate reabsorption increased by about 20% as plasma pH was reduced from 7.4 to 7.2 and fell by about 40% as plasma pH was raised from 7.4 to 7.8. By reducing P_{CO_2} bicarbonate and chloride reabsorption fell in molar ratios of 1:1.98. By administering acetazolamide bicarbonate and chloride reabsorption was reduced in a molar ratio of 1:2.16. Constant molar ratios indicate proportionality between trans- and paracellular transport. Thus, acetazolamide administration and changes in P_{CO_2} exert additive effects on the bicarbonate and NaCl reabsorption remaining after ethacrynic acid infusion.

48.9

CONDUCTIVE HCO_3^- EXIT ACROSS THE BASOLATERAL MEMBRANE OF RABBIT PROXIMAL CONVOLUTED TUBULES (PCT). C.A. Berry, S. Sasaki*, and M. Baum*. Dept. of Physiol., Univ. of Calif., San Francisco, CA 94143

The mechanism of HCO_3^- exit in the rabbit PCT is not known. HCO_3^- could exit either neutrally in exchange for Cl^-, or conductively down its electrochemical gradient. To examine whether HCO_3^- exit is neutral or conductive, we perfused PCT in vitro with ultrafiltrate-like solutions and measured net volume absorption (J_v) with inulin and net total CO_2 absorption ($JNTCO_2$) with microcalorimetry.

The hypothesis that HCO_3^- exit is neutral, in exchange for Cl^-, was tested by measuring the effect of Cl^- replacement with isethionate. Removal of Cl^- did not affect $JNTCO_2$ (82.6 ± 15.0 vs. 83.4 ± 13.6 pmol/mm•min, n=5) or J_v (0.68 ± 0.10 vs. 0.65 ± 0.09 nl/mm•min, n=5). Conductive HCO_3^- exit was tested by measuring the effect of 2 mM bath Ba^{++} which is known to depolarize the basolateral membrane by 50%. Bath Ba^{++} reduced $JNTCO_2$ 41% from 96.4 ± 13.4 to 56.4 ± 7.9 pmol/mm•min (n=9) and J_v 31% from 0.95 ± 0.11 to 0.65 ± 0.13 nl/mm•min (n=9). When PCT were perfused with high Cl^-, low HCO_3^- solutions J_v (due entirely to NaCl absorption) was unaffected by 2 mM bath Ba^{++} (0.68 ± 0.10 vs. 0.69 ± 0.08 nl/mm•min, n=10). Thus, the Ba^{++} effect was specific for $JNTCO_2$.

In summary: (1) $JNTCO_2$ is Cl^- independent. (2) Bath Ba^{++} inhibits $JNTCO_2$ 41%. (3) Bath Ba^{++} does not inhibit Na^+-K^+ ATPase. From these data we conclude that HCO_3^- exits conductively down its electrochemical gradient. (NIH Grant R01 AM 26142).

49.1

NITRENDIPINE ACTIONS ON TRANSMEMBRANE CA^{++} AND K^+ CURRENTS IN ISOLATED ATRIAL CELLS. W. Giles*, Y. Momose*, and E. Shibata* (Spon. C.E. Hall). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

A single microelectrode voltage clamp technique has been used to study the effects of nitrendipine (Nt) on transmembrane currents carried by Ca^{++} and K^+ in single cardiac cells which are enzymatically isolated from segments of bullfrog atrium. In the dose range 10^{-9} to 10^{-6}M, Nt produces a strong inhibition of i_{Ca}. Measurements of the blockade of i_{Ca} (2.5 mM) (Ca^{++}_o) elicited by 100 msec pulses from -80 to 0 mV yield a K_D of approximately $5x10^{-8}$M if the data are fitted assuming that Nt binds to a single saturable site.

The selectivity of this i_{Ca} blockade was assessed by studying effects of Nt on two different potassium currents: (1) an inwardly-rectifying K^+ current which gives rise to the resting potential; and (2) a slow time- and voltage-dependent outward current (delayed rectifier) which initiates repolarization. At 10^{-8}M to 10^{-7}M, Nt produces a significant increase in the inwardly-rectifying K^+ current in approximately 50% of our experiments. However, it has no effect on the delayed rectifier. Supported by DHHS HL-27454, AHA 81-835, and an AHA Established Investigators Award (W.G.)

49.2

CHANGES IN MEMBRANE RESISTANCE DURING THE PACEMAKER CURRENT IN PURKINJE FIBERS. Mario Vassalle, Hiroshi Kotake* and Cheng-I Lin*. SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203.

The pacemaker current and associated changes in membrane resistance were studied in sheep cardiac Purkinje fibers using a two-microelectrode voltage clamp technique. It was found that the amplitude of current flowing during small hyperpolarizing clamps decreased during the pacemaker current. Increasing $[K]_o$ from 2.7 to 5.4 mM reduced the membrane resistance and (during hyperpolarizing clamps) increased the instantaneous current and slowed the development of the pacemaker current. Adding barium (Ba, 0.05 mM) increased the membrane resistance and the holding current, reduced the instantaneous current on hyperpolarization, abolished the initial dip, and accelerated the development of the pacemaker current especially at high K. Cesium (Cs, 2 mM) reduced the holding current, had little effect on the instantaneous current on hyperpolarization, eliminated the pacemaker current and the associated membrane resistance changes. Cs reduced the holding current even at 10.8 mM K. If the current was reversed, Cs decreased but did not abolished it. When Cs was applied in the presence of Ba, the pacemaker current was eliminated or reduced depending on the extracellular K. It is concluded that cesium abolishes the pacemaker current by abolishing a time-dependent change, presumably a decrease in potassium conductance and that barium enhances the pacemaker current by reducing the background K conductance and more so at high K. (Supported by NIH grants HL27038 and HL17451).

49.3

ON THE MECHANISM UNDERLYING THE OSCILLATORY CURRENT IN CARDIAC PURKINJE FIBERS. Cheng-I Lin*, Hiroshi Kotake* and Mario Vassalle. SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203

The mechanism of the oscillatory current (I_{OS}) has been investigated in sheep cardiac Purkinje fibers with a voltage-clamp technique. The results show that: 1) I_{OS} can be initiated not only by repolarizing but also by depolarizing clamps, provided that the preparation is preloaded with calcium and that the depolarizing test clamp initiates I_{Si}; 2) the I_{OS} initiated by a depolarizing test clamp is usually smaller and has a longer time to peak than the I_{OS} initiated by a repolarizing clamp; 3) brief depolarizing clamps can be followed by an I_{OS} in fibers preloaded with calcium; 4) the amplitude of I_{OS} induced by a test clamp diminishes as the interval from the conditioning clamp increases; 5) no I_{OS} is initiated by repolarizing or depolarizing clamps to potentials positive to about -30 to -40 mV; 6) decreasing $[Na]_o$ enhances the I_{OS} but still this current does not appear at less negative potentials; 7) when a test clamp is applied at the peak of I_{OS} to potentials positive to about -30 mV, the current disappears; 8) with 10^{-6}M strophanthidin, current oscillations appear with depolarizing or repolarizing clamps; 9) the membrane resistance during I_{OS} increases. It is concluded that the repolarization initiates I_{OS} indirectly, that I_{OS} is due to a calcium-triggered release of calcium and that the released calcium induces I_{OS} by an electrogenic Na-Ca exchange.

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49.4

CELLULAR MECHANISM OF LIDOCAINE'S ANTIARRHYTHMIC ACTION IN SHEEP CARDIAC PURKINJE FIBERS. S-S. Sheu* and W.J. Lederer. Univ. of Maryland Sch. Med., , Baltimore, MD 21201

We examined the action of therapeutic concentrations of lidocaine (20uM) on the time-course of the changes of action potential duration (APD), twitch tension and intracellular sodium activity (a^i_{Na}) in sheep cardiac Purkinje fibers. The addition of lidocaine to the $4K_o$, $2Ca_o$ Tyrode's superfusion solution, resulted in a reduction of a^i_{Na} in fibers stimulated at 1 Hz. This reduction of a^i_{Na} was preceeded by a rapid reduction of APD. This observation, when combined with the experimental results of Eisner, Lederer & Sheu (J.Physiol, 1983, In Press) suggests that lidocaine first blocks a portion of the component of I_{Na} that contributes to the APD and it is this reduction of Na-influx that is responsible for the decline of a^i_{Na}. If a preparation was first exposed to the cardiotonic steroid strophanthidin (10^{-7}-10^{-5} M), a^i_{Na} is markedly elevated and arrhythmogenic transient depolarizations (TD) are observed. In many fibers the addition of lidocaine under such conditions leads to a similar rapid shortening of the APD and a gradual reduction of a^i_{Na}. The amplitude of the TDs is diminished as the APD is reduced and, as a^i_{Na} falls, there is a further reduction of the TD amplitude. We conclude that at least part of the anti-arrhythmic action of lidocaine (at therapeutic concentrations) must be due to a reduction of inward Na^+ current as reflected in the shorter action potentials and the fall of a^i_{Na}.

49.5

ON THE MECHANISM OF ACTION OF LOW Na SOLUTION ON THE PACEMAKER CURRENT. Hiroshi Kotake*, Cheng-I Lin* and Mario Vassalle. SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203.

It has been reported that the pacemaker current (Ip) disappears in the absence of $[Na]_o$, but not much is known about the mechanism of such an effect. Thus, in the present study, the role of an increased membrane conductance in Ip change induced by solutions with different Na or Ca concentrations was investigated by means of a two-microelectrode voltage clamp technique in cardiac sheep Purkinje fibers. The following results were obtained: 1) Reducing $[Na]_o$ to 80.8 mM increased the instantaneous current and decreased the amplitude of Ip to a slight degree. 2) When all the NaCl was omitted (and $[Ca]_o$ was lowered to 0.0245 mM), the instantaneous current was larger. At the same time, the membrane potential at which the current during the hyperpolarizing clamp was reversed shifted to a less negative potential. 3) Adding Ba (5×10^{-5}M) decreased the instantaneous current and restored an inward current during the hyperpolarizing clamp and the membrane potential at which the current was reversed. 4) Increasing $[Ca]_o$ to 8.1 mM increased the instantaneous current, induced an oscillatory current and reversed the current during the hyperpolarizing clamp. 5) Reduction of $[Ca]_o$ to 0.54 mM decreased the amplitude and slowed the time course of Ip. It is concluded that the disappearance of Ip in a low Na solution may be related to an increased potassium conductance that exaggerates depletion phenomena. (Supported by NIH grants HL27038 and HL17451).

49.6

EFFECTS OF ACETYLCHOLINE ON THE SINO-ATRIAL NODE OF NEONATAL AND ADULT GUINEA PIGS. Zeljko J. Bosnjak, and John P. Kampine. Medical College of Wisconsin and Wood VA Medical Center, Milwaukee, WI 53193

The purpose of this study was to examine the sensitivity of the neonatal (0-7 days old) and adult (over 5 weeks old) sino-atrial (SA) node to acetylcholine (Ach) by recording intracellular potentials from SA nodal fibers in vitro under identical conditions. Ach was introduced by switching to Kreb's superfusate for 15 minutes containing graded concentrations of the drug ($1X10^{-7}$ to $1X10^{-4}$M). Under these experimental conditions, the spontaneous rate of the SA node from the adult guinea pig decreased an average of 29% from the control, while the neonatal SA node exhibited a 63% decrease. During the introduction of Ach the following changes in the action potential were observed: the slow diastolic depolarization was markedly depressed with an average decrease in the phase 4 dV/dt from 139 mV/sec to 56 mV/sec, action potential duration was shortened from 70 ms to 42 ms while Ach-induced hyperpolarization has led to a pronounced increase in the phase 0 dV/dt of the action potential from 3 V/sec to 15 V/sec. These data indicate that the neonatal primary pacemakers in the SA node are more sensitive to the negative chronotropic effects of Ach than the adult SA node. (Supported in part by NIH grant GM 29641 and the Veterans Administration).

49.7

EFFECT OF ADENOSINE ANALOGS ON RAT VENTRICULAR AUTOMATICITY. L. J. Heller, L. J. Sunnarborg*, and R. A. Olsson. U Minnesota, Duluth, MN 55812 and U. So. Florida, Tampa, FL 33620.

Adenosine's interaction with extracellular membrane receptors that either inhibit (R_i) or activate (R_a) adenylate cyclase may account for many of the effects of adenosine in a variety of tissues. The availability of receptor-selective adenosine analogs permits identification of receptor types in biological systems. Previous studies have shown that adenosine suppresses rat ventricular automaticity. In the present study the relative potency of adenosine analogs was assessed in order to identify the type of receptor involved in the negative chronotropic effect. Isolated rat hearts with atrial tissue removed were perfused at constant flow via the aorta with modified Krebs-Henseleit solution. Varying doses of adenosine (ADO), S- or R-N^6-phenylisopropyladenosine (S-PIA, R-PIA) or 5'-N-ethylcarboxamideadenosine (NECA) were added to the perfusate and spontaneous beating rate was measured. The order of potency was R-PIA > NECA > S-PIA > ADO with mean ED_{50}'s of 7.8×10^{-9} M, 4.2×10^{-8} M, 7.2×10^{-8} M, and 4.4×10^{-6} M respectively. S-PIA was only a partial agonist with a maximum effect of 40% which may suggest receptor-stereoselectivity. Theophylline added to the perfusate competitively inhibited the negative chronotropic effects of adenosine and its analogs, producing 5 to 10 fold increases in the ED_{50}'s. These data suggest that adenosine's effect on ventricular automaticity may be mediated by R_i receptors.

49.8

DIRECT NEGATIVE CHRONOTROPIC EFFECT OF LOW DOSE ATROPINE ON SINUS NODE AUTOMATICITY. G.E. Musgrave. Dept. of Medicine and Pharmacology, Medical College of Virginia and V.A. Medical Center, Richmond, VA 23249

Low dose atropine (ATR) is associated with bradycardia in man and animals. Although this negative chronotropic effect (NCE) has been attributed to both central and peripheral effects of ATR, the mechanism for peripheral effect has not been determined. Experiments were conducted using the isolated perfused canine sinus node, a model devoid of extra cardiac influences, to determine whether the NCE is caused by potentiation of the action of acetylcholine (ACh), or by a direct depressant action of ATR on sinus node automaticity. A log concentration chronotropic response curve for the effect of graded concentrations of ATR (12.5-400 pg/ml) on sinus node automaticity was determined. Log concentration-response curves for ACh (16-1020 ug/ml) in the absence and in the presence of ATR (50 pg/ml) were then constructed. ATR alone elicited a biphasic response pattern. Sinus rate decreased by a maximum of 12 depolarizations per minute at 50 pg/ml and subsequently increased to the baseline at 125 pg/ml. Further increments in ATR concentration caused progressive sinus acceleration. No potentiation of the NCE of ACh was observed in the presence of the concentration of ATR associated with maximal sinus slowing. These results suggest that there is a peripheral effect of low concentration ATR that is, in part, responsible for the bradycardia observed in man and animals. The mechanism for the NCE of low concentration ATR seems to be a non specific depressant effect of ATR on sinus node automaticity.

49.9

DIFFERENTIAL PARASYMPATHETIC INNERVATION OF CONDUCTILE TISSUE IN THE CANINE MYOCARDIUM. Jeffrey L. Ardell* and Walter C. Randall. Loyola University Medical Center, Maywood, IL 60153

Anatomical projections of parasympathetic nerves onto the canine heart have been described (*Fed. Proc. 42*: 1112, 1983), allowing functional denervation of the sinoatrial (SA) node while leaving the atrioventricular (AV) nodal parasympathetic supply intact. Utilizing selective intrapericardial surgical ablation procedures, we further examined the predominant vagal pathways innervating the SA and AV nodal regions. From a transthoracic approach (T4-T5), the heart was suspended in its pericardium allowing exposure of the superior vena cava (SVC), inferior vena cava (IVC), pulmonary artery (PA), and pulmonary vein (PV) complex. Right (RCV) and left (LCV) cervical vagal trunks and right (RSS) and left (LSS) ansae subclavia were electrically stimulated (10-20 Hz-5 msec-5v) before and after each denervation step. Nerve stimulations were repeated with and without atrial pacing. Rt vagal pathways to SAN included those accompanying the Rt superior PV, Rt middle PV, and SVC-azygos complex. Input to SAN from the Lt vagus courses by way of Lt superior PV, and along superior surface Lt atrium via the Lt PA. AVN input from RCV was via Rt PV complex while that from LCV was eliminated by dissection at the root of the Rt PA. These denervation procedures preserved major sympathetic inputs from the left side, and at least 50% of the right sympathetics to the conductile tissues of the canine heart. (Supported by NIH grants HL 27664 and HL 27595)

49.10

MAPPING BEAT TO BEAT EXCITATION OF THE HEART DURING ARRHYTHMIAS. Abigail Tischler*, David Auslander*, Penelope Boyden*, Reidar Bornholdt* and Andrew L. Wit. Department of Pharmacology, Columbia University, New York, N.Y. 10032.

Mapping excitation of the heart during arrhythmias is often necessary to elucidate the mechanism (reentry or automaticity). Accurate excitation maps require that electrograms be recorded from a large number of sites; studies on mechanisms of nonsustained and multiformed arrhythmias require that all electrograms be recorded simultaneously. We have designed and implemented a recording system which fulfills these requirements; two hundred bipolar or unipolar electrograms can be recorded simultaneously. Electrograms are preamplified by 200 programmable gain amplifiers controlled by a microprocessor, enabling automatic gain control. Amplified signals stream into a multiplexing/analog to digital conversion system. Sampling frequency of the multiplexer is 2 kHz per data channel. Multiplexed and digitized signals in the form of eight bit, parallel TTL, binary words are led into an Ampex high bit rate (HBR) pulse code modulation (PCM) tape recorder. Activation times at each recording site are determined with a PDP 11/34 computer. Unix controlled software demultiplexes digitized electrograms and displays them 10 at a time on a Tektronix 4012 graphics terminal. Moments of activation are marked by a thumbwheel cursor, and then are displayed as isochronal activation maps. Using this system we have produced detailed activation maps showing reentrant excitation during ventricular tachycardia and during atrial flutter in canine hearts (Supported by Grant HL 12738).

49.11

EFFECTS OF REVERSIBLE COOLING ON REENTRANT TACHYCARDIAS IN CANINE INFARCTION. N. El-Sherif, R. Mehra*, W.B. Gough*, R. Zeiler* Downstate and VA Medical Centers, Brooklyn, N.Y.11209

Monmorphic ventricular tachycardias (MVT) were reproducibly induced in 13 dogs 3-5 days following ligation of the left anterior descending coronary artery. Isochronal maps of ventricular activation were constructed from 64 close bipolar electrograms utilizing a computerized multiplexing technique. The induced tachycardias were due to reentrant circuits (RC) in the surviving epicardial layer overlying the infarction. The RC had a figure of 8 activation pattern in the form of 2 wave fronts around 2 arcs of functional conduction block that coalesced into a slow common reentrant wave front (CRWF) before reexciting an area on the other side of the arcs of block. Cooling (0-5°C for 10-30 sec by a Spembley-Amoils TCC 42 cryounit) resulted in characteristic changes in conduction of the reentrant wave front. The RC could be consistently interrupted when cooling was applied to the distal part of the CRWF proximal to the site of earliest reactivation. Localized cooling of the site of earliest reactivation usually failed to interrupt reentry because the CRWF reactivated other sites close to the original reactivation site. The study: 1) Fulfills Mines' criteria that circus movement reentry is the mechanism of the induced rhythms in this canine experimental model. 2) Identifies the critical site along the reentrant circuit where cryothermal ablation (or surgical interruption) of reentrant activation could be successfully accomplished.

49.12

VENTRICULAR ARRHYTHMIAS RESULTING FROM INTRACORONARY ALCOHOL INFUSION IN CONSCIOUS DOGS. K.J. Dormer, R. Mahnken#, B. Watrous# and L. Goforth#. Univ. of Okla., Okla. City, OK 73190

Sudden cardiac death from ventricular arrhythmias often occurs in patients who are asymptomatic for alcohol heart muscle disease (AHMD). This study seeks to model AHMD and associated arrhythmias in the conscious dog by aqueous ethanol infusion into the patent left circumflex coronary artery (LCA). Anesthetized mongrels (halothane 1-1.5%, n=6) 19-30 kg were instrumented by sterile proceedure to provide left ventricular pressure (LVP), LCA flow velocity (Doppler), ECG, coronary sinus sampling and LCA infusion. A 17% ethanol solution in normal saline (1500 units/ml heparin) was infused (1-8 ml/min) twice daily for 2 weeks into the LCA while the ECG, LVP, $dLVP/dt$ and heart rate (HR) were recorded. Sinus blood alcohol concentrations during infusion ranged from 150 to 458 mg%. During infusion LVP and $dLVP/dt$ decreased 5-17%, LCA flow velocity increased 20-35% but the HR response was variable due to arrhythmias which persisted for days afterward. First, second and third degree heart block, asystole, ventricular premature contractions (VPC), tachycardia and ventricular fibrillation have been observed during infusions. Ventricular tachycardia often persisted or was readily evoked and preceded 2 instances of sudden death. Histopathology indicated posterior wall injury but not infarction was associated with arrhythmias. These data indicate that sudden death by alcohol-induced lethal arrhythmias may be modeled in conscious dogs. Supported by HL 20857.

50.1

EFFECTS OF UNILATERAL AND BILATERAL SYMPATHETIC STIMULATION ON CEREBRAL BLOOD FLOW (CBF) DURING NORMOCAPNIA. David W. Busija. Dept. Physiol. & Biophysics, Univ. Tenn. Ctr. Hlth. Sci., Memphis, Tennessee 38163

The distribution of sympathetic innervation to cerebral vessels is primarily ipsilateral, although there is overlap in basal and medial areas of the brain. During profound cerebral vasodilation by hypercapnia, bilateral activation of sympathetic nerves reduces CBF more than unilateral stimulation (Circ. 60: IV-232, 1981). However, the functional importance of bilateral innervation has not been examined at lower levels of CBF. In the present study, effects of unilateral and bilateral stimulation of sympathetic nerves were compared during normocapnia ($PaCO_2$ = 30-35 mmHg) in anesthetized rabbits. CBF was measured using 15 μm microspheres during control and after 1-5 minutes of electrical stimulation (8-10 Hz) of one or both superior cervical ganglia. In the former group (N=5), CBF was 31±4 ($\bar{X}$±SEM) ml/min per 100 g and unilateral stimulation did not change blood flow. In the latter group (N=7), CBF was 33±4 ml/min per 100 g and bilateral stimulation reduced CBF by 25±5% ($P<0.05$, compared to unilateral stimulation). Also, bilateral stimulation, but not unilateral stimulation, reduced blood flow to cerebral grey matter (30±6%), cerebral white matter (35±11%), brainstem (19±6%), and cerebellum (17±6%) ($P<0.05$, compared to unilateral stimulation for all comparisons). In summary, during normocapnia, bilateral sympathetic stimulation reduces CBF more than unilateral stimulation.

50.2

THE FETAL CARDIOVASCULAR(CV) AND CATECHOLAMINE (CAT) RESPONSE TO HYPOXEMIA(HYP) FOLLOWING CHEMICAL SYMPATHECTOMY(CS). William M. Sischo* and Alan B. Lewis*, (Spon: T.G. Keens). University of Southern California School of Medicine, Childrens Hospital of Los Angeles, Department of Pediatrics, Los Angeles, CA 90027.

The normal fetal response to HYP is cardiovascular hypertension. This response is the result of both circulating CAT and increased sympathetic neuronal activity (SYM). However, the relative contribution of these two factors in controlling the CV response to HYP is unclear. The effect of CS on CV responses to HYP was investigated in fetal lambs in utero. CS was produced by infusion of 6-hydroxydopamine and confirmed by the lack of a CV response to tyramine. Heart rate (HR), blood pressure (BP), plasma norepinephrine (NE), and plasma epinephrine (E) were compared between CS fetal animals and non-CS fetal animals.

	N	PO_2-Hyp	HR	BP	NE pg/ml	E pg/ml
non-CS	12	11.4±.5	100±3	86±3 58±2	2416±419	2017±749
CS	8	12.6±.3	118±4	86±3 61±2	1550±261	244±42* *p<.05

The fetal CV and NE response to HYP was similar in CS and non-CS animals, but the CS animals had a significantly reduced E level in response to HYP. These results demonstrate that the CS fetus is able to mount an appropriate response to HYP despite the absence of SYM and a reduced level of circulating E. (Research supported by the Norris Foundation).

50.3

PROPRANOLOL INCREASES OXYGEN AVAILABILITY DURING HYPOXIA IN DOGS. H.J. Khambatta, J.G. Stone,* E. Khan.* Dept. of Anesth., Coll. of Phys. & Surg., Columbia Univ., New York, N.Y. 10032.

Propranolol has been reported to shift the O_2 dissociation curve to the right. In a previous report we studied the effect of propranolol on O_2 transport in hypoxic dogs. We then demonstrated that $\dot{V}O_2$ during hypoxia (measured by examining expired gas with a mass spectrometer and an automatic spirometer) was higher in the propranolol-treated group as compared to the untreated one. O_2 extraction ($a\text{-}\bar{v}\ O_2$) during hypoxia was also higher in the propranolol-treated group, but O_2 content was obtained indirectly using standard nomograms. The purpose of this study was to validate these findings by measuring O_2 content directly with a Lexington O_2 content analyzer. Cardiac output was determined by thermodilution and $\dot{V}O_2$ calculated. The hypoxic mixture contained 10% O_2. Ten dogs were studied, five of whom received propranolol, 0.5 mg/kg, prior to hypoxia. In the untreated group during hypoxia, PaO_2 decreased from 91 ± 5 to 29 ± 2 mmHg, $\dot{V}O_2$ decreased from 126 ± 4 to 100 ± 3 ml/m^2/min and $a\text{-}\bar{v}\ O_2$ from 3.5 ± 0.2 to 2.5 ± 0.2 vol %. In the propranolol-treated group, PaO_2 decreased from 93 ± 1 to 29 ± 2 mmHg, $\dot{V}O_2$ decreased from 124 ± 4 to 110 ± 3 ml/m^2/min and $a\text{-}\bar{v}\ O_2$ from 4.0 ± 0.6 to 3.3 ± 0.3 vol %. The data show that for the same degree of hypoxia, with similar inspired and arterial O_2 tension, both oxygen consumption and extraction were higher in the propranolol-treated group. We believe that propranolol shifts the O_2 dissociation curve to the right and thus increases O_2 availability and transport.

50.4

EFFECT OF CEREBELLECTOMY ON THE INCREASE IN CEREBRAL BLOOD FLOW AND THE PRESSOR RESPONSE DURING HYPOXIA. L. O. Lutherer and D. G. Davies. Department of Physiology, Texas Tech Univ. Health Sci. Center, Lubbock, Texas 79430.

Acute exposure to hypoxia induces a pressor response and increase in cerebral blood flow. Cerebellectomy or specific lesions of the fastigial nucleus (FN) interfere with many pressor responses. Electrical stimulation of the FN produces both a pressor response and an increase in cerebral blood flow. This study was undertaken to determine if the FN mediate these responses during hypoxia. Cats of either sex were anesthetized with cloralose-urethane and instrumented for measurement of arterial pressure and cerebral blood flow (CBF) using the radioactive microsphere technique. Each animal was exposed to 10% O_2 for a 10 min period both before and after cerebellectomy (Cx) Ventilation was adjusted to maintain a constant end-tidal Pco_2 throughout the experiment. Cerebellectomy did not affect resting arterial pressure at 21% O_2 (119+6 vs 114+8 mmHg) nor did it block the pressor response to hypoxia (= 24+4 vs 25+6 mmHg). In 4 of the 6 cats Cx led to a decrease in CBF during normoxia (mean decrease in CBF for all animals was 7.3%). Furthermore, during hypoxia the increase in CBF observed in Cx animals was only 34% of that seen in intact cats. These results suggest that the FN play no role in the pressor response to this level of hypoxia, but may mediate a portion of the increase in CBF seen with hypoxia. This research supported by NIH grant HL 25984.

50.5

REGIONAL VASCULAR CONDUCTANCES DURING ARTERIAL HYPOXIA: ROLE OF CHEMOREFLEX HYPERVENTILATION. George J. Crystal, H. Fred Downey, Fouad A. Bashour, and Judith M. Metzger. University of Texas Health Science Center, Dallas, Texas 75235

It is established that chemoreflex hyperventilation affects vasomotor responses during arterial hypoxia (AH), but its regional influence requires elucidation. Thus, changes in regional vascular conductance were compared in free-breathing (FB; n=7) and artificially-ventilated (AV; n=7), chloralose-anesthetized dogs after 5 min AH, induced with 5% O_2 gas. Regional flows (15 μ microspheres) were used to calculate conductances. In FB, AH reduced arterial PO_2 to 16.9±0.9 mmHg and PCO_2 to 23.3±3.5 mmHg, and raised pH to 7.57±0.04. In AV, AH reduced PO_2 similarly, but PCO_2 and pH remained near control. % changes in regional vascular conductance (table) indicate that, during severe arterial hypoxia, chemoreflex hyperventilation 1) nullifies dilation in brain and constriction in kidney and muscle, 2) attenuates constriction in spleen, pancreas, and skin, and 3) has no influence on vasculature in myocardium, duodenum, and liver. Supported by NIH (HL-21657; HL-25897) and AHA, TX Affiliate.

	FB	AV
Cerebral Cortex	+39±30†	+290±53*
Myocardium (LV)	+451±34	+531±140
Kidney Cortex	-6±8	-70±11*
Spleen	-44±4	-61±7*
Pancreas	-47±15	-71±7
Duodenum	-29±9	-33±12
Liver (Hep. a.)	+2±20	-5±30
Sk. muscle	+23±36	-92±3*
Skin	-54±6	-79±5*

†Mean ± SE; *$P< 0.05$, FB vs AV

50.6

5'-NUCLEOTIDASE ACTIVITY IN CANINE ADIPOSE TISSUE. Sharon E. Martin, Lisa M. Schwartz*, and Emma L. Bockman. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814

Studies of factors affecting adenosine production in vivo have focused primarily on changes in cellular metabolism that result in changes in concentrations of substrate, inhibitors, and/or stimulators of 5'-nucleotidase (5-N). The present study indicates that 5-N activity, measured in vitro under optimal conditions, may also be affected by events occurring in vivo. Vascularly isolated, inguinal fat pads of female dogs were treated with saline (control; N=4), norepinephrine alone (NE; N=5), theophylline alone (THEO;N=3) or NE-THEO combined (N=3). Tissues were frozen in situ, extracted with acetone, homogenized in 50 mM phosphate buffer (PB), and dialyzed for 24 hr against PB. Tissue supernates were incubated in the presence or absence of AOPCP (5-N inhibitor). 5-N activity (AOPCP-suppressible) was calculated as nmoles AMP converted/min/g. Control activities averaged 60±12 ($\bar{X}$±SE). Tissues treated with NE had activities (18±5) significantly lower than control, and those treated with THEO had activities (106±3) significantly higher than control. Activities in NE-THEO tissues (72±9) were not different from control. Because tissues were treated with acetone and dialyzed, it is unlikely that NE or THEO was present at the time of 5-N assay. Thus, drug effects on the enzyme are apparently occurring in vivo. These findings indicate that changes in enzymatic activity, as well as substrate concentrations, may have to be taken into account. (Supported by USUHS Grant R07639)

50.7

OUABAIN BLOCKS ACTIVE HYPEREMIA IN GRACILIS (WHITE) BUT NOT SOLEUS (RED) MUSCLE OF CATS. Emma L. Bockman. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814

The release of K^+ correlates with active hyperemia in gracilis but not soleus muscles of cats (Physiologist 24:76, 1981). The purpose of the present study was to determine whether ouabain, which inhibits the Na-K pump, affects active hyperemia. Muscles were vascularly isolated, perfused under free-flow conditions, and stimulated to contract isometrically. Ouabain (60-70 µg/kg;i.v.) administration resulted in a decrease in resting blood flow (in ml/min/100g) from 4.1±1.0 to 2.2±0.2 in gracilis (N=4) and from 6.5±0.7 to 4.2±0.5 in soleus (N=4). After 15 min of stimulation, blood flow was 2.9±0.6 in gracilis and 18±6 in soleus. In control preparations not receiving ouabain, stimulation increased blood flow from a control value of 6.2±0.4 to 21±5 in gracilis (N=4) and from 6.4±1.4 to 22±5 in soleus (N=4). Initial muscle performance (developed force x frequency) was similar in ouabain-treated and control preparations. Gracilis muscles receiving ouabain fatigued such that performance after 15 min of contraction was 14±2% of the initial value. In soleus, performance was 76±8% of the initial value in ouabain-treated muscles and 85±3% in control muscles. These findings indicate that ouabain administration blocks active hyperemia in gracilis but not in soleus muscles and are consistent with a role for K^+ as a mediator of active hyperemia in gracilis but not soleus muscles of cats. (Supported by USPHS Grant HL26345)

50.8

MITOCHONDRIAL DISTRIBUTION IN HAMSTER CREMASTER MUSCLE. B. Klitzman, Dept. of Physiology & Biophysics, Louisiana State University School of Medicine, Shreveport, Louisiana 71130.

In virtually all models of O_2 transport to tissue, O_2 is assumed to be consumed homogeneously throughout the tissue. Since most O_2 is consumed by discrete mitochondria, the assumption of consumption homogeneity must be incorrect. The distribution of mitochondria has been described qualitatively for skeletal muscle as being dense near capillaries (Hoppeler et al, 1981). Although this would decrease the diffusion distance for O_2, it would increase the diffusion distance for ATP. Since ATP has a lower diffusion coefficient than O_2, this would not seem to be an optimal location for mitochondria. The mitochondrial distribution in heart has been described quantitatively as being sparse near capillaries and increasing radially, reaching a peak density at 8µm from the capillaries (Kayar and Banchero, 1982). Using electron microscopy and sterological techniques, the mitochondrial volume density in hamster cremaster muscle was determined as 3.16%. Although there were a few dense mitochondrial aggregates in close proximity to capillaries, most mitochondria are located on the I-bands of sarcomeres, evenly distributed throughout the muscle cells. A consistent preferential distribution toward the capillaries was not observed. This described mitochondrial distribution would significantly alter the predicted O_2 tension profile from that predicted assuming homogeneous oxygen consumption. (Supported by the American Heart Association and the American Heart Association, Louisiana Affiliate)

50.9

PATTERN OF PERFUSED CAPILLARIES IN RAT GASTROCNEMIUS. S.R. Kayar, A.J. Lechner and N. Banchero. Univ. Colorado Medical School, Denver, CO 80262.

We studied the gastrocnemius of rats to determine the density and spatial arrangement of perfused capillaries after different time intervals following injection of a plasma-carried fluorescent dye. A bolus of Thioflavine S, which marks the endothelium of vessels, was injected into the aorta via a carotid catheter. After 10, 15 or 30 sec, the gastrocnemius was removed and flash-frozen. Muscle cross-sections 10-15 µm thick were transilluminated with blue light (430nm), examined for fluorescence at 530nm, and photographed. The same muscle sections were then treated by the myosin ATPase method to stain all capillaries, and rephotographed under white light. After 10 sec, there were patches of tissue that contained fluorescent capillaries surrounded by areas with no fluorescence. Within the patches with fluorescence, 30% of all capillaries were fluorescent and their arrangement was ordered (Kayar et al., Microvasc. Res. 24:326, 1982). After 15 sec, 40% of all capillaries were fluorescent, they appeared evenly distributed across the muscle section, and their arrangement was ordered. After 30 sec, all capillaries were fluorescent. Thus within one mean transit time through these tissues, all capillaries were perfused for some time. These data indicate that flow to capillaries is intermittent and that the pattern of perfused capillaries is always ordered, suggesting that control mechanisms permit complete perfusion over time. Supported by NIH-HL18145 and HL06527.

50.10

REGIONAL VARIATIONS OF HEMATOCRIT IN DOGS. R.Y.Z. Chen, R.D. Carlin, K.M. Muraszko* and S. Chien. Columbia Univ., P and S, NY, NY 10032 and Fairleigh Dickinson Univ., Hackensack, NJ.

Regional distribution of red cell volume (CV_r) and plasma volume (PV_r) in various organs were determined in seven pentobarbitalized dogs. ^{51}Cr-RBC and ^{125}I-albumin were injected 30 min and 10 min, respectively, prior to sacrificing the animal with intravenous KCl solution. Organs were immediately removed following ligation of major vessels, fixed for 24 hrs, dissected, weighed, and counted for radioactivities. CVr and PVr (ml/100g) were calculated from the ^{51}Cr and ^{125}I activities in tissues and blood, and the arterial hematocirt (Ha, microcentrifugation, corrected for plasma trapping), which averaged 36.0 ± 1.5 percent. Regional hematocrit (Hr) was calculated as 100CVr/(CVr+PVr). F_{cells} factors (=Hr/Ha) for various organs were as follows:

	Mean±SEM		Mean±SEM
Lung	1.35±0.10	Cerebral cortex	0.92±0.06
Liver	1.44±0.06	Endocardium	0.68±0.03
Spleen	1.27±0.07	Epicardium	0.60±0.03
Choroid plexus	1.79±0.10	Adipose tissue	0.55±0.07
Pancreas	1.16±0.12	Kidney	0.44±0.08
Omentum	0.49±0.06	Skeletal muscle	0.69±0.06
Mesentery	0.54±0.09	Parotid gland	0.70±0.04

These results indicate that there is a remarkable regional variation of Hr which may be related to microvascular architecture and flow conditions. (Supported in part by NHLBI Grants HL07114 and HL16851).

50.11

MICROVASCULAR HEMODYNAMICS IN SYSTEMIC HEMOCONCENTRATION AND DILUTION. H. H. Lipowsky, J. C. Firrell*, S. Usami and S. Chien. Dept. of Physiology, College of Physicians and Surgeons, Columbia Univiversity New York, N.Y. 10032.

Intravascular pressure, red cell velocity and microhematocrit were measured in the mesenteric microcirculation (cat) during successive isovolemic exchange of packed red cells or plasma from donor animals. Regional resistance, R, was calculated as the pressure drop from paired arteriole to venule divided by arteriolar flow, $\Delta P/Q$, for arcuate microvessels 30-55 µm in diameter. Red cell volumetric flux was calculated as the product of bulk flow and microhematocrit, QHmicro. These parameters were examined as a function of the value of systemic hematocrit normalized with respect to its control (pre-exchange) value, H* = Hsys/Hsys-con, and revealed: (1) R/Rcontrol increased from 0.3 to 2.8 as H* ranged from 0.2 to 2.0, with a plateau of relatively constant magnitude in the range $0.7 < H^* < 1.4$., (2) arteriolar flow decreased monotonically as H* increased, (3) arteriolar Hmicro followed H* linearly, and (4) arteriolar QHmicro varied as an inverse parabolic-like function of H*, with a maximum value occurring in the range $0.8 < H^* < 1.3$. For H* below or above this range, QHmicro fell dramatically, supporting the concept of an optimal systemic hematocrit which maximizes red cell flux at the microvascular level. (Supported by NIH Research Grants HL-16851, HL-25305 and HL-28381, RCDA HL-00594 for HHL and Traning Grant HL-07114 for JCF).

50.12

POWER DISSIPATION: A NETWORK APPROACH TO PERIPHERAL VASCULAR RESISTANCE. Jeffrey L. Borders* and Harris J. Granger. Texas A&m Univ., College Station, Tx. 77843.

Attempts to identify regions of peripheral vascular resistance have been hampered by the complex mesh that the microcirculation presents. Previous work has attempted to isolate sites of resistance by examining pressure profiles, mean diameters, and counting vessels. These techniques do not consider the network properties and their influence. The interpretation of their findings strongly depends on hypothetical network models. This study examines a network independent property, power dissipation - Pd, as a measure of network resistance. Since Pd is network independent, problems with vessel classification, identification, and sampling techniques are unimportant.

Resistance changes associated with the onset of hypertension were studied using this parameter in WKY and SHR rats in the cremaster muscle. The animals were weight matched with an equivalent age of 6-8 weeks. Muscle flow was reduced in the SHR group from 11.6 - WKY to 9.2 - SHR, ml/min-100g. Despite this reduction, power dissipation was elevated over a wide range of vessel segments. Pd was significantly elevated in arterial vessels showing flows from .08 to 80 nl/sec. Venous Pd was significantly elevated for vessels with flows >8 nl/sec. The elevated Pd is due to a network averaged reduction in cross-sectional area. The reduction occurs from a reduced mean diameter rather than rarefaction of vessel segments.

Supported by NIH HL-25387 and HL-06576.

51.1

CAROTID BODY CHEMOSENSORY FUNCTION IN CHRONIC HYPEROXIA. K. H. McGregor*, M. Pokorski*, A. Mokashi* and S. Lahiri. University of Pennsylvania School of Medicine, Phila.,PA 19104

Factors such as high blood flow, possibly a large tissue PO_2 gradient, high oxygen consumption, and catecholamine metabolism are expected to predispose the carotid body to oxygen toxicity. In our preliminary studies to develop a suitable animal model for studies of carotid body oxygen toxicity, we exposed 8 cats to 50% O_2 at sea level for 15 to 24 days. The cats were anesthetized with α-chloralose and surgically prepared. The activity of a single or a few afferent fibers from the carotid body were recorded throughout an experiment. The steady-state activity at 3 levels of $PaCO_2$ (range 29 to 66 torr) and at 2 to 5 levels of PaO_2 (30 to 400 torr) were measured. The carotid bodies were fixed by perfusion with gluteraldehyde solution and the ultrastructure was studied. The arterial blood $PaCO_2$-pH relationship and hematocrit did not change from the normal control values. In general the chemosensory activity in hyperoxia at a given arterial $\{H^+\}$ was greater in the chronically hyperoxic group than the control. The responses to hypoxia did not change significantly, although the shape of the response curve changed, indicating a depressed pattern. There were no obvious ultrastructural changes. We conclude that oxygen poisoning was not clearly expressed in the structure and function of carotid body in the chronically hyperoxic (PaO_2 = 200 Torr) cats.
(Supported in part by NIH grants HL--08899 and HL-19737).

51.2

INTRA-CAROTID INFUSION OF NICOTINE ENHANCES VENTILATORY RESPONSE TO HYPOXIA. L.-Y. Lee. Dept. of Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536

The effect of intra-carotid infusion of nicotine (N) on the ventilatory ($\dot{V}_E$) response to hypoxia was studied in 8 anesthetized dogs (avg. wt. 23.8 kg). Three levels of arterial oxygenation were induced for 8-10 min periods in each dog: hyperoxia (PaO_2 > 340 mmHg), mild hypoxia (MH) (58-79 mmHg) and relatively severe hypoxia (SH) (42-53 mmHg). Catheters were inserted bilaterally into lingual arteries and advanced retrogradely until the tips were 10 cm proximal to the carotid bifurcation. N solution (0.0015%) was infused at a constant rate (1.23 ml/min each side) for 1 min after the isocapnic $\dot{V}_E$ response to each step change in PaO_2 had reached a steady state. Infusion of N increased $\dot{V}_E$ from 11.6 ± 1.5 (M ± SEM) to 25.2 ± 5.9 l/min during SH, from 5.4 ± 0.4 to 13.0 ± 3.6 l/min during MH and from 5.4 ± 0.4 to 9.8 ± 1.6 l/min during hyperoxia. The $\Delta\dot{V}_E$ induced by N was greater during SH than during either MH or hyperoxia in each dog tested. Ligation of both internal carotid arteries in 3 dogs either increased or did not affect the response, while denervation of carotid bodies in 2 dogs completely abolished the effect of N. These results indicate that: 1) intra-carotid infusion of N enhances $\dot{V}_E$ response to isocapnic hypoxia, and 2) the enhancing effect may result from a positive interaction of these two stimuli at carotid body chemoreceptors. (Supported by grants from Kentucky Tobacco Research Board 4F012 and NIH HL 25089.)

51.3

VENTILATION IN THE CAROTID SYMPATHECTOMIZED AND DENERVATED AWAKE CAT. P.C. Szlyk and D.B. Jennings. Queen's University, Department of Physiology, Kingston, Ontario, Canada K7L 3N6.

In 4 awake cats, ventilation ($\dot{V}_E$), tidal volume (V_T), and frequency (f) were measured with a plethysmograph during successive inhalation of 0%, 2%, and 4% CO_2 in air over 90 min. In intact cats breathing air, $\dot{V}_E$ ranged from .11 to .22 l/min·kg, V_T from 3.2 to 7.5 ml/kg, and f from 18 to 56/min. After bilateral sympathectomy (SD) of the carotid bodies, the range of $\dot{V}_E$ observed during air breathing was similar to that of intact cats, but V_T increased and f decreased at a given $\dot{V}_E$. Carotid sinus nerve section (CSX) had no additional effect on f, V_T, or $\dot{V}_E$ while breathing air compared to SD. Inhalation of 2% and 4% CO_2 resulted in a progressive increase in $\dot{V}_E$ in intact, SD and CSX cats. In intact cats breathing CO_2, a decrease in f and an increase in V_T were observed at any $\dot{V}_E$ level relative to air. In comparison to the intact response to CO_2, SD resulted in a greater reduction in f and increase in V_T at each level of $\dot{V}_E$. Surprisingly, CSX had no additional effect on the range of $\dot{V}_E$ during CO_2 inhalation, but tended to increase f and decrease V_T relative to the SD responses. Our data suggest that in the awake cat, the carotid bodies regulate respiratory pattern rather than the level of $\dot{V}_E$. This role of carotid chemoreceptors appears to be, in large part, determined by their sympathetic innervation. (supported by Medical Research Council and Canadian Heart Foundation).

51.4

THE EFFECT OF AGE ON THE LARYNGEAL RESPONSE TO HYPOXIA AND HYPERCAPNIA. Thomas V. McCaffrey and Daniel J. Blum.* Mayo Clinic, Rochester, Mn. 55905

In order to define the effect of maturation on the sensitivity of chemoreceptor control of laryngeal resistance anesthetized dogs ranging in age from one day to adulthood were studied. Laryngeal resistance was derived from constantly monitored measurements of laryngeal flow and laryngeal pressure. Simultaneously tidal volume, respiratory flow, respiratory rate, end expiratory CO_2, inspired O_2, and blood pressure were monitored. Laryngeal resistance at FIO_2 of .05, .10, .20, .50 and 1.0 and $FECO_2$ of .05, .06, .07, .08, .09, .10 were compared on the basis of age (grouped at one day, 10 days, 30 days, 50 days, 75 days and adult). With incremental decreases in FIO_2, laryngeal resistance decreased from 100 percent of control at FIO_2 of .20 to 70 percent of control at FIO_2 of .05 in one-day-old pups as compared to a decrease from 100 percent at FIO_2 of .20 to 50 percent of control at FIO_2 of .05 in adults. As $FECO_2$ increased from .06 to .09 laryngeal resistance decreased from 100 percent at 85 percent of control in one-day-old pups, whereas a decrease from 100 percent to 40 percent of control was noted over the same range of $FECO_2$ in adults. The relationship between laryngeal resistance and both $FECO_2$ and FIO_2 is curvilinear. The slope of these relationships increases with increasing age in dogs. This study demonstrates that in the process of maturation laryngeal resistance changes in response to O_2 and CO_2 are enhanced.

51.5

VENTILATORY RESPONSE TO CO_2 BELOW EUPNEA IN ANESTHETIZED DOGS W. Hwang*, D. Sedlock*, S.M. Yamashiro and F.S. Grodins. Biomedical Engineering, USC, Los Angeles, CA 90089-1451.

The shape of the V_e-CO_2 response curve below eupnea remains controversial. We measured hyperoxic CO_2 responses below and above the eupneic level in pentobarbital anesthetized dogs. Pa_{CO_2} below eupneic level was controlled using extracorporeal gas exchangers and in separate dogs using high frequency ventilation (HFV). CO_2 responses above eupnea were measured by CO_2 inhalation (3%-5%). Blood from the jugular veins and inferior vena cava was shunted through gas exchangers to the superior vena cava. No significant difference in the CO_2 response slopes above and below eupnea was found in six dogs using gas exchangers although high variability may have obscured any difference. Airway CO_2 unloading was performed by HFV which allowed simultaneous breathing in an attempt to reduce variability. The peak sinusoidal airflow at 5Hz was adjusted to produce apnea while inhaling 100% O_2. Mean tracheal pressure was 0 ± 1 cm H_2O. In five dogs, CO_2 responses to HFV and conventional inhalation above eupnea and to HFV below eupnea were not significantly different in either slope or intercept. In two animals the slope below was twice that above, and a tendency for periodic breathing near apnea was observed. In general, lower variability of responses was observed during HFV permitting a closer examination of response characteristics below eupnea.
(Supported by: NIH grants HL16390, HL07012, GM23732).

51.6

RESPONSE OF AERIAL VERSUS AQUATIC GAS EXCHANGE TO HYPERCAPNIA IN AMPHIUMA TRIDACTYLUM, SIREN LACERTINA, AND SIREN INTERMEDIA. Beth A. Braun. Univ. of Oklahoma, Norman, OK 73019

In animals with more than one respiratory surface, the capacity for increased CO_2 release is an important factor in the control of blood acid-base balance. This study compares the limits of aerial versus aquatic contribution to CO_2 loss in one bimodal (A. tridactylum) and two trimodal (S. lacertina S. intermedia) breathers. Acclimated to 20°C and LD 12:12 photoperiod, animals were subjected to normoxic normocapnia in both media until steady-state gas exchange was established; CO_2 was then introduced into one phase. Under aquatic hypercapnia (P_{CO_2} 40-100 torr), pulmonary CO_2 elimination increased over control values by 35X in Amphiuma, 14X in S. lacertina, and 2.1X in S. intermedia. With aerial hypercapnia (3% or 6% CO_2) cutaneous CO_2 loss fell by 1% of control values in Amphiuma and cutaneous plus branchial CO_2 loss increased by 26% in S. lacertina and 3.9% in S. intermedia. Pulmonary versus aquatic respiratory exchange ratios (R) increased by 543% vs. 88% in Amphiuma, 1111% vs. 14.8% in S. lacertina, and 391% vs. 60% in S. intermedia. These results demonstrate 1) a much greater capacity for increased CO_2 loss across the lung than across skin or skin and gills and 2) the ability of the lung to eliminate excess CO_2 at levels above those encountered in nature. Thus, pulmonary gas exchange does not limit the ability of these animals to control blood acid-base balance during hypercapnia in the field.

51.7
EVIDENCE FOR A PERIPHERAL VASCULAR ORIGIN OF THE ISOPROTERENOL-INDUCED HYPERPNEA IN THE DOG. A. Oren*, A. Huszczuk*, M. Pokorski*, P.H. Ferrer*, B.J. Whipp, and K. Wasserman. Harbor-UCLA Medical Center, Torrance, CA 90509.

An intravenous bolus injection of Isoproterenol (ISO) induces a hyperpnea which has been attributed to a "cardiodynamic" mechanism or, alternatively, direct stimulation of the carotid bodies. In a previous study, which demonstrated that both mechanisms can operate (Fed. Proc. 40:568, 1981), we noted that the initial hyperpnea began with a latency (~18 s) which appeared too long for direct cardiac mediation. In an attempt to clarify this mechanism, we injected a 10 μg bolus of ISO, into the following vascular sites (in 12 anaesthetized dogs, Nembutal, 20 mg/kg i.v.): 1) right atrium, 2) descending thoracic aorta, 3) abdominal aorta (~5 cm above iliac bifurcation) and 4) a single iliac artery. In all cases the cardiovascular response (an initial vasodilatation followed by a tachycardia) preceded the hyperpnea. There was no significant difference between the latencies of the hyperpneic responses to right atrial or either aortic injection sites (~14 s). In contrast, the response to the iliac injection was appreciably delayed (~24 s), attributable to a similarly delayed cardiovascular response. However, when half the dose of ISO was injected simultaneously into each iliac artery, both the cardiovascular response and the hyperpneic latency were shortened to that of the right atrial and aortic sites. We therefore conclude that the "cardiodynamic" component of the isoproterenol hyperpnea results from an initial peripheral vascular mediation.

51.8
THE REGULATION OF "ALVEOLAR" AND END-TIDAL GAS TENSIONS AT THE ONSET OF EXERCISE. Susan A. Ward*and Brian J. Whipp. UCLA, Los Angeles & Harbor-UCLA Medical Center, Torrance, CA.

The "cardiodynamic" hypothesis of the exercise hyperpnea posits that rapid changes in pulmonary blood flow ($\dot{Q}$) at the onset of exercise induce an hyperpnea which regulates "alveolar" gas tensions and the gas exchange ratio (R) during the first 15 s of moderate exercise in man. The maintenance of end-tidal gas tensions and R at resting levels in this phase is commonly cited as support for the concept. Recently, however, we have documented a steepening of the alveolar profiles of both PCO_2 and PO_2 at the start of exercise (J. Physiol., May, 1983), resulting presumably from an increased $\dot{Q}$. We have therefore characterized the relationship between mean alveolar ($P\bar{A}CO_2$, $P\bar{A}O_2$) and end-tidal gas tensions during the initial phase of constant-load cycling (100 W) in 17 normal subjects. The mid-points of the alveolar phase of the expired gas tension profiles were used to estimate $P\bar{A}CO_2$ and $P\bar{A}O_2$. The abrupt hyperpnea at exercise onset was associated with stable end-tidal gas tensions throughout the initial phase of the work. However, invariably, $P\bar{A}CO_2$ fell and $P\bar{A}O_2$ rose systematically (by up to 5 torr). Consequently, mean alveolar, and presumably arterial, gas tensions in this phase of exercise may not be readily inferred from the end-tidal tensions. And, as the initial phase of the exercise hyperpnea is typically hyperventilatory, then it may be less precisely coupled to the pulmonary blood flow changes than has previously been assumed.

*Senior Investigator of the American Heart Association (GLAA)

51.9
VENTILATION STUDIED WITH CIRCULATORY OCCLUSION DURING TWO INTENSITIES OF EXERCISE. W.C. Clark-Stanley*, W.R. Lee*, G.A. Brooks. Exercise Physiology Laboratory, University of California, Berkeley, CA 94720

Respiratory gas exchange was measured breath-by-breath at two intensities of exercise with circulatory occlusion in the legs. Eight male subjects exercised on a cycle ergometer at 300 and 600 kg·m/min for 12 mins; circulation to the legs was occluded by thigh cuffs (200mmHg) for two mins after 6 mins of unoccluded exercise. Mean 15 s values were statistically compared to the preocclusion baseline. $PETCO_2$ and $\dot{V}O_2$ decreased significantly during occlusion at both workloads. Occlusion elicited marked hyperventilation, as evidenced by sharp increases in $\dot{V}E$, $\dot{V}E/\dot{V}CO_2$, and $\dot{V}E/\dot{V}O_2$. An inflection in $PETCO_2$ was seen after cuff release in all subjects at 7.3 ±.7 and 3.4 ±.9 s at 300 and 600 kg·m/min, respectively. At 300 kg·m/min a post-occlusion inflection in $\dot{V}E$ was seen in 7 subjects 23.2 ±6.1 s after the $PETCO_2$ inflection. Three subjects showed an inflection in $\dot{V}E$ at 600 kg·m/min 25.0 ±7.7 s after the $PETCO_2$ inflection. There were significant increases in $PETCO_2$, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$ following cuff release. $\dot{V}E$ tracked $\dot{V}CO_2$, not $\dot{V}O_2$, post-occlusion. It is concluded that 1) neural ventilatory drive increased during occlusion, 2) the lag time between the $PETCO_2$ and $\dot{V}E$ inflections suggests the CO_2 ventilatory drive is mediated by the carotid bodies, and 3)no evidence for a pulmonary CO_2 receptor was found.

51.10
THE RESPONSE OF THE NEWBORN VS. THE MATURE INFANT MONKEY TO AN EXTERNAL FLOW RESISTANCE. W.A. LaFramboise*, T.A. Standaert* R.D. Guthrie* and D.E. Woodrum. Univ. of Washington, Seattle WA 98195

Six-48 hr. old and six-21 day old Macaca nemestrina were studied to assess developmental changes in their response to an acute inspiratory flow resistive load. Resistances 4X and 10X the normal pulmonary resistance for these infants were added to the inspiratory port of a 2-way valve to which the unanesthetized infants were connected via a tracheostomy tube. $\dot{V}_E$ fell immediately in the 2 day old neonates upon presentation of either load and remained depressed for the 10' exposure (baseline:325±33ml/kg;4Xload:257±71,$p<.025$;10X:240±95,$p<.05$) while pressures obtained from end expiratory airway occlusions ($P_{0.1}$,$P_{0.2}$,P_{max}) did not change. The fall in $\dot{V}_E$ was due to a drop in respiratory rate with a prolongation of inspiratory time (T_I). Mean arterial O_2 fell (-5 torr) and CO_2 rose (+ 2 torr) slightly with loading. The older infants responded to loading by uniformly increasing occlusion pressures ($P_{0.2}$=baseline:4.5±1.8cm H_2O;4X:7.4±4.3, $p<.05$;10X:12.9±6.5, $p<.001$) and defending minute ventilation and arterial blood gases from any significant change. Again, T_I was increased with each load and a small drop in frequency was offset by a slightly larger V_T. We conclude that the neonatal compensatory response to an inspiratory resistive load is absent or markedly diminished relative to the older infant and that postnatal maturation is critical in the development of load compensation. (Supported by NIH #HL19187 and #RR00166).

51.11
PERCEPTION OF ADDED RESPIRATORY LOADS IN PATIENTS WITH RESTRICTIVE LUNG DISEASE. N.K. Burki, and R. Lorenz*. University of Kentucky Medical Center, Lexington, KY 40536

The magnitude perception of added suprathreshold resistive (ΔR) and elastic (ΔE) loads was studied in 8 patients with restrictive lung disease (RLD), and compared to a control, age and sex matched group of 7 healthy, normal nonsmoking subjects. Total lung capacity was significantly ($p<0.05$) reduced in the RLD patients compared to the normal group, and total thoracic elastance (E_{tot}) was significantly increased (mean E_{tot} 13.9 ± 2.1 $cmH_2O.L^{-1}$). The magnitude perception measured by handgrip dynamometry, of a range of added resistive loads was not significantly different between the 2 groups when expressed as the relationship between log ΔR and log handgrip (RLD, mean slope = 0.51; normals, mean slope = 0.65); however, there was a significant difference when perception was expressed as the relationship of log mouth pressure (PM) to log handgrip response (RLD mean slope 0.71; normals, mean slope = 1.15, $p<0.01$). There were no significant differences between the 2 groups in the log-log relationship between handgrip response and ΔE or Pm generated with the ΔE load. These results imply that patients with RLD, with an increased thoracic elastance, have an altered perception of added resistive loads compared to normal subjects, but do not exhibit differences compared to normal subjects in the perception of added elastic loads. (Supported in part by USPHS NIH grant HL 24412).

52.1

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE PRESENCE OF HISTAMINE H_2 RECEPTORS ON GUINEA-PIG MYENTERIC NEURONS. P. R. Nemeth* and J. D. Wood. University of Nevada, Reno, NV 89557

Intracellular methods were used to record electrical activity of myenteric neurons from guinea-pig ileum in vitro. Tissues were isolated in a 1 ml bath and superfused at a rate of 8 to 12 ml per min with carboxygenated Krebs solution at 37°C. Histamine (100 μM) or dimaprit (100 μM) in Krebs solution was applied to neurons by microejection from fine pipettes with controlled nitrogen pressure pulses of 5 to 750 msec at 20 psi. Antagonists were applied in the superfusion solution. Application of histamine or dimaprit resulted in a long-lasting, 5 to 60 sec, depolarization of AH/type 2 neurons by 10 to 15 mV. In most neurons, there was an increased excitability during depolarization. Change in neuronal excitability was determined by a significant change in the spikes evoked per pulse of current injected into a neuron through the recording electrode. Superfusion with the H_2 receptor antagonist, cimetidine (10 μM), reversibly abolished both the depolarization and increased excitability elicited by histamine or dimaprit. Superfusion with the H_1 receptor antagonists, diphenhydramine (10 μM) and pyrilamine (10 μM), and with the serotonergic antagonist, methysergide (10 μM), failed to diminish the response to histamine or dimaprit. None of the antagonists displayed local anesthetic activity at the concentrations used. We conclude that histamine affects the electrical behavior of myenteric neurons by stimulating H_2 receptors. (Supported by NIH grants NS17363 and AM26742.)

52.2

SIMULTANEOUS RECORDINGS OF MECHANICAL AND INTRACELLULAR ELECTRICAL ACTIVITY FROM CANINE ILEOCOLONIC SPHINCTERIC SMOOTH MUSCLE IN VITRO. I. MacKenzie* and J.H. Szurszewski. Mayo Foundation, Rochester, MN 55905

These preparations (7 x 2 mm) exhibited spontaneous tone which assumed minor variability. The intracellularly recorded resting membrane potential averaged -55 ± 0.8 mV (n=50). In some recordings the cell membrane potential was stable, but the majority of cells exhibited small rhythmic oscillations in membrane potential. Spontaneously-occurring spikes were rarely superimposed on these slow waves. In the absence of drugs, electrical field stimulation (1-20 Hz for 1 s; 0.5 ms pulse width) consistently elicited prolonged relaxation of the muscle (7-35 s). With longer stimulation periods (up to 60 s), relaxation was maintained and there was no subsequent "rebound contraction". Mechanical inhibition was accompanied by a hyperpolarization in the resting membrane potential (inhibitory junction potential, IJP) whose magnitude was frequency-dependent. Stimulation frequencies of 1, 5 and 10 Hz produced IJPs of 2.6, 8.6 and 13.9 mV (n=10), respectively. At all frequencies of stimulation, the duration of the IJP was shorter than the duration of relaxation. Mechanical responses and IJPs were abolished by tetrodotoxin (1 μM). Neurogenic responses were not antagonized by atropine (1 μM), alone or together with guanethidine (3.4 μM) and apamin (0.1 μM). These data suggest that the predominant intrinsic innervation of the canine ileocolonic sphincter is supplied by non-adrenergic, non-cholinergic inhibitory neurons. Support - NIH AM 17238.

52.3

SLOW WAVES OF ANTRAL CIRCULAR MUSCLE CELLS NEAR THE MUCOSA DIFFER FROM THOSE NEAR THE LONGITUDINAL MUSCLE. A.J. Bauer,* N.G. Publicover* and K.M. Sanders. University of Nevada School of Medicine, Reno, NV 89557

Muscle cells from the circular layer are thought to be homogeneous in their electrical and mechanical activities and in their responsiveness to drugs. We tested this hypothesis with a novel in vitro preparation in which the antral muscle wall was viewed in cross section. Muscles from anesthetized dogs were removed from the gastric antrum and separated from the mucosa. Strips of muscle (1 mm x 10 mm) were cut parallel to the circular muscle fibers. The strips were pinned on edge in a recording chamber, and superfused with warmed, oxygenated Krebs solution. Membrane potentials of circular muscle cells (n=62) at various distances from the longitudinal layer were recorded. Slow waves from cells near the longitudinal border had average upstrokes of 31.9 mV, plateau potentials of 28.6 mV, and durations of 6.32 sec. Near the mucosal border slow waves had average upstrokes of 24.0 mV, plateau potentials of 16.2 mV, and durations of 5.5 sec. The differences were statistically significant ($p < .001$). The muscles were exposed to prostaglandin E_2, 10^{-6} M. Cells near the mucosal border were more responsive to PGE_2. In summary, the excitability mechanisms and the sensitivity to PGE_2 appear to differ as a function of the position of the cells in the circular layer. (Supported by NIH grants: AM 32176, AA05168 and AA 05883.)

52.4

ANATOMICAL LOCALIZATION OF CHOLECYSTOKININ RECEPTORS TO THE PYLORIC SPHINCTER: PHYSIOLOGICAL IMPLICATIONS. Gregory T. Smith*, T.H. Moran*, J.T. Coyle*, M.J. Kuhar*, T.L. O'Donohue* and P.R. McHugh, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, and NIH, Bethesda, MD 20205.

Infusions of cholecystokinin (CCK) produce an inhibition of gastric emptying and result in behaviors associated with satiety. In an effort to elucidate target sites within the gastrointestinal (GI) tract through which CCK may directly exert its effects, the distribution of CCK receptors was mapped by in vitro autoradiography in the rat.

Receptor sites for CCK were labelled with ^{125}I-Bolton Hunter CCK_{33} (^{125}I-CCK_{33}). Binding was carried out on 25μ slide-mounted serial cross-sections sampling the esophagus, cardia, fundus, corpus, antrum, pyloric canal, pyloric sphincter, proximal duodenum, and terminal ileum. Binding conditions were the same as those demonstrated to provide saturable, reversible, and high affinity binding (K_d = 0.45nM) in brain sections with specific binding of 80-90%.

Autoradiographs revealed specific CCK receptor binding to be localized to the circular smooth muscle layer of the pyloric sphincter. Receptors were concentrated in discrete banding patterns at the distal most portion of the pyloric sphincter. Negligible binding was observed in oblique, circular or longitudinal muscle layers at the other GI levels sampled. The restriction of CCK receptors to the pyloric sphincter suggests this location as the site through which CCK inhibits gastric emptying. (Supported by NIH grant 2-R01-AM19302).

52.5

OSMOTIC INHIBITION OF GASTRIC EMPTYING IN RELATION TO PLASMA LEVELS OF NEUROTENSIN,SOMATOSTATIN AND GIP IN THE RAT.
M Moqard*G Mylander & O Flaten*Karolinska Hospital,Sweden and Ulleval Hospital Norway. (SPON JH Walsh)

The osmolality of the gastrointestinal contents exerts a marked influence on several GI functions.The regulating mechanisms are not known.

METHODS:Rats were fed by intragastric instillation of solutions with different osmolality containing an isotope-labelled non-absorbable marker.After fixed time intervals the rats were sacrifized and the distribution of the isotope along the GI canal was determined.Candidate inhibitory peptides were measured by RIA.

RESULTS:A hyperosmolar solution,1200 mOsm/kg,inhibited the gastric emptying by 16%/h,$p < 0.05$.

Osmolality mOsm/kg	Neurotensin pmol/l	GIP pmol/l	Somatostatin pmol/l
300	3.7 + 0.6	24 + 2	29 + 2
1200	2.9 + 0.8	15 + 2	25 + 2
$p < 0.005$	ns	s	ns

SUMMARY: An osmotic inhibitory mechanism of gastric emptying has been demonstrated in the rat.This mechanism is not related to elevated plasma levels of neurotensin,GIP or somatostatin.

52.6

INTRINSIC PROPULSIVE BEHAVIOR OF GUINEA PIG ILEAL SEGMENTS AND ITS RELATION TO THE "PERISTALTIC REFLEX." W.A. Weems, L.D. Scott* and N.W. Weisbrodt. U.TX Med.Sch.Houston,TX 77025

The intrinsic ability of guinea pig terminal ileum to propel luminal fluid was determined and compared to that of cat ileum. The oral and aboral ends of ileal segments were attached in vitro to a propulsion evaluation system that imposed input-output conditions of constant capacitance (0.025 ml cm^{-1} of H_2O) and negligible resistance. When basal intraluminal pressure was increased to 5 cm of H_2O, segments spontaneously produced propulsion complexes at an average frequency of 17.3 hr^{-1}, a frequency 2.3 times greater than that observed in cat ileal segments. The temporal pattern of pressure and volume changes of these complexes also differed from cat ileum. These complexes consisted of a primary expulsion of fluid that occured simultaneously from both the oral and aboral ends with superimposed oscillations of fluid ejections occuring at an average frequency of 9.5 min^{-1}. Net aboral expulsion of fluid during each complex was observed in 66% of the segments. Propulsion was abolished by atropine sulfate (10^{-6} g ml^{-1}). Nicotinic antagonists, however, only reduced the frequency and duration of complexes. It was concluded that (1) intrisic propulsion behavior of guinea pig ileum differs from cat ileum; (2) the susceptibility of propulsion behavior to nicotinic antagonists is different between the two species, and (3) the rapid fluid oscillations of the complex are analogous to the peristaltic reflex. (Supported by NIH Grant AM23038)

52.7

IS INTESTINAL TRANSIT IN FASTED, BYPASSED RATS RELATED TO INTESTINAL MYOELECTRICAL ACTIVITY? C. Eeckhout* and N.W. Weisbrodt, The University of Texas Medical School at Houston, Houston, TX 77030.

