THE PHYSIOLOGIST

The American Physiological Society was founded in 1887 for the purpose of promoting the increase of physiological knowledge and its utilization. The APS Constitution and Bylaws appears in the FASEB Membership Directory. Officers: *President,* Howard E. Morgan, Pennsylvania State University, Hershey, PA; *Past President,* John B. West, University of California, La Jolla, CA; *President-Elect,* Franklyn G. Knox, Mayo Medical School, Rochester, MN; *Council,* Shu Chien, Harvey V. Sparks, Jr., Norman C. Staub, Aubrey E. Taylor; *Executive Secretary-Treasurer,* Martin Frank, 9650 Rockville Pike, Bethesda, MD 20814.

A Publication of the American Physiological Society, Martin Frank, Editor — Volume 28, Number 4 — August 1985

Editorial

Public Affairs

APS News

LSRO Report

Announcements

The Physiology Teacher

36th Annual Fall Meeting

The Physiologist (ISSN 0031-9376) is published bimonthly by the American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814. Subscriptions: Distributed with the Physiology Teacher to members as part of their membership; nonmembers and institutions, $60.00 per year in the United States; elsewhere $75.00. Single copies and back issues, including Fall Abstracts issue, when available, $12.00 each. The American Physiological Society assumes no responsibility for the statements and opinions advanced by contributors to *The Physiologist.*

Cover: William Beaumont, M.D., in front of Fort Niagara where he served as a young man.

Editorial

Reflections

During the past several months while preparing to receive the baton from Orr Reynolds and thus become the fourth Executive Secretary-Treasurer of APS, I have had the opportunity to think about the history of the Society and the lessons to be learned. I felt that reflection on APS' historical precedents might provide a means to facilitate a smooth transition and a means to anticipate the needs of the Society membership.

In 1887 when the five founders of APS organized the first meeting, they were seeking a vehicle for exchange of scientific knowledge and research findings. To that end, APS has fulfilled the objectives of the founders through its journals, meetings, and educational activities.

However, in its interactions with other Societies and its responsiveness to growing physiological subgroups, APS has not been entirely successful. The early Society consisted of a heterogeneous group of investigators in the area of physiology, biochemistry, pharmacology, pathology, histology, and many others. For these early members, physiology's promise might have been viewed as its integrative nature, allowing for investigators with different backgrounds and experiences to strive for an understanding of the systems of the body. As APS grew in size, it provided the seeds for new societies such as the American Society of Biological Chemists (1906) and the American Society of Pharmacology and Experimental Therapeutics (1908). While APS spawned new societies, it also worked to ensure that it could exchange knowledge with its children. As a result, the three societies agreed in 1912 to form a federation, the Federation of American Societies for Experimental Biology. To this day, FASEB has provided a forum for investigators in different disciplines to exchange information relative to common scientific questions.

Over the years, APS has continued to spawn new societies, in many cases because the governance of the Society did not respond to the needs of maturing subdisciplines. As with our own offspring, communications and scientific exchange with these newer societies has often been stilted. However, as the parent society, we must consider ways to increase communications between APS and groups such as the Society for Neuroscience, Biophysical Society, and Society of General Physiologists. We must look to them to help us understand how biological systems operate. If we, as physiologists, are to understand how biological systems operate at the organ and systems level, we must understand the processes at the cellular and molecular level as well. The Society and its meetings must be forward looking, not rooted to an organ-and-systems approach to physiology, but designed to encourage participation of cell physiologists, biophysicists, and molecular biologists. Just as our understanding cannot be complete until we understand the workings of the cellular and molecular components of an organ system, the biophysicists and molecular biologists must be able to integrate their findings into the larger biological systems.

As we approach our Centennial and start a second century of progress in physiology, I urge our membership to encourage cell physiologists, biophysicists, and molecular biologists to join with us to provide an integrative approach to biomedical understanding. As Phil Knauf stated in his recent editorial announcing the conversion of *AJP: Cell Physiology* to a monthly, "one of the most exciting aspects of cell physiology is the way in which experimental information from different sources can be combined to provide a picture of cell function that could never have been derived from one approach alone." We should remember that the same can be said about organ-and-systems physiology. While we maintain the traditional aspects of physiology, we must encourage the participation of investigators utilizing the new techniques and approaches.

It is my view that APS is a healthy and viable organization that will be rejuvenated by the forthcoming Centennial. As we move into the second century, the membership might expect to see a revised program structure for the Fall meeting, a modified governance structure reflective of the sectionalization of the Society, and broader participation by investigators attempting to understand the larger system by studies at the cellular and molecular level.

I hope that you will take the time to share your views of APS and physiology with me so that I might be able to assist our elected representatives as they lead the Society into its Second Century of Progress.

Martin Frank
Executive Secretary-Treasurer

American Physiological Society Endowment Fund

The APS Endowment Fund was established in 1977 to support programs for the development of physiologists and physiology; to encourage communication with other disciplines of science and the public; and to foster scientific and cultural relations with other parts of the world.

The APS Endowment Fund was established to encourage tax deductible contributions or bequests to the Society at any time and in any amount, for specific or general purposes. Upon request, the Society will provide to a donor or institution contributing a memorial gift a replica of the plaque bearing the name of the individual living or deceased in whose honor the gift was made. The family of, or the individual being honored by a donation to the fund will be advised formally of the donor's name, unless the contributor specifically requests that the donation be anonymous.

Donations to the APS Endowment Fund or queries should be addressed to the fund at 9650 Rockville Pike, Bethesda, Maryland 20814.

House Bill Seeks Penalties for Break-ins at Research Facilities

A bill that sets federal penalties for break-ins and destruction of research facilities using animals has been introduced by Rep. George E. Brown, Jr. (D-CA) as a companion bill to his "Improved Standards for Laboratory Animals Act of 1985." Both bills, if enacted, would amend provisions of the Animal Welfare Act.

The need for federal penalties for break-ins and destruction of research laboratories was presented by the American Physiological Society last September during Congressional oversight hearings to consider possible changes to the Animal Welfare Act. The proposal by APS has since been endorsed by FASEB, the Association of American Medical Colleges, and the American Psychological Association.

The primary purpose cited by APS in proposing the amendment to the Animal Welfare Act is the need to provide a mechanism to enable authorities to pursue vandals across state lines.

In introducing the bill (HR 2654), entitled "Penalty for Destruction of Research Facilities," Brown said, "There have been a number of break-ins of research facilities which have included malicious destruction of property, vandalism, as well as the theft of animals.

"While I sympathize with the goals of these (animal rights) activists to expose the public to the moral questions regarding the use of animals in research, I cannot and will not condone the use of vandalism and malicious destruction as a legitimate means for achieving policy changes.

"Because of this increase in vandalism to laboratories . . . I consider this (both bills) a package, but in light of the severity of this action, (I) will introduce it (HR 2654) separately to allow appropriate Congressional consideration of this provision."

Brown also noted that one of the recent break-ins—the University of California at Riverside—was in his district and that the theft of 406 animals and $700,000 in damages to the facility has caused the loss of the benefits gained of the research being conducted and that several projects had been set back or had to be started again.

The maximum penalties proposed by Brown are a fine $250,000, imprisonment of five years, or both. In an offense where a person's life is placed in jeopardy, the maximum imprisonment would be 20 years.

The companion bill introduced by Brown (HR 2653) would provide for three changes in the Animal Welfare Act.

One change would add to the current standards a provision for exercising all dogs used in laboratory programs and a requirement that researchers give assurances that alternatives have been considered to practices which may cause pain and distress to animals.

Another proposed change would be a requirement that every facility using laboratory animals must appoint an Institutional Animal Committee that will be responsible for inspecting at least twice a year all animal study areas and animal facilities and to review all practices involving pain to animals as well as the condition of the animals.

The committee is to be composed of at least three members, one of whom must be a veterinarian and one a person not affiliated in any way with the institution.

The third change being proposed is the creation of a national database on current and completed experiments, which will be made available to researchers on a voluntary basis. The database is to be operated by the National Agricultural Library, working in cooperation with the National Library of Medicine.

Because oversight hearings were conducted last year, the House Subcommittee on Departmental Operations, Research, and Foreign Agriculture is not expected to conduct hearings on the changes proposed in HR 2653. However, the Subcommittee and the House Judiciary Committee are expected to have hearings on HR 2654.

Dole Sponsors Senate Bill Identical to Brown's Proposal

Sen. Robert J. Dole (R-KS) has introduced in the Senate a bill identical to Rep. Brown's "Improved Standards for Laboratory Animals Act of 1985." Both Dole and Brown sponsored similar legislation in the last Congress.

Because of Congressional hearings on the Animal Welfare Act in the last Congress the Senate Committee on Agriculture, Nutrition, and Forestry is not expected to conduct hearing on the Dole bill (S 1233).

A bill calling for federal penalties for breakins and destruction of research laboratories has not been introduced as yet in the Senate.

William M. Samuels, CAE

The Myth of Stabilization

In February 1980, Donald Fredrickson, the then NIH Director, testifying before the Appropriations Subcommittee, expressed the Administration's desire to fund at least 5,000 research grants in fiscal year (FY) 1981 in order to stabilize the science base. This stabilization strategy was designed to reduce the fluctuations that occurred in the numbers of new and competing research grants that could be funded each year. This policy was to apply not only to individual investigator-initiated research projects but also to other support mechanisms. As stated in the 1981 House Appropriations Committee report language, "The effectiveness of the overall NIH programs will be promoted if there is stability of funds available for other research activities as well as research project grants."

Unfortunately, as is painfully evident to all, NIH's stabilization policy has been accomplished at the expense of other support mechanisms, such as research centers, research resources, contracts, and training. In essence, the stabilization policy has resulted in a loss of flexibility and a loss of NIH's ability to allocate resources among the many competing program demands.

As we are all aware, the past several years have seen the stabilization floor become, instead, a ceiling. That is, until the FY 1985 appropriation provided for over 6,500 awards, which brought relief and excitement to the research community. The excitement, however, was short-lived thanks to David Stockman and OMB's action. As a result of the timing of the cutback to 5,000 awards, NIH has been forced to fund at the stifling rate of approximately 4,000 new and competing research grants for the last two council rounds.

While the question still remains whether NIH will fund 5,000, 6,000, or 6,500 research grants in FY 1985, it is important to note that, whatever the number, the traditional research community will be shortchanged. The poorly publicized reason is that Public Law 97-219, an amendment to the Small Business Act, requires the PHS agencies, as well as others, to set aside a specified amount of their research and development budgets for the Small Business Innovation Research (SBIR) program. The awards made under this program are included as part of the new and competing investigator-initiated research project grant total. These awards represent an increased percentage of NIH's research and development budget, rising from 0.2% in FY 1983 to 1.25% in FY 1986 and 1987. Since for-profit organizations can already compete for traditional project grants, the setting aside of funds for the SBIR program effectively limits NIH's flexibility and reduces the amount of money available to the investigative community based in universities and research institutes.

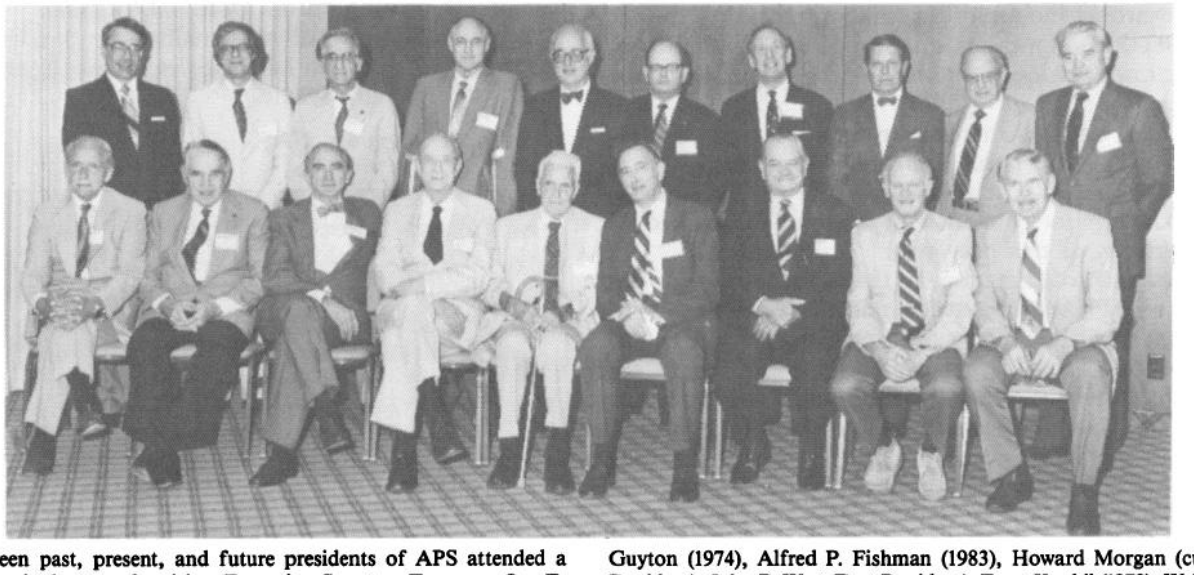
As indicated below, these awards represent an increasing percentage of the total, a total that both Congress and the White House quote as a reflection of their generosity.

Grants FY	1980	1981	1982	1983	1984	1985
Total Research*	4,785	5,109	5,027	5,389	5,497	6,000‡
SBIR†	–	–	–	123	246	375§

*Represents grants coded R01, R22, R23, R43, R44, P01, and U01. †Represents grants coded R43, R44. ‡Probable total. §Approximate.

Martin Frank
Executive Secretary-Treasurer

Gathering of APS Past Presidents



Eighteen past, present, and future presidents of APS attended a reception in honor of retiring Executive Secretary-Treasurer Orr E. Reynolds held on Tuesday evening, April 23, 1985, during the Federation meeting in Anaheim, CA. The above photograph was taken to commemorate this special occasion. The year in parentheses is the year in which the president's term began.

First row, left to right: David F. Bohr (1978), Earl H. Wood (1980), A. Clifford Barger (1970), Hermann Rahn (1963), David Bruce Dill (1950), Robert E. Forster (1966), Orr E. Reynolds, Robert M. Berne (1972), Ewald E. Selkurt (1976).

Second row, left to right: Franklyn Knox (President-Elect), Harvey Sparks (member of Council), Francis J. Haddy (1981), Arthur C. Guyton (1974), Alfred P. Fishman (1983), Howard Morgan (current President), John B. West (Past-President), Ernst Knobil (1979), Walter C. Randall (1982), William F. Ganong (1977).

Daniel C. Tosteson (1973) was also present at the reception. Other past presidents – Eugene M. Landis (1952), Edward F. Adolph (1953), Horace W. Davenport (1961), Hymen S. Mayerson (1962), John R. Pappenheimer (1964), John M. Brookhart (1965), Robert W. Berliner (1967), C. Ladd Prosser (1970), John R. Brobeck (1971), and Bodil Schmidt-Nielsen (1975) – while unable to attend, were present in spirit, having contributed autographed photographs and letters to the album presented at the reception to Dr. Reynolds.

Honors and Awards

MacArthur Foundation Award

Jared M. Diamond, a member of APS since 1965, was among the 25 latest recipients of the John D. and Catherine T. MacArthur Foundation awards announced on June 18. As a MacArthur Fellow, he will receive an annual stipend for the next five years to use however he chooses. Dr. Diamond was granted a D.Phil. in physiology from Cambridge University in 1961 and has, since 1966, been a member of the Department of Physiology, UCLA Medical School. In addition to research on membrane transport, he is known for his ecological studies of bird life in New Guinea and other southwest Pacific islands and for his work on speciation mechanisms and the dynamics of island communities.

Passano Foundation Award

APS member, **Howard Green,** M.D., Professor and Chairman of the Department of Physiology and Biophysics at Harvard Medical School, was chosen as the recipient of the 1985 Passano Foundation Award for his pioneering research in cell biology. His original contributions in several areas have had a major impact on medical science. In the course of studies with human-mouse hybrid cells, Dr. Green and his colleagues discovered that the rapid loss of specific human chromosomes from hybrid cells was correlated with the loss of specific gene markers. This approach enabled them to make chromosomal assignments for the location of several genes, a principle which has since been widely used as the basis for mapping human chromosomes. Dr. Green's research on cell growth control and differentiation produced model cell systems for epidermal and preadipose cell development which faithfully mimic these processes in the living animal, but which can be investigated under cell culture conditions. Using an important application of his basic work with epidermal cells, Dr. Green and his associates have developed techniques whereby epidermal cells from small skin biopsies can be rapidly cultivated to form coherent sheets of epithelium which are not only suitable for laboratory studies, but can serve as skin grafts. In collaboration with plastic surgeons, Dr. Green has successfully utilized such sheets of autologous cells grown in vitro to regenerate epidermis covering more than half the body surface of severely burned children.

APS Members Elected to the Institute of Medicine

Two members of APS, **John W. Eckstein**, M.D., and **Thomas F. Hornbein**, M.D., were among the 29 new active members recently elected to the Institute of Medicine of the National Academy of Sciences. Dr. Eckstein, Professor of Internal Medicine and Dean, College of Medicine, University of Iowa, has specialized in cardiovascular physiology, with a special emphasis on peripheral blood flow. That work led to his selection by the American Heart Association as an Established Investigator. He was elected to APS in 1960. Dr. Hornbein, Professor and Chairman of the Department of Anesthesiology and Professor of Physiology and Biophysics, University of Washington School of Medicine, has made contributions to the areas of respiratory control and high altitude physiology. He ascended Mount Everest in 1963. Elected to APS in 1965, he has served as a member of the Editorial Board of the *Journal of Applied Physiology.*

Bernard Fisher, M.D., Professor of Surgery, University of Pittsburgh School of Medicine and a member of APS since 1956, has been elected a senior member of the Institute of Medicine. Noted for advancing the treatment of breast and colon cancer, his interests also include immunology and cytology.

Committee Reports

Porter Development

The report of the Porter Development Committee to the Society provides the opportunity of informing the members that fellowship funds are available for able minority students both at the pre- and postdoctoral level. Two of the former Porter Development Postdoctoral Fellows have recently returned to the Department of Physiology at the University of Puerto Rico: Dr. Nelson Escobales, who was a fellow in the laboratory of Dr. Mitzi Canessa in the Department of Physiology and Biophysics at Harvard Medical School; and Dr. Jose E. Garcia-Arraras, who was a fellow in the laboratory of Dr. Nicole Le Dourain at the Institut d'Embryologie in the Centre National de la Recherche Scientific at Nogent-sur-Marne, France. We are also continuing the support of Dr. Jorge R. Mancillas, who is a postdoctoral fellow in the laboratory of Dr. Floyd E. Bloom at Salk Institute; Ms. Jean A. King, who is a candidate for the Ph.D. degree in the Department of Biology at New York University in the laboratory of Dr. Fleur L. Strand; Ms. Darlene K. Racker, who is a candidate for the Ph.D. degree in the Department of Physiology and Biophysics at Chicago Medical School in the laboratory of Dr. Warren W. Tse.

The Committee has continued funding for the Atlanta consortium, a program organized with the assistance of the Department of Physiology at Emory Medical School. Two former Porter Development Committee Fellows, Drs. Pamela Gunter-Smith and John C. S. Fray, have been Visiting Porter Lecturers in the Atlanta Program. The Dillard Program in New Orleans has been assisted by the staff of the Departments of Physiology at Louisiana State University and Tulane University.

The Committee is also providing support for a Minority Student Research Internship Program in the Department of Physiology at Michigan State University and for a Summer Student Research Program for Native Ameri-

can Indians in the Department of Physiology and Biophysics at the College of Veterinary Medicine and Biomedical Sciences at Colorado State University. Mr. Errol Williams of Princeton University was a summer fellow in the laboratory of Dr. George M. Langford at Woods Hole. Dr. Harris Mackey, who was a Porter Development Fellow in Dr. John G. Hildebrand's Laboratory in the Department of Biological Sciences at Columbia University, has recently been awarded a Johnson and Johnson Fellowship.

We again express our appreciation to the Harvard Apparatus Foundation for its continuing support of the Porter Development Program.

A. C. Barger and E. W. Hawthorne

Program

By any criterion the 1985 Spring Meeting was among our most successful. APS programmed 2,117 contributed papers and 42 symposia. In both cases the society achieved new highs in the level of its activity in the scientific program.