Intestinal transit in rats three days after jejunoileal bypass is rapid through the in-continuity segment (ICS) and slow through the bypassed segment (BPS)(Am. J. Physiol. 241: G256, 1981). To study the mechanisms responsible for the transit patterns, myoelectric activity of the ICS and BPS was recorded after bypass in six rats implanted with monopolar electrodes. As early as three days after bypass, migrating complexes were seen in all areas of the intestine in all animals. In six hours of recording (1 hr. from each rat) the total numbers of complexes were 9, 19, and 8 for the proximal ICS, distal ICS, and BPS respectively. The numbers recorded from each animal ranged between 0-4, 0-5, and 0-3 in each of the three segments respectively. Migration of the activity fronts on each segment did not always occur. In contrast, 20 activity fronts were seen during a one hour recording from each of six control rats, thus suggesting a decreased number in the proximal ICS and BPS. Our data indicate that the rapid transit through the ICS may be due to the presence of early activity fronts. The slow transit in the BPS is not due to absence of activity fronts but perhaps to the presence of abnormal contents in the lumen of the BPS. (Supported by a Grant AM 19886 from NIH)

52.8

β-ENDORPHIN AND ITS METABOLITES STIMULATE GASTROINTESTINAL MOTILITY. Thomas P. Davis, A.J. Culling*, J.J. Galligan*, H. Schoemaker* and T.F. Burks. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Previous studies have demonstrated that β-endorphin (βE) and enkephalins are released into the systemic circulation by the pituitary and adrenal medulla respectively. To determine if the small intestine could be a target for circulating βE, segments (5cm) of mid-jejunum were removed from anesthetized dogs and perfused with Krebs bicarbonate buffer containing βE (1 μg/ml), while motility was recorded and venous effluent collected in 1 min. fractions (23 ml). βE significantly increased ($P< 0.002$) motility of intestinal segments. HPLC analysis of the venous effluent identified, amongst others, several α- and γ-type endorphins. The identified peptides were then perfused through intestinal segments to determine their motility effects. α-Endorphin (αE), γ-endorphin (γE), des-tyrosin-α-endorphin and des-tyrosine-γ-endorphin, significantly increased motility. Responses were characterized by an increase in phasic contractions of constant amplitude and frequency. To determine regional specificity and site of βE metabolism during perfusion, we studied *in vitro* time-course processing of βE in membrane-bound mucosal and muscularis homogenates. Mucosa was much more enzymatically active than muscularis. These studies demonstrate that the small intestine can metabolize βE into a number of active fragments which increase motility and suggests a regional specificity of enzymatic processing.

52.9

EFFECT OF PREGNANCY ON GALLBLADDER MOTILITY IN VITRO. J.P. Ryan, Temple Univ. School of Med., Phila., PA. 19140

Studies were done to determine the reason for the decreased contractility of the gallbladder (GB) to acetylcholine (Ach) and the octapeptide of cholecystokinin (OP-CCK) during pregnancy (P). Two hypotheses were tested. First, that the overall contractility of the GB is reduced during P. This was examined by studying the in vitro motor response to Ach, OP-CCK, and KCl of GB from P and non-P guinea pigs. Second, that GB contraction involves both internal and external calcium (Ca) stores, and that P is associated with a decrease in the contribution of the internal pools. This was tested by examining the contractile responses to Ach, OP-CCK, and KCl in the presence and absence of extracellular calcium. The results were as follows: 1) the contractile responses to Ach and OP-CCK, but not to KCl, were significantly reduced during P; 2) Ach and OP-CCK, but not KCl, elicited contractions in a 0-Ca solution. The responses from pregnant animals, however, were significantly reduced (Ach, 3.7%; OP-CCK, 4.1%) when compared with control (Ach, 10%; OP-CCK, 24.2%). The data are expressed as a percent of the maximal control response in a Ca-containing solution (2.5mM). It is concluded that both Ach and OP-CCK utilize extra- and intracellular stores of Ca for contraction. P is associated with a decrease in the contribution of the intracellular pools, rather than with a generalized decrease in GB contractility. (Supported by NIH Grant HD16132).

52.10

PRESSURE MEASUREMENT IN THE DISTAL ESOPHAGEAL SPHINCTER (D.E.S.): A NEW METHOD. Gordon H. Bryant (SPON: J.R. Claybaugh). Department of Clinical Investigation, Tripler Army Medical Center, Honolulu, Hi. 96859

The elegant simplicity of the Hennist Winans perfused side hole motility catheter has rendered difficult the design of an acceptable device of equal utility, but without the attendant disadvantages involved when prolonged DES pressure recordings are attempted. These include the hydrostatic pressure changes produced by patient movement, as well as water loading and the necessity for syringe refilling. A technique suitable for all DES pressure measurements has been devised in this laboratory. In principle, change of electrical resistance of an electrolyte column as external pressure varies the area of cross section, is the essence of the technique. In the prototype sections of latex tubing were joined by segments (<1cm) of hypodemic tubing selected to be a tight fit in the latex. A thin copper wire was soldered to each and the whole joint to make a continuous length. After partial filling with 0.4 M NaCl, a plug was placed in the proximal end, while the distal end was maintained at a higher elevation. A half bridge circuit with an external half bridge is not an essential feature; potentiometric methods serve equally well, and the only other requirement is the selection of a tubing of suitable elasticity.

53.1

THE VALIDATION OF A FIELD TEST OF MAXIMAL AEROBIC POWER
D.W. Michielli* Davidson, L.* Falvey, J.* Huey, S.* (Sponsor: George Fried) Laboratory of Work Physiology, Brooklyn College of the City University of New York, Brooklyn, NY 11210

The purpose of this study was to develop an inexpensive, submaximal test that accurately assesses maximal aerobic power. Twenty-three healthy subjects (13 men, 10 women) $\bar{x}$ age = 21± 3 years, volunteered for the study. Subjects were randomly assigned to one of two conditions for their initial test. One condition consisted of directly measuring the maximum oxygen consumption ($\dot{V}O_2max$) on a motor-driven treadmill for each subject. The second condition required that each subject run as far as possible for five minutes on an outdoor 400-meter track. A measuring wheel accurate to 1.27cm was used to measure the total distance run. Results indicated that the reliability of the distance run in five minutes based upon a test re-test analysis was r = 0.85. There was a highly significant correlation, r = 0.89 ($p<.001$), between the distance run in five minutes and the criterion measure, $\dot{V}O_2max$. A linear regression analysis of Y = -1.742 + 0.043x with a standard error of estimate = 4.73 was established to predict $\dot{V}O_2max$. $\dot{V}O_2max$ varied from 34.8 to 75.7ml/Kg/min with a $\bar{X}$ = 49.5 ± 10.3 ml/Kg/min. The mean distance run in five minutes was 1203.5 ± 215.3 meters and the range was from 889 to 1718 meters. In conclusion, a submaximal five minute run for distance is an inexpensive, reliable and valid field test for assessing maximal aerobic power in young adults.

53.2

SUPERFICIAL SHELL INSULATION IN COLD WATER DURING SEVERE EXERCISE: ROLE OF CORE AND SKIN TEMPERATURE. A. Veicsteinas, G. Ferretti, R. Perini and D.W. Rennie. Dept. Biomedical Sciences and Technologies, U. Milan, Italy and Depts. Physiology, SUNYAB, Buffalo, NY 14214 and Brescia, Italy.

During immersion in water at critical temperature (CWT), the in-series insulation provided by skin and fat (I_{ss}, $^{o}C \cdot m^2 \cdot W^{-1}$) are maximal (vasoconstriction is complete) at rest and while exercising up to a metabolic rate 3 times higher than resting (3 MET). From temperature measurements of subcutaneous fat (Tsf), skin surface (Tsk) and direct skin heat flux (H), I_{ss} was calculated as (Tsf-Tsk)/H in 4 men immersed head out in a well stirred bath. In constant water temperature (range 22-32°C) after 40-60 min of rest to achieve a rectal temperature (Tre) of about 37.0°C, subjects exercised for 45-70 min at 8 MET until Tre reached 38.3°C. At rest I_{ss} was 0.043±20% $^{o}C \cdot m^2 \cdot W^{-1} \cdot cm^{-1}$ and independent of Tre. During exercise: 1) Tre, Tsf and H increased with time reaching a steady state value in 20-40 min; and b) the threshold for decrease of I_{ss} (vasodilation threshold, VT) depends upon both Tre and Tw as Tw = -6.9 Tre + 289; r = -0.91. Thus, the thermoregulatory perfusion of the superficial shell in water depends on combined thermal inputs from skin and core, as is true for shivering and sweating. (Supported by USPHS grant HL-28542)

53.3

EFFECT OF THERMISTOR POSITION ON THE MEASUREMENT OF TAIL-SKIN TEMPERATURE IN EXERCISING RATS. Frank G. Shellock*, Stanley A. Rubin, Alberto Rabines*, Linda Tabak*, H.J.C. Swan. Cedars-Sinai Medical Center, Los Angeles, CA 90048.

The tail is the predominant thermoregulatory effector organ for the exercising rat and, therefore, tail-skin temperature is commonly evaluated in investigations of rat temperature regulation. We studied six young female Sprague-Dawley rats (225±19gm) during steady-state treadmill exercise (Ex, 20m/min) in an ambient temperature of 22-25°C. Tail-skin (Tsk) temperatures were measured with accurate thermistors placed at the base of the tail in the following positions: ventral (V)-over the ventral arterial bundle, lateral (L)-over the lateral vein, and at a point equidistant (E) between these two positions. Colonic temperature (Tc) and oxygen uptake (mean maximum of 6.9±0.3ml/min·100gm) were also measured.

	Tc	TskV	TskE	TskL
Rest	38.49±0.30	29.14±0.47	28.32±0.71	27.92±0.76
Ex	40.39±0.31	36.81±0.71	36.01±0.87	35.35±0.71

There were significant ($p<0.01$) differences between tail-skin temperatures measured in the three positions at rest and during Ex. In addition, the onset of tail vasodilation (defined as an increase in Tsk of ≥1.5°C/2min) for TskV preceded TskL by 1.5±0.5 min and the corresponding Tc and Tsk were also significantly different for each of the three tail-skin positions. We conclude that Tsk measurements of rats at rest and during exercise are position and time dependent and, therefore, consistent placement of tail-skin thermistors are necessary when temperature regulation studies are performed in rats.

53.4

CARDIOPULMONARY EFFECTS OF INTERMITTENT EXERCISE IN 100 ppm CARBON MONOXIDE. Milan J. Hazucha*, David J. Davin*, Mitchell Friedman and George L. Goldstein*. Ctr. Environ. Health & Med. Sci., Univ. of North Carolina at Chapel Hill, NC 27514.

Occupational exposures to 100 ppm CO for more than 1 hour commonly occur. To study the possible adverse effects of CO exposure on cardiopulmonary function during intermittent exercise, normal healthy males (19 to 27 yrs old) were exposed for 2 hours in an environmental chamber (22°C, 40% RH) to either air or 100 ppm CO using a crossover study design. During the exposure period, the subjects were studied during a 15 min rest period and then during two 15 min periods of graded exercise on a bicycle ergometer ($\dot{V}_E$ 18 and 24 l/min, respectively). This cycle was repeated twice and followed by a 30 min rest period. Total $\dot{V}_E$ was measured using a pneumotachograph, while cardiac output ($\dot{Q}_T$) and diffusing capacity for $C^{18}O$ (DL_{CO}) was obtained using a multiple gas rebreathing method. During exposure to air or CO, mean $\dot{Q}_T$ during exercise increased by an average of 41% (range 32% to 51%) over the preexercise values. Average DL_{CO} increased by 11% during exercise compared to preexercise value during exposure to air but only increased an average of 6% during CO exposure. The changes with exercise in $\dot{Q}_T$ and DL_{CO} between the air and CO exposures were not significantly different. Blood levels of COHb averaged 9.96 ± 0.26% SEM by the end of the CO exposure day. Thus it appears that, despite high blood COHb levels caused by exposure to 100 ppm CO, the cardiopulmonary response in exercising normal subjects is not affected significantly. (Supported by the US Army MBR & D Labs.).

53.5

STATIC MUSCLE CONTRACTION IN CATS CAUSES REFLEX TRACHEAL RELAXATION. J.C. Longhurst. UCSD, La Jolla, CA 92093

Static contraction of skeletal muscle is associated with increased ventilation. Although we have shown that chemical stimulation of hindlimb afferents causes relaxation of tracheal smooth muscle (TR), it is not known if skeletal muscle contraction also causes TR. Therefore, in 9 chloralose anesthetized cats we examined the hemodynamic and tracheal smooth muscle responses to hindlimb contraction induced by stimulating L_7 and S_1 ventral spinal cord roots. Isometric tension was measured in the transverse cervical trachea. During contraction which increased gastrocnemius tension from 0.7±0.1 to 7.0±1.5 kg, blood pressure and heart rate increased (106±9 to 137±9 mmHg; 198±14 to 209±16 beats/min) and tracheal tension fell (19.6±0.4 to 17.3±0.9 g)(all $p<.02$). Comparing unilateral to bilateral hindlimb contraction in 3 cats, tracheal tension fell (20.5 to 19.1 g vs 20.0 to 17.1 g) while the total gastrocnemius tension increased (0.7 to 6.7 kg and 1.2 to 11.1 kg). There was a strong correlation between average developed gastrocnemius tension and change in tracheal tension (r=0.83). Transection of L_7 and S_1 dorsal roots in 6 cats reduced the extent of tracheal relaxation from 2.5±0.38 to -0.02±0.31 g while peak developed gastrocnemius muscle tension was not altered (6.3±1.5 vs 7.1±1.4 kg). Thus, static hindlimb contraction reflexly lowers tracheal tension in cats. This response is related to muscle mass and tension generated by the contracting skeletal muscle. (Supported in part by NIH NINCDS NS 20165 and AHA 80-784)

53.6

THE REPRODUCIBILITY OF MAXIMUM VENTILATORY RESPONSES TO PROGRESSIVE EXERCISE. C.S. Garrard*, C. Emmons* and M. Lopata. University of Illinois at Chicago, IL 60612.

Progressive exercise testing in 1 minute, 30 watt increments was performed to the maximum tolerated level in 6 healthy volunteers (aged 19-22 yrs), twice a day (am and pm) for 3 days. Tests of forced expired airflow and whole body plethysmography (E. Jaeger Inc.) were made immediately before each exercise test. No significant diurnal variations were demonstrated in FEV1, FVC, RV, FRC, TLC, or Raw although FVC, RV and TLC were significantly greater on the first compared to subsequent days (am and pm combined). The group mean maximum oxygen uptake ($\dot{V}O2$) achieved was 3.96 ± 1.38 l/min, max. tidal volume (Vt) 2.68 ± 0.97 l., max. breath frequency (BF) 40 ± 7 breaths/min, max. minute ventilation (MV) 107.6 ± 46.7 l/min and the max. heart rate (HR) 173 ± 13 beats/min. The intrasubject coefficients of variation for max $\dot{V}O2$ were 8.8%, max VT 7.3%, max BF 9.0%, max MV 12%, and max HR 3.7%. Analysis of variance showed no significant diurnal or day to day variation in grouped exercise data while in two subjects max MV showed significant intrasubject diurnal variation due to small and reciprocal changes in Vt and BF. Thus although the ventilatory response to exercise appears to be generally reproducible occasional intrasubject variations in max MV may be observed which cannot be attributed to changes in pulmonary function tests.

53.7

VENTILATORY RESPONSE TO STEP INCREMENTS IN WORKLOAD WHILE BREATHING AIR OR 4.25% CO_2. Lawrence J. Folinsbee, John F. Bedi* and Steven M. Horvath. Institute of Environmental Stress, University of California, Santa Barbara, CA 93106

We studied the ventilatory responses of 6 young males to 100 W step increases in cycle ergometer power output while these subjects were breathing either air (A) or 4.25% CO_2, 24.6% O_2 in N_2 (C). Each subject pedaled for 2 min at 16 W and then for 4 min at 106 W. Ensemble averages were calculated from 6 repetitions of each condition. The time required to reach new steady-state $\dot{V}O_2$ (78 s [A]; 80 s [C]) and new steady-state heart rate (90 s both) was similar in both conditions. Ventilation reached steady state more rapidly with air breathing (105 s) than with CO_2 (125 s) and in both cases closely paralleled the change of $\dot{V}CO_2$. The ventilatory response was nearly isocapnic (+1.4 Torr $PaCO_2$) in air but was hypercapnic ($PaCO_2$ increased from 46.9 to 52.2 Torr) in the CO_2 series. A new steady-state $PaCO_2$ was reached in just over 100 s (C). The relative changes in inspiratory duration (-12.5%, -14.4%), expiratory duration (-25.7%, -23.4%) and frequency (25.1%, 23.8%) were similar (A and C respectively) despite the initially higher ventilation with CO_2. The relative increase in tidal volume was greater with A (56 vs 41%) although the absolute increases were similar. The establishment of a ventilatory steady state following a step workload increment while breathing CO_2 is delayed because of the concurrent rise in CO_2 stores. (NHLBI HL26034).

53.8

EFFECTS OF FORMALDEHYDE ON PULMONARY FUNCTION OF EXERCISING DOGS. William J. Mautz*, Michael T. Kleinman*, Peter Reischl, Charles Bufalino*, and T. Timothy Crocker*. University of California, Irvine, Ca. 92717.

Female Beagle dogs were exposed to 8 ppm formaldehyde (HCHO) during exercise on a refrigerated treadmill at 5 km h^{-1} and 7% grade. Dogs wore a low deadspace mask with an esophageal balloon. Inspiratory and expiratory flow rates, respiratory gas fractions, skin and rectal temperatures, and transpulmonary pressure were continuously recorded for breath-by-breath computation of $\dot{V}_E$, $\dot{V}_{O2}$, $\dot{V}_{CO2}$, breath time, inspiratory and expiratory times, and a dynamic measure of airway resistance and lung compliance. Clean air exposures were conducted 4 days prior and 1 day following HCHO exposure using identical exercise protocols. HCHO exposures (n=9) for 120 min of exercise resulted in pulmonary function changes including a 35% increase in breath time, a progressive increase in expiratory proportion of breath time to 5% of control at end exercise, a 25% increase in airway resistance, and a 9% decrease in lung compliance. $\dot{V}_E$ decreased by 14% and was accompanied by declines in both $\dot{V}_{O2}$, $\dot{V}_{CO2}$, and air convection requirements ($\dot{V}_E/\dot{V}_{O2}$ and $\dot{V}_E/\dot{V}_{CO2}$). We conclude that HCHO inhalation induces reflex changes in breathing pattern and pulmonary mechanics. The reduced minute ventilation and gas exchange might follow from reduced work of breathing secondary to change in breathing pattern or from modification of running behavior. Supported by EPA R808267.

53.9

HYPOCAPNIA DURING EXERCISE IN BEAGLE DOGS. P.S. Clifford*, J.T. Litzow*, R.L. Coon, and J.P. Kampine. Dept. of Physiology and Anesthesiology, The Medical College of Wisconsin and Wood VA Center, Milwaukee, WI 53193.

CO_2 delivery to the lung has been postulated to be the mechanism mediating the hyperpnea of exercise. The underlying assumption of this theory is that there is arterial isocapnia due to a tight coupling of ventilation with pulmonary CO_2 flow. Pan et al. (Fed. Proc. 42(4):740, 1983) have recently shown that in the pony there is not isocapnia during exercise. During the transition period between rest and exercise they observed a progressive hypocapnia which persisted into the steady-state. Because of the potential importance of such a finding, we chose to investigate this phenomenon in 10 beagle dogs in which arterial blood sampling could be accomplished via exteriorized carotid artery loops. All dogs had been familiarized with the laboratory and the procedures employed. $PaCO_2$ was measured at rest and at 15 sec intervals during the first minute of unrestrained treadmill exercise at 5.0 km/hr, then at 2 min and 3 min at the same workload. Mean resting $PaCO_2$ was 37.1 mmHg. At the onset of exercise $PaCO_2$ fell progressively to a nadir of 34.6 mmHg during the 30-45 sec sampling period. Arterial samples at 2 min and 3 min remained hypocapnic (PCO_2=34.8 mmHg). The hypocapnia exhibited in this group of beagle dogs at the onset of exercise and during the steady-state suggests that there is not a tight coupling of ventilation with pulmonary CO_2 delivery. (Supported by the VA Medical Research Service)

53.10

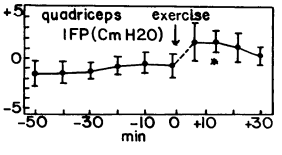
THE EFFECTS OF INSPIRATORY MUSCLE TRAINING ON EXERCISE PERFORMANCE IN NORMAL SUBJECTS. Hsiun-ing Chen* and Bruce Martin. Physiol. Sec. Med. Sci. Program, Indiana Univ., Bloomington, IN 47405

Although respiratory muscle fatigue may occur in heavy exercise, it is still unknown if ventilatory muscle training can improve exercise performance in normal subjects. To investigate this question, 18 young, healthy subjects (10 males, 8 females) were divided into control and experimental groups in single-blind fashion. All subjects' resting pulmonary function (FVC, $FEV_{1.0}$, and 12-s MVV), ventilatory muscle (VM) strength (determined by maximal inspiratory mouth pressure, PIM_{max}, at FRC and at RV), VM endurance (determined by the sustained time at 60% PIM_{max}FRC), and $\dot{V}O_{2max}$ on the treadmill were measured on a first visit. The experimental group trained inspiratory muscles by breathing against a resistive load for 15 min, twice daily, for 4 wks. Afterwards, all of the tests were repeated on both groups. The experimental group alone significantly improved their VM endurance (3.3 vs. 5.1 min; $p<0.01$) after training. Treadmill work time in the experimental group also showed improvement (410 vs. 435 s; $p<0.05$). In contrast, FVC, $FEV_{1.0}$, 12-s MVV, VM strength, peak exercise ventilation and $\dot{V}O_{2max}$ were unchanged in both groups. We conclude that inspiratory muscle training greatly improves inspiratory muscle endurance, and that it improves severe, short-term exercise performance without changing $\dot{V}O_{2max}$ in normal persons. (Supported by NIH Grant HL 26351 from the NHLBI)

53.11

INTERSTITIAL FLUID PRESSURE AND FLUID SHIFT AFTER SHORT BOUTS OF EXHAUSTIVE EXERCISE. Vahid Mohsenin* and Richard R. Gonzalez. John B. Pierce Foundation Laboratory and Yale Medical School, New Haven, CT 06519.

Six subjects exercised on a cycle ergometer 3 min. The work load demanded 105% of peak O_2 uptake resulting in a 17% ± 1% reduction in plasma volume based on changes in Hb & Hct, accompanied by an increase in plasma Na from 142.6±0.5 to 148.1±1.0 mMl^{-1} ($p<0.005$), Cl from 101.8±0.6 to 104.6±0.9 mMl^{-1} ($p<0.005$), lactate from 1.43±0.21 to 14.05±1.48 mMl^{-1} ($p<0.005$), and osmolality from 283±2 to 299±3 mOS/kg H_2O ($p<0.005$) within 2 min after cessation of exercise. Interstitial fluid pressure (IFP) (wick method) in the exercising muscles increased from a base line value of -1.0±0.9 cm H_2O to +1.5±1.1 cm H_2O, 14 min after exercise p = 0.02. Plasma colloid osmotic pressure increased from 25.1±0.6 mmHg to 30.6±1.4 mmHg, p = 0.003 and plasma protein increased from 7.0±0.1 to 8.1±0.2 (p = 0.001). The results suggest that further reduction of plasma volume during supramaximal exercise is prevented by both an increase in plasma colloid osmotic pressure and increase of interstitial fluid pressure. In addition plasma hyperosmolality may cause fluid shifts from inactive tissues. (NIH Grants #HL-17407 and #OH00836).



53.12

VOLUNTARY DEHYDRATION AND ALLIESTHESIA FOR WATER. R.W. Hubbard, B.L. Sandick*, W.T. Matthew*, R.P. Francesconi, M.J. Durkot and O. Maller*. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760

The purpose of this experiment was to explore the complex relationship between fluid consumption and consumption factors (thirst, voluntary dehydration, water alliesthesia, palatability, work-rest cycle) during a simulated, 9 mile, desert walk (treadmill, 1.34 m • sec^{-1}, 5% grade, 40°C d.b./26°C w.b. and wind speed of 2.5 mph). Twenty-nine subjects were tested (30 min • h^{-1}, 6 h) on each of two nonconsecutive days. Ss were randomly assigned to 1 of 3 groups: tap water (n=8), iodine-treated tap water (n=11) or iodine-treated, flavored, tap water (n=10). The temperature of the water was 40°C during one trial and 15°C on the other. Mean sweat losses (6 h) were similar and averaged 3.9 ± 0.06 kg. Fluid consumption (6 h) varied between 1.4 kg (warm, iodine-treated; 232 ± 44 g • h^{-1}) and 3.0 kg (cool, iodine-treated, flavored; 509 ± 50 g • h^{-1}). Warm drinks were consumed at a slower rate than cool drinks (negative and positive alliesthesia). This decreased consumption resulted in the highest percent body weight losses (2.8 and 3.2%). Cooling and flavoring effects on consumption were additive and increased the rate of intake by 120%. The apparent paradox between reduced consumption concomitant with severe dehydration and hyperthermia is attributed to negative alliesthesia for warm water rather than an apparent inadequacy of the thirst mechanism. The reluctance to drink warm, iodine-treated water resulted in significant hyperthermia, dehydration, hypovolemia and in two cases, heat illness.

54.1

EFFECT OF NIFEDIPINE ON MYOCARDIAL BLOOD FLOW IN DOGS WITH PARTIAL OBSTRUCTION OF CORONARY ARTERY. Arthur G. Williams*, Fouad A. Bashour, George J. Crystal, and H. Fred Downey. Univ. of Tex. Hlth. Sci. Ctr., Dallas, Tex. 75235

Partial obstruction of left anterior descending coronary artery (LAD) was produced just below its first major branch in chloralose-anesthetized, open-chest dogs. Nifedipine (NIF) was infused at constant rate (2.5 µg/kg/min i.v.) for 10 min. In Group I aortic pressure fell 25%, whereas in Group II it was held constant. Regional myocardial blood flow (15 µ microspheres) was measured before (control) and at 10 min NIF infusion. Results (ml/min/g):

		Group I (n = 7)		Group II (n = 7)	
		Control	10 min NIF	Control	10 min NIF
LAD	Epi	0.64±0.17	0.49±0.12	0.30±0.04	0.36±0.08
	Endo	0.45±0.09	0.33±0.06	0.23±0.06	0.29±0.08
LC	Epi	0.74±0.03	2.09±0.28*	0.93±0.16	2.26±0.34*
	Endo	0.87±0.05	1.68±0.23*	0.93±0.13	1.76±0.21*

Mean ± SE. * P< .05 from control.

NIF increased markedly blood flow in normal myocardium supplied by left circumflex coronary artery (LC), but did not significantly alter flow in ischemic region. There was no evidence of coronary steal during NIF. Supported by the Cardiology Fund.

54.2

FUNCTIONAL COLLATERALIZATION INDUCED BY REPEATED REVERSIBLE MYOCARDIAL ISCHEMIA IN PONIES. Harold E. Garner, Karla Rugh*, Joe Miramonti* and Dan Hatfield*. Dalton Research Center, University of Missouri-Columbia 65211

Four ponies were chronically instrumented with pairs of ultrasonic crystals positioned circumferentially in the left ventricular basal and apical endocardium, a left ventricular micromanometer and a Doppler flow meter on the left anterior descending coronary artery proximal to a hydraulic occluder. After recovery from surgery, 2 minute occlusions (CO) at 30 minute intervals were initiated. After an average of 239 (range 114 to 440) occlusions, CO resulted in a 1.2% (range -6.5 to 5.9) reduction in segmental systolic shortening compared to 82.3% (range 65.5 to 106.8) mean reduction at the beginning of CO studies. Three of the ponies were then similarly occluded during treadmill exercise sufficient to double resting heart rate. After an average of 170 (range 38-260) additional exercise occlusions, CO during exercise decreased segmental shortening by an average of 24.4% (range-6.5 to 55.9) compared to 68.3% (range 36.3 to 96.9) average at the beginning of treadmill studies. Thus, repeated reversible myocardial ischemia in ponies stimulates coronary collateral perfusion adequate for resting metabolic requirements. Coronary collateral development is further enhanced by continued stimulus during exercise. Funded by NIH grant # 1-RO1-HL29007-01 and the Missouri Heart Association.

54.3

CELLULAR ELECTROPHYSIOLOGIC EFFECTS OF HISTAMINE FOLLOWING MYOCARDIAL INFARCTION IN THE GUINEA PIG. John S. Cameron, Marion S. Gaide*, Cynthia B. Altman*, Javier Cuevas*, Robert J. Myerburg* and Arthur L. Bassett. Univ. of Miami School of Medicine, Miami, FL 33101

Histamine (H) is readily released from mammalian myocardium by drugs or surgical manipulation. To test the hypothesis that H plays a role in generating cellular electrophysiologic abnormalities following myocardial infarction (MI), we studied its effects in isolated guinea pig left ventricle. Microelectrodes were used to monitor transmembrane action potentials after acute (1-hr), subacute (24-hr) and healed (4-6 wks) MI was induced by ligation of multiple distal branches of the left coronary artery system. Normal and sham-operated ventricles were studied as controls. In all preparations, H (10^{-9}M to 10^{-5}M) increased action potential amplitude, maximum diastolic potential and maximum upstroke velocity ($\dot{V}_{max}$), while local refractory period and action potential duration were reduced. H also caused marked concentration-dependent increases in normal automaticity. At threshold concentrations in control (10^{-8}M) and MI (10^{-8}M) ventricles, H induced abnormal automaticity including premature ventricular depolarizations, irregular rhythms and repetitive tachyarrhythmias. Cimetidine (10^{-5}M) reduced automaticity when given alone and blocked ventricular responses to low concentrations of H (10^{-8}M to 10^{-6}M). These data suggest that H acts at H_2-receptors to alter electrical characteristics and induce arrhythmias and that tissue sensitivity to H is increased after MI. (SK&F Labs.; NIH: HL19044, HL27680, HL21735; AHA, Fla. Aff.)

54.4

CREATINE KINASE IN CARDIAC LYMPH OF CONSCIOUS DOGS. Lloyd H. Michael, Robert M. Lewis*, Robert Roberts and Mark L. Entman. Baylor College of Medicine, Houston, TX 77030.

Plasma levels of creatine kinase (CK) are elevated after coronary artery occlusion and have been used to assess extent of myocardial injury. A precise role for cardiac lymph in CK transport as well as a quantitative, temporal relationship of this transport to extent of myocardial injury in the conscious animal remains unclear. Thus, our conscious dog model, with indwelling cardiac lymphatic cannula, circumflex coronary artery (CFX) flow probe and occluding device, allowed serial sampling of cardiac lymph for CK analysis over several days and also optional CFX occlusion. In all dogs (n=9) cardiac lymph CK (Units/L) ranged from 8800-12,800 at surgery (day 1) and decreased to 110-21 on day 4. CK levels in 2 dogs followed subsequently for 8 days, with no CFX occlusion, did not deviate from the day 4 range. In 2 dogs, CFX occlusions, for 75 min or permanent, elevated cardiac lymph CK by 792 and 880 Units/L, respectively, within the first hour of occlusion; elevations in plasma CK occurred several hours later. In 5 animals with CFX occlusions lasting 15 min, 30 min or 1 hr followed by reperfusion, cardiac lymph CK was elevated within minutes and reached levels above control of 1730, 25,150 and 77,118, respectively, within 30 minutes of reperfusion. In summary, the lymphatics appear to play a major role in the transport of CK. In animals with CFX occlusion, CK appears in cardiac lymph several hours before increased levels in plasma; and, with reperfusion, the increase in cardiac lymph CK occurs immediately.

CARDIAC DYNAMICS II

55.1

HEMODYNAMIC CORRELATIONS (C) IN UNANESTHETIZED RATS. T.L. Smith, T.G. Coleman, W.R. Murphy,* and K.A. Stanek.* University of Mississippi Medical Center, Jackson, MS 39216

A systemic study of C between hemodynamic variables was performed in unanesthetized rats (8, male, Sprague-Dawley, 304-383 grams) to assess hemodynamic (HD) interactions. Data were collected once per minute for 24 hr by computer in rats previously instrumented with electromagnetic flow probes and arterial catheters. Cardiac index (CI), mean arterial pressure (MAP), heart rate (HR), stroke volume index (SVI), peak aortic flow (PAF) and total peripheral resistance index (TPRI) were analyzed. C were performed using linear regression analysis on 24 hr data (1300 data pairs), on 24 hr data averaged over 2 hr intervals (long-term, 12 data pairs), and on minute-to-minute (MTM) data from a 5 hr (short-term) period of stable HD (300 data pairs). On a MTM basis, MAP vs TPRI, MAP vs CI, CI vs SVI, CI vs HR and MAP vs HR were positive C whereas SVI vs HR and TPRI vs CI were negative C. MTM observations over a few hours illustrate basic short-term HD control. Long-term positive C included MAP vs CI, CI vs SVI, CI vs HR, SVI vs PAF, PAF vs HR, SVI vs HR and MAP vs HR. MAP vs TPRI, TPRI vs HR and TPRI vs CI were negative C on a long-term basis. Long-term C reflected diurnal/metabolic influences on HD. Five hr MTM C and averaged long-term C yield more information than composite 24 hr C (1300 data pairs) since the composite 24 hr C reflects coalesced short-term and long-term HD control. Supported by HL 11678 and HL 07270.

55.2

DECREASED $^{45}Ca^{++}$ UPTAKE IN HEARTS OF CHRONICALLY DIABETIC RATS. D.R. Bielefeld, D.A. Berkich, J.R. Neely*. The Milton S. Hershey Medical Center, Hershey, PA 17033.

Previous studies have shown that the aortic output of isolated hearts from chronically diabetic rats is more sensitive to decreased perfusate Ca^{++} concentrations than hearts obtained from normal rats. To assess whether this phenomenon is reflected by a difference in Ca^{++} influx, the uptake of $^{45}Ca^{++}$ by isolated perfused hearts obtained from normal and diabetic rats was determined. Rats injected with streptozotocin (45 mg/kg) developed plasma glucose concentrations of 400-600 mg %, plasma insulin levels of 11 µ U/ml, and were maintained without treatment for 3 months. Hearts were perfused in the non-working mode at 30°C with a Krebs-Henseleit buffer containing 11 mM glucose and were paced at 220 beats per min. The hearts were exposed to perfusate containing $^{45}Ca^{++}$ for various times up to 30 min at a constant pressure of 60 mm Hg and extracellular $^{45}Ca^{++}$ was washed out for 5 min with ice cold buffer containing no Ca^{++} and 0.5 mM EGTA. In hearts from normal animals, tissue $^{45}Ca^{++}$ increased during the first 10 min and remained essentially the same for an additional 20 min when perfusate Ca^{++} was 1.25 mM. Hearts from chronic diabetic animals when perfused for 3 min with 1.25 and 0.75 mM Ca^{++} had a 25% and 38% decrease respectively in the $^{45}Ca^{++}$ influx compared to normal hearts (n=6). Our results further suggest that there may be a chronic diabetes related defect in regulation of a Ca^{++} sensitive reaction in myocardial tissue. Supported by J.D.F. and NIH grant #HL20484.

55.3

THYROID AND GROWTH HORMONE IN CARBON MONOXIDE-INDUCED CARDIOMEGALY. David G. Penney, Bernd G. Barthel* and Joseph C. Dunbar, Jr.* Dept of physiol, WSU, Detroit, MI 48201

4 groups of adult male rats were used in Exper 1: 1) normal (N)/AIR, 2) N/CO, 3) thyroidectomized (TX/AIR, and 4) TX/CO. CO rats inhaled 500 ppm CO continuously for 42 days. TX rats received 1% Ca++, as Ca lactate, and .02% N-6-propylthiouracil in drinking water. While hematocrit (Hct) increased in TX/CO, it was significantly lower than in N/CO. Hct of TX/AIR was also lower than N/AIR. Combined ventricular wt (2V):body wt (BW) ratio of TX/CO was the same as N/AIR, while those of the N/CO and TX/AIR gps were signif greater and smaller, respectively. Because BW's of TX rats were about 60% that of N's, comparison of 2V wt was made using predictions based on BW, rather than on actual wt. 2V wts of TX/CO and TX/AIR rats were 12% and 23% smaller than predicted, respectively. On the other hand, 2V wts of N/CO and N/AIR rats were 29% and 0% larger than predicted, respectively. TX increased the growth of left ventricle (LV), relative to right ventricle (RV). CO reversed this, ie. RV/LV+S ratio increased, as occurred in N/CO. The results of Exper. 2, carried out identically, were similar. In addition, T_4 and growth hormone (GH) assayed by radioimmunoassay in TX were less than 20% and 30%, respectively, that in N's. GH was not stimulated in TX/CO, as it was in N/CO. Thus, TX produces overall cardiac atrophy. Even CO-induced "cardiomegaly" in TX yields hearts smaller than in N's. This is perhaps related to the diminished metabolic demands on the heart in TX, or to lack of T_4 and/or GH.

55.4

EFFECTS OF 500 ppm CARBON MONOXIDE ON THE NEONATAL RAT HEART. Fred J. Clubb, Jr.* Dept of Path, UTHSCD, Dallas, TX 75235, David G. Penney. Dept of Physiol, WSU, Detroit, MI 48201, and Sanford P. Bishop.* Dept of Path, UAB, Birmingham, AL 35294

Groups of newborn rats inhaled 500 ppm carbon monoxide continuously for 32 days (CO), after which development continued in ambient air. At birth, 6, 15, 28 and 200 d the following were studied: body wt (BW), total heart wt (HW), left ventricle + septum (LV+S) and right ventricle (RV) wet and dry wt, volume % myofibers from perfusion fixed hearts, and % binucleation (2N), cell length, and cell volume from isolated myocardial cells (MC) obtained by perfusion with Ca++ free media containing 0.1% collagenase. HW/BW increased sharply after birth, peaked at 15 d, and then fell progressively. CO % 2N LV+S and RV was significantly less than controls at 6 d (34% vs 42%), but not at 15, 28 or 200 d. CO 2N cells in LV+S at 15 and 28 d were consistently longer than in the control gp (A). However, by 200 d, CO LV+S, compared to A, had significantly shorter cells. CO LV+S cell volume to BW was significantly greater than A at 6, 15 and 28 d. At 200 d the CO LV+S had a significantly smaller MC volume than A ($27x10^3$ fl vs $37x10^3$). By 15 d the CO group had more total MC than A, demonstrating hyperplasia; however at 28 and 200 d there was no difference in MC numbers ($45x10^6$). We conclude that CO-induced cardiomegaly in the early transitional growth phase results from MC hyperplasia, and in the hypertrophic growth phase results in MC hypertrophy, whereas following regression, cell numbers return to normal, although cell length and volume remain smaller.

55.5

EFFECTS OF 200 ppm CARBON MONOXIDE ON THE PERINATAL RAT HEART. Sanford P. Bishop.* Dept of Path, UAB, Birmingham, AL 35294, Fred J. Clubb, Jr.* Dept of Path, UTHSCD, Dallas, TX 75235, and David G. Penney. Dept of Physiol, WSU, Detroit, MI 48201

To study effects of CO exposure on perinatal myocardial cell (MC) development, pregnant rats were maintained in room air or exposed to 200 ppm CO from 7 d gestation through 28 d postpartum. A control gp (A/A) was exposed to room air pre- and postpartum; an A/C gp inhaled CO postpartum only; a C/C gp received CO pre- and postpartum; and a C/A gp was exposed to CO pre- and room air postpartum. From birth through 28 d, the following were studied: body wt; total heart wt; left ventricle + septum and right ventricle (RV) wet and dry wt; volume % MC from perfusion fixed hearts; and % binucleated MC; and MC volume from isolated MC obtained by perfusion with Ca++ free media containing 0.1% collagenase. These data were used to calculate MC numbers. CO exposure in utero resulted in cardiomegaly at birth and an increase in RV MC numbers ($6.8x10^6$ C/C & C/A vs $5.8x10^6$ A/A & A/C). Continued CO-exposure postpartum, resulted in cardiomegaly and high MC numbers in both heart regions (28 d=50x 10^6 C/C vs $32.7x10^6$ A/A). CO exposure after birth only, resulted in MC hyperplasia during the transitional growth phase; however, during the hypertrophic growth phase, MC length increased and MC numbers decreased. CO-exposed rats, 6 d after birth, had fewer binucleated MC. In early neonatal growth, MC volumes were smaller than controls; those of the C/C gp being the smallest. Thus pre- and postnatal CO exposure at 200 ppm has profound MC effects, and implications for human health.

55.6

EXERCISE TRAINING AND CARDIAC PARAMETERS. M.A.B. Frey, B.M. Doerr*, and D.S. Miles. The Bionetics Corp., Biomedical Research Laboratories, Kennedy Space Center, FL 32899 and Wright State University, Dayton, Ohio 45435

Changes in cardiac function subsequent to a 10-wk training program were evaluated in seven men (26±3.6 yr) trained with a cycle ergometer, three 30-min sessions/wk at 75% max HR reserve. Before and after training, cardiovascular parameters were measured during seated rest and while exercising at power outputs of 300 and 600 kpm/min. Changes in myocardial function were evaluated by systolic time interval analysis and impedance cardiography.

	REST		300 kpm/min		600 kpm/min	
	Pre	Post	Pre	Post	Pre	Post
Cardiac Output (CO,l/min)	4.66	5.06	9.67	8.64	12.7	12.3
Stroke Volume (SV,ml)	61.3	72.4+	87.7	87.2	85.5	98.3+
Heart Rate (beat/min)	77	72	111	99+	150	124+
Electromechanical Systole (ms)	351	354	296	312	240	267+
Left Ventricular Ejection Time (LVET,ms)	240	243	233	243	193	219+
Pre-ejection Period(PEP,ms)	111	111	64	69	47	48
PEP/LVET	0.467	0.456	0.277	0.295	0.248	0.227

+ $P<0.05$, post-training values differ from pre-training

Post-training CO is maintained by increases in SV. Stability of PEP with increased filling time indicates sympathetic outflow is decreased. After a moderate intensity training protocol, both chronotropic and inotropic aspects of cardiac function become more efficient during moderately light exercise.

55.7

THE USE OF CLONIDINE IN THE TREATMENT OF SYSTOLIC HYPERTENSION IN THE ELDERLY.

Robert L. Wolf, Chuanshu Ji,*Ralph Craft,*and Martin Kaplan.* The Cardiovascular Center, New York, New York 10029.

The systolic (sitting) hypotensive effects of clonidine hydrochloride (Catapress (CA)) in varying doses (0.2 mg to 0.6 mg daily) and a fixed daily dose of chlorthalidone (CH) (Set I) were compared to placebo and to the same fixed daily dose of CH (Set II) employing a double blind, parallel program with elderly patients at least 60 years of age. The results prove: A) that Set I reduced the systolic blood pressure (SBP) ($\triangle \hat{u}=43$) twice the amount of Set II ($\triangle \hat{u}=20$) compared to CH alone, B) that, when tested by analysis of variance, the twice greater amount of SBP reduction of Set I compared to Set II is supported by an alpha-level of 0.01 and a power of 0.7. On the basis of the present sample, further major significant SBP reductions are obtained in the elderly, CH-treated population with the addition of CA to the treatment program without the penalty of adverse effects. Moreover, in 75% of the patients, the large SBP reduction of Set I was achieved with a total daily CA dose of only 0.2 mg.

56.1

A METHOD FOR CONTINUOUS ON-LINE COMPLIANCE (C) MEASUREMENT OF ISOLATED VASCULAR SEGMENTS. E.J. Zuperku, L.B. Bell*, J.L. Seagard and J.P. Kampine. Depts. of Anesthesiology & Physiology, Medical College of Wisconsin, Milwaukee, WI 53193

A method has been developed which provides an on-line (1/sec) measurement of elastance (E=1/C) in a perfused isolated vessel segment in situ. Values of E can be obtained as a function of perfusion pressure (P) or in response to drug interventions. Non-compliant stainless steel tubes are used as inflow and outflow cannulae. The segment P is obtained from a short, small-bore, stainless steel catheter and miniature pressure transducer. During constant flow through the inflow cannula, an adjustable outflow resistance is used to set the segment P. The simultaneous activation of solenoid pinch valves, for 200 msec, compresses inline silastic tubing next to each cannula and serves: 1) to isolate the segment and 2) to inject a small volume into the segment (ΔV=0.04 ml). This ΔV results in a step increase in segment pressure (ΔP). Since ΔV is constant over the P range of 0-200 mmHg, $\Delta P \propto E$, and $1/\Delta P \propto C$. C as determined by this method was found to be linearly related (r=.997) to the calculated C of a test cell air bubble (range:0.01<C< 0.07 ml/mmHg; resolution: 0.001 ml/mmHg). A similar linear relationship was obtained with a fluid filled elastic tube of various lengths. Comparison of C measured by this on-line method and that of ramp volume-pressure method for a segment of dog carotid sinus-artery resulted in a linear relationship (r=.939). Thus, this method appears capable of high resolution on-line C measurement. (Supp. by Am. Heart Assoc. and the VA).

56.2

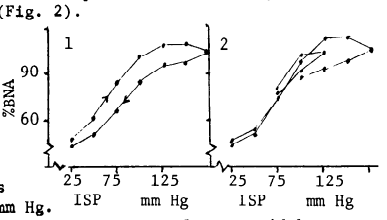
ON-LINE COMPLIANCE CHANGES IN THE ISOLATED DOG CAROTID SINUS DURING CHANGES IN PERFUSION PRESSURE. L.B. Bell*, E.J. Zuperku, J.L. Seagard and J.P. Kampine. Depts. of Anesthesiology & Physiology, Medical College of Wisconsin, Milwaukee, WI 53193.

The purpose of this study was to determine the effect of changes in mean carotid sinus (CS) pressure on CS compliance (C) over a range of 50-200 mmHg. A technique for in situ on-line C measurement in vascular vessels has been developed (Zuperku,Bell, et.al; companion abstract) and has been employed in this study in the isolated CS of the anesthetized dog. Mean CS pressure, arterial pressure, ECG, CS afferent nerve activity (CSNA) from the desheathed, intact CS nerve, and on-line CS C were measured during step increases and decreases in mean CS pressure of 25 mmHg, ramp increases in CS pressure (produced by a continuous volume infusion, 0.573 ml/min) and sustained step changes in CS pressure (50 mmHg for 10 min). Changes in CSNA were directly related to CS pressure, while changes in CS C were inversely related to CS pressure (range: 3>C>0.5 μl/mmHg; resolution: 0.06 μl/mmHg). Sustained step increases or decreases in CS pressure produced step decreases and increases, respectively, in CS C which were maintained for the duration of the change in CS pressure. Recent studies have shown, however, that CSNA resets to changes in CS pressure within 5 min (Physiologist 25(4):322, 1982). This study demonstrates that: 1) CS C is a function of CS pressure, 2) CS C decreases as CS pressure increases within a physiological range and 3) that the change in CS C secondary to a step change in CS pressure does not appear to adapt over a 10 min period. (Supp. by AHA and the VA).

56.3

CAROTID BARORECEPTOR NERVE ACTIVITY/SINUS PRESSURE HYSTERESIS: CRITICAL PRESSURE. H.O. Stinnett, F.A. Tello*, C.L. Roller*, and T.K. Akers. UND Sch. Med., Grand Forks, ND 58202

In 14 pentobarbital anesthetized rabbits the right carotid sinus was vascularly isolated, intrasinus pressure (ISP) was controlled and multifiber baroreceptor nerve activity (BNA) recorded. BNA was integrated at each 25 mm Hg steady state (> 1 min) pressure step in ISP (range 25 to 175 mm Hg). Normalized relationship curves of integrated BNA to ISP demonstrated well known hysteresis; as in Fig. 1. Shorter ISP ranges of 25 to 100, 75 to 125 and 100 to 175 mm Hg demonstrated that hysteresis was not present until steady state ISP exceeded 100 mm Hg (Fig. 2).



With intact efferent innervation of the right sinus, occlusion of the left common carotid artery resulted in a decrease in BNA at ISPs above 50 mm Hg. However, the onset and degree of hysteresis was unaltered at ISP > 100 mm Hg. Results demonstrate that the major factor for carotid baroreceptor "accomodation" or "resetting" can be attributed to the "creep" or "stress-relaxation" properties of the sinus. Support in part AHA Dak. Aff. DA-G-04 and 510.

56.4

EFFECTS OF CAROTID BARORECEPTOR RESETTING ON PERIPHERAL CIRCULATORY AND THERMAL RESPONSES IN THE COLD-EXPOSED CAT HINDLIMB. Carl A. Ohata. US Army Research Institute of Environmental Medicine, Natick, MA 01760

A carotid sinus was vascularly isolated in order to determine responses to increasing carotid sinus pressure at 50 mm Hg steps for 15 minutes from 50 to 250 mm Hg in 10 cats anesthetized with chloralose. The influence of other baroreceptors was diminished by sectioning the vagus nerves and the contralateral carotid sinus nerves. Mean arterial pressure, heart rate, mean femoral blood flow, footpad temperature and heat loss, and calculated femoral arterial vascular resistance and footpad thermal insulation were monitored at the different carotid pressures when the hindlimb was exposed to room air and during immersion in a 0^{0}C bath. Arterial pressure varied inversely to carotid pressure in both room and cold conditions. A sigmoidal relationship of tachycardia at low carotid pressures and bradycardia at high carotid pressures was observed only when the hindlimb was exposed to room air. With increasing carotid pressures, femoral arterial resistance increased, femoral arterial blood flow decreased, footpad thermal insulation increased, footpad temperature decreased, and footpad heat loss decreased. These trends were similar in both room and cold conditions, but a relatively greater level of vasoconstriction in the cold-exposed hindlimb resulted in a lower blood flow and temperature. In summary, carotid baroreceptors were shown to reflexly affect peripheral circulatory and thermal responses during room air and cold exposure. These carotid baroreflexes were modulated by other cardiovascular reflexes elicited by the cold stimulus.

56.5

ROLE OF THE AORTIC WALL IN BARORECEPTOR ACUTE RESETTING P.A. Munch and A.M.Brown, Univ. of Texas Medical Branch, Galveston, Texas 77550.

Baroreceptor (BR) pressure-frequency (P-F) curves show rapid (5 min) and reversible resetting to changes in mean arterial pressure (MAP). The effect is independent of efferent nervous discharge or circulating hormones. Since they are mechanically coupled to vessel wall structures, the resetting may be due to changes in the vessel wall. We examined this in an in vitro aortic arch/aortic nerve preparation from normotensive WKY rats using single, regularly discharging BRs. The arch was perfused 15-30 min at different MAP levels. The latter were interrupted at 5 min intervals by slow pressure ramps in order to construct pressure-diameter (P-D) and P-F curves which were compared before and after the more prolonged step change in MAP. In some experiments 10^{-6}M sodium nitroprusside (NP) was added to the internal perfusion system to relax the vascular smooth muscle. With increased MAP no consistent changes in the P-D relationship occurred; diameter changes that occurred were smaller than predictions based on P-F curves. In NP, P-F and P-D curves initially shifted to lower pressures, then stabilized. When MAP was raised BRs reversibly reset to higher pressures whereas P-D curves remained unchanged. These results indicate that whole-wall dimensional changes in the aorta do not play a primary role in the mechanism of acute resetting. (Supported by HL-16657 & GM-07856).

56.6

Correlations of Baroreceptor Discharge to Wall Strain in Individual Rats. Michael C. Andresen, Dept.of Physiology and Biophysics, Univ. of Texas Med. Branch, Galveston, TX 77550.

Baroreceptors (BR) do not respond directly to blood pressure, but rather to changes in vessel wall distention caused by changes in transmural pressure (P). In earlier studies, it was found that the discharge (FR) relationship was much more linear when plotted against circumferential wall strain E than P-FR plots. These studies used average E based on measurements of four representative rats and neural recordings in other rats. This report concerns a series of paired neural and mechanical measurements made on individual in vitro aortic arch-aortic nerve preparations (Andresen et al. 1978, Circ. Res. 43:728). In normotensive rats (WKY, n=15) and Spontaneously Hypertensive Rats (SHR, n=9) an average of five regularly discharging BRs were recorded in each rat. FR was measured during slow ramp increases in pressure from 20 to 200 mmHg. E-FR plots of these BR populations are substantially linear. Overall, however, no consistent pattern emerged for the observed deviations from linearity. Many curves were slightly sigmoidal. Some relationships flattened at high or low FR. Aside from the displacement of their E-FR curves to lower ranges of E, SHR's appeared similar to WKY's. In summary, BR discharge is certainly better correlated to E than to either P or stress over a wide range of input, but significant nonlinearities exist suggesting that other factors contribute to the determination of discharge. Supported by Amer. Heart Assoc., TX.

56.7

SUMMATION OF CARDIOVASCULAR RESPONSES DURING AIRWAY IRRITANT RECEPTOR STIMULATION FOLLOWED BY AORTIC NERVE ACTIVATION. D. Fred Peterson, Oral Roberts University Medical School, Tulsa, OK 74171

Eleven rabbits were anesthetized with sodium pentobarbital and instrumented to measure femoral arterial blood pressure (AP), ECG, heart rate (HR) and respiratory movements (R). The left aortic nerve (LAN) was carefully isolated from other tissue in order to stimulate it electrically. Insertion of two mid-tracheal cannulae allowed spontaneous respiration of room air while permitting passage of cigarette smoke across upper airway irritant receptors and out the nares. Resting values were: AP, 103 ± 2mmHg, mean ± SEM; HR, 275 ± 8 b/min. Supramaximal stimulation of LAN had no affect on respiration but caused a fall in BP (-21 ± 2mmHg) and a fall in heart rate (-35±4 b/min). Passage of 50 ml of smoke through the upper airways produced apnea (24±1 sec) a rise in BP (+27±3 mmHg) and a fall in HR (-175±18 b/min). When LAN stimulation was started during apnea but after BP and HR changes had stabilized there was further reduction in HR of (-54±6 b/min) and a fall in BP (-43±5 mmHg). Both of these changes were significantly greater than during aortic nerve stimulation alone. It appears that irritant receptor stimulation potentiates the fall in heart rate and blood pressure resulting from supramaximal LAN stimulation. This increased sensitivity in the baroreflex must occur centrally since electrical stimulation bypasses the peripheral receptors. This work supported by Oral Roberts University Intramural funds.

56.8

AORTIC BARORECEPTOR CHARACTERISTICS IN DOGS WITH CHRONIC VOLUME OVERLOAD. I.H. Zucker, M.J. Niebauer*, M.J. Holmberg*. Dept. Physiol., Univ. Nebraska Coll. Med., Omaha, NE 68105.

It has been shown that the arterial baroreflex is attenuated in chronic heart failure. It was the purpose of this study to determine if any of the altered baroreflex in heart failure could be attributed to alterations in afferent discharge characteristics. Single unit aortic baroreceptor activity was recorded from 5 normal dogs and from 7 dogs with chronic aorto-caval fistulas (AVF). At the time of the acute experiment mean arterial blood pressure (ABP) was similar in the two groups of dogs however systolic BP was higher in the AVP dogs (131.6 ± 8.8 mmHg vs 113.7 ± 4.8 mmHg $P < .05$). LVEDP was higher in the AVF dogs (29.6 ± 2.7 vs. 5.8 ± 1.4 mmHg $P < .0005$). AVF dogs had elevated heart wt/body wt. ratios. The relationship of systolic aortic pressure to systolic discharge was examined (pressure changed with aortic and vena caval occluders). The peak gain (normalized to the maximum discharge) averaged 2.22 ± .38 in the normal dogs compared to 1.15 ± .09 for the AVF dogs ($P < .01$). Saturation pressures and maximum discharge rate were greater in the AVF dogs although the threshold pressures were not different in the two groups. These data suggest that there is an attenuated response of aortic baroreceptor discharge in dogs with chronic volume overload and this abnormality may partly be responsible for the abnormal baroreflex in heart failure. (Supported by NIH grant #HL22594).

56.9

A COMPARISON OF FOUR METHODS OF ANALYSIS OF EFFERENT NERVE ACTIVITY. F.A.Hopp*, J.L.Seagard, J.L.Osborn, and J.P.Kampine. Depts. Anes. and Physiol., Med. Col. Wis., Milw., WI 53193

Four currently used methods of analysis of efferent nerve activity were compared using both pulse trains and renal efferent nerve activity (RENA). The methods were 1) counting spikes/100 mS (S) above a threshold; 2) integrating (I) the full wave rectified (FWR) RENA and measuring the time between resets; 3) averaging (A) FWR RENA using a leaky integrator (τ=140 mS); and 4) averaging FWR RENA using a voltage to frequency converter (VFC) and counting pulses/100 mS. Analysis of RENA by I, A, and VFC gave similar results which found RENA to vary inversely with arterial blood pressure. RENA analyzed using S did not show the same instaneous response as the other methods. Pulse trains analyzed using I, A, and VFC showed that each responded to frequency, amplitude, and duration modulation of spikes, while S responded only to frequency modulation and therefore did not provide as much information as the other methods. The effect of using a threshold to window out noise was also studied. Larger changes in RENA in response to blood pressure changes were observed when noise was removed with a window. However, some signal was also lost in this process. Since the window threshold was set somewhat arbitrarily, an alternative method to eliminate noise was developed. A recording of system noise generated by shorting the inputs of the amplifier to ground was made prior to nerve recording and this level of input was then subtracted from the signal during analysis. (Supp. by GM 29641 and the VA).

56.10

EFFECTS OF ISOFLURANE ON CHRONICALLY-RECORDED SYMPATHETIC EFFERENT NERVE ACTIVITY. J.L.Seagard, F.A.Hopp*, J.L.Osborn, and J.P. Kampine. Departments of Anesthesiology and Physiology, Medical College Wis., Milwaukee, WI 53193

Chronic recordings of renal sympathetic efferent nerve activity (SENA) were used to determine the effects of the anesthetic isoflurane (I) on SENA. The left renal artery and adjacent nerves were exposed via a flank incision and fine platinum electrodes were coiled around an isolated renal nerve. The nerve-electrode preparation was embedded in silicone and the electrodes and a femoral artery cannula were exteriorized in the cervical region. Five hours after recovery from anesthesia, nerve activity was recorded in the conscious (resting) state; during induction (4%I) and intubation; after 20 minute exposure to 1.5% and 2.5% I; and during recovery and extubation. SENA and arterial pressure (AP) were recorded on a FM tape recorder and analyzed as % control (conscious) using a PDP 11/ 10 computer. Thirty seconds of SENA for each procedure was analyzed as voltage to frequency converted averaged nerve activity (NA) and as spikes/100mS.

Anes. Level	NA	Spikes	AP	
Conscious	100	100	114±9	n=3
1.5% I	71.4±18.8	113.3±32.9	85±8*	*p<.05 vs
2.5% I	44.3±25.8*	47.7±11.2*	73±8*	conscious

Renal SENA showed an attenuation at 2.5% I, in spite of accompanying hypotension. This indicates both central depression of SENA, and possible blunting of baroreflex control of sympathetic tone. (Supported by GM 29641 and the VA).

56.11

MORE EVIDENCE FOR CARDIAC REFLEXES MEDIATED BY THE STELLATE AND MIDDLE CERVICAL GANGLIA USING ^{14}C-2-DEOXYGLUCOSE. D.R. Kostreva and J.A. Armour. Depts. Anesthesiol. & Physiol., Med. Col. Wis. and Wood VA., Milw., WI. 53193 & Dept. Physiol. Dalhousie U., Halifax, Nova Scotia B3H 4H7.

Stimulation of cardiopulmonary afferents produce localized increases in glucose utilization (GLU) of specific regions of decentralized stellate (SG) and middle cervical ganglia (MCG) and the heart. Dogs, 3-4 kg were anesthetized with sodium pentobarbital (35 mg/kg i.v.), intubated and ventilated. The left SG and MCG were decentralized except for the ansae subclaviae and cardiopulmonary nerves. The left vagal trunk was sectioned above the heart and the central end stimulated electrically (10 Hz, 0.5 ms, 5 mA). A single bolus of ^{14}C-2-Deoxyglucose was given i.v., and the nerve was stimulated periodically for 45 min. The SG, MCG and the heart were frozen and sectioned at (20 um), then covered with film. After 12 days exposure, the autoradiographs were analyzed quantitatively with a computerized densitometer, and the measurements were converted to GLU using Sokoloff's equation and lumped constants derived for dog brain. Compared to non-stimulated control animals, vagal trunk afferent stimulation resulted in increases in GLU of the entire MCG and SG, with the SG being more active than the MCG. However, the caudal half of the SG utilized much more glucose than the rostral half. In addition, the endocardium of the left ventricle showed marked increases in GLU. (Supp. by Dept. of Anesth., VA, NIH RCDA 00959, HLBI 27968 and NINCDS 18037).

56.12

MODIFICATION BY HYPOTHALAMIC GABA ADMINISTRATION OF FREQUENCY DEPENDENT CARDIOVASCULAR CHANGES ELICITED FROM THE AMYGDALA. L. K. Clarke, R. A. Galosy and C. D. Barnes, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

Extensive electrophysiologic and autoradiographic tracing studies have demonstrated pathways to the hypothalamus from the amygdala. There is evidence that some of these fibers may be GABAergic and that anesthetic agents impair neurotransmission in these fibers. In conscious animals, stimulation of the basolateral amygdala produces a pronounced pressor response in contrast to the depressor response which obtains in the anesthetized preparation. In the present study, microinjections into the ventromedial hypothalamus of 1 µl of GABA in doses of .5 to 4 µg significantly reduced the amplitude of the depressor responses at low frequency amygdaloid stimulation and changed the depressor responses obtained at 50 and 100 Hz to significant pressor responses. These cardiovascular changes were dose-dependent. This suggests a possible role of hypothalamic GABA in cardiovascular responses elicited from the amygdala. This research was supported by NIH grant HL 07474.

57.1

OPTIMIZATION OF A 2-DIMENSIONAL VIBRATING PROBE VIDEOSYSTEM TO MEASURE BIOLOGICAL CURRENTS. John A. Freeman, Paul B. Manis*, and Philip Samson*, Vanderbilt Univ., Nashville, TN, 37232. Recently we developed a circularly oscillating microprobe capable of detecting extremely small biological currents. In order to further extend the ability to measure such currents, which have been shown to be associated with a variety of developmental and regenerative events, we have made a number of improvements . We constructed a new probe, using piezoelectric drivers instead of miniature loudspeakers, and implemented a convolution algorithm on an inexpensive microprocessor which allows the on-line computation of two important vectors: the principal current density, J, and $\nabla|\vec{J}|$, a vector which points towards a current source, and which can be used to rapidly detect "hot spots" of current in biological memebranes. The use of a computer to detect, quantitate, and display current vectors was found to be greatly superior to the use of a lock-in amplifier. Finally, we implemented several video techniques, using a frame buffer and a color monitor to superimpose time-lapse polar plots of measured currents on a microscopic image of the structures producing them. The combination of these techniques greatly increases the resolution with which biologically important currents may be detected and displayed. We have used these methods to measure ionic currents generated by growth cones, as well as injured and regenerating neurites of goldfish optic nerve axons. Supported by NIH Grants # EY01117 and NS18103 to J.A.F.

57.2

INTRACELLULAR RECORDINGS OF THE EFFECTS OF DOPAMINE ON MEMBRANE PARAMETERS AND CELLULAR EXCITABILITY IN RAT HIPPOCAMPUS STUDIED IN VITRO. Valentin K. Gribkoff* and John H. Ashe, Department of Psychology, University of California, Riverside, CA 92521.

Brief exposure of area CA_1 neurons to dopamine (DA) results in a robust and long-lasting enhancement of population responses to afferent stimulation (Gribkoff and Ashe, submitted for publication). The present study investigates the corresponding intracellular effects of brief DA applications on hippocampal neurons.

Intracellular recordings were obtained from 35 CA_1 area neurons (average resting potential -68.4±5.9mV; input resistance 32.4±3.6Mohm). The predominate effect of microtopical DA application was an initial membrane hyperpolarization (HP; 4.1 ±2.3mV) with a decrease in membrane resistance (Rm; 21.0±7.8%). The percentage decrease in Rm was significantly related to the magnitude of the membrane HP.

These early actions of DA were followed by a late depolarization (DP; 5.4±2.9mV) along with an increase in Rm (15.8± 6.1%). The increase in Rm was abolished by manually clamping the membrane potential at resting levels. The late DP was accompanied by an increase in spikes elicited by orthodromic volleys or depolarizing current pulses. Simultaneous recordings of extracellular potentials exhibited a corresponding pattern of excitability change. DA effects similar to the above were observed in granule cells of the dentate gyrus. (Supported by NIH grant BRSG-RR07010-17 to JHA).

57.3

MOTOR DISORDER FOLLOWING ADRENOCORTICOTROPIN 4-10 INJECTION IN THE RAT BRAINSTEM. M.L. Leavitt, M.E. Combs*, and S.E. Thompson*. Biology Department, Southwest Missouri State University, Springfield, MO. 65804

Adrenocorticotropin (ACTH) affects norepinephrine turnover and is present in cell bodies of hypothalamic neurons which project to the locus cereleus (LC). The LC is a brainstem nucleus which may be involved in the dopamine deficient condition manifested in Parkinson's disease. The purpose of the present study was to determine whether the 4-10 fragment of ACTH would induce any movement or posture disorder upon injection into the LC. A 22g or 30g stainless steel guide cannula was implanted into male rats (300-350 gm) with the tip positioned 2mm dorsal to the right LC. Following at least one week of recovery rats were immobilized for injection of between 2.5×10^{-8} and 7.5×10^{-8} moles of ACTH 4-10 via an injection cannula extending 2mm beyond the tip of the guide cannula. Immediately following injection postural asymetry occurred and persisted for 30 min. Rats leaned to their right i.e., to the same side which received the injection but demonstrated a strong grip on all four paws. At the highest dose a rapid rotation ipsilateral to the injection side was observed. Other known effects of ACTH (stretching and yawning) were not seen, thus suggesting the motor effects are specific to the LC. These results confirm and extend the recent findings of Jacquet and Abrahms (1982) that fragments of ACTH produce in rats a condition resembling the human movement disorder of dystonia.

57.4

DETECTION OF ANGIOTENSIN PEPTIDES IN BRAIN TISSUE BY RADIOIMMUNOASSAY (RIA) AND BIOASSAY. Robin Barraco, Mike Moron,* Nancy Mumford,* and Howard Normile.* Wayne State University Detroit, MI 48201.

Although angiotensin peptides and angiotensin receptors have been detected in the brains of a variety of species by bioassay and immunoassay, the results have been extremely variable. One possible explanation for this variability may be the high activity of brain angiotensinases. To abridge this difficulty, nephrectomized mice were sacrificed by microwave irradiation. Whole brain was hemogenized in dilute buffer and, following ultracentrifugation, the supernatant was fractionated on Sephadex G-50. The eluate was monitored by RIA and bioassay. The fraction containing bioactive and immunoactive peptides also corresponded to the Ve of synthetic angiotensin and its amide. The angiotensin fraction produced sustained contractions in rabbit bladder serosal strips which were diminished by saralsin. Following further fractionation on Biogel P-2, the immunoactive fraction was lyophilized and resolubilized in vehicle; 0.25%/g brain of the isolated material produced a potent drinking response when injected into the third ventricle.

57.5

Choline Acetyltransferase Activity in the Cochlea of the Rat. D.A. Godfrey*, J.L. Park*, J.D. Dunn and C.D. Ross, Depts. Physiol. and Anat., Oral Roberts Univ., Tulsa, OK 74171

Microscopic samples from freeze-dried rat cochleas were isolated for analysis of choline acetyltransferase (ChAT) activity, as a marker of cholinergic structures. Samples of the organ of Corti, where highest activities were found, were subdivided into inner and outer parts and according to location in apical, middle or basal turn. Activities for these regions are presented as mean ± SEM (no. of samples, no. of rats):

	apical	middle	basal
inner	936±230(17,4)	5463±721 (8,3)	3029±909(7,2)
outer	206±25(37,7)	2074±226(29,6)	1701±319(9,3)

The ChAT activities in the inner part of the middle turn organ of Corti are ten times those of average rat brain and approach those of cholinergic tracts such as the facial root.

In two rats in which the olivocochlear bundle was cut on one side in the brainstem, ChAT activities in the organ of Corti were reduced to less than 5% of control side values in two days, and to zero in seven days. The results are consistent with other evidence that cholinergic synapses in the cochlea are derived from the olivocochlear bundle, and they further imply that all cholinergic structures in the cochlea derive from its centrifugal innervation. The high ChAT activities in the inner part of the organ of Corti imply that olivocochlear synapses under the inner hair cells are cholinergic as are those under the outer hair cells.

(Supported by ORU Intramural Funds and NIH Grant #NS17176.)

57.6

TWO AMINERGIC PROJECTIONS TO LUMBAR CORD FROM LOCUS COERULEUS IN THE CAT. Y.-Y. Lai and C. D. Barnes, Department of Physiology, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

Previous findings from our laboratory have demonstrated that stimulation of the locus coeruleus (LC) in the decerebrate cat results in facilitation of both extensor and flexor monosynaptic reflexes in the hind limb. These effects were further shown to be blocked by alpha adrenergic blockade but more completely by generalized aminergic blockade. The present study was undertaken to determine if the indoleaminergic as well as the catecholaminergic neurons present in the cat LC might also project to the cord. Under pentobarbital anesthesia, 0.5 ul of Evans blue (10% w/v) was injected into the L_7 ventral horn of adult cats. After 4 days, the cat was sacrificed, perfused with Faglu solution, the brain stem and spinal cord removed, the tissues cut into 12 µm slices and placed in Faglu solution, then transferred to a microslide. Fluorescence was examined under a fluorescence microscope where the Evans blue could be seen in both catecholamine and serotonin neurons, thus demonstrating both catecholaminergic and indoleaminergic neurons within the LC projected to the spinal cord. This work was supported by NIH grant NS 17040 and the Tarbox Parkinson's Disease Institute at TTUHSC.

57.7

REPRESENTATION OF TOOTH PULP STIMULI IN THE TRIGEMINAL NUCLEUS (P.INTERPOLARIS). M.A. Biedenbach, Physiol.,Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284.

Sensory axons from tooth pulp terminate in all trigeminal subnuclei, with p. interpolaris receiving a significant projection. Pars interpolaris is likely to play an important role in pulpal pain mechanisms, particularly since pulpal pain is not abolished following trigeminal tractotomy. In chloralose anesthetized cats unit potentials were recorded in p. interpolaris while stimulating cannine tooth pulps with bipolar electrodes. Pulp-driven neurons were tested for additional input from mechanoreceptors in orofacial regions. Of 150 pulp driven neurons tested, approximately 10% responded only to pulpal stimulation but the majority also to stimulation of mechanosensitive fields in orofacial regions. Responses to tooth pulp stimulation in the pulp-specific neurons were similar to those in the nonspecific neurons, except in the latter mean discharge latency was longer (20 vs. 4msec. for the first spike) and on repeated stimulation the response was more variable. In nonspecific neurons, comparison of pulp and mechanosensitive field stimulation showed also longer latencies and more variable discharges for the pulp-evoked responses. Tooth pulp stimuli, relatively pure noxious stimuli if compared with noxious cutaneous stimuli, are represented centrally by two main neuron groups, a pulp-specific and a larger nonspecific group. (Supported by NSF grant BNS 78-06953).

57.8

RETROGASSERIAN GLYCEROL INJECTION IN CATS AND IN CLASSICAL TRIGEMINAL NEURALGIA PATIENTS. Marvin H. Bennett, L. Dade Lunsford* and Julio A. Martinez*. University of Pittsburgh, Pittsburgh, PA 15261.

Trigeminal Evoked Potentials (TEP) and sensory threshold following stimulation of the maxillary gum were obtained in patients before, and six weeks following retrogasserian glycerol injection. Trigeminal brainstem evoked responses were obtained in cats before and four weeks following retrogasserian glycerol injection. Cats nerve and ganglion were then prepared for histological analysis. The major post-injection findings in patients were an average reduction in latency and threshold on the affected side, most patients with normal N_{20} latencies following treatment (10) were relieved of their pain (8/10) and a ninth was improved. Seven of the eight patients with no pain and a normal N_{20} latency had a reduced or unchanged sensory threshold. In cats, the average latency of the evoked potentials were increased on the glycerol injected side compared to the control side. The average amplitude of the response was reduced on the glycerol side. A loss of myelinated fibers was seen on the glycerol injected side. No loss of fibers was seen on the control side. The histopathology ranged from no effect, a mild focal loss of fibers to extensive, diffuse axonal degeneration. Correlations between histological and electrophysiological findings were greatest for cats with frank axonal loss (5/6) and least for those with a slight loss (1/4). From these data it is concluded that glycerol has its clinical effect from destruction of damaged myelinated fibers.

57.9

EFFECT OF NOXIOUS RADIANT HEAT STIMULATION OF HIND PAW ON GLUCOSE UTILIZATION IN THE SPINAL CORD OF ANESTHETIZED CATS USING ^{14}C-DEOXYGLUCOSE. S.E. Abram* and D.R. Kostreva. Depts. Anesthesiol. & Physiol., Med. Col. of Wis. & Wood VA, Milwaukee, WI 53193

A study was designed to determine if the ^{14}C-2-deoxyglucose (DG) metabolic mapping technique of Sokoloff could be used to localize areas of increased metabolic activity in the spinal cord resulting from noxious stimulation. Cats 2.0-2.6 kg were anesthetized with sodium pentobarbital (35 mg/kg i.v.). The hindlimb footpad was stimulated on one side intermittently with radiant heat. Skin temperature was raised to 52°C for 20 sec at 30 sec intervals. At the beginning of stimulation, 125 uCi/kg of DG was injected intravenously. After 45 min of periodic stimulation, the spinal cord was removed, frozen, and sectioned at 20 um and prepared for autoradiography. After 12 days of exposure, quantitative scanning of the autoradiographs was carried out according to Sokoloff's method. At the L7 and S1 spinal cord levels, DG uptake was 24 to 30% higher on the stimulated side in the outer layers of the dorsal horn (lamina I,II). A 10 to 33% increase in DG uptake was seen in the ipsilateral ventral horn at low to mid-lumbar segments. This study suggests that DG is a useful technique for assessing localized metabolic responses in the spinal cord during noxious peripheral stimulation. (Supp. by Dept. of Anesthesiol., VA, NIH RCDA HLBI 00959, HLBI Grant 27968, & NINCDS Grant 18037).

57.10

LOCAL CEREBRAL GLUCOSE UTILIZATION IN FETAL AND NEONATAL SHEEP. R.M. Abrams and C. Kennedy*. Department of Ob-Gyn, Univ. of Florida, Gainesville 32610 and Dept of Pediatrics, Georgetown Univ., Washington, D.C. 20007.

The newborn mammalian brain of several species has been shown to have a lower average rate of energy metabolism and a narrow range of rates in its various components than is found at maturity. In a further study of cerebral energy metabolism during development we have employed the [^{14}C]deoxyglucose method for measuring local cerebral glucose utilization in fetal and neonatal sheep. After establishing the lumped constant to be 0.40 and finding the rate constants for the kinetic behavior of deoxyglucose in plasma and brain to be close to those in other species we measured the rates of glucose utilization in 44 regions of the brain. The rates were low and homogeneous in mid-gestation except for those of brain stem nuclei of the auditory and vestibular systems and for those of the hippocampus which were relatively high. In the last 7 weeks, local rates rose approximately threefold. After birth there was a further average increase of 50% above term levels. The study shows that cerebral energy metabolism rises in most structures during prenatal maturation, a time when sensory stimulation is at a relatively low level and behavioral responses are minimal. Supported in part by USPHS grant HD11911.

57.11

COMPARATIVE ACTIONS OF MECHOLYL AND PILOCARPINE ON HUMAN ECCRINE GLANDS. Kenneth Kraning, University of Washington, Seattle WA 98195

Since pilocarpine (PC) induced sweating is inhibited by atropine (AT), it is thought to act on muscarinic receptors of eccrine secretory cells. However, Edisen and Lloyd (J Physiol 211: 25P, 1970) showed PC-induced sweating in cat's paw elicited action potentials similar to adrenaline. In the present study sweating responses of normal volunteers to stimulation by PC and by the muscarinic agonist acetyl-beta-methacholine (MCh) were compared on 2 adjacent pairs of forearm skin areas. Sites were pretreated by iontophoresis (0.2 mA, 240 s): one of each pair with NaCl (CT) and the other with either propranolol (PR) in 9 subjects, AT in 3, or lidocaine (LC) in 5. Then, one pair was treated with PC; the other with MCh (0.2 mA, 90 s). Gland activity was measured with evaporative capsules and iodized paper impressions. 8 parameters describing sweating rate curves (SR) and active gland populations (AG) were analyzed. As expected, AT abolished activity of PC and MCh, and on all CT sites PC was less potent than MCh in terms of SR ($p<.02$) and AG ($p<.01$). Unexpectedly, peak SR time was 80% longer for PC ($p<.001$) and the decay $t_{1/2}$ was twice as long. With PC as agonist PR reduced AG by 30% ($p<.02$) and LC abolished (3) or restricted (2) all activity. Neither PC or LC altered MCh sweating. These data suggest a more complicated mode of sudorific action for PC in vivo than that described for pure muscarinic agonists.

57.12

THE EFFECT OF LIVER DENERVATION (LD) ON MEAL PATTERNS, BODY WEIGHT (BW), AND BODY COMPOSITION OF RATS. L.L. Bellinger, V. E. Mendel, F.E. Williams and T.W. Castonguay*. Baylor Coll. Dent., Dallas, TX 75246 and Univ. of Calif., Davis, CA 95616.

Neural liver glucoreceptors are proposed as a major controller of food intake (FI). Rats (~255g) were sham (n=16) operated (SO) or LD (n=21). LD rats had all tissue cut between the esophagus, stomach, upper 1 cm of the duodenum and the liver. The hepatic artery and surrounding tissue were removed, next the hepatic portal vein was stripped clean and phenol treated (see Bellinger and Williams, Physiol. Behav. 30:463, 1983). At no time after surgery did the daily FI or BW of the groups differ significantly. Meal size (MS) and frequency (MF) were determined for 6 days and averaged; LD vs SO: 24 hr FI, 19.7±0.6 vs 19.7±0.7g; 24 hr MF, 13.4±0.6 vs 13.7±0.8; 24 hr MS, 1.5±0.1 vs 1.5±0.1g; dark phase FI, 13.9±0.8 vs 13.2±0.9; dark phase MF 8.8±0.6 vs 8.6±0.7; dark phase MS, 1.6±0.1 vs 1.6±0.1g and light phase MS, 1.3±0.1 vs 1.3±0.1, all differences nonsignificant (NS). In addition to the average values daily meal parameters did not vary significantly, thus there was no transient effect of LD. During the next 6 months the FI and BW of the groups did not differ significantly. At sacrifice body composition was determined; LD vs SO: %$H_2$0, 59.4±0.9 vs 58.6±0.7; %Fat, 9.2±1.3 vs 7.4±1.6; %Protein, 24.5±0.5 vs 26.5±1.1 and %Ash, 5.1±1.2 vs 5.5±0.2, all comparisons NS. LD were confirmed histologically. The data do not support the concept that the liver glucoreceptors are a major controller of FI. Supported by BCD and UCD FI lab. research funds.

57.13

THE EFFECT OF PORTAL (PORT) AND JUGULAR (JUG) GLUCOSE (G), MANNITOL (MAN) AND SALINE (SAL) INFUSIONS ON FOOD INTAKE, PLASMA GLUCOSE AND INSULIN IN DOGS. F.E. Williams and L.L. Bellinger, Baylor College of Dent. Dallas, TX 75246.

Liver glucoreceptors have been proposed to be a major controller of food intake but controversy in the area exists (Bellinger, Appetite 2:144, 1981). In this study mongrel dogs (13-20 kg) were adapted to being fed 1 hr each day and then hepatic PORT and JUG cannulas were inserted. After recovery the dogs were infused with G at 2.4 (G-A) and 3.6 (G-B) gm/kg of 30% G at a rate of 10cc/min and fed 10 min after infusion stopped. SAL and MAN were used for volume and osmotic controls. Four to eight dogs were tested per infusate with infusions repeated 1-4 times. Data is expressed as a % of averaged non-infused control days. After G-A into the PORT the dogs ate 82.0±5.9; while with SAL, 74.6±12.6 and MAN, 81.9±18.9. After G-B into the PORT the dogs ate 94.6±2.9; while with SAL, 96.5± 12.5 and MAN, 62.9±23.6. With G-A into JUG the dogs ate 102.4± 21.8 and with SAL, 117.9±17.8. After G-B into the JUG the dogs ate 59.1±25.4 and with SAL 103.9±8.5. ANOVA revealed no significant differences [$F(9,38)=1.24$] between groups. After PORT G-A and G-B fasting plasma G increased from 86.9±5.0 to 457.8± 21.4 mg%, $P<0.01$ and 85.3±2.0 to 524.3±16.8 mg%, $P<0.001$, respectively, just prior to feeding. During this same time insulin concentrations had increased by 9-20 times. These data do not support the concept of liver glucoreceptors being a major controller of food intake.

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57.14

INFORMATION PROCESSING BY THE BRAIN(CNS)AND THE COMPUTER(C)(BASIC DIFFERENCES). Clara Torda. N. Y. Center Pa. Tr. Current address: P. O. B. 4866, Stanford Univ. Stanford, Cal., 94305

With the development of artificial intelligence the problem of differences in information processing by the CNS and the C emerged. Cs exceed human performance by speed, accuracy, consistency, total recall, lack of distractibility, near perfect logical relationship between input and output, and limitless ability for data manipulation. The CNS is far superior in quality, complexity and depth of knowledge acquisition, intuitiveness, flexibility, ability to set goals, creativity, decision making, ability to initiate, motivation, selfdescription, selfmodification, selfimprovement, repair of shortcomings and the complexity of metaknowledge. Metaknowledge is based on a complex data structure about another data structure combined with the potential to use this knowledge to improve performance by many methods. Plasticity, cognitive complexity and growth are tangible manifestations of the use of metaknowledge. Information processing methods of transformation of the energy forms of inputs, parallel processing, multiple and partial coding, elaboration of codes, memory and retrieval by the CNS have been thoroughly studied(reviewed by Torda, Memory and dreams, Walters, 1980; Information processing, Walters, 1982). Survival of a basically fully selfsupporting system requires a large metaknowledge bank. The use of metaknowledge by the C is in its infancy. (Doyle, Th-58, MIT. AI, 1980; Genesereth-Smith, Stanford, HPP-81-6, 1982; Lenat et al., ibid. 1981). A new metasystemis here presented.

57.15

COMPARATIVE PHYSIOLOGY OF THE GAP JUNCTION CHANNEL. D.C. Spray*, R.L. White* and M.V.L. Bennett, Dept. of Neuroscience, A. Einstein College of Medicine, Bronx, N.Y. 10461.

Cytoplasmic pH (pH_i) transjunctional voltage, or inside-outside voltage determine gap junctional conductance (g_j) in many tissues. In fish and amphibian blastomeres (normal pH_i 7.6-7.8) the g_j-pH_i relation is steep (Hill coefficient 4.5) and the apparent pK is 7.3. In crayfish axon (pH_i 7.1) the curve is less steep and the apparent pK is about 6.7. In amphibian embryos, a transjunctional voltage (V_j) of 14 mV reduces g_j by half; a similar g_j decrease in teleost embryos requires 28 mV. In pairs of dissociated adult cardiac myocytes of rat and in crayfish septate axon g_j is independent of V_j. In all four preparations g_j is independent of inside-outside voltage. In vertebrate embryos the action of pH_i and V_j on g_j are independent; when g_j is lowered by low pH_i the remaining g_j shows normal V_j dependence and certain drugs (retinoic acid, glutaraldehyde, octanol, formaldehyde) reduce sensitivity to pH_i or V_j selectively. In squid embryos, reducing g_j by low pH_i reveals dependence of g_j on V_j (large V_j increasing g_j) and on inside-outside potential (cytoplasmic negativity increasing g_j). These studies on gating mechanisms of gap junctions in various tissues illustrates a considerable range of properties possessed by comparable membrane channel proteins.

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58.1

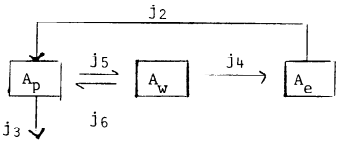
INTRACELLULAR pH IN DIABETES MELLITUS. R.L. Clancy, N.C. Gonzalez, M. Shaban* and V. Cassmeyer*. Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

The possibility that a decrease in intracellular pH (pH_i) contributes to impaired cellular processes in diabetes mellitus was examined. Male Sprague Dawley rats were made diabetic with alloxan or streptozotocin. pH_i was determined from the distribution of C-14 radiolabeled 5,5 dimethyloxazolidine-2,4-dione (DMO). Two day diabetic (D) rats had a marked extracellular acidosis (pH_e=7.07). pH_i's of cardiac muscle (CM), skeletal muscle (SM) and liver (L) were decreased 0.22, 0.28 and 0.14 units respectively. pH_e was normal in 2 and 4 wk D rats but pH_i's remained decreased: CM 0.28, 0.26, SM 0.23, 0.27 and L 0.16, 0.24 units at 2 and 4 wks respectively. Administering insulin for 4-5 hrs to 2 day D rats restored pH_i of CM and SM to normal while pH_e remained decreased. The resulting decrease in the transmembrane H^+ ratios suggested insulin increased H^+ efflux. This was examined using in vitro hemidiaphragm preparations from normal and 2 day D rats. Insulin (100 mU/ml) increased pH_i 0.1-0.25 units. Addition of amiloride ($5x10^{-4}$ M) blocked the insulin pH_i effect. These results indicate that in diabetes mellitus pH_i's of CM, SM, and L are decreased even when compensatory mechanisms have restored pH_e to normal. Secondly insulin restores pH_i to normal, in part via an active H^+ efflux. In skeletal muscle this appears to be mediated by a Na^+-H^+ exchange mechanism. (Supported by Kansas Aff. Am. Heart Assoc.)

58.2

IN VIVO CHARACTERIZATION OF ANTITHROMBIN IN THE RABBIT. A.C. Atencio and T.H. Carlson.* Dept. of Biochemistry, University of New Mexico, Albuquerque, New Mexico 87131.

Antithrombin III, AT_3 purified by heparin sepharose chromatography and labeled with radioiodine was injected intravenously. The behavior of $*AT_3$ was then monitored by radioactive measurements in serial blood samples and by whole-body counting. The disappearance of the AT_3 from the plasma followed three exponential kinetics. A three compartment model is described with the third compartment being in close proximity to the vascular compartment.



(A_p is the plasma; A_w - the vascular endothelial wall; A_e - extravascular; and the j's are rate constants.)

The size of the compartment were calculated to be: A_p = 0.283 ± 0.03; A_w = 0.193 ± and A_e = .524 ± .05. The fractional rates per day were j_2 = 1.30, j_3 = 0.697, j_4 = 5.42, j_5 = 19.9, and j_6 = 83.1.

58.3

IN VITRO METABOLISM STUDIES OF ANTICANCER DRUGS UNDER HYPOXIC AND OXIC CONDITIONS USING HUMAN TUMOR CLONOGENIC ASSAY (HTCA) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). Y-M Peng*, D.S. Alberts*, J.G. Einspahr*, C.V. Ludwig*, and T.P. Davis, Univ. of AZ, Cancer Center and Dept. of Pharmacology, Tucson, AZ 85724.

The HTCA has been widely accepted and used for testing the biological activity of anticancer agents. HPLC has been successfully used as an analytical tool to study the metabolism of these drugs. In the present study, we have combined these two methods to evaluate certain anticancer drugs and their metabolism both biologically and chemically. We have tested the cytotoxicity of mitomycin-C (MC), a bioreductive alkylating agent, against the murine EMT-6 fibrosarcoma cell line under hypoxic and oxic conditions in the HTCA. We have examined, using HPLC, the metabolism of MC by homogenates of this cell line. We have demonstrated that EMT-6 cells were more sensitive to MC and metabolized MC at a more rapid rate under hypoxic than oxic conditions. We have also utilized a rat liver S-9 preparation to study the metabolism of the new anthracene derivative bisantrene (B). We have shown that B was metabolized by the activated S-9 fraction under oxic but not hypoxic conditions. The HTCA showed that the biological activity of B decreased while the HPLC chromatographic profiles indicated at least three metabolites formed. Therefore, MC may require bioactivation in a hypoxic environment, whereas B probably requires an oxic environment leading to the formation of relatively inactive metabolites.

58.4

EFFECTS OF OSMOLALITY ON RED CELL DEFORMABILITY. Walter H. Reinhart* and Shu Chien. Columbia Univ. College of Physicians and Surgeons, New York, NY 10032

Changes in osmolality alter the red cell volume (MCV) and the intracellular hemoglobin concentration (MCHC). We studied the influence of variations in osmolality on red cell filterability. Fixed numbers of washed red cells (10^6 RBC/μl) were suspended in Ringer solution with 8 different osmolalities between 172±3 mOsm (Mean±S.D.), which led to RBC swelling (MCV 149±9 fl, MCHC 23.7±0.8 g/dl), and 665±28 mOsm, which caused cell shrinkage (MCV 67±10 fl, MCHC 55.9±3.9 g/dl). The viscosity of the intracellular fluid varies in the same direction as MCHC. These suspensions were filtered through Nuclepore polycarbonate filters with pore diameters of 2.6, 4.5 and 6.9 μm at a constant flow of 0.82 ml/min and the pressure was recorded. The relative resistance (β) of a RBC in a pore to cell-free Ringer solution was calculated. For each sized pore, the β exhibited a minimum value at a different osmolality. The osmolality for minimum β was 400, 250 and 200 mOsm, respectively, for 2.6, 4.5 and 6.9 μm pores. The results suggest the following conclusions: The passage of RBC through pores < 3μm is mainly determined by the cell volume, whereas the transit through pores > 5 μm is primarily influenced by the internal viscosity of RBCs. Changes in osmolality thus have opposing effects on the flow of red cells in small blood vessels and their passage through narrow pores, e.g. in the spleen. (Supported by NHLBI Grant HL 16851 and Swiss National Science Foundation).

58.5

OSMOTIC STABILITY OF ERYTHROCYTES IN THE RENAL CIRCULATION REQUIRES RAPID UREA TRANSPORT. Robert I. Macey and Lenore Wadzinski Yousef, Dept. of Physiology-Anatomy, Univ. of California, Berkeley, California 94720.

Urea transport by the human red cell occurs via a facilitated diffusion system with high K and high v_{max}; the equivalent permeability in the limit of zero urea concentration is approximately 10^{-3} cm/sec (Mayrand and Levitt, J.G.P. 81:221, 1983). A physiological role for this system is revealed by numerical integration of the Kedem Katchalsky equations which show that rapid urea transport is essential for red cell stability in passing through the renal medulla. The calculation compares two cells: cell A transports urea with permeability characteristics of normal red cells; cell B has urea permeability similar to lipid bilayers. Upon entering the hypertonic medulla, both cells shrink to a minimum volume. Both cells leave the medullary tip laden with urea; upon entering the isotonic cortex both will swell. The osmotic stress for B is far greater than for A. B is close to hypertonic hemolysis in the medulla and to hypotonic hemolysis in the cortex. B remains swollen for some time following its exit; the resulting decreased deformability presents a hazard if B re-enters the microcirculation. Further, by carrying urea, B may compromise osmotic gradients in the medulla. On the other hand, shrinkage of A is less in the medulla and upon exit, it hardly swells at all. (Supported by USPHS Grant #GM18819.)

58.6

EFFECTS OF S-ADENOSYLMETHIONINE ON NA^+,K^+-ATPase AND p-NITROPHENYL PHOSPHATASE OF DOG KIDNEY. George R. Henderson* (SPON: P.H. Brand). Medical College of Ohio, Toledo, Ohio, 43699.

S-Adenosylmethionine (S-AM), in a dose-dependent fashion, inhibits Na^+,K^+-ATPase and K^+-dependent p-nitrophenyl phosphatase (K^+-NPPase) activities of a purified enzyme prepared from dog kidney medulla. The sensitivity of K^+-NPPase to S-AM is much greater than the sensitivity of Na^+,K^+-ATPase. The IC_{50} for S-AM as K^+-NPPase inhibitor is $1x10^{-4}$ M. At a similar S-AM concentration, Na^+,K^+-ATPase is only inhibited 10%. The maximal inhibition attained for Na^+,K^+-ATPase was 25% using an S-AM concentration of $5x10^{-4}$ M; concentrations higher than $5x10^{-4}$ M could not be tested because of interference with the ATPase assay conditions. No inhibition of the basal Mg^{2+}-stimulated components of ATPase and NPPase reactions was observed. Further specificity of the S-AM inhibition was noted in that neither adenosine nor methionine, in similar concentration ranges, caused enzyme inhibition. The inhibition of Na^+,K^+-ATPase and K^+-NPPase was not time dependent and was completely reversed on washing the enzyme free of S-AM. pH profiles for Na^+,K^+-ATPase and K^+-NPPase demonstrated comparable inhibition by S-AM in buffered acidic, neutral and alkaline pH ranges. Kinetic analysis of the effects of S-AM on K^+-NPPase indicated competitive inhibition with respect to NPP and non-competitive inhibition with respect to K^+.

58.7

REGULATION OF GASTRIC MICROSOMAL (H^++K^+)-ATPase SYSTEM BY A CYTOSOLIC ACTIVATOR PROTEIN. Tushar K. Ray and Jyotirmoy Nandi. Department of Surgery, SUNY-Upstate Medical Center, Syracuse, NY 13210

The (H^++K^+)-ATPase activity associated with pig gastric microsomes was abolished within 10 min. of phospholipase A_2 (PLA_2) treatment at 21° or 37°C. About 60 and 80% of the microsomal PC was attacked by PLA_2 at 21° and 37°C respectively while 80% of the PE was hydrolyzed at both temperatures. Contrary to the PLA_2 treated microsomes at 21°C those digested at 37°C needed pretreatment with PC before assaying with the activator (AF) for maximal activity; PE was without any effect Similar to our previous reports with ethanol (ABB 202,8,1980) and trypsin (Life Sci 28,1969,1981) the present data reemphasized a critical role for the AF in gastric (H^++K^+)-ATPase function. The steady-state ^{32}P-intermediate level (p.mol/mg +SD) was reduced to 225+7 after PLA_2 treatment from the control (527+17) but elevated after AF reconstitution (398+43). The new steady-state levels in presence of 5 mM K^+ were control, 258+22; PLA_2, 174+54 and PLA_2+AF, 210+24. The turnover of the PLA_2 treated enzyme was reduced by about two orders of magnitude compared to the control and reconstituted enzymes. The data suggest that (1) the AF is an extrinsic protein and (2) the AF appears to be essential for both the kinase and phosphatase steps of the overall (H^++K^+)-ATPase reaction.

58.8

ATP DRIVEN H^+ TRANSPORT SYSTEMS IN RENAL PROXIMAL TUBULE AND COLLECTING DUCT. E. Kinne-Saffran* (SPON: R. Kinne). Albert Einstein College of Medicine, Bronx, N.Y. 10461

Brushborder membrane vesicles from rat cortical proximal tubules (BBM) and plasma membrane vesicles from bovine papillary collecting ducts (CDM) were isolated and preloaded with ATP and an ATP regenerating system. Intravesicular ATP hydrolysis was monitored for 10 min at 37°C in the presence of .5 µg oligomycin/mg protein under the conditions shown in the table:

	BBM	CDM
control	100 %	100 %
0°C	4 %	4 %
CCCP	200 %	197 %
pH gradient: Δ pH = 1	C %	94 %
Δ pH = 2	n.d.	76 %
valinomycin, $K_i = K_o$	116 %	155 %
DCCD (2 µM/mg protein)	32 %	26 %
DCCD + CCCP	39 %	29 %

These findings suggest that both, proximal tubular brushborder membranes and collecting duct (luminal) plasma membranes contain an ATP driven proton translocating system, sensitive to DCCD but insensitive to oligomycin. The electrogenicity of the system and its ability to operate against pH gradients across the membrane are more pronounced in the collecting duct than in the proximal tubule.

CCCP: Carbonyl-cyanide m-chlorophenylhydrazone; DCCD: Dicyclohexyldiimide.

Supported by NIH grant AH 29927

58.9

NUCLEOTIDE SPECIFICITY OF THE GASTRIC (H^++K^+)-ATPase. J.G. Forte and W.W. Renstra*. Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720

Nucleotide (NTP) hydrolysis and exchange reactions of the gastric (H^++K+)-ATPase were studied. NTP/ADP exchange was monitored by incorporation of ^{14}C-ADP into ATP. The reaction required Mg^{+2} and had a K_m^{ADP} for ADP of 40 µM at 0.2 mM ATP. K_m^{ADP} increased with increasing [ATP], in general agreement with Rabon et al. (BBA 688:515), but unlike their results, showed little K^+ stimulation (< 10%) over a wide pH range. Other NTP's also acted as phosphate donors for the exchange reaction, with the sequence ATP(1) > ITP(0.8) > CTP(0.5) = GTP. Mg^{+2}-dependent NTPase was about the same for all NTP's; only ATP showed K^+-stimulated hydrolysis, characteristic of the (H^++K^+)-ATPase, and H^+ transport activity. The addition of ADP (0.1 mM) caused K^+-stimulated hydrolysis of all the NTP's. H^+ transport by vesicles equilibrated with 100 mM K^+ occurred only with ATP; however, when external K^+ was reduced, H^+ uptake was seen with other nucleotides. These results are interpreted in terms of an initial rapid E-P formation sequence,

$$NTP + E \rightleftarrows E{\cdot}NTP \rightleftarrows E\text{-}P{\cdot}NDP \rightleftarrows E\text{-}P + NDP$$

where all NTP's can act as Pi-donor. If this reaction occurs in the (H^++K^+)-ATPase, then E-P formation can not be the intermediate that leads to the K^+-stimulated, proton translocating steps, but may be an intermediate for the "non-productive" hydrolysis. The effects of ADP in stimulating, e.g., K^+-stimulated ITPase, suggest a specific role for adenine nucleotides.

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58.10

CONCERNING THE POST-TRANSLATIONAL MECHANISM OF ALDOSTERONE ACTION: THE BARNACLE MUSCLE FIBRE AS A PREPARATION. E. Edward Bittar, Jude Nwoga*, Linda Chiang* & Geoffrey Chambers*. Univ. of Wisconsin, Madison, WI 53706.

One explanation of increased sensitivity of ouabain-poisoned, aldosterone-preexposed fibers to the injection of $GTPNa_2$ (or Gpp(NH)p) is slowing in the reassociation of the cAMP-protein kinase holoenzyme as the result of a raised internal ATP level caused by the steroid. Supporting evidence for this is provided by measurements with firefly and enzymic fluorimetry indicating a rise in ATP_i in preexposed fibers e.g. with firefly: 6.47 ± 0.36 (SE) (n=20) vs 4.81 ± 0.38 mMoles/kg water (n=15), P being < 0.01. Additional evidence pointing to a raised ATP_i comes from experiments showing that (1) unexposed fibers suspended in 10^{-3}M-adenosine-ASW undergo a rise in ATP_i and a fall in ArP_i, whereas preexposed fibers undergo a less pronounced rise. (2) Unexposed fibers injected with 0.1M-$ADPNa_2$ undergo a more pronounced rise in ATP_i than preexposed fibers. And (3) A rise in ATP_i and fall in ArP_i is seen following the injection of ADP into fibers preexposed to aldosterone and cycloheximide. The question now is: Does injection of cAMP into preexposed fibers lead to kinetic results resembling those obtained by injecting GTP? The answer is 'no', and no because of the presence in these fibers of a brisk peripheral PDE system. Whether inactivation of this system with 1-propyl-3-methyl-7-(5-hydroxylhexyl)-xanthine will make a kinetic analysis possible and whether a raised ATP_i does retard dissociation of the holoenzyme by cAMP remains to be seen.

58.11

SOLUBILIZATION OF AN ACTIVE (H^+,K^+)ATPase. A. Soumarmon, M.J.M. Lewin INSERM U10, Hop Bichat, 75018 Paris, FRANCE.

Membranes enriched in (H^+,K^+) ATPase activities (ATP phosphohydrolase, p nitrophenyl phosphatase and phosphorylation of a 95,000 daltons peptide) were prepared from hog gastric mucosa and treated by 1 - 2 % n octylglucoside. Soluble ATPase represented at least 32% of the native enzyme (1). Its activity was stable and specifically stimulated by potassium. Elution on a S400 sephacryl column and sedimentation on glycerol gradient were consistent with a 350,000-390,000 structure. Elution from the column destroyed the K^+ sensitivity but this was restored after reconstitution onto asolectin vesicles (2).
As compared to Triton X100, solubilization by n octylglucoside promoted limited depolymerization that was characterized on glycerol gradients. Three active forms were separated that had different yield of ATPase pNPPase and phosphorylating activities.

1. Soumarmon A., Grelac F. and Lewin MJM, Biochim. Biophys. Acta. in press.
2. Soumarmon A., Grelac F., Lewin MJM. (1983) In : Physico-Chimie des Mouvements Ioniques Transmembranaires. Elsevier/Biomedical Press, Amsterdam, in press.

58.12

CALCIUM INVOLVEMENT IN CARBACHOL-INDUCED ACID SECRETION. Shmuel Muallem* (SPON: G. Sachs). CURE Membrane Biology, UCLA School of Medicine and VA Wadsworth, Los Angeles, CA 90073.

Ca^{2+} fluxes associated with carbachol-induced acid secretion were measured in rabbit gastric glands pretreated with inosine. Pretreatment with inosine markedly reduced the $[Ca]_{in}$ without an apparent effect on the ability of the glands to accumulate acid or to be stimulated by either carbachol, histamine or DB-cAMP. The aminopyrine ratio (which reflected the glands to medium ΔpH) of carbachol stimulated glands was increased for about 20 min and then declined gradually toward the resting level. The same glands showed net Ca^{2+} accumulation over a period of 50 min, followed by a fall of $[Ca]_{in}$. The kinetic behavior of net Ca^{2+} influx suggested that a carbachol induced, La^{3+} sensitive Ca^{2+} pathway led to Ca^{2+} accumulation into intracellular organelles, presumably mitochondria. This Ca^{2+} accumulation continued until saturation of the intracellular Ca^{2+} buffers while further increases in $[Ca^{2+}]_{in}$ activated a Ca^{2+} extrusion mechanism which was sensitive to high medium $[La^{3+}]$. The La^{3+} effects on Ca^{2+} and aminopyrine accumulation together with the measurements of Ca^{2+} influx and efflux establish a direct connection between Ca^{2+} metabolism and carbachol-induced acid secretion.

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58.13

Cl^- REQUIREMENT OF ACID SECRETION IN ISOLATED GASTRIC GLANDS. Danuta H. Malinowska* (SPON: G. Sachs). UCLA Dept. Med., CURE Membrane Biology, VA Wadsworth, Los Angeles, CA 90073.

The dependence of acid formation on medium and intracellular Cl^- was investigated in resting and stimulated rabbit gastric glands. Acid formation was measured by aminopyrine accumulation (AP acc.) and intracellular Cl^- (Cl^-ICW) by steady state distribution of 36-Cl^-. Medium Cl^- induced AP acc. in stimulated glands with a $K_{0.5}$ of 10 mM, equivalent to 18 mM Cl^-ICW, with little effect in resting glands. With normal Na^+ICW and K^+ICW maintained, similar results were seen in stimulated glands treated with amphotericin and ouabain (ampho+ouab), where Cl^- pathways in the basolateral membrane were confirmed to be short-circuited. In ampho+ouab treated resting glands, AP acc. increased linearly with increasing Cl^-_i, i.e. no saturation was seen. Inhibitory effects of Na^+ICW were observed. Removal of Na^+ reduced the $K_{0.5}$ for Cl^- from 17.5 to 10 mM in stimulated glands, and from > 100 mM to 17 mM in resting glands. At a physiological Cl^-ICW (60 mM) and in the absence of Na^+, the $K_{0.5}$ for K^+ICW decreased from 19.5 to 12 mM on stimulation. Thus, stimulation seems to activate both K^+ and Cl^- components in the secretory membrane of the parietal cell and Na^+ may play an important regulatory role in this process. The characteristics of the Cl^- requirement of acid formation were, thus far, only partially reproducible in resting and stimulated digitonin permeabilized gastric glands.

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58.14

SEPARABLE K^+ AND Cl^- PATHWAYS IN GASTRIC VESICLES. John Cuppoletti* (Spon: G. Sachs) UCLA Dept. Med., and CURE Membrane Biology, VA Wadsworth, Los Angeles, CA 90073.

Membrane vesicles derived from histamine stimulated rabbit gastric mucosa exhibit H^+ transport dependent upon KCl. In contrast to vesicles from cimetidine treated rabbits, these vesicles do not require KCl preloading or valinomycin (val) treatment for maximal H^+ transport. Thus, stimulation results in the expression of an altered KCl permeability. The K^+ pathway was studied independently of the Cl^- pathway by using an impermeant anion, gluconate (Glu). Vesicles equilibrated in 100 mM K-Glu were diluted into sucrose media. Addition of 5 uM TCS led to rapid acidification of the vesicle interior, as followed by acridine orange, which was fully reversible by external K-Glu. When K-Glu loaded vesicles were diluted into media containing the potential probe, $DiSC_35$ (4 uM), a fluorescent signal (interior negative) was observed. The permeability of K^+ relative to Glu is high, as judged by a small additional valinomycin response. Upon treatment with 10 uM MgVanadate, a potent inhibitor of the H-K ATPase, the intrinsic K^+ permeability is inhibited. Thus, the K^+ pathway may be associated with a peptide of the H-K ATPase. Cl^- movements were studied similarly. In the absence of K^+, an inwardly directed Cl^- gradient caused H^+ uptake only in the presence of TCS. Potential ($DiSC_35$) signals generated by inward Cl^- gradients could be dissipated by K^+ addition. Thus, gastric vesicles exhibit K^+ and Cl^- conductive pathways by two different dye techniques. Supported by NIH AM32931.

58.15

MONOCLONAL ANTIBODY IMMUNOASSAY OF THE GASTRIC H^++K^+ ATPase. Adam Smolka* (SPON: G. Sachs). CURE Membrane Biology, VA Wadsworth, Los Angeles, CA 90073.

Monoclonal antibodies against the K^+-dependent ATPase responsible for acid secretion in the hog gastric mucosa were generated by hybridoma technology. One antibody (HK 111), shown earlier to bind selectively a major sub-unit of the ATPase, and to label tubulovesicles and secretory canaliculus of rabbit parietal cells, was used to develop a sensitive (<0.5 ug) immunoassay for the ATPase. Enzyme samples were covalently bound to the wells of PVC microtitration plates, then incubated sequentially with monoclonal antibody HK 111, isotype-specific goat anti-mouse Ig coupled to alkaline phosphatase, and p-nitrophenyl phosphate. Development of yellow coloration in the wells was monitored spectrophotometrically at 410 nm. Plotting the A_{410} values against standard amounts of ATPase bound to the wells gave an exponential dose-response curve which, in a semilogarithmic plot, was approximately linear over the range 0.25 to 10 ug ATPase. The enzyme-linked immunoassay has proven useful in monitoring H^++K^+ ATPase distributions in gastric mucosal cell fractions obtained during purification of rabbit zymogen granules.

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58.16

EXTRACTION AND SOLUBILIZATION OF THE HOG GASTRIC ATPase. E.C. Rabon*, R.D. Gunther* and A. Soumarmon* (SPON: G. Sachs). CURE Memb. Biol., UCLA School of Medicine and VA Wadsworth, Los Angeles, CA 90073, and INSERM U.10, Bichat Hospital, Paris, France.

Protected K^+ stimulated ATPase from hog microsomal vesicles was extracted and solubilized in enzymatically active forms using 1-O-n-Octyl-B-D-glucopyranosid (n-Octylglucosid). The extracted material, defined as that which settled, but did not pellet at 100,000 x g for two hours, was retarded on a Sepharose Cl-4B-200 column (Mol. exclusion 20 x 10^6 daltons). At a detergent/protein ratio of 1.4 (1.4%/1.0% w/v), approximately 70% of protein was extracted, demonstrating 50-100% of control K^+ stimulated specific activity upon dilution. At a selected detergent/protein ratio of 1.7 (2.4%/1.4% w/v), approximately 30% of the protein remained in the clear supernatant. This solubilized protein demonstrated approximately 50% of control specific activity and accounted for about 15% of total K^+ stimulated ATPase activity.

Supported by NIH AM32931.

61.1
INWARD AND OUTWARD CURRENT IN ISOLATED NON-SPIKING DENDRITES. Maurizio Mirolli. Medical Sciences Program, Physiology Section, Indiana University, Bloomington, IN 47405

Segments 1 to 2 mm long of the dendrites of the coxal receptors of Portunus sanguinolentus and of Callinectes sapidus were isolated by ligatures. The following currents could be recognized by voltage clamping: a) A fast inward current, peaking 1 to 2 msec after clamp onset, carried by sodium. b) A slow inward current peaking at 0.1 to 0.5 seconds, carried by calcium. c) A fast outward current, peaking withing 5 msec, carried by potassium. d) A slow outward current, peaking within 20 to 40 msec, also carried by potassium. e) A slow outward current peaking in half to 1 second, the carrier of which has not been identified. Although these currents can be recognized in segments cut from either the proximal or the distal parts of the dendrites, the slower ones are larger in segment cut from the proximal part which is closer to the point where the dendrites are presynaptic to other fibers (presumably axons of motor neurons). (Supported by NSF grant no. 48-830-09).

61.2
COMPARISON OF GLYCINE AND GABA CHANNELS IN CNS NEURONS OF THE LAMPREY. Michael R. Gold and A. R. Martin. Univ. of Colorado Sch. of Medicine, Denver CO 80262

Glycine-activated Cl^- channels in lamprey reticulospinal neurons (Muller cells) have mean open-times of 33 msec at 5°C and single channel conductances of 73 pS. Moreover, conductance decreases rapidly as intracellular Cl^- is raised above normal (Nature 299: 828-830). We now show that GABA-activated Cl^- channels in these same cells have very different properties. Mean open times are longer (42 ± 5 msec, mean ± S.D., n=15), and channel conductances are smaller (18 ± 4 pS) and unaffected by intracellular Cl^-. Additional studies revealed that the macroscopic conductance changes produced by glycine and GABA add algebraically, suggesting that the ligands do not compete for the same channels. The anion selectivities of the two channels were determined in order to assess the effective channel dimensions. Both channels were permeable to Cl^-, I^-, Br^-, NO_2^-, ClO_3^- and formate but impermeable to F^-, acetate and citrate.[3]These results indicate that glycine and GABA activate different populations of Cl^- channels on these cells, and that the larger conductance observed with glycine is unlikely to be due to a "wider" channel.
(Supported by grant NS-09660 from the NIH)

61.3
NEURALLY-MEDIATED ELECTRICAL AND MECHANICAL ACTIVITIES IN THE RAT TAIL ARTERY. D.W. Cheung (SPON: F. Sunahara) Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8 Canada.

Stimulation of the perivascular nerves elicited two types of electrical responses in the rat tail artery - the excitatory junction potential (e.j.p.) and the slow depolarization (s.d.). The e.j.p. is resistant and the s.d. is sensitive to α-blockade. The functional role of these two electrical components was investigated by simultaneous recording of the electrical activity and the isometric tension of ring segments of rat tail arteries. With increasing stimulus strength, e.j.p.s increased in amplitude until threshold was reached for action potentials. E.j.p.s per se did not generate any mechanical response. Fast phasic contractions ensued whenever an action potential was generated. The e.j.p., the action potential, and the resulting phasic contraction were resistant to α-antagonists. Slow tonic contraction associated with the s.d. and sensitive to prazosin and phentolamine could also be generated. However, the time course of the slow contraction and the s.d. were not the same. Study with exogenous noradrenaline also showed time discrepancy between the depolarization and the development of tension, suggesting that the slow contraction was independent of the s.d. but mediated by the same receptor.

61.4
STATISTICAL ANALYSIS OF SPONTANEOUS TRANSMITTER RELEASE USING A MODEL OF TEMPORAL STATIONARITY. M. D. Miyamoto* (SPON: R. Wondergem) E TN ST UNIV Col of Med, Johnson City, TN 37614

At motor nerve terminals, quantal transmitter release is described as a binomial event, where the no. of quanta released (m) is related to the probability of release (p) and the no. of release sites occupied by vesicles (n). However, due to temporal and spatial variation in n and p, binomial estimates of these parameters may be inaccurate. Temporal variation most likely results from 'turbulence' created by rapid depletion and replenishment of transmitter occurring between one nerve-evoked endplate potential (epp) and the next. If temporal variation is minimized, e.g. by using spontaneous release (which is low level and in steady-state), then spatial variation in p ($var_s p$) would be the only major factor. Miniature epps (mepps) were recorded from cutaneous pectoris muscles of Rana pipiens with standard microelectrode technics. The no. of mepps per oscilloscope sweep constituted one trial and 200 such trials used for each estimate of n and p. $Var_s p$ was computed using a 3rd moment with 3 simultaneous equations. Values of p obtained in 2.5 mM K were meaningless (Poisson) as expected, but values in 5 mM K were finite. Progressive elevation from 5 to 20 mM K increased both p and $var_s p$. The similarity of these results to those obtained with epps in high Mg suggests that this method may be useful for studying the effects of agents at the release mechanism, in the absence of complications due to transmitter depletion and mobilization. Supported in part by BRDG 1-S08-RR09171.

61.5
SPATIAL DISTRIBUTION OF SPINAL NERVE NETS. Edgar L. Gasteiger and Suzanne de la Monte*. Dept. and Sect. of Physiology, N.Y. State College of Veterinary Medicine and Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

Integrative mechanisms of the spinal cord can be characterized by the cord's slow wave spontaneous activity, particularly by the negative slow waves (NSWs) that occur at highest amplitude in the dorsal gray (Rexed's lamina IV). We studied the longitudinal and transverse distribution of these NSWs in the lumbar cord of decerebrate cats by averaging their amplitudes at different sites with respect to a trigger electrode set near the site of maximum amplitude. With longitudinal mapping, overlapping "nerve nets" were identified by their NSW activity, each having a field of peak activity located at or near the trigger electrode. For spinal levels L_6 and L_7 the amplitude of the NSWs declined linearly and symmetrically about the trigger point. When the trigger point was moved rostrally or caudally the linear decrement was maintained but the rate of decline was greater at the limits of the lumbar enlargement. Each nerve net encompassed approximately three spinal segments. Transverse mapping revealed uniformly high amplitude between the midline and rootline. Amplitude declined sharply outside the ipsilateral rootline and as the recording site was moved contralaterally. The distribution of nerve nets by this field mapping method is consistent with distribution studies of afferently driven postsynaptic cells in the dorsal horn. Supported by NIH grant RR 326.

61.6
THRESHOLDS FOR MEDIAN NERVE COMPRESSION UNDER NORMOTENSIVE AND HYPERTENSIVE CONDITIONS OF BLOOD PRESSURE. Alan R. Hargens, Robert M. Szabo* and Richard H. Gelberman.* Division of Orthopaedics and Rehabilitation (V-151), V.A. and University of California Medical Centers, San Diego, CA 92161.

Sensory and motor functions of the median nerve at wrist level were assessed during acute conditions of local compression. After measuring resting fluid pressure by the wick catheter (inserted during local anesthesia) in tissues which immediately surround the median nerve, the carpal tunnel of the nondominant hand was compressed by a rubber-mold apparatus. Thus, tissue fluid pressure was raised and maintained constant at levels between 30-70 mm Hg for periods up to two hours in 28 studies of 14 normotensive and 9 hypertensive volunteers. Sensory and motor functions were tested before, during and after compression. Compression thresholds for nerve dysfunction were consistently 30 mm Hg below diastolic blood pressure in all subjects. Sensory responses were completely blocked at threshold pressures of 40-50 mm Hg in normotensive subjects and 60-70 mm Hg in hypertensive subjects. Normal function returned in all subjects shortly after release of compression. These studies also identified sensibility tests which were the most sensitive to acute compression of peripheral nerve. The neurophysiologic results support the concept that ischemia is the primary cause of conduction block in low-pressure, nerve compression syndromes(Supported by the Veterans Administration and by USPHS/NIH grants AM-26344, AM-25501 and AM-00602).

61.7
SODIUM PENTOBARBITAL BLOCKS MORPHINE TOLERANCE AND POTENTIATION IN THE RAT. G.W. Terman*, J.W. Lewis* and J.C. Liebeskind. UCLA, Los Angeles, CA 90024.

Repeated exposure to footshock potentiates subsequent morphine analgesia in rats. It has been suggested that this phenomenon, as well as morphine tolerance, depend on the association of environmental cues with drug administration. To test this hypothesis, we compared morphine (2.5 mg/kg, s.c.) analgesia in awake and anesthetized (pentobarbital, 55 mg/kg, i.p.) rats previously subjected for 7 days to morphine (10 mg/kg, s.c.), footshock (4 min of continuous 2.5 mA 60 Hz sine waves) or no treatment. Pain responsiveness was assessed by the tail-flick test before and after the test dose of morphine.

Groups did not differ in tail-flick latencies prior to morphine on the test day. After morphine, unanesthetized animals previously given this drug showed significant analgetic tolerance, and unanesthetized animals previously exposed to footshock showed significantly potentiated morphine analgesia. In contrast, anesthetized animals, regardless of prior treatment, did not differ from untreated controls. Thus, pentobarbital does not affect morphine analgesia in naive rats, as measured by the tail-flick test, but does block both development of tolerance to morphine's analgesic action and potentiation of morphine analgesia by prior stress, possibly by preventing the perception of environmental cues important for these phenomena. (Supported by NIH grant NS 07628 and a gift from the Brotman Foundation)

61.8
SHORT and LONG-TERM EFFECTS of EXTRACELLULAR FIELDS on EXCITABILITY in the HIPPOCAMPAL SLICE. S.M. Bawin*, A.R. Sheppard*, M.D. Mahoney* and W.R. Adey. VA Medical Center and Loma Linda University, Loma Linda, CA 92357

Field potentials evoked in the CA1 cell layer were studied during and following stimulation with sinusoidal currents applied to the perfusing solution. The electric fields in the bath(10-140mV/cm, p-p)were of the order of EEG field gradients. Stimulation at 5Hz, a frequency representative of hippocampal theta activity, was compared with 60Hz, which is in the range of frequencies capable of inducing long term potentiation in the hippocampus, and is often used in kindling procedures. Both 5 and 60Hz fields(5-30s) could induce long-term increase of the evoked potential. Fields at 60Hz, but not at 5Hz, also induced short-term decrease of the response. Short lived post-field excitation was also seen more often following 60Hz stimulation than 5Hz fields. Prolonged(180s) stimulation during which the test pulses were phase-locked to the applied sinusoidal field confirmed this frequency dependent plasticity of the response. The field effects were independent of the position of the test pulse on the sine wave and of the direction of the current in the bath(either perpendicular or parallel to the CA1 cell layer). These findings argue against a cumulative polarizing effect of the fields at the cell membrane. These effects support concepts of a role for EEG-level fields in modulation of cerebral excitability, shown here in the absence of more powerful membrane potential oscillations that follow synaptic activation. (Supported by: Dept. of Energy and Southern Calif. Edison Co.)

61.9
VISUAL MOTION DISPLACEMENT SENSITIVITY OF CAT X, Y, AND W CELLS by R. P. Scobey, P.L.E. van Kan*, and L. Toepfer*, Dep. of Neurol., Sch. of Med., Univ. of California, Davis, CA 95616, U.S.A.

The motion sensitivity of single units in and around the LGN of anaethetized and paralysed cats was determined by measuring their displacement thresholds in response to a line stimulus. A displacement threshold was defined as the distance that a initially stationary line must move from a location A to a location B at constant velocity to evoke a criterion response from the cell. The computer adjusted the A-B distance on successive trials using a variable staircase paradigm until 8 reversals at the minimum stepsize could be averaged to define the displacement thresholds. The range of sensitivity for on- and off-center cells of the X, Y, and W classes overlapped with one another. On-center X cells tended to have smaller displacement thresholds than all other cell types and off-center W cells tended to be least sensitive. In conclusion, we found all cells to be sensitive to small displacements, suggesting that cells of all classes can potentially contribute to the detection of small displacements of narrow line stimuli.

61.10
A SUBSTANCE P-LIKE PEPTIDE MODULATES PHOTOSENSITIVITY IN THE LATERAL EYE OF LIMULUS. Jorge R. Mancillas, Dept. of Neurosciences, Univ. of Calif. San Diego, La Jolla, Calif. 92093.

Substance P-like immunoreactivity is contained in discrete cell populations throughout the length of the central nervous system of Limulus, including 6 pairs of bilaterally-symmetrical cell clusters in the circumesophageal connectives (CEC's) and subesophageal mass (SEM). Two of those pairs of clusters send efferent fiber projections to the lateral eye, where they innervate several components of the ommatidia, including the retinular cells, eccentric and distal pigment cells.

Subcorneal injection of synthetic substance P ($10^{-9}$$10^{-7}$m) into the lateral eyes causes reversible increases in the photoreceptors sensitivity that are dose-dependent and mimic those occuring spontaneously in a circadian fashion. Substance P also alters the endogenous circadian rhythm in photosensitivity by increasing the amplitude of the nocturnal plateau, while injection of D-pro^2, D-phe ^{7}, D-trp^9 substance P in the afternoon slows the rise in sensitivity and depresses the nighttime levels. Application of the antagonist after the nocturnal plateau has been reached, causes a reversible, short-term drop to diurnal levels. Our results are consistent with an involvement of a substance P-like peptide in circadian rhythms of photosensitivity. The 6 clusters in the CEC's and SEM are postulated to constitute a generalized, level-setting system with multiple targets, which can be driven by a circadian clock, as well as by other systems responsible for integrated organismic responses.

61.11
EVIDENCE FOR A RECEPTOR MEDIATED MODULATION OF 5HT UPTAKE IN CNS. E. Costa, M.L. Barbaccia*, O. Gandolfi*, & D.M.Chuang* Lab. Preclinical Pharmacol., NIMH, Saint Elizabeths Hosp., Washington, D.C. 20032

For several years the reuptake that terminates the synaptic action of monoamines was viewed as an energy dependent process whose efficiency depends on the energy available. This view must now be reconsidered. We reported (Science 215: 1112, 1982) that the high affinity brain recognition sites for imipramine discovered by Langer et al. (Nature 281: 148, 1979) are located on serotonergic terminals and probably are connected functionally with the 5HT reuptake system. 5HT displaces imipramine from its binding sites with low affinity and low Hill coefficient, indicating that an allosteric process links the imipramine binding site to the 5HT recognition site of the reuptake mechanism. Two daily injections of imipramine for 3 weeks down regulate Bmax of imipramine binding but increase the Vmax of neuronal reuptake for 5HT by hippocampal minces. Moreover in these minces the efficiency of imipramine as a blocker of the 5HT uptake is diminished. Hence, the high affinity binding sites for ^{3}H-imipramine may modulate 5HT reuptake physiologically through the action of an endogenous effector. A heat stable nonpeptidic molecule capable of displacng ^{3}H-imipramine from its high affinity site and of inhibiting the 5HT uptake in a dose related manner has been extracted from rat brain. Its partial purification will be reported.

61.12
EFFECTS OF CATECHOLAMINES AND AMMONIA ON BRAIN AMINO ACIDS AND NEUROTRANSMITTERS IN DOGS. D.R. Strombeck, D.R. Harrold*, and Q.R. Rogers. University of California, Davis, CA 95616

Hepatic encephalopathy is associated with changes in plasma amino acid (AA) concentrations which are in part a result of changes in plasma concentrations of glucagon and insulin. They are also produced by increased plasma catecholamine (cat) levels (Castroenterology 84:1399, 1983). The purpose of this study was to examine the effects of cat infusions with and without NH_3 on brain AA and neurotransmitters in normal dogs. Three groups of 4 dogs were infused for 5 hr with 4 μg/kg BW/min epinephrine + 2.3 μg/kg BW/min norepinephrine (E+N), E+N with 5mmol NH_3 (hr 4) and 2.5mmol NH_3/kg BW (hr 5), (E+N+NH_3), or saline (C). After 5 hr brain was collected and 5 regions were assayed for AA and neurotransmitters. During hr 4 and 5 plasma levels were decreased to an average of 45% of 0 hr for Ala, Arg, Asp, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. Mean brain levels of these AA except Asp and Trp were 76% (E+N) and 91% (E+N+NH_3) of C. Reductions were significant for Arg, His, Ile, Leu, Met, Phe, and Ser. In E+N mean Tau increased (P<.03). In E+N+NH_3 mean brain Gln increased to 235% of C (P<.001), mean brain Trp increased to 195% (P<.05), mean brain serotonin increased 175% (NS), mean HIAA increased 173%, and mean norepinephrine was unchanged. These data indicate brain levels of Trp increased with hyperammonemia despite a 58% reduction in plasma Trp. This suggests that NH_3 stimulates Trp movement into the brain and may be the basis for increased brain serotonin levels.

62.1

CENTRAL FACILITATION OF THE ARTERIAL BAROREFLEX FOLLOWING ACTIVATION OF BARORECEPTOR AFFERENTS. Cheryl M. Heesch^ and Francois M. Abboud. Univ. of Iowa, Iowa City, IA 52242

We reported acute resetting of carotid sinus (CS) baroreceptors (increased pressure threshold and rightward shift of pressure-discharge curve) when CS pressure is elevated by 90 mmHg for 5 minutes (Fed. Proc. 42:309, 1983). In this study we tested the hypothesis that a central facilitation of the baroreflex may compensate for the resetting of the CS baroreceptors. In 9 chloralose anesthetized dogs both vagi (including aortic depressor nerves) and the right CS nerve were cut and the left CS was vascularly isolated. Reflex reduction in renal nerve activity and in arterial pressure during elevation of left CS pressure from 50 to 250 mmHg averaged -0.18 ± 0.05 impulses/sec and -0.62± 0.09 mmHg per mmHg increase in CS pressure respectively. These reflex responses were repeated after the central end of the right CS nerve had been stimulated (20 Hz, 0.1 msec, 10V) for 5 min. to provide a constant "baroreceptor" inhibitory input to the brain. After this "conditioning stimulus" the corresponding reflex responses averaged -0.36± 0.13 impulses/sec and -0.84± 0.11 mmHg. This augmentation of baroreflex responses was reversed within 10-20 minutes. Thus it appears that brief periods of stimulation of arterial baroreceptors may provoke 2 opposing effects: peripheral resetting of baroreceptors which would tend to increase sympathetic outflow and central facilitation of the reflex which would tend to compensate for the peripheral resetting.

62.2

DOES PREGANGLIONIC SPROUTING LIMIT CARDIAC CHOLINERGIC SENSITIVITY FOLLOWING CHRONIC UNILATERAL VAGOTOMY? D. V. Priola, C. Anagnostelis*, R. Anaya* and D.C. Smith. Univ. New Mexico, School of Medicine, Albuquerque, NM 87131.

In a previous study, we found that no clear pattern of cardiac nicotinic supersensitivity was detectable 1-2 wks after unilateral cervical vagotomy (VGX). This lack of change in the sensitivity of the intrinsic cardiac nerves might have been caused by preganglionic sprouting from the remaining vagus. To test this, we compared cardiac responses to vagal stimulation in control animals to those with chronic VGX done either 1-2 wks or 8-12 wks previously. Atrial and ventricular isovolumic pressure responses to cervical vagal stimulation were evaluated in animals on total cardiopulmonary bypass. Stimulations were done at 0.5-30 Hz, 5 ms and 6-8 v. Positive responses were blocked by intracoronary timolol (8 mg) and hearts were A-V paced at constant rate. There were no significant differences between the frequency response (F/R) curves of any chamber in the control and 1-2 wk VGX groups. However, the 8-12 wk VGX animals showed F/R curves less sensitive than control, in some cases failing to respond at all. We could find no functional evidence of preganglionic sprouting in animals subjected to either short- or long-term VGX. Either sprouting does not occur or, if it does, it is not functionally important. In the long-term animals, degeneration of preganglionic fibers from the intact side may have taken place. (Supported by NHLBI Grant #HL-18517 and MBRS Grant #RR-08139).

62.3

INITIAL HEMODYNAMIC RESPONSE TO EXERCISE IN DOGS BEFORE AND AFTER BILATERAL CERVICAL VAGOTOMY. A. M. Booth, D. A. Gerasch*, M. E. Anderson*, C. R. Swayze* and I. J. Fox
University of Minnesota, Minneapolis, Mn. 55455

To test the effect of bilateral cervical vagotomy (Vx) on the hemodynamic response to exercise, one min. treadmill exercise periods (9 kph, 0% grade) were studied in two 25 kg mongrel dogs instrumented with solid state pressure transducers in the left ventricle (LV) and descending aorta, and an electromagnetic flow probe on the ascending aorta. Vx, along with vocal cord section and pyloromyotomy (to permit longitudinal study), were performed and the exercise repeated. Following Vx, an 18 ± 2% fall in mean aortic pressure (MAP) was observed 5 sec. after onset of exercise as compared to no fall in MAP in the intact state, which then returned toward control ($P < 0.01$, n = 17 and 16). Also following Vx, aortic flow rose 26 ± 2% at 5 sec. vs 50 ± 4% in the intact state ($P < 0.01$), but by 30 sec. this flow equalled the 60 ± 4% increase in the intact state. Resting heart rates were greater following Vx (166 ± 3 vs 128 ± 2 per min. $P < 0.01$). This may explain why increases in LV peak dP/dt and in heart rate were significantly higher throughout exercise in the intact state (40 ± 3 and 20 ± 3% vs 15 ± 2 and 10 ± 2%, respectively at 30 sec., $P < 0.01$). Thus, bilateral cervical vagotomy results in significant hemodynamic alterations early in exercise. Supported by NIH Grant HL23947.

62.4

THE ROLE OF INTERSTITIAL POTASSIUM LEVELS IN THE EXERCISE PRESSOR REFLEX. K.J. Rybicki,* M.P. Kaufman,* J.L. Kenyon* and J.H. Mitchell. Univ. of Texas Health Science Center, Dallas, TX 75235

Static muscular contraction reflexly increases cardiovascular function. Substantial evidence has been gathered to support the hypothesis that these reflex increases are caused by the excitation of thin fiber muscle afferents whose endings are stimulated by the build-up of metabolites trapped in the contracting muscle. Potassium (K+) has been suggested as one of the "metabolic stimuli" that activates these muscle afferents. Therefore, in chloralose anesthetized dogs, we measured gracilis muscle interstitital K+ "on-line", using an ion-selective electrode, while we either statically contracted the gracilis muscle, or infused K+ (1-5 mEq) into the arterial supply of this and adjacent muscles. In 3 dogs we found that statically contracting only the gracilis muscle increased interstitial K+ from a control level of 3.4±.5 mM to 6.8±.6 mM. In 9 dogs we found that increasing gracilis muscle interstitial K+ levels (from 3.1±.1 mM to 5.4±.5 mM), by infusion, significantly increased mean arterial pressure (MAP) (8±2 mmHg) and heart rate (HR) (7±3 bpm). Moreover, these increases were abolished by cutting the obturator nerve, demonstrating that these responses were a reflex. Thus, we have shown that elevating gracilis muscle interstitial K+ to levels occurring during static contraction reflexly increases MAP and HR. We have therefore provided further evidence that potassium release may play a role in the exercise pressor reflex.

62.5

EVIDENCE FOR AN AFFERENT PRESSOR PATHWAY IN THE VENTRAL SPINAL CORD. G.A. Iwamoto,* B.R. Botterman* and T.G. Waldrop* (SPON: J.H. Mitchell). Univ. of Texas Health Science Center, Dallas, TX 75235

It has long been held that the afferent spinal path for pressor reflexes evoked by somatic stimuli ascend the spinal cord in the vicinity of the dorsolateral sulcus (e.g., Ranson, 1916). However, there have been two reports of experiments using dorsolateral spinal cord lesions possibly suggesting otherwise (Johansson, 1962; Kozelka et al., 1981). In addition, the presumed afferent pathway to lateral reticular nucleus mediating the exercise pressor reflex is held to be in the ventral spinal cord (Iwamoto et al., 1982). It therefore seemed likely that an alternative afferent pressor pathway could exist. Adult cats were midcollicularly decerebrated under halothane. The anesthesia was removed following surgery. The L_7 and S_1 ventral roots were exposed so that stimulation of the peripheral cut ends would give rise to muscular contraction which in turn evoked a pressor response. In 7 of 7 cats in which a dorsal hemisection of the spinal cord was made at the T_{13}-L_1 level, significant increases in blood pressure (20±2 mmHg, $p<.001$) and heart rate (6+3 beats/min, $p<.05$) were observed on stimulating the ventral roots (3X motor threshold, 0.1 msec duration, 50 Hz). Further lesions revealed the bilateral nature of the afferent pathway. These data indicate the existence of a bilateral ventrally located spinal cord afferent pressor pathway from the hindlimbs which plays a role in the exercise pressor reflex.

62.6

INFLUENCE OF VARIABLE HEADGEAR LOADING ON BLOOD PRESSURE AND HEART RATE. Chandler A. Phillips and Jerrold S. Petrofsky. Wright State University, Dayton, OH 45435

An extensive series of experiments have been conducted to quantify the stress and fatigue of neck muscles as measured by cardiovascular responses. The neck muscles were dynamically and statically loaded by systematic variation of fifteen headgear configurations consisting of five different combinations of centers-of-gravity (forward-low, center-low, center-high, right-lateral-low and aftward-low) and three different weights (3.2 lbs., 5.0 lbs. and 9.0 lbs.). Six subjects would rotate their heads laterally (from side-to-side) for 30 minutes in each of the 15 headgear loading combinations. Immediately thereafter, the subject would position his head in an isometric head dynamometer and exert a sustained right lateral neck contraction or forward neck contraction at 70% of his maximum strength, during which the systolic and diastolic blood pressures rose an average 25%, diastolic blood pressures rose an average 40% and heart rates increased 20% (on the average) at the fatigue end-point (compared to resting levels). The results confirm that there is a significant cardiovascular response associated with fatiguing isometric neck muscle contractions. (This work was supported by U.S. Army contract DAMD17-80-C-0089).

62.7

ROLE OF BRADYKININ-INDUCED GASTRIC CONTRACTION IN CARDIOVASCULAR REFLEXES. C.S. Stebbins* and J.C. Longhurst. University of California, San Diego, La Jolla, CA 92093

Topical application of bradykinin (BK) to the serosal surface of the stomach reflexly activates the cardiovascular system. While stimulation of chemoreceptors may cause this response, it also has been suggested that smooth muscle contractions caused by BK may activate mechanoreceptors in the visceral wall. To test this hypothesis we compared mean arterial pressure and isometric longitudinal wall tension in 17 anesthetized cats during topical application of BK and bethanecol (BTH) to the stomach. Mean arterial blood pressure and max dP/dt rose to significantly higher levels ($p<.01$) in response to BK (5µg) than to BTH (100µg)(103±5 to 133±5 vs. 101±4 to 117±5 mmHg)(3444±142 to 3941±179 vs. 3388±146 to 3641±151 mmHg/sec). However, significantly greater isometric wall tension ($p<.01$) was achieved following BTH treatment (5.8±0.7 to 55.8±4.9 g) than following BK application (6.4±0.5 to 17.5±2.4 g). In 3 animals, no changes in wall tension occurred during BK stimulation. Similar cardiovascular responses to BTH occurred with isotonic contractions. However, application of atropine to the stomach abolished the cardiovascular response. These data suggest that smooth muscle contractions evoked by BK applied to the gastric serosa are not a major stimulus for reflex activation of the cardiovascular system. Additionally, the reflex cardiovascular response to bethanecol may be caused by activation of muscarinic receptors which are located on the afferent nerve endings. (NIH NINCDS NS 20165)

62.8

RESPONSES OF RETICULAR FORMATION NEURONS TO EPICARDIAL BRADYKININ. Robert W. Blair, Dept. of Physiology, Univ. of Okla. Health Sciences Center, Okla. City, OK 73190

Noxious information from the heart is transmitted to spinal neurons projecting to the medullary reticular formation (RF). The purpose of this study was to determine whether RF neurons respond to noxious stimulation of the heart with bradykinin (BK). Cats were anesthetized with chloralose (40-50 mg/kg) and paralyzed with pancuronium (70 mg/kg bolus doses as required). A bipolar electrode was placed on the cardiac nerve and caudal ansa subclavia. Only cells responsive to electrical stimulation of these nerves were studied further. BK was injected (2 ug/kg) into the left atrium via a catheter (14 cells), or was dripped onto the epicardium (40 ug in 1 cc, 11 cells). The floor of the 4th ventricle was exposed, and extracellular potentials of single neurons in RF recorded. Seven cells increased their discharge rates from 8 ± 3.2 (SEM) to a peak of 20 ± 8.2 spikes/s in response to BK. One cell decreased its rate in response to BK. The remaining cells were unaffected. Latency to onset of cell response occurred 20 ± 2.8 s, and latency to peak activity 33 ± 6.7 s, following application of BK to the heart. Mean spontaneous activity of cells responsive to BK (8 ± 3.2 spikes/s) was significantly ($p<.001$) greater than that of non-responding cells (0.1 ± 0.04 spikes/s). Results indicate RF neurons are sensitive to noxious cardiac stimulation. (Supported by NIH grant HL29618 and Oklahoma Heart Grant G-82-11).

62.9

CONTROL OF ARTERIAL BLOOD PRESSURE BY THE PARAVENTRICULAR NUCLEUS IN THE SHR. D.O. NELSON and CAROL A. GRAHAM*. Northwestern Medical School, Chicago, IL 60611.

Recent physiological and neuroanatomical evidence suggest that the paraventricular nucleus (PVN) may play an important role in the central autonomic regulation of the cardiovascular system. Considering that altered central mechanisms have been implicated in the development and maintenance of spontaneous hypertension, we examined the effects of microstimulation and lesioning of the PVN on blood pressure (BP) and heart rate (HR) of spontaneously hypertensive rats (SHR) and normotensive control WKY rats. Urethane anesthetized animals, 12 weeks of age, were catheterized for measurement of BP and HR and small, bipolar concentric electrodes were stereotaxically positioned bilaterally in the PVN. Low frequency microstimulation produced significant increases in BP, accompanied by mild tachycardia, above resting levels of each strain. SHR showed a higher treshold and slightly attenuated sensitivity to PVN stimulation compared to WKY controls. Increases in BP and HR were not affected by parasympathectomy but were eliminated by pharmacological or surgical sympathectomy. Bilateral PVN lesions produced a 24 ± 8 mm Hg (mean ± S.D.) decrease in BP and a significant reduction in HR in the WKY. Lesions in SHR produced a 60±15 mm Hg decrease in BP and a larger bradycardia (80-100 beats/min) compared to WKY controls. These data support the suggested importance of the PVN in blood pressure control and suggest that the PVN may contribute to the hypertensive state in the SHR. (Supported by AHA Grant-in-Aid 82659)

62.10

ABOLITION BY HYPOTHALAMIC LESIONS OF CARDIOVASCULAR RESPONSES TO CHRONIC SALINE INGESTION. **Ruben D. Bunag and Susumu Sasaki*. Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas, 66103.**

In rats made to drink isotonic saline solution instead of water for 5 weeks, systolic pressure and heart rate, whether recorded indirectly from the tail or directly from indwelling femoral catheters, rose slightly. When the ventromedial hypothalamus (VMH) was stimulated electrically with graded currents, increases in arterial pressure and sympathetic neural firing were larger in saline-drinking than in water-drinking rats. By contrast, pressor responses to injections of norepinephrine or tyramine were unaltered thereby indicating that cardiovascular reactivity was not generally enhanced. Following bilateral destruction of the VMH in other rats, none of the effects of chronic saline ingestion occurred. Although neither the site nor the mechanism causing sympathetic overactivity was determined our results are in accord with the interpretation that salt loading elevates blood pressure, at least in part, by stimulating the VMH to increase sympathetic activity. **(Supported by NIH Research Grant AM 27660)**

62.11

EFFECTS OF CENTRALLY APPLIED d-and l-PROPRANOLOL ON ACTIVE MUSCLE VASODILATATION (AMV) IN RABBITS. S. Shimada* & J. Stitt. J. B. Pierce Lab. & Yale Univ. Sch. Med., New Haven, CT. 06519.