The Program Advisory Committee met in Anaheim to develop proposals for the 1986 Spring Meeting. Forty-five symposia were proposed by the section representatives and guest societies. The following list gives the symposia approved for presentation in the APS program by the Program Executive Committee, together with the name of the organizer and sponsor section.

The Arteriole—Functional Unit of the Peripheral Circulation; B. Duling; Cardiovascular Section

Leukocytes and the Microcirculation in Ischemia; R. Engler and B. Lucchesi; Cardiovascular Section

Control of the Coronary Circulation and Myocardial Function by Eicosanoids; G. Kaley; Cardiovascular Section

Myosin Polymorphism in Striated Muscle, S. Winegard; Cardiovascular System

Physiology and Biophysics of Chloride and Cation Co-Transport Across Cell Membranes; P. Lauf; Cell and General Physiology Section

Ion Transport Across Epithelial Tissues: New Insight from Single Channel Measurements; D. Eaton; Society of General Physiology

Aging and Exercise: Physiologic Interactions; J. Holloszy; Environmental, Thermal and Exercise Section

Regulation of the Cutaneous Circulation; J. Johnson; Environmental, Thermal and Exercise Section

Use of Cellular and Molecular Biology Techniques in Neuroendocrinology; M. Smith and P. Conn; Endocrinology and Metabolism Section

Expression and Function of Neuropeptides within Endocrine Tissues; S. Leeman; Endocrinology and Metabolism Section

Application of the Techniques of Molecular Biology to the Study of Ion Transport Mechanisms; J. Gargus; Epithelial Transport Group

Ionic Regulation of Gene Expressions; I. Arias; Gastrointestinal Physiology Section

The Single Cell as a Physiologic Model; M. Lieberman; Muscle Physiology Group

Prospectives in Neuroscience; J. Lipton; Neurophysiology Section

Physiologic Role of Carotid Body Chemoreceptors; C. Eyzaguirre; Neurophysiology Section

Mechanism of Chloride Transport along the Nephron; P. Aronson; Renal Section

Direct Assessment of Renal Microcirculatory Dynamics; G. Navar; Renal Section

Bronchial Circulation; J. Butler and R. Johnson; Respiratory Physiology Section

Pulmonary Vagal Receptors: Current Controversies; A. Pack and J. Widdecombe; Respiratory Physiology Section

Transport Properties of Pulmonary Vascular Endothelial Monolayer; A. Malik; Respiratory Physiology Section

Morphometry and Video Analysis of Histologic Sections Applied to Physiologic Research; J. Gil; Respiratory Section

Body Fluid Volume and Blood Pressure Regulation; J. Hall; Water and Electrolyte Homeostasis Section

Atrial Natriuretic Factor; G. Cahill; Clinical Physiology Section

Transport Abnormalities in Cystic Fibrosis; P. Quinton; Program Executive Committee

Dietary Fat Calories and Disease; D. Kritchevsky; Society for Experimental Biology and Medicine

In addition, the 1986 Spring Meeting will include an intersociety theme on pH to be organized by Walter Boron, of APS.

In contrast to the Spring Meeting, which continues to advance in strength, the Fall Meeting is the subject of some concern. At the 1984 Fall Meeting in Lexington, Council charged the Program Executive Committee to develop a proposal for improving the Fall Meeting. Following review by the Long-Range Planning Committee, the proposal was presented to and approved by Council in Anaheim. In developing this proposal the Program Committee addressed three factors: time, location, and content. It was recommended that the meeting should be held during October in cities, such as San Diego or New Orleans, known to be attractive to members. With respect to program content, it was proposed that advantage should be taken of the Society's sectional organization and that the sections should be encouraged to develop independent thematic meetings under the APS umbrella. The first opportunity to present a Fall Meeting in the new format will come in 1986 when the meeting is scheduled to be held in New Orleans.

Because this is my last report as Program Committee Chairman, I will take the opportunity for some personal comments. In particular, I wish to record my sincere appreciation of the assistance provided by Dr. Joe Saunders of the Membership Services Office. His exceptional organizational skills facilitate every phase of program development and presentation. Also, I would like to thank the members of the Program Advisory and Executive Committees for their efforts and support. The new chairman is Carl Gisolfi. I hope he will find the position as stimulating and satisfying as I have.

M. J. Jackson, Chairman

Senior Physiologists

The principal function of this committee is simply that of maintaining contact with the members of our Society who are 70 years old or older. This is carried out primarily through correspondence. Most of the business of the committee is also carried out by correspondence and telephone.

There have been two meetings of the committee during the past 5 years, one in San Diego in October 1982 and the other in St. Louis in April 1984. In each instance only three members of the committee attended.

Although the Society operates on a July 1–June 30 year, this committee functions on a calendar-year basis. Late in the calendar year the Membership Office prepares a computer printout of members who will become 70 years of age during the next calendar year plus all members who are already 70 or older.

At the beginning of 1984 there were 556 names on our list, approximately 90 correspondents for each of our 6 members. (Dr. Code had resigned leaving us with only 6 members.) At the end of 1984 we had 536 names. The addition of 65 names of those whose birthday is in 1915 increased the list to 601 at the beginning of 1985. With the addition of Drs. Selkurt and Zweifach and the loss of Dr. Alexander, we now have, on average, 86 correspondents per committee member.

In 1983, 52 responses from senior physiologists were published in *The Physiologist*. Last year the number was 37. Beginning with the February 1984 issue, a new logo for the "From Senior Physiologists" column appeared in *The Physiologist*. Most of us on the committee consider it to be an improvement.

E. B. Brown, Jr., Chairman

Animal Stress

The American Physiological Society is pleased to announce the publication of a second book on animal welfare. *Animal Stress*, edited by Gary P. Moberg, is described in the preface as follows:

Society is currently engaged in an emotional debate concerning the moral justification for the use of animals in various human pursuits, including laboratory experimentation. This has resulted in increased pressure for legislation and regulation intended to protect animals against the infliction of unwarranted pain and distress and to establish guidelines that assure the well-being of laboratory animals. The problem in developing such guidelines is to define what constitutes well-being for animals. Without such a definition, legislation concerning care and use will be based primarily on emotional reaction rather than objective information.

Arriving at a universally acceptable definition of animal well-being is probably impossible, because how people define the quality of animal life depends on their personal experiences and views. However, most people will agree that if an animal is under stress, its well-being is threatened. Conversely, if the animal is living under conditions that it finds nonstressful, its well-being probably is not at risk. Therefore, stress, by its presence or absence, provides us with an acceptable and meaningful way to define animal well-being.

Because of the impact of stress on the health and well-being of both humans and animals, the biology of stress has been the subject of considerable research over the past several decades. Nevertheless, researchers still cannot precisely define stress or even accurately measure its effects on the individual. Our failure reflects the complexity of the biological response to stress—a response that includes behavior, physiology, immunology, and nutrition. Clearly, any meaningful advances in the study of stress require a multidisciplinary effort. Therefore, in July 1983 a symposium sponsored by the college of Agriculture and Environmental Sciences at the University of California, Davis, brought together experts in behavior, nutrition, physiology, immunology, and human and animal medicine to discuss the problems involved in defining and measuring stress in animals. The conference provided the opportunity for these individuals to discuss the problems and methods of studying stress and to suggest possible directions for research. It is hoped that such research will provide the biological basis for the establishment of meaningful guidelines and legislation for protecting the well-being of animals.

The book is divided into four sections. Determining Animal Well-Being reviews what constitutes well-being in animals, the evolutionary and ontogenetic determinants of animal suffering, and the use of the biological responses to stress a way to assess well-being in animals. Stress in Animals examines the various biological responses to stress and possible ways to monitor stress in animals. Effects of Stress on Well-Being addresses how stress can threaten an animal's health, disrupt normal reproduction, and influence growth and metabolism. Well-Being of Laboratory Animals emphasizes the importance of this topic to the development of guidelines regulating the use of animals in scientific research.

Animal Stress (332 pages, 45 figures) is available to APS members for $34.00 (list price $42.50) when ordered from the Society Business Office, 9650 Rockville Pike, Bethesda, MD 20814.

APS Sections

History of Physiology

Twenty-four members and friends of the APS Section on the History of Physiology met at a luncheon meeting held on April 24, 1985, during the Federation meeting in Anaheim, CA. The luncheon speaker was Dr. Robert G. Frank, Jr., Medical History, UCLA, who gave a slide presentation under the title, " 'Innocents Abroad?' American Physiologists in European Laboratories." This paper dealt with the experiences in European laboratories of the charter members of APS and those elected in the first five years and of the importation to America of "the ideal of a systematic, laboratory-based, experimental physiology."

Dr. Norman Staub, PAC representative, announced that there is now a history category on the abstracts form for the Fall meeting and for the FASEB Spring meeting. Any member of the Society may now submit an abstract for a paper on the history of physiology. Dr. Staub prefers poster sessions for history papers and has obtained permission from the Program Committee to have history posters on display for a longer period than the usual 3 hours. Two history posters are scheduled for the Fall meeting and will be on display in the Learning Resources Center area. Members of APS are encouraged to make use of the history category. If you have questions regarding format of presentations or posters, please contact Dr. Staub.

Several events of historical interest are planned for the APS Fall meeting. November 21, 1985, is the two hundredth birthday of William Beaumont. At the Anaheim meeting, Beaumont was commemoriated by a symposium entitled "Roots and Shoots of Experimental Physiology," at which representatives of each FASEB Society spoke on the relevance of Beaumont's work to later developments in some aspects of their fields. The location of the Fall meeting at Niagara Falls and Buffalo is particularly appropriate because Beaumont was stationed at Fort Niagara in 1925-26 and carried out four sets of experi-

ments on Alexis St. Martin there and in Plattsburgh, NY. The Section is sponsoring an historical symposium on Beaumont, entitled "William Beaumont's World," chaired by Dr. Robert J. T. Joy on Wednesday afternoon, October 16. Among the speakers will be one of the Society's most prolific historians, Dr. Horace Davenport, William Beaumont Professor Emeritus of the University of Michigan. There will be a small Beaumont exhibit put together by Robert E. Johnson and Toby Appel on display in the Learning Resources Center area. Attention is called to the Tutorial Lecture entitled "A Tribute to Sid Robinson: Pioneer in Experimental Physiology" scheduled for Wednesday morning, October 16. A feature of special interest to historians at the Fall meeting is the 25-year-old collection of over a thousand photographs of physiologists on display in the hallway of the Department of Physiology of the University of Buffalo (*Physiologist* 28: 133, 1985).

T. A. Appel, Secretary

Nervous System

The participation of the Section on the Nervous System in APS activities has been continuing to increase over the past few years. At the spring FASEB meeting, the section, in conjunction with the Cardiovascular and Autonomic Controls sections, sponsored a four-part symposium, "Update in Cardiovascular Neurobiology." The Gould Corporation provided some of the financial support for this symposium and has expressed an interest in sponsoring an annual lecture in cardiovascular neurobiology.

At the Fall meeting in October, there will be an unusually large number of "neuro" posters and slide presentations due to the participation of our colleagues in the Canadian Physiological Society.

As of September 1985, members of the Steering Committee are Lorne Mendell, Charles Edwards, Janett Trubatch, Evelyn Satinoff, James Lipton, and James Houk – and three people will have to be elected to fill the slots vacated by Don Humphrey, Jim Blankenship, and Ian Phillips.

The ballots for the Steering Committee election will go out in August. If you are interested in serving on the Committee or in organizing a symposium, FASEB summer conference, tutorial refresher course, or any other "Neuro" related APS activity, please let us know by filling in the appropriate space on your ballot. If you do not receive a ballot but are interested in being part of the Section on the Nervous System, just call the membership services and tell them to include you (there is no cost or obligation).

The next meeting of the Steering Committee will occur at the Society for Neuroscience meeting in Dallas, in October 1985. See the program for specific time and place. Everyone is welcome.

Don't forget the Neuro Mixer next April in St. Louis.

J. Trubatch, Chair

George A. Feigen Memorial Lectureship

Contributions to the George A. Feigen Memorial Lectureship (see *Physiologist* 28: 31, 1985) can be made to K. B. Taylor, MD, Dept. of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

H. Lowell Stone (1936–1984)

This is a tribute to a person who died at the prime of his life and who was recognized nationally and internationally for his significant contributions to physiology. Here are some highlights of the career and life of Dr. H. Lowell Stone.

Dr. Stone was born in 1936 in the heart of Cajun Country in Louisiana. Those of us who knew him recognized that his Cajun blood and heritage flowed in him throughout his life. After completing his undergraduate education at Rice University and his graduate training at the University of Illinois in 1961, he joined Dr. Arthur Guyton's Department at the University of Mississippi.

After working for seven years at the School of Aerospace Medicine at Brooks Air Force Base in San Antonio, he was appointed Professor of the Department of Physiology and Biophysics and Chief of the Cardiovascular Control Section of the Marine Biomedical Institute of the University of Texas at Galveston in 1971. In 1977 Lowell made the decision to accept the Chair at the University of Oklahoma Health Sciences Center. This Department had been without a chairman for six years and was discouraged and understaffed. In the seven subsequent years Lowell built his Department up to national recognition in both teaching and research. For the past few years the Department of Physiology and Biophysics had the largest extramural research budget in the College of Medicine, even considering the clinical departments. His leadership provided an environment where members of the Department could establish themselves in research and teaching. Lowell also had the ability to unite the faculty into a tightly knit well-integrated group of people.

Lowell was recognized nationally and internationally for his scientific achievements in study of the cardiovascular system and neural regulation during exercise and his leadership in many societies and organizations. Lowell also was recognized for his achievements by his own institution. In 1984 he received an appointment as a George Lynn Cross Research Professor from the University of Oklahoma. This professorship is bestowed upon a faculty member for leadership and outstanding research. He also received many other awards and honors. He wrote chapters in several books, including one in the recent Annual Review of Physiology. As a member of the American Physiological Society he served on the editorial board of *Journal of Applied Physiology* from 1977 through 1984 and at the time of his death had recently been appointed an Associate Editor. He was also Secretary of the Association of Chairmen of Departments of Physiology.

Our profession has lost a dynamic leader and outstanding scientist, but his legacy will live on. To maintain this legacy, our Department has established an "H. Lowell Stone Memorial Fund," the purpose of which is to give a monetary award to a graduate student or postdoctoral fellow who presents the best paper in "Exercise and Neural Control of Circulation" at the Federation Meetings in the Spring. Persons wishing to contribute to this fund may send contributions to H. Lowell Stone Memorial Fund, Dept. of Physiology and Biophysics, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.

R. D. Foreman

Satellite Symposium on Environmental Physiology

Hyatt Regency, Buffalo, New York
October 11-12, 1985

The Department of Physiology, State University of New York at Buffalo, is organizing a satellite symposium on Environmental Physiology, co-sponsored by APS, to be held at the Buffalo Hyatt Regency Hotel, October 11-12, 1985. Three half-day sessions are planned on the physiology of adaptation to *1*) altitude; *2*) diving and elevated pressure; and *3*) elevated G-force; and a fourth half-day session on comparative physiology will conclude the satellite symposium. Registration is Thursday, October 10, 7:00-9:00 P.M., and Friday, October 11, 8:45-9:00 A.M. *Information*: Environmental Symposium, c/o Dr. Charles Paganelli, 124 Sherman Hall, SUNY at Buffalo, Buffalo, NY 14214.

Session I. Physiology of Adaptation to Altitude
Chaired: S. M. Tenney

Introduction; S. M. Tenney; Dartmouth Med. Sch.
Structure and Function of Carotid Body at High Altitude; Lahiri; Univ. of Pennsylvania Med. Sch., Philadelphia
Mechanisms of Ventilatory Acclimatization; J. A. Dempsey; Univ. of Wisconsin, Madison
Severe Pulmonary Hypertension in the Newborn Calf at High Altitude: Proliferation of Adventitia; J. T. Reeves; Univ. of Colorado Med. Ctr., Denver
Muscle Function Impairment in Man Acclimated to Chronic Hypoxia; P. Cerretelli; Univ. of Geneva, Switzerland
Hypoxic Insomnia; J. R. Pappenheimer; Harvard Univ. Med. Sch.

Session II. Physiology of Diving and Exposure to Elevated Pressure
Chaired: T. Yokoyama

Introduction; T. Yokoyama; Keio Univ. Sch. of Med., Japan
Breathing Under Water; C. E. G. Lundgren; SUNY at Buffalo
Resistance and Inertance when Breathing a Dense Gas; H. Van Liew; SUNY at Buffalo
Diffusive Gas Mixing in the Lungs; A Possible Factor Limiting Alveolar Gas Exchange at Depth; Y. Ohta; Tokai Univ., Japan
Water Exchange in Hyperbaria; S. K. Hong and C. Paganelli; SUNY at Buffalo
On the Use of a Bubble Formation Model to Calculate Nitrogen and Helium Diving Tables; D. Yount; Univ. of Hawaii at Manoa
Diving Physiology of the Antarctic Weddell Seal; W. Zapol; Massachusetts Gen. Hosp.

Session III. Physiology of Exposure to Altered G-Force
Chaired: A. H. Smith

Introduction; A. H. Smith; Univ. of California, Davis
Development of Anti-G Suits; E. Wood; Mayo Fndn. and Med. Sch.
Human Physiologic Limitations to G in High Performance Aircraft; R. Burton; Brooks AFB.
Effects of Weightlessness on Human Fluid and Electrolyte Physiology; C. Leach; Houston
Cardiopulmonary Function at High and Low Gravity; L. E. Farhi; SUNY at Buffalo
Study of Altered Gravitational Environments Using Computer Simulations; R. J. White; NASA, Washington, DC

Session IV. Comparative Physiology
Chaired: C. Lenfant

Introduction; C. Lenfant, NHLBI, NIH
Aquatic Life and the Transition to Terrestrial Life; P. Dejours; Ctr. Natl. de Recherche Sci., Strasbourg, France
Gas Exchange Efficiency of Fish Gills and Bird Lungs; J. Piiper; Max Planck Inst. Exp. Med. FRG
Bimodal Gas Exchange of the African Catfish; A. Ar; Tel Aviv Univ., Israel
Homeostasis: Embracing Negative Feedback Enhanced and Sustained by Neuromodulation; H. T. Hammel; Scripps Inst. of Oceanography
Life Span, Metabolism and Body Size; H. Rahn; SUNY at Buffalo

APS Council

President
H. E. Morgan
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

President-Elect
F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

Past President
J. B. West
Dept. of Med.
Univ. of California
La Jolla, CA 92093

Exec. Secy.-Treas.
Martin Frank (ex officio)
American Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

Councillors
S. Chien
Dept. of Physiol.
Columbia Univ.
New York, NY 10032

N. C. Staub
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

H. V. Sparks, Jr.
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

A. E. Taylor
Dept. of Physiol.
Univ. of South Alabama
Mobile, AL 36688

APS Committees, Their Principal Functions and Membership (1985-86)

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.

I. J. Fox, *Chairman*
Dept. of Physiol.
Univ. of Minnesota
Minneapolis, MN 55455

J. F. Amend
Dept. of Vet. Science
Univ. of Nebraska
Lincoln, NE 68583-0905

J. H. U. Brown
Dept. of Biol.
Univ. of Houston
Houston, TX 77004

D. N. Granger
Dept. of Physiol.
Univ. of South Alabama
Mobile, AL 36688

T. N. Pullman
VA Lakeside Hosp.
Northwestern Univ.
Chicago, IL 60611

L. Ramazzotto
Dept. of Physiol.
Fairleigh-Dickinson Univ.
Hackensack, NJ 07601

I. H. Zucker
Dept. of Physiol./Biophys.
Univ. of Nebraska
Omaha, NE 68105

N. B. Marshall (ex officio)
Therap. Res.
The Upjohn Co.
Kalamazoo, MI 49001

W. M. Samuels (ex officio)
American Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

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.

R. J. Traystman, *Chairman*
Research Labs.
Johns Hopkins Hosp.
Baltimore, MD 21205

C. M. Gregg
Dept. of Physiol.
Pennsylvania State Univ.
Univ. Park, PA 16802

D. M. MacCanon
Div. of Vascular Disease
NHBI, NIH
Bethesda, MD 20205

S. A. Nunneley
USAF School of Aerospace Med.
Brooks Air Force Base
San Antonio, TX 78235

J. Van Liew
Dept. of Physiol.
State Univ. of New York
Buffalo, NY 14215

C. E. Vreim
Div. of Lung Diseases
NIH, NHLBI
Bethesda, MD 20205

R. C. Webb
Dept. of Physiol.
Univ. of Michigan
Ann Arbor, MI 48109

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.