AMV in rabbits can be elicited by electrical stimulation in the perifornicular area of the hypothalamus. While attempting to identify the peripheral neurotransmitter for AMV we found that large doses of d,l-propranolol could block AMV. However, it took over 1 hr to establish this blockade, and the doses required were in excess of those necessary to block peripheral β receptors. Because propranolol crosses the blood-brain barrier, we reasoned that the drug may have acted in the brain. Therefore, we injected into the brain both d- and l-propranolol to see if: 1. small quantities of drug could block AMV, and 2. there was a difference in potency between the two isomers. The drugs were dissolved in 10 µl sterile saline and infused into the lateral cerebral ventricle. To reduce an 80% maximal AMV response by half, we found that only 0.0062 ± .0026 µg l-propranolol were necessary, whereas 2.51 ± 0.95 µg d-propranolol were required to produce the same attenuation. These results suggest that there is a central β-adrenergic mediation of AMV. AMV is a component of the multifaceted defense reaction, thought to be naturally elicited in response to fear and emotional stress. Since high levels of stress are linked to some forms of persistent hypertension, perhaps propranolol's clinical effectiveness as an antianxiety and antihypertensive agent can be explained by an antagonism of a central β-adrenergic mediation not only of AMV, but of the entire defense reaction as well. (Supp. by NIH grant HL26426).

63.1

EFFECTS OF NECK EXTENSION (NE) ON DENSITY DEPENDENCE (DD) OF MAXIMAL EXPIRATORY FLOW (MEF) IN NORMAL HUMANS. R. Castile*, R. Ingram, Jr. and J. Mead. Children's Hospital Medical Center, Brigham and Women's Hospital and Harvard School of Public Health, Boston, MA 02115

Melissinos and Mead (J.A.P. 43:537-544, 1977) reported that NE increased MEFs at high lung volumes in some individuals. They attributed these increases in MEFs to increases in longitudinal tension at tracheal choke points. We postulated that increases in tracheal elastance produced by NE would diminish HeO_2 to air gas related differences in choke point geometry and thereby increase DD in subjects with tracheal choke points. Ten normal subjects with plateaus in their flow-volume curve (FVC) configurations (indicative of tracheal choking) were identified. While seated in a pressure compensated volume displacement body plethysmograph, each subject performed forced expiratory maneuvers with the neck in a normal posture and during NE, breathing air and HeO_2. Three air and 3 HeO_2 FVCs in each neck position were filtered, averaged, and compared. NE produced variable changes in airflows on the FVC plateau, but consistently increased HeO_2 MEFs more than air MEFs resulting in increases in DD. Both NE and HeO_2 tended to shorten the FVC plateau and move "bumps" in FVC configuration to higher lung volumes. There was a small (2%) but consistent decrease in vital capacity related to NE. We conclude that increases in elastance at tracheal choke points caused by NE increase DD of MEF. (Supported by HL 19170-07).

63.2

THE USE OF MAGNETOMETERS FOR MEASURING CHEST-WALL MOTION DURING HIGH-FREQUENCY OSCILLATION. M.J. Ackerman,* J.R. Clarke and M.E. Bradley. Naval Medical Research Institute, Bethesda, Md. 20814.

A chronic problem in studies of high-frequency oscillation (HFO) has been the paucity of quantitative measurements of gas volumes delivered to the chest. Magnetometers have been used as a noninvasive method of measuring tidal volume during exercise. Here we describe the use of magnetometers in man to measure noninvasively total ventilation during HFO, and to separate tidal breathing from the superimposed HFO generated by an Emerson diaphragm ventilator. Four pairs of magnetometers are used across the chest and abdomen, both anterior-posterior and lateral. The magnetometer signals are sampled by a PDP-11/03 computer. The HFO component of the signal is separated from the tidal volume component by a digital filter. Volume is derived from these two signals by curve fitting to previously calibrated volume signals. The quality of the signal is judged by comparison with accelerometer measurements of chest-wall motion. Transfer characteristics across the chest wall, including coherence, are determined by comparison of the magnetometer signal to tracheal pressure measured by a Millar catheter pressure transducer. (Supported by NMRDC Work Unit M0099PN.01B.0009.)

63.3

GAS TRAPPING CAN UNDERESTIMATE FUNCTIONAL RESIDUAL CAPACITY (FRC) BY BOYLE'S LAW. L.J. Becker*, G. Crawford*, G.R. Long*, I. Mayers*, P.T. Schumacker*, P. Ruygrok*, L.D.H. Wood.*, and P.H. Breen*. (SPON: A. Leff). Sec. of Pulmonary and Critical Care Med., University of Chicago, Chicago, IL, 60637.

During inspired effort against a closed glottis, FRC $\propto \Delta FRC/\Delta Palveolar$. In dogs with severe gas trapping, this method underestimated FRC when Δ Palv was estimated by either Δ Pao (airway opening) or Δ Ppl (pleural). We considered that severe gas trapping at high transpulmonary pressure (Pl) renders those lung units stiff, decreasing their contribution to ΔFRC. In 6 dogs studied by volume displacement plethysmography, right (R) and left (L) lungs were synchronously and separately ventilated with a double lumen endotracheal tube, bilateral Pao were measured, and Ppl was estimated by an esophageal balloon. The table summarizes measurements during FRC determinations (ml) before and after inflating R or L to Pl$\geq$25 cm H_2O: (means$\pm$SD).

	ΔPl-L	FRC (PaoL)	ΔPl-R	FRC (PaoR)
Baseline	-0.9±3.5	1136±332	1.3±2.7	1230±385
L + 489ml	12.0±7.8	-	-0.4±1.4	1327±400
R + 824ml	-2.8±3.1	1178±385	14.7±11.2	-

Thus, at high volume and Pl where the compliance (C) of the lung approaches the C of the gas within it, lung volume changes little in response to ΔPpl, so the increase in FRC is underestimated. We considered that ΔPpl might not be equal bilaterally, especially with asymmetric expansion of the chest wall. However, introduction of bilateral pleural catheters with small pneumothoraces in 2 dogs showed equal bilateral ΔPpl, and confirmed underestimation of FRC in the presence of gas trapped in lung units at high Pl.

63.4

MEASUREMENT OF RESPIRATORY MECHANICS IN NEWBORN INFANTS WITH RESPIRATORY DISEASE. SJ England, PN LeSouef*, and AC Bryan Respiratory Physiology, Hosp for Sick Children, Res. Inst. Toronto, Canada.

Accurate measurements of respiratory mechanics are difficult to obtain in critically ill newborn infants. Recent evidence suggests that such measurements can be obtained from the pressure measured during a brief end-inspiratory occlusion and assessment of the flow-volume curve of the subsequent passive expiration. With this method, respiratory system time constant (T), and its two components, compliance (C) and resistance (R) were measured in 22 premature infants with respiratory disease. Infants normally inspired before their passive expiration reached zero flow, thus maintaining an end-expiratory lung volume which increased with increasing T. C was lower in severely ill infants requiring intermittent positive pressure ventilation than in non-intubated infants or those on continuous positive airway pressure. R did not differ in the two groups of intubated infants but fell markedly immediately following extubation, indicating that the endotracheal tube contributed signficantly to R. We conclude that accurate assessments of respiratory mechanics can be easily obtained in critically ill infants using this method and that respiratory system time constant is an important determinant of the ability to maintain lung volume in the premature infant.

Supported by Grant MT5609 Canadian Medical Research Council

63.5

MAXIMUM INSPIRATORY PRESSURE-FLOW RELATIONSHIP (MIPF) IN MAN DURING SUBMAXIMAL NEUROMUSCULAR BLOCKADE (SMNB). L. David Pengelly, J.R.A. Rigg*, G.R. Buick*, and R. Lemieux*. Department of Medicine, McMaster University, Canada.

The isovolume MIPF has been used to define the active dynamic characteristics of the respiratory system[1,2]. The major determinants of this characteristic are the passive resistance of the respiratory system, and the force-velocity relationship of inspiratory muscles. From previous studies[2] we predicted that the slope of the MIPF (intrinsic resistance, R') would fall under conditions of muscle weakness produced by SMNB. In 6 normal human subjects we studied MIPF during maximum inspiratory efforts through a set of linear resistances. Measurements were made of pressure and flow at the mouth. Studies were repeated in the same subject during steady-state muscle weakness produced by infusion of d-tubocurarine. Data were analysed at 0.5 l above FRC, and fitted a linear model with $R^2 > 0.7$. Average slope of the relationship was 14.0 ± 3.9 cm H_2O/l/sec, under control conditions. During SMNB, in all but 2 subjects the slope decreased by > 50%, and in those two by > 30%. These data support the hypothesis that R' is decreased when muscle activation is reduced by SMNB.

1 Agostoni and Fenn. J.Appl.Physiol. 15:349, 1960.
2 Pengelly et al, J.Appl.Physiol. 30:797, 1971.
(Supported by MRC of Canada).

63.6

REGIONAL DIFFERENCES IN ABDOMINAL PRESSURE SWINGS IN SUPINE DOGS. Marc Decramer*, Suzanne Kelly*, André De Troyer and Peter Macklem. Meakins-Christie Labs, McGill Univ. Montreal.

We measured swings in abdominal pressure in the stomach (Pgas), below the costal (Pcos) and crural diaphragm (Pcru) and between the intestinal loops (Pab) with identical air-filled balloons, in eight supine anesthetized dogs. During mechanical ventilation, all pressures increased but both Pcos and Pcru increased considerably more than Pgas, whereas only a very small change was found in Pab (Pcos 5.0 ± 0.6, Pcru 4.5 ± 0.8, Pgas 2.9 ± 0.4 and Pab 1.0 ± 0.3 cmH_2O). During bilateral stimulation of the costal diaphragm Pcos invariably increased more than Pgas and Pab, whereas almost no change was observed in Pcru (Pcos 6.7 ± 0.8 vs. Pcru 0.3 ± 0.6 cmH_2O). During bilateral stimulation of the crural diaphragm Pcru invariably increased more than Pgas, Pab and Pcos (Pcru: 3.1 ± 0.8 vs. Pcos 1.4 ± 0.4 cmH_2O). During abdominal muscle stimulation, as during external compression, Pab always increased more than Pcos and Pcru (e.g. internal oblique: Pab 10.4 ± 1.9 vs. Pcos 5.3 ± 0.9 cmH_2O). During sternocleidomastoid stimulation all pressure swings were negative, but the change in Pab was always smaller than in Pcos, Pcru and Pgas (Pab 0.3 ± 0.2 vs. Pcos 2.9 ± 0.6 cmH_2O). We conclude: 1) In all experimental circumstances important regional differences in abdominal pressure swings were present 2) The abdomen consequently does not behave as a fluid-filled container 3) These regional differences allow the costal and crural diaphragms to develop different tensions. (Supported by MRC of Canada).

63.7

IN SITU SHORTENING VELOCITY OF CONTRACTING CANINE DIAPHRAGM. Cobern V. Peterson,Jr. and A.B. Otis. University of Florida, Gainesville, Florida, 32610.

Length changes of right costal, left costal and left crural diaphragmatic muscle segments were recorded from gauges composed of miniature Hall generators and magnets which had been sutured to the abdominal side of the diaphragm in 8 anesthetized dogs. Generators and magnets were placed so the distance between the two decreased as the muscle shortened thereby decreasing the electrical output. The abdomen was closed with sutures after placing the gauges. Cervical nerve roots to the phrenic nerve were isolated bilaterally. External airway and abdominal resistive loads were applied during repeated trials of supramaximal electrophrenic stimulation. Muscle segment shortening velocity was measured as the slope of the initial length change immediately after supramaximal phrenic stimulation. Shortening velocities of the right, left and crural regions ranged from 2.7 to 5.1, 4.4 to 5.9, and 4.2 to 7.0 initial lengths per second, respectively. Contractile velocities were not substantially decreased by increasing the external load, even with total airway occlusion. These observations suggest that the compliance of the respiratory system in the dog is distributed such that the diaphragm can shorten despite a variety of external loads. Supported by NIH Grant HL 28263.

63.8

MORPHINE SULFATE INHIBITS BRONCHOCONSTRICTION IN SUBJECTS WITH MILD ASTHMA WHOSE RESPONSES ARE INHIBITED BY ATROPINE. W.L. Eschenbacher*, R.A. Bethel*, H.A. Boushey, and D. Sheppard*, SFGH, CVRI, UCSF, SF, CA. 94110.

To determine whether morphine alters the bronchoconstriction to inhalation of distilled water, we gave 13 subjects with mild asthma 0.15 mg/kg morphine or saline intravenously followed by inhalation of increasing volumes of nebulized water. We measured specific airway resistance (SRaw) after each output and by interpolation determined the output of the nebulizer that would have resulted in a 50% increase in SRaw (PO_{50}). On a separate day we had the subjects inhale 2.0 mg. of atropine one-half hour before the inhalation of water. We compared the bronchoconstriction after morphine or after atropine to the response after saline by using the ratio of PO_{50}'s. For 7 of the 13 subjects, the ratio of PO_{50} after atropine to the PO_{50} after saline was ≥2.0: atropine was considered effective in inhibiting bronchoconstriction. For the remaining 6 subjects, the ratio of PO_{50} after atropine to the PO_{50} after saline was <2.0: atropine was considered ineffective. When bronchoconstriction after morphine was compared for these two groups, the atropine-effective subjects were significantly more inhibited by morphine than the atropine-ineffective group (p=0.002). In the three morphine-sensitive subjects tested, naloxone reversed morphine's inhibitory effect. We conclude that opiate receptor stimulation by morphine causes inhibition of the vagally-mediated component of water-induced bronchoconstriction.

63.9

COMPARATIVE EFFECT OF VERAPAMIL ON HISTAMINE- AND ANTIGEN-INDUCED BRONCHOSPASM IN A CANINE ASTHMA MODEL. D. H. Moore*, A. D. Jensen*, J. B. Walter*, M. Puckett and G. A. Rinard. Dept. Physiol. Emory Univ. Atlanta, GA 30322.

Greyhound dogs anesthetized with pentobarbital were challenged with increasing aerosol doses of verapamil. At the highest dose (30 mg/ml), pulmonary resistance (R_L), dynamic compliance (Cdyn) and pAO_2 were unchanged; however, a significant decrease in arterial blood pressure (ABP) was observed. Animals were then challenged with half-log increasing aerosol doses of A. suum antigen (ASA) or histamine, nebulized together with 10 mg/ml verapamil. R_L, Cdyn and ABP were measured and the results compared to control challenges without verapamil. The pattern of verapamil inhibition of bronchoconstriction was markedly dissimilar for histamine vs. ASA. The dilution of ASA producing a doubling of basal R_L and a 35% decrease in basal Cdyn averaged 10^{-4}w/v (threshold dose). A 10^{-3}w/v dilution of ASA produced an average four-fold increase in basal R_L (N=5). After verapamil, this dose was ineffective in producing even a threshold response. In contrast the threshold dose of histamine was unchanged after verapamil, but the max. measured increase in basal R_L was attenuated from 640±130% to 335±40% (N=7). Verapamil can abolish antigen-induced bronchospasm while only attenuating a maximal histamine response. We conclude that Verapamil is acting not solely on airway smooth muscle, but may block histamine release from mast cells, or depress vagal reflex activity. Other mediators may be responsible for antigen-induced bronchospasm.

63.10

EFFECT OF AN H_1 ANTIHISTAMINIC ON EXERCISE AND COLD AIR INDUCED BRONCHOSPASM. S.M. Walden*, P. Mason*, and E.R. Bleecker. The Johns Hopkins Medical Institutions, at Baltimore City Hospitals, Baltimore, Maryland 21224.

Airway cooling and drying with hyperventilation during exercise are important factors responsible for post-exercise induced bronchospasm (EIB). If bronchospasm produced by hyperventilation with cold dry air (CA) and EIB are caused by the same mechanisms, then pharmacologic agents should effect these challenges in a similar manner. Since histamine may be released during EIB and CA, we studied the effects of H_1 histamine blockade with diphenhydramine (DPH) (50mg orally) on EIB and CA in 6 asthmatics. EIB consisted of 6 min of steady state exercise, breathing compressed air (0% humidity, 21 ± 1° C). CA consisted of breathing conditioned cold air (0% humidity, -20 ± 1° C) for 3 min with stepwise increases in minute ventilation (Ve) until the FEV_1 fell 20% (Ve_{20} FEV_1). DPH increased FEV_1 from 2.95 ± .74L ($\bar{x}$ ± S.E.) to 3.37 ± .43L. The effects of DPH on EIB and CA are summarized below:

(*p<0.05, **p<0.01)	EIB % fall FEV_1	CA (at max bsl Ve) Ve_{20} FEV_1 L/Min
Baseline (bsl)	55 ± 13	38 ± 14
Post-DPH	57 ± 14	7 ± 5 ** 121 ± 59*

Thus, H_1 histamine blockade partially or completely blocked CA but did not effect EIB. Therefore, despite similarities in airway responses to cooling and exercise, different mechanisms may be important in causing exercise and CA induced bronchospasm.

63.11

ENDOGENOUS PROSTAGLANDINS ARE DETERMINANTS OF HISTAMINE SENSITIVITY OF ISOLATED CANINE TRACHEAL SMOOTH MUSCLE (CTSM). S.A. Shore*, W.S. Powell*, and J.G. Martin*, (SPON: J. Milic-Emili) Meakins-Christie Labs., McGill University and Endocrine Research Labs, Royal Victoria Hospital, Montreal, Canada.

We studied the role of endogenous prostaglandins(PG) in determining the histamine (H) sensitivity of CTSM in-vitro. High pressure liquid chromatography established PGI_2, through its metabolite 6-oxo-$PGF_{1\alpha}$ as the predominant PG produced by CTSM. Radioimmunoassay showed that H (10^{-4}M) increased 6-oxo-$PGF_{1\alpha}$ production from 6.5±1.9 to 12.5± 3.2 ng/g muscle/min (mean±SE) ($p< 0.01$;n=10). The isometric tension produced by H (10^{-4}M) was inversely linearly correlated with the concentration of 6-oxo-$PGF_{1\alpha}$ in the tissue bath(r=0.71;p<0.01). Indomethacin (INDO) (10^{-5}M) shifted the H dose-response curve of CTSM, increasing the maximum tension (Tmax) from 1.14±0.15 kg/cm^2 in control to 1.59±0.20 kg/cm^2 in INDO pretreated samples ($p < 0.05$;n=9). The dose of H required to produce 50% of Tmax (ED_{50}) decreased from $2.0x10^{-5}$M to $5.9x10^{-6}$M($p < 0.005$). The magnitude of the decrease in ED_{50} with INDO was linearly correlated with the H sensitivity of the CTSM (r=0.72;p<0.02). INDO also decreased the standard deviation of log ED_{50} among the 9 dogs studied from 0.49 to 0.22 (p<0.025). Our results show that 6-oxo-$PGF_{1\alpha}$ production increases during H induced contraction of CTSM and suggest an important role for endogenous bronchodilating PG's in determining both the H sensitivity of CTSM in-vitro and its variability among individual animals. (Supported by MRC Canada and the Can. Lung. Assoc.).

63.12

DIFFERING RESPONSE OF ASTHMATICS TO SO_2 EXPOSURE WITH CONTINUOUS AND INTERMITTENT EXERCISE. H. Kehrl*, L.J. Roger*, M.J. Hazucha* and D. Horstman (SPON: V. Ranga). Clin. Studies Branch, US EPA, Chapel Hill, NC 27514

Sheppard et al. have reported (ARRD 125:151, 1982) that asth-matics exposed repetetively develop short-term tolerance to SO_2-induced bronchoconstriction.We reported (AARD 127:160, 1983) a similar pattern of response in a group of asthmatics performing 3 cycles of 10 minutes of treadmill exercise (V_E = 41 l/min) followed by 15 minutes of response testing at rest during a 75 minute chamber exposure (26°C,70% RH) to 1.0 ppm SO_2. In a study to compare the effects in asthmatics of SO_2 exposure with continuous versus intermittent exercise, ten of our subjects returned several weeks later and were exposed to 1.0 ppm SO_2 while treadmill walking for 30 minutes under the same environmental chamber conditions and exercise intensities. Changes in specific airways resistance (SRaw) measured before and after each exercise period were:

1.0 ppm SO_2	Baseline	10 min	30-35 min	60 min
Intermittent Exercise	5.39	14.67	12.23	11.12
Continuous Exercise	5.20	-	17.41	-

The change in SRaw after 30 minutes of intermittent exercise was significantly less than that observed after either the first exercise period ($p < 0.01$) or 30 minutes of continuous exercise ($p < 0.005$). From present knowledge of the SO_2-induced bronchoconstriction of moderately ventilating asthmatics, it appears that this response develops rapidly and is still maintained at the end of a 30 minute exposure to SO_2.

64.1

OMEPRAZOLE INCREASES K^+ NET FLOW DURING STIMULATED CONDITIONS IN THE MAMMALIAN GASTRIC MUCOSA IN VITRO. H. Larsson* and B. Ryberg* (SPON: B. Johansson). Research Laboratories, AB Hässle, S-431 83 Mölndal, Sweden.

A Proposed model for the initiation of acid secretion involves activation of a KCl symport pathway in the apical membranes of the parietal cell. K^+ is then exchanged for H^+ by the H^+,K^+-ATPase resulting in a net secretion of HCl. Provided that the KCl pathway is unaffected, an inhibition of the ATPase would lead to an increased secretion of KCl. Using omeprazole (OME) ($5x10^{-6}$M) as a proton pump inhibitor, this hypothesis was tested in the isolated guinea pig gastric mucosa. As references SCN^- ($5x10^{-3}$M) cimetidine ($5x10^{-5}$M) and p-chloromercuribenzene sulfonic acid (pCMBS) (10^{-4}M) were used. Effects on basal as well as on stimulated (histamine, $2x10^{-5}$M, or IMX, 10^{-4}M + db-cAMP, 10^{-3}M) secretion were studied. All compounds inhibited histamine-stimulated acid secretion by 60-90%. OME and, to a minor degree, pCMBS increased net flow of K^+ to the mucosal side, whereas it was unchanged after SCN^- and cimetidine treatment. The OME-induced increase in K^+ flow was also seen during inhibition of db-cAMP-stimulated but not of basal acid secretion. Our results are in accordance with the proposed model for stimulated acid secretion.

64.2

TRANSPORT BY CULTURED MONOLAYERS OF GASTRIC SURFACE EPITHELIAL CELLS. M.J. Rutten, S.Ito,* D. Rattner,* W.Silen. Harvard Medical School, and Beth Israel Hospital, Boston, MA.

Guinea pig gastric surface epithelial cells were isolated, enriched and cultured in collagen gel cups and put into Ussing chambers. Formed monolayers retained their morphology like that of intact tissue. Maximal R of 216 to 265 $\Omega \cdot cm^2$ and PD of -2.5 to -4.0 mV (apical negative) was seen between 4-9 days (n=22). Membrane capacity data and light microscopy showed cultures of $R < 125\ \Omega \cdot cm^2$ were not confluent and were discarded. NaCl gradients across the monolayer gave asymmetrical dilution potentials with the side of lower chemical potential becoming electrically negative (n=10). Calculations gave a Cl/Na permeability ratio (β) of 1.09. Current-voltage relationships were asymmetrical and nonlinear (n=12). Amiloride (0.1-1.0 uM) had no effect on PD or R from the apical or basal side (n=18). Amiloride (1.0-0.1mM) increased R when given apically and was Na dependent (n=12). DIDS (1.0-.01mM) also increased R from the apical side only (n=14). DIDS and amiloride apically increased the R higher than the sum of their individual responses (n=8). Ouabain was effective only basally and abolished the PD. HCO_3-free Ringer on both sides had little effect on PD or R but 1.0 mM acetazolamide reduced the PD to -0.5 mV (n=8). These findings suggest R is not dominated by the paracellular pathway and that the PD involves an apical Na^+/H^+-Cl^-/HCO_3 exchange mechanism. Supported by NIH AM-30303 and AM-31158.

64.3

A RE-EVALUATION OF THE HSCN BACKFLUX HYPOTHESIS IN BULLFROG GASTRIC MUCOSA. W.W. Reenstra and J.G. Forte, Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Gutknecht and Walter (BBA 685:233) have proposed that SCN^- inhibits gastric acid secretion by the backflux of HSCN from an acidic luminal space across the apical membrane. We have previously demonstrated (Biophys. J. 41:164a) that SCN^- in the nutrient (N) or secretory (S) solution produced similar decreases in the N→S flux of H^+ and Cl^-; subsequent addition of 20 mM imidazole to the N side increased H^+ (Sanders et al. AJP 234:E120) and Cl^- fluxes in parallel. Because the observed flux of SCN^- was small relative to Cl^-, and based on a demonstrated Cl^-/SCN^- exchange, we proposed that the backflux hypothesis requires an anion pool within the gland lumen that is equilibrated with oxyntic cell cytoplasm. We have now tested buffers added to the S solution, to ascertain whether they might neutralize glandular H^+ and prevent HSCN/Cl backflux. As H^+ and Cl^- fluxes always changed in parallel, we have used Cl^- flux as a monitor of H^+ flux. Addition of imidazole to S side, at 55 mM, reversed the inhibitory effects of SCN^- on Cl^- flux. However, other buffers (phosphate, HCO_3^-, histidine) were without effect. In the broad framework of the backflux hypothesis, these data suggest that HSCN is formed in a restricted compartment at the apical secretory surface. Possibilities that the restriction is the result of bulk flow and/or membrane barrier will be discussed. (Supported by USPHS Grant #AM10141.)

64.4

ANGIOTENSIN IS A PHYSIOLOGICALLY IMPORTANT NEUROMODULATOR OF JEJUNAL FLUID ABSORPTION. Nigel R. Levens* (Spon: R. Klabunde). West Virginia University, Morgantown, WV 26506

The purpose of this study was to determine if the increase in small intestinal fluid absorption observed following extracellular fluid (ECF) reduction is mediated by angiotensin II (AII). Infusion of AII at doses which increase plasma levels of the hormone within the physiological range stimulate jejunal fluid absorption in-vivo. In contrast, at pharmacological doses which result in plasma levels of AII unlikely to be encountered normally, the hormone inhibits absorption and/or stimulates jejunal secretion. The AII stimulation of jejunal fluid absorption is potentiated by nephrectomy suggesting that the endogenous levels of AII are related to and have an important role in the regulation of the cellular level of its own receptors. Extracellular volume reduction as a result of sodium depletion, non-hypotensive hemorrhage, or water deprivation increases jejunal fluid absorption 30-40% above control. This increase in jejunal absorption following ECF reduction is not affected by adrenalectomy but is abolished by nephrectomy, either alone, or in combination with adrenalectomy. Captopril, prazosin and peripheral sympathectomy also abolish the increase in jejunal absorption following ECF depletion. It is suggested that AII is generated following ECF reduction and increases jejunal fluid absorption by facilitating the release of norepinephrine from enteric sympathetic nerves. Supported by AM-30941-01.

64.5

INTRALUMINAL DISTENSION PRESSURE ON SEROSAL TRANSUDATION, LYMPH FLOW, AND LYMPH PRESSURE DURING WATER ABSORPTION FROM RAT JEJUNUM. Jui S. Lee. Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Holtzman rats (380-460 gm) fasted for 24 hr were anesthetized by ip injection of pentobarbital. After the abdomen was opened by crisscross incisions, jejunal segment (~20 cm long) was placed in a warm chamber for collection of transudation. The lymph duct was cannulated for determination of lymph flow (J_L) and lymph pressure (P_L). After introduction of Krebs-glucose (28 mM) solution into the lumen of the segment at various distension pressures (DP), water absorption rate (J_V), transudation rate (J_T), J_L, and P_L were determined. Under basal conditions (non-absorptive), J_L, P_L and J_T were 21±3 µl/cm·h, 1.6±0.1 mmHg, and 24±10 µl/cm·h, respectively (mean ± S.E., N = 22). During absorption, the excess $J_L(J_L^e)$ or excess $J_T(J_T^e)$ was the difference between total J_L and basal J_L or between total J_T and basal J_T, respectively. When distension pressure was 0,3,20 or 70 mmHg, J_V was 77±5, 154±6, 211±19 or 175±18 µl/cm·h; J_L^e was 25±5, 30±6, 9±4, or 3±2; J_T^e was 0, 9±3, 43±10, 102±9; J_L^e/J_V was 32±4, 19±3, 4±2, or 2±1%; and J_T^e/J_V was 0, 6±2, 20±4 or 58±2%, respectively. P_L increased by ≈0.7 mmHg above the basal P_L, and was not affected by either J_L^e or DP. It appears that a significant fraction of the lymph may be transferred into the blood or to the serosal side as transudation at elevated DP. Supported by NIH Grant 18085.

64.6

A COMMON H^+-REGULATED, NA^+-DEPENDENT SO_4 TRANSPORT SYSTEM FOR BRUSH BORDER VESICLES FROM RABBIT INTESTINE AND KIDNEY. Gregory A. Ahearn and Heini Murer. Dept. of Physiology, University of Zurich, Zurich, Switzerland.

Epithelial brush border vesicles were made from rabbit ileum and kidney cortex using Mg-precipitation. In ileum, SO_4 influx (J_{oi}^{SO4}) from outside to inside the vesicles was Na-dependent and a hyperbolic function of $[SO_4]_o$. At pH 7.4 (both sides), ileal J_{oi}^{SO4} was a sigmoidal function of $[Na]_o$ at high (4.0 mM) and low (0.2 mM)$[SO_4]_o$. At bilateral pH 6.0 both tissues also displayed sigmoidal Na-dependent J_{oi}^{SO4} which was composed of two transport components: one with a high Na affinity, the other a low Na affinity. Hill plots of J_{oi}^{SO4} at this pH had a slope near 1.0 at low $[Na]_o$ and a slope near 2.0 at high $[Na]_o$. At bilateral pH 8.0, the two tissues exhibited hyperbolic Na-dependent J_{oi}^{SO4}, only showed a single transport component, and had Hill plots with a slope near 1.0. For ileum, electrogenicity of J_{oi}^{SO4} at pH 8.0 for high and low $[Na]_o$ was shown using valinomycin and a K diffusion potential. At bilateral pH 6.0, electrogenic J_{oi}^{SO4} occurred at low $[Na]_o$, while anion transport was electroneutral at high $[Na]_o$. A common model is proposed for the two tissues for proton regulation of sodium-SO_4 cotransport where flux stoichiometry is controlled by $[H]_i$ and Na binding affinity is modified by $[H]_o$. Supported by U. S. National Science Foundation grant number PCM 81-18366 and Schweiz. Nationalfonds grant 3.226.082.

64.7
TRANSCELLULAR MECHANISMS OF L-ALANINE UPTAKE BY DISTAL RAT ILEUM IN SITU. Anwar B. Bikhazi and Michel N. Abu Salbi*. Dept. Physiology, American University of Beirut, Lebanon.

A study on the transcellular transport mechanisms of L-alanine through distal rat ileum. Approximately 10 cm distal ileum was exposed, freed from mesenteries and perfused at a rate of 1.5 $ml.min^{-1}$ with buffer. The perfused segment was then incised, homogenized, digested in HNO_3 and assayed for (1-C^{14})L-alanine. Steady state of L-alanine absorption was observed at 10 min perfusion time and was in the range of 0.30±0.17 ng/mg protein/cm^2 of intestine. In Na-free choline Krebs Ringer, Na-Krebs Ringer + 1 mM ouabain, Na-Krebs Ringer + 1 mM preloaded ouabain, Na-free choline Krebs Ringer + 1 mM ouabain, and Na-free choline Krebs Ringer + 1 mM preloaded ouabain, the amount of L-alanine absorbed was 0.078±0.027, 0.20±0.09, 0.10±0.03, 0.066±0.014 and 0.045±0.008 ng/mg protein/cm^2 of intestine respectively. A 5, 10 and 25 times increase in luminal L-alanine concentration in Na-free choline Krebs Ringer preloaded with ouabain resulted in increase of amino acid absorption of the same order of magnitude. Therefore, a. extracellular luminal Na is indispensable for L-alanine absorption, b. Na and L-alanine co-transport at the mucosal site is not linked to metabolic reactions, c. a ouabain sensitive Na-alanine co-transport pump exists at the serosal site, d. the L-alanine pump at the serosal site is Na-independent and yet ouabain sensitive, e. 15% of absorbed L-alanine is passively transported. (Supported by Grant 18-5205 from the Faculty of Medicine Research Funds.)

64.8
INHIBITION OF ALANINE INTESTINAL TRANSPORT BY PROCAINE, COLCHICINE AND VINCRISTINE. C.F. Nassar, M.E. Haddad*, A. Jurjus* and E. Sarru*. Depts. of Physiology and Human Morphology, American University of Beirut, Lebanon.

The effect of procaine, colchicine and vincristine on steady-state accumulation and unidirectional influx of alanine was evaluated in the rat and turtle small intestine. Procaine at a concentration ranging from 1-20 mM in the incubation medium caused a significant inhibition of alanine uptake by the mucosal strips of the rat intestine (P<.01). However, the same concentrations of procaine did not show any change in alanine uptake by the turtle intestinal cells. Moreover, colchicine and vincristine at a concentration of $5x10^{-4}$M and $1.5x10^{-6}$M respectively, produced a significant decrease (P<.01) in alanine accumulation in the rat mucosal strips with no effect noticed in the turtle. The unidirectional influx of alanine across the rat small intestine was significantly reduced (P<.01) by the presence of procaine, colchicine or vincristine in the preincubation medium. This effect however, is not noticed in the turtle. A morphological study of the treated rat intestinal strips showed well-preserved villi after procaine and vincristine treatment, and a completely disrupted architecture of the villi when the strips were incubated with colchicine. The results would suggest that procaine, colchicine and vincristine inhibit alanine transport across the rat small intestine by limiting its entry step into the intestinal cell which is probably due to alterations in the membrane structure.

64.9
RELATIONSHIP BETWEEN DIETARY-INDUCED INCREASE OF INTESTINAL DISACCHARIDASE ACTIVITIES AND DISACCHARIDE DIGESTION AND ABSORPTION IN THE ADULT RAT. J. Leichter*, T. Goda*, S.D. Bhandari* and O. Koldovský. Div. Human Nutrition, Univ. British Columbia, Vancouver, Canada, and Departs. Pediatrics and Physiology, Univ. of Arizona, Tucson, Arizona.

To study the relationship between dietary-induced increase in intestinal disaccharidase activities and disaccharide absorption, adult male rats were either fed a high starch low fat diet or a low starch high fat diet for 3 days. Food intake, body weight changes, and amount of protein per intestinal segment were similar in the two groups of animals. The intestinal sucrase and lactase activities were significantly higher ($p < 0.01$) in the high starch than in the low starch group. This was reflected in the significantly higher absorption of sucrose and lactose ($p < 0.01$) by the rats fed the high starch diet as determined in vitro by the everted sac technique of Wilson and Wiseman. In addition to the increased disaccharide absorptions glucose transport was also higher in the high starch animals. Our findings indicate that the increase in sucrase and lactase activities induced by feeding a high starch diet to adult rats results in an increased functional capacity to hydrolyze lactose and sucrose, and absorb the constituent monosaccharides.

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64.10
MODULATION OF THE VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN AND CALMODULIN IN PIGS ADAPTED TO A LOW CALCIUM INTAKE. Susan C. Vendeland*, Robert H. Wasserman and James F. Zimmer*. Cornell Univ., Ithaca, NY 14853

Young pigs were fed diets containing either 1.1% Ca and 1.0% P (NCaNP) or 0.1% Ca and 1.0% P (LoCa NP) for 5-6 weeks. ^{47}Ca absorption was determined in duodenal, jejunal and ileal segments by the in situ ligated loop technique. Vitamin D-dependent calcium-binding protein (CaBP) was measured by radial immunoassay and calmodulin (CaM) by radioimmunoassay. The greatest degree of absorption and the highest concentrations of CaBP occurred in the duodenum and jejunum. In these segments, however, ^{47}Ca absorption and CaM levels were unaffected by diet, but CaBP was 25% greater in the LoCaNP group. In the ileum, ^{47}Ca absorption was 2-fold greater in the LoCaNP group than the NCa NP group, and mucosal CaM was increased by 70% in the low Ca pigs. CaBP in ileal mucosa was undetectable in the NCaNP group but its synthesis was stimulated by the low calcium intake. These data indicate that, in this species, the distal part of the small intestine is of more importance in maintaining calcium homeostasis during periods of inadequate calcium intake. (Supported by NIH Grant AM-04652.)

64.11
CARNITINE UPTAKE BY SMALL INTESTINE OF RAT. Dileep S. Sachan*, Robin A. Ruark* and Gary W. Randall* (Spon: R.M. Bagby). Dept. of Nutr. & Fd. Sci., Coll. of Home Econ. & Agr. Exp. Sta., Univ. of Tenn., Knoxville, TN 37996-1900.

Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is an essential biocatalyst which may become dietary essential under certain pathophysiological conditions. The purpose of these studies was to determine rate and site of carnitine absorption from the small intestine (SI) of the rat. In situ preparations of SI of male Sprague-Dawley rats starved for about 15 hours were used for these experiments. DL-[methyl-^{14}C] carnitine (2 μ moles) in Earle's buffer was continuously infused for 1-2 hours. The rate of carnitine uptake by SI was 0.50 ± 0.15 nmoles/min/g of SI tissue as determined by the loss of radioactivity from the infusate as well as the appearance of the radioactivity in the SI tissue. The rate of carnitine uptake by in situ isolated SI sacs was comparable to the rate obtained by continuous infusion. Uptake of carnitine in these sacs was consistent throughout the SI with a significant decrease toward the distal end (40%). Uptake of carnitine was dependent on the concentrations of carnitine in the infusate as well as the time of its exposure to the SI mucosa. The L-carnitine uptake was higher than that of DL-carnitine at equimolar concentrations. (Supported by CRGO, USDA).

64.12
MORPHOMETRIC RESPONSE OF THE INTESTINE TO FEED RESTRICTION SCHEDULES IN YOUNG SWINE. Jerome C. Pekas. USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933

This study was to investigate by morphometry the basis of the gastrointestinal tract weight reduction during feed restriction. Pigs (84 days of age; 27 kg body weight) were assigned to three treatments (two pigs/treatment) and fed to gain 14 kg over 70 days. Treatment HL gained the first 35-day period (P1) and lost weight the last 35 days (P2); MM gained slowly in P1 and P2; LH lost weight in P1 and gained in P2. Specimens of intestine were collected at three sites and analyzed by morphometry procedures. The results are expressed as tissue volume (TV) per unit intestine length (L) (cm^3/cm L). The value accounted for both the composition and cross-sectional size of the intestinal wall. The results (mean of two animals, three sites) are as follows: HL, MM, LH treatment, respectively, and the probability (P) — total small intestine weight (g), 1747, 2384, 2996 (P<.01); intestine TV (cm^3/cm L), .375, .428, .514 (P<.10); muscle TV (cm^3/cm L), .100, .087, .104 (NS); mucosa TV (cm^3/cm L), .221, .289, .364 (P<.05); tissue matrix TV (cm^3/cm L), .053, .052, .046 (NS). It was concluded that the intestine response was principally because of a response in the mucosa. The changes observed, HL relative to LH, are as follows: the total TV decreased 0.139 cm^3/cm L; mucosa decreased 0.143 cm^3/cm L; muscle decreased 0.004 cm^3/cm L; tissue matrix increased 0.007 cm^3/cm L. Mucosa accounted for 103% of the intestine decrease whereas muscle accounted for only 3% and the tissue matrix increased 5%.

65.1
SALIVA FLOW IN HEAT-STRESSED RATS DURING BEHAVIORAL THERMOREGULATION. Ingrid Schmidt*, Randy Kaul* and Eckhart Simon. Max-Planck-/W.G. Kerckhoff-Institut, D-6350 Bad Nauheim, FRG

We have developed a method for collecting thermoregulatory saliva produced by the submandibular glands of behaviorally active heat-stressed rats. A loop of PP 50 tubing, with perforations straddling the submandibular papillae, penetrates the floor of the mouth on either side of the lower incisors, and the ends are led subcutaneously to emerge at the neck. These chronically implanted catheters do not block normal salivation. During heat exposure saliva was continuously aspirated from 5 female Wistar rats (250-300 g) restrained in a tubular cage and trained to adjust mean ambient temperature ($\overline{T_a}$) by pressing a lever for cold reinforcement (RF). $\overline{T_a}$, colon temperature (T_c) and saliva flow were recorded over 10-min intervals at various RF-magnitudes and during extinction. With large RF-magnitudes, rats controlled $\overline{T_a}$ and T_c close to the salivation threshold. Reduction of RF-magnitude resulted in a higher controlled $\overline{T_a}$; T_c and saliva flow both increased. From the individual steady state regressions of saliva flow on T_c ($r = 0.80 \pm 0.04$, $\bar{x} \pm SE$, N=5), a threshold T_c for salivation of $38.1 \pm 0.3°C$ and a T_c of $40.1 \pm 0.2°C$ at a saliva flow of 50 $\mu l \cdot min^{-1}$ were calculated. For 4 rats saliva composition at high flow rates (40-80 $\mu l \cdot min^{-1}$) was analyzed: osmolality = 115 ± 3 mOsm/kg H_2O, $|K^+| = 54 \pm 3$, $|Na^+| = 9 \pm 3$, and $|Cl^-| = 14 \pm 1$ meq$\cdot l^{-1}$. This method should assist the analysis of salivation, as well as of autonomic and behavioral interaction.

65.2
ROLE OF BROWN ADIPOSE TISSUE (BAT) ON THE HYPERTHERMIC RESPONSES OF RATS TO MORPHINE. J.A. Thornhill and M. Desautels*, University of Saskatchewan, Saskatoon, Sask. Canada S7N OWO

Experiments were conducted in male, Sprague-Dawley rats to determine if the increase in core temperature induced by morphine sulphate (MS) is mediated by select activation of BAT. BAT (T_{BAT}), rectal (T_R) and tail (T_t) temperatures were measured for 3 hr in unrestrained rats following intraperitoneal (i.p.) injection of MS or other drugs. Selective activation and inhibition of BAT was observed following i.p. injection of isoproteronol (0.5 mg/kg) and propranolol (10 mg/kg), respectively, to control rats kept at 22°C. I.p. injection of 2 or 10 mg/kg MS evoked parallel increases in both T_{BAT} and T_R. T_t followed those temperature changes seen in T_R and T_{BAT}. Naloxone HCl (10 mg/kg i.p.) given at the time of peak morphine-induced hyperthermia caused a precipitous fall in T_{BAT} and T_R. Injection of MS (10 mg/kg i.p.) to cold-acclimated (4°C) or fasted rats did not alter the T_{BAT} and T_R from those seen in control rats kept at 22°C given MS, suggesting that morphine hyperthermia is independent of the amount and capacity of BAT for heat production. Moreover, GDP binding of BAT mitochondria (known to increase with BAT activation) was not enhanced by i.p. injection of 10 mg/kg MS. Inhibition of adrenergic β receptors with 10 mg/kg propranolol i.p. during the peak hyperthermic response of rats to MS (10 mg/kg i.p.) caused only a slight, transient fall in T_R. The results suggest that the acute hyperthermic response of rats to MS is not due to specific activation of BAT. Supported by MRC Canada

65.3
THE EFFECT OF AMBIENT TEMPERATURE ON THE FEBRILE RESPONSES OF RATS TO ENDOGENOUS PYROGEN (EP) John T. Stitt & Steven C. Shimada* John B. Pierce Fdn Lab. Yale Univ. Sch. of Med. New Haven, CT 06519.

Rats generally exhibit poor febrile responses to pyrogens even at ambient temperatures (Ta) above thermoneutrality, and it is believed that at low Ta's, they are completely incapable of generating any febrile response. Indeed, we have shown that I.V. doses of EP that can elicit fevers in rats exposed to Ta=26°C, have no effect on body temperature (Tre) when the animals are exposed to Ta=3°C, (1). However, we have recently discovered that the febrile responses of rats to EP can be enhanced markedly by pretreating the rats with immunological adjuvant substances. Thus, at Ta=26°C, the control febrile response of untreated rats to EP (2ml/kg i.v.) was ΔTre=0.51 ± 0.09°C (n=6). After adjuvant treatment, these animals responded to the same dose of EP with increases in Tre of 1.15 ± 0.05°C. When these fever-enhanced rats were exposed to Ta=3°C and injected with EP, significant febrile responses were obtained; but they were also significantly attenuated, ΔTre=0.55±0.04°C. We conclude that rats are capable of developing fever in the cold. However, under normal circumstances their febrile responses are so small that the attenuating effects of low ambient temperatures mask any potential increase in Tre. The mechanism by which cold temperatures attenuate fever in rats is unexplained at this time. (Supported by NIH Grant NS 11487) (1) Shimada, Stitt, Thomas & Bernheim, Fed Proc. V42, 463, 1983.

65.4
DIURNAL RHYTHM OF MENOPAUSAL HOT FLASHES: SUBJECTIVE REPORT AND PHYSIOLOGICAL CORRELATION. Fredi Kronenberg* and John A. Downey. Dept. Rehab. Med., College of Physicians and Surgeons, Columbia University, New York, NY 10032

Patterns of hot flashes in post-menopausal women with frequent flashes were studied to see if there is a diurnal rhythm of flash frequency. Core (vaginal or rectal) and skin temperatures were recorded with an ambulatory temperature monitor at 30-sec or 2-min intervals for 24-hour periods during which subjects kept a written record of the time of their hot flashes. There was a high correlation between report of a hot flash and a fall in core temperature and skin temperature (due to sweat evaporation). A significant correlation was found between core temperature and frequency of hot flashes, flashes being most frequent when body temperature was highest. In addition, one subject recorded only the time of her hot flashes for 60 consecutive days. The occurrence of her hot flashes exhibited a diurnal rhythm, with the lowest frequency occurring during the hours of the day when body temperature is typically at its lowest. It is not clear whether core temperature itself influences flash frequency, or whether some other factor, such as sleep or the circadian pattern of a hormone may be causally related to the observed hot flash pattern.
(Supported by NIH grant AG 03367)

65.5
SIMULATION OF SHIVERING THERMOGENESIS: A MODEL BASED ON THE ACTIVITY OF THERMOSENSITIVE NEURAL STRUCTURES. Igor B. Mekjavic* and James B. Morrison*. (Spon: T.J. Smith) Dept. Kinesiology, Simon Fraser University, Burnaby, B.C., V5A 1S6, Canada.

Existing stimulus-response expressions predicting shivering thermogenesis are based on static peripheral, core and central temperatures. These expressions predict a linear increase in metabolic heat production in response to displacements of either central or core and peripheral temperatures from set-point temperature values. A model of shivering thermogenesis has been developed incorporating the non-linear static and dynamic characteristics of thermosensitive neural structures in the body. The parameters defining receptor activities, dynamics of response, regional summation and thermoneutral set points are based on data published in the literature. Predictions of metabolic heat generation (in units of ml O_2 kg^{-1} min^{-1}) are derived by integrating excitatory and inhibitory thermogenic drives, resulting from thermoreceptor stimulation in various regions of the body. The relative gains of the thermogenic drives were determined by least-squares regression analysis using empirical data from a series of cold water immersions. The present model demonstrated improved simulations of shivering thermogenesis during cooling and rewarming, when compared to existing stimulus-response predictive expressions.

65.6
BODY TEMPERATURE ELEVATION TO REPEATED EXERCISE IN DOGS: ADRENERGIC IMPLICATIONS. J. E. Greenleaf, H. Kaciuba - Uscilko*, B. Kruk*, K. Nazar*, and S. Kozlowski* Polish Academy of Sciences, 00730 Warsaw, Poland and NASA, Ames Research Center, Moffett Field, CA 94035

The effect of i.v. propranolol (0.25 mg/kg) on the interaction of metabolic variables (blood lactate [LA], glucose, plasma free fatty acid [FFA], and osmotic concentrations), with the progressive elevation of muscle (T_m), rectal (T_{re}), and hypothalamic (T_{hy}) temperatures, was measured in 9 male mongrel dogs (14.4 to 21.0 kg) during 4 consecutive 30-min treadmill exercise-bouts (1.3 m/s, 12° slope) separated by 30-min rest periods that allowed temperatures to return to resting levels. From bout I to bout IV, ΔT_m increased (*P<0.05) from 1.9 to 2.4°C,* ΔT_{re} from 1.3 to 1.7°C,* and ΔT_{hy} from 0.8 to 1.0°C (NS). In the 4 exercise-bouts Δheart (H_f) and Δrespiratory (R_f) frequencies were unchanged, [LA] and [FFA] increased progressively (P<0.05), [glucose] decreased progressively (P<0.05), and osmolality was unchanged. The only significant (P<0.001) correlations among the body temperatures and metabolic variables were between ΔT_m and resting [FFA] (r=0.68) and ΔT_m and post-exercise [FFA] (r=0.59). During successive exercise bouts, β-adrenergic blockade with propranolol completely inhibited the normal increases in T_m, T_{re}, [FFA], and [LA], significantly attenuated H_f, and had no effect on R_f. It was concluded that progressive increase of body temperatures during repeated exercise-bouts is, in part, due to metabolic effects of catecholamines. (PAS Project No. 10.4)

65.7
ACTIVATION OF COLD-DEFENSE EFFECTORS DOES NOT INDUCE ACUTE-PHASE RESPONSES IN RABBITS. Llanos-Q.*, J., R.A. Ahokas*, T.A. Mashburn, Jr., W.S. Hunter, and C.M. Blatteis. Univ. of Tenn. Ctr. Hlth. Sci., Memphis, TN 38163

We have demonstrated previously that certain blood chemical changes characteristic of the acute-phase reaction (APR) to systemic endotoxin (LPS) or intracerebral endogenous pyrogen (EP) are not stimulated merely by a rise in body temperature. To determine whether the activation of the thermoeffectors of fever production per se, i.e., without a concomitant fever, could mediate the APR, we exposed conscious, New Zealand white rabbits for 3.5 h to a cold environment (T_a = 3±1°C) and measured their colonic (T_{co}) and ear skin (T_{sk}) temperatures, rates of O_2 consumption ($\dot{V}_{O_2}$), and selected indices of their APR (plasma Fe, Zn, Cu, and sialic acid levels) before, during, and at appropriate times after the onset of the cold exposure; the effectors evoked in defense against overcooling are the same as those activated in the production of fever. The T_{co} of these animals was maintained at 39.5±0.3°C, their T_{sk} fell to 9.3±1.1°C, and their $\dot{V}_{O_2}$ increased 2.1±0.8 W/kg (ca. 61%) during this cold exposure, but the plasma levels of the acute-phase reactants measured were unaffected. The acute-phase blood responses to LPS/EP, therefore, probably are not mediated by the concomitantly induced thermogenic and/or vascular effectors of fever. (Supported by F.O.E. of Illinois and BRSG 81RCFFP-001)

65.8
EFFECT OF FEVER ON REGIONAL BLOOD FLOW IN TERM-PREGNANT RABBITS. R.A. Ahokas*, D.W. Busija, W.S. Hunter, J. Llanos-Q.*, and C.M. Blatteis. U. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163

Circulatory adjustments occur at term of pregnancy which optimize the growth and survivability of the offspring during this critical time before birth. To determine whether a superimposed fever might cause competitive, undue shifts in the distribution of the cardiac output, we measured the regional blood flow of nonpregnant (NP) and pregnant (P) does with 15 µm microspheres before and during the rising phase of fever, 35 min after the iv injection of 2 µg/kg of S. enteritidis endotoxin (LPS). LPS induced decreases in the blood flow to brain, tongue, mammary gland, small intestine and ear, and increases to masseter muscle, bone, and liver; blood flow to kidneys, spleen, uterus, and ovaries did not change. There were no demonstrable differences in these vascular responses between NP and P rabbits, excepting that a greatly reduced blood flow to the placentas also occurred in the P rabbits. Fever heights and courses and mean arterial blood pressures and gases were not significantly different in the two groups during fever. These results indicate that, during the rising phase of fever, opposing changes in blood flow occur in different vascular beds, the pattern of which is not altered at term of pregnancy in rabbits. We conjecture that this redistribution of cardiac output during fever might cause fetal stress. (Supported by F.O.E. of Illinois, BRSG 81RCFPP-001, and HL 30260 and 30521)

65.9
CHOLINESTERASE INHIBITION: EFFECTS ON ENDURANCE AND THERMOREGULATION DURING WORK IN THE HEAT. R.P. Francesconi, R.W. Hubbard, and M. Mager. US Army Res. Inst. Environ. Med., Natick, MA 01760.

To determine the effects of various levels of plasma cholinesterase inhibition on the ability to work (level treadmill, 9.14 m/min) in the heat (35°C), adult, male rats were treated with malathion (M) or pyridostigmine (P). Treatment with M effected a 35% inhibition of circulating cholinesterase levels while P elicited a 64% decrement. M treatment had no significant effects on endurance when rats were run to hyperthermic exhaustion (Tre = 43°C) while endurance was decreased by 33% (23.09 min vs 34.68 min) in P-treated rats. In M-treated animals and saline-treated controls, rates of heat gain were similar, but P treatment elicited significant increments in rates of heat gain (.20°C/min vs .14°C/min) during exercise in the heat. While weight (water) loss during exercise in the heat was unaffected by M administration, P induced significantly increased fluid loss (.49 g/min vs .32 g/min). Following exercise in the heat, significantly increased elevations in several clinical chemical indices of heat/exercise injury were noted in P-treated rats. We concluded from these studies that moderate cholinesterase inhibition can be endured with no effects on endurance or thermoregulation. However, subsequent to marked cholinesterase inhibition, both thermoregulation and endurance are compromised. We hypothesize that the increased cholinergic activity due to the more intense cholinesterase inhibition resulted in increased fluid loss and more rapid onset of hyperthermic exhaustion.

65.10
EFFECT OF PH ON LIVER MITOCHONDRIAL SUCCINOXIDASE ACTIVITY OF HIBERNATING AND ACTIVE HAMSTERS AND GROUND SQUIRRELS. Jane C. Roberts. Dept. of Biology, Creighton Univ., Omaha, NE 68178.

Succinoxidase activity (SO) of liver mitochondria (M) is significantly depressed during hibernation in both hamsters and ground squirrels (GS). Since hibernating (HIB) mammals tend to regulate pH relatively constant as body temperature (T_B) changes, experiments were designed to determine whether this depression in SO could be due to either hibernation or temperature related changes in pH optimum. State 3 SO was assayed polarographically at 12, 25 and 37 C over the pH range 5.0 - 8.2. In hamsters, the pH optimum at 12 C was 7.5 for both active and HIB animals. At 37 C the pH optimum was more acidic for M from HIB than active animals, and SO of M from HIB hamsters was maximal at pH 6.5 where it equaled that from controls. In GS, at 12 C pH had no significant effect on SO of HIB liver M, while for Fall active GS the pH optimum was between 7.35 - 7.5. At 25 and 37 C the pH optimum was the same for SO from HIB and Fall GS. In both species, the pH optimum for SO was more acidic at 37 C than at 12 C for both active and HIB animals. These data suggest that at low T_B changing pH between 6.8 - 7.8 would have little effect on SO activity in HIB GS and, except for the sharp peak at 7.5, would have little effect in HIB hamsters. However, during arousal from hibernation, the combination of rising T_B and a fall in pH, due to shivering-induced lactic acid production, might facilitate reversal of the suppression of SO seen in hibernation thus facilitating arousal. Supported by NSF Grant No. PCM80-21895.

65.11
TEMPERATURE DEPENDENCE OF OXYGEN TRANSPORT AND CRITICAL PO_2 IN THE DOMESTIC PIG. David C. Willford, Esther P. Hill, Francis C. White, William Y. Moores*, and Brian D. Guth*. University of California, San Diego, La Jolla, CA 92093

The effect of temperature on O_2 transport to peripheral tissues and critical PO_2 was studied in 2 groups of pigs (37 and 30-31 C.) Pigs were anesthetized, ventilated, thoracotomized, instrumented with an electromagnetic flow probe and a left ventricular pressure transducer (to measure cardiac function). Metabolic rate (VO_2), cardiac output (Q), arterial and venous blood gases, saturation, and lactate concentrations were measured as the inspired O_2 concentration (and thus the arterial PO_2) was lowered until the animals exhibited cardiac failure. The critical PO_2 was defined as the break point at which the VO_2 and lactate concentration became PO_2 dependent. Group I animals were kept at 37°C and pH 7.4. Group II animals were cooled to 30-31°C and maintained at pH 7.5 (constant relative alkalinity) using combined surface cooling and cooling of jugular venous blood with a small external heat exchanger. Temperature has a profound effect on lowering P_{50} of the dissociation curve and thus venous PO_2, but the critical PO_2 was also lower at the lower temperature, indicating that the tissues are better able to tolerate the lower venous (and presumably tissue) PO_2. A decreased critical PO_2 might be expected at low temperature because a smaller oxygen driving gradient is needed at the lower VO_2. (Supported by NIH Grant HL07212).

65.12
OLDER WOMEN MAY BENEFIT DURING COLD EXPOSURE FROM READILY AVAILABLE CIRCULATING ENERGY SUBSTRATES. Jeames A. Wagner, Kaoru Kitagawa* and Steven M. Horvath. Inst. of Environ. Stress, Univ. of California, Santa Barbara, CA 93106

Men and women between the ages of 20-30 yrs and 51-72 yrs, wearing minimal clothing, rested for 2 h in 28°C, 20°C, 15°C and 10°C room temperatures (T_a). Only the group of older women maintained their central body temperatures during the coldest exposure, partially due to rapid initial increases in metabolism. Hemoconcentration occurred in all groups in the cold but decreases in plasma volume tended to be greater in women than men. Regardless of T_a, blood glucose levels were higher in older than younger women during the first h of the exposures. Lactate levels were generally higher in both older men and women than in younger people. Free fatty acid levels were higher in older women than in any other group. Epinephrine levels were higher in men than in women, regardless of age, and cortisol levels were higher younger than older subjects regardless of gender. All these blood measurements, as well as norepinephrine, were elevated during the 10°C cold exposure, regardless of age or gender. These data indicate that the rapid increases in metabolism in older women during cold exposure may have been facilitated by the availability of energy substrates circulating in their blood. The higher lactate levels in older people indicated greater involvement of anaerobic energy sources than the young, even in a thermoneutral environment.

66.1

SODIUM AND POTASSIUM DEPENDENT CHLORIDE UPTAKE BY PLASMA MEMBRANE VESICLES FROM THE THICK ASCENDING LIMB OF HENLE'S LOOP.

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A microsomal membrane fraction was prepared via differential centrifugation from cells isolated from the medullary thick ascending limb of Henle's loop (TALH) from rabbit kidney. Cl^{36} uptake into the plasma membrane vesicles was measured by a rapid filtration technique. At 25 mM Na_2SO_4, 25 mM K_2SO_4, and 6 mM Cl^{36}, chloride uptake at 15s. was 1.93 pmol/mg protein and was inhibited 35% by 1 mM Bumetanide. Removal of sodium resulted in a 34% inhibition of chloride uptake - addition of Bumetanide to sodium free media had no effect on chloride uptake. When potassium was removed, chloride uptake also decreased to the level obtained in the presence of Bumetanide - again, no effect of Bumetanide was seen in the potassium free media. Addition of 10^{-4}M SITS(4-acetamido-4' isothiocyanatostilbene-2,2' disulfonic acid) to the medium produced no change in chloride uptake. These experiments provide the first direct evidence that the movement of chloride into TALH plasma membrane vesicles can be driven by sodium and potassium and inhibited by Bumetanide, a conclusion heretofore reached only indirectly in sodium and rubidium flux and tracer exchange experiments using the same vesicle preparation. These data strongly support the operation of a sodium, potassium, chloride cotransport system in active chloride reabsorbtion in the thick ascending limb of Henle's loop. Supported by NIH AM29927, NIH T32GM7288, NIH AM27441 and the Max Planck Institute.

66.2

ACTIVE SODIUM AND PASSIVE CHLORIDE ION TRANSPORT IN PROXIMAL CONVOLUTED AND STRAIGHT TUBULES PERFUSED UNDER OIL.

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Proximal convoluted (PCT) and proximal straight (PST) tubules perfused under oil with artificial ultrafiltrate (AUF) or simple electrolyte solution (SES) develop transepithelial gradients for Na^+ and Cl^- between the luminal and absorbed fluids (Fed Proc 42:304, 1983). To determine if these ion gradients demonstrate active Na^+ and Cl^- transport, the electrical potential (PD) was measured (lumen reference).

	PCT		PST	
	AUF	SES	AUF	SES
PD (mV)	-0.5	+0.2	-0.8	-0.2
M ΔNa^+ (mM)	-4.6	+3.4	+4.4	+6.8
P ΔNa^+ (mM)	-2.7	+1.2	-4.9	-1.0
M ΔCl^- (mM)	-21.0	-15.9	-11.7	-10.1
P ΔCl^- (mM)	+2.3	-1.3	+3.3	+1.0
JV	1.07	0.14	0.34	0.14

Δ = absorbate - mean luminal, M (measured), P (predicted using Nernst equation), JV volume absorption (nl min^{-1} mm^{-1}). It is clear Cl^- is absorbed down an electrochemical gradient in all cases. Active Na^+ transport is demonstrated in PST perfused with AUF and SES, and PCT perfused with SES. However, in PCTs perfused with AUF Na^+ transport is down an electrochemical gradient. It appears this "downhill" Na^+ flux in the PCT results from rapid volume absorption, due to perferentially absorbed solutes. (Support: NIH AM 25519)

66.3

OSMOTIC WATER PERMEABILITY OF THE IN VIVO RAT PROXIMAL CONVOLUTED TUBULE (PCT).

Patricia A. Preisig and Christine A. Berry. Univ. of Calif., San Francisco, CA 94143

Recent studies in the rabbit PCT find the transepithelial osmotic water permeability (P_f) is so high (3560 μ/s) that spontaneous volume absorption (J_v) can be explained by less than 6 mOsm of luminal hypotonicity. The purpose of our studies was to determine the P_f and driving force needed to explain J_v in the rat PCT. P_f was determined from J_v and the log mean osmotic gradient ($\Delta \pi$). Rat PCT were perfused at 49 nl/min with an ultrafiltrate-like artificial solution made hypoosmotic to rat plasma by the removal of 50 mM NaCl. This solution also contained 8.33 mM Na-cyanide which we have found to inhibit active transport along the entire perfused segment. J_v was measured with ^3H inulin and $\Delta \pi$ was calculated from measured osmolalities, obtained with a Ramsay-Brown type osmometer, of perfusate, collected fluid, and plasma. The P_f is 1196-1554 μ/s, assuming a reflection coefficient for NaCl of 0.7-1.0. Green and Giebisch (Kidney Intern. 23:256A, 1983) have published a luminal hypotonicity of 3.8 mOsm and a J_v of 0.88 nl/mm•min when tubules and capillaries were perfused with 154 mM NaCl at 45 nl/min. Using our P_f this amount of luminal hypotonicity is inadequate as the sole driving force for J_v. These results therefore suggest that lateral intercellular space hypertonicity is required to provide an additional driving force for J_v. (This work supported by National Institutes of Health grant RO1 AM 26142.)

66.4

INFLUENCE OF PHOSPHATE DEPRIVATION ON RENAL PHOSPHATE TRANSPORT IN RESPIRATORY ACIDOSIS AND ALKALOSIS.

Aviad Haramati and David Nienhuis*, Dept. of Physiology, Mayo Clinic, Rochester MN 55905

Hypercapnia increases plasma (P) phosphate (Pi) and the fractional excretion (FE) of Pi in rats fed a normal Pi diet. We tested whether these changes would occur in Pi-deprived rats that avidly retain Pi. Respiratory acidosis (R ACID) or alkalosis (R ALK) was induced in 14 acutely TPTX rats fed a low Pi diet (0.07%) for 4-7 days. All rats were hyperventilated at 92 BPM. Different acid-base states were achieved by varying the mixture of CO_2 in inspired air. Following baseline clearances the filtered Pi load was increased with successive Pi infusions to assess the transport maximum of Pi reabsorption (Tm_{Pi}/GFR). Results:

	N	PCO_2 mmHg	Baseline P_{Pi} (mM)	Baseline FE_{Pi}(%)	Tm_{Pi}/GFR (μmol/ml)
R ALK	(4)	26±2	1.8±.2	0.4±0.1	5.1±.3
CONTROL	(4)	40±3	2.1±.4	0.3±0.1	5.3±.5
R ACID	(6)	90±4	3.9±.2	0.7±0.3	5.2±.3

Although P_{Pi} was higher in R ACID than either control or R ALK, baseline FE_{Pi} was less than 1% in all groups, and there were no differences in Tm_{Pi}/GFR. (Tm_{Pi}/GFR in normal Pi diet rats = 3.6 μmol/ml). We conclude that in Pi deprivation, hypercapnia increases P_{Pi}, but the high Tm_{Pi}/GFR prevents an increase in FE_{Pi}. (Supported by NIH grant AM 19715 and by the Reimann Foundation)

66.5

RENAL ACID SECRETION IN RESPONSE TO ACUTE RESPIRATORY ACIDOSIS AND ALKALOSIS.

Y.L. Chan, P.P. Shah*, B.P. Lee*, and S.T. Chiang. Univ. of Illinois Col. of Medicine, Chicago, Il. 60680 and National Yang Ming Medical Col. Taipei, ROC.

The effect of acute hypocapnia and hypercapnia on urinary acidification was study in the rat kidney. Glomerular filtration rate was determined by clearance of inulin. Urine minus blood pCO_2 gradient (U-B pCO_2) and urinary deta pCO_2 were assessed as an index of distal tubular acidification. Microperfusion method was used to examine bicarbonate absorption ($JHCO_3$) and net fluid flux (Jv) in the proximal convoluted tubule (PCT). Bicarbonate was measured as total CO_2 by microcalorimetry. Experiments in rats with acute hypercapnia demonstrated an increase of deta pCO_2 and a decrease of U-B pCO_2. $JHCO_3$ in the PCT was increased by 15%, that could not completely account for a decreased fractional excretion of bicarbonate. A 35% increase in Jv may be responsible for a reduction of urinary flow rate. On the other hand, acute hypocapnia resulted in an increase of fractional excretion of bicarbonate, that was reflected by decreases of $JHCO_3$, deta pCO_2 and U-B pCO_2. Results suggest that both proximal and distal acidification processes participate in renal responses to acute respiratory acidosis and alkalosis. (Supported by Chicago Heart Association and National Research Council, ROC).

66.6

INHIBITION OF PROXIMAL ACIDIFICATION BY AMILORIDE: EFFICACY AND SELECTIVITY.

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The sodium/proton antiporter identified in brush-border membrane vesicles prepared from rabbit proximal tubules is inhibited by amiloride. The effect of a high concentration of amiloride on proximal acidification and another sodium-dependent reabsorptive process, that of D-glucose, was examined in vivo.

Rat superficial proximal convoluted tubules were perfused at ~ 15 nl/min with a glomerular ultrafiltrate-like solution. Rates of volume absorption (Jv), acidification (JTCO2) and glucose absorption (J*glu) were estimated from the concentrations of 3H inulin, total CO2 (by picapnotherm), and 14C D-glucose in perfusate and collected samples and from the length of the perfused segment. The effect of 1 mM luminal amiloride is compared to controls in the following table:

	Jv nl/mm•min	JTCO2 pmol/mm•min	J*glu pmol/mm•min
Control	2.0 ± 0.2	103 ±7	27 ±3
Amiloride	1.6 ± 0.1	81 ± 5	28 ± 3
Significance	$p < 0.05$	$p < 0.05$	NS

The absence of an effect on D-glucose efflux despite a reduction in Jv is consistent with selective inhibition of the sodium/proton antiporter. The inhibition of acidification however, is far less than that predicted by simple transposition of the kinetic data derived from the vesicle studies. (This work was supported by the NKF, the NKF of No. Calif. and N.I.H. grant AM 27045.)

66.7

NEURAL REGULATION OF FLUID AND BICARBONATE ABSORPTION IN THE RAT KIDNEY. P.P. Shah*, M. LaPointe*, C. Sabo*, M. Laski* and Y.L. Chan. Univ. of Illinois Col. of Medicine, Chicago, Il. 60680

Previous study in this laboratory has shown that Norepinephrine could stimulate bicarbonate and fluid reabsorption in the rat proximal convoluted tubule (PCT) through alpha adrenergic receptor mechanism. This study was designed to examine the effect of acute denervation on fluid and bicarbonate absorption in the rat PCT and cortical Na-K-ATPase. In situ microperfusion of PCT was performed in the acutely denervated as well as sham operated kidney. Bicarbonate was measured as total CO_2 by microcalorimetry. Acute denervation resulted in a reduction of fluid absorption by 66% and bicarbonate absorption by 39% as compared to predenervation. There was no change in either parameter in sham operated kidney. In a separate series of experiments, microsomal Na-K-ATPase activity was determined in cortical homogenate obtained 30 minutes after denervation. No consistent decrease of activity was observed as compared to control, although our previous study indicated a decrease of Na-K-ATPase activity one day after denervation. These data suggest that the inhibitory effect of acute denervation on bicarbonate absorption in the PCT might be secondary to a reduction of apical sodium entry. (Supported by NIH Grant AM27691)

66.8

ACIDIFICATION IN SEMINIFEROUS TUBULES OF THE RAT TESTIS. Carlton R. Caflisch and Thomas D. DuBose, Jr. Departments of Medicine, Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Limited observations using antimony microelectrodes (Sb_e) in vivo and in vitro have suggested that the pH of seminiferous tubule fluid (STF) was 7.30 units. Assuming a PCO_2 equal to systemic arterial blood (35 mmHg) values for bicarbonate ($[HCO_3]$) in STF were calculated to be 20 mM. The purpose of this study was to measure pH and, for the first time PCO_2 using recently developed glass membrane microelectrodes. Seven Sprague-Dawley rats were prepared for testis micropuncture. All animals were maintained on a respirator and had normal blood values (pH=7.39 ± 0.01, PCO_2=37 ± 1.8 mmHg, $[HCO_3]$=22 ± 1.2 mM). In situ STF pH was 6.89 ± 0.01 (n=46) when pH was corrected for the observed electrical potential difference of -8.1 ± 0.5 mV (n=25). The PCO_2 in STF was 53.1 ± 1.0 mmHg (n=50) and exceeded arterial PCO_2 by 16.3 mmHg ($p < 0.001$). The $[HCO_3]$ in STF calculated from the observed pH and PCO_2 values was 9.2 mM. In conclusion: 1) STF pH measured with glass membrane microelectrodes is more acid than reported previously and can be attributed to the alkaline errors typical of Sb_e, 2) PCO_2 in STF is 16.3 mmHg above arterial blood and may result from a combination of metabolic production and diffusion of CO_2 between venous and arterial systems, 3) $[HCO_3]$ in STF calculated from in situ pH and PCO_2 measurements is half that previously described. The cellular mechanism of acidification in STF cannot be stated with certainty.

66.9

CONTINUOUS RAPID MEASUREMENT OF BLOOD PCO_2 AND pH. Stephen A. Katz, Allan C. Roth* and Eric O. Feigl, Dept. of Physiology & Biophysics, Univ. of Washington, Seattle WA 98195

Continuous measurement of blood PCO_2 and pH may be useful for the examination of physiological changes which might not otherwise be detected with static measurements. A carbon dioxide electrode and cuvette system have therefore been developed for the continuous measurement of blood carbon dioxide tension. Blood from a vessel of interest is delivered to the cuvette system at a rate of 1 to 15 ml/min by means of an occlusive roller pump. The cuvette maintains the electrode and blood at 37°C and also directs a continuous jet of blood against the electrode surface. The cuvette design permits frequent gas calibration. The electrode is fabricated from a 1.5mm diameter flat tip pH glass electrode covered by a film of carbonic anhydrase solution, over which a 25µm thick dimethyl-silicon membrane is attached. Porous ceramic filled with 20% polyacrylamide equilibrated with 100mM NaCl and 5mM $NaHCO_3$ serves as a salt bridge between a Ag/AgCl reference electrode and the pH glass surface. The 90% response time of the system to a step change of CO_2 tension is 3.0 sec for liquids and 1.3 sec for gases. Without the carbonic anhydrase the response times are approximately doubled. Removal of the silicon membrane yields a pH electrode which can continuously measure blood pH. When joined with a PO_2 electrode (J. Appl. Physiol. 30:909, 1971) continuous rapid blood PCO_2, pH and PO_2 may be recorded. (Supported by NIH grant HL 16910.)

66.10

PROSTAGLANDIN ANALOG INDUCED RENAL VASODILATION WITHOUT INCREASES IN SODIUM EXCRETION AND INTERSTITIAL PRESSURE. J.A. Haas, T.G. Hammond*, E.H. Blaine, F.G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester, MN 55905; Merck Institute for Ther. Res., West Point, PA 19486.

A newly synthesized prostaglandin analog, 4-{3-[3-[2-(1-hydroxycyclohexyl)ethyl]-4-oxo-2-thiazolidinyl]propyl}benzoic acid, increases renal blood flow without increasing sodium excretion. To investigate the role of renal interstitial hydrostatic pressure (IP) in this dissociation, comparisons were made with prostaglandin E_2 (PGE_2), a natural prostaglandin that increases renal blood flow and is natriuretic. Renal blood flow (RBF), interstitial pressure, fractional sodium excretion (FE_{Na}%) and blood pressure (BP) were measured before and after sequential intrarenal infusion of PGE_2 and the prostaglandin analog (0.15 µg/kg/min).

	RBF (ml/min)	IP (mm Hg)	FE_{Na} (%)	BP (mm Hg)
Control 1	125±15	7.2±1.4	0.3±0.2	141±6
PGE_2	188±12†	12.9±1.7†	3.8±1.0†	135±8
Control 2	122±12	6.0±1.6	1.0±0.4	136±9
PG Analog	216±13†	4.9±1.1	0.9±0.3	133±9

(n=6; mean ± SEM; †$p < .05$ vs control)

The prostaglandin analog is similar to PGE_2 in that it increases RBF, but differs in that it does not increase interstitial pressure or sodium excretion. This observation suggests that renal interstitial pressure is a crucial factor linking renal hemodynamics to sodium excretion in the dog.

66.11

RENAL TUBULAR HANDLING OF NOREPINEPHRINE IN THE DOG. L.R. Willis,* W.B. Zimmerman,* J.H. VanHuysse* and D.P. Henry* (SPON. G.A. Tanner). Dept. of Pharmacology, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Renal tubules of rat and rabbit secrete norepinephrine (NE), but fractional NE clearances in the dog (<1.0) suggest either tubular reabsorption (TR) alone, or tubular secretion (TS) with net TR. These possibilities were examined with renal surface application and tubular microinjection techniques. To examine TS, a 5ul saline droplet containing ^{3}H-NE or ^{3}H-p-aminohippurate (PAH) and ^{14}C-Inulin (In) (^{3}H:^{14}C =10) was applied to a decapsulated portion of the left kidney of pentobarbital-anesthetized, mannitol-treated dogs. Serial 1-min urine samples were collected from each kidney. ^{14}C-In recoveries from each kidney were equal; thus, increases in urinary ^{3}H/^{14}C suggest TS. In 7 dogs, the ^{3}H-NE/^{14}C-In ratio was increased significantly, but by only 2.9±0.3-fold (X±SE). ^{3}H-PAH/^{14}C-In ratios, in contrast, were increased 34±6-fold vs the droplet ratio ($p<0.01$, n=4), but only 7±1-fold after treatment with probenecid ($p<0.01$). Thus, little if any TS of NE is evident. Preliminary data from early proximal tubular microinjections (3nl) in 2 dogs and 3 rabbits are:

% Recovery	dog	rabbit
^{14}C-In	98.1±0.1	100.7±1.7
^{3}H-NE	74.4±0.4	91.1±1.7

The data suggest that the renal tubule of the dog reabsorbs, but may not secrete, NE. (Am. Heart Assoc., Ind. Aff.)

67.1

PLASMA PROSTANOID CONCENTRATIONS FOLLOWING LIPOXYGENASE INHIBITION IN A RABBIT MODEL OF ENDOTOXIC SHOCK. J.T. Flynn and A.J. Glick*. Thomas Jefferson Univ., Phila., PA 19107.

Endotoxic shock was produced in rabbits with 1 mg/kg E. coli endotoxin. Sham group rabbits received vehicles for both endotoxin and nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor. Eight of 8 endotoxic rabbits receiving the NDGA vehicle died. NDGA pretreatment of 8 rabbits with a 2 mg/kg iv bolus plus a 5 hr constant infusion at 2 mg/kg/hr post-endotoxin did not improve survival. Prostacyclin and thromboxane concentrations in venous plasma were (ng/ml):

	Minutes after endotoxin			
I. 6 keto-PGF$_{1\alpha}$:	Base	60	120	180
sham	0.84±0.31	1.04±0.34	1.16±0.36	1.13±0.89
endotoxin	0.47±0.12	5.29±2.35	4.40±1.33	3.12±1.13
endo & NDGA	0.87±0.24	4.48±0.96	3.89±0.78	3.47±0.63
II. TxB$_2$:				
sham	0.91±0.17	1.18±0.30	0.98±0.23	0.95±0.21
endotoxin	0.76±0.25	1.71±0.29	1.46±0.19	1.21±0.38
endo & NDGA	0.60±0.18	2.40±1.13	2.35±1.25	1.98±0.95

Lipoxygenase inhibition was confirmed by incubating platelets from vehicle and NDGA treated rabbits with ^{14}C arachidonic acid. These data suggest that lipoxygenase inhibition in the rabbit LD100 model of endotoxic shock has no effect on the rate of survival. There was no statistically significant indication that substrate shunting into either the prostacyclin or thromboxane pathways occurred. Supported by GM 28023 and Shriner's Burns Hospital.

67.2

DEXAMETHASONE ATTENUATION OF ENDOTOXIN-INDUCED ALTERATIONS IN LUNG GLUCOSE METABOLISM. Diane M. Klein. Dept. of Physiology, Loyola University Medical Center, Maywood, IL 60153.

Glucose utilization as indicated by glucose oxidation (GO) is depressed in lungs of guinea pigs (GP) in endotoxic shock. The purpose of this study was to determine if dexamethasone attenuated this metabolic derangement. GO, expressed as the conversion of U-^{14}C-D-glucose to $^{14}CO_2$, was measured in lung slices from GP receiving one of the following treatments: saline injected controls (C); 10 mg/kg S. enteritidis endotoxin (E); 500ug/kg dexamethasone (DEX) 30 min. prior to either saline or prior to endotoxin (DEX+E). Lung glycogen was also measured in each group. Results are as follows: (mean ± S.E.; N=5-17/group).

	C	E	DEX	DEX+E
Glucose Oxidation (dpm $^{14}CO_2 x 10^3$/gm/2hr)	818±82	634±43[a]	582±47[a]	778±61[b]
Glycogen (mg/gm)	1.7±0.1	6.1±1.4[a]	1.6±0.1	3.6±0.4[b]

[a] $p < 0.05$ compared to control values by analysis of variance
[b] $p < 0.05$ compared to E group by analysis of variance

The data indicate that GO is depressed and glycogen is increased in lungs of endotoxic GP. This suggests a preferential use of substrate for glycogen synthesis rather than mobilization or utilization for energy production. Although DEX depresses GO in controls, it attenuates the effects of E on both GO and glycogen suggesting that its protective action may involve modulation of pathways of glucose utilization in the lung. (Supported by Loyola Univ. Dean's Development Fund.)

67.3

LEFT-VENTRICULAR DYSFUNCTION IN ENDOTOXIN SHOCK. J.L. Parker†*, H.R. Adams* and N.B. Watts* (SPON: C.E. Jones†), Dept. Pharm., Univ. Tex. Hlth. Sci. Ctr., Dallas, TX.; Dept. of Physiology, Tex. College of Osteopath. Med.†, Fort Worth, Texas, 76107.

Isolated, perfused heart preparations and left-ventricular (LV) papillary muscles were used to evaluate cardiac effects of endotoxin (ET) shock in guinea pigs. In vivo administration of ET (4mg/kg;IP) 16 hrs prior to sacrifice produced circulatory shock and inotropic dysfunction of hearts perfused using a modified Langendorff technique. Shock hearts (n=8)exhibited reduced ($P<0.02$) peak systolic contractile force, + and -dF/dt and heart rate,when compared to control hearts (n=8); electrical pacing at equivalent control heart rates failed to restore the shock-induced contractile depression. LV function curves (n=8) demonstrated that LV performance of shock hearts remained depressed as LV end-diastolic pressure (EDP) was incrementally increased (isovolumic LV balloon technique) from -5 to 20 mmHg. At LVEDP=10 mmHg, control and shock hearts generated peak systolic pressures of 85.0±1.6 and 53.3±7.6 mmHg, respectively, ($P<0.001$); + and -dP/dt were similarly reduced. Length-tension curves of isolated LV papillary muscles (PM) confirmed the shock-induced LV dysfunction; contractile tension developed by 10 control and 10 shock LVPM at L_{max} averaged 1.06±.22 and 0.35±.06 g/mm^2 ($P<0.01$),respectively. These studies provide evidence of primary LV myocardial dysfunction in endotoxin shock, using cardiac preparations contracting independently of complicating in vivo influences. (Supported by NIH-23423, American Heart Assoc., and Burroughs Wellcome Fund).

67.4

LYMPH FLOW (LF) AND PROTEIN FLUX IN ENDOTOXIN SHOCK. JOEL D. Hubbard* and Herbert F. Janssen. Depts. Physiol. and Ortho. Surg., Tx Tech Univ. Health Sci. Ctr. Lubbock, Tx 79430

Capillary permeability and LF increase with I.V. administration of endotoxin. This study attempted to determine the rate and extent of these changes in the dog following a bolus injection of E. Coli endotoxin. The thoracic duct was cannulated caudally (PE-90) catheter through a surgical opening made in the 8th intercostal space. An experimental group (n=6) received 0.5 mg/kg endotoxin in saline via the femoral vein, and a control group (n=6) received only saline. Within 10 minutes post-endotoxin, LF rate in the thoracic duct had increased five-fold, returning toward baseline values by 3 hrs. By 5 min. after endotoxin, increases were also seen in total protein lymph/plasma (L/P) ratio, plasma protein clearance (ml/min), and plasma to lymph protein flux (mg/min). Serum and lymph protein electrophoresis suggested a rise in the amounts of albumin, alpha, beta, and gamma globulins found in lymph 10 min. after endotoxin injection. These data suggest that I.V. endotoxin 1) increases LF in the thoracic duct, 2) increases the total amount of lymph proteins and 3) increases capillary permeability to larger proteins. Previous studies by others have shown increased lung LF with a decrease in L/P protein ratio after endotoxin, reflecting an increase in flow due mainly to increased capillary pressure. In contrast, these data show an increased LF and increased L/P protein ratio suggesting increased protein permeability in the hepatosplanchnic vasculature.

67.5

BLOOD VOLUME CHANGES IN ANAPHYLACTIC SHOCK. **E.M. Wagner*, W.A. Mitzner, P.L. Smith* and E.R. Bleecker. Johns Hopkins Med. Inst., at Baltimore City Hospitals, Baltimore, Md. 21224**

Using a model of canine anaphylaxis produced by Ascaris suum antigen (Ag), we examined peripheral circulatory variables contributing to shock. In 6 anesthetized dogs instrumented for right heart by-pass, cardiac output decreased 50% in shock due primarily to the 27% decrease in mean systemic pressure (Pms). Since systemic vascular compliance did not change, we concluded that Pms falls in anaphylactic shock due to either 1) a rightward shift of the pressure-volume curve implying changes in the elastic properties of the vasculature, or 2) a loss of plasma volume. In 3 additional animals, we performed splenectomies and made serial measurements of hematocrit and plasma oncotic pressure. Ten minutes after Ag, the hematocrit had increased from 45.5% (control) to 49% and plasma oncotic pressure had increased from 13.8 (control) to 14.8 mmHg. Assuming constant red cell volume, this change in hematocrit represents a 128 ml loss of plasma volume. However if one assumes constant protein levels, a plasma volume loss of 66 ml is obtained. This discrepancy can be explained by 1) protein loss in addition to plasma volume, with 66 ml representing a minimum volume loss and 2) dilation of the vasculature during shock resulting in a release of sequestered red cells, with 128 ml representing a maximum volume loss. Despite this range, the relative magnitude of the volume loss is sufficient to explain the decrease in Pms observed during anaphylactic shock.

67.6

PLASMA ELECTROLYTE AND ENZYME ALTERATIONS DURING CHRONIC HYPER DYNAMIC SEPSIS IN THE DOG. J.X. Thomas, D. Defily*, D.A. Gibbons* and R.M. Raymond. Depts. of Surgery and Physiology Loyola Univ. Med. Ctr., Maywood, IL. 60153 and The V.A. Hosp. Hines, IL 60153

Arterial electrolytes and enzyme profiles were monitoried daily in a chronic (14-21 days) hyperdynamic peritonitis model. This specific septic model developed an increased cardiac output, decrease total peripheral resistance, increased heart rate, normal or slightly decreased mean arterial blood pressure and increased body temperature. All animals became anorectic and exhibited signs of decreased sensorium. Listed below are the mean concentrations from five animals.

	DAYS					
	CONTROL	1	5	9	13	17	21
Ca++(mg/dl)	10.2	8.7	9.0	8.2	7.9	7.4	7.6
Phosphorous(mg/dl)	3.7	3.5	5.1	9.4	9.3	9.6	9.2
Mg++(mEq/L)	1.3	1.4	1.7	1.7	1.6	1.6	1.7
Na++(mEq/L)	15.4	146	140	150	146	141	144
K+(mEq/L)	4.4	4.0	5.0	4.6	4.7	4.3	4.4
cl^- (mEq/L)	117	108	109	113	107	112	110
SGOT (Iu/L)	25	243	86	45	32	36	47
SGPT (Iu/L)	50	46	27	27	26	18	20

In regard to Ca++ and Mg++ alterations, these changes could be responsible for a variety of metabolic alterations reported to occur during sepsis and shock.

67.7

CARBOHYDRATE AND HORMONAL CHANGES ASSOCIATED WITH HYPERDYNAMIC SEPSIS IN THE DOG. R.M.Raymond, T.E.Emerson and D.A. CIBBONS*. Depts. of Surgery and Physiology, Loyola Univ. Med. Ctr., Maywood, IL 60153, The V. A. Hospital,Hines, IL 60141 and The Cutter Group of Miles Laboratories, Berkeley, CA 94701

Recent reports from this laboratory have described glucose and insulin alterations associated with acute endotoxin shock. The present study was undertaken to document the glucose and hormonal changes during hyperdynamic sepsis in the dog. Following control metabolic and hemodynamic measurement, sepsis was induced by implanting 4X4 gauze sponge previously innoculated w/bacteria, amid the intestines, Daily hemodynamic and metabolic measurements were made for 21 days or until death of the animal. Sepsis induced a hyperdynamic state indicated by an increased cardiac output, increased heart rate and decreased total peripheral resistance. Plasma glucose concentration exhibited a biphasic response, increasing from 111 to 163 mg/dl during the first week, followed by a slow progressive decrease during the last two weeks (115 to 87 mg/dl).Plasma insulin increased from 16 to 90 uU/ml over the first three days of sepsis and waned to 34 uU/ml by 21 days. Glucagon increased from 47 to 180 pg/ml at the first day of sepsis and remained elevated throughout the septic period. Plasma catecholamines (Nor-Epinepherine, Epinepherine, Dopamine) increased to a maximum level at ten days of sepsis,then waned to near control by 21 days. These data demonstrated that sepsis is associated with a catabolic state which is maintained throughout the septic period.

67.8

MYOCARDIAL DYSFUNCTION DURING THE HIGH-FLOW PHASE OF SEPSIS. J.J. Spitzer, C.H. Lang and K.H. McDonough. LSU Med. Ctr., New Orleans, LA 70112.

Myocardial function was investigated during the high-flow phase of sepsis, when arterial blood pressure (MABP) was normal and cardiac output (CO) elevated 27%. Sustained sepsis was induced in conscious unrestrained rats by IP administration of a pooled fecal inoculum. Coronary blood flow (CBF) and CO were measured in vivo on days 1-4 postinfection, using radioactive microspheres. Septic rats had a consistently elevated (45%) CBF which was not linearly related to the CO. The hearts from septic rats also had a consistently elevated CBF over a range of myocardial work loads (estimated as CO X MABP) and failed to increase CBF in response to increased work. Myocardial dysfunction was further investigated using the in vitro isolated working heart preparation where preload and afterload were controlled. For these experiments, hearts were removed from control and septic rats 2 days postinfection (i.e., peak of high-flow phase), and perfused with varying preloads. CO and peak systolic pressure (PSP) were decreased (40% and 20%, respectively) in the septic hearts. The product, CO X PSP, was also decreased (50%). Finally, coronary flow was lower at every filling pressure, although the percent of the CO that perfused the coronary vasculature was unaltered. These studies indicate that there is an altered myocardial efficiency and a diminished myocardial reserve during the high-flow phase of sepsis. (Supported by grants HL 23157 and HL 07098).

PULMONARY VENTILATION, AIRWAY EPITHELIAL FUNCTION AND LUNG GENERAL

68.1

MULTIPLE INERT GAS VENTILATION PERFUSION RATIOS (VA/Q) IN PULMONARY ATELECTASIS. John K. Stene*, Juan Arrisueno*, Mark McCauley*, Peter Chodoff*, and Barry Burns. Critical Care Research/Anesthesia, Maryland Institute for Emergency Medical Services Systems, 22 South Greene Street, Baltimore, Maryland 21201

Multiple Inert Gas (MIG) VA/Q measurements were made on poly trauma patients in the intensive care unit on consecutive days with and without pulmonary lobar atelectasis. Informed consent was obtained from the patient or next of kin. Roentgenographic evidence of the appearance of atelectasis produced a distinct pattern in the MIG VA/Q measurements characterized by moderately increased shunt and a pronounced high VA/Q mode. The moderate increase in shunt with the development of atelectasis without an appreciable low VA/Q mode suggests little or no compensatory collateral ventilation. The sudden appearance of a high VA/Q mode on some patients suggest that atelectasis develops as an all or none phenomenom, e.g., when the airway becomes occluded, all ventilation to that region ceases and mechanically ventilated patients with a fixed minute ventilation develop a high VA/Q region as the ventilation is shifted from the atelectatic region to more compliant lung regions.

Support for this research was obtained from Merrell Dow Pharmaceuticals.

68.2

RECOVERY OF DISTRIBUTIONS OF VENTILATION, BLOOD FLOW, GAS AND TISSUE VOLUME BY ENFORCED SMOOTHING. Peter D. Wagner, Gwen E. Gale* and Harold Z. Bencowitz.* UCSD, La Jolla, CA 92093

Steady state inert gas retentions can be transformed into estimates of the distribution of $\dot{V}A/\dot{Q}$ by least squares methods with enforced smoothing. Nonsteady state inert gas washin data could be similarly processed to estimate the distribution of $\dot{V}A,\dot{Q}$, gas and tissue volume (since washin patterns depend on the distribution of these 4 variables). In this analysis, we selected 10 theoretical distributions and computed arterial and mixed expired concentration-time profiles at 5 points in time for a washin of the 6 gases SF_6, Krypton, Freon-12, enflurane, ether and acetone. Included were normal examples, and distributions with low $\dot{V}_A/\dot{Q}$ areas, shunt and high $\dot{V}_A/\dot{Q}$ areas, allocated various amounts of edema. Edematous areas delayed the approach to equilibrium of soluble gases while low $\dot{V}_A/\dot{Q}$ areas delayed insoluble gases. We used a least squares algorithm with smoothing to determine how well the original distributions could be recovered from the washin data. In spite of the enormous array processing task (62 equations compared to 7 in the steady state), the algorithm consistently recovered the original distributions if the smoothing coefficient (Z) was ≤ 40, and solutions required only 3 minutes computing time. Thus error-free washin data well-selected at 5 points in time for each of 6 gases can be accurately transformed into the representative distributions of $\dot{V}_A/\dot{Q}$, gas and tissue volume. The next step will be to evaluate effects of error on the stability and accuracy of the recovery process.

68.3

DISTRIBUTIONS OF VENTILATION, PERFUSION, TISSUE AND GAS VOLUME ESTIMATED FROM TRANSIENT INERT GAS DATA. Gwen E. Gale* and Peter D. Wagner, Univ. of Calif. San Diego, La Jolla, CA 92093

Transient changes in partial pressure of inert gases of different solubilities can be used to estimate the functional distributions of ventilation ($\dot{V}_A$), perfusion ($\dot{Q}$), gas volume (GVOL) and tissue volume (TVOL) in the lungs. In anesthetized dogs, mixed venous ($\bar{v}$) and arterial (a) washins of 6 gases (SF_6,Kr,freon-12,enflurane,ether,acetone) were measured online with a mass spectrometer (MS) following the start of an intravenous infusion (Physiologist 25:267,1982). Simultaneously, single breath mixed expired ($\bar{E}$) samples were collected in bags sealed to self-closing valves made to slide onto the expired port of the ventilator. These samples were analyzed by MS soon after completion of the on-line blood measurements. CO_2 was used to correct for dilution due to valve deadspace by multiplying the inert gas concentrations by the ratio $P_{\bar{E}}CO_2/P_{bag}CO_2$ (which averaged 1.15). Multiexponential fits were then determined for the $\bar{v}$, a and $\bar{E}$ washins. Using values from these fits as input data, a linear, least squares analysis with smoothing was used to estimate distributions of $\dot{Q}$, $\dot{V}_A$, TVOL and GVOL. Estimates of $\dot{V}_A/\dot{Q}$ distributions agreed with the steady state method. The residual sums of squares showed the data could be fitted by this approach and the total tissue and gas volumes calculated agreed with the measured values. This new transient inert gas method opens up a new area in gas exchange research because of the ability to estimate tissue volume (lung water) and gas volume distributions in relation to $\dot{V}_A/\dot{Q}$.

68.4

EFFECT OF POSTURE ON REGIONAL VENTILATION IN OBESE SUBJECTS. A.N.Hurewitz*, H.Susskind* and W.H.Harold*.(SPON:G.Smaldone). Medical Research Center at Brookhaven National Laboratory, Upton, N.Y. 11973 and SUNY/Stony Brook, N.Y. 11794.

Regional ventilation was determined in 3 massively obese patients(>150% ideal body weight) in both the supine and lateral decubitus positions. Changes in regional distribution of ventilation($\dot{V}$) and volume(V) were determined from in-vivo measurements with radioactive Kr-81m and Xe-127, respectively; posterior projections were used in each position. Ventilation per unit volume($\dot{V}/V$) was markedly reduced in the dependent lung(D) and relative to the non-dependent lung(ND) in both left and right decubitus positions. The D lung volume was reduced compared with its supine volume, while the ND lung volume was increased. A similar study of 3 non-obese, healthy subjects showed slightly increased $\dot{V}/V$ in the D lung. We conclude that the normal distribution of $\dot{V}$ in the lateral decubitus positions is reversed in obesity such that the gradient of $\dot{V}/V$ increases from the most dependent portion of the lung to the least dependent portion. This may be due to compression of the dependent lung by the abdominal contents and mediastinum and result in airway closure. This could result in marked alteration of ventilation/perfusion matching and subsequent hypoxemia when obese patients lie on their sides. (USDOE#DE-AC02-76CH00016, NHLBI#HL00439, BRSG#S07RR05736-09).

68.5

THE ALVEOLAR UPTAKE OF HALOTHANE USING HIGH FREQUENCY OSCILLATION. E. Jane McCarthy*, P.H. Abbrecht, J. Langston*, W.E. Keefe*, S. Muldoon. Departments of Physiology, Medicine, and Anesthesiology, Uniformed Services University, Bethesda, Maryland 20814

The objective of this study was to determine the effect of high frequency oscillation (HFO) on pulmonary delivery of halothane. HFO (Emerson vibrating diaphragm pump producing a half cycle volume displacement of 25 ml at 12-23 Hz) was delivered to anesthetized dogs (16-25 kg) via a triple port endotracheal tube inserted through a tracheostomy. Compressed air (10-15 l/min) containing 0.5% halothane was delivered to the tip of the endotracheal tube via an inner channel. Serial blood halothane concentrations were measured during the 20 min period following onset of halothane administration. Halothane content was determined by gas chromatography. In each dog, separate runs were done at frequencies of 16, 25, and 34 Hz, with 20 min of room air ventilation being given between each run. Blood halothane concentration was expressed as the ratio of arterial to fresh gas halothane partial pressure (P_a/P_I). In all animals, P_a/P_I increased progressively ($P < 0.05$) with increasing frequency at each time studied. The results of this study show that alveolar uptake of halothane increases with increasing HFO frequency. This study supports the hypothesis that HFO operates via the mechanism of enhanced diffusivity. (Supported by USUHS Grant TO 7661)

68.6

REFLEX INCREASE OF CANINE TRACHEAL GLAND SECRETION EVOKED BY STIMULATION OF PULMONARY C-FIBERS. H. Schultz, A.M. Roberts, C. Bratcher,* H.M. Coleridge, J.C.G. Coleridge, B. Davis. CVRI, UCSF, San Francisco, Calif. 94143.

Stimulation of bronchial C-fibers evokes reflex increase in secretion by tracheal submucosal glands. We have carried out experiments to determine whether pulmonary C-fibers have similar effects. In anesthetized dogs with open chests, we ventilated the lungs through the lower trachea, opened the upper trachea in the midline and retracted the cut edges. We sprayed powdered tantalum onto the exposed mucosa. Secretions from the openings of submucosal gland ducts caused elevations (hillocks) in the tantalum layer which we recorded on videotape with a television camera connected to a microscope. We counted the number of hillocks on an area (1·2 cm^2) of trachea at 10 s intervals for 1 min before and 1 min after injecting capsaicin (10-20 µg/kg) into the right atrium (an injection known to stimulate pulmonary C-fibers). In 9 dogs, capsaicin increased the maximum rate at which hillocks appeared from 3±1 to 15±3 hillocks/10 s (mean±SE; n=17; $p<0.001$). Cutting the vagi or cooling them to 0°C abolished this response in 6 dogs. Capsaicin (10-20 µg/kg) injected just beyond the pulmonary circulation into the left atrium in 4 dogs caused no increase in tracheal gland secretion. We conclude that stimulation of afferent pulmonary C-fibers reflexly increases secretion from tracheal glands. (Supported in part by USPHS NIH grants HL-24136 and HL-07192 and a grant from Cystic Fibrosis Research Inc.)

68.7

ELECTRICAL PROPERTIES OF MONOLAYER CELL CULTURES OF DOG TRACHEAL EPITHELIUM. D.L. Coleman,* I.K. Tuet,* and J.H. Widdicombe, CVRI, UCSF, San Francisco, Calif. 94143.

Isolated cells from dog tracheal epithelium were suspended in a 50/50 mixture of Dulbecco's modified Eagle medium and Ham's nutrient F12, containing 5% fetal calf serum, and cultured on polycarbonate filters coated with human placental collagen. Confluent monolayers formed 5-6 days after plating at 2.5 x 10^5 cells/cm^2. When mounted in Ussing chambers, the monolayers (19 filters, 2 dogs) showed a spontaneous potential difference (PD) of 4.1±0.8 mV (lumen negative) (range 0.1-13.8 mV), and a resistance of 438±47 Ω·cm^2 (range 60-743 Ω·cm^2). Amiloride (10^{-4}M) decreased PD by 7.9±1.4% when applied to the serosal side and by 12.4±4.5% when applied to the luminal side. PD decreased by 25.1±4.0% after serosal bumetanide (10^{-4}M) and by 40./±5.6% after 10^{-3}M luminal MK 196 (6,/-dichloro-2-methyl-1-oxo-2-phenyl-5 indanyloxy acetic acid). Serosal ouabain (10^{-4}M) abolished the PD. Luminal ouabain (10^{-4}M) had no effect. Serosal isoproterenol (10^{-5}M) increased PD by 313± 62% after 5 sec, followed by a decline to a steady state PD that was 153% above the original. Similar changes were seen after dibutyryl cAMP (10^{-3}M). Serosal $PGF_{2\alpha}$ (10^{-5}M), VIP (10^{-7}M), PGE_2 (10^{-5}M), methacholine (10^{-4}M), and bradykinin (10^{-5}M) each increased PD. These effects of drugs resemble their actions on intact dog tracheal epithelium. Thus, airway epithelial cells can be maintained in monolayer culture and retain electrical properties that resemble those of the original tissue. (Supported in part by USPHS PPG HL-24136)

68.8

ALVEOLAR PERMEABILITY IN GROWING LAMBS. Arlene A. Hutchison*, Kenneth J. McNicol* and Gerald M. Loughlin*. (SPON: Marc Jaeger) Univ. of Florida, Gainesville, FL 32610.

The purpose of this study was to measure alveolar permeability (AP) in awake newborn and adult sheep, and to assess AP longitudinally in growing lambs. Awake sheep were intubated with a nasotracheal tube using a flexible bronchoscope. A solution of ^{51}Cr-EDTA and ^{125}I-antipyrine (8:1 concentration ratio in 0.25 cc/kg saline) was injected into the trachea via the nasotracheal tube. Arterial blood was drawn at 1,3,5,7, 10,13,15,20 and 25 min post injection. The ratio of the counts of $^{51}Cr/^{125}I$ at 7,10 and 13 min were averaged for each animal. Eleven lambs were studied longitudinally at days 0,5,10,20,30, 60 and 90. Six adult sheep were studied on one occasion. The mean ± SD of the AP ratio for the 6 adult sheep was 0.011 ± 0.006. Within the first 24 hours of life the AP ratio was 0.036 ± 0.013 which was significantly greater ($p < 0.05$, Mann-Whitney) than the adult value. At 5 days there was no significant difference ($p > .05$) between lambs and adult sheep. Using all data (lambs and adults) there was no significant correlation ($p > .05$) between age and AP ratio. Using data from the first week of life only, there was a significant linear correlation (n=20, r=-0.49, $p < .05$) between AP ratio and age. In conclusion, 1) AP can be assessed in awake sheep using the method of Jones et al., 2) newborn lambs have a significantly higher AP ratio, 3) AP reaches the adult level within days, 4) there is a significant linear correlation between the AP ratio and age during the first week of life.

68.9

INTRAVENOUS ARACHIDONIC ACID DOES NOT INCREASE LUNG EPITHELIAL PERMEABILITY. Jan Stevenson*, Richard J. Lemen, Stuart F. Quan*, Hugh Roseberry*, George McNeill* and Dennis Patton*. Departments of Pediatrics, Physiology and Nuclear Medicine, University of Arizona, Tucson, Az. 85724.

Prostaglandins have been implicated in noncardiogenic pulmonary edema. We studied lung clearance of ^{99m} Technetium diethylenetriamine pentaacetic acid (^{99m}Tc DTPA) as an index of lung epithelial permeability in 7 controls and 9 arachidonic acid (AA) infusion rabbits. Rabbits were anesthetized, paralyzed and artificially ventilated. Half-times ($T_{½}$) of ^{99m} Tc DTPA were measured using a computerized gamma camera imaging system over 10 minute periods during baseline or AA infusions. Plasma 6-keto-F1α and thromboxane (TxB2) levels were measured by radioimmunoassay. Data ($\bar{X}$±SE) are summarized:

		AA Infusion (µg/kg/min.)		
	Baseline	250	300	350
^{99m}Tc DTPA ($T_{½}$, min)	39±4	48±7	62±19**	54±15
6-keto-F1α (ng/ml)	3.3±0.6	4.3±0.9	5.9±1.6**	5.5±0.7**
TxB2 (ng/ml)	17.8±4.2	38.1±6.9*	34.1±8.9**	68.5±42.1
Platelets$_3$(x10^3)	288±22	217±58	153±47*	198±69
WBCs (x10^3)	4.9±0.7	5.7±1.8	5.3±1.1	5.6±1.5

*$p<0.05$ or **$p<0.10$ by Student's unpaired t-test compared with baseline.

We conclude that AA infusions do not increase, and may decrease, pulmonary epithelial permeability in spite of increases in 6-keto-F1α and TxB2 concentrations. Supported in part by a grant from the Arizona Lung Association.

68.10

BRADYKININ STIMULATION OF ACTIVE CHLORIDE TRANSPORT IN CANINE TRACHEAL EPITHELIUM. C.D. Leikauf,* I.F. Ueki,* J.A. Nadel, and J.H. Widdicombe, CVRI, UCSF, San Francisco, Calif. 94143.

We studied the effects of bradykinin (Bk) on canine tracheal epithelium mounted in Ussing chambers. Bk increased the mean short-circuit current (SCC) when added either to the mucosal (K_D=0.7 nM; ΔSCC_{max}=25.3 µA·cm^{-2}) or submucosal bath (K_D=108 nM; ΔSCC_{max}=27.8 µA·cm^{-2}). The mucosal response was more transient than the serosal; therefore 10^{-5}M Bk was added to the submucosal bath in further studies. In 7 tissue pairs, Bk significantly increased ($p<0.05$) net ^{36}Cl flux to the mucosa from 2.13±0.36 to 3.07±0.67 µEq·h^{-1}·cm^{-2} (X±SE), while net ^{22}Na flux to the submucosa and the tissue conductance were unchanged (0.43±0.16 to 0.19±0.14 µEq·h^{-1}·cm^{-2}; 3.17±0.18 to 3.46±0.16 mS·cm^{-2}). Inhibitors of Cl^- transport significantly reduced ($p<0.05$) the maximal change in SCC (µA·cm^{-2}; n=5: control = 24.0±4.2; Cl^- free (gluconate)=10.5±2.9; Cl^- free (iodide)=2.2±1.0; bumetanide (10^{-4}M) = 8.2±2.7. Involvement of intramural nerves was ruled out because increases in SCC induced by Bk were not inhibited by phentolamine-propranolol-atropine (all 10^{-5}M) or by tetrodotoxin (10^{-6}). Indomethacin (10^{-5}M) was a potent inhibitor of the response (control ΔSCC = 29.3±3.5; indomethacin ΔSCC = 7.1±1.9 µA·cm^{-2}, n=10). Thus, bradykinin increases chloride secretion by canine tracheal epithelium, possibly due to an increase in prostaglandin production. (Supported by USPHS PPG HL-24136)

68.11

CYCLIC ADENOSINE MONOPHOSPHATE IS A MODULATOR OF THE BIOELECTRIC PROPERTIES OF ALVEOLAR TYPE II CELL MONOLAYERS. G.R. Cott*, K. Sugahara*, R.J. Mason. National Jewish Hospital, Denver, CO, 80206

The alveolar epithelium may play a major role in lung water homeostasis by actively transporting electrolytes across its surface. The factors controlling this process are unknown. As an in vitro model, rat alveolar type II cells were cultured on collagen coated micropore filters and studied in an Ussing chamber. These monolayers demonstrated spontaneous potential differences (PD), short circuit current (Isc), and electrical resistance (R). The addition of 10^{-3}M 8-bromo-adenosine 3':5' cyclic monophosphate (8-Br cAMP) resulted in an increased Isc from 2.9 ± 0.71 $\mu a/cm^2$ (Mean ± S.E., n=8) to 6.9 ± 0.67 $\mu a/cm^2$ (n=8) at 35 minutes ($P<.005$). After an initial rise in R_t values fell from 265 ± 30 Ω/cm^2 (n=8) to 135 ± 16Ω/cm^2 (n=8) at 60 minutes ($P< .001$). The addition of agents thought to increase intracellular cAMP resulted in similar changes. 3-Isobutyl-1-methyl xanthine produced the largest response and cholera toxin, terbutaline and vasopressin lesser responses. The addition of secretory neuropeptides (substance P, bombesin, and vasoactive intestinal peptide) resulted in minimal changes whereas the addition of aldosterone, histamine, bradykinin, and carbamylcholine produced no effect. In summary, we have demonstrated an increase in Isc and a drop in R across alveolar type II cell monolayers with 8-Br-cAMP and agents thought to increase intracellular cAMP.

68.12

EVALUATION OF SHEEP AND DOG TRACHEAS AS A SOURCE OF ISOLATED SUBMUCOSAL GLAND CELLS. W.E. Finkbeiner* and C.B. Basbaum* (SPON: J.A. Nadel), CVRI, UCSF, San Francisco, Calif. 94143.

Cellular heterogeneity and diffusional barriers impede direct study of secretion mechanisms in tracheal gland explants. To overcome these problems, Culp and Marin (Fed Proc 41:1510, 1982) recently developed a preparation of isolated gland cells from cat trachea. Cell yields ranged from 6-12 x 10^6. We attempted to increase yield by isolating cells from larger species, i.e., sheep and dog. After removing the epithelium, soft tissue was dissected from cartilage rings, minced, enzymatically digested and treated with EDTA. Following digestion, gentle mechanical disruption yielded 2.4-19.3 x 10^6 cells (n=8 dogs) and 21.2-27.1 x 10^6 cells (n=2 sheep). Viability as assessed by dye exclusion was 56.6%±6.8 (dogs) and 54.2%±2.55 (sheep). Alcian blue/PAS staining revealed intact granulated cells containing neutral and/or acidic glycoprotein. Electron microscopy confirmed the presence of isolated serous and mucous cells. Uptake of ^{35}S was not time-dependent but reached values ranging from 10,000-30.000 cpm/mg protein (n=2). Studies to determine whether cells can secrete ^{35}S are in progress. We conclude that it is possible to obtain higher cell yields from larger tracheas, but viability is compromised, perhaps due to denser connective tissue in larger animals. (Supported by USPHS PPG Grant HL-24136)

68.13

TESTS OF A MODEL OF HIGH FREQUENCY VENTILATION (HFV) IN A LONG STRAIGHT TUBE. W. Mitzner, C. Weinmann, & S. Permutt. The Johns Hopkins Medical Institutions, 615 N. Wolfe St., Baltimore, MD 21205.

We have recently proposed a model of HFV which states that gas transport during this unique form of ventilation is proportional to the product of frequency (f), tidal volume squared (V^2) and the square of the coefficient of dispersion of transit times through the dead space $(\sigma_t/\bar{t})^2$ (Ann. Biomed. Eng. 11:81, 1983). In the present study we measure the steady state transport of CO_2 through straight tubes between 2 fixed concentration sources. Two tubes were studied of lengths and cross-sectional areas (A) equal to 74.5cm/1.13cm^2 and 200cm/1.27cm^2, respectively. Each tube was connected between a small piston oscillator and a 100 liter box filled with 4.7% CO_2 in air. Close to the oscillator outlet a small gas flow was drawn through a CO_2 meter and then pumped through a sodalime cannister back into the tubing close to the withdrawal site. Pump frequency was adjusted between 3 and 30 Hz, and tidal volume was adjusted to give mean penetration lengths (V/A) between 5 and 60% of the total tube length. For each tube, direct measurements of $\sigma_t/\bar{t}$ were also made by drawing different steady flows from the box after washout of the tubes with CO_2 free gas. For both tubes gas transport during oscillation was proportional to fV^2, and the calculated $\sigma_t/\bar{t}$ for long and short tubes were 0.12 and 0.25, respectively. The respective $\sigma_t/\bar{t}$ measured directly for these tubes were 0.11 and 0.18, supporting the model predictions.

68.14

EFFECTS OF CHEMICALS ASSOCIATED WITH COTTON DUST ON RAT ALVEOLAR MACROPHAGE FUNCTION. V. Castranova, C.A. Glance*, and M.J. Reasor*. NIOSH and WVU, Morgantown, WV 26505.

Workers exposed to cotton dust often develop respiratory dysfunction, i.e., byssinosis. Since alveolar macrophages serve to cleanse the lungs of inhaled particulates, it is necessary to understand the effects of cotton dust on these phagocytes. Attempts to study the effects of cotton dust and aqueous extracts of cotton dust on oxygen consumption and superoxide anion release from rat alveolar macrophages proved impossible due to interference with these assays. As an alternative, we investigated the actions of chemical constituents of the cotton plant (gossypol, rutin, and catechin) on macrophage function. Rat alveolar macrophages were preincubated in the absence or presence of these cotton chemicals at 37°C for 15 minute prior to conducting various bioassays. These chemicals had no effect on cellular volume of rat alveolar macrophages, i.e., they did not cause membrane leakiness. However, inhibitory effects were observed with other physiological parameters of these phagocytes, such as, resting and zymosan-stimulated oxygen consumption, zymosan-stimulated chemiluminescence, phagocytic rate, and of acid phosphatase activity. In general, gossypol was found to be the most toxic of the cotton chemicals tested while catechin was the least toxic. Thus, chemicals found in cotton dust can compromise alveolar macrophage function. Further investigation is required to relate these findings to the etiology of byssinosis. (NIOSH-USDA 58-7B30-M28).

68.15

SURFACE TENSION ACTIVITY OF COTTON DUST COMPONENTS. K.C. Weber, J. Ma* and C. White*, LIB, DRDS, NIOSH, Morgantown, W.V. 26505

We have previously shown that some surface active material is contained in cotton dust and that this material can be leached onto the air-liquid interface and affect interfacial tension. Gossypol, rutin and catechin (major components of cotton) all showed surface activity when spread onto the aqueous subphase of a Wilhelmy balance. The surface tension-surface area hysteresis produced by 25 ug of gossypol (dissolved in chloroform and layered on the subphase) was equal to that of 8 ug dipalmitoyl lecithin (DPL). A minimum of 5 mg of rutin and 30 mg of catechin (both dissolved in aqueous buffer) was needed to produce any hysteresis. 30 mg of rutin and 140 mg of catechin were needed to produce 50% and 30% of the hysteresis of 8 µg of DPL. Minimum surface tensions obtained with gossypol (250µg), rutin (30 mg) and catechin (140 mg) were 20, 30 and 30 dynes/cm respectively. The effect on DPL of the components in powder form was checked by sprinkling each of the components onto the DPL film, when DPL was spread on the subphase as a control. When gossypol was sprinkled onto the DPL film, the hysteresis of DPL increased. When 5 mg of rutin was sprinkled onto DPL, the hysteresis decreased to 25% and then increased with additional rutin. Catechin abolished the hysteresis of the DPL film when 30 mg was used. The results indicate that gossypol may be the component of the cotton dust responsible for the surface activity. (NIOSH-USDA 58-7B30-M28).

68.16

AN IMPROVED METHOD FOR ISOLATION OF TYPE II ALVEOLAR EPITHELIAL CELL SUSPENSIONS. B.E. Goodman, S.E.S. Brown *, J.R. Wells* and E.D. Crandall. University of California, Los Angeles, CA.

We have developed a method for obtaining a satisfactory yield of high purity Type II alveolar epithelial cell suspensions from rat lungs that does not require adherence to a surface. The technique involves buoyant density gradient sedimentation (after Dobbs et al, BBA 618:510-523, 1980), followed by unit gravity velocity sedimentation. Briefly, isolated, lavaged and perfused rat lungs are inflated first with albumin-fluorocarbon emulsion and then with elastase solution (37°C, 20 min each). The lungs are minced and sequentially filtered through gauze, 175 µm and 35 µm nylon cloth. The resultant crude cell mixture (45% Type II cells) is layered onto a discontinuous metrizamide gradient (ρ=1.040, ρ=1.090) and spun at 200xg (20 min, 4°C). The Type II cells are found in a broad band throughout the gradient while AMs, PMNs, and clumped RBCs pellet. The suspended cells (65% Type II cells)are loaded onto a continuous 2-4% Ficoll gradient containing 1% FBS in a cell separator (Wescor CelSep). Cells sediment at unit gravity for 2.5 h at 25°C. The peak fractions contain about 10^7 cells/rat of >90% Type II cells which are >90% viable by trypan blue dye exclusion. The major contaminant is small lymphocytes. These Type II cells form domes in 3 days when plated on plastic. This preparative method results in high purity, adequate yield, viable Type II cell suspensions, and has the advantage of not requiring adherence to a surface. (AHA-GLAA IG1-783, HL 26223.)

68.17

DEPLETION OF PLASMA KININOGEN AND INCREASED CONVERTING ENZYME (CE) ACTIVITY DURING ACUTE PULMONARY EDEMA. P.S. Verma* and R.G. Adams* (SPON: J.A. Gagnon). Walter Reed Army Inst. of Res., Washington, DC 20307 and Howard Univ. Med. Sch., Washington, DC 20060

Intravenous administration of oleic acid is known to impair the capillary endothelium and cause pulmonary edema. Kinins are potent edematogenic agents in systemic tissues, producing an increase in vascular permeability to proteins and water. We therefore decided to investigate the relationship of kinins and CE during oleic acid induced pulmonary edema in the dog. Mongrel dogs of either sex were anesthetized with pentobarbital sodium, mechanically ventilated, and catheters placed into the carotid artery, pulmonary artery, superior vena cava, and femoral vein. Oleic acid (35 mg/kg) was infused into the femoral vein, and arterial and venous blood samples removed at 30 min intervals. Plasma kininogen levels were measured as an index of the rate of kinin production. The base line arterial plasma kininogen values were 3.04 ± 0.25 μg bradykinin (BK)/ml (mean ± S.D.). Within 30 min of oleic acid infusion, plasma kininogen depletion reached 0.59 ± 0.09 μg BK/ml. The levels of plasma kininogen stayed close to 0.3 μg BK/ml for the rest of the experiment. The arterial serum CE value prior to the treatment was 1.76 ± 0.46 units and increased to 118% after 30 min treatment. These findings suggest increased liberation of kinins into the circulation and increased CE activity may play a role in the development of acute lung injury.

68.18

LIPOPROTEIN DISTRIBUTION IN SHEEP LUNG LYMPH. T.M. Forte*, C.E. Cross*, R.A. Gunther* and G.C. Kramer* (SPON: J.C. Schooley). Donner Laboratory, Univ. of Calif., Berkeley 94720 and Departments of Physiology, Medicine, and Surgery, Univ. of Calif., Davis 95616

Lipoprotein distribution in lung lymph was studied in order to determine whether there was selectivity of lipoprotein transport across the lung capillary bed and whether modification of lipoproteins occurred in the lymph. Mature sheep lung lymph was collected over several hours and lipoproteins recovered from lymph compared with those of plasma. Lymph and plasma triglyceride levels were 6.1 ± 1.3 and 12.0 ± 3.0 mg/dl, respectively, with a lymph to plasma (L/P) ratio of 0.50. Lymph and plasma cholesterol values were 18.8 ± 5.8 and 42.1 ± 10.5 mg/dl, respectively, L/P ratio = 0.45. Very low density lipoproteins were absent from both plasma and lymph. High density lipoproteins (HDL), d = 1.063-1.21 g/ml and low density lipoproteins (LDL), d < 1.063 g/ml were isolated from lymph and plasma. Lymph HDL and LDL levels were approximately 50% those of plasma but were present in the same general proportions: lymph HDL = 37 ± 8 mg/dl and LDL = 26 ± 8 mg/dl while plasma HDL = 84 ± 16 mg/dl and LDL = 52 ± 17 mg/dl. The LDL fraction of lymph contained particles which were enriched in phospholipid and free cholesterol. These particles had unusual electron microscopic morphologies and were not seen in plasma. Studies suggest that lipoproteins transported in the lung lymph undergo physical and chemical modifications in this fluid compartment.

68.19

UNRELIABILITY OF THE THERMODILUTION MEASUREMENT OF LUNG WATER IN HYDROCHLORIC ACID LUNG INJURY. P.V. Carlile*, P.M. Butler*, M. Mojarad* and B.A. Gray. Dept. of Medicine, VA Medical Ctr. and Univ. of Oklahoma, Oklahoma City, OK 73104

We investigated the relationship between the extravascular volume of distribution for thermal indicator (ETV) and extravascular lung mass (ELM) in dogs with focal lung injury produced by the bronchial instillation of hydrochloric acid (group I) or diffuse injury produced by alloxan or ANTU (group II). Lung injury in group I was either bilateral (IA) or restricted to one lung with a tracheal divider (IB) to permit measurement of the percentage of blood flow to the injured lung (Qi) with ^{99}Tc labelled macroaggregated albumin. ETV was determined from the difference in mean transit times of thermal and dye dilution curves recorded in the aorta after right and left atrial injections. ETV and shunt were measured four hours after lung injury. ELM and extravascular wet to dry weight ratio (W/D) were determined postmortem. The W/D was 9.12 ± 1.29 (SD) for Group I and 10.98 ± 2.2 for Group II. The ETV/ELM ratio was 0.31 ± 0.14 for Group I and 1.09 ± 0.16 for Group II ($P < 0.01$). Qi averaged 18 ± 6% (normal 40-45%). Shunt was 18 ± 12% for Group I and 44 ± 22% for Group II ($P < 0.01$). We conclude that the thermal dilution method fails to detect edema in focal lung injury characterized by redistribution of blood flow and low shunt. (Supported by Medical Research Svc. of the V.A., N.I.H. Grants HL 07207, HL 07524 & HL 30450 and the Parker B. Francis Fndn.)

69.1

TUBULOGLOMERULAR FEEDBACK MEDIATED REDUCTIONS IN DIRECTLY MEASURED GLOMERULAR CAPILLARY PRESSURE IN RESPONSE TO INCREASED DISTAL VOLUME DELIVERY L. G. Navar, M. Reddington*, P.D. Bell and D.W. Ploth. Univ. of Alabama in Birmingham, Birmingham, AL, 35294

Increases in flow rate to the macula densa segment stimulate the tubuloglomerular feedback mechanism to decrease glomerular filtration rate. While it is generally thought that there are associated decreases in glomerular capillary pressure (GP) resulting from increases in afferent arteriolar resistance, a recent study (Am J Physiol 243:F447, 1982) indicated that directly measured GP in Munich Wistar rats (MWR) does not decrease as long as the filtration process is not interrupted. To evaluate this issue further, GP was measured in MWR during increases in flow rate achieved by perfusing an isotonic electrolyte solution into an unblocked late proximal convolution. Arterial pressure averaged 115±5 mmHg and proximal tubule pressure was 14±1 mmHg. In 21 tubules from 10 rats, perfusion at 24 nl/min decreased GP from 57±1.3 mmHg to 47±2 mmHg. Upon cessation of perfusion, GP returned to 56±1.5 mmHg. The statistically significant decrease in GP averaged 10±1.7 mmHg. These results demonstrate that GP does decrease during increased distal delivery even when the tubule is not blocked and support the hypothesis that changes in afferent arteriolar resistance are primarily responsible for feedback mediated reductions in GFR. (Supported by NIH Grant HL18426)

69.2

TUBULOGLOMERULAR FEEDBACK RESPONSES DURING CALMODULIN INHIBITION WITH TRIFLUOPERAZINE IN THE RAT. P. Darwin Bell and Mary Reddington*. University of Alabama in Birmingham, Birmingham, AL 35294.

In recent studies, we have suggested that cytosolic calcium within the macula densa cells serves to transmit signals from distal tubular fluid to vascular elements. Since calcium binding proteins, such as calmodulin, have been implicated in the intracellular actions of calcium, we tested the effects of inhibition of calmodulin activity with trifluoperazine (TFP) on stop flow pressure (SFP) feedback responses. In the rat, SFP was measured during retrograde microperfusion for up to 5 minutes at 15 nl/min. Perfusion with a isotonic Ringer's solution (IRS) resulted in a decrease in SFP of 10.7±0.9 mmHg (n=18). With the addition of 50 μM TFP to IRS, SFP was decreased by 11±1.1 mmHg (n=25); with 75 μM TFP, SFP feedback responses were 13±0.5 mmHg (n=16). The rate of decrease in SFP in response to the initiation of perfusion with TFP was the same as with IRS alone. After cessation of perfusion, however, recovery of SFP to preinfusion values was significantly prolonged in tubules perfused with TFP (69±7 vs 160±32 sec). Accordingly, these results suggest that calmodulin does not directly mediate the effects of macula densa cytosolic calcium to transmit vasoconstrictor signals. In contrast, since calmodulin can stimulate calcium transport, the delay in the return of SFP to control values may be due to an impaired ability of the macula densa cells to lower cytosolic calcium.

69.3

ANTINATRIURETIC ACTION OF ANGIOTENSIN II AND ANGIOTENSIN III IN SODIUM DEFICIENT DOGS DURING CAPTOPRIL INDUCED NATRIURESIS. R.E. McCaa, M.D. Crawford*, B.E. Brown*, D.K. Roman*, and R.T. Morris*. Univ. of MS. Med. Ctr., Jackson, MS. 39216

Captopril administration inhibits angiotensin II formation and produces natriuresis and hypotension in sodium deficient dogs. Angiotensin II infusion (3 ng/kg/min) into dogs treated with captopril restores urinary sodium excretion and arterial pressure within 3 days to levels observed in untreated sodium deficient dogs. In the present study we evaluated the effects of des-asp^1-angiotensin II (A-III) on arterial pressure (AP), plasma aldosterone concentration (PAC), plasma renin activity (PRA), urinary sodium excretion ($U_{Na}V$), effective renal plasma flow (ERPF), and glomerular filtration rate (GFR) in sodium deficient dogs treated with captopril (400 mg/day). After 7 days of captopril treatment, AP decreased from 108 to 78 mm Hg, PAC decreased from 32.3 to 12.4 ng/dl, PRA increased from 3.87 to 9.98 ng/ml/hr, $U_{Na}V$ increased from 0.85 to 8.85 mEq/day, ERPF increased from 197 to 259 ml/min, and GFR decreased from 84 to 76 ml/min. In response to A-III infusion (5 ng/kg/min) AP, PRA, and PAC failed to change significantly. However, $U_{Na}V$ decreased from 8.85 to 2.35 mEq/day, ERPF decreased from 259 to 201 ml/min, and GFR decreased from 76 to 70 ml/min. These data demonstrate that A-III, like A-II, produces parallel decreases in ERPF and $U_{Na}V$ in sodium deficient dogs during captopril treatment. However, unlike A-II, A-III has little or no effect on arterial pressure or aldosterone secretion. (Supported by USPHS NIH Grant HL-09921.)

69.4

NATRIURETIC AND ANTINATRIURETIC ACTIONS OF ANGIOTENSIN II (AII): ROLE OF RENAL ARTERY PRESSURE AND PROXIMAL TUBULAR REABSORPTION. M.E. Olsen*, J.E. Hall, J-P. Montani* and A.C. Guyton. Univ. Miss. Med. Ctr., Jackson, MS. 39216

The aim of this study was to determine the role of changes in renal artery pressure (RAP), renal hemodynamics, and tubular reabsorption in mediating the natriuretic and antinatriuretic actions of AII. AII formation was blocked with SQ-14225 and AII was infused iv at rates of 5,15,45,135,405, and 1215 ng/kg/min for 30 min at each dose while RAP was either servo-controlled at the normal level or permitted to increase. At AII infusion rates of 45 ng/kg/min and below, urinary Na excretion ($U_{Na}V$), fractional Na excretion (FE_{Na}), and renal blood flow (RBF) decreased markedly, while filtration fraction (FF) increased. When RAP was permitted to increase during AII infusion at 135 ng/kg/min and above, $U_{Na}V$ and FE_{Na} increased while fractional reabsorption of lithium (FR_{Li}), an index of proximal tubular Na reabsorption, decreased. When RAP was servocontrolled, AII infusion at rates of 5-1215 ng/kg/min decreased $U_{Na}V$ and FE_{Na}, while increasing FR_{Li} even though RBF, GFR, and FF were not significantly different from the values observed when RAP was allowed to increase. These data indicate that in the intact kidney, even very large doses of AII cause antinatriuresis when RAP is prevented from increasing. The natriuretic effect of high doses of AII is caused by increased RAP which appears to decrease proximal tubular Na reabsorption. (Supported by NIH grants HL 23502, HL 11678 and by the Miss. Heart Assoc.).

69.5

ANGIOTENSIN II (AII) CONSTRICTS PREGLOMERULAR VESSELS IN THE PRESENCE OF ADENOSINE. John E. Hall, Joey P. Granger and Andre J. Premen. Univ. Miss. Med. Ctr., Jackson, Miss. 39216

The present study was designed to test the hypothesis that high renal levels of adenosine (Ado) may greatly enhance the vasoconstrictor action of AII primarily in preglomerular vessels. In normal dogs, AII infusion (20 ng/kg/min iv) with renal artery pressure (RAP) maintained constant decreased renal blood flow (RBF) to 61±3% of control, increased filtration fraction (FF) to 173±21% of control, and did not change GFR. After intrarenal infusion of 1 μmol/min of Ado, AII (20 ng/kg/min iv) decreased RBF and GFR to 61±5% and 64±6% of control, respectively, due to large increases in calculated preglomerular (R_A) and postglomerular (R_{PG}) resistances. After inhibition of tubuloglomerular feedback (TGF) by occluding the ureter during mannitol diuresis, AII increased R_{PG} markedly but did not alter R_A. However, after intrarenal Ado infusion and inhibition of TGF, AII raised R_A and R_{PG} to 213±27 and 155±7% of control, respectively, while decreasing RBF to 59±5% of control. These data indicate that while the direct renal vasconstrictor effect of AII is normally localized in postglomerular vessels, AII may increase R_A markedly when renal levels of adenosine are elevated. This interaction between Ado and AII could play a role in lowering GFR when renal Ado and AII levels are both elevated, as in renal ischemia. (Supported by NIH grants HL 23502 and HL 11678 and by the Miss. Heart Assoc).

69.6

CONTROL OF ARTERIAL PRESSURE AND RENAL HEMODYNAMICS DURING CHRONIC INTRARENAL ADENOSINE INFUSION. A.J. Premen, J.E. Hall and J.P. Granger. Univ. Miss. Med. Ctr., Jackson, Miss. 39216

Acute intrarenal infusion of adenosine (Ado) has been reported to cause hypertension in conscious dogs, possibly by activation of the sympathetic nervous system via intrarenal chemoreceptors. The primary aim of the present study was to examine the chronic effects of elevated intrarenal Ado levels on renal hemodynamics and mean arterial pressure (MAP). In 6 normal dogs, intrarenal Ado infusion (2 μg/kg/min) for 6 days caused no significant changes in MAP, which averaged 94±3 mmHg during the control period and 91±2 mmHg during Ado infusion. Chronic Ado infusion decreased GFR and filtration fraction (FF) and transiently lowered plasma renin activity. To examine the role of suppressed angiotensin II (AII) formation in preventing hypertension during chronic Ado infusion, we also studied the effects of intrarenal Ado infusion after infusion of AII (1.0 ng/kg/min) to prevent renal levels of AII from decreasing. But even when renal AII levels were maintained constant, Ado infusion (2.0 μg/kg/min for 4 days and 10 μg/kg/min for 5 days) failed to alter MAP, which averaged 104±6 mmHg during the control period, 106±5 mmHg during infusion of 2 μg/kg/min, and 105±5 mmHg during infusion of 10 μg/kg/min. These data indicate that chronic elevation of intrarenal Ado causes sustained reductions in GFR and FF, but does not elevate MAP. (Supported by NIH grants HL 23502 and HL 11678 and by the Miss. Heart Assoc.).

69.7

DOSE RESPONSIVENESS OF THE RENAL VASCULATURE TO SELECTIVE ADENOSINE RECEPTOR AGONISTS. Robert D. Murray and Paul C. Churchill, Hypertension Research Lab., Henry Ford Hospital and Dept. of Physiology, Wayne State Univ. Detroit, Michigan 48202

We have previously reported (Fed. Proc. 42(5):1261, 1983) that 1 μM N-ethylcarboxamideadenosine (NECA) increased flow rate when infused into an isolated rat kidney under conditions of constant pressure. 1 μM cyclohexyladenosine (CHA) had no effect on flow rate. In the present studies, we have determined the dose-dependency of total renal resistance to CHA, a selective A_1 adenosine receptor agonist, and to NECA, a selective A_2 adenosine receptor agonist. Kidneys isolated from female Sprague-Dawley rats were perfused at a constant flow rate (initial perfusion pressure = 100 mm Hg) and increasingly higher doses of agonist (10^{-12} through 10^{-4} M). Perfusion pressure was monitored for four minutes following initiation of each dose. CHA (n = 4) in doses of 10^{-9} to 10^{-7} M caused increases in perfusion pressure (vasoconstriction). The perfusion pressure declined to values below baseline (vasodilation) at concentrations of 10^{-5} and 10^{-4} M. NECA (n = 4) had no apparent vasoconstrictive effect in most of the experiments, but was vasodilatory at doses of 10^{-8} M and higher. These results support the hypothesis that A_1 and A_2 adenosine receptors are present within the renal vasculature and that adenosine receptor agonists may either increase or decrease renal vascular resistance in a dose-dependent manner.

Supported by NIH grant HL 28982-01

69.8

EFFECTS OF CHANGES IN BLOOD VISCOSITY ON RENAL HEMODYNAMICS AND PLASMA RENIN ACTIVITY. S. Simchon*, R.Y.Z. Chen, R.D. Carlin, F.C. Fan, K.M. Jan and S. Chien. Columbia U. Coll. Physicians and Surgeons, New York, N.Y. 10032 and Fairleigh Dickinson Univ., Hackensack, NJ.

The effects of induced changes in blood viscosity on renal hemodynamics and plasma renin activity (PRA) were studied in dogs anesthetized with sodium pentobarbital. Blood viscosity was altered either by changes in red blood cell concentration (hematocrit, Hct) or by an increase in plasma viscosity (dextran administration). Arterial blood pressure and renal blood flow (RBF) remained relatively constant when the blood viscosity was elevated. In hyperviscosity induced with dextran administration, the increase in PRA correlates with the renal vasodilation (decrease in renal vascular hindrance) in a linear manner, with a coefficient of correlation of 0.968 ($P<0.005$). The increases in PRA with hemoconcentration up to 65 percent Hct can also be correlated with renal vasodilation, while the inhibition of renin release with hemodilution down to 25 percent Hct can be correlated with renal vasoconstriction. The renin release found following extreme levels of hemoconcentration (above 65 percent Hct) and hemodilution (below 25 percent Hct), despite the renal vasoconstriction, reflects the overriding effect of beta-adrenergic activation. (Supported by NHLBI Research Grant HL-16851 and NRSA Grant HL-07114)

69.9

ACTION, AND MECHANISM OF ACTION, OF VERATRINE ON RENIN RELEASE P. C. Churchill, M. C. Churchill, and F. D. McDonald. Wayne State University. Detroit, Michigan 48201

It is known that increased activity of the renal nerves stimulates renin secretion by a beta-adrenergic mechanism. The goal of these experiments was to develop an in vitro model of neurally-mediated renin secretion. Veratrine produces an Na-dependent depolarization of nerve terminals resulting in neuro transmitter release. Rat renal cortical slices were incubated at 37°C in a buffered, oxygenated, physiological salt solution containing various concentrations of veratrine. As can be seen in the table below, veratrine stimulated renin secretion in a concentration-dependent manner. Denervation of the kidneys 3-5 days prior to the experiments nearly abolished the stimulatory effect of veratrine. Similarly, 9×10^{-7} M timolol (a beta-adrenergic antagonist) nearly abolished the stimulatory effect of 10^{-4}M veratrine (6.4 ± 0.5 GU/g/30 min versus 5.4 ± 0.4 for 0 veratrine). These results are consistent with the hypothesis that veratrine stimulates renin secretion by inducing release of norepinephrine from nerve terminals in the preparation.

Veratrine, M	Renin Secretory Rate, GU/g/30 min
0	5.4 ± 0.4
1×10^{-5}	5.9 ± 0.4
2×10^{-5}	8.6 ± 0.6
5×10^{-5}	10.4 ± 0.8
1×10^{-4}	10.9 ± 0.6

69.10

EFFECT OF PROSTAGLANDIN F_2(PGE_2) ON IN VITRO RENIN RELEASE IN SODIUM LOADED AND SODIUM DEFICIENT RATS. G. A. Lopez, K. Khalighi*, A. Ebneshahidi*, A. Appleton*, E. Gonzalez*, S. Ko*, S. Jaramillo*, S. Rivas* and A. Weiss* California State University, Los Angeles, CA 90032

This study investigated whether the effect of PGE_2 on in vitro renin release (RR) involves tissue c-AMP (Tc-AMP) changes and if it is modified by dietary sodium manipulation. Resting RR in renal cortical slices from sodium deficient (SD) rats was greater than in slices from sodium loaded (SL) rats. 10^{-5}M PGE_2 significantly stimulated RR and Tc-AMP content in both SD and SL groups of slices while 10^{-7}M and 10^{-9}M doses were ineffective, although RR in response to 10^{-7}M PGE_2 in the SD group was significantly greater than that caused by the same dose in the SL group. The PG-synthetase inhibitor indomethacin (I, 10^{-4}M), reversed the effect of 10^{-7}M PGE_2 alone on RR in the SD group from non-stimulation to significant stimulation, without affecting Tc-AMP content. The phosphodiesterase inhibitor theophylline (T, 10^{-4}M) reversed the effect of 10^{-7}M PGE_2 alone on both RR and Tc-AMP content in the SD group of slices. These data indicate that: 1) Resting RR is enhanced in the SD state; 2) PGE_2 can directly stimulate RR by a c-AMP dependent mechanism; 3) RR responses to PGE_2 administration are enhanced in the SD state; 4) I potentiates the RR responses to ineffective PGE_2 doses in the SD group by an effect on release only and; 5) T inhibition of Tc-AMP degradation is more effective in the SD state. (Supported by NIH #5-S06-RR08101).

69.11

EVOLUTION AND REGRESSION OF JUXTAGLOMERULAR HYPERPTROPHY FOLLOWING CAPTOPRIL ADMINISTRATION. Harley D. Sybers, Baylor College of Medicine, Houston, Texas, 77030. Merrill L. Overturf* and Sheryl A. Smith,* University of Texas Medical School, Houston, Texas, 77025.

Morphologic analysis of the kidneys was done on control rabbits and those fed normal diet plus 5.7 mg/kg captopril daily for up to 7 months. Blood pressure, plasma renin activity (PRA) and angiotensin I converting enzyme were determined monthly. After 3 months groups of rabbits were sacrificed at monthly intervals. After 7 months of captopril the drug was stopped and the remaining rabbits sacrificed at monthly intervals for 6 months. Renal tissue was examined by light and electron microscopy. Hypertrophy of the juxtaglomerular apparatus (JGA) was prominent at 3 months. At 7 months, the cross sectional area averaged 10,342 ± 2,373 um^2 while that of the controls was 3,221 ± 631 um^2. Regression of JGA was noted as early as 2 months after cessation of captopril but slight hypertrophy was still ocassionally seen at 4 mo. post cessation. These results indicate that interruption of the normal inhibitory feedback of the renin-angiotensin system results in marked stimulation of the JGA with consequent hyperplasia and hypertrophy which can be reversed by cessation of captopril.