A. P. Fishman, *Chairman*
Dept. of Med.
Univ. of Pennsylvania Hosp.
Philadelphia, PA 19104

O. E. Reynolds
Task Force Director
American Physiol. Soc.
Bethesda, MD 20814

P. A. Chevalier
Medtronic, Inc.
3055 Old Highway Eight
Minneapolis, MN 55440

J. S. Cowan
Dept. of Physiol.
Univ. of Ottawa
Ottawa, Ont. CN KIN 9A9

D. L. Gilbert
Lab. Biophys.
National Inst. of Hlth.
Bethesda, MD 20205

R. J. T. Joy
Dept. of Military Med. Hist.
Uniformed Services Univ.
Bethesda, MD 20814

R. Kellogg
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

L. L. Langley
Univ. of Missouri
Sch. of Med.
Kansas City, MO 64109

S. Ochs
Dept. of Physiol.
Indiana Univ. Med. Ctr.
Indianapolis, IN 46202

A. B. Otis
Dept. of Physiol.
Univ. of Florida
Gainesville, FL 32610

M. C. Shelesnyak
674 Chalk Hill Rd.
Solvang, CA 93463

H. V. Sparks, Jr.
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

N. C. Staub
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

P. C. Johnson (ex officio)
Dept. of Physiol.
Univ. of Arizona
Tucson, AZ 85724

Committee on Committees

Serves as an advisory committee to Council for the purpose of making recommendations for nominees to the standing committees.

H. V. Sparks, Jr., *Chairman*
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

B. P. Bishop
Dept. of Physiol.
State Univ. of New York
Buffalo, NY 14214

A. W. Cowley
Dept. of Physiol.
Med. Coll. of Wisconsin
Milwaukee, WI 53226

W. H. Dantzler
Dept. of Physiol.
Univ. of Arizona
Tucson, AZ 85724

R. E. Fellows
Dept. of Physiol./Biophys.
Univ. of Iowa
Iowa City, IA 52242

M. J. Fregly
Dept. of Physiol.
Univ. of Florida
Gainesville, FL 32610

J. E. Greenleaf
Biomed. Res. Div.
NASA/Ames Res. Ctr.
Moffett Field, CA 94035

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.

C. L. Prosser, *Chairman*
Dept. of Physiol./Biophys.
Univ. of Illinois
Urbana, IL 61801

F. J. Haddy
Dept. of Physiol.
Uniformed Services Univ.
Bethesda, MD 20814

B. Schmidt-Nielsen
Biological Labs.
Mt. Desert Inst.
Salsbury Cove, ME 04672

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.

J. A. Spitzer, *Chairman*
Dept. of Physiol.
Louisiana State Univ.
New Orleans, LA 70112

F. L. Abel
Dept. of Physiol.
Univ. of South Carolina
Columbia, SC 29208

M. Anderson-Olivio
Dept. of Biol. Sci.
Clark Science Ctr.
Northampton, MA 01063

H. G. Hempling
Dept. of Physiol.
Med. Univ. of South Carolina
Charleston, SC 29425

H. I. Modell
Virginia Mason Res. Ctr.
1000 Seneca St.
Seattle, WA 98101

H. S. Pitkow
Pennsylvania Coll. of Podiatric Med.
Philadelphia, PA 19107

C. F. Rothe
Dept. of Physiol.
Indiana Univ.
Indianapolis, IN 45223

M. J. Siegman
Dept. of Physiol.
Jefferson Med. Coll.
Philadelphia, PA 19107

W. S. Spielman
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

M. Frank (ex officio)
Education Officer
American Physiol. Soc.
Bethesda, MD 20814

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.

F. J. Haddy, *Chairman*
Dept. of Physiol.
Uniformed Services Univ.
Bethesda, MD 20814

N. R. Alpert
Dept. of Physiol./Biophys.
Univ. of Vermont
Burlington, VT 05405

J. D. Wood
Dept. of Physiol.
Ohio State Univ.
Columbus, OH 43210-1238

Financial Development Committee

Develops and implements programs designed to attract financial support aimed at reducing Society dependence on member dues payments.

J. B. West, *Chairman*
Dept. of Med.
Univ. of California
La Jolla, CA 92093

E. H. Blaine
Merck Inst. for Therap. Res.
West Point, PA 19486

T. Cooper
Exec. Vice President
The Upjohn Co.
Kalamazoo, MI 49001

D. W. Rennie
Dept. of Physiol.
State Univ. of New York
Buffalo, NY 14214

A. Robert
The Upjohn Co.
Kalamazoo, MI 49001

C. A. Sanders
Exec. Vice President
Squibb Corporation
Princeton, NJ 08540

Honorary Membership Committee

Recommends to Council candidates for nomination to Honorary Membership, distinguished scientists who have contributed to the advancement of physiology.

B. Schmidt-Nielsen, *Chairman*
Biological Labs.
Mt. Desert Inst.
Salsbury Cove, ME 04672

R. W. Berliner
Yale Univ.
Sch. of Med.
New Haven, CT 06510

E. Knobil
Dept. of Physiol.
Univ. of Texas
Houston, TX 77030

International Physiology Committee

Keeps Council informed of activities of the U.S. National Committee for the International Union of Physiological Sciences (IUPS), handles all matters pertaining to international physiological affairs on consultation with Council, and informs APS members of IUPS activities.

A. P. Fishman *Chairman*
Dept. of Med.
Univ. of Pennsylvania Hosp.
Philadelphia, PA 19104

F. J. Haddy
Dept. of Physiol.
Uniformed Services Univ.
Bethesda, MD 20814

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

H. E. Morgan
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

K. Schmidt-Nielsen
Dept. of Zool.
Duke University
Durham, NC 27706

J. B. West
Dept. of Med.
Univ. of California
La Jolla, CA 92093

Future Meetings

1985	
Joint APS/The (British) Physiological Soc Mtg	Sept. 12-14, Cambridge (UK)
APS Fall Meeting	October 13-18 Niagara Falls/SUNY, Buffalo
1986	
FASEB Annual Meeting	April 13-18, St. Louis
IUPS Congress	July 12-20, Vancouver, Canada
APS Fall Meeting	October 5-10, New Orleans
1987	
*FASEB Annual Meeting	March 29-April 3 Washington, DC
APS Fall Meeting	October 11-16, San Diego

*APS Centennial Celebration

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.

N. B. Marshall, *Chairman*
Therap. Res.
The Upjohn Co.
Kalamazoo, MI 49001

P. T. Beall
Ciba-Geigy Pharmaceutical
Ardsley, NY 10502

R. Beeuwkes, III
Dept. of Pharm.
Smith Kline & French Labs
Philadelphia, PA 19101

S. F. Flaim
Dept. of Biol. Res.
McNeil Pharmaceutical
Spring House, PA 19477

A. M. Lefer
Dept. of Physiol.
Jefferson Med. Coll.
Philadelphia, PA 19107

N. Sperelakis
Dept. of Physiol.
Univ. of Cincinnati
Cincinnati, OH 45267

J. C. Strand
Pharmaceutical Dept.
Penwalt Pharmaceuticals Co.
Rochester, NY 14523

C. V. Gisolfi (ex officio)
Dept. of Physiol./Biophys.
Univ. of Iowa
Iowa City, IA 52242

J. A. Spitzer (ex officio)
Dept. of Physiol./Med.
Louisiana State Univ.
New Orleans, LA 70112

R. J. Traystman (ex officio)
Research Labs.
Johns Hopkins Hosp.
Baltimore, MD 21205

Long-Range Planning Committee

Advises and reports annually to Council and interacts with the Section Advisory Committee. Analyzes past and present societal performance; conducts periodic reviews of APS' relationship with other organizations; and devises specific goals and objectives pertinent to the future scientific mission of APS and American physiology.

W. C. Randall, *Chairman*
Dept. of Physiol.
Loyola University
Maywood, IL 60153

R. M. Berne
Dept. of Physiol.
Univ. of Virginia
Charlottesville, VA 22908

E. O. Feigl
Dept. of Physiol.
Univ. of Washington
Seattle, WA 98195

J. P. Granger
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

G. A. Hedge
Dept. of Physiol.
West Virginia Univ.
Morgantown, WV 26506

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

X. J. Musacchia
Graduate Prog. & Res.
Univ. of Louisville
Louisville, KY 40292

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.

A. E. Taylor, *Chairman*
Dept. of Physiol.
Univ. of South Alabama
Mobile, AL 36688

N. M. Buckley
Dept. of Physiol./Biophys.
Albert Einstein Coll. of Med.
Bronx, NY 10461

S. Lahiri
Dept. of Physiol.
Univ. of Pennsylvania
Philadelphia, PA 19104

C. Levinson
Dept. of Physiol.
Univ. of Texas Med. Sch.
San Antonio, TX 78284

J. A. Schafer
Dept. of Physiol./Biophys.
Univ. of Alabama
Birmingham, AL 35294

L. C. Weaver
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

Perkins Memorial Fund Committee

Selects recipients for visiting scientist family support awards and administers the Fund.

J. R. Pappenheimer, *Chairman*
Dept. of Physiol.
Harvard Univ. Med. Sch.
Boston, MA 02115

D. F. Bohr
Dept. of Physiol.
Univ. of Michigan
Ann Arbor, MI 48109

J. N. Diana
Dept. of Physiol./Biophys.
Louisiana State Univ.
Shreveport, LA 71130

N. C. Staub
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

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.

A. C. Barger,
Co-Chairman
Dept. of Physiol.
Harvard Univ. Med. Sch.
Boston, MA 02115

T. G. Coleman
Dept. of Physiol./Biophys.
Univ. of Mississippi
Jackson, MS 39216

E. L. Ison-Franklin
Dept. of Physiol.
Howard Univ.
Washington, DC 20059

G. Kaley
Dept. of Physiol.
New York Med. Coll.
Valhalla, NY 10595

E. W. Hawthorne
Co-Chairman
Dept. of Physiol.
Howard Univ.
Washington, DC 20059

J. W. Manning
Dept. of Physiol.
Emory Univ.
Atlanta, GA 30322

E. L. Pautler
Dept. of Physiol./Biophys.
Colorado State Univ.
Fort Collins, CO 80523

J. Stinson
Dept. of Physiol.
Meharry Med. Coll.
Nashville, TN 37208

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.

C. V. Gisolfi, *Chairman*
Dept. of Physiol./Biophys.
Univ. of Iowa
Iowa City, IA 52242

P. D. Harris
Dept. of Physiol.
Univ. of Louisville
Louisville, KY 40292

B. L. Umminger
Reg. Biology Program
National Sci. Fndn.
Washington, DC 20550

M. L. Entman (ex officio)
Sec. Cardiovascular Sci.
Baylor Coll. of Med.
Houston, TX 77030

F. G. Knox (ex officio)
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

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.

Cardiovascular
A. W. Cowley
Dept. of Physiol.
Med. Coll. of Wisconsin
Milwaukee, WI 53226

J. W. Downey (ex officio)
Dept. of Physiol.
Univ. of Alabama
Birmingham, AL 36688

Cell & General Physiology
P. J. DeWeer
Dept. of Physiol.
Washington Univ. Med. Sch.
St. Louis, MO 63110

Clinical Physiology
G. F. Cahill, Jr.
Harvard Med. Sch.
H. Hughes Med. Inst.
Boston, MA 02215

Comparative Physiology
L. I. Crawshaw
Dept. of Biol.
Portland State Univ.
Portland, OR 97207

Endocrinology & Metabolism
G. A. Hedge
Dept. of Physiol.
West Virginia Univ.
Morgantown, WV 26506

Environmental, Thermal & Exercise Physiology
L. B. Rowell
Dept. of Physiol./Biophys.
Univ. of Washington
Seattle, WA 98195

Epithelial Transport Group
J. S. Handler
Lab. of Kidney/Electrolyte Metabolism
NIH, NHLBI
Bethesda, MD 20205

Gastrointestinal Physiology
L. Lichtenberger
Dept. of Physiol.
Univ. of Texas Med. Sch.
Houston, TX 77025

History of Physiology
N. C. Staub
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

Muscle Physiology
M. J. Siegman
Dept. of Physiol.
Jefferson Med. Coll.
Philadelphia, PA 19107

Nervous System
J. Trubatch
Assoc. Vice Pres. for Res.
Univ. of Chicago
Chicago, IL 60637

Neural Control & Autonomic Regulation
R. D. Foreman
Dept. of Physiol./Biophys.
Univ. of Oklahoma
Oklahoma City, OK 73190

Renal Physiology
W. F. Boron
Dept. of Physiol.
Yale Sch. of Med.
New Haven, CT 06510

Respiratory Physiology
A. J. Berger
Dept. of Physiol./Biophys.
Univ. of Washington
Seattle, WA 98195

Water & Electrolyte Homeostasis
R. E. Shade
Southwest Fndn. for Research & Education
San Antonio, TX 78284

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.

J. T. Shepherd, *Chairman*
Board of Development
Mayo Fndn.
Rochester, MN 55901

K. D. Gardner, Jr.
Div. of Renal Diseases
Univ. of New Mexico
Albuquerque, NM 87131

J. A. Nadel
Dept. of Med./Physiol.
Univ. of California
San Francisco, CA 94143

N. B. Marshall (ex officio)
Therap. Res.
The Upjohn Co.
Kalamazoo, MI 49001

W. M. Samuels (ex officio)
American Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

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.

S. F. Gottlieb
Graduate School
Univ. of South Alabama
Mobile, AL 36688

L. K. Miller
Inst. of Arctic Biology
Univ. of Alaska
Fairbanks, AK 99701

P. C. Johnson
Dept. of Physiol.
Univ. of Arizona
Tucson, AZ 85724

G. S. Campbell
Dept. of Surg.
Univ. of Arkansas
Little Rock, AR 72201

E. A. Rhode
Sch. of Vet. Med.
Univ. of California
Davis, CA 95616

D. Robertshaw
Dept. of Physiol./Biophys.
Colorado State Univ.
Fort Collins, CO 80523

R. W. Berliner
Yale Univ.
Sch. of Med.
New Haven, CT 06510

F. E. South
Sch. of Life & Hlth. Sci.
Univ. of Delaware
Newark, DE 19711

M. E. Freeman
Dept. of Biol. Sci.
Florida State Univ.
Tallahassee, FL 32306

D. R. Humphrey
Dept. of Physiol.
Emory Univ. Sch. of Med.
Atlanta, GA 30322

M. D. Rayner
Dept. of Physiol.
Univ. of Hawaii Med. Sch.
Honolulu, HI 96822

T. H. McKean
Dept. of Zool. (WAMI)
Univ. of Idaho
Moscow, ID 83843

B. A. Curtis
Dept. of Basic Sci.
Peoria Sch. of Med.
Peoria, IL 61656

E. E. Selkurt
Dept. of Physiol.
Indiana Univ. Sch. of Med.
Indianapolis, IN 46223

R. E. Engen
Coll. of Vet. Med.
Iowa State Univ.
Ames, IA 50010

J. L. Voogt
Dept. of Physiol.
Univ. of Kansas Med. Sch.
Kansas City, KS 66103

H. R. Hirsch
Dept. of Physiol./Biophys.
Univ. of Kentucky
Lexington, KY 40503

T. H. Dietz
Dept. of Zool./Physiol.
Louisiana State Univ.
Baton Rouge, LA 70803

J. M. Norton
Univ. of New England
Coll. of Osteopathic Med.
Biddeford, ME 04005

T. R. Hendrix
The Johns Hopkins Univ.
Sch. of Med.
Baltimore, MD 21205

D. M. Philbin
Dept. of Anaesthesia
Massachusetts Gen. Hosp.
Boston, MA 02114

H. V. Sparks, Jr.
Dept. of Physiol.
Michigan State Univ.
East Lansing, MI 48824

I. J. Fox
Dept. of Physiol.
Univ. of Minnesota
Minneapolis, MN 55455

M. Petrini
Dept. of Physiol./Biophys.
Univ. of Mississippi
Jackson, MS 39216

D. M. Griggs, Jr.
Dept. of Physiol.
Univ. of Missouri
Columbia, MO 65212

J. A. McMillan
Dept. of Biol.
Montana State Univ.
Bozeman, MT 59717

C. M. Moriarty
Dept. of Physiol./Biophys.
Univ. of Nebraska
Omaha, NE 68105

J. D. Wood
Dept. of Physiol.
Univ. of Nevada Med. Sch.
Reno, NV 89557

H. Valtin
Dept. of Physiol.
Dartmouth Med. Sch.
Hanover, NH 03755

G. F. Merrill
Dept. of Physiol.
Rutgers Univ.
Brunswick, NJ 08903

S. Solomon
Dept. of Physiol.
Univ. of New Mexico
Albuquerque, NM 87131

R. E. Dutton
Dept. of Physiol.
Albany Coll. of Med.
Albany, NY 12208

M. L. Wolbarsht
Dept. of Ophthalmology
Duke Univ. Med. Ctr.
Durham, NC 27710

T. K. Akers
Dept. of Physiol.
Univ. of North Dakota
Grand Forks, ND 58202

J. J. Curry
Dept. of Physiol.
Ohio State Univ.
Columbus, OH 43210

K. J. Dormer
Dept. of Physiol./Biophys.
Univ. of Oklahoma
Oklahoma City, OK 73190

A. J. Rampone
Dept. of Physiol.
Univ. of Oregon Med. Sch.
Portland, OR 97201

J. R. Neely
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

H. F. Cserr
Dept. of Physiol./Biophys.
Brown Univ.
Providence, RI 02912

B. T. Cole
Dept. of Biol.
Univ. of South Carolina
Columbia, SC 29208

W. W. Winder
Dept. of Physiol./Pharm.
Univ. of South Dakota
Vermillion, SD 57069

J. C. Ross
Dept. of Med.
Vanderbilt Univ.
Nashville, TN 37203

C. Desjardins
Dept. of Zool.
Univ. of Texas
Austin, TX 78712

J. H. Petajan
Univ. of Utah
3E512 Med. Ctr.
Salt Lake City, UT 84132

N. R. Alpert
Dept. of Physiol./Biophys.
Univ. of Vermont
Burlington, VT 05405

S. Price
Dept. of Physiol.
Med. Coll. of Virginia
Richmond, VA 23298

H. D. Patton
Dept. of Physiol./Biophys.
Univ. of Washington
Seattle, WA 98195

G. A. Hedge
Dept. of Physiol.
West Virginia Univ.
Morgantown, WV 26506

J. A. Will
Vet. Med. Sch.
Univ. of Wisconsin
Madison, WI 53706

S. L. Lindstedt
Dept. of Zool./Physiol.
Univ. of Wyoming
Laramie, WY 82071

APS member, **Janett Trubatch**, the Chair of the Section on the Nervous System will be assuming the position of Associate Vice President for Research at the University of Chicago on July 15, 1985. Her new address: Director, Office of Sponsored Programs, University of Chicago, 970 East 58th St., Chicago, IL 60637.

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.

P. C. Johnson, *Chairman*
Dept. of Physiol.
Univ. of Arizona
Tucson, AZ 85724

W. F. Ganong
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

L. R. Johnson
Dept. of Physiol.
Univ. of Texas
Houston, TX 77025

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

J. McE. Marshall
Div. of Biol. & Med. Sci.
Brown Univ.
Providence, RI 02912

Senior Physiologists Committee

Maintains liaison with senior and emeritus members.

E. B. Brown, *Chairman*
51 Sheneman Dr.
Bella Vista, AR 72712

E. F. Adolph
Dept. of Physiol.
Univ. of Rochester
Rochester, NY 14520

R. O. Greep
Lab. of Human Reprod.
Harvard Univ. Med. Sch.
Boston, MA 02115

W. A. Himwich
6679 Buckstone Ct.
Columbia, MD 21044

A. B. Otis
Dept. of Physiol.
Univ. of Florida
Gainesville, FL 32610

E. E. Selkurt
Dept. of Physiol.
Indiana Univ.
Indianapolis, IN 46223

B. W. Zweifach
Professor of Bioengineering
Univ. of California
La Jolla, CA 92093

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.