69.12

HEPATIC EXTRACTION OF RENIN (R) ALTERED BY CAPTOPRIL (C). Joan A. Keiser, Terry J. Opgenorth, and Juan C. Romero, Mayo Clinic and Foundation, Rochester, MN 55905

We hypothesized that R extraction by the liver may be modulated by circulating angiotensin II (AII). In the present study hepatic extraction of R was measured in anesthetized dogs before and after administration of C (1 mg/kg·hr). During 1.5 hr post-surgical stabilization a sulfobromophthalein (BSP) infusion was initiated. BSP extraction by the liver was used to calculate hepatic plasma flow (HPF). In 8 dogs plasma renin activity (PRA) averaged 9.9±3.1 ng/ml·hr during the control period and rose to 20.5±8.4 after 1 hour of C infusion. Hepatic extraction of R averaged 45.7±4.8% under control conditions. Administration of C resulted in a significant fall in R extraction to 31.9±4.4 ($P<.05$), however, HPF was unchanged (21.9±2.7 to 22.5±2.9 ml/kg·min). Mean arterial pressure (MAP) fell by 18 mmHg post-C. Time control dogs undergoing an identical protocol (except C-infusion) had R extractions of 41.5±2.4 and 43.4±4.0% during the two periods, respectively. In several dogs AII was infused in addition to C at a dose sufficient to restore MAP to pre-C levels, samples were collected after an additional 30 min. The AII infusion did not alter HPF (22.1±4.4) and depressed PRA to control values (9.0±2.8), however, R extraction remained blunted (36.2±4.2%). From the present experiments we conclude that converting enzyme inhibition does alter R metabolism, although the mechanism remains unclear. (Supported by NIH Grant HL-16496)

69.13

PLASMA RENIN ACTIVITY (PRA) AND ANGIOTENSIN CONVERTING ENZYME (ACE) LEVELS ARE DETERMINED BY THYROID FUNCTION L.M. Resnick* and J.H. Laragh, Cardiovascular Center, Cornell University Medical College, NY, NY 10021

In order to study thyroid function and the renin-angiotensin system in human hypertension (HiBP), we measured FT4, T3(RIA), TSH, PRA, and ACE before and after 500 mcg. IM of thyrotropin releasing hormone (TRH) in 20 euthyroid normotensive (NL-BP) and 24 HiBP subjects. Mean FT4 and T3(RIA) were normal for NL-BP (FT4 2.14±0.16, T3(RIA) 143±15 ng%) and HiBP (FT4 2.32±0.45, T3 (RIA) 157±37 ng%). ACE levels were higher in HiBP than NL-BP (23±1.4 vs. 17±1.4 U/ml, p<0.001). Results (mean±SEM) for HiBP were:

Group (ΔTSH, U/ml)	T3(RIA) (80-220 ng%)	PRA (ng/ml/h)	ACE (11-35 U/ml)
Hyperresponsive (77±20)	106 ±7.7	0.91 ±0.16	18 ±2.7
NL-Responsive (18±2)	138* ±7.7	2.6** ±0.8	24 ±1.2
Non-Responsive (1.1±0.5)	190** ± 32	9.3*** ±3.5	35.0** ±6.2

*p<0.05, **p<0.02, ***p<0.005 vs. hyperresponsive

PRA was directly related to T3(RIA) in all patients (r=0.81, p<0.001), as were ACE levels (NL-BP, r=0.76, p<0.005; HiBP, r=0.92, p<0.001). We conclude 1) PRA and ACE are highly significant functions of circulating thyroid hormones, 2) ACE levels are higher in HiBP than in NL-BP, and 3) subclinical thyroid disease may exist in a significant fraction of HiBP.

69.14

CHRONIC SELECTIVE BLOCKADE OF BRAIN ANGIOTENSIN II RECEPTORS AND BRAIN ANGIOTENSIN CONVERTING ENZYME IN THE RAT. C.A. Bruner* and G.D. Fink* (SPON: G.L. Gebber), Michigan State Univ., E.Lansing, MI 48824.

Experiments were designed to develop a method to produce chronic selective pharmacological blockade of brain angiotensin II (AII) receptors or brain angiotensin converting enzyme (ACE). The pressor responses to sequential 10 min iv infusions of AII (10, 30, 100 ng/min) and to an intraventricular (ivt) bolus injection of AII (150 ng) were recorded in conscious Sprague-Dawley rats that had been instrumented with arterial and venous catheters and a lateral cerebral ventricular cannula. After a 5-day ivt infusion of ^{1}Sar-^{8}thr-AII (sarthran), pressor responses were retested. In a separate group of rats, the pressor responses to iv and ivt administration of angiotensin I (AI) were measured before and after 5-day ivt infusion of the ACE inhibitor teprotide. After ivt infusion of sarthran at 1 µg/hr for 5 days, the pressor response to ivt injection of AII was totally blocked while the dose response curve to iv AII was slightly diminished. Teprotide (10 µg/hr, ivt) for 5 days produced significant depression of the pressor response to ivt AI but had no effect on the dose response curve to iv AI. In addition, ivt sarthran infusion produced no measurable agonistic effects in rats on high sodium intake. The results of the present experiments demonstrate that 5-day ivt infusion of sarthran (1 µg/hr) or teprotide (10 µg/hr) will selectively block brain AII receptors or brain ACE, respectively. These methods may be useful in determining the role of the central effects of AII in models of experimental hypertension in the rat. (Supported by USPHS grant HL24111.)

69.15

EFFECT OF ELEVATED BLOOD PRESSURE, ALDOSTERONE AND RENIN ON RENAL SODIUM RETENTION. Jamil N. Bitar*, Anwar B. Bikhazi and Adil E. Birbari*. Dept. Physiology, Faculty of Medicine, American University of Beirut, Lebanon.

A study on the effect of elevated blood pressure, renin and aldosterone on renal Na retention in 2-kidney Goldblatt hypertensive rats. The technique involves retrograde kidney perfusion from the renal veins via the kidneys, and then through the renal arteries and dorsal aorta. Sodium retention in 7 and 30-60 days post-stenosis hypertensive rats was 70% greater than in normotensive sham operated rats. No significant change was observed in Na retention in the clipped kidney of the 1 day post-stenosis. However, the contralateral kidney retained 25% more Na in the hypertensive rats. The 1 and 7 days post-stenosis rats had higher plasma aldosterone concentrations than the controls while the 30-60 days post-stenosis rats showed lower aldosterone plasma levels. The plasma renin activity of the 1 day post-stenosis rats showed 66% higher activity than that of the sham operated control rats with no significant change in the 30-60 days post-stenosis. Therefore Na retention may be mediated by aldosterone in the 7 days post-stenosis rats. Natriuresis in the non-stenosed kidney of both the 7 and 30-60 days post-stenosis may be modulated by an increase in single nephron glomerular filtration rate in hypertrophied kidneys and by the washout of the medullary gradient. (Supported by Grant 38-5706 from the Lebanese National Research Council).

69.16

EFFECTS OF CAPTOPRIL ON THE GENESIS OF NOREPINEPHRINE-INDUCED MALIGNANT HYPERTENSION. Thomas E. Lohmeier, Robert G. Carroll, Alison J. Brown* and Larry J. Tillman*. Univ. of Miss. Med. Ctr., Jackson, MS 39216

Infusion of progressively higher rates of norepinephrine (NE) for 6 days--.05,.1,.2,.3,.4, and .5 µg/kg/min on days 1-6, respectively, into the renal artery of a solitary kidney consistently produces malignant hypertension (MHT) in the dog (Fed. Proc. 42:985, 1983). Further, the transition from benign to MHT occurs either on day 5 or 6 and is associated with marked increases in both mean arterial pressure (MAP) and plasma renin activity, and a severe decrease in renal function. To determine whether the renin angiotensin system (RAS) is critically involved in the transition to MHT, 4 unilaterally nephrectomized dogs maintained on 50 mEq Na were infused with the angiotensin converting enzyme inhibitor Captopril for 4 days prior to and throughout NE infusion. During Captopril infusion alone, MAP fell from 96±8 to 71±8 mmHg, effective renal plasma flow increased from 144±21 to 157±25 ml/min, and GFR was unchanged. Subsequently, and in marked contrast to dogs with a functional RAS, MAP increased only slightly (to 88±7 mmHg) during intrarenal NE infusion; however, severe renal impairment occurred after only 3 days of NE. Thus, in this model of NE-induced malignant hypertnsion, Captopril prevents the genesis of hypertension but accelerates the development of renal insufficiency. (Supported in part by NIH Grant HL 11678).

69.17

NATRIURETIC RESPONSE TO β-BLOCKADE IN DOCA-SALT HYPERTENSIVE DOGS. T.J. Burke, B.R. Walker, and A.L. Erickson.* Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

It has been shown that renal denervation causes a natriuresis in animals receiving DOCA-salt and may delay the onset of hypertension in this model. The current experiments were designed to test for the possible importance of α- and β-sympathetic pathways in sodium retention observed in DOCA-salt hypertension. Five dogs receiving DOCA and salt for 5 weeks were studied in the conscious state. Mean arterial pressure averaged 137±6 mmHg. After control studies, dogs received one of the following intravenously: the β-blocker Servier 2395 [dl(hydroxy 2-t-butylamino-3' propyloxy)8-thiochromane] (50 µg/kg, n=5), propranolol (50 µg/kg, n=2), the α-blocker phenoxybenzamine (0.5 mg/kg, n=2), or saline vehicle (n=5). Experiments were separated by a minimum of 3 days. Both β-blockers increased urine flow (V) and sodium excretion ($U_{Na}V$); glomerular filtration rate (GFR) and renal plasma flow (RPF) were unaltered. The peak natriuretic effect was comparable for both β-blockers, however, Servier 2395 caused a more prolonged effect (>80 vs 30 min). α-blockade or vehicles resulted in no change in V, $U_{Na}V$, GFR or RPF. Therefore, only β-blockade results in a profound natriuresis in this model. β-sympathetic activity exerts a tubular effect in the DOCA-salt hypertensive animal and may contribute to sodium and water retention.

69.18

Histamine H-1 Receptors and Ureteral Occlusion-Induced Renal Vasodilation in the Dog. R.O. Banks and E.D. Jacobson. Department of Physiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

We evaluated the mechanism by which ureteral occlusion (UO) causes ipsilateral renal vasodilation. Five adult dogs (19-23 kg) were anesthetized with pentobabital. Renal arterial pressure (RAP) was measured with a femoral arterial catheter. Isotonic saline was administered ($1 ml \cdot kg^{-1} \cdot min^{-1}$ for 15 min then $0.25 ml \cdot kg^{-1} \cdot min^{-1}$) via a femoral vein. The left ureter was cannulated with PE100 tubing. Left renal blood flow (RBF) was measured with a flow probe positioned on the artery via a flank incision. Saline or the histamine H-1 receptor antagonist, chlorpheniramine (Ch, 10^{-5}M/min) was infused into the artery via a 25g needle. RAP was decreased experimentally with an aortic ligature positioned above the left renal artery. Prior to Ch infusion, RBF averaged 3.10±0.38 (SE) $ml \cdot min^{-1} \cdot g$ kidney $wt.^{-1}$ and 4.17±0.48 (P<0.001) before and during UO, respectively (RBF was stable after 15 min UO). Following release of UO and restabilization, Ch infusion was begun. After 5 min RBF was 3.62±0.46. The ureter was occluded and RBF increased to a stable value of only 3.67±0.49. Ch infusion was then stopped and RBF promptly rose to 4.42±0.47 (P<0.001). RBF displayed good pressure autoregulation during control periods but pressure autoregulation was abolished during UO, UO+Ch and Ch alone. These data suggest that UO causes intrarenal histamine release and a resultant vasodilation via H-1 receptors. Supported by NIH Grant #HL29021.

69.19

DOES PROLACTIN REGULATE SODIUM TRANSPORT BY THE KIDNEY? Sidney Solomon, University of New Mexico, Albuquerque, New Mexico 87131

Previous studies have indicated that prolactin is able to play a role in regulation of renal reabsorption of sodium. Most preparation of prolactin are contaminated by antidiuretic hormone (ADH) and oxytocin (OXY), at least. It was decided to test if these contaminants were responsible for the apparent action of prolactin. Effectiveness of ADH and OXY was eliminated by use of antibodies or by treatment of prolactin preparations with serum obtained from pregnant women. Rats were volume expanded with saline (2.5% of body weight) and the added volume and sodium excretion compared between groups which were untreated, were given a constant infusion of prolactin or of prolactin with either inactivators of ADH and OXY. Whereas untreated prolactin caused a reduction in the renal response to blood volume expansion, if the action of the contaminants was eliminated the effects of prolactin on renal function was inhibited. It is concluded that the response to volume expansion is not influenced by prolactin per se but that its reputed action in inducing increased sodium reabsorption during volume expansion by saline is caused by contaminants in prolactin preparations. (Supported by a grant from NSF - PCM 7815383).

69.20

EFFECT OF URINARY ALKALINIZATION ON INTRARENAL FORMATION OF KININS. J. Brukman,* O. Carretero, P. Churchill and A. Scicli. Henry Ford Hosp., Detroit, MI 48202.

Since the optimum pH for kallikrein and kininases is alkaline, we studied whether increasing urinary pH in the presence or absence of kininase II inhibition affects intrarenal kinin formation. Urine was collected from both ureters of 30 anesthetized rats during control (ctl) (0.15M NaCl infusion) and experimental (exp) periods of 40 min each. During exp periods, group 1 (time control) was infused with 0.15M NaCl, group 2 and 3 with 0.3M $NaHCO_3$, and group 4 with 0.3M NaCl, all at a rate of 0.113 ml/min. Group 3 was pretreated with captopril (40 mg/kg). Urinary kinins (Ukin), kallikrein (Ukal), pH(UpH), volume (UV), and inulin (GFR) and PAH (RPF) clearances were measured. During ctl and exp periods, Ukin were: 10±4.0 and 31±9.0 ($p<0.01$); 9.0±1.9 and 61±14.6 ($p<0.001$); 14±4.2 and 84±10.9 ($p<0.001$); and 7.75±1.9 and 34.4±10.9 pg/min/kg ($p<.05$), in groups 1, 2, 3 and 4, respectively; UpH was 5.8±0.09 and 6.1±0.11, ($p>.05$); 5.8±0.11 and 7.2±0.06, ($p<.0009$); 5.8±0.08 and 7.2±0.05 ($p<0.001$); 5.7±0.9 and 6.2±.08 ($p<.005$); and UV was 67±10 and 114±15 ($p<0.005$); 60±9 and 222±19 ($p<0.001$); 71±7 and 206±15 ($p<0.001$) and 46±5 and 186±24 µL/min/kg ($p<.005$). Ukal, GFR, and RPF did not change in any of the groups. Ukin correlated with UV ($r=0.62$, $p<0.001$) and UpH ($r=0.73$, $p<0.001$). We conclude that urinary alkalinization increases intrarenal formation of kinins. (Supported by NIH HL28982.)

69.21

GUANINE NUCLEOTIDE REGULATION OF THE RAT KIDNEY VASOPRESSIN RECEPTOR. L. E. Cornett and J. S. Norris*, Depts. of Physiology-Biophysics and Medicine, Univ Arkansas Med Sci, Little Rock, Arkansas 72205

The primary action of arginine^8-vasopressin (AVP) in the mammalian kidney is to increase the permeability of the collecting duct to water, an action mediated by cyclic AMP. We have examined the effects of guanine nucleotides on 1) binding of (^{3}H)-AVP to rat renal medullary membranes and 2) stimulation of adenylate cyclase by AVP. (^{3}H)-AVP binding to rat renal medullary membranes is saturable and of high affinity (Dorsa et al, Peptides-in press). Guanine nucleotides had a biphasic effect on (^{3}H)-AVP binding. At low AVP concentrations (10^{-10} M to 10^{-8}M) binding of (^{3}H)-AVP was reduced by approximately 10% in the presence of either 100µM GTP or 100µM Gpp(NH)p while at high AVP concentrations (10^{-7}M to 10^{-5}M) binding of (^{3}H)-AVP was not affected. Stimulation of renal medullary adenylate cyclase by AVP was guanine nucleotide dependent. Using App(NH)p as the substrate in the absence of a regenerating system, basal activity was 344 pmoles cAMP/mg/10 min, 388 pmoles/mg/10 min with 100µM GTP and was 564 pmoles/mg/10 min with 100µM Gpp(NH)p. Generation of cAMP by 10µM AVP was 452 pmoles/mg/10 min in the absence of guanine nucleotides and was 536pmoles/mg/10 min with 10µM GTP and was 776 pmoles/mg/10 min with 100µM Gpp(NH)p. These results indicate that guanine nucleotides regulate rat renal medullary AVP receptor function and suggest that the AVP receptor is associated with a GTP binding regulatory subunit.

69.22

AMELIORATION OF CIS-PLATINUM NEPHROTOXICITY. James S. McNeil,* Benjamin Jackson,* Oscar Taylor,* Angela Allen* and Donald E. Butkus. Walter Reed Army Institute of Research, Washington, D.C. 20307.

Cisplatinum (CP) nephrotoxicity is the major limiting factor in the therapeutic use of this agent in cancer chemotherapy. We assessed the protective effects of dithiothreitol (DTT) 30.8 mg/kg, 5'5'dithiobis-2-nitrobenzoic acid (DTNB) 247 mg/kg, or 2,3 dimercapto-1-propanesulfonic acid (DMPS) 200 mg/kg in male Sprague-Dawley rats given a single injection of CP, 7.5 mg/kg IV and compared their renal function to rats given CP alone. Each group was subdivided into two groups receiving ad lib H_2O with or without supplimental saline (S) 7 ml/kg BW q6h to prevent volume depletion. CP decreased GFR from 1.7 ± 0.16 to 0.13 ± 0.05 ml/min by day 5. Saline loading, and all three agents ameliorated the CP-induced decrease in GFR as exemplified by day 5 (Table I):

	GFR day 5 (% baseline)	
	H_2O	H_2O + SALINE
CP	7.6	27
CP + DMPS	22	51.5
CP + DTNB	26.7	56.4
CP + DTT	37	60.1

DMPS, DTNB and DTT also ameliorated the CP-induced polyuria and decrease in Uosm and had no affect on GFR, UV or Uosm when administered alone. These observations suggest that volume depletion contributes to the decrement in GFR in this model of ARF and that these agents can significantly ameliorate the reduction in GFR, with or without volume expansion, and may be of potential therapeutic benefit in preventing nephrotoxicity. Their affects on the tumorocidal activity of CP requires further study.

69.23

AGE-DEPENDENCE OF THE RENIN RESPONSE TO VOLUME CONTRACTION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C. Rodriguez-Sargent,* J.L.Cangiano and M. Martinez-Maldonado, Veterans Hospital and University of Puerto Rico Sch. of Medicine, San Juan, Puerto Rico 00936.

We previously found that plasma renin activity (PRA) is low and does not respond to furosemide-induced volume contraction in adult SHR. In the present study we evaluated the renin response to furosemide in 18 SHR and 18 Wistar-Kyoto rats(WKY) at the age of 12 and 24 wk. All rats were kept in metabolism cages throughout each study. After a 3 day balance study the rats were bled for the determination of basal PRA. Five days later, the rats were given a single dose of furosemide (4mg/kgBW, ip)and returned to their metabolism cages for an 8h balance study. Afterwards, each rat was again bled for the determination of PRA. Basal PRA was similar in 12wk-old SHR(10.2 ±0.6) and WKY (9.0±0.6). At this age PRA rose in both SHR (15.9±2.3) and WKY (14.1±0.8) in response to furosemide. In contrast, basal PRA was lower in SHR (5.2±0.8) than in WKY (13.1±1.2) at 24 wk of age. Moreover, in response to furosemide, PRA increased in WKY (23.3±1.0) but not in SHR (5.6±0.9) at this age while the diuretic and natriuretic response was greater in SHR. These data suggest that low renin hypertension in SHR may be associated exclusively with the maintenance stage of blood pressure elevation. In addition changes in PRA with age may partly explain conflicting reports regarding the status of renin activity in SHR. (Supported by a Merit Review grant of the Veteran's Administration)

70.1
AN APPROACH TO REINFORCING COMMON THEMES IN PHYSIOLOGY.
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Because many students view their educational environment primarily as one in which facts associated with each course are to be memorized and recalled, they often fail to recognize that common themes exist in physiology around which a unifying conceptual framework can be built. In developing a set of independent study materials to reinforce common themes in physiology, we have taken the approach illustrated below using the principle of conservation of mass (CM). Each exercise in the set deals with one concept and consists of 3 components: a microcomputer based simulation, a written procedure for the model, and a problem set. The CM model consists of a reservoir having several possible inlets and outlets, the number of which are defined by the student's choice of organ system and specific conditions. The student provides values for known volume flows and mass concentrations and specifies whether steady state or transient data are desired. The model returns numerical data and a visual aid describing the quantity of mass entering, contained in, and leaving the reservoir. The written procedure describes, in the context of the model, applications of the concept to several physiological systems (e.g., pulmonary, c-v, renal) and includes sample input values for each case. The problem set consists of latent image materials arranged in a format that presents a problem and allows the student to selectively view the correct numerical answer, additional information, or the complete solution to the problem. The problems illustrate and reinforce the CM concept as it relates to various organ systems.
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RECEPTOR REGULATION

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Early biochemical studies examining adaptation in the central nervous system concentrated on presynaptic events. This work demonstrated that modifications in membrane transport systems, transmitter synthesis, metabolism and release are important in the maintenance of homeostasis. Because of technological advances it is now possible to examine changes in neuronal activity brought about by alterations in postsynaptic elements. Particular emphasis has been placed on characterizing membrane receptors for neurotransmitters. These efforts have led to a number of significant advances in the understanding of receptor-mediated phenomena in general, and synaptic transmission in particular. The present communication is designed to highlight and summarize some of the more fundamental concepts relating to receptor regulation that have resulted from these studies.

Receptor Components

Neurotransmitter receptor proteins are synthesized in the neuron, transported in small vesicles and inserted into the plasma membrane. Eventually the receptors are returned to the cytosol by endocytosis and destroyed by lysosomal enzymes. A common endpoint for neurotransmitter receptor function is a change in membrane permeability to ions. The basic units of a receptor are the transmitter recognition site and an ion channel. It is unclear whether the recognition site and ion channel are parts of the same molecule, or are separate constituents of a supramolecular complex. Evidence suggests that ion channels may be associated with more than one type of recognition site. There are at least two ways in which recognition site activation leads to channel opening. One is through a direct coupling between the recognition site and the ion channel, and the other by way of a cyclic nucleotide system (Fig. 1).

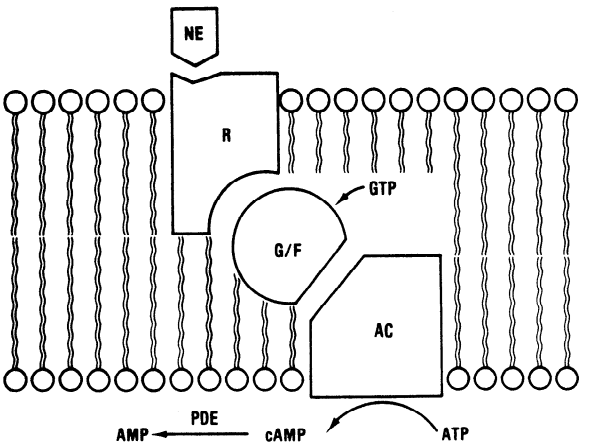

Figure 1. Schematic representation of the relationship between the various components of the β-adrenergic receptor-coupled adenylate cyclase system.

It also appears that there are membrane constituents that can regulate recognition site number and affinity. These phospholipid or protein modulators are capable of rapidly altering the availability of receptors for neurotransmitter. Because receptors are composed of a number of interacting units there are a number of ways in which they can be modified.

Recognition Site Regulation

Radioligand binding assays have provided a great deal of information about the molecular characteristics of receptor recognition sites. The basic principle of these assays is that the amount of radioligand bound to plasma membrane fragments at equilibrium is a function of recognition site number and affinity. Several criteria must be fulfilled before a binding assay may be considered valid for a particular recognition site. Paramount among these are saturability and specificity. By exposing membranes to increasing concentrations of ligand it should be possible to demonstrate that the binding plateaus (saturates) at a biologically

relevant concentration (Fig. 2A). Scatchard analysis of saturation data reveals the equilibrium binding dissociaton constant (K_d) of the receptor for the ligand, and the binding site concentration (B_{max}). In the Scatchard plot shown there appear to be two binding compounds, a relatively high affinity site having a K_d of 1 nM and a B_{max} of 1 pmole/mg protein, and a lower affinity component with a K_d of 20 nM and a B_{max} of 5 pmole/mg protein (Fig. 2B).

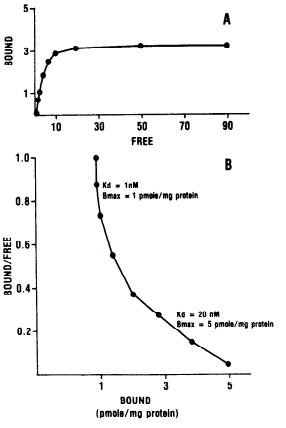

Figure 2. A. Representative saturation curve for radioligand attachment to a neurotransmitter receptor.

B. Scatchard plot of equilibrium binding saturation data. (Adapted from Bylund, D.B., in Receptor Binding Techniques, D.B. Bylund, ed., pp. 70-99, Society for Neuroscience, Washington, D.C. 1980)

Further proof that the ligand is attaching to the relevant site is provided by defining the specificity of the ligand-membrane interaction. Binding should be greatest in those organs, tissues, and subcellular fractions known to have the greatest density of receptors for the radioligand. Furthermore, only physiologically active receptor agonists and antagonists should inhibit ligand attachment, and relative potencies as inhibitors should be similar to relative potencies in physiological tests. Binding assays have now been developed for virtually all neurotransmitter candidates.

Ligand binding assays have revealed that the recognition site can be regulated either directly or indirectly. For example, it has been demonstrated that recognition site number is a function of receptor activity. An increase or decrease in synaptic activity will eventually lead to a decrease or increase, respectively, in recognition site number. Likewise receptor number can be modified by chronic administration of direct-acting recognition site agonists or antagonists. These alterations are viewed as an adaptive response to compensate for the relative excess or deficiency of nerotransmitter.

Recognition site affinity can be altered by inorganic ions, guanine nucleotides and protein modulators. These alterations occur almost instantaneously, providing the receptor with the capacity to make rapid adjustments in response to a change in demand.

Recognition site number or affinity may also be altered by activation of a separate receptor system. Thus, GABA receptor activation increases the affinity of the benzodiazepine binding site, and benzodiazepines modify the number and affinity of GABA receptors. This finding suggests that receptors can influence membrane sensitivity to other neurotransmitter substances.

Receptor Coupling

Modifications in recognition site-coupled components can also alter receptor responses. In regard to the β-adrenergic system, it has been proposed that norepinephrine (NE) activation of the recognition site (R) causes a conformational change that favors the attachment of a regulatory protein (G/F) to the recognition site (Fig. 1). Binding of GTP to G/F activates the enzyme adenylate cyclase (AC) and catalyzes the formation of cyclic AMP from ATP. The cyclic AMP activates protein kinases, one of which may be a constituent of the ion channel. This results in channel opening and an alteration in the polarity of the cell. The cyclic AMP is subsequently converted to AMP by the enzyme phosphodiesterase (PDE). Receptor activity is diminished by a sequential change in the affinities of the various receptor components. Thus, GTP is hydrolysed to GDP, returning the G/F-AC complex to an inactive state and lowering the affinity of the recognition site for neurotransmitter. The transmitter is released and the R-G/F-AC complex separates. Such a receptor system can be modified in a variety of ways. These include alterations in the coupling between the components due to a change in the GTP or receptor recognition sites, or modifications in the amount, or activity, of AC or PDE. In the latter two cases receptor function would be altered even though a normal complex is formed.

Less is known about the coupling mechanism in systems where the recognition site is directly linked to the ion channel. However, it appears that perturbation of the channel can influence the affinity of the recognition site, suggesting that there is a reciprocal relationship between the two.

These data indicate that receptor function can be altered in a variety of ways, and that such changes need not be reflected by a modification in the recognition site. Conversely, alterations in the recognition site do not necessarily result in a modification in receptor function since there may be compensatory changes that occur in the recognition site-coupled components. These possibilities illustrate the importance of directly relating biochemical changes to functional alterations before drawing conclusions about biological consequences.

Conclusions

Neurotransmitter receptor sites are composed of a variety of constitutents, including the recognition site, ion channel and, in some case, a cyclic nucleotide system. A number of factors are capable of modifying the biochemical properties of these various components. Certain modifications, such as a change in receptor number, may take days or weeks to become apparent, whereas others occur quite rapidly. This makes it possible for the cell to adapt immediatley to a change in demand and to make long-term adjustments that may be necessary for functioning. Thus, neurotransmitter receptors are dynamic entities that play an ongoing role in maintaining homeostasis in the central nervous system.

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STRUCTURAL AND FUNCTIONAL ASPECTS OF THE RECEPTORS FOR INSULIN AND THE INSULIN-LIKE GROWTH FACTORS

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Receptor Subunit Structures

The event that initiates the cellular actions of insulin is its recognition and binding by specific receptors in the target cells. The insulin receptor is also the vehicle for internalization and degradation of receptor-bound ligand. Receptors for insulin are present in almost every cell type in chordates and the general structure of these receptors has been preserved for at least 500 million years of evolution (1). Relatively high amounts of these receptors are present in human placenta, rat liver and rat adipocytes, and in the cultured cell lines 3T3-L1 adipocytes and IM-9 human lymphoblasts and for this reason these tissues and cell lines have been extensively used in biological and structural studies of the insulin receptor. Like many other types of hormone receptors, insulin receptors exhibit a K_d for insulin in the low nanomolar range. A complication of the binding of insulin to its receptor is that the kinetics of this process do not reflect a simple interaction with one single class of binding sites, but rather a more complex type of interaction. This atypical behavior has been attributed to either "negative cooperativity" among insulin binding sites (2), interaction of insulin with a heterogenous population of receptors (3), or conformational and kinetic changes that occur in the receptor upon insulin binding (4), and in many cases it probably reflects the summation of various of these, and perhaps other factors. The insulin-like growth factors I and II (IGF-I and IGF-II) are peptide hormones with extensive amino acid sequence with insulin (5,6). The biological actions of insulin, IGF-I and IGF-II include activation of cellular metabolism and macromolecule synthesis (7,8). They also exhibit mitogenic activity on selected cell types (7,8). These actions are thought to be initiated by the interaction of the growth factors with specific cell surface receptors. The molecular basis of the events immediately triggered by the interaction of these hormones with their respective receptors is as yet unknown, and represents a major question in the area of cellular physiology. Critical clues to address this question have been recently provided by the rapid progress in identification and molecular characterization of receptors for insulin and these factors. Our laboratory has developed an affinity-labeling methodology using chemical crosslinkers that allows the identification of hormone receptor components on cell surfaces and membranes (9). Using this methodology, it was possible to establish a model for the structure of the insulin receptor (10). We proposed that the insulin receptor consists of the symmetrical heterotetrameric structure (β-S-S-α)-S-S-(α-S-S-β) in which the α (Mr=125,000) insulin receptor subunits and the β (Mr=90,000) insulin receptor subunits are all linked by disulfide bonds (10,11). This model was simultaneously formulated by Jacobs and Cuatrecasas (12) on basis of their work on insulin receptor purification by affinity chromatography (12,13). In addition to this predominant insulin receptor structure, free α and β insulin receptor subunits, partially reduced ($\alpha\beta$) and $(\alpha)_2$ receptor fragments, and receptor complexes containing biosynthetic precursors of the α and β subunits have been identified in various cell types by receptor affinity-labeling (14) and immunoprecipitation with anti-insulin receptor antibodies (15). An intriguing possibility based on the multiple chemical and biological similarities between IGF-I, IGF-II and insulin is whether the respective receptors for these hormones also share structural and biological properties. Using the affinity labeling methodology mentioned above we (16,17) and others (18,19) recently crosslinked many types of intact cells and membranes from human and rodent tissues to cell- or membrane-bound ^{125}I-IGF-I and ^{125}I-IGF-II. These studies revealed the existence of two distinct types (type I and type II) of the high-affinity receptors for IGF-I and IGF-II (17). The type I IGF receptor is a disulfide-linked, multisubunit complex strikingly similar to the insulin receptor in molecular size and subunit structure. Thus, the type I receptor consists of Mr=130,000-140,000 α subunits and Mr=97,000 β subunits, all linked in an $(\alpha\beta)_2$ configuration like the insulin receptor (17). The β subunits in the type I IGF-I receptor contain a site in β the middle of their amino acid sequence very susceptible to cleavage by elastase-like enzymes, in analogy to a similar site in the subunit of the insulin receptor (17,20). However, the type I IGF receptor exhibits a higher affinity for IGF-I than for IGF-II, and a low affinity for insulin (17). We refer to this receptor type as IGF-I receptor. The type II IGF receptor consists of a single peptide chain (Mr=250,000-270,000) on dodecyl sulfate-polyacrylamide gels that is not disulfide linked

to any other membrane component. This receptor type has been affininty-labeled with either ^{125}I-IGF-I, ^{125}I-IGF-II (17,18) or ^{125}I-multiplication stimulating activity (rat IGF-II) (16). The type II IGF receptor is also termed IGF-II receptor because it exhibits a higher affinity for this ligand than for IGF-I (17).

These studies have led to the conclusion, schematically shown in Fig. 1, that three receptor structures account for all of the binding and biological signalling by insulin, IGF-I and IGF-II. Peptide mapping of the receptors for insulin and IGF-I indicate extensive homology, but further work will be required to determine the presence or extent of actual protein sequence homology.

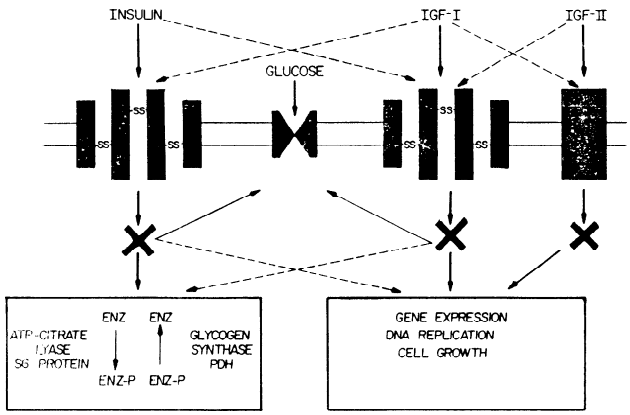

Figure 1. Schematic representation of proposed structures and some interrelationships among the receptors for insulin, IGF-I and IGF-II. The ligand with highest affinity for each of the three receptor types is shown with a vertical arrow, and the cross-reactivities with diagonal arrows. Other subunit forms or states of the receptors shown also appear to exist. The receptors for insulin and IGF-I are both capable of activating hexose transport and the rapid enzyme changes exemplified by the box at the left. The IGF-II receptor does not have this capability.

IGF Receptor Purification

While current methods to purify the insulin receptor are available, purification methods for the IGF-I receptor are just now being developed. Recently, we have developed methodology to fully purify large quantities of the IGF-II receptor (21). The membrane receptor for insulin-like growth factor II (IGF-II) has been purified to near homogeneity from rat placenta by chromatography of crude plasma membranes solubilized in Triton X-100 on agarose-immobilized IGF-II. Elution of the IGF-II receptor from the matrix at pH 5.0 in the presence of 1.5M NaCl resulted in a receptor purification of 1100 fold from isolated plasma membranes, or 340 fold from the Triton extract, with an average yield of about 50% in five separate purifications. Analysis of ^{125}I-IGF-II binding to the solubilized receptor in the Triton extract and in purified form by the method of Scatchard demonstrated no change in receptor affinity (K_d=0.72nM). Sodium dodecyl sulfate electrophoresis of the purified receptor showed one major band at Mr=250,000 with only minor contamination. Affinity labeling of the receptor in isolated placenta membranes and in purified form using ^{125}I-IGF-II and the crosslinking agent disuccinimidyl suberate resulted in covalent labeling of only the Mr=250,000 band. Such labeling was abolished by unlabeled IGF-II but was unaffected by insulin, consistent with the previously reported specificity of IGF-II receptor (17). These results establish a one-step affinity method for the purification of the type II IGF receptor that is rapid and highly efficient.

Insulin Action on the IGF-II Receptor

The binding of IGF-II to its own receptor in rat adipocytes and hepatoma cells has been reported to be markedly stimulated by physiological concentrations of insulin, presumably acting through the insulin receptor (22,23). Incubation of intact rat adipocytes with physiological concentrations of insulin stimulates binding of insulin-like growth factor II (IGF-II) to its receptor by 3 to 10 fold. The effect is temperature and dose dependent, with 0.1nM insulin giving half-maximal stimulation. Scatchard analysis of IGF-II binding to intact adipocytes indicates that this effect is due to an apparent increase in receptor affinity, from K_d=63nM in the absence of insulin to K_d= 5.8nM in the presence of 10nM insulin, with no apparent change in the number of cell surface binding sites (220,000 per cell).

Scatchard analysis of ^{125}I-IGF-II binding to isolated membrane fractions demonstrated that all IGF-II receptors in plasma membranes and low density microsomes from control cells are converted during homogenization to the high affinity form (K_d=2-6nM) seen in insulin-treated intact adipocytes. No significant difference in affinity was observed between low density microsomes from control or insulin-treated cells. However, in apparent contrast to the results obtained in intact adipocytes, the number of binding sites is increased in the plasma membrane fraction from insulin-treated cells by an average of 60%, while the number of receptors is decreased by 40% in low density microsomes from insulin-treated cells compared to control cells. These results were confirmed by direct visualization of the Mr=270,000 IGF-II receptor band on dodecyl sulfate gels following affinity labeling with ^{125}I-IGF-II and the crosslinker disuccinimidyl suberate. Scatchard analysis of the total cellular membranes showed no difference in the total number of binding sites between control and insulin-treated cells.

These results demonstrate that insulin has two effects on the IGF-II receptor in adipocytes: (1) it rapidly increases the apparent affinity of the receptor in the intact

cell without changing the apparent number of receptors on the cell surface; and (2) it induces a redistribution of the high affinity IGF-II receptor between plasma membranes and low density microsomes upon homogenization of cells and preparation of membranes. The latter effect closely parallels the insulin-induced membrane redistribution of the glucose transporter that occurs in the rat adipocyte by an unknown mechanism.

The Insulin Receptor as a Protein Kinase

A recent discovery by Kasuga and co-worker (24,25) showed that insulin stimulated the incorporation of ^{32}P into the β subunit of its own receptor in cultured human lymphocytes and rat hepatoma cells. Subsequent studies have shown that insulin also increased the phosphorylation of the insulin receptor in rat adipocytes (26), human placenta (27-29), 3T3-L1 adipocytes (27) and rat adipocytes (30). In cell free systems, the interaction of insulin with its receptor in the presence of γ-^{32}P-labeled ATP also resulted in increased phosphorylation of the insulin receptor (26-35). The increased phosphorylation of the insulin receptor in insulin-treated cells was predominately due to an increase in the level of phosphoserine with the simultaneous appearance of phosphotyrosine (5), whereas the increase by insulin added to detergent extracts of membranes or cells was primarily due to phosphotyrosine (27,29,31,33).

Petruzzelli and co-workers (27) demonstrated that insulin activated a tyrosine-specific protein kinase in Triton X-100 extracts of 3T3-L1 adipocytes and human placenta. The insulin-activated tryosine specific kinase was capable of phosphorylating added subtrates such as histone and casein. The insulin-stimulated tyrosine specific kinase co-purified with the insulin receptor until the final elution from insulin-agarose with urea at pH 6. It is not clear from these studies whether the loss of the kinase activity was due to the denaturing effect of the harsh conditions used to elute the insulin receptor or to the loss of a distinct kinase that does not absorb to the insulin-agarose matrix. Thus a critical question is whether the kinase activity is intrinsic to the insulin receptor structure in a manner analogous to the EGF receptor (36) or is associated with a distinct protein.

This hypothesis that the insulin receptor complex contains intrinsic protein kinase activity was tested by assaying in our laboratory the receptor, specifically immobilized on insulin-agarose, for enzyme activity. Human placental membranes solubilized in Triton X-100 were absorbed with wheat germ agglutinin-agarose and eluted with N-acetylglucosamine. The eluent was incubated with insulin-agarose in the absence and presence of free native insulin which blocks receptor absorption to the immobilized insulin. After absorption the insulin-agarose preparations were incubated at 24° with [γ-^{32}P] ATP and type II as histone followed by boiling in sodium dodecyl sulfate and analysis on polyacrylamide gels. Five endogenous proteins of Mr=180,000, 130,000, 93,000, 53,000 and 45,000 as well as the added histone incorporated ^{32}P to a much greater extent in the reaction mixture containing insulin agarose that had absorbed membrane extract in the absence of free insulin. The Mr=93,000 protein corresponds to the β subunit of the insulin receptor, while the other endogenous proteins are contaminants absorbed non-specifically to the insulin-agarose. This conclusion was documented by: (1) inhibition of absorption of the β subunit was specific for insulin, while desoctapeptide insulin only partially inhibited and cytochrome c was without effect, (2) little or no β subunit was absorbed when the experiment was performed with boiled insulin agarose, (3) absorption of the extracts with boiled insulin-agarose prior to absorption with insulin-agarose resulted in a greatly reduced background of absorbed Coomassie blue stained bands, while the β subunit was the major labeled protein absorbed under these conditions. The receptor-mediated increase in phosphorylation of the Mr=180,000 band, the β subunit of the insulin receptor and histone was due to the increase in phosphotyrosine content. We conclude that the insulin receptor itself or a protein that remains bound to the receptor in Triton X-100 solution contains intrinsic protein kinase activity.

Experiments in progress in our laboratory and others are designed to test whether the IGF receptors may also be associated with protein kinase activity. The key question related to the insulin receptor kinase activity is its possible relevance to biological signalling or other receptor functions. This question is under intensive investigation.

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LIPOPROTEIN RECEPTORS AND THEIR ROLE IN CHOLESTEROL METABOLISM

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I. Plasma Lipoproteins

Plasma lipoproteins transport triglycerides, cholesterol, and phospholipids to and from various tissues of the body. The major classes of lipoproteins, along with their functions and properties, are described in Table 1. Much attention has been focused on the protein constituents of lipoproteins, referred to as apoproteins, because of their central role in regulating the metabolism of lipoproteins. There are nearly a dozen different apoproteins. The major ones are shown in Fig. 1. Two of these proteins, apoproteins B and E, mediate the specific interaction of lipoproteins with various lipoprotein receptors on cells.

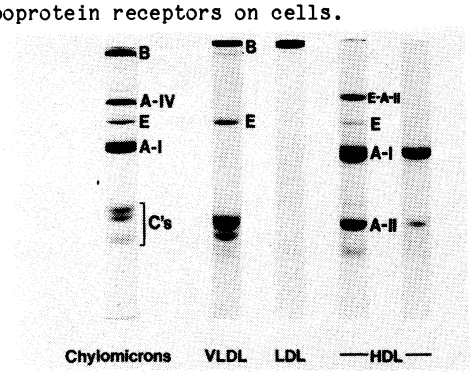

Figure 1. The apoproteins of the major classes of lipoproteins separated on SDS-polyacrylamide gels (from Ref. 8).

II. Lipoprotein Receptors

There are several different lipoprotein receptors on cells that have thus far been described. The most extensively characterized receptor is the LDL (apo-B,E) receptor, which was initially described by Goldstein and Brown (1,2). Their classic work on the subject defined familial hypercholesterolemia as a condition in which LDL receptors are defective or absent, and highlighted the importance of specific cell surface receptors in the regulation of lipoprotein metabolism. These receptors are expressed in several tissues, both hepatic and extrahepatic.

A second major lipoprotein receptor appears to be present only in the liver. This receptor, referred to as the apo-E or chylomicron remnant receptor, has been shown to mediate the uptake of cholesteol-rich chylomicron remnants and certain other apo-E-containing lipoproteins, but not the apo-B-containing LDL (2,3,4). The liver is the organ of paramount importance in regulating total body cholesterol homeostasis. Cholesterol is primarily eliminated from the body via the liver through the bile.

Lipoprotein receptors are also present on macrophages (5,6,7). It is now appparent that macrophages have receptors, or high affinity binding sites, for two different types of lipoproteins: 1) cholesterol-rich β-VLDL of patients with type III hyperlipoproteinemia and β-VLDL (chylomicron remnants) induced by diets high in fat and cholesterol, and 2) negatively charged LDL that can be generated by a chemical modification of its apoprotein constituent. The macrophage receptors may be of particular importance, in that macrophages are one of the progenitors of arterial wall foam cells that occur in atherosclerotic lesions.

A. LDL (Apo-B,E) Receptors

The LDL (apo-B,E) receptor has been described in several reviews (1,2,8). The general properties of apo-B,E receptors are compiled in Tables 2 and 3. In addition, Fig. 2 shows

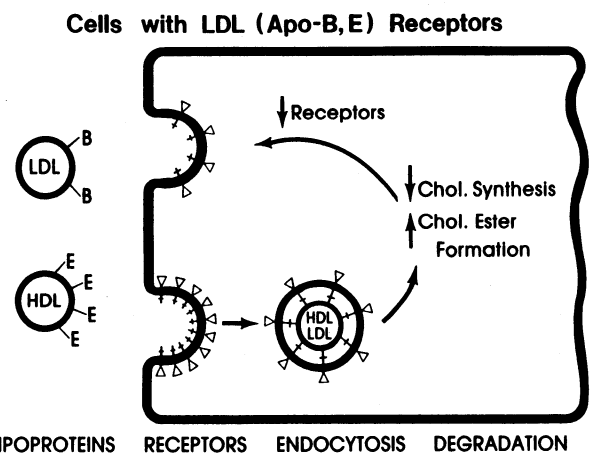

Figure 2. Diagram of a cell possessing apo-B,E (LDL) receptors, e.g., fibroblasts, smooth muscle cells, etc. The apo-B-E-containing lipoproteins can interact with these specific receptors, initiating internalization and degradation of the lipoproteins and regulation of intracellular cholesterol metabolism. (From Mahley, Arch. Pathol. in press.)

how lipoproteins binding to these receptors in coated pits are internalized and degraded in the lysosomes. Three intracellular processes are initiated by this internalization process: 1) suppression of HMG CoA reductase, the rate-limiting step in cholesterol biosynthesis, 2) stimulation of ACAT for cholesterol esterification and intracellular storage of cholesterol and 3) the down-regulation of the expression of the apo-B,E receptors.

Initial studies demonstrated that only LDL containing apo-B interact with these receptors. However, it is now known that apo-E containing lipoproteins, such as HDL-with apo-E, also interact with apo-B,E receptors (8). The HDL-without apo-E do not bind to these apo-B,E receptors. It is now understood that apo-B and apo-E are the determinants responsible for the receptor interaction (hence, the receptor is referred to as the apo-B,E receptor).

The importance of these apoproteins in the interaction between lipoproteins and apo-B,E receptors was established by selective chemical modification of specific amino acid residues. The chemical modification of a limited number of arginyl residues by 1,2-cyclohexanedione, and of lysyl residues by acetoacetylation, reductive methylation, and carbamylation, not only established the importance of the apoproteins, but also demonstrated the significance of these particular amino acid residues in the recognition process. The modification of relatively few arginyl or lysyl residues totally prevents LDL or HDL-with apo-E from binding to cell surface receptors on fibroblasts (8).

The interaction of apo-B- and apo-E-containing lipoproteins with apo-B,E receptors is identical except for one important property. It has been established that HDL-with apo-E (apo-E HDL_c) bind to apo-B,E receptors of fibroblasts with a much higher affinity (20 to 25 times higher) than that observed for the binding of LDL. The equilibrium constant (K_d) for HDL_c and LDL binding are $\sim 0.1 \times 10^{-9}$ M and $\sim 2.8 \times 10^{-9}$ M, respectively. In addition, 4 times more LDL particles are required to saturate all available apo-B,E receptor sites on fibroblasts. In a typical study, it requires 90,000 to 100,000 LDL particles to saturate the receptors, as compared to 25,000 HDL_c particles (a 4:1 ratio). The LDL and HDL_c are very similar in chemical composition and particle size. Based on these and other observations, it has been proposed that the apo-B,E receptor posesses multiple binding sites (shown schematically in Fig. 3). This model is compatible with the higher affinity of HDL_c binding and the decreased number of HDL_c particles necessary to saturate all the binding sites. The simplest model would predict that one HDL_c particle binds to four binding sites and that each LDL particle binds to a single site (8).

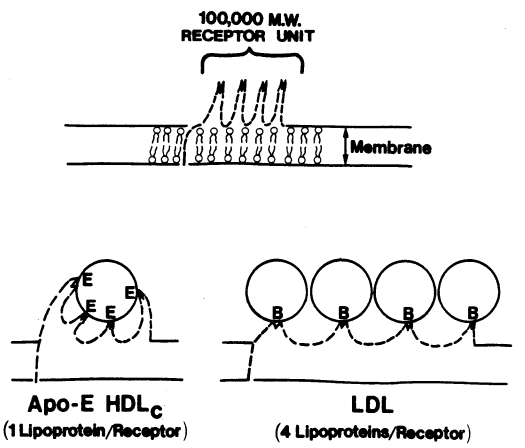

Figure 3. Schematic diagram showing hypothetical structure of the apo-B,E receptor unit with multiple binding sittes. The M_r 100,000 to 110,000 M_r receptor unit inserted in the cell membrane is capable of binding either one apo-E HDL_c particle, with its four binding sites, or four individual LDL particles (from Ref. 9).

The multiple binding site model proposed for apo-B,E receptors has been backed up by studies using the radiation inactivation technique (8,9). These studies have demonstrated that the receptor complex binding either the one LDL particle or 4 HDL_c particles is a single binding unit with an average functional molecular weight of $\sim$110,000 daltons. The purified LDL (apo-B,E) receptor has been shown to have an apparent molecular weight of 164,000. In a series of eloquent studies, it was demonstrated that the apo-B,E receptor is actually synthesized as a precursor protein molecule of 110,000 to 120,000 daltons and that it possesses lipoprotein binding activity (10,11,12). Subsequently, the receptor molecule is processed (apparently by posttranslational glycosylation) into a mature form with a molecular weight of $\sim$160,000 daltons. The functional molecular weight determined by radiation inactivation agrees quite closely with the molecular weight of the precursor form of the receptor.

Detailed analyses of the structure of the 34,000 dalton apo-E molecule, and of several mutant forms of apo-E that have been shown to be defective in receptor binding, have resulted in the identification of the binding domain for this ligand (8,13,14). As shown in Fig. 4, the binding domain is the region near the center of the molecule, which is enriched in lysyl and arginyl residues. Naturally occurring mutants, resulting from single amino acid substitutions involving lysyl and arginyl residues occur at residues 142, 145, 146, and 158. These mutants are variably defective in receptor binding activity and have been associated with type III hyperlipoproteinemia. In addition, studies using chemically and enzymatically cleaved

fragments of apo-E along with apo-E monoclonal antibodies, have defined the receptor binding region as being in the vicinity of residues 140-150.

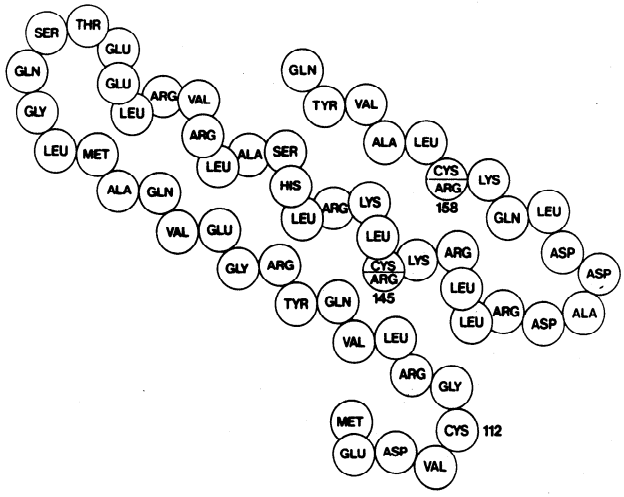

Figure 4. A portion of the apo-E sequence from the region of the apo-E molecule determined to be involved in receptor binding. Two sites of substitution know to affect binding are at residues 145 (W.M. apo-E2) and 158 (D.R. apo-E2) (from Ref. 16).

B. Hepatic Lipoprotein Receptors (Apo-B,E Receptors and Apo-E Receptors)

The liver possesses at least two different high affinity receptors, the apo-B,E receptor and the apo-E receptor (2,3,4). The LDL (apo-B,E) receptor in the liver appears to be very similar to the apo-B,E receptor in extrahepatic tissues, ie., they are both capable of interacting with apo-B-and apo-E-containing lipoproteins. However, the second hepatic receptor, the apo-E receptor, is unique to the liver and interacts with apo-E-containing HDL and chylomicron remnants but not with LDL. The ligand mediating the interaction between lipoproteins and this receptor is apo-E. The apo-E receptor has been described in the livers of dogs, swine, baboons, and man (3,4,8). (For summary see Table 3.)

Regulation of the hepatic apo-B,E and apo-E receptors appears to be under independent contol (8,15). The apo-B,E receptors are prominent in the livers of immature, growing animals, but are expressed at low levels in adults. Furthermore, the apo-B,E receptors are markedly - and in some cases very rapidly - down-regulated. Cholesterol feeding in animals, or the infusion of lymph lipoproteins or bile salts, suppress the expression of this receptor to almost undetectable levels. However, treatment of animals with cholestyramine (a bile-acid binding resin) or estrogen results in a marked induction of the expression of hepatic apo-B,E receptors. Under all these conditions, which markedly modulate the expression of the apo-B,E receptors, the expression of the apo-E receptors remains at a relatively constant level. This observation is compatible with its proposed role in lipoprotein metabolism.

It appears that the apo-E receptor functions to clear chylomicron remnants from the plasma (8). In addition, the apo-E receptor (also referred to as the chylomicron remnant receptor) seems to be genetically distinct from the apo-B,E receptor. Patients with familial hypercholesterolemia, who lack apo-B,E receptors or have defective receptors, do not have difficulty clearing chylomicron remnants from their plasma. This reflects the apparent normal activity of the apo-E receptor in these subjects. Chylomicron remnants (β-VLDL) which possess an abnormal form of apo-E, accumulate in the plasma of patients with type III hyperlipoproteinemia. This accumulation reflects the defective receptor binding activity of the abnormal apo-E in these subjects.

C. Macrophage Receptors

Macrophages are capable of taking up two distinctly different types of lipoproteins by high affinity processes, including β-VLDL and chemically modified LDL (8). (For summary see Table 3.) The only naturally occurring plasma lipoprotein that these cells take up are β-VLDL. These lipoproteins occur in the plasma of patients with type III hyperlipoproteinemia (a genetic disorder characterized by hypercholesterolemia and accelerated vascular disease) and in animals fed diets high in fat and cholesterol. The β-VLDL occuring under these conditions are at least partly derived from cholesterol-enriched chylomicron remnants.

The β-VLDL receptor on macrophages is distinct from other lipoprotein receptors (8). Unlike the apo-B,E receptor, it is poorly regulated, and internalization of β-VLDL results in excessive cholesteryl ester uptake and accumulation. The cholesteryl ester content of macrophages can increase 100- to 200-fold following incubation with β-VLDL from cholesterol-fed dogs, rats, rabbits, or monkeys. The β-VLDL from type III hyperlipoproteinemic subjects also cause excessive cholesteryl accumulation in macrophages. The uptake is mediated by the β-VLDL receptor.

Chemical modification of LDL by a variety of procedures, including acetylation, acetoacetylation, and malondialdehyde treatment, causes these lipoproteins to be taken up by macrophages via a high affinity process (5,6,7). The high affinity binding sites are clearly distinct from the β-VLDL receptors, apo-B,E receptors, or apo-E receptors described above. The uptake is unregulated and results in massive intracellular cholesteryl ester accumulation.

The uptake of β-VLDL and chemically modified LDL is of potential physiological significance in the development of atherosclerosis. Macrophages are one of the cell types within the arterial wall that accumulate cholesterol. The observation that

diet-induced β-VLDL can cause excessive cholesteryl ester accumulation in these cells may represent a direct link between diets high in fat and cholesterol and accelerated atherogenesis. Furthermore, the association of β-VLDL in the plasma of type III patients with accelerated atherosclerosis may reflect the propensity of these lipoproteins to deliver cholesterol to macrophages. In addition, it has been postulated that LDL may become chemically modified as they circulate in the plasma or perfuse the extracellular space of various tissues and that these modifications allow their uptake by macrophages, including those of the arterial wall. Foam cells within atherosclerotic lesions have been shown to possess receptors for β-VLDL and chemically modified LDL (8).

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TABLE 1
HUMAN PLASMA LIPOPROTEINS

			Physical and Chemical Characteristics				
	Origin	Major Function(s)	Density of Flotation	Electrophoretic Mobility	Particle Size	Major Lipids	Major Proteins
Chylomicrons	Intestine	Transport triglyceride and cholesterol from intestine to plasma.	d<1.006	Origin	>2000Å	Triglycerides	B,E,A-I, A-IV, C
Chylomicron Remnants	Derived from chylos following lipolysis	Transport cholesterol to the liver (uptake mediated by apo-E receptors.)	d<1.006	Origin to β	>1000Å	Cholesterol	B,E
Very Low Density Lipoproteins (VLDL)	Liver	Redistribution of triglyceride from liver to various tissues utilizing fatty acids.	d<1.006	Pre-β	300-900Å	Triglycerides	B,E,C
Low Density Lipoproteins (LDL)	Derived from VLDL following lipolysis	Provide cholesterol to various tissues for: 1. membrane biosynthesis 2. steroid hormone production. Uptake mediated via apo-B,E receptors.	d=1.006-1.063	β	~200Å	Cholesterol	B

TABLE 1 (cont)
HUMAN PLASMA LIPOPROTEINS

	Origin	Major Function(s)	Physical and Chemical Characteristics: Density of Flotation	Electrophoretic Mobility	Particle Size	Major Lipids	Major Proteins
High Density Lipoproteins (HDL)							
I. HDL-without apo-E	Liver, intestine ?	Reverse cholesterol transport (acquisition of chol. from various tissues)	d=1.063-1.21	α_1	80-100A	Phospholipid	A-I, A-II, C
II. HDL-with apo-E	Apparently derived from HDL-without apo-E by acquisition of cholesterol & apo-E	Redistribution of cholesterol to hepatic and extrahepatic tissues mediated via apo-B,E and apo-E receptors.	d=1.063-1.21	α_1, α_2	100-150A	Phospholipid, Cholesterol	E, A-I, A-II, C

TABLE 2

PROPERTIES OF THE LDL (APO-B,E) RECEPTORS

Localization:	Fibroblasts, smooth muscle cells, leukocytes, adrenal cortex, testes, ovaries, liver
Protein determinants for binding:	Apo-B, apo-E
Binding specificity:	LDL HDL-with apo-E, including HDL_C Chylomicron remnants VLDL (especially hypertriglyceridemic VLDL) Lp(a)
Binding affinity (K_d):	LDL = 2.8×10^{-9}; HDL_C = 0.1×10^{-9}
Receptors per cell (fibroblasts):	50,000 to 100,000
Entry of LDL into cell (fibroblasts):	$t_{1/2} \cong 5$ minutes
Turnover of receptors (fibroblasts):	$t_{1/2} \cong 20$ hours
Molecular weight of receptors:	Isolated and purified (adrenal) — 164,000 daltons By radiation inactivation (fibroblasts) — 106,000 daltons
Enzymatic sensitivity:	Pronase, trypsin, pepsin
Binding characteristics:	a) Requires divalent cations b) Abolished by chemical modification of lysine and arginine residues of apo-B and apo-E

TABLE 3

SUMMARY OF LIPOPROTEIN RECEPTORS

Receptors	Cells or Tissues	Lipoproteins Bound	Ligands Involved	Receptor Regulation	Functional Roles
LDL (apo-B,E)	Fibroblasts Smooth muscle cells Liver Adrenal cortex Ovaries Testes Adipocytes Lymphocytes Macrophage-monocytes	LDL HDL-with apo-E (HDL_C) VLDL Chylomicron remnants Lp(a) β-VLDL (cholesterol induced)	Apo-B Apo-E	Regulated by delivery of lipoprotein cholesterol	Regulation of LDL levels. Redistribution of cholesterol by apo-B and apo-E lipoproproteins to various tissues. Cholesterol utilized for membrane or hormone production.
Apo-E (Chylo remnant)	Liver	Chylo-remnants HDL-with apo-E (HDL_C)	Apo-E	Not subject to marked down-regulation	Uptake of chylomicron remnants and cholesterol-loaded HDL-with apo-E. Delivery of cholesterol to the liver for excretion.
β-VLDL	Macrophage	β-VLDL (from Type III subjects and induced by cholesterol)	?	Poorly regulated	Potential role in foam cell production in atherogenesis. Uptake of diet-induced lipoproteins (link between diet and atherosclerosis).
Chemically-modified lipoprotein	Macrophage	Modified LDL	Charge-modified apo-B	Not regulated	Potential role in foam cell production in atherogenesis.

CALCIUM CHANNEL INHIBITORS

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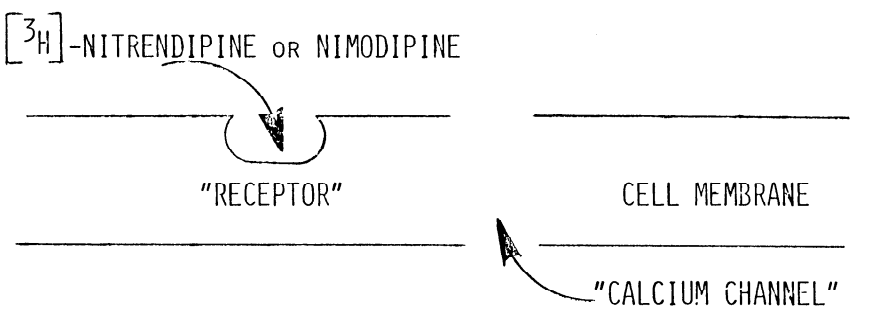
University of Cincinnati College of Medicine

The calcium antagonists, verapamil (V), nifedipine (N), and diltiazem (D), or slow channel inhibitors, are extremely interesting and important new therapeutic agents for the treatment of cardiovascular diseases. The diverse chemical structures of the calcium antagonists do not permit the assignment of a specific receptor or a structure-activity relationship.

D,N, and V shorten the Action Potential Duration 50 (APD 50), but V also lengthens the APD 50. In the doses used to dilate the coronaries, D and V also lengthens the APD 50. In the doses used to dilate the coronaries, D and V prolong A-H interval, but N has no effect. All three produce negative inotropy on isolated ventricular, atrial and Purkinje tissue, with a potency $N^{o}V>D$. D has a much greater effect on coronaries (particularly collaterals) than it does on the peripheral vascular system or veins, while both N and V are very potent peripheral vasodilators. In conscious animals, equipotent doses of D,N and V that lower blood pressure by about 15μmHg, result in increase in LV dp/dt by N, decrease by V and no change by D. D markedly protects ischemic myocardium by dilating coronaries and preventing calcium-induced damage to the gap junctions and to mitochondria. The calcium antagonists protect the heart and relieve angina by affecting the "supply" side to a greater extent than they affect "demand". H^{3}-nitrendipine (NTD) and nimodipine (NMD) bind specifically to cell membranes and perhaps junctional SR. Kinetics and pharmacologic correlates will be discussed.

HOW DO THE CALCIUM ANTAGONISTS WORK? IS THERE A SPECIFIC SINGLE RECEPTOR AND/OR CHANNEL OR ARE THERE MULTIPLE SITES?



Binding parameters of nitrendipine and of plasma membrane markers to membranes from pig coronary arteries and dog ventricular muscle. The dissociation constant (K_D) and the maximal binding (B_{max}) were estimated from Scatchard plots by linear regression analysis, in several membrane preparations (N). S.E.M. is indicated where relevant.

Tissue	[^{3}H]Ligand	Temperature (°C)	N	K_D (nM)	B_{max} (fmol/mg)
Pig coronary arteries (microsomes)	Nitrendipine	37	3	1.6 ± 0.5	35 ± 2
	Dihydroalprenolol	30	3	0.7 ± 0.3	40 ± 8
	Quinuclidinyl benzoate	37	2	1.4 ± 0.9	80 ± 7
Dog ventricular muscle (sarcolemma)	Nitrendipine	30	3	0.11 ± 0.01	230 ± 10
	Dihydroalprenolol	30	3	6.5 ± 0.9	2,700 ± 640
	Quinuclidinyl benzoate	37	1	0.15	21,000
	Ouabain	37	3	32 ± 6	370,000 ± 34000

DISPLACEMENT OF [^{3}H] -NITRENDIPINE

BINDING TO PIG CORONARY ARTERIES

LABEL CONCENTRATION: 0.3 nM

DRUG	CONCENTRATION	% BINDING
NITRENDIPINE	10^{-9}M	51
NIFEDIPINE	10^{-8}M	43
DILTIAZEM	10^{-7}M	118*
	10^{-6}M	152*
VERAPAMIL	10^{-7}M	64
	10^{-6}M	37
D600	10^{-7}M	51
	10^{-6}M	52

*STIMULATION

Stimulation by diltiazem of [^{3}H]nimodipine binding to cardiac membranes. The lower panel shows a typical experiment (triplicate determinations); the specific binding was analyzed according to Scatchard (Insert). Upper panel: effect of diltiazem expressed as percentage of control specific binding in 4 to 13 different preparations (± S.E.M.).

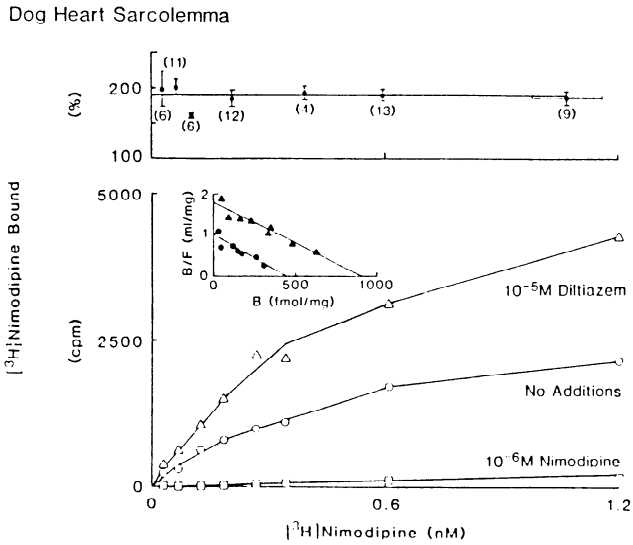


Figure 1

Effect of d-cis-diltiazem (0) and 1-cis-diltiazem (□) on [^{3}H]nitrendipine binding to microsomes from pig coronary arteries.

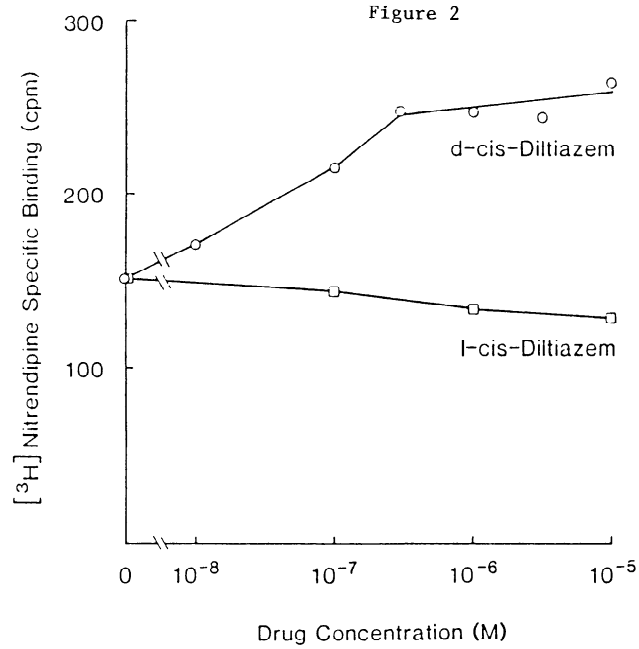


Figure 2

Drug-induced relaxation of depolarized pig coronary arteries. Cumulative doses of nitrendipine (Δ), d-cis-diltiazem (0) or 1-cis-diltiazem were (□) added to the incubation medium after maximum tension development in response to 35 mM KC1. The data represent the tissue response in percent of initial maximum active tension. Decay of the maximum active tension was observed in simultaneously paired control tissue rings exposed to carrier solution alone. Appropriate corrections were made. Each point represents 4-12 coronary rings (± S.E.M). In separate experiments, single doses of nitrendipine produced the same response at equilibrium as cumulative doses.

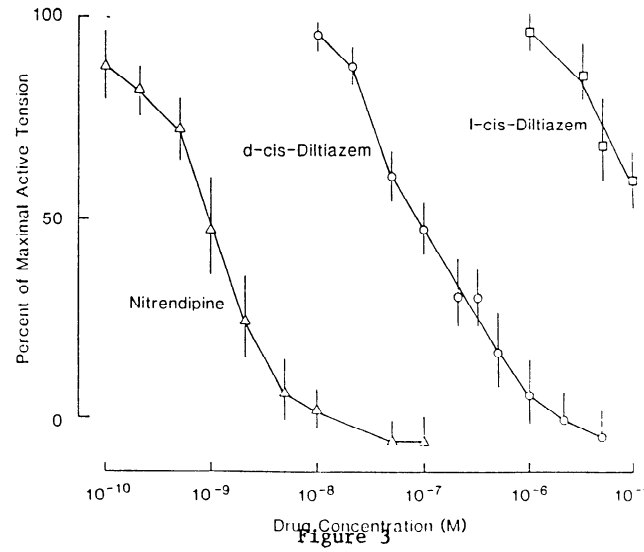


Figure 3

[^{3}H]nimodipine specific binding to isolated myocytes (washed cells). The data represent means (± S.E.M.) from experiments with 4 different myocytes preparations (1-2 x 10^4 cells per ml). Same conditions as described in the legends to Fig. 2. Inset: Scatchard plot.

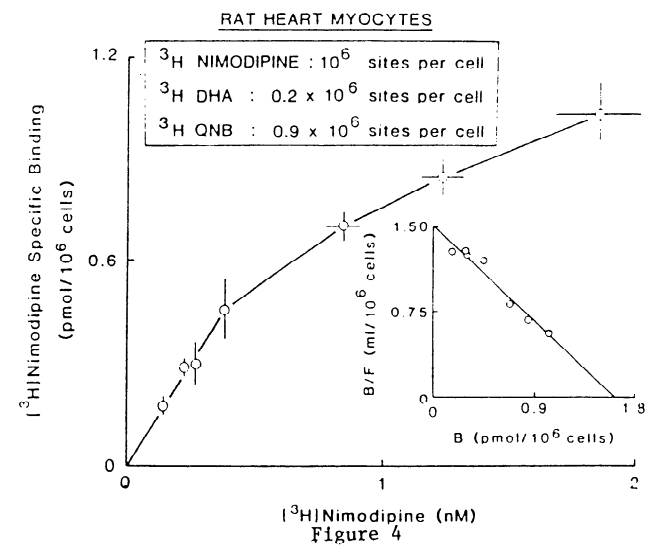


Figure 4

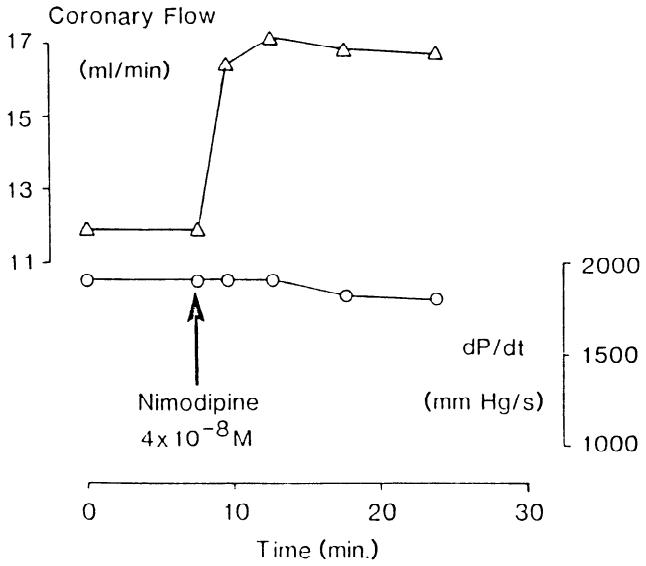


Figure 5

NORMAL VENTRICULAR ACTION POTENTIAL
MONOPHASIC AND IONIC CHARACTERISTICS
AND ACTION OF Ca CHANNEL BLOCKERS

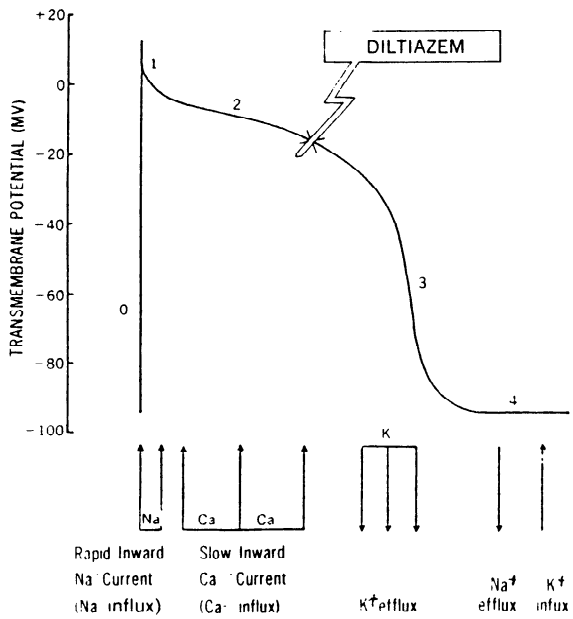


Figure 6

NORMOXIC

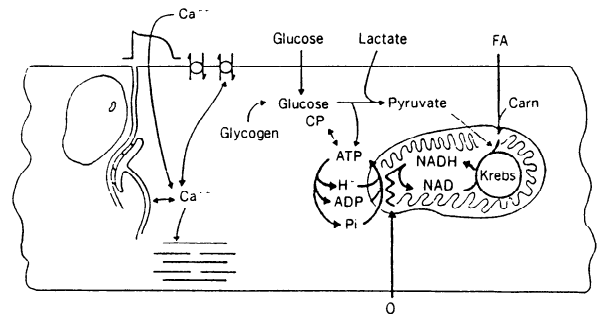


Figure 7

EARLY ISCHEMIC

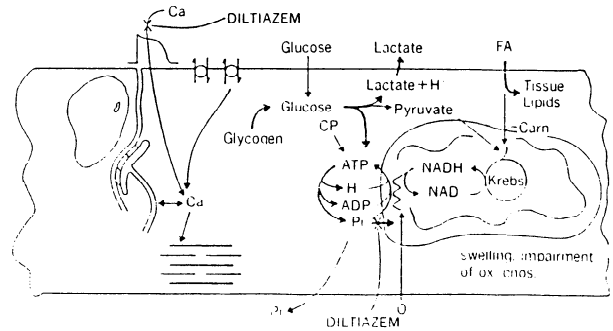


Figure 8

DILTIAZEM MAY LIMIT AMOUNTS OF CALCIUM ENTERING THE DISEASED HEART

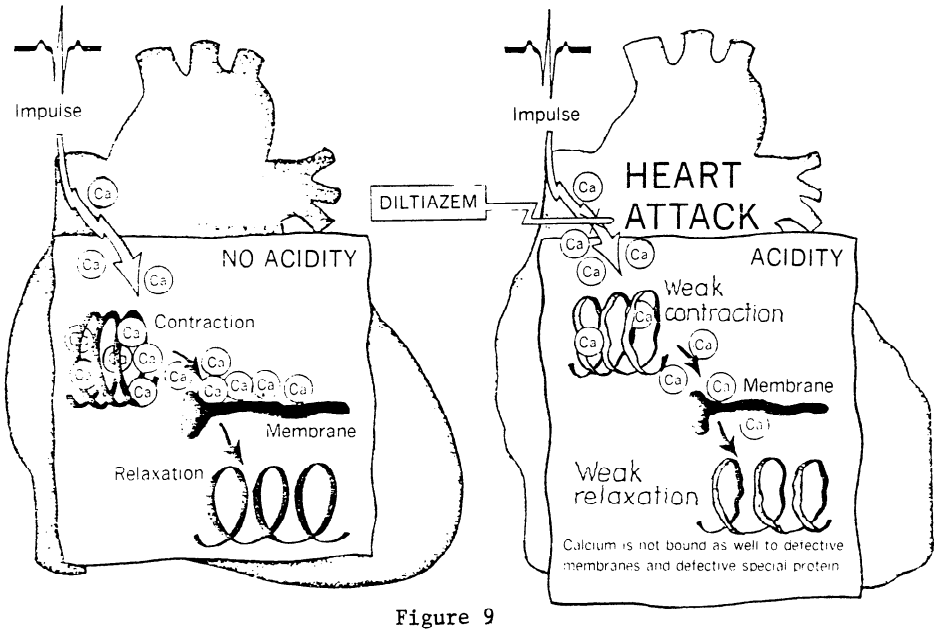
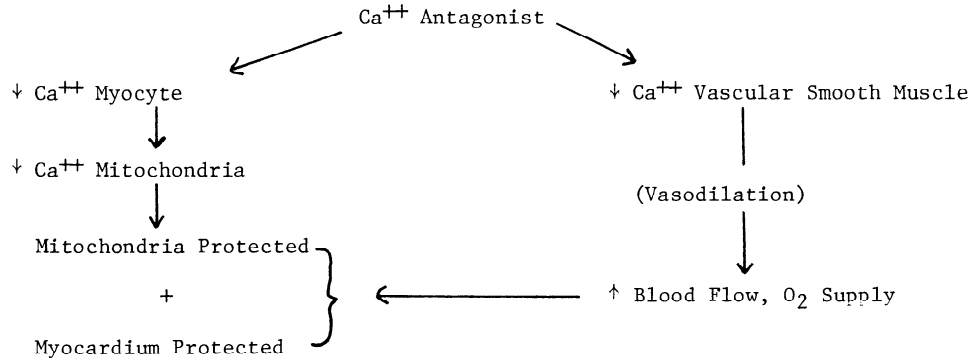


Figure 9

HOW MIGHT DILTIAZEM PROTECT?



Ca^{2+} REGULATION IN VASCULAR SMOOTH MUSCLE

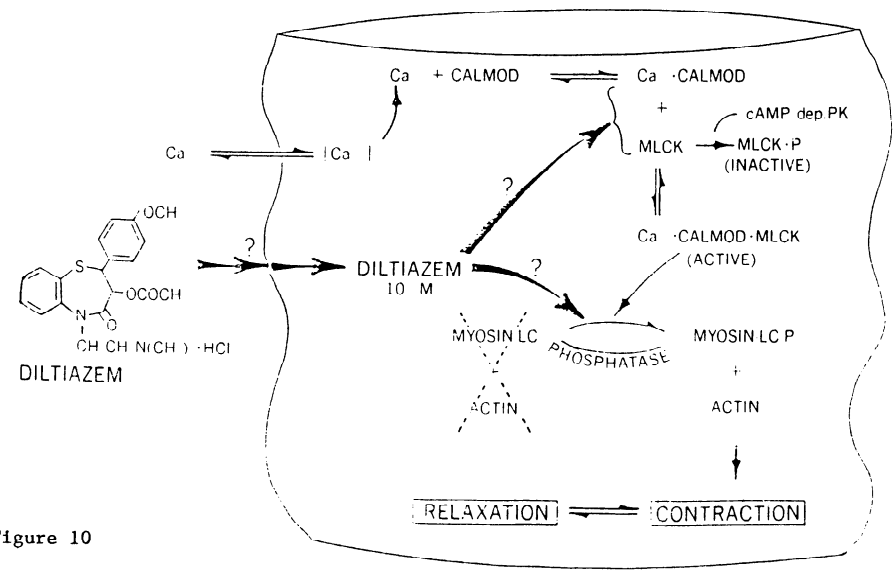


Figure 10

Cardiac Muscle

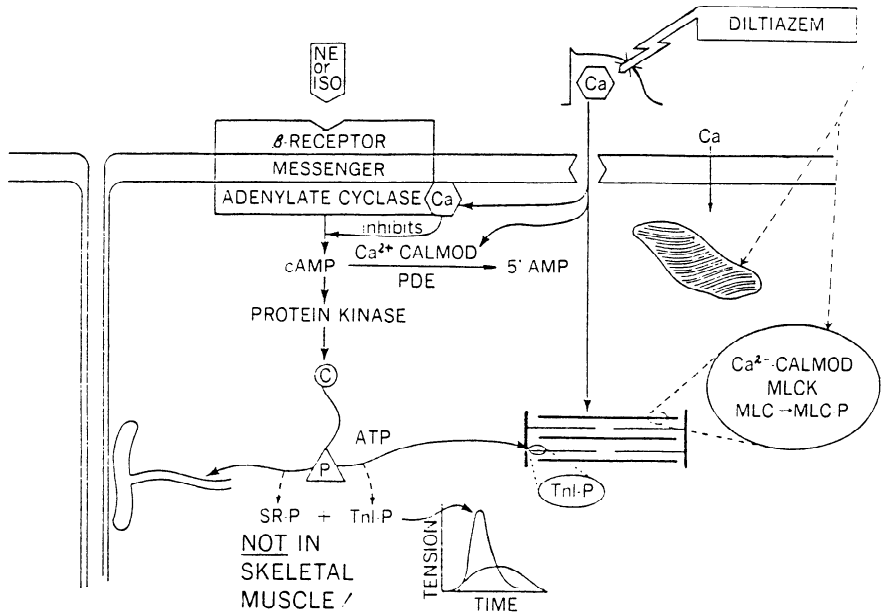


Figure 11

Proceedings of the Symposium Teaching Cardiovascular Physiology Outside the Lecture Hall

The 33rd Annual Fall Meeting of the
American Physiological Society
with
The Latin American Association of
Physiological Sciences

October 12, 1982
San Diego, California

Edited by
Joel A. Michael
Allen A. Rovick

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Teaching Cardiovascular Physiology Outside the Lecture Hall

Introduction

The easiest and least expensive way to teach physiology (or most other disciplines) is to hire a small number of lecturers, fill a hall with students, and let each lecturer "do his thing". If the lecturers are skilled their presentations will be logically organized, well illustrated and interestingly presented. Lectures are an efficient method of teaching basic physiology. In fact, this is how most of physiology is now taught.

However, the plethora of alternative techniques that have been developed, each of which takes the student and the faculty out of the lecture hall, clearly indicates that teachers think that something more is needed to properly educate students in physiology. Each of the non-lecture approaches is thought to do something that lectures don't do as well; something that is achieved by having students actively manipulate the facts, concepts and the anatomical substance that compose physiological systems; something that requires students to apply the ideas, components and relationships of physiology to solve problems. Alternative educational experiences help students to learn to solve qualitative problems (i.e. involving cause and effect relationships) as well as quantitative (numerical) problems.

In lectures it is difficult to convey many important features of physiological systems with verbal descriptions, conjured up thought experiments or mental images. Phenomena such as biological variation, sources of error, the stability and the mortality of living systems and the beauty that's revealed by the interaction of the components of a physiological system are examples of topics that "suffer" from presentation in a simple lecture format. Further, in the lecture hall, such topics are usually discussed piecemeal. Using other approaches, they may be experienced and directly appreciated and thus may be understood as a unified whole.

Students often have no difficulty in repeating the facts or relationships that they have heard in a lecture or read in a textbook. But, if faced with the need to explain the cause(s) of a particular state of an organism, they may not even know where or how to begin such an analysis. Exposure to similar challenges helps to develop in them the problem-solving "sense" that is needed.

Non-lecture exercises slow the frenetic pace that lecture series often assume. They give students time to think, to learn and to ask questions, while they give faculty time to explain and to guide. They reduce the student/teacher ratio and help to re-humanize the instructional process while guaranteeing that an essential ingredient in students' education, learning to use physiology, is not missing.

Alternative teaching techniques come in many guises. Each provides its own characteristic and often unique educational benefits. None alone supplements all of the weaknesses of lectures or, for that matter, is without deficiencies of its own. However, each makes a contribution toward the objective of a complete education in physiology.

We hope that the pluses and minuses of a number of non-lecture approaches will become clear in this volume. The techniques discussed are not the only ones now in use. The back issues of the Physiology Teacher contain many additional approaches. The papers assembled here deal with variations on traditional non-lecture teaching formats as well as some that are not so old.

The symposium was divided into three parts. First, there was a group of papers on laboratories. This started with a review of the results of the third national survey of laboratory teaching activities of departments of physiology in US medical schools (C. Rothe, published separately in The Physiologist 26, No. 3, 1983). This was followed by three novel approaches to "wet" labs. In the second part there were three papers on techniques for small group or individual instruction using clinical or pathophysiological problems. Finally, there was a group of papers on computer-based educational methods.

The papers are derived from a symposium that was held on October 12, 1982 at the combined Fall meeting of the American Physiological Society and the Latin American Physiological Society in San Diego, California. We organized the symposium at the suggestion of Arthur Vander, then Chairman of the Education Committee of APS and we chaired it along with W.T. Beraldo of Brazil.

Our sincere thanks to Glenda Keaton, who prepared all of the manuscripts and who typed endless versions of our papers.

Joel Michael
Allen Rovick

A MECHANICAL MODEL OF THE CARDIOVASCULAR SYSTEM FOR EFFECTIVE TEACHING

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A circulation model effectively teaches interrelationships. Live animal studies provide a better realization of the beauty and complexity of living systems than do models, but animals are becoming more and more expensive to purchase and to use in the laboratory. Computer simulation is more precise than either, but such models do not seem as real for the student as a physical model.

The model is shown in Fig. 1. Except for the annual replacement of the rubber tubing, little maintenance has been required. The students are warned to keep their fingers from being caught under the cam.

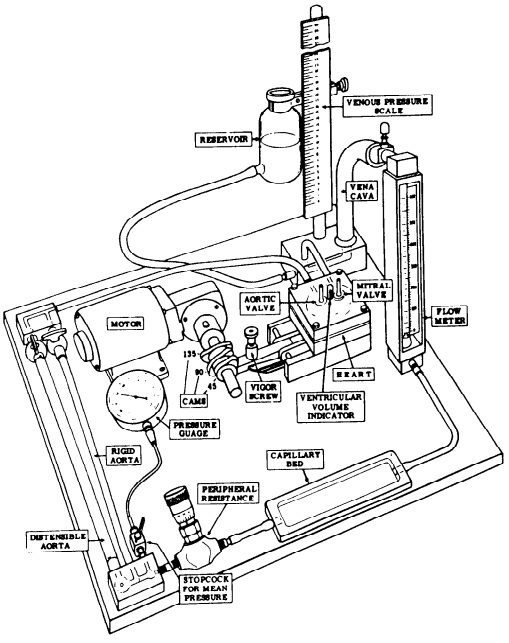

Figure 1. Model of cardiovascular system. (From Rothe, C.F., J. Appl. Physiol. 17: 156-158, 1962).

Important Features

Passive filling and dependence upon ventricular afterload are the most important features of the model. Because filling of the ventricle is dependent upon filling pressure (the height of the reservoir), the Frank-Starling relationship is simulated. Because the lever between the cam follower and the plunger can bend (Fig. 2), the amount of fluid pumped during the time available for systole depends upon the pressure load. The tension of the leaf spring is adjustable to mimic changes in vigor of contraction. Three heart rates (45,90,135 beats per minute) are available by moving a 1- to 3-lobe cam along the drive shaft.

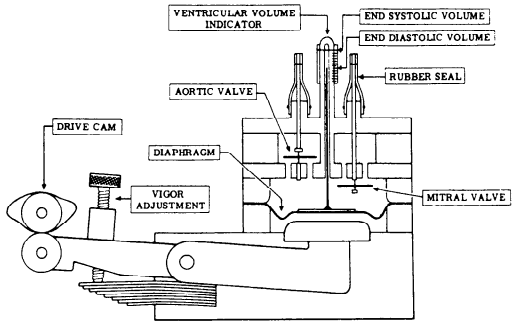

Figure 2. Cross-sectional diagram of ventricle showing diaphragm, valves and ventricular volume indicator. (From Rothe, C.F., J. Appl. Physiol. 17: 156-158, 1962).

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Aortic distensibility, simulated with a piece of 1/4 inch diameter thin-wall rubber tubing inside a piece of 3/8 inch diameter tubing, provides the student a tactile example of arterial pulsations. By switching to a piece of semi-rigid vinyl tubing, stiffening of the arterial bed reduces the mean flow and arterial pressure, because the ventricle is outflow-pressure dependent. The systolic arterial pressure does not increase appreciably, but the pulse pressure increases. No reflex or intrinsic compensatory mechanisms are represented in the model.

Ventricular volume changes are indicated by a plunger attached to a disk that rests on top of the ventricular diaphragm (Fig. 2). The plunger moves along a millimeter scale and the student estimates the minimum and maximum values ("end systolic" and "end diastolic" volumes). Zero is set by a rubber O-ring at the highest point (minimum end systolic volume) with the cam follower manually depressed far enough so that the cam tips just touch the cam follower. A movement of 1 mm is equivalent to about 1.5 ml of stroke volume. (The alternative method for calculating stroke volume is cardiac output divided by heart rate. Discrepancies provide the basis for some subsequent laboratory discussion.)

Valvular lesions may be studied because the valves, constructed from disks of stainless steel, can be held open to simulate insufficiency or held closed to simulate stenosis (Fig. 2).

Laboratory Exercise

Groups of 2-4 students spend about three hours with the model. A day or so later in laboratory conferences, groups of about 20 students spend another two hours discussing their data under the guidance of a faculty member.

We ask the student first to make qualitative observations of arterial pulsation, venous collapse, motion of valves and ventricular filling. By gently lifting up the plunger from the end of the "ventricle" opposite to the cam follower, the students observe how easily cardiac tamponade impedes filling and so cardiac output. The students then systematically vary the vigor of contraction, a peripheral resistance needle-valve, heart rate, and venous filling pressure. Flow, arterial pressure (mean, systolic and diastolic) and atrial filling pressure measurements provide the basis for calculation and understanding. Data manipulation involves plotting mean flow and the arterial pressures as functions of heart vigor, peripheral resistance, heart rate and venous filling pressure.

The students are expected to discover that with either inlet or outlet valve insufficiency, the actual stroke volume is large compared to the stroke volume calculated from flow and heart rate. They also should note that aortic valve problems are associated with a large heart (large end diastolic volumes), whereas with mitral (filling valve) problems, the heart tends to be small (small end systolic volume).

The effects of cardiac tamponade, arteriosclerosis, heart failure and hemorrhage are mimicked. The students are asked to consider possible reflex compensation or therapeutic interventions. They try various forms of compensation and then discuss limitations of the attempted compensation, as well as the reality of the model.

Medical and graduate students are also asked to interrelate the influence of venous pressure and heart rate on cardiac output. Data are plotted for the 3 heart rates, with venous pressure as the independent variable and flow as the dependent variable. They "discover" that with bradycardia, flow tends to plateau, and further increases in filling pressure have little influence because end diastolic volume is limited. With high heart rates, an increase in venous pressure has a marked influence on flow. At normal venous pressures, flow is greater at the normal heart rate than with either bradycardia (too few strokes per minute) or tachycardia (limitation due to filling time). On another graph, flow versus heart rate is plotted using the data from 3 different venous pressures. With high venous filling pressures, flow tends to be proportional to heart rate. At low filling pressure, heart rate, beyond a minimum, has little influence on flow.

The model described has been used by 3,000 medical, 2,000 dental, and several hundred graduate students at Indianapolis over the past twenty years. (It was described in J. Appl. Physiol. 17: 156-158, 1962). When asked to evaluate their teaching laboratory experiences, our students usually consider the exercise with the circulation model to have been the most effective. Copies have been constructed for use in over 30 other institutions throughout the world. Additional copies can be fabricated at reasonable cost in our machine shops.

In summary: Physical models provide an effective teaching tool with distinct advantages compared to lectures, reading, animal experiments, or computer-based aids. However, because they are only models, students must be warned of their limitations.

A SEPARATE COURSE FOR EXPERIMENTS IN CARDIOVASCULAR PHYSIOLOGY

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For the past decade, students at University of Texas Medical Branch, in Galveston, have enjoyed the benefits of several interdisciplinary courses in addition to the traditional basic science courses. The interdisciplinary courses were introduced by the Faculty of Medicine as part of an overall curricular revision. The objective of the interdisciplinary courses is to draw the various basic science courses together while reducing the student's total contact hours. The Integrated Functional Laboratory, together with endocrinology and neuroscience comprise the interdisciplinary courses.

Curricular Organization: The organization of the various courses in the curriculum is illustrated in Figure 1. The curriculum is built on a structure of four, 16 week blocks or terms. The Integrated Functional Laboratory is taught during the second and fourth terms. During the second term the course runs simultaneously with medical physiology which contains lectures on cardiovascular physiology

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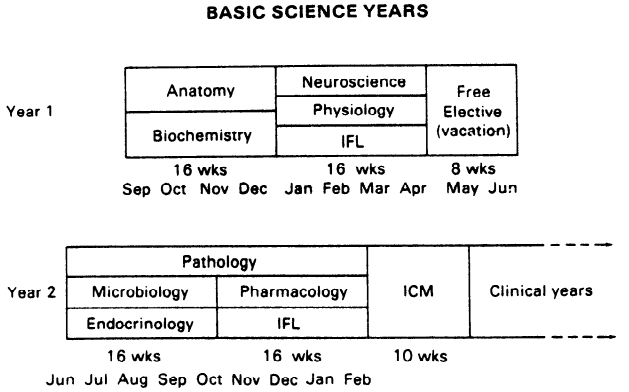

Figure 1. The Basic Science Years. This is a diagramtic representation of the various courses taught during the basic science portion of the curriculum at the University of Texas Medical Branch. The Integrated Functional Laboratory (IFL) is taught during the second and fourth term blocks. We begin teaching the sophomore class in October and the freshman class in January. There is an overlap of several weeks. We end the year in April when we teach the last classes to the freshmen.

and with the neuroscience course, in which the anatomy and the physiology of the autonomic nervous system were presented.

The second-half of the Integrated Functional Laboratory is taught simultaneously with pharmacology and the second-half of pathology. Both of these courses contain a great deal of information related to the function of the cardiovascular system. In addition, in the interim term (term three) students take the first-half of pathology and endocrinology and are introduced to the hormonal influences on the cardiovascular system as well as the factors in the immune system which affect cardiovascular function and other cardiovascular pathology. It is advantageous to teach some cardiovascular physiology at the end of the basic science years since it offers the unique opportunity to integrate and reinforce the students' knowledge through review and problem solving experiences.

Course Organization: The course is organized by an interdisciplinary committee appointed by the Dean of Medicine and chaired by the director of the interdisciplinary laboratories. The committee solicits and evaluates experiments for use in the laboratory, determining placement of experiments in sequence in the overall arrangement of the course and oversees the grading of the students. The day to day operation is handled by the director and his staff.

The laboratory is usually taught to well over 200 students as there are 203 Medical Students and Graduate Students also take the course. For laboratory, the class is divided into two sections, with half of the class taking laboratory on one day and half on another. Each of these sections is then divided into six groups. Each of these groups would eventually then be doing the same experience, be in the same room, and have the same instructor. (An explanatory note is necessary. Associated with each experiment is a conference period in which the experimental results are discussed. We have adopted the term "experience" to pertain to the combination of experiment and conference.) Each of the groups of sixteen to eighteen students is divided into four teams of four or five students. Each team would thus operate with the same animal model or other laboratory experience. Each member of these teams is designated as team leader on a rotating basis.

The manner in which the experiments are taught is somewhat different in our course compared to traditional laboratory courses. Rather than have the entire class do the same experiment during a given laboratory period we usually have six experiences being taught simultaneously. Each group of students then rotates from experience to experience on a weekly basis. At the end of six weeks all the students have completed all of the experiments. At this time six new experiments are then instituted and the rotations begin once more.

The teaching of the laboratory in this way allows us to make better use of faculty expertise and equipment. If we have only one person sufficiently knowledgeable in a given area to conduct laboratories on that topic he may teach all 12 laboratory experiences over 6 weeks. With two authorities, each would conduct six experiments which could either run serially over six weeks or could be run simultaneously so that all students rotated through the experiment in only three weeks. The same principle applies to equipment. Where adequate quantities of equipment are available, experiments may be "double-up". There are 24 laboratory exercises: 12 experiments in term two and 12 in term four. The term two experiments are listed in Figure 2; the cardiovascular experiments performed in this term are underlined.

TERM II EXPERIMENTS

Compound action potential
Spinal cord reflex
Erythrocyte glucose-6 phosphate dehydrogenase deficiency
Hepatic necrosis and biliary obstruction
Cardiac muscle
Events in the cardiac cycle
Intestinal secretion and absorption
Hemorrhagic shock
Human pulmonary function
Acid base balance
Genetic counseling
Neuromuscular transmission

Figure 2. The Term II Experiments. We teach twelve laboratories in each term. During this term there are three cardiovascular laboratories. The titles are underlined; note also that the cardiovascular labs are taught at the same time as the laboratory on Acid Base Function.

* * * * * * * *

Term Two Experiments: One of the first experiments the freshman take is entitled The Events of the Cardiac Cycle. The objectives of this experiment are to acquaint the students with the equipment, working with living preparations and to illustrate cardiac principles presented in the physiology course. The experiment is performed using dogs which have been anesthesized with pentobarbital. Their left ventricles are catheterized via the common carotid artery. This trace is displayed on a cathode ray oscilloscope of a VR-6 recorder (Electronics for Medicine). The aortic pressure is likewise obtained from the aorta via the femoral artery and displayed on the screen along with lead II of the electrocardiogram and heart sounds. The students thus look at the time relationship between these events. They manipulate these pressures by giving drugs which affect the afterload. They also manipulate the electrical activity of the heart, first by giving large doses of epinephrine and then by opening the thoracic cavity and the pericardium and creating atrial fibrillation with a stimulating electrode. Ideally, students should anesthetize the animals and perform the surgical procedures. But because the students' time is limited we have hired technicians to prepare the animals. Since this is part-time work we have attracted a remarkably well qualified staff including one dentist and several registered nurses who do not want full time employment.

The influence of the respiratory system on the cardiovascular system is also examined in the cardiac cycle experiment, and the right and left vagi are stimulated to show the effect that these nerves have on the cardiac cycle. At the end of the experiment the students fibrillate the ventricles and subsequently defibrillate them using a clinical defibrillator. One of the major benefits of this experiment is a subtle one: students see--many for the first time--living tissue "in action". They are impressed with how it differs from cadaver tissue and with the "feel" of the heart in normal systole and in fibrillation.

Running simultaneously with the cardiac cycle experiment is a second experiment on contractility of the cardiac muscle. This gives the students a look at the isolated right ventricular strip of a rat. This preparation has previously been described in the PHYSIOLOGIST by another group (2). In this experiment the students look at the Frank-Starling relationship and then modify contractility by changing the ionic content of the bath solution as well as administering positive and negative inotropic drugs.

The last cardiovascular experiment to be performed in this term is somewhat more sophisticated than the other two and consequently has been placed at the end of the term. This allows the students to have learned to operate the polygraph and other equipment and to have overcome what fear they may have had of working with a live animal, before conducting the more involved experiment. This experiment is done on unanesthetized sheep which have previously been prepared for chronic study. The animals are used continuously in the laboratory, not only in this particular experiment, but in two others which are taught in term four. This is possible because term two immediately follows term four. In fact, there is a three week overlap of the two terms. The preparation of the sheep involves placement of a Swan-Ganz thermodilution catheter into the pulmonary artery via the femoral vein. An arterial catheter is placed in the descending aorta via one femoral artery. A two mm i.d. polyethylene catheter is placed in the inferior vena cava via the opposite femoral vein. The catheters are filled with 1,000 units of heparin and they are flushed and re-heparinized on a weekly basis. On the day of the experiment the animals are brought to the student laboratories in mobile metabolic cages. They are connected aseptically to intensive care monitors (Electronics for Medicine, OM-9 recorder); the aortic, pulmonary artery and left atrial pressures are displayed. The cardiac output is de-

termined by the thermodilution technique using an Edwards Computer. Blood gases are determined by an Instrumentation Laboratories Blood Gas Analyzer. Once the baseline data have been obtained the students begin removing blood from the animal into sterile plastic bags. This blood is heparinized for reinfusion. As the animal reaches a new steady state following the withdrawal of the blood, variables are again measured. This sequence is repeated three times and then the animals are reinfused and a final set of data is obtained. The conference is utilized to look at the data which have been obtained during the experiment and are plotted on transparencies. These data are also discussed in conjunction with a syllabus on hypovolemia.

Between term two and term three in our curriculum, the students have an eight week free elective which approximately ten percent of them use to persue research activity. In the autumn the students enter into their term four classes and we begin the second half of the Integrated Functional Laboratory.

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TERM IV EXPERIMENTS

Temperature regulation
The effects of drugs on respiration
The effects of exercise on cardiovascular reflexes
Endotoxic shock
Immunology
Renal drugs
Glucose tolerance test
Anesthesiology
Effects of drugs on cardiovascular reflexes
Studies on the contractile state of the myocardium
Uterine responsiveness to hormones
Renal excretion of salicylates

Figure 3. The Term IV Experiments. Of the twelve experiments taught during this term, four are cardiovascular experiences. These are underlined in the figure. There are also several experiments that are related to cardiovascular physiology: notably the experiment on the uterus which includes a general discussion of smooth muscle, the experiment in anesthesiology where the students see anesthesia induced and there is a discussion of the relationship between uptake and distribution of anesthetic agents and cardiac output, and renal drugs in which a discussion of the relationship of the diuretic agents in hypertension are presented.

Term Four Experiments: **The term four experiments are listed in Figure 3. As can be seen, during this term there are four cardiovascular experiments. In the first six weeks we teach an experiment on the effects of exercise on cardiovascular function and another on endotoxic** shock. The first experiment uses the student as a subject. EKG electrodes, for the standard leads, as well as stress leads are connected to a VR-6 recorder. Their EKGs and blood pressure are monitored in the resting, supine state. The students then do standard exercises either on a treadmill or bicycle ergometer. The recovery process is observed and analyzed.

The endotoxic shock experiment is performed in instrumented sheep as described above. In this instance the sheep are injected with a dosage of 0.5 µg/kg of endotoxin and its cardiovascular and pulmonary- responses are monitored, again using the OM 9 Recorder and Edwards Cardiac Output Computer apparatus. While the endotoxin is taking effect a dialogue is carried out between the instructor and the students involving problem solving types of exercises. A syllabus on gramnegative sepsis is included in the packet for this experience.

The second-half of the term contains two cardiovascular experiences. One of these deals with cardiovascular reflexes. Here the subject is an anesthetized dog. These animals have a strain-gauge arch sewn onto their right ventricles, the femoral artery is cannulated for recording arterial pressure. The common carotid arteries are isolated and the vagal nerves are exposed and prepared for both central and peripheral stimulation. Students inject various sympathomimetic drugs and their antagonists and determine how all these affect the baroreceptor reflexes by occluding the carotid arteries. The last cardiovascular experiment that is performed is entitled The Effects of Drug on Myocardial Contractility. The left ventricles of dogs are cannulated via the carotid arteries. The signal from the catheter tip manometer recording the left intra-ventricular pressure is amplified and then fed into an electrical circuit which develops an index of contractility known as the peak (dP/dt)/P students monitor this index while they change afterload by injecting an α-adrenergic agonist. They change preload through volume loading; and they modify myocardial contractility with catecholamines, cardiac glycosides, and ß-adrenergic antagonists.

Student Evaluations: Each of these experiments is evaluated by the students at the end of each laboratory experience. The evaluation is on a scale from 0 to 12; 12 being excellent. A table of the ratings of individual experiments appears in Figure 4. The Medical Students' overall evaluation of these cardiovascular experiments has been between 9 and 10. This is slightly higher than the overall evaluation of the cardiovascular lecture series in physiology. In addition, the laboratory staff as well as the Integrated Functional Laboratory Committee, periodically evaluates the experiments. Since we collect student evaluations on a weekly basis we can monitor all experiments during the six weeks they are taught and reorganize, restaff, or re-equip any experiment in which a problem is identified. We monitor the

student's comments and attempt to make improvements suggested by the comments. For instance, an instructor who has failed to make clear one of the objectives for the experience can quickly identify this problem from the student evaluation. Or if there is continued comment that an instructor is difficult to understand or that there has been a continuous failure of item of equipment during lab period, this can be rapidly corrected. The Integrated Functional Laboratory Committee, based upon input from several sources including the students, periodically reviews the experiences as well as reviewing proposed experiments which have been submitted by the faculty. In addition, many innovative changes in experiments are the result of developments in technology. Through the years, as a result of this particular fine tuning, the experiments have become progressively more perfect. On the average, we make one major modification of an experiment a year. Most recently, this has involved the utilization of computer technology as a part of our experimental experience. To date our experience with this innovation is not sufficient to include here.

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Figure 4

	CV_1	CV_2	CV_3	CV_4	CV_5	IF_2	IF_3
1. Rate your understanding of the objectives of this experiment.	9.14	10.04	9.42	9.08	9.46	9.20	9.40
2. Rate the adequacy of the experiment and lab preparations to carry out the objectives.	9.72	10.40	10.37	9.63	8.62	10.01	9.64
3. Rate the extent to which the experiment reinforced materials in other courses.	9.49	10.46	10.17	9.73	9.70	10.12	9.83
4. Rate the quality of the instructor's explanations (clarity, organization, helpfulness).							
a. in lab?	9.04	10.41	9.78	9.97	10.39	10.22	9.83
b. in conference?	9.15	10.45	9.72	10.11	10.43	10.35	9.92
5. Rate the instructor's skill at arousing your interest and participation.							
a. in lab?	8.89	10.40	9.51	9.79	10.07	9.72	9.64
b. in conference?	8.91	10.32	9.36	9.84	9.98	9.91	9.60
6. Overall impression of experiment.	9.14	10.46	9.92	9.79	9.21	9.87	9.31
7. Overall impression of effectiveness of instructor.	9.14	10.51	9.73	10.00	10.44	10.15	9.74

CV_1: Studies of the contractile State of the Myocardium; CV_2: Events of the Cardiac Cycle; CV_3: Hemorrhagic Shock; CV_4: Effects of Exercise on Cardiac Function; CV_5: Cardiac Muscle; IF_2: Endotoxic Shock; IF_3 Effects of Drugs on Cardiovascular Reflexes

Figure 4. Student Evaluations. This figure is a copy of our student evaluation form. These are handed out to the students and they are asked to evaluate each experience as they do it. These are usually given them to be filled out at the very end of the experience and they then turn them in within a few minutes of the ending of their laboratory period. These forms can be rapidly processed into a computer and weekly printouts are given to each instructor which evaluate his daily performance. The evaluation comments are also available to the instructor. We have summarized the evaluations which the students gave to the various experiments during the 1981-82 school year.

THE SELECTIVE LAB: AN ALTERNATIVE TO COOKBOOK EXPERIMENTS

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INTRODUCTION

It has been noted that there has been a general downward trend in the use of "wet" labs as a teaching tool in medical education (3). There are undoubtedly many factors that have contributed to this phenomenon; e.g., the need to streamline courses due to an ever increasing knowledge base vis-a-vis reduced teaching time. However, as Eichna (1) has pointed out, medical education should be, but for the most part is not, a thinking, problem solving process. In a basic science discipline, such as physiology, the optimal setting for problem solving is the laboratory. Unfortunately, the aforementioned reduction in teaching labs has resulted in remaining laboratory experiences being mainly "cookbook" exercises in which students follow prescribed procedures, the outcomes of which are usually known in advance. Although such laboratory exercises do provide valuable experiences such as working with live tissues and directly observing temporal phenomena (e.g., the action potential), the traditional cookbook labs do not provide students with the opportunity to work through all aspects of a laboratory problem, e.g., design and performance. We feel that this is a definite deficiency in medical education since physicians are constantly faced with the task of evaluating research information in professional journals, etc. If a physician's only experience with medical science laboratories is with "tried and true" exercises, then he or she has no substantial knowledge base with which to evaluate the research of others.

The purposes of the selective laboratory described herein were to 1) enable students to gain experience in all aspects of medical science experimentation (e.g., design, performance, evaluation, and presentation); and 2) to provide them with an opportunity for creativity and individual expression. This was approached by providing one selective laboratory experience in addition to six regular wet labs given within the framework of a one semester 7 credit hour course in medical physiology. At the University of Kentucky, physiology is given during the first year of medical school to a class of about 110 students. This manuscript is based on the results from our 1981 and 1982 courses.

METHODS

The underlying method of the selective lab was to change the role of the laboratory instructor (faculty) from a source person who authors and supervises specific laboratory excercise (Fig. 1), to a resource person who provides guidance in helping students design and carry out their own experiments (Fig. 2). In this context, the various faculty participating

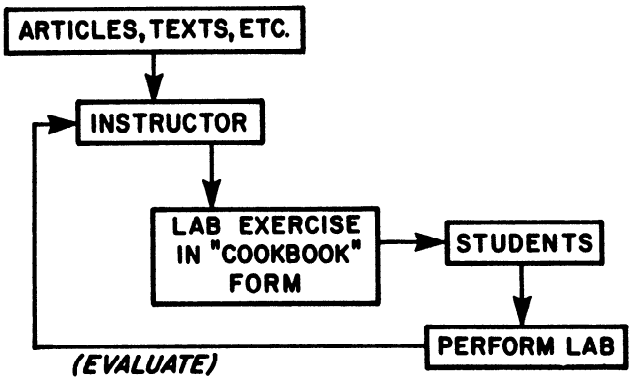

Fig. 1 Flow diagram of the steps involved in a traditional "cookbook" lab.

in the medical physiology course each provided one or two topics, or problems, along with brief descriptions. The topic list is given in Table 1. Examples of the descriptions are presented in Table 2 for an animal model and a human study. After allowing the class about two weeks to consider the list, each student was required to select one of the topics. The number of students selecting a particular topic was limited by equipment and space; e.g., the cardiac contractility topic was limited to 8 students (Table 1). Once the quota for a given topic had been filled, the faculty resource person responsible for the topic met with the students to discuss the general aspects of the subject, present them with articles to read, and if need

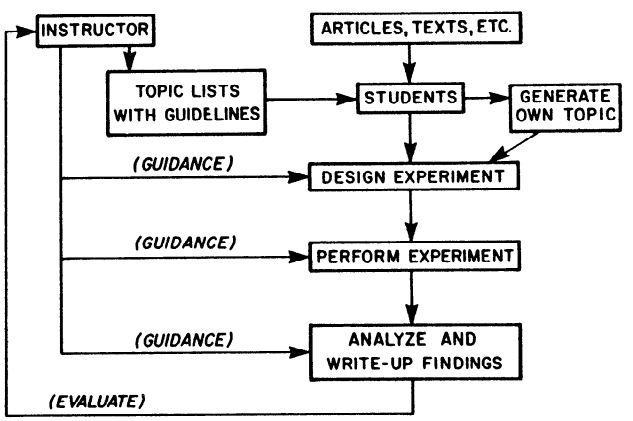

Fig. 2. Flow diagram of the steps involved in the selective lab procedure.

be, divide them up into teams for the actual performance of the experiments. The number of students working as a team depended on available space and equipment. For most of the experiments, students worked in teams of two or three, but for the more complex topics requiring extensive animal preparation and/or sophisticated equipment, the students worked in teams of four.

Table 1
Selective Lab Topic List

1) Aerobic Conditioning
   Faculty: Drs. Richardson and Kearney
   Limit: 10 students

2) Cardiac Contractility
   Faculty: Dr. Randall
   Limit: 8 students

3) EEG and Evoked Potentials
   Faculty: Drs. Frazier and Lastimosa
   Limit: 20 students

4) Ionic Mechanisms of Nerve
   Faculty: Dr. Peretz
   Limit: 16 students

5) Muscle Blood Flow in Humans
   Faculty: Dr. Richardson
   Limit: 8 students

6) Regulation of Heart Rate
   Faculty: Dr. Randall
   Limit: 8 students

7) Renal Diuresis
   Faculty: Dr. Ott
   Limit: 20 students

8) Pulmonary Mechanics
   Faculty: Drs. Lee and Zechman
   Limit: 20 students

* * * * * * * *

If a student or group of students wished to generate their own topic or problem independent of the list, they were free to do so within the limits of our facilities and provided they could recruit a faculty sponsor. The results of one such experience led to a presentation by one of the student groups at a national meeting (2).

Subsequent to the initial contact between students selecting a particular topic and the associated faculty person, that faculty member met with the students individually and/or as a group to assist them in the design, performance and analysis of their experiments (Fig. 2). The degree of faculty assistance in each of these areas, as well as the total time the students spent on their selective lab, depended upon the nature of the experiments. For example, the topic dealing with aerobic exercise conditioning was spread out over a six week period, whereas the topic concerned with ionic mechanisms of nerve was concentrated over a two week interval.

The number of times a given student team actually performed an experiment depended upon

Table 2
Samples of Selective Lab Descriptions

Topic 2 - Cardiac Contractility

The objective of this study will be to measure the effects of the following on regional cardiac contractility in the anesthetized dog: 1) increases in sympathetic activity; 2) changes in preload and afterload of the heart; 3) hypoxia; and 4) coronary occlusion.

Equipment available will include ventricular force transducers, catheters and pressure transducers for the measurement of intraventicular and systemic pressures, nerve stimulators, and sympathetic agonistic and antagonistic drugs.

Topic 5 - Muscle Blood Flow in Humans

The objective of this study will be to examine relative values of "active" and "reactive" hyperemia in the skeletal muscle circulation of humans.

For these experiments forearm blood flow will be measured by placing a Doppler ultrasonic flow probe over the brachial artery. Active hyperemia will be induced by static and by rhythmic hand grip contractions. Reactive hyperemia will be induced by occluding brachial arterial blood flow via a pneumatic cuff.

Possible questions to answer:

1) How does reactive hyperemia following a period of ischemia compare with active hyperemia following an equal period of static muscle contraction at different levels of muscle force?

2) Are active and reactive hyperemia additive?

3) How does active hyperemia following a period of static exercise compare with that following a period of rhythmic exercise at different contraction frequencies?

4) Etc., etc., etc. (Students doing these experiments should think of some more questions).

Equipment will include: a Doppler flow meter, pressure cuffs, ECG equipment, and a hand dynamometer for rhythmic and static hand grip exercise.

* * * * * * *

the nature of the experiments. Those requiring modest equipment and preparation (e.g., renal diuresis, human muscle blood flow) were performed several times; whereas, those requiring extensive preparation (e.g., cardiac contractility) were usually performed only once. The location of the laboratories used also depended upon the nature of the experiments. Some experiments (e.g., ionic mechanisms of nerve, aerobic conditioning,

human muscle blood flow) were performed in the faculty member's research laboratory using his/her own equipment; whereas other experiments (e.g., pulmonary mechanics) used the regular teaching labs and equipment.

At the conclusion of their selective laboratory experience, each student independently prepared a written report in journal format. These reports were graded subjectively by the faculty responsible for the particular topic and given a score ranging 1 to 30. This constituted a maximal 10 percent of the total points used in determining a student's final grade. The other 270 possible points were derived from objective multiple choice tests on lecture material.

RESULTS AND DISCUSSION

Both quantitative and qualitative types of analysis were used to evaluate the selective laboratory experience. Quantitative analysis involved statistical comparisons of points earned on the selective lab write-up with points earned for the total course (exams plus lab). Qualitative analysis was achieved via a questionnaire given to the students in which they evaluated the selective lab vis-vis the regular wet labs and the lecture portion of the course.

Quantitative Evaluation: Figure 3 presents class means for percentage scores (number of points as a percentage of maximum possible) for the selective write up and for the total course. Both the 1981 and 1982 classes did slightly, but not significantly, better in the selective lab compared to the total course. Thus, for the class as a whole, the selective lab tended to help rather than hurt with regard to a final grade. Note also that both classes performed essentially the same in the selective lab and the total course . This demonstrates that selective lab grades, which are determined subjectively via written reports, are reproducible between classes that are academically matched in terms of objective matching graded exams.

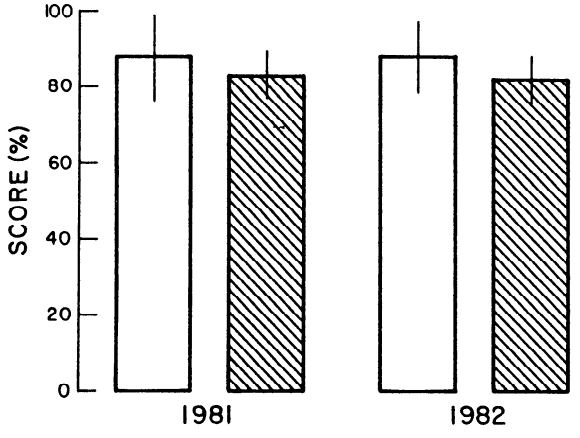

Fig. 3 Class means ± SD for percentage scores received for the selective lab write-ups (open bars) and for the total course, lab plus exams (solid bars).

The data presented in Figure 3 shows that in terms of a whole class, students performed about equally in the selective lab and in the total course. To determine if this was generally true for individual students, the correlation between selective lab and total course scores was determined. This information is presented in Figure 4 for the 1982 class. There is a general linear trend between selective lab and total course scores, but the regression coefficient (r) was low. The 1981 class was similar but with an r value of 0.403. Therefore, performance of a student in the selective lab was not generally reflective of total course performance. In terms of an overall education, this is probably more beneficial than detrimental since it points out that a student who does not do well in objective grading may still have the ability and motivation to learn through laboratory experimentation and the communication skills necessary to express what he or she has learned. In this regard, 35 percent of the students from both classes combined, scored better than 10 percentage points higher in their selective lab write-ups compared to total course scores, while only 6 percent of the students had selective lab scores 10 percentage points or more lower than their total course scores.

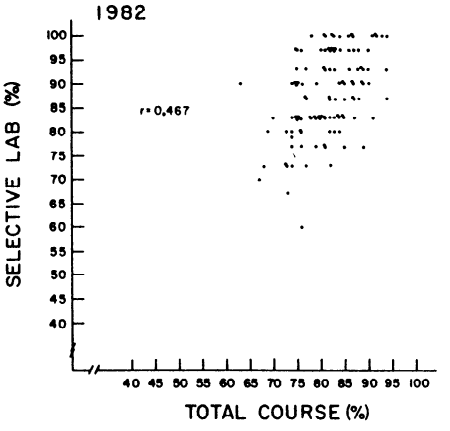

Fig. 4. Relationship between selective lab and total course scores, as percentage of maximum possible points, for the 1982 class. r= linear regression coefficient.

Qualitative Evaluation: At the end of the semester the students were given a questionnaire in which they were to rate a variety of aspects of the physiology course (e.g., quality of the instructors, the examinations, the labs, etc.) as good, fair, or poor. Figure 5 gives the combined

1981 and 1982 class ratings of the selective labs, the traditional (cookbook) labs and the course as a whole. Neither the selective lab nor the traditional labs received high ratings compared to the total course. However, the higher ratings of the selective lab, compared to the traditional labs, clearly indicates that providing students with the opportunity for a complete and creative laboratory experience is a move in the direction of a more positive attitude towards wet labs in medical education.

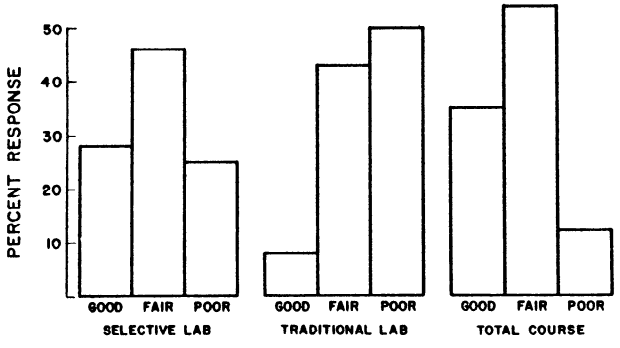

Fig. 5 Qualitiative evaluation of the selective lab, the traditional lab and the total course. The percent response refers to the 1981 and 1982 classes combined.

SUMMARY

In overall scope, the addition of at least one laboratory that enables students to experience all aspects of laboratory science adds several valuable dimensions, which compliment the traditional labs given within the framework of a classical physiology course. To provide such an experience, The Selective Lab procedure was developed and added to a one semester course in medical physiology. The following paragraphs summarize and compare some of the advantages and disadvantages of The Selective Lab vis-a-vis our experience with the 1981 and 1982 classes.

The major advantages of the selective lab were: 1) the opportunity for individual expression in an otherwise objective, impersonal course presentation; 2) higher motivation on the part of the students by allowing them some degree of personal preference in the labs they select; 3) the opportunity for the students to experience all phases of experimental science, and to delve deeper into a given topic than would be possible in a cookbook lab format; and 4) the opportunity to be graded on a subjective basis.

The disadvantages were: 1) a decrease in topic breadth, (with the present limits of equipment, space, faculty personnel, and time, all possible topics simply cannot be covered), and 2) logistics. The need to accommodate 110 students with a limited number of selective labs does create problems with regard to sharing equipment, working in teams rather than individually, and finding the time to perform complex experiments.

On balance, both the students and the faculty felt that The Selective Lab was a positive experience.

ACKNOWLEDGEMENTS

The author would like to thank the Division of Educational Development for their assistance in evaluating The Selective Lab.

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PROBLEM-BASED STUDENT-CENTERED LEARNING OF THE CARDIOVASCULAR SYSTEM USING THE PROBLEM-BASED LEARNING MODULE (P.B.L.M.)

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The Clinical Reasoning Process

Research into the actual behavior of physicians has revealed certain fundamental steps in the process of clinical reasoning (1-6). There is no a priori reason to suppose that the process of clinical reasoning in medical problem-solving is any different than the reasoning process invoked by any experts when confronting a problem in their respective disciplines (7).

In summary the process contains the following steps illustrated here within the framework of clinical problem solving (see figure 1): The physician or the student confronts the patient, perceives cues from the environment and the subject, and assembles an initial mental formulation of the problem. Very rapidly multiple hypotheses are generated as possible explanations of the problem. An inquiry strategy is engaged which involves, among other things, the use of clincal skills to acquire data which may be used to refine the initial problem formulation. The refined problem formulation is then compared to the hypotheses generated. These hypotheses are retained, discarded, or enlarged. Further inquiry is pursued until a particular hypothesis attains sufficient confidence to form the basis of management decisions.

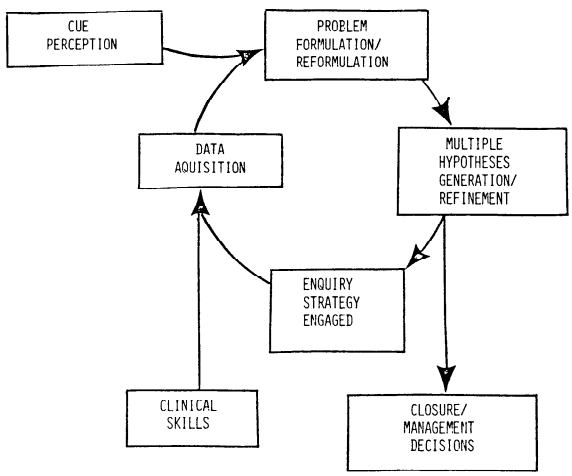

Figure 1. Schematic representation of the clinical reasoning process.

Self-Directed Learning

At any stage in the reasoning process progress may be obstructed by lack of appropriate knowledge or skills. When progress or understanding is impeded, learning issues may be identified. A wide variety of learning resources may be brought to bear upon the problem in a context of immediate application (8). (see Figure 2): This self-directed learning may be summarized as follows In the clinical reasoning process, at any stage in the cycle (see figure 1), students may find progress obstructed by ignorance or lack of understanding and skills. When this occurs the student notes the obstruction as a learning issue and continues to confront the problem. When learning issues accumulate to the point where progress is frustrated the process is stopped. The student then designs a learning prescription, under faculty guidance, which considers both acute learning needs and objectives for the relevant phase of the curriculum. Learning resources are then consulted by the student to meet the dictates of the prescription. These resources may be any of a wide variety-tests: journals, faculty, slide tapes, etc. After relevant study the student then returns to the problem either at the point left off or to begin anew better equipped for the process.

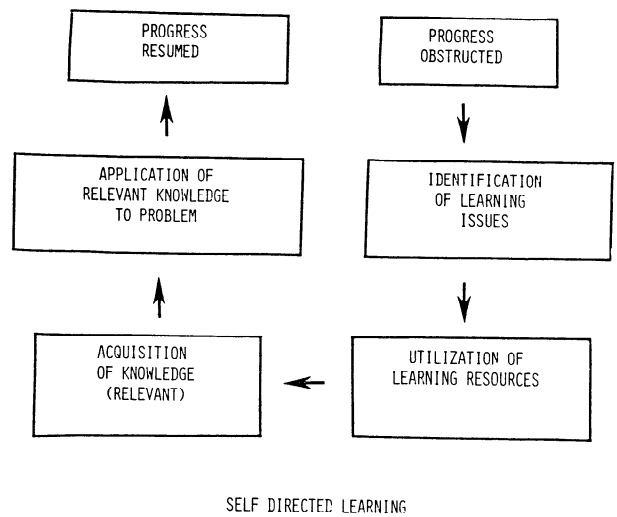

Figure 2. Schematic representation of the self-directed learning process related to patient problem solving using the P.B.L.M. as a patient problem simulation.

Utility of the Physician's Knowledge

The practicing physician must have a copius store of recallable factual knowledge as well as a battery of clinical skills which can be relied upon in the context of medical problems. This is so because the professional consultation cannot, as a rule, be interrupted while the physician consults a textbook or a journal article or perhaps makes inquiries of colleagues or professors. In some cases, of course, these things must be done and it may be better for patient rapport if they are accomplished without the patient's acute knowledge. Naturally, in some instances, the patient will be better served in the certain knowledge that the physician is consulting other sources of expertise.

A large fraction of the essential knowledge which a physician must have in recallable form or effective practice is physiology. The most widespread and relentless form of health failure or disease is cardiovascular disease. For these reasons a sound recallable background of cardiovascular physiology is essential to the effective physician. Recallable knowledge is not enough. It must also be recallable in the context of a problem to which it may be applied. For example, it is of no consequence to know the Reynolds number for blood if the implications of turbulant and laminar blood flow are not understood. The relationship and character of heart sounds within the cardiac cycle are of no value if the association between valve stenosis or regurgitation and hypertrophy of the myocardium is not recognized. The list of examples may be endless. The point is that the massive accumulation of facts and concepts out of context is either lost or not reliably useful (9, 10). Numerous investigations have now demonstrated that most of the material memorized by students for examinations is forgotten or not usefully recallable within a short time (11,12). It is known that most students in clerkships cannot pass even the clinically relevant portions of their first year basic science exams if they are readministered (13).

Fortunately, this turns out not to be a serious problem for the health of the nation because the physicians in training as clerks and residents or later in practice relearn the relevant information in the context of patient problems where it may be brought to bear upon other clincial problems to which practitioners are exposed. The tragedy lies in the time and resources wasted in the teaching by faculty and the memorizing by students of vast amounts of data which is either forgotten or not recallable within a practical context. The student would be better served learning how to acquire relevant knowledge in a context of the professional task environment. The faculty would better serve the academic community by devoting their time to their reseach and their particular fields of expertise. When faculty are consulted by students in pursuit of learning resources for the acquisition of knowledge relevant to a professional task situation, the student benefits greatly and the faculty suffer only a minor inconvenience. The faculty talents are accurately used and the student acquires useful current information. The student gains in education and learns early in the career the methods of self-education which must be relied upon for a lifetime of continuous education.

Sources of Problem-Based Learning Material

All of the foregoing may be well recognized but, where do we obtain patients for first year medical students to use in a professional task situation while they generate learning issues and gain practice in using faculty and other learning resources?

As a rule, we don't. However, we can obtain such patients if we use simulations. A variety of patient simulations are available. The simulated patient is one (14). In this case a person is trained to simulate a patient problem so exactly that expert physicians cannot tell them from real patients (15). These simulations are relatively expensive and most appropriate for clinical skills training; physical examination and interview skills--the benefits for the advancing student are obvious. Another simulation is the Problem-Based Learning Module--P.B.L.M. (16). In this simulation a real patient problem is recorded on paper in an easily accessible format. The PBLM permits free inquiry. Any action, which was or could have been taken by the real physician, can be taken by the student. The patients' actual response is obtained. Actions can be taken in any order-or disorder for that matter. Items from the interview, physical examinations, laboratory tests, specialist consultations are available from the simulation.

Problem Based Learning in Practice

Students encounter the problem (PBLM) in a group of six. This number may range from five to seven. Four is too few to ensure an adequate pool of background knowledge for first year students and eight is too many to maintain a uniformity of participation. There is a faculty tutor who ensures that the group adheres to the reasoning process. The tutor does not normally serve as a resource person although he or she may be specifically requested to do so. Learning resources are not normally consulted in the course of a group meeting except for brief interruptions to consult a medical dictionary or a ready reference. The students engage the problem "cold" without prior study. Their pooled knowledge always permits some progress. As they progress they encounter areas of ignorance which obstruct their progress. As they are first year students, in this case, their objectives are to explain and understand problems in terms of basic mechanisms--anatomical, physiological, biochemical and behavioral mechanisms. As these obstacles (areas of ignorance) are encountered, they are noted on a blackboard. When finally no

progress can be made because of the lack of knowledge, the group process is stopped. This usually occurs after about two hours. The students, with the help of the group and the tutor, design an educational prescription which takes into account each student's existing knowledge and skills (8). They may partition the topics and exchange investigated resources. At a later date, usually a few days, the group reconvenes and brings their newly acquired relevant knowledge to bear on the problem either beginning anew or continuing from where they left off. The student-directed learning is active. The relevance of the basic science learned is perceived directly, and vocabulary, skills and problem-solving abilities are enhanced simultaneously. The student learns in the professional task environment. The faculty tutor, once having gained socratic tutorial skills, does not have to prepare for the session and when consulted in session or out, it is as an expert in a particular discipline area where no extra preparation is required.

Results of a Problem Based Learning Trial

Two classes of first year medical students were compared. One class, the control group, received the Cardiovasclar section of a system based curriculum in a modular format. They were given written modules containing specific learning objectives, official sources of reference material for each objective and lectures or laboratory exercises on each topic. The problem based learning class in the following year received seven Problem Based Learning Module simulations of patients with cardiovascular disease. They received no written objective modules, no specified learning resources, but the same lectures and laboratory exercises. The two classes had similar backgrounds with Grade Point Averages from prior college work and Medical College Admission Test scores which were essentially comparable. The means for both estimators of success in medical school were slightly higher for the control group (see Table I).

Both classes were evaluated for subject matter content knowlege with the same objective multiple choice exam encompassing Anatomy, Behavioral Science, Biochemistry, Pharmacology and Physiology. There was no significant difference between the two classes in the scores obtained in any of the disciplines examined (see Table II). The correlations among scores (in the sense that the score in any one discipline may be a predictor of performance in another discipline) were significantly improved in the problem based learning class (see Table III).

Twelve weeks after the cardiovascular systems examination both classes were reevaluated using the identical subset of the original examination. Again, there was no significant difference between the scores obtained by the two classes. However, when the absolute level of the mean scores was considered the rate of decline of score was significantly less (twice as slow) than in the PBLM group (see Figure 3).

DISCUSSION

It is evident that there are deficiencies in the current widespread teacher-centered subject-based curricula popular in most medical schools. Student-centered problem-based learning has been proposed as an alternative.

In this limited trial, which was admittedly retrospective to the extent that it was not devised until the control class had progressed to the second year, some positive evidence in support of problem based learning has been accumulated.

In the objective sense of discipline exams problem-based learning students perform just as well as traditional subject-based learning students. The observation that correlation among discipline exam scores is significantly enhanced could be interpreted as better integration of knowledge by problem based learning students. The rate of decline of scores after the course was less than half as fast in the problem based learning class suggesting that knowledge is better retained by this group.

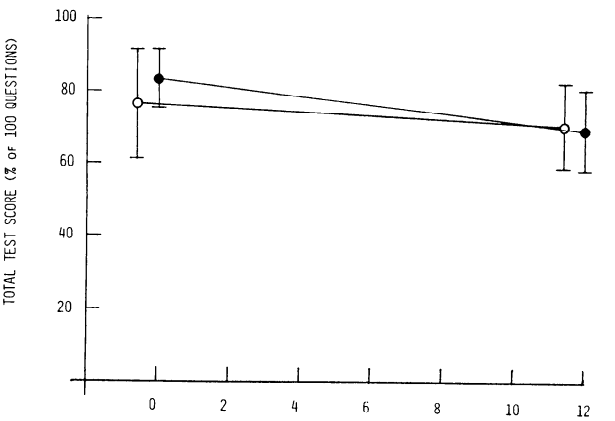

Figure 3. Decline in test score over 12 weeks following initial exam. There were 72 students in each group. (●)represents the control group having an objectives based curriculum only while (o) represents the group having objectives based curriculum with P.B.L.M's. The rate of decline of score was 1.114%/Wk vs. 0.454%/Wk for the two groups respectively.

It should be mentioned that all was not "roses" with the problem based learning group. The level of apprehension among faculty and students was greater than usual. Some faculty were concerned that the four to six hours per week spent as tutorial group facilitators was an unwarranted extra bruden on their time. Many

students were uncertain that they were learning enough of the appropriate material when they were not told exactly what to learn and where to read about it. The faculty concern can only be addressed by administrative recognition of tutorial facilitation efforts as valid teaching time. In this university that recognition has been extended. The student concern will be addressed only if future behavior vindicates the anxieties expressed during the course. For future classes who may have the experience of an entirely problem-based curriculum familiarity should considerably reduce anxiety.

In summary it cannot be overlooked that problem-based learning students learned at least as well as subject-based predecessors, forgot at less than half the rate, and indicated improved integration of knowledge among basic science disciplines.

Finally, it must be recognized that problem based learning students had the additional benefit of gaining experience in patient problem solving, the repeated practice of discovering their own learning needs, and the opportunity to apply their knowledge in the professional task environment of their vocation.

TABLE I

ADMISSION CRITERIA FOR MEDICAL CLASSES REPORTED IN STUDY

SCORE CATAGORY	OBJECTIVES BASED CURRICULUM ONLY	OBJECTIVES BASED WITH P.B.L.M.'S
GRADE POINT AVERAGE	3.479 ± 0.047 *	3.368 ± 0.048
M.C.A.T. SCORES		
PB	9.465 ± 0.146 *	8.890 ± 0.190
BI	9.324 ± 0.193	9.068 ± 0.207
CH	9.408 ± 0.183 *	9.068 ± 0.207
PH	9.070 ± 0.202 *	8.671 ± 0.222
RD	8.634 ± 0.186	8.781 ± 0.174
QT	8.845 ± 0.182	8.384 ± 0.245
MEAN M.C.A.T.	9.124 ± 0.036 *	8.747 ± 0.025

The values reported are class means and standard error of the mean. There were 72 students in each group with column one representing the class completing first year in 1981 and column two representing the class completing first year in 1982. (*) indicates a significant difference between means at the $P<0.05$ level. If there was any advantage derived from college preparation or aptitude for medical school implied by these estimators it was in favor of the Control group.

TABLE II

MULTIPLE CHOICE INFORMATION CONTENT EXAM SCORES

DISCIPLINE	OBJECTIVES BASED CURRICULUM ONLY	OBJECTIVES BASED WITH P.B.L.M.'S
ANATOMY	13.60 ± 1.88 / 17	14.83 ± 2.37 / 17
PHARMACOLOGY	10.97 ± 2.32 / 14	10.69 ± 2.26 / 14
PHYSIOLOGY	47.57 ± 5.40 / 56	40.63 ±10.49 / 56
BIOCHEMISTRY	5.42 ± 0.76 / 6	4.90 ± 1.24 / 6
BEHAVIORAL SCIENCE	5.40 ± 0.89 / 7	5.09 ± 1.44 / 7
TOTAL (AT END OF COURSE)	83.03 ± 8.68 / 100	76.14 ±15.23 / 100

In all disciplines except Physiology curriculum was derived from faculty prescribed objectives for both groups. In Physiology, curriculum was derived from student-generated learning issues associated with the solving of simulated patient problems (P.B.L.M.'S). The standard multiple choice (National Board Format) exam was the same for both classes and consisted of the number of questions indicated to the right of each discipline mean score in the two columns above. There were 72 students in each group and variability is expressed as standard deviation. There was no significant difference between any of the scores obtained by the two groups at the $P<0.05$ level.

TABLE III

CORRELATION MATRIX FOR DISCIPLINE EXAM AND TOTAL SCORES

	ANATOMY	PHARMACOLOGY	PHYSIOLOGY	BIOCHEMISTRY	BEHAV. SCI.	TOTAL
ANATOMY	1.00	0.32	0.50	0.14	0.29	0.64
PHARMACOLOGY	0.64*	1.00	0.57	0.10	0.31	0.72
PHYSIOLOGY	0.62*	0.52	1.00	0.36	0.43	0.95
BIOCHEMISTRY	0.43*	0.34*	0.60*	1.00	0.26	0.38
BEHAVIORAL SCIENCE	0.50*	0.34*	0.61*	0.51	1.00	0.52
TOTAL	0.76*	0.66	0.97*	0.66*	0.68*	1.00

The upper right half of the matrix shows correlation coefficients relating test scores in each discipline and the total score to every other discipline and the total score for the class having only a faculty prescribed objectives based curriculum. The lower left half of the matrix shows the same data for the class having the same curriculum except for student-generated learning issues in Physiology and patient problem solving using P.B.L.M.'S. The *'s in the lower left half of the matrix indicate increased correlation associated with the use of P.B.L.M.'S and student-generated curriculum in Physiology.