H. J. Cooke, *Chairman*
Dept. of Physiol.
Ohio State Univ.
Columbus, OH 43210-1238

B. C. Hansen
Dept. of Physiol.
Southern Illinois Univ.
Carbondale, IL 62901

C. B. Tai
5417 Wehawken Rd.
Bethesda, MD 20816

P. S. Timiras
Dept. of Physiol./Anat.
Univ. of California
Berkeley, CA 94720

H. D. Van Liew
Dept. of Physiol.
State Univ. of New York
Buffalo, NY 14215

Genetic Engineering

A short course on Genetic Engineering will be held on 15-17 November, 1985, at the Hyatt Regency O'Hare Chicago, IL. *Information*: J. E. Bailey, BRE Systems, 1665 E. Mountain St., Pasadena, CA 91104 (818/356-4116).

APS Representatives to Other Organizations (1985-86)

American Association for Accreditation of Laboratory Animal Care

L. Ramazzotto
Dept. of Physiol.
Fairleigh-Dickinson Univ.
Hackensack, NJ 07601

American Association for the Advancement of Science

W. Chavin
Dept. of Biol.
Wayne State Univ.
Detroit, MI 48202

H. L. Conn, Jr.
Dept. of Med.
Rutgers Univ. Med. Sch.
Piscataway, NJ 08854

American Institute of Biological Sciences

M. Frank
Am. Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

Council of Academic Societies of the Association of American Medical Colleges

G. A. Hedge
Dept. of Physiol.
West Virginia Univ.
Morgantown, WV 26506

J. L. Kostyo
Dept. of Physiol.
Univ. of Michigan Med. Sch.
Ann Arbor, MI 48104

Federation of American Societies for Experimental Biology

Board

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

H. E. Morgan
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

J. B. West
Dept. of Med.
Univ. of California
La Jolla, CA 92093

Executive Committee

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

Executive Officers Advisory Com.

M. Frank
Am. Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

Education Committee

J. A. Spitzer
Dept. of Physiol.
Louisiana State Univ.
New Orleans, LA 70112

Federation Proceedings Ed. Bd.

M. L. Entman
Sec. Cardiovascular Sci.
Baylor Coll. of Med.
Houston, TX 17030

Life Sciences Advisory Com.

W. B. Severs
Dept. of Pharm.
Pennsylvania State Univ.
Hershey, PA 17033

Meetings Committee

C. V. Gisolfi
Dept. of Physiol./Biophys.
Univ. of Iowa
Iowa City, IA 52242

Program Committee

M. Frank
Am. Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

Public Affairs Committee

J. T. Shepherd
Board of Development
Mayo Fndn.
Rochester, MN 55901

Public Information Committee

M. M. Cassidy
Dept. of Physiol.
George Washington Univ.
Washington, DC 20037

Publications Committee

L. R. Johnson
Dept. of Physiol.
Univ. of Texas
Houston, TX 77025

Research Conference Advis. Committee

C. V. Gisolfi
Dept. of Physiol./Biophys.
Univ. of Iowa
Iowa City, IA 52242

M. S. Smith
Dept. of Physiol.
Univ. of Pittsburgh
Pittsburgh, PA 15260

US National Committee for International Union of Physiological Sciences

F. G. Knox
Dept. of Physiol./Biophys.
Mayo Med. Sch.
Rochester, MN 55901

J. B. West
Dept. of Med.
Univ. of California
La Jolla, CA 92093

H. E. Morgan
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

APS Sections

How to Become Affiliated

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 Record Questionnaire.

Endocrinology and Metabolism. 23(5): 8, 1980. Same as Cardiovascular.

Environmental, Thermal and Exercise Physiology. 20(6): 14, 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.

History of Physiology. 27(4): 160, 1984. Same as Cardiovascular.

Nervous System. 21(3): 25, 1978. Indicate a primary or secondary Interest Area 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 Area Code 32 on the Membership Records Questionnaire.

Water and Electrolyte Homeostasis. 25(3): 143, 1982. Same as Cardiovascular.

Officers

Cardiovascular Section

B. R. Duling, *Chairman*
Dept. of Physiol.
Univ. of Virginia
Charlottesville, VA 22901

V. S. Bishop, *Secretary*
Dept. of Pharm.
Univ. of Texas
San Antonio, TX 78284

L. B. Rowell, *Treasurer*
Dept. of Physiol./Biophys.
Univ. of Washington
Seattle, WA 98195

Cardiac Mechanics Subsection
J. S. Janicki
Michael Reese Hosp.
Univ. of Chicago
Chicago, IL 60616

Splanchnic Circulation Subsection
D. N. Granger
Dept. of Physiol.
Univ. of South Alabama
Mobile, AL 36688

Cell and General Physiology Section

J. McE. Marshall, *Chairman*
Div. of Med. Sci.
Brown Univ.
Providence, RI 02912

M. Lieberman, *Councillor*
Dept. of Physiol.
Duke Univ. Med. Ctr.
Durham, NC 27710

C. S. Pace, *Secretary-Treasurer*
Diabetes Hosp.
Univ. of Alabama
Birmingham, AL 35294

A. V. Somlyo, *Councillor*
Pennsylvania Muscle Inst.
Univ. of Pennsylvania
Philadelphia, PA 19104

Comparative Physiology Section

R. B. Reeves, *Chairman*
Dept. of Physiol.
State Univ. of New York
Buffalo, NY 14214

D. C. Jackson, *Councillor*
Div. of Biomedical Sci.
Brown Univ.
Providence, RI 02912

W. K. Milsom *Secretary*
Dept. of Zool.
Univ. of British Columbia
Vancouver, BC V6T 2A9
Canada

Endocrinology and Metabolism Section

W. F. Ganong, *Chairman*
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

J. E. Gerich, *Councillor*
Endocrine Res. Inst.
Mayo Clinic
Rochester, MN 55901

L. S. Jefferson, *Secretary-Treasurer*
Dept. of Physiol.
Hershey Med. Ctr.
Hershey, PA 17033

S. E. Leeman, *Councillor*
Dept. of Physiol.
Univ. of Massachusetts
Worcester, MA 01605

Environmental, Thermal and Exercise Physiology Section

L. B. Rowell, *Chairman*
Dept. of Physiol./Biophys.
Univ. of Washington
Seattle, WA 98195

P. B. Raven, *Steering Com.*
Texas Coll. of Osteopathic Med.
Camp Bowie at Montgomery
Fort Worth, TX 76107

M. Kluger, *Steering Com.*
Dept. of Physiol.
Univ. of Michigan
Ann Arbor, MI 48109

D. Robertshaw, *Steering Com.*
Dept. of Physiol./Biophys.
Colorado State Univ.
Fort Collins, CO 80523

Epithelial Transport Group

R. A. Frizzell, *Chairman*
Dept. of Physiol./Biophys.
Univ. of Alabama
Birmingham, AL 35294

D. C. Dawson, *Steering Com.*
Dept. of Physiol.
Univ. of Michigan
Ann Arbor, MI 48109

P. Aronson, *Steering Com.*
Dept. of Med.
Yale Univ. Sch. of Med.
New Haven, CT 06510

E. M. Wright, *Steering Com.*
Dept. of Physiol.
Univ. of California
Los Angeles, CA 90024

Gastrointestinal Section

N. Weisbrodt, *Chairman*
Dept. of Physiol./Cell Biol.
Univ. of Texas Med. Sch.
Houston, TX 77025

H. J. Cooke, *Councillor*
Dept. of Physiol.
Ohio State Univ.
Columbus, OH 43210-1238

H. S. Ormsbee III, *Secy.-Treas.*
Pharmacol. R&D
Smith Kline & French Labs.
Philadelphia, PA 19101

J. A. Williams, *Councillor*
Mt. Zion Hosp. Med. Ctr.
Cell Biol. Lab.
San Francisco, CA 94120

History of Physiology Section

J. B. West, *Chairman*
Dept. of Med.
Univ. of California
La Jolla, CA 92093

R. Kellogg, *Steering Com.*
Dept. of Physiol.
Univ. of California
San Francisco, CA 94143

T. Appel, *Secretary*
Am. Physiol. Soc.
9650 Rockville Pike
Bethesda, MD 20814

Nervous System Section

J. Trubatch, *Chairman*
Assoc. Vice Pres. for Res.
Univ. of Chicago
Chicago, IL 60637

M. I. Phillips, *Steering Com.*
Dept. of Physiol.
Univ. of Florida
Gainesville, FL 32610

J. E. Blankenship, *Steering Com.*
Dept. of Physiol.
Univ. of Texas
Galveston, TX 77550

J. C. Houk, *Steering Com.*
Dept. of Physiol.
Northwestern Univ.
Chicago, IL 60611

D. R. Humphrey, *Steering Com.*
Dept. of Neurophysiol.
Emory Univ.
Atlanta, GA 30322

J. Lipton, *Steering Com.*
Dept. of Physiol.
Univ. of Texas Hlth. Sci. Center
Dallas, TX 75235

L. N. Mendell, *Steering Com.*
Dept. of Neurobiol./Behavior
SUNY at Stony Brook
Stony Brook, NY 11794

E. Satinoff, *Steering Com.*
Dept. of Psychol.
Univ. of Illinois
Champaign, IL 61820

Neural Control and Autonomic Regulation Section

A. D. Loewy, *Chairman*
Dept. of Anat./Neurobiol.
Washington Univ.
St. Louis, MO 63110

P. G. Schmid, *Councillor*
Univ. of Iowa
Coll. of Med.
Iowa City, IA 52240

M. D. Thames, *Secretary-Treasurer*
Cardiovascular Section
McGuire VA Medical Ctr.
Richmond, VA 23249

Renal Physiology Section

L. P. Sullivan, *Chairman*
Dept. of Physiol.
Kansas State Univ.
Kansas City, KS 66103

W. Arendshorst, *Secretary*
Dept. of Physiol.
Univ. of North Carolina
Chapel Hill, NC 27514

E. H. Blaine, *Treasurer*
Merck Inst. for Therap. Res.
West Point, PA 19486

Respiratory Physiology Section

J. A. Nadel, *Chairman*
Cardiovascular Res. Inst.
Univ. of California
San Francisco, CA 94143

J. Butler, *Councillor*
Dept. of Med.
Univ. of Washington
Seattle, WA 98195

P. Wagner, *Secretary-Treasurer*
Dept. of Med.
Univ. of California
La Jolla, CA 92093

R. W. Hyde, *Councillor*
Radiation Biol. & Biophys.
Univ. of Rochester
Rochester, NY 14642

Water and Electrolyte Homeostasis

A. W. Cowley, *Chairman*
Dept. of Physiol.
Med. Coll. of Wisconsin
Milwaukee, WI 53226

L. Share, *Councillor & Secretary*
Dept. of Physiol./Biophys.
Univ. of Tennessee
Memphis, TN 38163

W. H. Dantzler, *Councillor*
Dept. of Physiol.
Univ. of Arizona
Tucson, AZ 85724

News From Senior Physiologists

Philip Kramer to E. B. Brown:

In a sense, I retired as of January 31, 1985, from practice, teaching, and research. However, I am still active in medicine in that I have been appointed to the Admissions Committee of Boston University School of Medicine where I am presently Professor Emeritus. Other activities consist of writing scientific papers, reporting previous research, attending hospital medical grand rounds, research conferences, medical staff meetings, etc., as well as being on the University Hospital Patient Care Committee and Practice Privileges Committee. The intellectual stimulation resulting from interaction with trainees and colleagues, the letters of appreciation from former trainees and colleagues, and the laudatory comments at my retirement reception given by the Department of Medicine have more than counterbalanced the stresses, some disappointments, hard work and long hours, and at times inadequate compensation inherent in an academic career. Research not only satisfies one's intellectual curiosity but makes one feel that they have made some contribution, albeit small, to society and thereby justifying one's existence.

University Hospital at Boston
University Medical Center
Boston, MA 02118

Clifford Nelson to E. B.:

I was pleased to receive your letter inquiring about my activities. I have been in the Research Department of Maine Medical Center in Portland since 1956 except for a sabbatical year in the Cardiac Department of the Royal Melbourne Hospital, and the Baker Medical Research Institute in Australia. I retired in 1983 as a Research Career Awardee from the National Heart, Lung, and Blood Institute.

I am still working mornings in the same job, however, and still attending conferences and writing papers. I have one great frustration. We have proved both theoretically and experimentally that it is necessary to include (not correlate with) thorax dimensions when determining the dipole moment of the heart (heart-vector). Although this would eliminate the variable of body size in studies in hypertrophy and so forth, I have not been able to get this point across. This concept has been used by only two or three groups in the world. I shall have to continue working, therefore, until I can get this point across. Afternoons, I generally spend working around our 1771 house and 40 acre tree farm in Gorham, Maine.

Dept. of Research
Maine Medical Center
Portland, ME 04102

Carl A. Bunde to Roy Greep:

My professional activities for the past several years are with Hill Top Research, Inc., a contract research organization located in Cincinnati. I am Chairman of the Institutional Review Board, back up Medical Director to Cintest, Inc., their Clinical Pharmacology unit, consultant to the President, and a member of the Board of Directors. All that averages about one day per week. It does stimulate my reading. I have not participated in any publishable research in the last four years. I attend two

or three meetings a year including Fall meetings of the American Physiological Society. The FASEB meetings are too much. I except to be at the Niagara Falls meeting. I am also attending the Diabetes and Endocrinology meetings in Baltimore. We should meet at at least one of these.

3738 Donegal Drive
Cincinnati, OH 45236

Chuck Shilling to Roy:

My first retirement was from the U.S. Navy after 28 years of service in 1955. I retired in order to go to the Atomic Energy Commission as a civil servant, Deputy Director, Division of Biology and Medicine. Because of the dual compensation law which would not allow me to draw any of my Navy retirement, at the end of five years when I could retire under the "high five" law, I retired from AEC to go on the faculty of the Medical School of the George Washington University. Then when I got too old for GW, I retired and came to my present job as Executive Secretary/Treasurer of the Undersea Medical Society.

In a good many ways this is old home week because my entire career in the Navy was submarine and deep sea diving and research related to this, and the present job started out as being a Society interested in the biomedical aspects of diving and exposure to high pressure air. As a natural extension we are now in the field of hyperbaric oxygen therapy because we are using the same pressure chamber, the same chamber operators, the same oxygen, the same doctors. The only difference is the patient; instead of being one with air embolism or decompression sickness may be a patient with multiple sclerosis.

There are all of the usual Society duties with an international group and with a large number of contracts, but I am enjoying myself immensely even though one of these days I may have to retire again—this time finally to do nothing.

Undersea Medical Society
9650 Rockville Pike
Bethesda, MD 20814

Richard J. Bing to Ewald Selkurt:

I am not retired. Although I am Professor Emeritus at The University of Southern California, I am Director of Experimental Cardiology and Scientific Development of the Huntington Medical Research Institutes. The Institutes have added a new building fully equipped with nuclear magnetic resonance magnets, for both imaging and spectroscopy. I am beginning to work on spectroscopy of the heart under certain conditions, and we hope to use surface coils for this purpose. I also continue my work on the endothelial-derived relaxing factor and on the microcirculation through the brain. We have published, or have in print, nine papers dealing with these matters. Because of that I continue to write papers. I am planning to finish my book on *Essays in Cardiology* by the end of this year. Fortunately, I still find time to write music, and I have had several performances of my music in Australia, as well as in this country and in Germany. I have a nice album which the Germans have put out. As far as wisdom to pass on to younger colleagues, I can only say that each one of us has a choice between political and status appointments on one hand and doing good work on the other. I am all for the latter.

Huntington Memorial Hospital
100 Congress Street
Pasadena, CA 91105

Gerhard Brecher to Ewald:

The following is a brief story about my work in my last year in Cleveland before I moved to Columbus. This story may be entitled, "History of the Kay-Cross rotating disc oxygenator for open heart surgery." At the time, 1955 to early 1956, I developed this oxygenator as consultant for Drs. Kay and Cross. This work gave me the idea of writing the book, *Heart-Lung Bypass* with Galletti in 1962. As mentioned in the book I had asked my friend from my year at Duke in 1929-30, Murray C. Miller, to improve our English. He did a perfect job with us two foreigners. Right now, I am writing my life story which I am sending to Murray Miller in Philadelphia. Is it not unusual that this friendship has lasted 56 years?

If you should ever need me for some special lecture or seminar I could talk about "How does the heart fill?" or "Physiologist behind the Swastika Curtain." My talk about the Kay-Cross pump oxygenator did very well at the University of Eastern North Carolina in 1982, arranged by Hannis Latham, my roommate at Duke, now living in Washington, NC.

7708 Rumsey Road
Oklahoma City, OK 73132

Frank Craig to Ewald:

Thanks for your note of 25 March and the reminder of the good years in Homer Smith's department. I can't forget the view of First Avenue from the student laboratory and the man who used to lie in the gutter at the gates of Belleview until the ambulance from St. Vincent's came by to pick him up.

My only recent professional activity was to review a paper for the *Journal of Applied Physiology*. My research interests have shifted smoothly from physiology to genealogy. Having been brought up in a strict school of publish or perish, I was relieved to see my name in print again; this time in the October 1984 issue of the New England Historical and Genealogical Register.

Mary and I continue in good health. Last year we moved to Broadmead, a life care community. Among the residents are Mary Hardy, elected to APS in 1933, Harry F. Dowling, Chairman Emeritus of the Department of Medicine, University of Illinois, and Douglas Marsland, former Professor of Biology at N.Y.U.

13801 York Rd., G-5
Cockeysville, MD 21030

Samuel L. Leonard to Ewald:

Thank you for your letter inquiring about the activities of retired members of APS. Just as in the past, I go to my office mornings, when in town, to read and consult with graduate students but am not active in research or writing. During the summer, my wife and I travel about the country in a recreational vehicle including visits to our children. My research field is Endocrinology and Reproductive Physiology, and my work base has been in Zoology Departments. In the early days, much of the advances in these fields was made by Zoologists

and Anatomists. I took my degree with Dr. Frederick Hisaw at Wisconsin and then was a National Research Council Fellow under Dr. Philip E. Smith in the Anatomy Department of the College of Physicians and Surgeons, Columbia. As these subjects became more physiologically oriented, I became a member of APS. Dr. Walter Meck was on my doctoral committee, and I took his courses in the Physiology Department in the Medical School at Wisconsin. During the years, I have published in the APS journals, in fact, my doctoral thesis was published there in 1931.

Section of Genetics & Development
Cornell University
Ithaca, NY 14853

Oscar Richards to Ewald:

After retiring from American Optical Corporation Research in 1967, I taught Environmental Vision at Pacific University College of Optometry, retiring as Professor Emeritus in 1982. I still do some consulting on human vision problems and am trying to catch up with previous work. Last summer I revisited Sunset Bay, OR, to learn what changes in animal life occurred since the previous research there during summers 1924-25. Recently, I published two papers on human color vision deficiency problems. My wife and I live with my son Richard and his wife on a 40-acre park. It is proving very pleasant and successful.

O & R Laboratories
Route 1, Box 79F
Oakland, OR 97462

Isaac Starr to Ewald:

I am still working, but not full-time. I work about 5 hours/day 5 days a week. I still have my small office and laboratory in the University Hospital. Recently I have been greatly assisted by the Shared Ride Service of the Yellow Cab Company. This service for senior citizens is funded by the Lottery of the State of Pennsylvania. It takes care of my transportation from my apartment at Cathedral Village, to my office at the University of Pennsylvania Hospital and back, a service invaluable to my work and almost as good as having a private chauffeur. After arriving at my office I spend most of my time writing. I share a secretary with others at the Hospital. I am at work on a review requested by the editor of a medical periodical. I have just finished an obituary on "Dr. William Stadie," written at the request of the National Academy of Sciences.

University of Pennsylvania
University Hospital
Philadelphia, PA 19104

Bruce Dill to Ewald:

Hearing from you, a member of the committee on Senior Physiologists, refreshes some old memories. It was 1950 that I proposed to the Council the formation of such a committee. They did so and appointed me chairman. The letters exchanged with many of the old-timers of the Society were a delight to members of the committee mingled with sadness when we learned of their departure.

I have a tie with UNLV which is formalized by the title of Research Professor and salary of one dollar/month. I have an office at home and a part-time typist, Susan Cummings, who has uncanny skill in transcribing my handwriting. With her help I have composed the draft of a small book, which was put on a word processor at UNLV by Laurie Vincent, who looks after my interests with skill and devotion. The book based on our desert studies and titled by Baird Hastings, *The Hot Life of Man and Beast*, is due for publication by Thomas as one of their series "Lectures in Environmental Physiology."

I can quote honestly a phrase Dennis Jackson used at age 97, "I have no aches nor pains worth mentioning!" My main handicaps are loss of LBM, lethargy, decline of VO_{2max}, and poor vision and hearing. I am scheduled to present a 10-minute paper at Anaheim illustrating by VO_{2max} on the treadmill from age 37 to 93. For the measurement at age 93 I am indebted to my friends at Brooks AFB, especially Loren Myhre, who has been a colleague since 1961 at Bloomington.

Dept. of Biological Sciences
University of Nevada
Las Vegas, NV 89154

Edwin Hiatt to Ewald:

When I retired from Ohio State I made sure I would not be at loose ends by taking on two medical jobs, one as the county coroner and the other as plant physician for a local factory. The coroner job was interesting, but it interfered with my farming and traveling, so I gave it up after four years. Now I spend only about 10 hours a week as factory physician.

With regard to scholarly activities, I have given up the research lab and scientific writing, but I still do a little teaching. I have been given a nonpaying appointment in the Dept. of Medicine at Cincinnati University so that I can teach third and fourth year students, one or two at a time when they spend a month at our local hospital.

Now it is almost spring and I intend to revel in it this year. As the grass comes on I can quit feeding my 50 beef cows and pay attention to gardening and fishing with occasional obstetrical services to the cows.

754 Hoskins Road
Wilmington, OH 45177

Kenneth G. Kohlstaedt to Ewald:

In 1977 we moved from Indiana to Palm Springs to escape the cold of the midwest. I have been fortunate in that I have been able to participate in educational and research activities at the Eisenhower Medical Center which is located in Rancho Mirage, a community near Palm Springs. The Eisenhower Medical Center is a unique institution. In addition to the hospital and buildings for offices of physicians and clinical laboratories, it includes the Annenberg Center for Education in Health Sciences, the Hal Wallis Research Laboratory, and the Betty Ford Center for Treatment of Alcoholism and Drug Abuse. The Eisenhower Medical Center receives generous support from private sources in this community. Presently I am serving as a member of the IRB at the Eisenhower Medical Center and as a member of the advisory committee for the Annenberg Educational program. It has been exciting to participate in the development of this free standing institution which is an example of what can be accomplished by free enterprise.

Although I have not been on Indiana University campus since 1982, I am aware of some of the changes. The new Riley Hospital addition will really by wonderful. Indianapolis certainly has undergone some great changes.

1430 Paseo de Marcia
Palm Springs, CA 92262

Henry S. Badeer to Edward Adolph:

I am still actively engaged in teaching cardiovascular physiology to medical students and cardiovascular and respiratory physiology to dental and nursing students. Karger has published my text on *Cardiovascular Physiology* (1984), which is essentially a synopsis for medical students. Currently, my interest is with regard to the role of gravity on hemodynamics, a subject that has been often misunderstood.

So far I have enjoyed good health and hope to continue my interest in cardiovascular physiology. During my spare time I keep busy with yard work and fixing and repairing things that need attention at home.

Dept. of Physiology
Creighton University
Omaha, NE 68178

John Boylan to Edward:

Looking back over nearly forty years of association with Physiology I realize that my greatest pleasure and satisfaction came in the teaching of that gentle science to first year medical students—especially in the laboratory, when the laboratory was the guts of every Physiology course. Those were the years in Buffalo, first with Fred Griffith, then with Hermann Rahn and the great bunch he assembled in the late '50's.

Physiology was the key to so much that was memorable in my life; the years with McCance in Cambridge, years of a wonderful friendship with Kurt Kramer in Göttingen and Munich; Homer Smith and the years at Mount Desert Island Biological Laboratory; years of a great working partnership with your former student, Judith (Litchfield) Van Liews.

I am finishing a second career, as Chief of Staff of a Veterans Medical Center in the heart of Connecticut. While the running of a hospital is interesting and at times exciting, it does not compare to "the excitement and fascination of science." I have managed to keep a laboratory going and am exploring characteristics of the red cell membrane in the spontaneously hypertensive rat.

Veterans Administration Medical Center
555 Willard Avenue
Newington, CT 06111

Frederick A. Fuhrman to Edward:

I was surprised to learn that I am now a *Senior* Physiologist. I have been retired from teaching and laboratory research for several years. After many years of living both here (Stanford) and by the ocean near Monterey, Gerry and I have recently given up our home in Pebble Beach and are now living full-time near Stanford. At present I am at work on a historical review of tetrodotoxin and related toxins for a forthcoming New York Academy of Sciences symposium on the Molecular Biology of the Sodium Channel.

Dept. of Physiology
Stanford University
Stanford, CA 94305

Bone Demineralization During Space Flight

SUE ANN ANDERSON
Life Sciences Research Office
Federation of American Societies
for Experimental Biology
Bethesda, Maryland 20814

STANTON H. COHN
Brookhaven National Laboratory
Upton, New York 11973

Physiological adjustments to the space environment involve most tissues and organs including the skeletal system. Bone demineralization, the excessive elimination of mineral or inorganic salts from the skeleton, is a consequence of space flight. Sustained or serious bone loss is a matter of concern because low bone mass is recognized as a factor in the complex etiology of fracture (19). In addition to bone demineralization, changes in calcium metabolism reported during space flight include hypercalciuria and increased fecal loss along with possible increased potential for formation of calcium-containing renal stones.

In United States space flights lasting as long as 3 months, neither loss of bone mineral nor the resultant hypercalciuria impaired functional capacities of astronauts. However, concern for the health, effectiveness, and safety of space crews during and following extended or repeated space flights requires that deficiencies in knowledge of bone demineralization be identified. To enhance its research and analysis programming on bone demineralization during space flight, the National Aeronautics and Space Administration (NASA) requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) prepare a report[1] to review and evaluate NASA's ongoing research effort and provide suggestions for future directions of research in this area.[2] The study was done with the guidance of an ad hoc Working Group.[3]

[1]Copies of the report, entitled "Research Opportunities in Bone Demineralization" are available from the Special Publications Office, FASEB, 9650 Rockville Pike, Bethesda, MD 20814, at a cost of $8.00 (prepaid).

[2]The NASA Biomedical Research Program is conducted intramurally by the NASA Research Centers and by means of extramural grants and contracts. Qualified scientists interested in learning more about the program should write to Chief, Space Medicine Branch/EBM, Life Sciences Division, NASA Headquarters, Washington, DC 20546.

[3]Participants were Stanton H. Cohn, Ph.D., Senior Scientist, Brookhaven National Laboratory, Upton, NY; Solomon Epstein, M.D., Head, Division of Endocrinology and Metabolism, Albert Einstein Medical Center, Philadelphia, PA; Gregory R. Mundy, M.D., Head, Endocrinology and Metabolism, University of Texas Health Science Center, San Antonio, TX; Charles Y. C. Pak, M.D., Chief, Section of Mineral Metabolism, University of Texas Southwestern Medical School, Dallas, TX; A. Michael Parfitt, M.B., B. Chir., Director, Bone and Mineral Research Laboratory, Henry Ford Hospital, Detroit, MI; G. Donald Whedon, M.D., Clearwater, FL.

This article summarizes the LSRO report to acquaint scientists with the problem of bone demineralization associated with manned space flight and to encourage research directed toward resolution of the problem.

Calcium Losses and Endocrine Responses in Humans During Space Flight

Metabolic balance studies conducted during space flights indicate that exposure to weightlessness results in negative balances of calcium and phosphorus as well as magnesium and nitrogen (33, 47, 62). Metabolic data collected for 5 days during the 12-day Apollo 17 flight suggested that large fecal losses of calcium were the primary cause of the negative calcium balance observed in the three astronauts. Additional studies of calcium and phosphorus balance conducted during the Skylab 2, 3, and 4 missions of 28, 59, and 84 days duration, respectively, confirmed the occurrence of negative calcium and phosphorus balance in the nine astronauts participating in these space flights. In contrast to the very large increases in fecal calcium and phosphorus excretion reported for the Apollo 17 astronauts (47), smaller overall increases in fecal calcium and phosphorus losses were observed in the crewmen of the Skylab flights (45, 62). Urinary excretion accounted for the greater proportion of calcium and phosphorus lost during the three Skylab flights. Urinary calcium excretion rose steadily during the first 2–4 weeks of flight and remained at levels 80–100% greater than preflight levels for the remainder of the flights.

The relative importance of routes of calcium loss during space flight remains unresolved. Although overall increases in fecal calcium and phosphorus were smaller than increases in urinary calcium losses during the Skylab flights, calculation of fecal calcium losses as a function of flight duration indicated a continuous increase with no tendency to plateau (44). Such an analysis suggests that increased fecal calcium excretion during space flight might represent the major route of calcium loss. However, a subsequent analysis of these data indicated that the changes in fecal calcium excretion, net calcium absorption, and relative calcium absorption (net absorption expressed as a percentage of dietary intake) were not statistically significant. These observations suggest that the negative calcium balance of space flight resulted from an increase in urinary calcium excretion (42).

Measures of bone density of astronauts corroborate the evidence for bone calcium loss implied by the balance studies during space flight. Mineral content of the radius, ulna, and os calcis of 18 astronauts was determined by photon absorptiometry immediately before and after the Apollo 14, 15, and 16 missions and the Skylab 2, 3, and 4 flights. No significant losses of bone

mineral were reported in the radius or ulna of the astronauts from any flight (46, 55). Two crewmen from the Apollo 15 mission (duration 14 days) showed loss of os calcis mineral (46). Although no loss of mineral from os calcis was detected in crewmen following the Skylab 2 mission (28 days), significant losses of os calcis mineral (−7.4%, −4.5%, and −7.9%) were reported in three of the six crewmen of the Skylab 3 and 4 missions. These crewmen also had the largest increases in urinary calcium loss and the greatest negative shifts in calcium balance (55). Similar mineral losses (5–8%) from os calcis were reported for cosmonauts following the 175-day Salyut-6-Soyuz flight (40).

Endocrine responses have been measured before and after the Apollo flights and before, during, and after the manned Skylab missions (24, 28, 62). Calcitropic hormones showed no changes that would account for the mobilization of bone mineral during space flight.

Serum parathyroid hormone (PTH) concentrations did not differ before and after the Apollo missions (24) and fluctuated above and below the preflight values during the Skylab flights (27). Although there was a tendency toward a slight increase in PTH levels during the third month of the Skylab 4 flight, only three samples were available for analysis and the increases were not statistically significant (27). Plasma calcitonin levels were too low to be measured by the assay used and were therefore considered not elevated to any clinically significant extent (27, 28, 61). Plasma levels of 25-hydroxycholecalciferol were not different before and after the 28- and 59-day Skylab flights but were decreased by ~9% immediately following the 84-day Skylab mission. Total concentrations of calcium and phosphate in plasma rose slightly but significantly during the Skylab flights and returned to preflight values during the postflight observation period (27). During space flight, levels of plasma calcium and phosphate remained within ranges considered normal (14).

Plasma levels of some other hormones related to calcium metabolism were either increased (human growth hormone) or decreased (insulin) during space flight. Thyroid-stimulating hormone (TSH) and thyroxine (T_4) concentrations in plasma were increased postflight when compared with preflight values; however, no change occurred in triiodothyronine (T_3) resin uptake postflight. Plasma and urinary corticosteroids were generally elevated in astronauts during space flight (24, 26, 27, 28). Evidence indicating interactions of glucocorticoids with PTH (8, 64) and vitamin D (9, 15, 54) has made these compounds of particular interest.

Mineral Losses and Endocrine Responses in Humans During Simulated Weightlessness

Bed-rest studies of healthy men have been used as the ground-based model of weightlessness for evaluation of mineral metabolism in humans during space flight.

Hypercalciuria persists throughout periods of bed rest as long as 36 weeks (10, 12, 17). In these studies, concentrations in healthy persons began to rise immediately, peaked after 6–7 weeks, and then began to subside. Fecal calcium excretion is also elevated during bed rest (12, 17). Studies of calcium kinetics have suggested increased endogenous secretion of calcium into the gut; however, measurement of intestinal calcium absorption after 5 weeks of bed rest showed decreased, increased, or unchanged absorption among the subjects (32). Overall, hypercalciuria plus increased fecal losses of calcium during bed rest produced a negative calcium balance of ~ −200 mg/day, corresponding to a loss of total body calcium of ~ 0.5%/month (12, 17). These losses are similar to the losses calculated for astronauts during space flight.

Immobilization is also associated with the development of renal stones (1, 29, 59). Several factors associated with immobilization may increase the risk of development of renal stones during bed rest and possibly during space flight; these include impaired drainage of the kidneys, increased phosphate excretion, and increased urinary pH (43).

Measurements of bone mineral content showed no losses of bone mineral in the radius but substantial losses (25–40%) in weight-bearing os calcis during bed rest studies of 24–36 weeks (12, 17). Studies of calcium kinetics suggested that rates of bone accretion and resorption were both increased, with a greater increase in bone resorption (32). The rate of loss from the os calcis (~4% each month) was greater than the overall rate of loss of body calcium (0.5%/month); however, loss of calcium from the os calcis accounted for only a small proportion of the total amount of calcium lost (44). Bone mineral losses of the lumbar vertebrae of 0.9%/week during bed-rest periods of 11–61 days (mean 27 days) suggest a rate of loss of bone mineral from the lumbar vertebrae similar to that of the os calcis during bed rest (21).

The changes in calcium balance and in mineral content of os calcis and lumbar vertebrae of subjects during voluntary or therapeutic bed rest may be reversible. Remineralization of os calcis began immediately upon reambulation and occurred at a rate similar to the loss (12, 17). Similarly, vertebral losses were nearly restored 4 months after therapeutic bed rest of 11–61 days (21). However, Rambaut and Johnston (44) referred to unpublished studies of unspecified duration in which calcium balance returned to zero balance in middle-aged men before all bone mineral could have been replaced.

Serum levels and urinary excretion of hormones directly or indirectly influencing calcium metabolism have not shown changes that account for the losses of calcium during bed rest. Studies of serum levels of PTH were inconclusive (16, 50), and measurements of calcitonin and 1,25-dihydroxycholecalciferol in healthy volunteers during bed rest are not yet available. Urinary excretion of glucocorticoids is not significantly elevated during bed rest (23, 25, 49), in contrast to the reports of elevated plasma levels and urinary excretion of glucocorticoids associated with space flight (24, 26–28). Insulin response to glucose challenge was exaggerated during bed rest (11, 31, 49). Increased insulin levels have been associated with increased urinary excretion of calcium (2).

Mechanisms of Bone Loss During Space Flight and Simulated Weightlessness

Information on mechanisms of bone loss and histologic changes in bone during weightlessness has been obtained from studies of rats flown aboard spacecraft, ground-based immobilized animals (rats and monkeys),

and patients exhibiting bone loss following spinal cord injury. Histologic change in bone during exposure to weightlessness and its cause have not been studied in human subjects or in an animal model having a bone remodeling system similar to that of man (e.g., monkeys, dogs, miniature pigs).

Collaborative studies between the U.S. and U.S.S.R. of skeletally immature male rats flown onboard Cosmos biosatellites have provided information concerning changes in bone during space flight (37, 60, 66). Parameters associated with periosteal bone formation were significantly decreased in weight-bearing bones of space-flown rats where intrinsic muscle forces did not act on the bone (56, 65). However, it is not yet clear how the presence or absence of mechanical forces influences the processes of bone modeling and remodeling. The histologic changes observed in the bones of rats following space flight are similar to those seen in ground-based studies of rats subjected to simulated weightlessness (36) or following administration of corticosteroids (18). Adrenal glands were enlarged in space-flown rats (37, 38), and it was suggested that increased glucocorticoid levels might contribute to bone changes. However, the specificity of loss from weight-bearing bones may imply that local rather than systemic factors mediated the bone loss.

Breaking strength was reduced in femurs of rats exposed to weightlessness for 19 days during space flight but returned to normal by 25 days postflight (57). However, breaking strength was not reduced in femurs of rats maintained in a 1-G centrifuge during the flight. Ground-based studies of chair-restrained monkeys (*Macaca nemestrina*) suggested that the axial skeleton (vertebrae) is more adversely affected than the appendicular skeleton (tibia, radius, and ulna) by extended (6 months) immobilization (7). Striking changes were observed in studies of metabolic, endocrine, and histomorphometric alterations of calcium metabolism and bone in *M. mulatta* immobilized in full-body casts in an upright position for 14 days (6, 20, 35, 48, 53). These changes were similar to those observed in rats flown aboard the Cosmos biosatellites.

Countermeasures

Methods for prevention of the calcium losses observed during space flight have focused on dietary modifications, physical techniques, and pharmacological intervention. Supplements of calcium and phosphate given to healthy subjects during bed rest were associated with less negative calcium balance for 12 weeks but calcium losses increased after this time (16). Similarly, daily administration of 10 mg fluoride did not prevent calcium losses during bed rest (34).

Exercise regimens have been associated with slower bone loss in patients with osteoporosis (3, 22) and with increased bone density in athletes (39). In healthy subjects otherwise at bed rest, 4 hours/day of controlled ambulation on a measured course was found necessary to alleviate completely negative calcium balance during a 6-week study (52). In U.S.S.R. space flights, 1.3–2.5 hours of exercise (warm-up exercises, rowing, bungee cord exercises, treadmill running, and bicycling) are provided for the cosmonauts. In some flights these protocols have been associated with decreased calcium losses, and it has been stated that changes in bone can be associated with changes in contractile properties of corresponding muscles (13, 41).

Diphosphonate compounds have been used as pharmacological agents to reduce calcium loss in healthy subjects at bed rest (32, 51). However, subsequent identification of serious side effects in patients receiving these drugs (4, 5) has contraindicated their further use.

Unresolved Questions

A number of questions concerning bone demineralization during space flight remain unanswered by the studies conducted to date during space flight and in ground-based models. Suggestions on research approaches that address questions on bone demineralization of potential utility to NASA's needs are listed in Table 1. These suggested research topics related to the major points identified in the following paragraphs.

Metabolic studies showing negative calcium and phosphorus balances and noninvasive measures of bone density changes have shown that bone mineral losses occur during space flight. Such findings indicate a net difference between anabolic and catabolic processes but provide little information concerning the mechanisms involved under conditions of weightlessness.

The fundamental processes underlying changes in bone of normal human subjects are poorly understood not only during space flight but also during prolonged bed rest. Study of bone changes in human subjects during simulated as well as actual weightlessness is essential to gain understanding of the pathogenesis of bone loss during space flight. Understanding of the mechanisms of bone loss during space flight in both human subjects and an animal model having a bone-remodeling system similar to that of man is a critical research goal. Knowledge of the types of changes occurring in human bone is essential for validating any model of immobilization bone loss, for predicting reversibility and consequences of immobilization bone loss, and for developing effective countermeasures. Studies of male and female human volunteers during bed rest should be expanded. Histomorphometric bone changes should be investigated and related to changes in bone as evaluated by noninvasive methods and to changes in hormonal profiles.

The studies of plasma levels of PTH, 25-hydroxycholecalciferol, and calcitonin during Skylab flights did not indicate consistent changes of a magnitude that would ordinarily be associated with increased mobilization of bone and general systemic effects cannot explain the local and preferential demineralization of weight-bearing bones. However, the ad hoc Working Group did not dismiss possible effects of PTH, 1,25-dihydroxycholecalciferol, or calcitonin on bone mobilization during space flight. At the times hormone levels were measured during previous space flights, assays for a number of hormones were not well defined and it is difficult to draw conclusions from those data. Comparisons of changes in levels of calcitropic hormones need to be made using state-of-the-art methods and compared with histomorphometric changes in bone during bed rest or space flight.