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CV PATHOPHYSIOLOGY PROBLEMS IN SMALL GROUP TUTORIALS

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INTRODUCTION

Almost every medical student can properly state the Fick equation, tell you that the outputs of the right and left hearts are equal, or define the notion of cardiac reserve. However, if we ask this same student to calculate cardiac output using data from the cardiac catheterization laboratory...or discuss how a patient can have a pulmonary blood flow of 15 L/min with a systemic flow of 5 L/min...or explain how a patient might have normal pressures throughout his cardiovascular system but be unable to play a round of golf...we are likely to find that the student can not give us an answer without considerable coaching.

The lesson here is well known to most of us. An instructor in front of an audience in a lecture hall is a system well suited for the passive, one way transmission of "facts". It does a much poorer job of assisting students to become active thinkers about physiology. That is to say, it is not much good at encouraging integration of information or the development of problem solving skills. Nevertheless, lectures remain the dominant instructional mode in most of our physiology courses.

However, medical students, and ultimately physicians, need to be more than passive regurgitators of stored "knowledge". In fact, it is fair to say that:

MASTERY OF PHYSIOLOGY=ACQUISITION OF "FACTS"+THE ABILITY TO "SOLVE PROBLEMS" WITH THESE "FACTS".

Therefore, recognizing the limitations of the lecture hall in fostering problem solving, then we need to devise other teaching/learning formats that will encourage more active "thinking about" physiology.

In Table I are listed some of the processes that may be involved in "problem solving" in physiology. It equally well describes the thought processes that are involved in clinical diagnosis and patient management.

TABLE I
Problem-Solving Processes That May Be Involved in Physiology (Or Medicine)

PROBLEM-SOLVING MAY INVOLVE:

1. "Translation"
2. Interpretation
3. Calculation
4. Integration
5. Analysis
6. Prediction
7. Hypothesis Generation

Laboratory exercises, whether they be "wet" labs in which the students prepare an experimental animal, or "dry" labs in which they only "exercise" a paper and pencil or a computer terminal, are one kind of experience in which we seek to develop the problem solving ability that is necessary for a truly complete mastery of physiology.

At Rush we use the lecture hall in the conventional way. We also schedule a variety of laboratories, one of which will be described later in this volume. However, we utilize a third kind of teaching exercise that we call the tutorial.

THE SMALL GROUP TUTORIAL

The tutorial is simply an instructor and a small number of students (ideally no more than 20) meeting together to consider a set of problems. These might be numerical problems to be solved by calculation. Or the problems may be the output of a system to be explained by describing the set of physiological interactions that generates it. The only requirement for these problems is that they not be answerable by simply memorizing numbers or facts.

One of our most successful tutorials utilizes data from three patients obtained in the cardiac catheterization laboratory at our hospital. And we must extend our thanks to Neil Ruggie, MD, the Director of the Cardiac Catheterization Laboratory, for providing us with case reports we have used. For each patient the data have been put together in a structured way that encourages the students to think through these problems in a series of logical steps. In this way they can be led to an explanation of how a cardiovascular system with relatively discrete pathology can produce the patient's condition.

A tutorial is most likely to help in the development of problem solving skills if student input to the discussion is maximal. This ought not be the occasion for still another lecture to be received passively by the students. The instructor's role is to guide the discussion, serve as a resource person, and see to it that as many of the students as possible take an active part in the exercise. This latter function usually requires that the instructor gently coerce participation from those reluctant to speak (perhaps because of a fear of being wrong "in public")

while not actively discouraging the more vocal.

One reason for the success of this exercise is the students' perception of the relevance of pathophysiology. To them stenosed valves or a-v shunts are "medicine" in a way that Na and Ca channels in the myocardium are not. (Never mind the clinical relevance of these channels, the issue is the students' perception of such phenomena.) While we should not try to "sell" every piece of physiology we want to teach by documenting its immediate and direct applicability to medicine, it is clear that the clinical flavor intrinsic to our pathophysiology problems encourages student involvement in a significant way.

Let us add that there is no reason to think that the appeal of pathophysiology problems is limited to medical students; everyone, undergraduate, graduate or medical students alike finds problems about "real people" fascinating.

THE STRUCTURED PATHOPHYSIOLOGY PROBLEM

The key to the success of this approach to teaching problem solving is the structuring of the problems and the discussion to channel the students' thinking without obviously suggesting the "right" answer. This may be achieved in a number of ways.

First, one must carefully select the problems to be used. Even the most "classical" case is likely to contain some data that is difficult to reconcile with the rest of the physiology at work. Judicious editing yields a case report that is understandable and clinically realistic while remaining "solvable" by first-year medical students. This does not mean that all ambiguities need to be removed; they can often be used to generate lines of discussion that are particularly instructive.

A second tactic that is helpful is to generate within the problem a required sequence of steps, i.e. a structure. As this will represent the major focus for the rest of this paper, we will briefly defer comment on this.

Finally, a plan for instructor "involvement" is developed, the Faculty Guide. This consists of a sequence of questions to be put to the students, or issues to be raised at certain points in the discussion. While these should not be taken as the only inputs to be provided by the instructors, they are a minimal set that ought to be discussed to derive adequate benefit from the material contained within each problem.

The Faculty Guide serves two additional purposes. It insures some minimal level of uniformity in the discussions that occur in tutorial sessions held at different times or by different faculty. And it allows faculty other than the current lecturer (or the authors of the pathophysiology problems) to participate effectively in the tutorials.

Students encountering complex pathophysiology problems initially need considerable guidance. They are often completely baffled and need to learn how to approach such problems in a logical manner. This guidance is provided by structuring the material they are given. Information in the tutorial write-up is presented in a carefully determined sequence. Calculations are requested at appropriate places as are questions to be answered.

Table II illustrates the over-all format that we have adopted for structuring our cardiovascular pathophysiology problems. We begin with a brief case history in which the patient's signs and symptoms are presented. Next, the students are asked to examine some general descriptive data obtained during the cardiac catheterization procedure and to make some simple but important calculations. Then static pressure values from various locations are presented, followed by an example of the actual pressure tracings. Finally, the resistances are calculated and the use of "indices" as a means of comparing results across a population is introduced.

TABLE II
The Structure Of The Cardiovascular Pathophysiology Problems Used In Small Group Tutorials

Signs and Symptoms
  Hypotheses?
General Data Describing Pt Condition
  Calculate: Flows Thru Heart
  Identify: Abnormalities
  Hypotheses?
Pressure Values
  Identify: Abnormalities
  Hypotheses?
Cardiac Cath Tracings
  Identify: Abnormalities
  Hypotheses?

  Calculate: Vascular Resistances
  Hypothesis?

  Calculate: "Indices"
  Hypothesis?

Diagnosis?

Explanation?

* * * * * * *

At every stage the students are asked to consider the data presented, or the values of parameters calculated, to look for abnormal values or patterns of values. They are encouraged to generate hypotheses about the pathophysiology that might give rise to such data and to reject untenable hypotheses as more data becomes available. Eventually the students ought to be able to "home in" on a most probable explanation of what is going on in that patient.

We can examine two components of this sequence in a little more detail. In Table III we can see an example of the initial data from one of the patients. With this information the students ought to be able to do a Fick calculation to determine left and right ventricular outputs and then to calculate stroke volume. The students are asked to examine these data to see if any of the values are abnormal or if they suggest additional hypotheses about the pathophysiology that is present. Can any of the initially posed hypotheses be discarded at this point?

TABLE III
Example Of Initial Cardiac Cath Data
Presented In Pathophysiology Problems;
Used To Calculate Right And Left Ventricle
Outputs And Stroke Volume

Heart rate (HR-beats/minute)	76
Ventilation (L/min)	5.16
O_2 consumption (cc/min)	255
Hemoglobin (gm%)	13.6
Pulmonary a-v O_2 difference (vol%)	4.4
(Pulmonary artery O_2 - Systemic artery O_2)	
Systemic a-v O_2 difference (vol%)	4.4
(Aorta O_2 - Pulmonary artery O_2)	

* * * * * * *

Figure 1 contains pressure tracings from the same patient. The ECG is used for timing purposes and pressure pulses from the left ventricle and the aorta are presented. The students must carefully examine the tracings to determine what they reveal about the pathophysiology that is present. Do they recognize that the contours of the ventricular

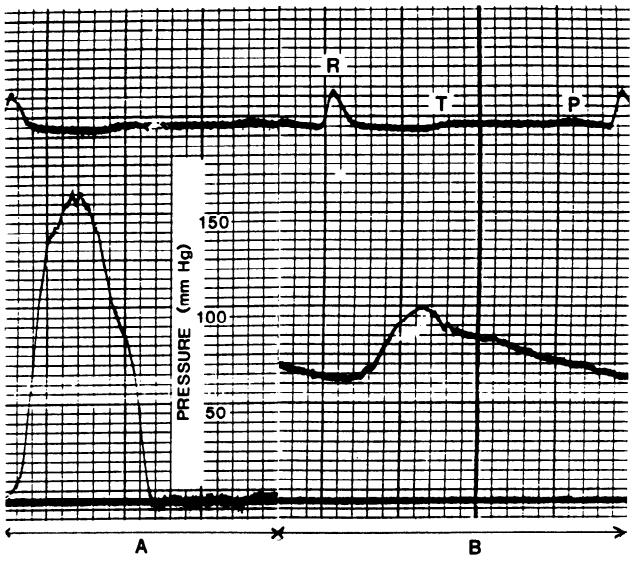

FIGURE 1
Sample of a pressure tracing obtained during cardiac catheterization from left ventricle (A) and aorta (B) in a patient with aortic stenosis. The top trace is an ECG record provided for timing purposes.

and aortic pressure pulses are quite different, that the rate of rise of pressure is much slower in the aorta and the peak pressure there is much lower. Do they know if this is normal? At this point, the student ought to be nearly certain about the nature of the patient's problem.

After examining all of the data and having made the requested calculations, the group should now be ready to present a final hypothesis about the pathophysiology that is present and should be able to explain how that gives rise to all of the findings (signs and symptoms, hospital data, cath lab data). One way of organizing and presenting such an explanation is to generate a flow chart (Figure 2) in which the cause and effect relationships arising from the initiating patholgy are diagrammed. Here we can see - step by step - the consequences of an aortic stenosis: increased outflow resistance; giving rise to a murmur, and an increased pressure drop across the valve; near normal mean pressure, but chronic sympathetic stimulation. All of the patient's signs and symptoms as well as the physical

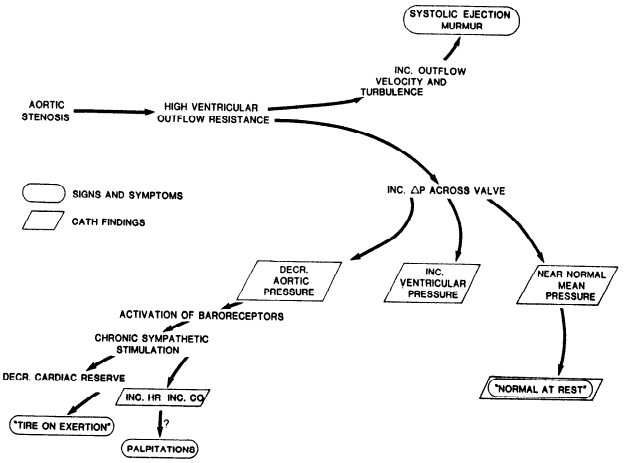

FIGURE 2
A flow chart illustrating the cause an effect relationships by which the consequences (signs and symptoms, physical findings) of an aortic stenosis manifest themselves. Students should be able to generate similar flow charts, or to verbally describe such relationships, for other pathophysiology problems, whether the problems involve the cardiovascular or other systems .

* * * * * * *

findings can be seen to arise from such a sequence of cause and effect relationships. It is this approach to the analysis of a complex physiological system or problem that the students need to master.

Having worked through a problem in this way the students have hopefully integrated many previously separate pieces of information about the cardiovascular system, and have begun to learn how to attack problems of this kind so as to arrive at a useful "solution".

SUMMARY

As in any good lecture, there must be a take-home message. It is this. Unless we do something about it, the majority of our students are likely to finish their physiology course as competent "regurgitators" of facts but much less competent physiological "problem solvers". These necessary skills are best learned by solving problems...but in a setting that permits immediate feed-back, reinforcement, and correction. The small group tutorial is one format that is well suited for such learning. And pathophysiology problems, by virtue of their perceived relevance, represent a "carrot" that we may take advantage of in seeking to make our students active, thinking learners.

NON-DIRECTIVE METHOD FOR TEACHING PHYSIOLOGY

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The traditional method of teaching, starting in the primary school, produces in the student a pedagogic distortion which we will call overprotection. Lessons that the children take home are supervised or even done by their parents. The result is that the young student becomes very dependent on his parents or siblings, refusing to make any effort to perform his job. A similar situation may occur at the secondary school level.

The examination for admission to the University depends on a lot of information which the student must keep in his memory. No test is applied in order to evaluate his initiative, attitude or problem solving ability.

At the University information is delivered to the student in "didactic" lectures. The student takes notes and during the examination reproduces exactly the words of the teacher. During the lectures, if he has questions he may be reluctant to interrupt the professor and thereby call attention to himself. However, the teacher, having heard no questions asked, feels happy thinking he has given a good lecture.

The questions presented to the students during the examination are similar to those of the previous year, according to the subject given in class.

Overprotection appears, clearly, when the teacher gives the lecture and the student takes notes on the lesson. A large number of students, receiving the information directly from the professor, remain passively waiting for the lecture in order to know what to study. So, it is not surprising that many students may finish the course without reading a single text-book of physiology.

During laboratory work, overprotection also occurs. The student attending a demonstration on the regulation of the blood pressure usually remains a passive spectator. He does not participate in the experiment; he does not anesthetize the animal and/or prepare the equipment to stimulate a nerve. Most importantly, he does not have an opportunity to commit errors or to discover, by himself, the way to reach the objectives of the demonstration.

When the student does perform the experiment he follows a laboratory manual containing more or less complete instructions and always works under the supervision of an assistant professor. The limitation of this approach to the laboratory experience is the student's anticipation that the results of the experiment can be found in the guide-book or revealed by the teacher. The student's pleasure in the discovery of knowledge is virtually precluded.

Conditioned by the traditional method of teaching, the student is not trained to take initiative and acquire self-confidence. At every step of the experiment he asks questions of the assistant that he could readily answer by himself. Questions such as the following are frequent: Is this the vagus nerve? Is the experiment correct?

Such questioning reveals the student's insecurity and is not in accord with the purpose of the experimental work. The student gets little benefit from the experiment, for the laboratory exercise is transformed into a mechanical act instead of serving to stimulate his curiosity to understand the physiological processes.

THE NON-DIRECTIVE APPROACH

The main purpose of the professor is to teach, that is, to create conditions for the student to learn. If the student does not learn, the professor has failed to reach his objective.

When we consider different methods of teaching, we are, indirectly, evaluating their efficacy and their costs. However, it is not easy to evaluate a teaching method. We think that the true teaching method is that which contributes to the formation of the student's personality, encouraging him to acquire new habits and attitudes, arousing his curiosity, and inducing him to think about the subject matter being studied. Obviously, however, these goals are usually not achieved.

In the great majority of courses in Physiology, the participation of the student is limited to receiving information from the professor during the lecture, while in the laboratory the experiments are conducted under the close supervision of the assistant professor.

In an attempt to find a method which gives the student the opportunity to develop his potentialities and acquire new abilities, in addition to facilitating personal growth, we decided to apply the non-directive method of psychotherapy (Rogers, 1951) to teaching physiology (Beraldo and Alvarenga, 1966).

With this approach the main role of the professor is to develop psychological conditions for the student so that, by his own effort, he can find solutions for his problems. This capacity to discern the true nature of a problem

(the elucidating glimpse or insight) cannot be transmitted by the professor; it can only be arrived at by the student himself.

Rogers (1951) suggested that the application of psychotherapeutic techniques for the removal of emotional blocks could facilitate an active and reflective learning process. Non-directive therapy seeks to replace the purpose of the therapist by that of the client. To achieve such a goal in an educational setting there must be created a friendly atmosphere in which the student feels happy and free to talk about his problems, whether directly or indirectly connected with his life in school.

The student is stimulated to find his own way, studying and discussing the proposed subject. The professor does not give the direct answer for questions put forward for discussion; the group by itself is able to find the solutions.

THE METHOD

Discussion Groups

Each group is formed by 15 students, after the application of a sociometric test. The student chooses his own group. In the class room the chairs are arranged in a circle and the coordinator takes any one of the chairs. Each member of the group receives a guide-book containing the subject to be discussed.

The Group Coordinator

The assistant professors are instructed by a psychologist in the techniques of coordinating the group. They are trained to listen to the student, accepting criticism, and learn how to stimulate the group to become self-confident and independent.

The coordinator must respect and accept the student as he is, notwithstanding his ideas, behavior or sentiment; however, acceptance does not mean approval. He should cultivate an atmosphere of understanding, tolerance and friendship. The group leader is not designated ahead of time; the leadership appears during the discussion, in accordance with individual participation and activity in the group, and therefore it changes from time to time. Whether an individual becomes a leader depends on his temperament and/or on the amount of information he has obtained from the text-book and the literature.

When a few members of the group monopolize the discussion the coordinator, carefully, invites the participation of other members of the group. In achieving this objective, the coordinator should not discourage dominant members of the group, but should try to stimulate the less active students to participate. The coordinator usually asks the opinion of each member of the group about the subject that has been discussed, starting, for example, with the student sitting on the right of the coordinator.

When the students do not begin the discussion the coordinator waits for 10 minutes, usually enough time for the discussion to begin.

The activity of the coordinator brings out the potentialities of the students, giving them more responsibility for the discussion. The group will rarely reach a wrong conclusion because of the restricted participation of the coordinator, because when a group of 15 students is discussing a subject many of them have already read several text-books of physiology (contrary to what occurs when the classical method is used).

Another interesting result of the non-directive method is that the enthusiasm of the students is increased, so that when the class finishes the discussion continues in the corridor and other non-classroom sites.

The good relationship between students in the group during the discussion class and laboratory work contributes to their progress in the course. We have observed that if, for any reason, one of the members of the group is transferred to another group, his previous colleagues feel his absence.

Laboratory Work

The orientation of work in the laboratory is also non-directive. The group of 15 is divided into sub-groups of 5 students. A typed guide is given to them containing only the essential information needed to perform the experiment. The student is encouraged to discover the results himself. It is not important that he finds the correct result; the effort, curiosity and enthusiasm for the experimental work are the qualities that we intend to develop with the non-directive method applied to the study of physiology.

STUDENTS' EVALUATION OF THE NON-DIRECTIVE METHOD

When the students were asked which answer best expressed their thinking, they responded:

1.	Did not study	2.6%
2.	Studied only for the exam	23.4%
3.	Studied to learn	87.0%

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A ROLE FOR MATHEMATICAL MODELS AND COMPUTER SIMULATION IN THE TEACHING OF PHYSIOLOGY?

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A variety of interesting mathematical models of biological phenomena have been developed in the past several years. These models, and the biological processes which they represent, range in scale from minute biochemical reactions buried within cells or even in parts of cells to interactions among organs within the body to the dynamics of whole populations. It is not clear at present whether or not these models will play an important role in the teaching of physiology in future years. Some of the most conspicuous positive and negative considerations are outlined below.

VOCABULARY

The use of terms such as "model" and "simulation" can lead to confusion. The term "model" generally refers to an imitation of an original, often in miniature, which bears a strong resemblence to the original. In science, "model" is often used to refer to an abstraction which faithfully captures the essence of the original but not necessarily details of lesser importance. As an example, constructs of plastic sticks and balls have often been successfully used to illustrate atomic interrelationships within molecules. Similarly water, reservoirs and elastic tubing have been used to build models that illustrate basic hemodynamic principles. In the present context, a more restrictive definition has been selected: "model" means "mathematical model" or a mathematical description of a biological process.

"Simulation" in general terms refers to imitating a real process using substitutes such as props and actors. Professional actors can be used to simulate patients as a teaching aid at the onset of clinical training. In the present context, however, a more technical and more limited definition must be used: "simulation" will mean "computer simulation" or the process by which mathematical models are analyzed using computers in order to reveal specific and general characteristics of the system under study. In a slightly expanded sense, the term "simulation" encompasses both mathematical model evaluation and the proceding steps of model building. Similarly, "modeling" is used herein to refer to the combination of mathematical model building and subsequent model evaluation.

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These definitions for "model", "modeling" and "simulation" are not wholly satisfactory, but they probably would have been even less appropriate two or three decades ago. Rapid advances in applied mathematics and computer technology have brought these rather specialized meanings to the forefront, without fostering the creation of additional, concise descriptors. Struggling with this lack of precision may be temporary, however. Our vocabulary may become enriched in the future as computer-generated mathematical analyses become more prevalent and stimulate the use of new descriptive terms. In fact, such advances are probably essential to proper use of and further growth of mathematical modeling since inadequate communication due to an underdeveloped lexicon can only foster confusion and skepticism. For instance, there are not terms in existence today that concisely distinguish between mathematical models built on established fact and those built principally on speculative relationships.

Currently, definitions-in-context must suffice with the caveat that carelessness can cause confusion and that inexactness is sometimes required.

MODELING IN PHYSIOLOGY TEACHING TODAY

Standing and Tidball (9) have recently critiqued physiological simulation. They argue that simulation can improve teaching efficiency and thereby reduce faculty work loads. Another direct consequence is that the learning experience for the student would be intensified. The analytical skills needed in clinical practice could be sharpened in simulated patient encounters that would surpass the rather constrained real encounters that are usually available to the medical student early in clinical training. Unfortunately, these insights primarily document a potential for modeling in the teaching of physiology rather than a realization of that potential.

The editorial board of The Physiology Teacher has been particularly receptive to papers describing mathematical models designed for teaching. Examples include an analysis of the renal excretory response to volume and osmolarity changes (5), an analysis of pulsatile hemodynamics in the aorta (4), an analysis of the evolution of the action potential and the subsequent refractory period (6) and an analysis of the determinants of cardiac output (8). These models generally demonstrate a single, well-founded physiological principle which also has an underlying computational or quantiative basis.

More complex physiological models also exist. These are generally described in internal reports of the agency of origin rather than in the scientific literature. Editorial constraints prevent detailed documentation of complex models in scientific journals. Examples of larger models include a cardiovascular model used primarily for research (3), a model of

respiratory control (2) and a model of the interactions of several organ systems (7).

Generally, both large and small models are used most extensively at the site of origin. The most notable exception is the model by Dickinson (2) describing respiratory control (called MacPuf) which has been written in straightforward FORTRAN and has been widely disseminated. A model by Randall and Coleman (7) describing the interaction of several organ systems (called "HUMAN") has been configured for use on several popular moicrocomputers and this has facilitated dissemination.

To summarize, mathematical models have had little impact to date on the teaching of physiology--but it's probably way too soon for a final judgement.

WHY MODELS MAY REMAIN UNIMPORTANT

Modeling will probably retain its insignificance until a clear superiority over traditional educational methods is demonstrated. If simulation is to supplement traditional methods, models must become easy to acquire and use, their internal structure must be clearly and fully documented, and the physiological phenomena being simulated must be scientifically interesting. At present these requirements are not being satisfied and there is no guarantee that they will be satisfied in the future.

Transportability

Models that are wedded to the peculiarities of a particular computer system cannot be easily transported to other systems, and this alone precludes widespread use. As an example, consider the generation of graphical images. There is such diversity in contemporary hardware that the simple act of changing from a computer terminal of one manufacturer to that of another often requires major reprogramming. Similarly, there are many dialects currently in existence for most high-level programming languages such as FORTRAN and software written on one computer system in one dialect is not easily transposed to another system and another dialect. Such restrictions discourage dissemination, yet it is not at all practical for each physiologist to develop his or her own teaching models just as it's not practical for each to write a physiology textbook.

The Rigors of Authorship

Developing analogies between teaching models and physiology textbooks seems natural enough. Each is used for communication, each has an author or authors, and so forth. Yet there are also some very striking differences which might prevent or at least slow the use of mathematical models in teaching. Certainly, the writing of texts is an attractive professional undertaking and authors are held in high regard by their colleagues; the same cannot be said (at this time at least) for the authors of models. And, the writing of models may be the more difficult of the two tasks. The author of a textbook must be familiar with the physiological concepts to be described and with the native language, namely English. The author of a model must be just as familiar with physiological concepts but he or she must also be skilled in the mathematical description of physical phenomena, computer programming and the numerical methods needed to produce accurate solutions in a reasonable amount of time. Hence, the pool of potential authors-for-texts is certainly much larger at this time than the pool of potential authors-for-models.

Ambiguity vs. Exactness

Some of the most interesting topics in physiology today are at the cutting edge of our scientific knowledge and therefore uncertainties and ambiguities must exist. The author of a text can list and discuss various explanations for a particular physiological phenomena without having to make an explicit evaluation of each possibility in terms of its relative and absolute importance. The author of a model is forced to quantitatively rank each and every documented and speculative physiological mechanism while the model is under construction. Misplaced emphasis in a text can remain hidden for years beneath the standard obfuscation; misplaced emphasis in a model readily becomes apparent as numerical solutions are generated and the solutions are quantitatively compared to laboratory and clinical observations. The thought processes of a model's author are placed in a scientific fishbowl, so to speak, and this exposure can be discouraging. One solution is to construct only models of relatively simple, straightforward, and well-understood phenomena. These phenomena can generally be described quite adequately using the format traditional to lectures and, therefore, modeling will offer only an alternative and not a superior alternative. Simplistic models cannot be expected to elevate simulation to the forefront since the greatest strength of simulation appears to be in analyzing complex processes and in minimizing ambiguity.

Hidden Conjecture

Models that describe and analyze broad phyisological topics tend to be suspect. Large-scale models, probably without exception, contain in their structure a blend of established fact and unsubstantiated conjecture. As long as conjecture is included, these models must be considered to be quantitative hypotheses (1) and not pure repositories for commonly accepted knowledge. Further, the conjectural or hypothetical relationships are not necessarily evident in the performance of the model; they are embedded in the underlying structure and are available only from explicit descriptions provided by the model's author. Teachers have certain prejudices and they will want to select models as an adjunct to teaching that contain

these same prejudices. The cataloging of established facts (and their source) and hypothetical relationships (and the rationale for selecting them) is a ponderous task and one that is usually not relished by model builders. However, this lack of proper documentation impedes the dissemination of models. A practical consideration is that model building and model documentation do not generally lead to publication in the usual scientific journals and this precludes professional advancement based on such publication.

The following section argues that models can have an important role in the teaching of physiology and that heretofore formidable technological barriers no longer exist, but it has been argued above that such enticements may not be sufficient to bring first-rank models into the mainstream of teaching.

WHY MODELING MAY BECOME IMPORTANT

Two arguments that support modeling and suggest that it may become an important part of the teacing of physiology in the future are: 1) Modeling has the potential to offer a student a learning experience available neither in the lecture hall nor the student laboratory and 2) Recent advances in computer science have overcome a variety of technological and financial hurdles.

Homeostasis and Learning Opportunities

The first of these two ideas is built upon the concept of homeostasis. Homeostasis is a dynamic process or, rather, the sum total of many dynamic processes. Three sequential steps are needed to fully describe each component of homeostasis. They are:

1. Defining the basic, underlying mechanisms.
2. Demonstrating the interaction among these mechanisms, particularly as a function of time and changing environmental influences.
3. Cataloging the external signs and symptoms created by such interactions.

Traditional classroom instruction generally cannot satisfy all of these requirements in a uniform way and deficiencies emerge as emphasis is shifted from one aspect to another.

Underlying mechanisms. A complete knowledge of basic, underlying concepts is often not available. Indeed, researchers are continually at work looking for new mechanisms, adding additional descriptive detail to known mechanisms, and evaluating known mechanisms for quantitative importance. At any given time then, knowledge is advancing and uncertainties exist. Because of these uncertainties, a potpourri of underlying mechanisms are presented to the student in didactic presentations, often without a concerted effort by the teacher to evaluate each for quantitative importance. Some mechanisms emerge later as interesting but of little causal importance. On the other hand, a "safe", short-list of underlying mechanisms (as is often used in introductory texts) can rely too heavily on conventional wisdom and fall wide of the mark created by contemporary research.

Simulation can make a contribution here by theoretically investigating one basic mechanism at a time; each can be tested in a mathematical model that explores interactions and predicts the ultimate contributions of the mechanism to homeostasis. Such a theoretical undertaking does not prove causal importance and should never be construed to do so. But it can provide a tentative listing of quantitative importance among competing explanations while we await convincing experimental demonstration.

It is the authors' opinion that many important questions in physiology (and science in general) fall into this category. For instance, many different mechanisms have been proposed as the cause of essential hypertension. Yet, it is not currently clear if any known mechanism, one single mechanism or many mechanisms are actually involved. This question remains in need of experimental clarification. Through the imaginitive use of experimental models, students might possibly become involved in the dialog that leads to clarification of unresolved scientific issues rather than being forced to accept an equivocal, unsubstantiated or over-simplified explanation on an interim basis.

Dynamic interelationships. A second consideration involves dynamic interactions among the basic mechanisms. Lectures can be used to provide detailed descriptions of static conditions and sequential events. But, homeostasis usually involves simultaneous, time-dependent interactions and these are not easily described using conventional language. Worse yet, such interactions cannot be rigorously analyzed using conventional language. In contrast, groups of differential-algebraic equations and their solutions can be used to accurately explore dynamic interrelationships. Hence, one of the strongest features of the use of simulation is found in the analysis of and communication of the dynamic interactions that exist within homeostasis. Many existing models emphasize this point: the evolution of the action potential (6), the dynamics of respiratory control (2), the dynamics of blood pressure control (3) and other physiological processes (7).

The student laboratory theoretically provides an opportunity to demonstrate the dynamic interactions and complexities of homeostasis, but practically this is not the case. Tidball (10) has summarized the most attractive features of student laboratories. Among other things, laboratories introduce the student (a) to the careful and humane treatment of experimental subjects, (b) to biological variation and (c) to the vicissitudes of biochemical determinations. Student laboratories are less successful in demonstrating dynamic

interactions. Firstly, there are time constraints. The relevant time constants must lie well within the three to four hours allocated for a typical laboratory session. Hence, chronic diseases and many other important pathophysiological phenomena cannot be explored.

This is not true with simulation where there are no conventional time constraints and such topics as the evolution of myocardial hypertrophy, management of chronic renal disease or long-term consequences of defects in calcium metabolism, for instance, could be simulated conveniently. A second constraint on the conventional student laboratory comes from limits placed on the type of and the frequency of experimental measurements that can be conveniently implemented. For instance, in the study of insulin-glucagon-glucose interactions, it would be desirable to have repeated and instantaneous measurements of the blood concentrations of these substances over a relatively short interval in investigating the transient response to an applied hormonal or metabolic disturbance. Yet, rapid assay is not possible. Results are usually available only long after the experiment has been completed and possibly forgotten. And, the expense is not trivial. On the other hand simulation can generally provide current values for all variables within a model as a function of time. Although the observed values are only theoretical, a strong sense of interdependency and time-dependency can be developed in the student by using simulation; this is less likely to be achieved using conventional laboratory procedures.

As another example, consider a popular laboratory protocol in the cardiovascular area which involves measuring pulsatile arterial pressure while short-acting vasoactive drugs are injected. This protocol satisfies the constraints of (a) relatively short time constants, (b) straightforward and instantaneous measurements and (c) reasonable costs. Yet, no one would argue that this protocol demonstrates one of the most important concepts in cardiovascular physiology--it's just convenient, that's all. Instead, with imaginative mathematical modeling, it might be possible to (theoretically) investigate the complex interactions of such cardiovascular phenomena as the acute and long-term control of blood volume, redistribution of blood flow in response to metabolic stimuli, neural-hemodynamic interactions or the long-term sequellae to reduced coronary blood flow. Such topics are outside of the purview of the traditional student laboratory at this time.

Descriptive vs. functional physiology. The third consideration in describing a dynamic process involves itemization of readily observable manifestations--that is, listing the signs and symptoms that are to become an important part of clinical medicine. Itemization does not absolutely require prior consideration of basic mechanisms and their interactions and, therefore, it can be undertaken in vacuo if desired. However, memorization of descriptive physiology is only partially effective at best. It has been reported by others at this symposium that a minimal amount of detailed information is retained by medical students several years after completion of a traditional medical physiology course.

When several complex systems are interacting, the total number of combinations of signs and symptoms must be close to uncountable. Hence, memorization of ultimate effects, while important, can also become mired in inefficiency in realistic situations. The debate concerning how much teaching emphasis should be placed on descriptive physiology and how much emphasis should be placed on functional relationships continues, but simulation may be used to emphasize and to strengthen the functional aspects of physiology and thereby offer some relief from the mental burdens associated with the descriptive components.

Technical Advances

Historically, it's been easier to build mathematical models than to solve them. Further, some of the most interesting features of real systems result from non-linearities; non-linear models (that is, families of non-linear differential-algebraic equations) are comparatively hard to solve.

The advent of the digital computer several decades ago and refinement of the necessary numerical methods shifted the status of all but the most elementary mathematical models from intractable to solvable, albeit with moderate to great difficulty in some cases. Models with severe non-linearities and widely ranging time constants can require considerable computer time to produce a single solution. Hence, large digital computers have traditionally been used most often in digital simulation and such computers are generally not readily available to physiologists--particularly for teaching-related use. Hence, the greatest successes to date have been found in aerospace and defense applications. One could argue that the use of simulation in physiological research and teaching has not yet been fairly and completely evaluated.

The historical picture is changing rapidly with several technological advances. Among other things, the number of solid-state computer elements that can be combined in a single package is increasing and the cost of computer memory is decreasing. In short, powerful computers are now available in compact configurations at reasonable prices. The potential impact of these developments on biomedical simulation is enormous. The current generation of small computers are inexpensive enough to be purchased by the teaching community in numbers which allow adequate student contact. These same machines are also powerful enough to solve complex mathematical models relatively rapidly.

Inexpensive display devices capable of generating detailed colored images are also now available and this may add to the attractiveness of simulation. Subsequent generations of small computers should be even more attractive. Hence, the utility of biological modeling may be about to get a fair test.

In 1978, Katz and colleagues (4) described a cardiovascular simulation for use in a student laboratory. Changes in arterial pressure were calculated in response to acute interventions and results were displayed on a polygraph. In addition to the requisite polygraph, a computing system costing approximately $70,000 was needed. It was also typical of that era that hardware configurations were not standardized and software could not be readily transported from one system to another. Today, this same simulation could be implemented on a $3,000 microcomputer having an increase in computing power of possibly 5- to 10-fold. Further, many popular microcomputers are available in large numbers and software can be transferred among similar machines with little or no alteration.

In total, many of the traditional computational and financial hindrances to successful simulation appear to be gone or disappearing rapidly. It remains to be seen whether or not this will be a decisive step in creating an important role for modeling in the teaching of physiology.

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HEARTSIM: A CARDIOVASCULAR SIMULATION WITH DIDACTIC FEEDBACK.

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INTRODUCTION

Computer simulations are a comparatively recent addition to the body of methods that are used to teach physiology. Simulations share with other non-lecture techniques the potential for helping students to be active learners. They provide students with a means to observe, analyze and understand the interaction between the components of complex systems by simulating "experiments" many of which are difficult or impossible to carry out in the teaching laboratory.

We have used computer simulations at Rush in scheduled, faculty-supervised "laboratories". In these labs the students work in groups. We suggest that they first follow a specific protocol that illustrates the most important principles before going off on their own, trying procedures that are more complex or less directly related to the course objectives. We encourage them to discuss each protocol step and try to arrive at a group consensus about the outcome of each procedure before they actually simulate it on the computer. The faculty circulate, question and challenge the students, and encourage group discussion. They point out phenomena or relationships that the students are likely to miss. They try to insure that the laboratory becomes an on-line learning experience.

However, there are some circumstances that make this process less than optimal. If the lab takes place before the students have studied the relevant material, they will be unable to make rational predictions or to explain or understand the behavior of the system being simulated. So, much of the teaching effectiveness of the exercise is lost. Also, some students feel hampered by having to work in a group or by having to work with peers who may be much faster or much slower than they. This discourages their participation and it may cause them to avoid the lab entirely.

For these and other reasons, we wanted to have the simulations available as free-standing units. Students could then use them whenever they felt ready. They could work alone if they wanted or in a group of their own choosing. However, we did not want the students to lose the positive benefits that derive from free discussion of the physiology, from the necessity of having to predict the outcome in advance, and from being challenged by a faculty member. We wanted to avoid the confusion that often accompanies using a simulation for the first time. We also wanted to make sure that the students were not tempted to slip into "cook-book mode", i.e., to carry out procedures and copy down the output data without trying to understand what is happening while it is happening. When students do this they honestly intend to review and make sense of the data later. However, they often do not or cannot do this. Then they have lost an opportunity to learn in this useful way and they have wasted their time.

To create our first stand-alone simulation we translated and reformatted the well known cardiovascular simulation MacMan (1,2) to run on PLATO. We then expanded the program in an important and we think unique way, by integrating an instructional component into it. This unit guides the students and provides didactic feedback based on the students' input, much like a teacher does. In fact the didactic component was designed to mimic the combined activity of a student group and an active, interested teacher in a laboratory setting.

We call this interactive version of the simulation HEARTSIM.

HEARTSIM

Heartsim starts by presenting a list of available procedures. These are stimuli to the CV system for which the program provides didactic feedback (Figure 1). The student starts by selecting one of these.

Briefly, what follows each protocol step is in sequence:

1) the student predicts the qualitative effect of the procedure,

2) the predictions are reviewed for logical consistency and to be sure that they agree with certain basic physiological relationships,

3) the program simulates the effects of the procedure providing both graphical and numerical output,

4) the qualitative results of the simulation are compared with the student's predictions, and finally

5) the program reacts to disagreements between the simulation output and the student's predictions, providing corrective and reinforcing feedback.

INSTRUCTIONAL INDEX

1. INTRODUCTION

Please select 2a if this is the first time you have used this exercise.

ARTERIAL RESISTANCE

2a. A sudden decrease to 50% of normal

2b. A patient with denervated baroceptors
- a decrease to 50% of normal R_a

3. CARDIAC CONTRACTILITY falls to 50% normal

4. VENOUS RESISTANCE increases to 200% normal

5. HEMORRHAGE
- a 1 liter hemorrhage
- an additional liter
- an additional 1/2 liter

6. INTRATHORACIC PRESSURE
- an increase to 0 mm Hg
- an increase to 4 mm Hg
- an increase to 8 mm Hg

Choose a topic and press NEXT.
BACK at any point will return you to this index.
SHIFT BACK for the main index.

Figure 1. The Instructional Index lists the procedures that have guided instruction, i.e. provide interactive feedback to the student user. All of the figures in this paper are prints from the monitor screen. The prompts listed at the bottom of the figure (for example NEXT, BACK, shiftNEXT) allow the user to control the direction of the lesson. All of the available prompts are not shown in later figures.

* * * * * * *

All of the feedback is organized around a matrix, the Predictions Table. Students enter their predictions into the table. To make these predictions a student must visualize how each procedure will impact the CV system, then must apply physiological principles to known relationships. Thus, in filling out the Predictions Table a student partially emulates the group discussion that we encourage when the simulation is used in the laboratory.

The first time that a student uses Heartsim, the program slowly leads him through the Predictions Table. The student is first asked to predict the direct physical effect (DR) of the experimental procedure on 8 variables: heart rate (HR), stroke volume (SV), cardiac output (CO), cardiac contractility (CC), arterial resistance, (R_a), mean arterial blood pressure (P_a), atrial pressure (P_{atr}), and capillary pressure (P_c). Entries are made by touching the monitor screen. One touch enters ↑, a second enters ↓ and the third enters 0 for increase, decrease and no change, respectively (see Figure 2). Usually all of the variables are listed in the table at the start of a procedure and the student must completely fill in the table before receiving any feedback. However, the first time that a student uses the lesson, the program provides definitions, instructions and reactive feedback immediately following each entry in the DR column.

When the DR column is completed, the student enters his predictions of the changes caused by the baroceptor reflex response (RR) in the second column and shows the changes that he expects to be present in the final steady state (SS) in the third column (see Figure 3).

PREDICTIONS TABLE

Parameters:	DR			
Heart Rate				
Art. Resist.	↓			

PROBLEM 2 a

Arterial Resistance is decreased to 50%

To enter a prediction, just touch the screen once for ↑, twice for ↓, and again for 0 (no change).

First, we're going to ask you to predict the qualitative changes that would result for the 50% ↓ R_a before a reflex can be activated, i.e. the direct physical consequence (DR) of the stimulus.

Press SHIFT NEXT when done.

Figure 2. Students must predict the effects of each procedure before the computer simulation is executed. Step 2a, a 50% reduction in arterial resistance (Ra), provides the student with detailed instructions on how to enter predictions into the Predictions Table. The figure shows the start of this process, in which the student enters the direct physical effects (DR) of this procedure in the first column.

The Predictions Table is organized in this way to condition the students to think in terms of the causal sequence that occurs in the intact organism following some stimulus to the CV system. It also helps students to understand the source of the system transients and to begin to get a feel for how long these reactions take.

PREDICTIONS TABLE

Parameters:	DR	RR	SS
Heart Rate	Ø	↑	
Stroke Vol.	Ø	↑	
Cardiac Out.	Ø	↓	
Card. Contr.	Ø	↑	
Art. Resist.	↓	↑	
Mean B.P.	↓	↑	
Atrial P.	Ø	↑	
Capillary P.	↑	↓	

Physiology relationships: $CO = HR \times SV$ $BP \approx CO \times R_a$
$P_{atr} \propto 1/CO$ when CO is an independent variable

PROBLEM 2 a

Arterial Resistance is decreased to 50%

Now let's do the last column.
This should show the change in each of the variables that will be present at the final steady state (SS). It is determined by the combination of DR and RR. If you don't understand this, press HELP. It must also be consistent with the relationships shown Press SHIFT NEXT when you have finished.

Figure 3. The student has entered the direct physical effects of lowering arterial resistance in column 1 of the Predictions Table (DR). The effects of the baroceptor reflex (RR) must now be entered into in column 2 and the final steady state change (SS) in each variable into column 3.

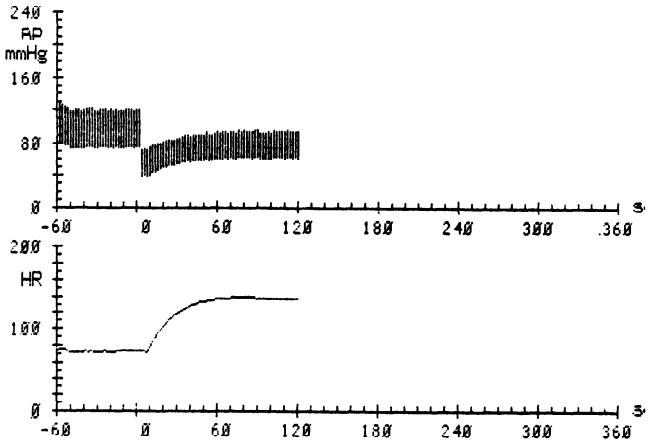
* * * * * * *

A student may make three kinds of errors in filling in the Predictions Table: 1) logical errors which mismatch transient and steady state changes, 2) errors which mis-state the relationship between two or more variables in a physiological relationship, and 3) errors which mis-predict the effects of the "experimental" procedure. The first two of these do not specifically relate to any particular CV stimulus. Hence they are reviewed immediately after the Predictions Table has been completed. The third relates to the specific procedure that is about to be carried out. These errors are reviewed after the computer has simulated the effects of the procedure.

The statement below the Predictions Table in Figure 3, "...the final steady state (SS)...is determined by the combination of DR [the direct response of a variable to the "stimulus"] and RR [the reflex response that follows....]," refers to the need for a student's predictions to have internal logic (consistency). For example, in Figure 3, the student predicted that a 50% decrease in arterial resistance caused no direct change in cardiac contractility (Card. Contr.) and that the subsequent reflex response caused contractility to increase (DR = 0, RR = ↑) . It would now be illogical for him to predict that in the steady state (SS) the cardiac contractility would be either decreased or unchanged. If the student does not understand this, he may request HELP (see bottom of Figure 3). The logical system would then be discussed and he would be led through a "truth table" which gives all possible correct combinations of DR, RR and SS.

A student's predictions must also conform to the physiological relationships given below the Predictions Table in Figure 3. Any errors must be corrected before the simulation is carried out.

Arterial Resistance is decreased to 50%
I feel faint when I try to stand up.



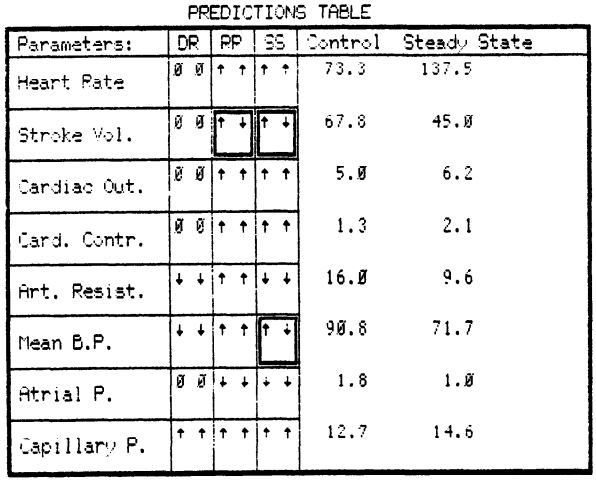
	Normal	Control	Steady State	
Time	Ø	Ø	120	sec
Heart Rate	73	73	138	bpm
Stroke Vol.	68	68	45	mL
Card. Output	4.96	4.97	6.19	L/min
Card. Contr.	1.3	1.3	2.1	L/min/mmHg
Art/Ven Rest.	16/2.2	16.Ø/ 2.2	9.6/ 2.2	mmHg/L/min
Mean B. P.	90	91	72	mmHg
RAP/Cap.P.	1.8/13	1.8/ 13	1.Ø/ 15	mmHg

Press NEXT to continue or LAB to see your predictions.
Press SHIFT NEXT to compare the results with your predictions.

Figure 4. The computer simulation of the effects of a 50% ↓ in Ra are shown. Arterial pressure and heart rate are plotted as a function of time. The table gives the normal, control and final steady state values of nine variables.

The simulation has two outputs (Figure 4). The graphic output in Heartsim is a plot of HR, systolic pressure (P_s) and diastolic pressure (P_d) against time. This gives students a view of how these events change in time and illustrates transients. Students often ignore or are unaware of transients in physiological responses. Heartsim's numerical output is a table with the normal, initial and steady state values of 9 variables.

Students may examine the simulation output at their leisure. Then the results are entered into the Predictions Table and disagreements between the student's qualitative predictions and the simulation's are outlined (Figure 5). Finally, the program reacts to each point of disagreement, providing appropriate feedback in each case.



PREDICTIONS TABLE

Parameters:	DR	RP	BS	Control	Steady State
Heart Rate	Ø Ø	↑ ↑	↑ ↑	73.3	137.5
Stroke Vol.	Ø Ø	↑ ↓	↑ ↓	67.8	45.Ø
Cardiac Out.	Ø Ø	↑ ↑	↑ ↑	5.Ø	6.2
Card. Contr.	Ø Ø	↑ ↑	↑ ↑	1.3	2.1
Art. Resist.	↓ ↓	↑ ↑	↓ ↓	16.Ø	9.6
Mean B.P.	↓ ↓	↑ ↑	↑ ↓	9Ø.8	71.7
Atrial P.	Ø Ø	↓ ↓	↓ ↓	1.8	1.Ø
Capillary P.	↑ ↑	↑ ↑	↑ ↑	12.7	14.6

The left sign in each box is your prediction.
The right sign is the Heartsim prediction.

3 of your predictions don't agree with the simulation.
Press SHIFT NEXT for a review of some of these errors.

Figure 5. The student's predictions are shown on the left and the simulation's on the right in each box. If the two are different, the box is outlined. Later, the physiological basis for the simulation's output in each of these instances will be reviewed with the student. The simulation's quantitative output is given in the right side of the Predictions Table.

The feedback may:

1) describe or discuss the underlying physiology,

2) test the student's understanding of specific facts or relationships, i.e. ask questions and respond to student answers,

3) summarize the changes observed in the simulation, or

4) at one point a small review lesson on the baroceptor reflex is provided. (This is one of a growing number of stand-alone, single topic computer lessons that we have written.)

When this review is completed, the student may select another (or the same) procedure from the protocol list.

CONCLUSIONS

Computer simulations can help students to be active learners. They provide a basis for students to integrate the components of systems and see the way that these parts interact. Heartsim provides an additional benefit. It can detect, on-line, student misconceptions about the system's function and errors in the student's thinking. It provides immediate feedback to correct these mistakes while reinforcing correct facts, ideas and problem solutions. Heartsim has enough variety in the available procedures to uncover many, if not most, of the users major misconceptions. Thus Heartsim's interactive didactic feature provides an added educational benefit over traditional simulations for the independent student learner.

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WHAT ARE WE DOING OUTSIDE OF THE LECTURE HALL AND WHY ARE WE DOING IT: A SUMMARY

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According to the recent survey on physiology teaching in the early 1980's (C. Rothe, The Physiologist 26, No. 3, 1983) lectures continue to be the dominant mode of instruction by a wide margin, but there is a great variety of other teaching methods that is used as well.

These non-lecture activities can be classified as labs ("wet" or "dry"), problem solving exercises, and various uses of computers. Regardless of the exact format, all seem to have as one of their goals the development of problem solving skills.

Three quite different approaches to laboratory instruction were described. In one (Richardson), the laboratory experiments are designed and carried out by students. This is surely the antithesis of the "cookbook" approach that conventional teaching laboratories often seem to foster. The active participation of all students would appear to be maximized in this way. In the second example (Traber et al) students carry out a series of experiments that integrate material across various organ systems and disciplines; the experience of dealing with "real", complex systems ought to provide the strongest possible message about the need to understand the function of an entire organism. Active participation is encouraged by the highly structured approach to the experiments and scheduled follow-up conferences. Yet a third kind of laboratory experience was described by Dr. Rothe a mechanical model of the heart of circulation is "exercised" by the students in order to gain an appreciation for the many interacting physical variables that determine system performance. Of interest is the experiential variety that is possible in the student laboratory.

The next three papers described non-laboratory approaches to problem solving. Dr. Coulson's problem-based learning modules represent a comprehensive approach to curriculum design which focuses on independent study in a problem solving environment. The papers by Michael and Rovick and by Beraldo and Alvarenga both discuss more limited problem-solving applications. Michael and Rovick describe the use of pathophysiology as a way of engaging student interest while focusing on integration (the interaction of system components) and problem solving. Beraldo and Alvarenga are concerned with the conditions that need to be created to encourage active, self-directed participation by the student in his own education. These two papers are complimentary in that the success of the tutorial sessions in which pathophysiology problems are considered requires exactly those conditions described by Beraldo and Alvarenga.

The three papers on computer-based exercises superficially appear to deal with quite different approaches to non-lecture teaching. Rovick and Brenner describe the use of a single system simulation (of the heart, circulation and baroreceptor reflex) as a stand-alone educational resource. The simulation is embedded in a program that requires students to predict system responses and provides appropriate feedback to correct errors. Hogan (Computer generated figures in physiology teaching; the "smart video slide") has developed a set of graphic displays of simple physiological systems that can be used either in a lecture/demonstration format, or in a laboratory in which the students may "exercise" the model. Coleman and Randall use of their experience with HUMAN, a very large model that encompasses most of the major organ systems to, discuss the benefits that can be derived from modelling.

But the seeming differences in these three approaches overlay a more important common feature. All of them encourage students to actively participate in the learning process and to focus on problem solving and the integration of information as key elements in the mastery of physiology.

In fact, these features characterize all of the non-lecture teaching exercises that have been described here. They are all designed to bring students out of their role as passive absorbers and memorizers of information (their behavior in the lecture hall). They all encourage students to become active problem solvers.

A variety of learning methods can be used to achieve these ends. For each we must carefully define the goals that we are seeking to achieve (and there are usually multiple goals for any teaching activity) and we must design the exercise to take advantage of features that will make the exercise attractive to the students and effective in working towards the stated goals; we must "human factor" all of our non-lecture activities if they are to be maximally beneficial.

Travel Grant Award Program XXIX International Congress of IUPS Sydney, Australia, Aug. 28–Sep. 3, 1983

The Physiologist, December 1981, contained an announcement of the XXIX International Congress of the International Union of Physiological Sciences to be held at the University of New South Wales, Sydney, Australia, August 28–September 3, 1983. In an effort to aid American scientists, who would not be able to attend without some financial assistance, the American Physiological Society submitted proposals to a number of Federal agencies and other organizations for travel grant awards designated specifically for the Congress. The APS proposed to handle the funds and administer the program by awarding the grants, with the US National Committee (USNC) of the IUPS sponsoring the program and selecting the award recipients. The travel grant award program was announced in *The Physiologist* (Vol. 25, No. 3, p. 153), June 1982, with a deadline date for receipt of applications of October 15, 1982—later extended to November 30, 1982, in conformance with an extension of deadline for Congress registration and abstract submission.

The USNC/APS travel grant program offered a limited number of travel awards to qualified physiologists, permanent residents of North America (US, Mexico, and Canada) and Hawaii, who required such assistance and who plan to participate fully in the XXIX Congress. The USNC/APS established a screening and selection committee, and the APS acted as fiscal agent and awarder of the travel grants. A major intention of the USNC/APS Selection Committee was to give particular attention to applications from younger physiologists.

Submission of proposals and allocation of funds by APS and USNC/IUPS resulted in $172,900 being available for travel grants, as follows:

National Institutes of Health	$81,400
Coordination of proposal review by the Fogarty International Center of NIH resulted in a number of NIH agencies and institutes providing joint funding of $81,400: Natl. Inst. of Child Health and Human Development, Natl. Inst. of General Medical Service, Natl. Inst. of Arthritis, Diabetes, and Digestive and Kidney Diseases, Natl. Heart, Lung, and Blood Inst., and Fogarty International Center	
National Science Foundation	$25,000
The Kroc Foundation	$ 7,500
American Physiological Society	$44,000
US National Committee/IUPS	$15,000

In response to the announcement of the availability of travel grant funds, approximately 600 requests for applications were received and 411 were returned in time to be reviewed. A Selection Committee of five members of the USNC (Dr. James B. Bassingthwaighte, Chairperson, and Drs. David H. Cohen, Francis J. Haddy, Charlotte P. Mangum, and Loren J. Mullins), representing all of the adhering societies as well as different areas of expertise, appraised and scored the applications. In the selection procedure priority in scoring was given to plenary lecturers, symposia chairpersons, and invited symposia speakers and, in particular and in accord with commitments to the IUPS and funding organizations, emphasis was placed on physiologists who had received their highest earned degree most recently—within the last 15 years.

The Selection Committee scored each applicant by applying various points to an applicant's credentials. These included: "scientific age" as defined by 15 points minus one for number of years beyond the year of the highest earned degree (including 15 points for those about to receive a degree); congress participation (letter of invitation was received as documentation), namely, plenary lecturers and symposia chairpersons, symposia speakers and participants, planned free communication; publications, for which the standard was two per year, for the past five years in good quality journals (not more than five, excluding abstracts and papers in press, were requested); abstract of the Congress presentation (250 words only); resume of purposes of the trip other than attending the Congress (other meetings, satellite symposia, laboratory visits, collaboration).

The results were averaged and rank-ordered, and awardees were selected and approved in early December 1982 so as to advise applicants of their acceptance before the deadline for submission of registration fees for the Congress (January 31, 1983). Most of the award recipients were notified formally by the APS by letter of December 27, 1982. The stipend for each awardee was calculated to be approximately $300 less than the "super-saver" round-trip airfare from an awardee's nearest major airport to Sydney, Australia. This fare in turn was based on a Los Angeles-Sydney (or San Francisco-Sydney) specially negotiated fare of $849, which included unlimited stopovers en route.

There were 169 travel grants awarded. Of these a total of 124 went to awardees who had earned their highest earned degree within the last 15 years (71 within 5 years, 30 within 10 years, and 23 within 13 years). There were

13 invited plenary lecturers and/or chairpersons and 71 invited symposia speakers.

In sponsoring travel grants, the Federal Government regulations restrict the expenditure of the monies to US residents and stipulate that, when flying from and to the United States, American air carriers must be used. Accordingly, the awardees were divided into two groups, those eligible for Federal grant funds and those who were not (non-US residents, US government employees). There were nine awardees who were US government employees and seven Canadian scientists. These 16 persons plus a few other special cases were funded by the private funds available from APS, USNC, and The Kroc Foundation sources (as noted above).

Membership held by applicants in any or none of the six societies was recorded from the applications submitted and evaluated. The following table shows the societies, the number of applicants per society, percent having received awards, the number of awardees per society, and percent having received awards.

Society	Applicants		Awardees	
	No.	% Awardees	No.	% Awardees
American Physiological Society	268	37%	99	58%
Society for Neuroscience	138	42%	58	34%
Society of General Physiologists	49	49%	23	14%
American Society of Zoologists	36	44%	16	9%
Microcirculation Society	28	36%	10	6%
Biomedical Engineering Society	10	20%	2	1%
Not a Member of any of the above societies	51	53%	27	16%

(Percentages add up to over 100% because of multiple memberships of individuals.)

The award recipients represented persons from 35 states, Puerto Rico, and Canada, as follows:

California	30
New York and Massachusetts	14 each
Maryland	11
North Carolina	9
Pennsylvania	8
Illinois, Ohio, and Texas	7 each
Virginia and Washington	5 each
Alabama, Florida, Connecticut, and Oregon	4 each
Arizona, Colorado, and Michigan	3 each
New Hampshire, Nevada, Wisconsin, Indiana, and Iowa	2 each
Arkansas, Kansas, Louisiana, Maine, Mississippi, Missouri, Nebraska, New Jersey, Puerto Rico Tennessee, Vermont, and Utah	1 each
Canada	5

To minimize administrative expense, since no allowance for overhead or indirect costs was included in the government grants, most recipients received their award in the form of a "credit" in their name with the travel agent (selected competitively by the US National Committee), Chevy Chase Travel, Inc., of Bethesda, MD. This use of a single travel agent for most awards allowed negotiations of a favorable airfare and blocking of space to assure awardees and other physiologists seats on appropriate schedules. Those awardees wishing to make travel arrangements independently were presented with travel vouchers to be submitted for reimbursement on completion of the travel.

Future Meetings

1983	
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 3, Lexington
1985	
FASEB Annual Meeting	Apr 21-26, Anaheim
*APS "Fall" Meeting	Aug 4-9, Buffalo
1986	
FASEB Annual Meeting	Apr 13-18, St. Louis
IUPS Congress	July 12-20, Vancouver, Canada

*Campus meeting

Symposia for 1984 Spring Meeting

Regulation of phosphoprotein photophatase activity
Organized by J. DiSalvo
Sponsored by Society for Experimental Biological Medicine

The Control of Cell Volume
Organized by A. L. Finn
Sponsored by Society of General Physiologists

Theoretical trends in neuroscience (2 sessions)
Organized by H. Lecar and J. Rinzel
Sponsored by Society for Mathematical Biology

Systems analysis of biocontrol
Sponsored by Biomedical Engineering Society

Histamine and the lung's circulation
Organized by A. L. Hyman
Cosponsored by Respiratory Physiology Section and the American Heart Association Cardiopulmonary Council

Capillary endothelium: cellophane wrapper or metabolic barrier?
Organized by H. V. Sparks, Jr.
Sponsored by Cardiovascular Section

Membrane ATPase function in vascular smooth muscle during hypertension
Organized by R. K. Hermsmeyer
Sponsored by Cardiovascular Section

Mediator mechanisms in shock
Organized by R. F. Bond
Sponsored by Cardiovascular Section

A history of neurophysiology and the latest developments
Organized by J. Trubatch
Sponsored by Nervous System Section

Sexual differences in neural development/sexual dimorphism of CNS
Organized by C. D. Toran-Allerand
Sponsored by Nervous System Section

Neurobiology of aging
Organized by C. E. Finch
Sponsored by Nervous System Section

Functional interaction of developing neurons with their target tissue
Organized by G. Pilar
Sponsored by Nervous System Section

Role of guanine nucleotide binding proteins in biology
Organized by A. G. Gilman
Sponsored by Endocrinology and Metabolism Section

Role of tyrosine phosphorylation in the action of hormones and growth factors
Organized by J. Avruch
Sponsored by Endocrinology and Metabolism Section

*Membrane biogenesis
Organized by G. Blobel

*Jointly sponsored by the following APS Sections: Cell and General Physiology, Epithelial Transport, Gastrointestinal, and Renal Physiology.

*Membrane component turnover
Organized by G. Ashwell
*Membrane modification by fusion events
Organized by Q. Al-Awqati
*Intestinal transport: structural and functional adaptive responses
Organized by H. J. Binder
*Renal transport: structural and functional adaptive responses
Organized by J. B. Wade
Regulation of renal phosphate transport
Organized by V. Dennis
Sponsored by Renal Physiology Section
Central mechanisms in the control of salt and water intake
Organized by D. J. Ramsay
Sponsored by Water and Electrolyte Homeostasis Section
Regulation in physiological systems during exercise in untrained and trained—five year update
Organized by F. W. Booth
Sponsored by Environmental, Thermal and Exercise Physiology Section
Thermogenesis: its role in the development and maintenance of obesity
Organized by J. S. Stern and B. A. Horwitz
Co-sponsored by Environmental, Thermal and Exercise Physiology Section and AIN
Skeletal muscle fiber regeneration following injury
Organized by J. A. Faulkner and B. M. Carlson
Sponsored by Environmental, Thermal and Exercise Physiology Section

Announcements

Comparative Physiology Section

The usual joint meeting of APS-American Society of Zoologists was scheduled for Fall 1984. Due to a scheduling conflict with an international symposium which will heavily involve ASZ, the next joint meeting will occur in the Fall of 1985 (information communicated by Dr. John Roberts on behalf of ASZ). Suggestions or proposals for symposia for Fall 1985 should be addressed to Dr. Donald C. Jackson, Program Officer for Comparative Physiology, Division of Biology and Medicine, Brown University, Providence, RI 02912.

Burroughs Wellcome Clinical Pharmacology Award

Advancements in the medical sciences have brought new therapeutic concepts and drug entities. The elucidation of disease processes, the development of new drugs and the determination of their relative efficacy and safety, and continued research to establish improved methods for measuring the effects of drugs in man require the skills of trained clinical pharmacologists. The unfilled needs for clinical pharmacology manpower to respond to these requirements, and also to function in teaching capacities, continue at a high level. In response to this need the Burroughs Wellcome Fund is offering a Clinical Pharmacology Award for 1984 in the amount of $200,000, payable in annual installments of $40,000. The aim of the Clinical Pharmacology Award is to support an individual who has chosen Clinical Pharmacology as a career objective and who will promote research, strengthen teaching programs, and attract young men and women interested in a career in this discpline. The Award recipient is known as a Burroughs Wellcome Scholar in Clinical Pharmacology. The Award is available to full-degree granting US medical schools to initiate and develop a new Division in Clinical Pharmacology or, alternatively, to provide for the salary of a faculty member in an established division. Invitations to apply for the 1984 Clinical Pharmacology Award are being sent to the Deans of medical schools and to the Chairmen of the Departments of Medicine and Pharmacology. *Deadline for applications nominating candidates for the 1984 Award:* November 1, 1983. The Award recipient, or recipients, will be announced in early Spring 1984. *For further information contact:* The Burroughs Wellcome Fund, Research Triangle, NC 27709. Phone: (919)248-3000.

Election to the American Academy of Arts and Sciences

APS member Dr. **Christina Enroth-Cugell** of the Departments of Engineering Sciences (Biomedical Engineering Division) and Neurobiology and Physiology of Northwestern University has been elected Fellow of the American Academy of Arts and Sciences. Professor Enroth-Cugell is a native of Finland and attended the Karolinska Institutet in Stockholm, Sweden, where she obtained her M.D. and doctorate degrees. Her research is devoted to physiological studies of the properties of the neural circuitry of the mammalian retina.

Second International Congress on Myocardial and Cellular Bioenergetics and Compartmentation

The Second International Congress on Myocardial and Cellular Bioenergetics and Compartmentation will be held February 16–18, 1984, at the University of Southern California, Los Angeles. Topics: Microcompartmentation and Energy Transport; Respiration Control—Cellular and Organ Level; Myocardial Preservation and Ischemia; Pathophysiology of Energy Compartmentation; Calcium, Magnesium and Bioenergetics; P^{31} NMR. There will be Free Communications (abstracts), 2 sessions; Poster Session, 1 session; and Poster Discussion Groups.
For more information contact: N. Brautbar, M.D., USC/LAC Medical Center, Dept. of Medicine, 2025 Zonal Ave., Los Angeles, CA 90033. Phone: (213)226-4768 or (213)226-7337.

APS Committees, Their Principal Functions and Membership (1983-1984)

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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E. E. Windhager
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Finance Committee

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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Education Committee

Conducts educational and teaching programs and develops teaching resource material that may be required by the Society. This includes naming tutorial lecturers, organizing teaching sessions and the refresher course conducted at APS meetings.

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Detailed functional statements for each committee are included in the APS Operational Guide distributed annually to officers and committee chairmen.

Program Executive Committee

Selects the scientific symposia and special sessions to be organized or supported by APS, develops policies regarding the conduct of scientific sessions and names the recipients of the Caroline tum Suden Travel Fellowship.

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Program Advisory Committee

Recommends to the Program Executive Committee scientific programs for APS meetings. Members of this committee also organize contributed abstracts into sessions, select session chairmen and introductory speakers, nominate candidates for the Caroline tum Suden Travel Fellowship.

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Public Affairs Executive Committee

Advises Council on all matters pertaining to public affairs that affect physiologists and implements public affairs activities in response to Council guidance.

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Public Affairs Advisory Committee

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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Ray G. Daggs Award Committee

Annually selects a member of the Society to receive this award in recognition of distinguished service to the Society and to the science of physiology.

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Membership Committee

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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Honorary Membership Committee

Recommends to Council candidates for nomination to Honorary Membership, distinguished scientists who have contributed to the advancement of physiology.

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Perkins Memorial Fund Committee

Selects recipients for visiting scientist family support awards and administers the Fund.

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Porter Physiology Development Committee

Selects recipients for visiting scientists and professorships; teaching and training fellowships, aimed at improving physiology departments of medical schools with predominantly minority enrollments. The Committee also supervises the administration of funds provided for this program.

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Animal Care & Experimentation

Maintains the APS "Guiding Principles in the Care and Use of Animals" by recommending changes for Council's consideration. Also provides other committees with consultation regarding animal experimental procedures and care.

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Committee on Committees

Serves as an advisory committee to Council for the purpose of making recommendations for nominees to the standing committees.

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Centennial Celebration Committee

Utilizes the activities of the 1987 Centennial Year to make the scientific community and lay public aware of the history, nature and contribution of physiology and physiologists. This committee will phase out not later than 1989.

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Public Information Committee

Furnishes guidance and advice to the Federation and APS Council regarding the dissemination of newsworthy information reported at APS scientific meetings. Recommends for Council's consideration and implements programs promoting public information concerning physiology.

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Senior Physiologists Committee

Maintains liaison with senior and emeritus members.

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Career Opportunities in Physiology

Provides Council with information regarding availability and needs for appropriately trained physiological personnel and recommends measures to assure proper balance in the supply and demand for physiologists.

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Financial Development Committee

Develops and implements programs designed to attract financial support aimed at reducing Society dependence on member dues payments.

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Liaison with Industry Committee

Fosters interactions and improved relations between the Society and industry. Develops new ways that the Society and industrial concerns can interact in mutually beneficial ways.

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Women in Physiology Committee

Deals with all issues pertaining to education, employment, and professional opportunities for women in physiology.

Provides the APS representative to the Federation of Organizations for Professional Women and, with two members of the Career Opportunities Committee, administers the Caroline tum Suden Professional Opportunity Award.

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In compliance with the Society's bylaws, a number of sections have been organized encompassing various physiological specialty interests. These sections advise the Society on matters of interest to the specialty represented by the section, assist the Society in organizing scientific meetings, and nominate individuals for membership on Society committees.

Membership in the sections is open to all members of the Society. However, the Statement of Organization and Procedures for each section establishes specific requirements for membership. APS members who wish to become affiliated with one or more of the listed sections should comply with the requirements noted following the named section. The reference shown beneath the name is the issue of *The Physiologist* where that section's Statement of Organization and Procedures has been printed.

Cardiovascular. 23(5): 5, 1980. Send a letter requesting affiliation to the Membership Services Department of APS.

Cell and General Physiology. 24(3): 35, 1981. Same as Cardiovascular.

Comparative Physiology. 20(6): 14, 1977. Indicate a primary or secondary Interest Area Code 10 on the Membership Records Questionnaire.

Endocrinology and Metabolism. 23(5): 8, 1980. Same as Cardiovascular.

Environmental, Thermal and Exercise. 20(6): 15, 1977. Indicate a primary or secondary Interest Area Code 13 or 14 on the Membership Records Questionnaire.

Gastrointestinal. 20(1): 5, 1977. Same as Cardiovascular.

Nervous System. 21(3): 25,1978. Indicate a primary or secondary Interest Code 25 on the Membership Records Questionnaire.

Renal Physiology. 20(2): 17, 1977. Attend Renal Dinner at the Spring Meeting.

Respiration Physiology. 23(5): 6, 1980. Indicate a primary or secondary Interest Code 32 on the Membership Records Questionnaire.

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