The Working Group suggested that an elevation in cortisol secretion may be an accessory factor in bone demineralization during exposure to weightlessness. However, most members of the Working Group observed that cortisol is probably not the sole stimulus for the in-

Table 1
Opportunities for Research in Bone Demineralization*

A. Pathogenesis

- Distinguish processes causing primary changes in bone during weightlessness from associated influences such as changes in hormone levels that may result from, rather than cause, changes in bone
- Analyze and compare quantitative histomorphometry of bones of human subjects exposed to real and simulated weightlessness to determine the gravity-dependent responses of bone itself
- Study effects of real and simulated weightlessness on histomorphometry of trabecular and cortical bone from different sites (axial and appendicular) in an appropriate animal model in inflight and ground-based studies to determine the type of bone preferentially mobilized
- Examine collagen at bone sites most severely affected by weightlessness to determine whether existing collagen is modified or whether newly synthesized collagen is defective
- Continue to study changes in bone density resulting from exposure to zero gravity. Examine changes at sites in addition to those previously observed to assess change in a more comprehensive manner
- Extend the length of follow-up studies of bone loss in astronauts and subjects at bed rest to determine whether effects of real and simulated weightlessness on bone demineralization are reversible
- Determine the results of weightlessness on local bone mechanisms, e.g., effects of removal of physical-mechanical stress and piezoelectric stimuli on bone, changes in influences of muscle action on bone, and consequences of altered circulation induced by weightlessness on bone cell metabolism
- Examine bone cell metabolism by tissue culture techniques to extend the definition of changes occurring during space flight

B. Endocrine Effects

- Determine endocrine changes simultaneously with bone changes in human subjects and appropriate animal models
- Determine and compare effects of weightlessness on plasma concentrations and urinary excretion of hormones in astronauts and subjects during bed rest
- Analyze plasma and urine samples from subjects of ground-based experiments to determine whether there is a substance that correlates to loss of bone mineral and could serve as a marker of bone demineralization

C. Models

- Study bone histomorphometry and associated biochemical and endocrine changes of astronauts during space flight to establish a basis for development and validation of a ground-based and space-flown animal model to evaluate bone demineralization, potential for formation of renal stones, and efficacy of countermeasures
- Expand the studies of subjects during bed rest. Compare the bone changes and biochemical and hormonal alterations in astronauts with those of healthy male and female subjects during bed rest
- Develop and validate a ground-based animal model exhibiting histomorphometric, biochemical, and hormonal changes that correspond to the changes observed in that species and in man during space flight
- Develop mathematical models for bone loss based upon observations of changes in bone during space flight in human subjects and in validated animal models

D. Integrated Research Approaches

- Examine the problem of bone demineralization from histomorphometric, endocrine, and biochemical aspects simultaneously. Integrate study of the potential for renal stone formation into these studies. Correlate bone loss during space flight and bed rest with loss of muscle mass and electrolytes
- Maximize the amount of information obtained from inflight experiments. Two suggestions for accomplishing this were increasing the number of analyses of blood and urine samples collected inflight as well as pre- and postflight and, whenever possible, combining studies of bone loss with studies of other physiological changes during space flight
- Continue to develop and refine newly emerging techniques for noninvasive measures of skeletal status. Expand application of these techniques for evaluation of bone loss

E. Countermeasures

- Evaluate the effect of aminopropylidene diphosphonate (APD) on bone loss and hypercalciuria during bed rest if safety tests of the drug prove satisfactory. Other drugs that might be considered for testing are fluoride (in larger doses than previously tested), human calcitonin administered on an intermittent schedule, and anabolic steroids
- Assess in a ground-based program the changes in bone and muscle occurring during an exercise program effective in counteracting calcium loss to help identify the influence of such factors as muscle pull, mechanical stress, piezoelectric stimulation, and altered circulation on bone demineralization
- Investigate in space flight, protocols for exercise that would place stress on weight-bearing bones equivalent in forces to 4 hours of walking on Earth.

*Listed in suggested decreasing order of priority within each of four separate categories. See text for detail.

creased bone mobilization observed during space flight. This opinion was based on observations that urinary cortisol excretion was not increased in subjects during bed rest studies, yet urinary calcium excretion is still elevated to a similar extent during bed rest as during space flight. Roles of other endocrine agents such as thyroid hormones, aldosterone, and prolactin were considered peripheral to the problem of bone demineralization during space flight.

Because physiological responses during weightlessness differ from those under gravity, it is possible that responses of bone cells to normal levels of hormonal agents in weightlessness differ from responses on the ground. This may be particularly evident in weight-bearing bones that lack their normal stimuli.

Effective measures to counteract bone demineralization and calcium loss during space flight have not been identified. A better understanding of the pathogenesis of this bone loss and the mechanisms by which it occurs will aid in the development of exercise regimens and/or pharmacological agents effective in preventing bone loss.

Bone demineralization and hypercalciuria may also be related to other health effects observed during space flight, i.e., muscle loss and deconditioning and an increased potential for renal stone formation. Although some effects of exercise on muscle atrophy in space flight have been described (30, 58, 63), the association among bone loss, muscle atrophy, and exercise needs additional critical study. To date, there are no reports of urolithiasis in astronauts during or following space flights. However, evidence from relatively short-duration inflight studies suggests the occurrence of changes in urine composition that may alter the tendency for renal stone formation.

The Working Group recommended that projects integrating several areas possibly related to bone demineralization be encouraged. Coordinated research projects to evaluate simultaneously histomorphometric changes in bone with endocrine changes, body composition changes (especially muscles), and tendency for renal stone formation were named as particular areas of research importance.

References

1. Albright, F., C. H. Burnett, O. Cope, and W. Parson. Acute atrophy of bone (osteoporosis) simulating hyperparathyroidism. *J. Clin. Endocrinol.* 1: 711–716, 1941.

2. Allen, L. H., G. D. Block, R. J. Wood, and G. F. Bryce. The role of insulin and parathyroid hormone in the protein-induced calciuria of man. *Nutr. Res.* 1: 3–11, 1981.

3. Aloia, J. F., S. H. Cohn, J. A. Ostuni, R. Cane, and K. Ellis. Prevention of involutional bone loss by exercise. *Ann. Intern. Med.* 89: 356–358, 1978.

4. Bone, H. G., III, J. E. Zerwekh, F. Britton, and C. Y. C. Pak. Treatment of calcium urolithiasis with diphosphonate: efficacy and hazards. *J. Urol.* 121: 568–571, 1979.

5. Bounameaux, H. M., J. Schifferli, J.-P. Montani, A. Jung, and F. Chatelanat. Renal failure associated with intravenous diphosphonates. *Lancet* 1: 471, 1983.

6. Cann, C. E., and S. B. Arnaud. Calcium metabolism and correlated endocrine measurements in nonhuman primates during hypokinesia. In: *A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates: A Compilation of Results,* edited by L. Kazarian, C. Cann, M. Parfitt, D. Simmons, and E. Morey-Holton. NASA Technical Memorandum 81268. 1981, pp. 29–33. (Available from NTIS, Springfield, VA)

7. Cann, C. E., H. K. Genant, and D. R. Young. Comparison of vertebral and peripheral mineral losses in disuse osteoporosis in monkeys. *Radiology* 134: 525–529, 1980.

8. Chen, T. L., and D. Feldman. Glucocorticoid receptors and actions in subpopulations of cultured rat bone cells. *J. Clin. Invest.* 63: 750–758, 1979.

9. Chesney, R. W., R. B. Mazess, A. J. Hamstra, H. F. DeLuca, and S. O'Reagan. Reduction of serum-1,25-dihydroxyvitamin-D_3 in children receiving glucocorticoids. *Lancet* 2: 1123–1125, 1978.

10. Deitrick, J. E., G. D. Whedon, and E. Shorr. Effects of immobilization upon various metabolic and physiologic functions of normal men. *Am. J. Med.* 4: 3–36, 1948.

11. Dolkas, C., and J. Greenleaf. Insulin and glucose responses during prolonged bed rest with isotonic and isometric exercise. *J. Appl. Physiol.* 43: 1033–1038, 1977.

12. Donaldson, C. L., S. B. Hulley, J. M. Vogel, R. S. Hattner, J. H. Bayers, and D. E. McMillan. Effect of prolonged bed rest on bone mineral. *Metabolism* 19: 1071–1084, 1970.

13. Gazenko, O. G., A. M. Genin, and A. D. Egorov. Major medical results of the Salyut-6-Soyuz 185-day space flight. *Congr. Int. Astronaut. Fed. 32nd Sess. D-5 Rome, 1981,* vol. II.

14. Guyton, A. C. *Textbook of Medical Physiology* (6th ed.). Philadelphia: Saunders, 1981, p. 976.

15. Hahn, T. J., L. R. Halstead, S. L. Teitelbaum, and B. H. Hahn. Altered mineral metabolism in glucocorticoid-induced osteopenia: effect of 25-hydroxyvitamin D administration. *J. Clin. Invest.* 64: 655–665, 1979.

16. Hantman, D. A., J. M. Vogel, C. L. Donaldson, R. Friedman, R. S. Goldsmith, and S. B. Hulley. Attempts to prevent disuse osteoporosis by treatment with calcitonin, longitudinal compression and supplementary calcium and phosphate. *J. Clin. Endocrinol. Metab.* 36: 845–858, 1973.

17. Hulley, S. B., J. M. Vogel, C. L. Donaldson, J. H. Bayers, R. J. Friedman, and S. N. Rosen. Effect of supplemental oral phosphate on the bone mineral changes during prolonged bed rest. *J. Clin. Invest.* 50: 2506–2518, 1971.

18. Hulth, A., and S. Olerud. The effect of cortisone on growing bone in the rat. *Br. J. Exp. Pathol.* 44: 491–496, 1963.

19. Johnston, C. C., Jr., and S. Epstein. Clinical, biochemical, radiographic, epidemiologic, and economic features of osteoporosis. *Orthop. Clin. North Am.* 12: 559–569, 1981.

20. Kazarian, L., and T. Collins. Strength characteristics of the isolated vertebral centrum. In: *A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates: A Compilation of Results,* edited by L. Kazarian, C. Cann, M. Parfitt, D. Simmons, and E. Morey-Holton. NASA Technical Memorandum 81268. 1981, pp. 4–15. (Available from NTIS, Springfield, VA)

21. Krølner, B., and B. Toft. Vertebral bone loss: an unheeded side effect of therapeutic bed rest. *Clin. Sci.* 64: 537–540, 1983.

22. Krølner, B., B. Toft, S. P. Neilsen, and E. Tøndevold. Physical exercise as prophylaxis against involutional vertebral bone loss: a controlled trial. *Clin. Sci.* 64: 541–546, 1983.

23. LaRochelle, F., C. Leach, and J. Vernikos-Daniellis. Effects of age and sex on hormonal responses to weightlessness simulation. *Physiologist* 25, Suppl.: S161–S162, 1982.

24. Leach, C. S., W. C. Alexander, and P. C. Johnson. Endocrine, electrolyte, and fluid volume changes associated with Apollo missions. In: *Biomedical Results of Apollo,* edited by R. S. Johnston, L. F. Dietlein, and C. A. Berry. Washington, DC: NASA, 1975, pp. 163–184.

25. Leach, C. S., S. B. Hulley, P. C. Rambaut, and L. F. Dietlein. The effect of bedrest on adrenal function. *Space Life Sci.* 4: 415–423, 1973.

26. Leach, C. S., P. C. Johnson, and P. C. Rambaut. Metabolic and endocrine studies: the second manned Skylab mission. *Aviat. Space Environ. Med.* 471: 402–410, 1976.

27. Leach, C. S., and P. C. Rambaut. Biochemical observations of long duration manned orbital spaceflight. *J. Am. Med. Women's Assoc.* 30: 153–172, 1975.

28. Leach, C. S., and P. C. Rambaut. Biochemical responses of the Skylab crewmen: an overview. In: *Biomedical Results from Skylab,* edited by R. S. Johnston, and L. F. Dietlein. Washington, DC: NASA, 1977, pp. 204–216. (NASA SP-377)

29. Leadbetter, W. F., and H. C. Engster. The problem of renal lithiasis in convalescent patients. *J. Urol.* 53: 269–281, 1945.

30. Leonard, J. I., C. S. Leach, and P. C. Rambaut. Quantitation of tissue lost during prolonged spaceflight. *Am. J. Clin. Nutr.* 38: 667–679, 1983.

31. Lipman, R. L., J. J. Schnure, E. M. Bradley, and F. R. Lecocq. Impairment of peripheral glucose utilization in normal subjects by prolonged bed rest. *J. Lab. Clin. Med.* 76: 221–230, 1970.

32. Lockwood, D. R., J. M. Vogel, V. S. Schneider, and S. B. Hulley. Effect of the diphosphonate EHDP on bone mineral metabolism during prolonged bed rest. *J. Clin. Endocrinol. Metab.* 41: 533–541, 1975.

33. Lutwak, L., G. D. Whedon, P. A. Lachance, J. M. Reid, and H. S. Lipcomb. Mineral, electrolyte and nitrogen balance studies of the Gemini-VII fourteen-day orbital space flight. *J. Clin. Endocrinol.* 29: 1140–1156, 1969.

34. Maheshwari, U. R., J. T. McDonald, V. S. Schneider, A. J. Brunetti, L. Leybin, E. Newbrun, and H. C. Hodge. Fluoride balance studies in ambulatory healthy men with and without fluoride supplements. *Am. J. Clin. Nutr.* 34: 2679–2684, 1981.

35. Mathews, C. H. E., S. P. Aswani, and A. M. Parfitt. Hypogravitational effects of hypodynamia on bone cell function and the dynamics of bone remodeling. In: *A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates: A Compilation of Results,* edited by L. Kazarian, C. Cann, M. Parfitt, D. Simmons, and E. Morey-Holton. NASA Technical Memorandum 81268. 1981, pp. 16–28. (Available from NTIS, Springfield, VA)

36. Morey, E. R. Spaceflight and bone turnover: correlation with a new rat model of weightlessness. *BioScience* 29: 168–172, 1979.

37. Morey, E. R., and D. J. Baylink. Inhibition of bone formation during space flight. *Science* 201: 1138–1141, 1978.

38. Morey-Holton, E. R., M. D. Bomalaski, E. Enayati-Gordon, M. R. Gonsalves, and T. J. Wronski. Is suppression of bone formation during simulated weightlessness related to glucocorticoid levels? *Physiologist* 25, Suppl.: S145–S146, 1982.

39. Nilsson, B. E., and N. E. Westlin. Bone density in athletes. *Clin. Orthop. Relat. Res.* 77: 179–182, 1971.

40. Oganov, V. S. Results of biosatellite studies of gravity-dependent changes in the musculo-skeletal system of mammals. *Physiologist* 24, Suppl.: S55–S58, 1981.

41. Oganov, V. S. Unpublished information presented during the 12th Meeting of the US/USSR Joint Working Group on Space Biology and Medicine, November 9-11, Washington, DC, 1981.

42. Parfitt, A. M. Bone effects of space flight: analysis by quantum concept of bone remodelling. *Acta Astronaut.* 8: 1083–1090, 1981.

43. Parfitt, A. M. Bone as a source of urinary calcium—osseous hypercalciuria. In: *Hypercalciuria States: Pathogenesis, Consequences, and Treatment,* edited by F. Coe. New York: Grune & Stratton, 1984.

44. Rambaut, P. C., and R. S. Johnston. Prolonged weightlessness and calcium loss in man. *Acta Astronaut.* 6: 1113–1122, 1979.

45. Rambaut, P. C., C. S. Leach, and G. D. Whedon. A study of metabolic balance in crewmembers of Skylab IV. *Acta Astronaut.* 6: 1313–1322, 1979.

46. Rambaut, P. C., M. C. Smith, Jr., P. B. Mack, and J. M. Vogel. Skeletal response. In: *Biomedical Results of Apollo,* edited by R. S. Johnston, L. F. Dietlein, and C. A. Berry. Washington, DC: NASA, 1975, pp. 303–322.

47. Rambaut, P. C., M. C. Smith, Jr., and H. O. Wheeler. Nutritional studies. In: *Biomedical Results of Apollo,* edited by R. S. Johnston, L. F. Dietlein, and C. A. Berry. Washington, DC: NASA, 1975, pp. 277–302.

48. Russell, J. E., and D. J. Simmons. The effect of postcranial immobilization on the maturation of matrix and mineral moieties in the rhesus monkey jaw. In: *A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates: A Compilation of Results,* edited by L. Kazarian, D. Cann, M. Parfitt, D. Simmons, and E. Morey-Holton. NASA Technical Memorandum 81268. 1981, pp. 50–57. (Available from NTIS, Springfield, VA)

49. Sandler, H., P. Webb, J. Annis, N. Pace, B. W. Grunbaum, D. Dolkas, and B. Newsom. Evaluation of a reverse gradient garment for prevention of bed-rest deconditioning. *Aviat. Space Environ. Med.* 54: 191–201, 1983.

50. Schneider, V. S., Principal Investigator. *Prevention of Disuse Osteoporosis of Bedrest: Effect of Disodium Dichloromethane Diphosphonate.* NASA Contract T-66D. Terminal Report, 1981, vol. III, pp. 263–324.

51. Scheider, V. S., and J. McDonald. Prevention of disuse osteoporosis: clodronate therapy (Abstract). In: *Osteoporosis: Recent Advances in Pathogenesis and Treatment,* edited by H. F. DeLuca, H. M. Frost, W. S. S. Jee, C. C. Johnston, Jr., and A. M. Parfitt. Baltimore: University Park Press, 1981, p. 491.

52. Schneider, V. S., L. Quan, and J. McDonald. *Prevention of Disuse Osteoporosis of Bedrest: Effect of Four Hour Daily Ambulation.* NASA Contract T-66D. Terminal Report, 1981, vol. II, pp. 160–192.

53. Simmons, D. J., and W. V. Walker. The effect of postcranial immobilization on the growth of the rhesus monkey jaw. In: *A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates: A Compilation of Results,* edited by L. Kazarian, C. Cann, M. Parfitt, D. Simmons, and E. Morey-Holton. NASA Technical Memorandum 81268. 1981, pp. 34–49. (Available from NTIS, Springfield, VA)

54. Slovik, D. M., R. M. Neer, J. L. Ohman, F. C. Lowell, M. B. Clark, G. V. Segre, and J. T. Potts, Jr. Parathyroid hormone and 25-hydroxyvitamin D levels in glucocorticoid-treated patients. *Clin. Endocrinol.* 12: 243–248, 1980.

55. Smith, M. C., Jr., P. C. Rambaut, J. M. Vogel, and M. W. Whittle. Bone mineral measurements—experiment M078. In: *Biomedical Results from Skylab,* edited by R. S. Johnston, and L. F. Dietlein. Washington, DC: NASA, 1977, pp. 183–190.

56. Spector, M., R. T. Turner, E. Morey-Holton, D. J. Baylink, and N. H. Bell. Arrested bone formation during spaceflight results in a hypomineralized skeletal defect. *Physiologist* 26, Suppl.: S110–S111, 1983.

57. Spengler, D. M., E. R. Morey, D. R. Carter, R. T. Turner, and D. J. Baylink. Effect of space flight on bone strength. *Physiologist* 22, Suppl.: S75–S76, 1979.

58. Thornton, W. E., and J. A. Rummel. Muscular deconditioning and its prevention in space flight. In: *Biomedical Results from Skylab,* edited by R. S. Johnston, and L. F. Dietlein. Washington, DC: NASA, 1977, pp. 191–197.

59. Tori, J. A., and L. S. Kewalramani. Urolithiasis in children with spinal cord injury. *Paraplegia* 16: 357–365, 1978.

60. Turner, R. T., E. R. Morey, C. Liu, and D. J. Baylink. Altered bone turnover during spaceflight. *Physiologist* 22, Suppl.: S73–S74, 1979.

61. Whedon, G. D., C. S. Leach, and P. Rambaut. Metabolic and endocrine hormone studies in manned space flights. In: *Molecular Proceedings of Endocrinology,* edited by I. MacIntyre, and M. Szelke. Amsterdam: Elsevier/North-Holland, 1979, pp. 229–250.

62. Whedon, G. D., L. Lutwak, P. C. Rumbaut, M. W. Whittle, M. C. Smith, J. Reid, C. Leach, C. R. Stadler, and D. D. Sanford. Mineral and nitrogen metabolic studies, experiment M071. In: *Biomedical Results from Skylab,* edited by R. S. Johnston, and L. F. Dietlein. Washington, DC: NASA, 1977, pp. 164–174.

63. Whittle, M. W. Caloric and exercise requirements of space flight: biostereometric results from Skylab. *Aviat. Space Environ. Med.* 50: 163–167, 1979.

64. Wong, G. L. Basal activities and hormone responsiveness of osteoclast-like and osteoblast-like bone cells are regulated by glucocorticoids. *J. Biol. Chem.* 254: 6337–6340, 1979.

65. Wronski, T. J., and E. R. Morey. Effect of spaceflight on periosteal bone formation in rats. *Am. J. Physiol.* 244 (*Regulatory Integrative Comp. Physiol.* 13:) R305–R309, 1983.

66. Wronski, T. J., E. Morey-Holton, and W. S. S. Jee. Cosmos 1129: spaceflight and bone changes. *Physiologist* 23, Suppl.: S79–S82, 1980.

New LSRO Studies Underway

Guidelines for Appropriate Use of Dietary Data from Large Field Surveys

Under terms of a contract with the Center for Food Safety and Applied Nutrition, Food and Drug Administration, the Life Sciences Research Office, FASEB, is assembling an Expert Panel to define guidelines for the appropriate analysis and interpretation of dietary data collected from large field surveys such as the Health and Nutrition Examination Surveys and the Nationwide Food Consumption Surveys. As part of the study, the Expert Panel will review past uses of the dietary data from large field surveys, discuss statistical and other methods for assessing the validity and accuracy of such data, and provide guidelines for data analyses. In addition, the Panel will be asked to identify appropriate and inappropriate uses of dietary data generated by different assessment methods (i.e., 24-hour recall, food frequency, etc.), and make recommendations for future collection of dietary data in large field surveys. Members of the Federation Societies with and interest in this study are invited to communicate with Dr. Sue Ann Anderson, Senior Staff Scientist, Life Sciences Research Office, FASEB, 9650 Rockville Pike, Bethesda, MD 20814 (301/530-7030).

Availability of Folate Report

An ad hoc Expert Scientific Working Group of the Life Sciences Research Office has completed a study of data on folate nutritional status collected during a recent national survey. Data analyses and interpretation of the results by the Expert Scientific Working Group form the basis of the report entitled "Assessment of the Folate Nutritional Status of the US Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976–1980." The report, sponsored by the Center for Food Safety and Applied Nutrition, Food and Drug Administration, provides a description of the methodologies used to determine the levels of serum and red blood cell (RBC) folate for a representative sample of the civilian, noninstitutionalized population of the United States, ages 6 months through 74 years. Estimates of the prevalence of low serum and/or RBC folate levels are given for several age/sex groups. The effects of factors such as race, education, poverty, smoking, fasting, use of vitamin/mineral supplements, use of aspirin, regular medication use, pregnancy, oral contraceptive use, and parity on the prevalence of low folate levels are examined, as well as some relationships between folate levels and hematological status. Recommendations for additional analyses of NHANES II data and for the conduct of future surveys of folate nutritional status are included in the report. *For copies of report*: FASEB Special Publications Office, 9650 Rockville Pike, Bethesda, MD 20814, at $12.00 postpaid.

Important titles from McGraw-Hill...

CARDIOVASCULAR PHYSIOLOGY, 2/e

Lois Jane Heller, Ph.D.
David E. Mohrman, Ph.D.

Thoroughly revised, Cardiovascular Physiology provides a concise yet complete presentation of the basic principles of cardiovascular physiology for beginning medical and physiology students, this book places particular emphasis on describing how the operation of the intact cardiovascular system is governed by the basic principles of cardiovascular physiology.

Cardiovascular Physiology has two significant features that make it attractive to students. Each chapter begins with a list of objectives that define the facts, concepts, and problems that the student is to master; used appropriately, this feature should enable the student to focus his or her attention in a productive manner. There are also a set of problems at the end of the book with answers to accompany each chapter.

AVAILABLE IN FEBRUARY 1986
1986 (27989-6) $17.95 (tent.)

ENDOCRINOLOGY: A LOGICAL APPROACH FOR CLINICIANS, 2/e

William Jubiz, M.D.

Now thoroughly revised, the Second Edition of this critically acclaimed text on endocrine diagnosis and management establishes a practical pathophysiologic approach to basic endocrine problems. All material has been updated to incorporate the latest research on the structure and function of the endocrine system. Several chapters have been extensively reorganized, and information on laboratory procedures and hormone therapy — previously presented independently in separate chapters — is now integrated in the sections dealing with the corresponding gland. Also new to the Second Edition — challenging case studies that enable readers to test their ability to apply basic concepts in a clinical context.

Endocrinology: A Logical Approach for Clinicians features:

- A preparatory review of basic concepts in endocrinology — detailing the organization of the endocrine system; the nature and action of hormones; and the impact of endocrine disorders on growth, metabolism, sexual development, and reproduction.
- A systematic survey of the glands of the endocrine system, covering anatomy and physiology, hormone secretion, pathophysiology, and the clinical manifestations, diagnosis, and treatment of specific endocrine syndromes.
- Extensive discussions on endocrine hypertension and endocrine syndromes caused by hormone production by nonendocrine tumors.

Throughout the book, emphasis is placed on our current knowledge of endocrine function and on following a practical and systematic approach to diagnosing and managing endocrinopathies. Recommended tests, medications, and therapeutic approaches are specific and cost effective.

Its clarity and outstanding organization make *Endocrinology: A Logical Approach for Clinicians* the text of choice for medical students. And its authoritative and up-to-date coverage makes it an invaluable resource for all residents, house staff, and clinicians who wish to review their knowledge of endocrine-related disorders.

1985 (33069-7) $22.00

PULMONARY PHYSIOLOGY, 2/e

Michael G. Levitzky, Ph.D.

This new edition of *Pulmonary Physiology* provides first year medical students, as well as respiratory therapy, nurse-anesthesia, and other students, with a complete and solid background in the aspects of pulmonary physiology essential for their understanding of clinical medicine.

Pulmonary Physiology is designed to encourage self-sufficiency by clearly stating objectives at the beginning of each chapter; by giving clear and concise explanations of physiological mechanisms, showing how they can be assessed by pulmonary function tests and how they apply to pathological states; by illustrating as many concepts as possible with clear figures by reviewing and relating concepts to other chapters in the book; and by giving the student a means of testing himself or herself with study questions and problems.

AVAILABLE IN JANUARY 1986
1986 (37468-6) $17.95 (tent.)

QUOTES FROM REVIEWS OF PULMONARY PHYSIOLOGY, 1/e

"I believe this book can be recommended as a primer."
...from a review in JAMA

"This manual easily meets its goal of providing a background in pulmonary physiology to the beginning student. It is a well written, concise review that would also be useful to both residents and fellows who are interested in acquainting themselves with vital physiology forgotten since medical school."
...from a review in Anesthesiology Review

CLINICAL PHYSIOLOGY OF ACID-BASE AND ELECTROLYTE DISORDERS, 2/e

Burton David Rose, M.D.

Like its highly successful predecessor, the Second Edition of this unique and innovative textbook effectively integrates the essentials of renal and electrolyte physiology with the common clinical disorders of acid-base and electrolyte balance. Thus, a review of the physiology of body fluids, how the kidney functions, and the effects of hormones on the kidney is followed by a discussion of the renal and extrarenal factors involved in the normal regulation of volume (sodium), water, acid-base, and potassium balance. The second half of the book then contains separate chapters on each of the major acid-base and electrolyte disturbances. Each chapter begins with a short summary of the pathophysiology of the specific disorder (with cross-references to more complete discussions in earlier chapters), and proceeds to examinations of etiology, symptoms, diagnosis, and treatment. Throughout, whenever possible, the physiologic discussions are correlated with situations in clinical medicine.

Dr. Rose has fully revised and rewritten the book both to incorporate the many changes that have occurred in the field over the last seven years and to refine and clarify existing information. It now includes more complete discussions of normal physiology and more clinical examples of how to interpret data. In addition, there is a wholly new chapter on the meaning and clinical applications of commonly ordered urinary tests, including the sodium, potassium, and chloride concentrations, pH, and osmolality. Problems designed to test understanding and to emphasize important concepts appear at the end of most chapters, and answers are provided at the back of the book. The book concludes with a summary of important equations and formulas. By providing the foundations for understanding how disease states can overcome the regulatory processes, the initial chapters of the book are admirably suited for the first-year course in renal physiology; residents and practitioners will find the clinical chapters an invaluable source for specific, practical, patient-oriented information.

1984 (53622-8) $26.95

"Rose's book is a well-written and successful attempt to cover all the topics included in the title....In addition to the clear writing style, a major strength of this book is its imaginative use of figures and mathematical calculations."
...from a review in The New England Journal of Medicine

CLIP AND MAIL COUPON TO REQUEST REVIEW COPIES

PATHOPHYSIOLOGY OF RENAL DISEASE

Burton D. Rose, M.D.

This concise book is intended especially for students, house officers, and practicing physicians who wish to acquire an understanding of the basic aspects of renal disease and hypertension. The book is not an exhaustive review; rather, it covers the core of information — diseases which are of concern to and seen and treated by the nonspecialist. Wherever possible, Dr. Rose discusses the mechanisms of disease as well as why medications may be effective. Each section reviews the clinical presentation of the disease and an approach to both diagnosis and therapy. Potential future developments are also considered.

The first two chapters present a broad overview of renal disease, including the common laboratory tests used to assess renal function and a general diagnostic approach to help determine what kind of renal disease is present. Subsequent chapters discuss acute types of renal failure, the different types of renal disease, the pathogenesis of uremia, treatment of end-stage renal failure, hypertension, nephrolithiasis, and drugs and the kidney.

The book is available in both hard and soft cover versions. It is an ideal companion volume to Dr. Rose's work, *CLINICAL PHYSIOLOGY OF ACID-BASE AND ELECTROLYTE DISORDERS.* **1981 (53615-5) (sc) $24.95 (53616-3) (hc) $34.00**

"This book should be valuable for medical students, house officers, and physicians in practice who are interested in patients with renal disorders and hypertension." ...from a review in JAMA

"I think Doctor Rose has done an outstanding job in presenting us with what house officers or non-nephrologist practicing physicians need to know when treating clinically manifest renal disease." ...from a review in Clinical Nephrology

RENAL PHYSIOLOGY, 3/e

Arthur J. Vander, M.D.

This thorough revision presents the essential core content of renal physiology for medical students, clinicians, and for students in other health sciences. Not intended as a reference book, but rather as a primary learning tool, the material is presented in a manner that permits the student to master the contents independently, i.e., with no (or very few) accompanying lectures by an instructor. Dr. Vander's primary goal of encouraging this self-sufficiency is explicitly enhanced and defined by —

- the identification of specific behavioral goals and objectives in each chapter which tell the student precisely what he/she should know and be able to do by the book's conclusion.
- a complete section containing study questions and answers (referenced in each chapter) which will give the student additional practice and feedback in difficult areas.
- a list of suggested readings keyed to each chapter to encourage further study of specific topics.
- the extensive use of flow-sheet diagrams to explain sequences of events which constitute the homeostatic control systems acting upon the kidneys.

Every section has been carefully revised and brought fully up-to-date to incorporate the most recent developments in the field. For example, the coverage of glomerular-filtration dynamics, mechanisms of sodium and chloride reabsorption, tubular handling of potassium, and nephron heterogeneity has been significantly expanded. In addition, new flow diagrams, study questions, and a number of suggested readings have been added. **1985 (66959-7) $16.95**

To Request Complimentary Examination Copies, CLIP AND MAIL TO:
McGRAW-HILL BOOK CO., 1221 AVENUE OF THE AMERICAS, HEALTH PROFESSIONS DIVISION, NEW YORK, N.Y. 10020, ATT: PATRICK HANSARD

Note: Your request for a complimentary copy will be reviewed by your local McGraw-Hill representative and is subject to acceptance by McGraw-Hill

- ☐ Complimentary copy for course adoption.
- ☐ Bill me, I will remit payment, including local sales tax, postage and handling, or I will return the book(s) postpaid.
- ☐ Heller/Mohrman: CARDIOVASCULAR PHYSIOLOGY, 2/e (27989-6) $17.95 (tent.)
- ☐ Jubiz: ENDOCRINOLOGY: A LOGICAL APPROACH FOR CLINICIANS, 2/e (33069-7) $22.00
- ☐ Levitzky: PULMONARY PHYSIOLOGY, 2/e (37468-6) $17.95 (tent.)
- ☐ Rose: CLINICAL PHYSIOLOGY OF ACID-BASE AND ELECTROLYTE DISORDERS, 2/e (53622-8) $26.95

Rose: PATHOPHYSIOLOGY OF RENAL DISEASE
- ☐ Softcover edition (53615-5) $24.95
- ☐ Hardcover edition (53616-3) $34.00
- ☐ Vander: RENAL PHYSIOLOGY, 3/e (66959-7) $16.95

Name _____
School _____
Course Title _____
Enrollment _____ Adoption Deadline _____
Address _____
City, State, Zip _____

72-X085-0068-1

Offer good only in U.S. All orders subject to acceptance by McGraw-Hill. All prices subject to change without notice.

The Vitamin D Endocrine System

ANTHONY W. NORMAN
Department of Biochemistry
University of California
Riverside, California 92521

Calcium and phosphorus are the most abundant of the inorganic elements in the body. As is detailed in Table 1, these two elements play key roles in a wide spectrum of biological processes. Obviously, a problem of major significance to the living organism is one of ensuring an adequate supply of calcium and phosphorus to meet these bodily needs. It is essential to life that calcium and phosphorus be supplied on a continuing daily basis. The process of calcium and phosphorus homeostasis involves the integrated actions at the site of uptake, the intestine, with those of the site of major deposition of these ions, the bone, and the major site of excretion of these minerals, the kidney. The coordinated operation of these three organs provides the minute-to-minute basis for calcium and phosphorus homeostasis. Calcium homeostasis is principally mediated by the intertwining biological actions of three hormones—the two peptide hormones, namely parathyroid hormone (PTH) and calcitonin, and the fat-soluble vitamin D and its metabolites.

The principal bodily stores of calcium are, of course, in the skeletal system. In a normal 70-kg man there should be at least 1.1 kg of calcium stored. Calcium also, of course, is maintained quite constantly in the blood and within quite narrow tolerable limits. The normal concentration of calcium in the blood is 2.5 mM or 10 mg Ca/100 ml. If calcium falls below ~7 mg or rises above 12 mg/100 ml, there will be adverse physiological effects promptly manifested. Calcium also, of course, has been shown to be important for muscle contraction, nerve pulse transmission, blood clotting, membrane structure, and serves as a metal cofactor for a number of enzymes including hemolase trypsinogen, lipases, and ATPases. And last, but not least of course, for *the chicken, the egg* shell is an important additional component of the "calcium story." In fact, in my laboratory we use the chicken as an experimental model to study vitamin D metabolism, and in the course of 1 year will use ~4,000–5,000 vitamin D-deficient chickens. The chicken is undoubtedly a superb experimental model for vitamin D research purposes because of the "intensity" of calcium metabolism present in the chicken due to the additional stresses on calcium metabolism that occurs during egg laying.

Table 1
Biological Calcium and Phosphorus

Calcium	Phosphorus
Body content: 70-kg man has 1,200 g [Ca^{2+}]	Body content: 70-kg man has 770 g P
Structural: bone has 95% of body Ca	Structural: bone has 90% of body P_i
Plasma [Ca^{2+}] is 2.5 mM, 10 mg/100 ml	Plasma [P_i] is 2.3 mM
Muscle contraction	Intermediary metabolism (phosphorylated intermediates)
Nerve pulse transmission	Genetic information (DNA and RNA)
Blood clotting	Phospholipids
Membrane structure	Enzyme-protein components (phosphohistidine, phosphoserine)
Enzyme cofactors (amylase, trypsinogen, lipases, ATPases)	Membrane structure
Eggshell (birds)	
Daily requirments (70-kg man)	
Dietary intake: 700	Dietary intake: 1,200
Fecal excretion: 300-600	Fecal excretion: 350-370
Urinary excretion: 100-400	Urinary excretion: 200-600

Figure 1 summarizes the "dynamic" view of calcium metabolism. In essence, this represents a 1-day balance study in a normal human subject who is consuming a diet containing 1,000 mg of calcium and 900 mg of phosphorus. Shown in shadowed arrows are the calcium fluxes in terms of milligrams per day of calcium and phosphorus that occur as the three calciotropic hormones affect calcium and phosphorus homeostasis. The three principal sites of action of vitamin D are the intestine, the bone, and the kidney. As shown here, there is a certain net absorption of calcium by the intestine that enters into the extracellular fluid compartment, which consists of 900 mg of calcium. In a given day, this extracellular fluid compartment is in communicaiton with the intracellular fluid compartment, which consists of ~11,000 mg of calcium.

At the skeletal level there are three important kinetic processes which can be identified. They are the "rapid exchange," the " slow exchange," and the processes of bone formation or accretion and bone resorption. Bone kinetically can be identified as having two major compartments—first the readily exchangeable bone compartment, which consists of ~4,000 mg of calcium and then the stable bone mineral compartment, which has ~1,000,000 mg of calcium. Kinetically, then the 900 mg of calcium that constitute the extracellular fluid will turnover ~11 times by the simple process of chemical exchange with the readily exchangeable bone compartment. In addition, there is a slow exchange process, which in any day involves a movement of only 300 mg of calcium. Neither the rapid exchange nor the slow exchange processes are believed to be affected by hormonal regulators. A principal action of the calciotropic hormones are on the two processes of accretion and resorption. It should be emphasized that the bone is a dynamic tissue which is constantly undergoing remodeling. Thus, in any given day ~300 mg of calcium will be incorporated into bone and an equivalent 300 mg of calcium will be reabsorbed. Both vitamin D and parathyroid hormone are believed to affect these processes. Under conditions of active growth, of course, net accretion would exceed net bone resorption.

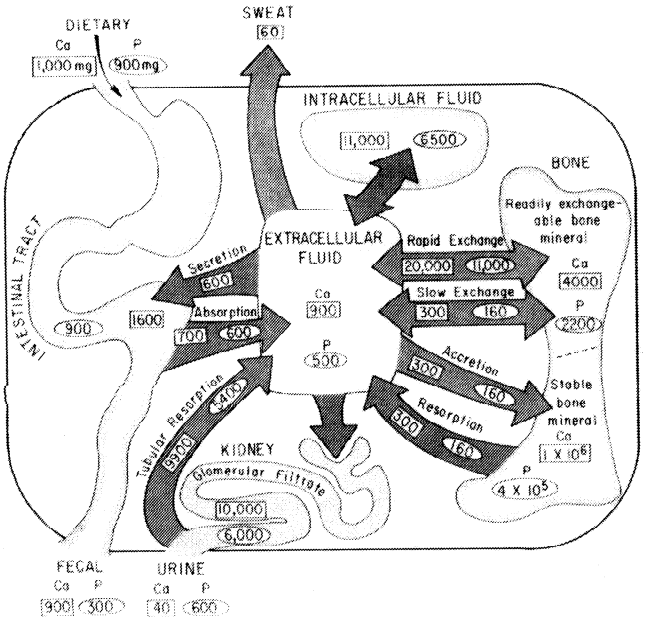


Figure 1
Dynamic view of calcium and phosphorus homeostasis.

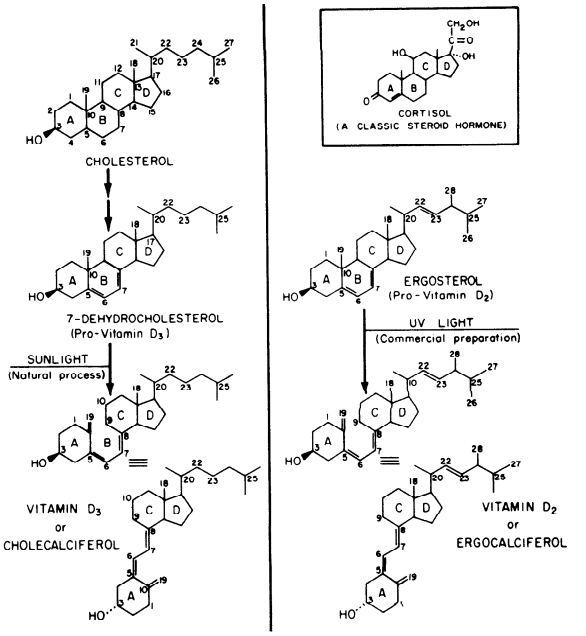


Figure 2
Production of vitamin D_3 from 7-dihydrocholesterol or vitamin D_2 from ergosterol mediated by ultraviolet or sunlight.

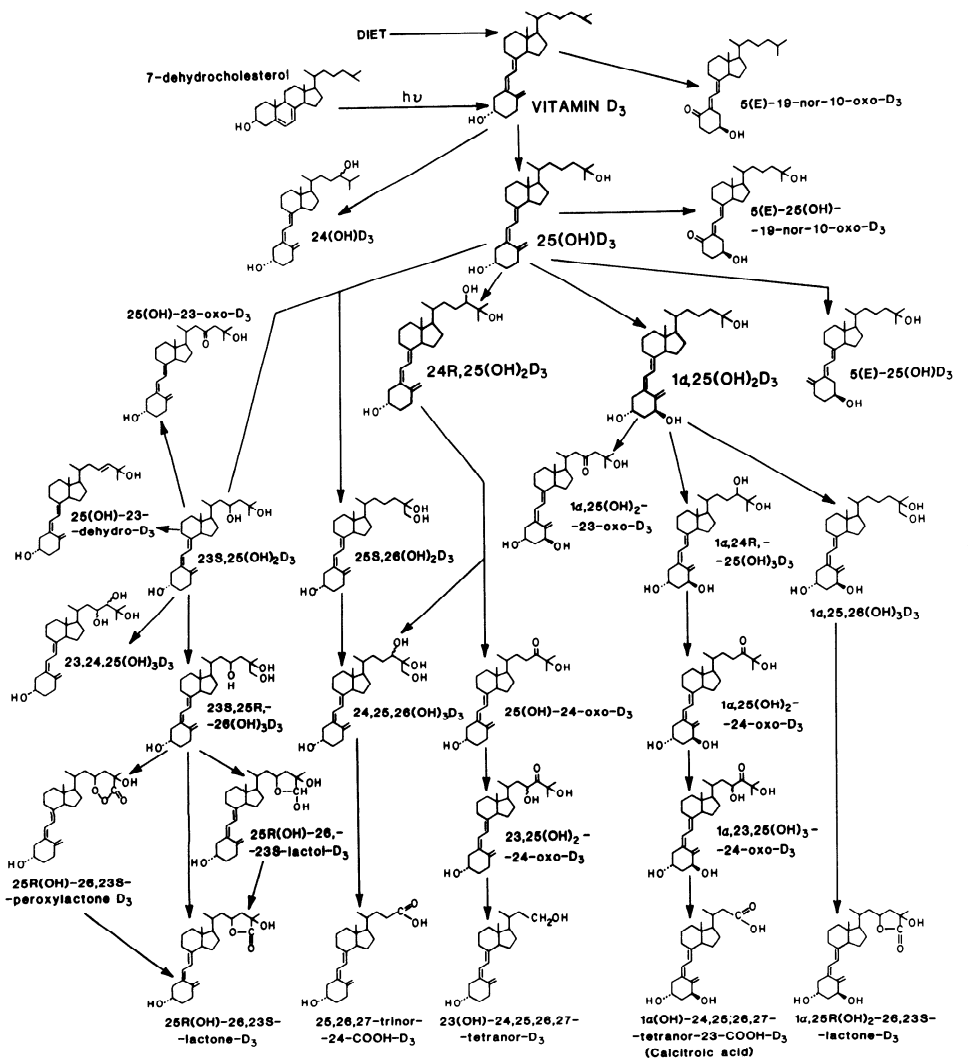


Figure 3
Summary of vitamin D metabolism.

The third side of vitamin D action is on the kidney. On any given day the kidney filters ~10,000 mg of calcium and 6,000 mg of phosphorus, but due to the very efficient renal tubular reabsorption process, ~99% of the calcium and 90% of the phosphorus is reabsorbed, and thus only a very small proportion of the renal filtrate is excreted in the urine.

In recent years an important concept has been developed regarding the role of vitamin D in calcium homeostasis: that vitamin D is in reality a steroid hormone and that its mode of action is analogous to that of other steroid hormones. It has further become evident that it is informative to analyze the role of vitamin D in calcium and phosphorus homeostasis from an endocrinological perspective. The remainder of this article, then, details some of the events surrounding the discovery of these concepts and their application to calcium and phosphorus metabolism.

Scientists and physicians have known since at least the 17th century of the correlation between the incidence of the bone disease rickets and lack of sunshine. These intuitive observations ultimately led to the brilliant studies by Sir Edward Mellanby, who devised the first experimental diet that was capable of producing rickets in puppies. An important but at that time unappreciated aspect of Mellanby's experimental protocol was that his puppies had no access to sunlight or ultraviolet light. These observations were soon followed by the demonstration of the existence of the antirachitic vitamin D or calciferol by several workers in the 1920's and 1930's. Once the connection among antirachitic activity, ultraviolet light, and the $\Delta^{5,7}$-unsaturated sterols such as 7-dehydrocholesterol and ergosterol, was appreciated, it was possible for Askew and Windaus independently to carry out a formal characterization of vitamin D_3 or cholecalciferol. It is a seco-steroid, formally known as 9,10-seco-5,7,10(19)-cholesta-dien-3β-ol. Seco-steroids are those in which one of the rings has undergone fission by breakage of a carbon-carbon bond; in the instance of vitamin D this is the 9,10-carbon bond of ring B of 7-dehydrocholesterol (see Figure 2). Vitamin D_3 is the naturally occurring form of the vitamin and is normally derived by exposure to sunlight of the precursor 7-dehydrocholesterol, which is present in the skin. Vitamin D_2 is produced synthetically via ultraviolet radiation of the sterol ergosterol. The primary biological functions of vitamin D are to mediate intestinal calcium absorption and permit normal skeletal development.

A formal definition of a " vitamin" is that it is a trace dietary constituent required to effect normal functioning of a physiological process. Thus vitamin D/calciferol is only a vitamin when the animal does not have access to sunlight or ultraviolet light. Under normal physiological circumstances all mammals, including humans can generate via ultraviolet photolysis adequate quantities of vitamin D. It is largely due to a historical accident that calciferol has been classified as a vitamin rather than as a steroid hormone.

Figure 3 summarizes our current understanding of the metabolic pathway for all the known vitamin D metabolites. If this figure had been drawn ~ 12 years ago, there would have been only one single entry, that of only vitamin D. Now there are 28 chemically characterized metabolites of vitamin D_3. The biologically active vitamin D metabolites, those which contribute to calcium and phosphorus metabolism, are shown in shadowed tones. They include 25(OH)D, which is produced in the liver, and $1,25(OH)_2D$ and $24,25(OH)_2D$, both of which are normally produced in the kidney.

Until recently little progress was made in understanding the mode of action of vitamin D. However, since 1965 there has emerged a new model for the mechanism of action of this important seco-steroid. The model is based on the concept that in terms of both chemical structure and mode of action, vitamin D is similar to other steroid hormones such as estradiol, testosterone, hydrocortisone, aldosterone, or ecdysone (see Figure 4). In fact it is now recognized that there is an endocrine system for processing the prohormone, vitamin D, into its hormonally active forms. Extensive evidence has been presented supporting the view that the steroid hormone $1,25(OH_2D_3$ is produced only in accord with strict physiological signals dictated by the calcium "demand" of the organism; a bimodal mode of regulation has been suggested. On a time scale of minutes, changes in the ionic environment of the kidney mitochondria resulting from accumulation or release of calcium or inorganic phosphate may alter the enzymatic activity of the 1-hydroxylase.

At the present time we have a much clearer understanding of both the biochemical mode of action of $1,25(OH)_2D_3$ and, as a consequence, its associated clinical implications. There are specific tissue receptors for $1,25(OH)_2D_3$in the three principal target organs classically associated with calcium homeostasis, namely the intestine, kidney, and bone. In addition, recent studies have indicated a number of secondary target tissues that also contain high-affinity low-capacity receptors for $1,25(OH)_2D_3$,e.g., parathyroid gland, pancreas, pituitary, parotid gland, mammary tissue, and placenta. In all of these tissues there is a high degree of structural homology in terms of specificity, affinity, and biological properties for the receptor of $1,25(OH)_2D_3$. These results suggest that the vitamin D endocrine system and its actions in calcium homeostasis are much more widespread than previously appreciated.

Currently, we do not have a detailed understanding of the role of $24,25(OH)_2D_3$. The concept has been advanced that $24,25(OH)_2D_3$ only works in conjunction with or in the presence of its sister dihydroxylated metabolite, $1,25(OH)_2D_3$. Evidence has been put forth to support their combined actions in mediating normal secretion of parathyroid hormone, normal egg hatchability in chickens, and normal bone development.

An important chemical "fact of life" concerning vitamin D seco-steroids is that they have a conformationally mobile A-ring (see Figure 5). That is to say, because the 9,10-carbon bond is broken during the photolysis that produces vitamin D from its provitamin, 7-dehydrocholesterol, a physical fact of life which emerges is that since the A-ring is no longer rigidly fused through the B-ring to the C-ring the A-ring acquires a conformational mobility. Thus one must remember that for every vitamin D seco-steroid, whether it be a biologically active one as shown here or one of the catabolites, as discussed previously, there is in essence two structural representations which reflect the end points of oscillation of the conformationally mobile A-rings.

Thus, focusing on vitamin D, one can see that in one conformational representation the 3β-OH is axial, or down, whereas in the other conformational representation, the 3α-OH is equatorial, or out. These two conformers interchange many thousands of time per second. Simi-

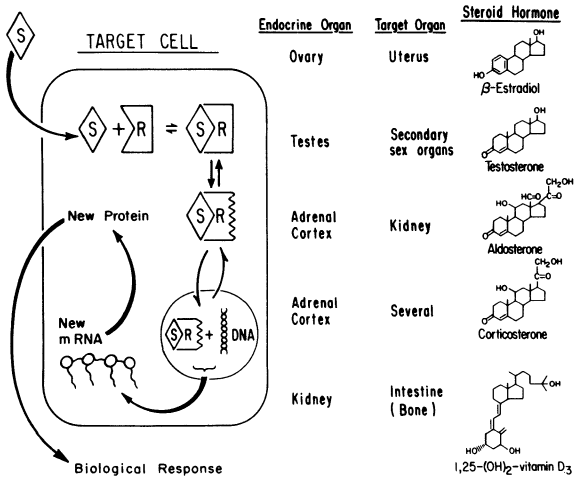


Figure 4
Model of steroid hormone action in target cells.

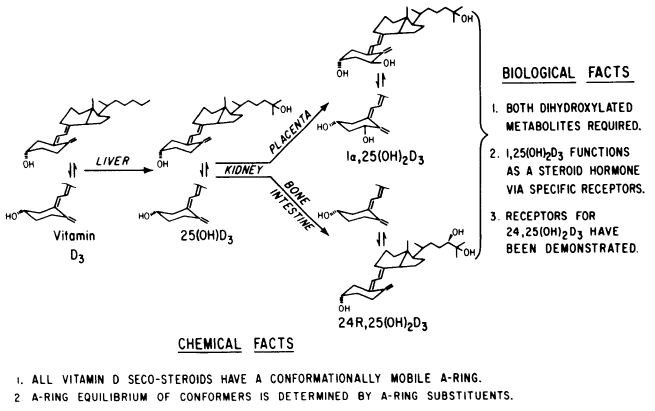


Figure 5
Seco-steroid have a conformationally mobile A-ring.

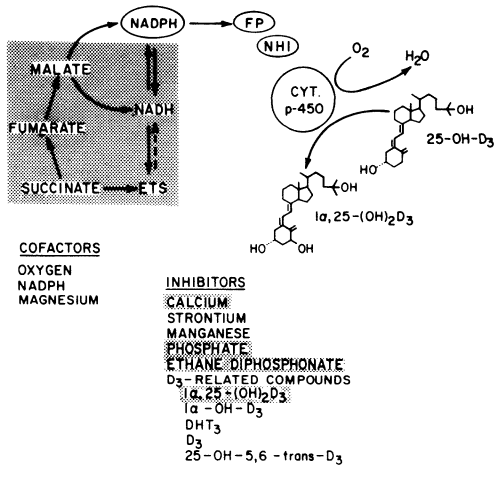


Figure 6
Schematic summary of kidney 25-hydroxyvitamin D-1-hydroxylase.

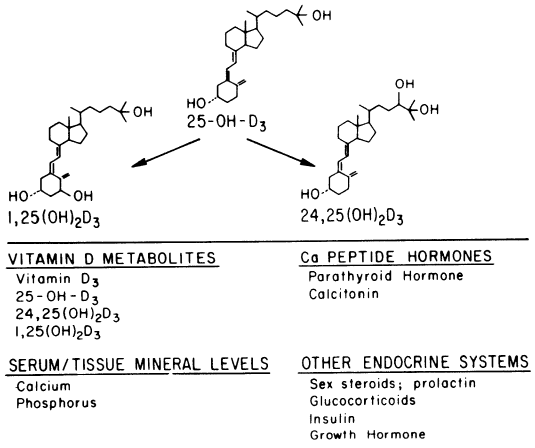


Figure 7
Physiological regulators of renal 1- and 24-hydroxylases.

larly, one can look at the hormonally active forms of vitamin D, namely $1,25(OH)_2D$, and $24,25(OH)_2D$, and can observe that in the A-ring there are again two conformers present for each metabolite. The equilibrium ratio of the two conformers for all known vitamin D metabolites is 1:1; i.e., they are each present in equal concentrations. However, to chemists an intriguing fact is that the equilibrium of the conformers can be perturbed by placing different functionalities on the A-ring. It is my expectation that this would be an active area of focus by chemists over the next decade, and it may be possible to develop metabolites with selective biological activity, which depends on the ratio of the two A-ring conformers that would be produced.

Now focusing on the biological facts, I wish to emphasize two points. First, it is my opinion that both dihydroxylated metabolites, namely $1,25(OH)_2D$ and $24,25(OH)_2D$, are both required to produce the complete spectrum of biological responses that one normally attributes to the parent vitamin D. Second, in terms of our biochemical understanding of the mode of action of $1,25(OH)_2D$, it is apparent that a major portion of its biological responses are produced as a consequence of it functioning like a steroid hormone in terms of its interaction with specific receptors present in specific target tissues.

Before turning our attention to a detailed consideration of our understanding of the hormonal mode of action of $1,25(OH)_2D$, as well as $24,25(OH)_2D$, it is appropriate to consider briefly some of the biochemical properties of the key enzymes which produce these two dihydroxylated metabolites. Figure 6 summarizes what is currently known about the biochemistry of the 1-hydroxylase, i.e., the enzyme responsible for producing $1,25(OH)_2D$. This work is largely the result of my collaborator and colleague, Dr. Helen Henry (6). The 1-hydroxylase is found in the nonpregnant animal exclusively in the kidney. In particular it is found in the proximal portion of the renal tubule. In terms of its subcellular distribution within the proximal renal tubule, it is found exclusively in the mitochondrial fraction of the cell. Thus Figure 7 then summarizes the biochemical properties of this renal 1-hydroxylase found in the mitochondria. It is clear that there is much similarity in the biochemical properties of this enzyme to the other more classic steroid hydroxylases concerned with cholesterol side-chain cleavage in the adrenal cortex or the insertion of hydroxyl functionalities on a variety of other steroids. In terms of the production of $1,25(OH)_2D$ it is known that the source of the oxygen on carbon 1 is derived from molecular oxygen. By definition, then, this classifies the 1-hydroxylase as being a "mixed-function hydroxylase." The electron transport chain that is responsible for delivering the reducing equivalents necessary to effect the reduction of the molecular oxygen down to that of a hydroxyl has as components a

cytochrome *P*-450 component as well as a nonheme iron or adrenodoxin-like component and a flavoprotein. The immediate source of reducing equivalence is from NADPH. Since this electron transport chain is localized in the mitochondria as shown here in shadowed part, a number of tricarboxylic acid cycle intermediates may effect the reduction of the NADPH. The simple cofactors, then, for the 1-hydroxylase are oxygen, NADPH, and magnesium.

Now, if one prepares a homogenate of mitochondria from the kidney and sets up a 1-hydroxylase assay wherein radioactive 25(OH)D is being converted to $1,25(OH)_2D$, if any of the substances listed here as "inhibitors" are added to the incubation media, there will be a prompt reduction in the output of $1,25(OH)_2D$. Those regulators highlighted in shadowed square, namely calcium, phosphate, ethane diphosphonate, or the product $1,25(OH)_2D_3$ itself, may be of physiological significance. That is to say, if the mitochondrial ionic environment changes by virtue of mitochondrial accumulation of calcium or phosphate, then there will result a decreased output of production of $1,25(OH)_2D$ by the mitochondria. This is in accord with physiological circumstances where under conditions of reduced serum calcium, i.e., a lowered calcium accumulation by the mitochondria, there would be an increased output of $1,25(OH)_2D$. Conversely, under conditions of normal to hypercalcemia, there would be an increased mitochondrial accumulation of calcium and might well be a reduced output of $1,25(OH)_2D$.

Figure 7 represents a summary of the known relationships of the renal 1-hydroxylases and the renal 24-hydroxylases. It should be emphasized that under normal physiological circumstances the $1,25(OH)_2D$ is an inducer of the 24-hydroxylase. That is to say, $24,25(OH)_2D$ can only be produced in the presence of $1,25(OH)_2D$. Under normal physiological circumstances, both dihydroxylated metabolites are being produced. Shown at the bottom of Figure 7 is the lengthy list of physiological regulators, including prolactin, growth hormone, estrogens and androgens, and insulin, known to affect the output capability of these dihydroxylated metabolites. It is beyond the scope of this lecture for me to describe in detail its operation.

Now, in Figure 8, we will turn our attention to the biological functions of vitamin D in trying to assign responsibility to one or other of the daughter biologically active metabolites. Of course, the classic responses to vitamin D are *1*) a stimulation of intestinal calcium and phosphorus absorption, *2*) an increased bone mineralization or conversely, under appropriate circumstances, a mobilization of bone calcium, and *3*) the renal tubular reabsorption of calcium and phosphorus. The remainder of this presentation will be addressed to assigning responsibility to the daughter metabolites for these biological actions.

Figure 9 summarizes what is our current understanding of the biological functions of $1,25(OH)_2D$. This secosteroid is known to be a superb stimulator of intestinal calcium and phosphorus absorption. $1,25(OH)_2D$ can also increase bone ash, mobilize bone calcium, modulate parathyroid hormone secretion, and stimulate in a wide variety of tissues, including the intestine, kidney, bone, cerebellum, pancreas, placenta, and pituitary, the production of a vitamin D-dependent calcium-binding protein

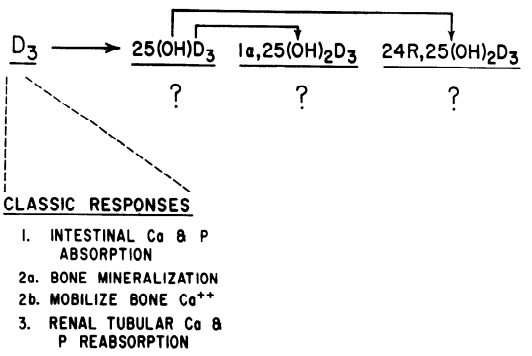


Figure 8
Vitamin D biological functions.

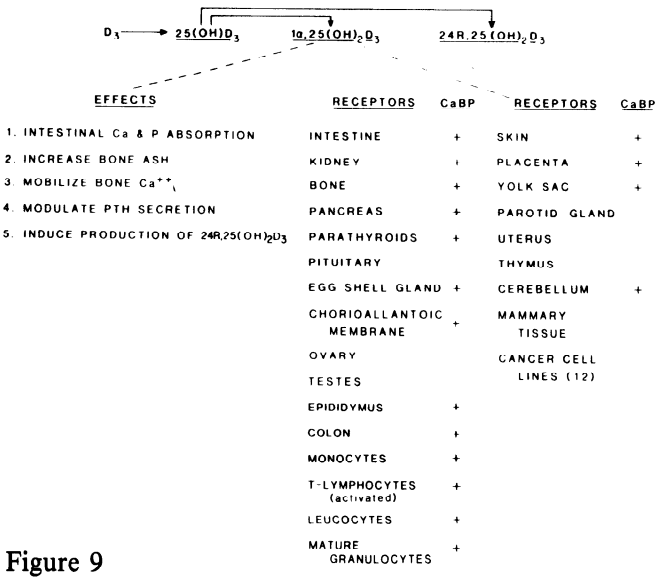


Figure 9
Biological functions of 1,25-dihydroxyvitamin D_3.

(CaBP). And lastly and most importantly, $1,25(OH)_2D$ is also known to induce the production of $24,25(OH)_2D$.

The five panels of Figure 10 summarize what is our present understanding of the mode of action of $1,25(OH)_2D$ in the intestine. The lower left-hand panel presents the general model of steroid hormone action. A target cell for a steroid hormone is defined as one that contains a specific receptor capable of binding the ligand which is passing by in the bloodstream with the formation of a steroid receptor complex. An "activation" step occurs, which then permits the complex to translocate to the nucleus of the cell. Here the steroid hormone receptor complex, in some as yet not well biochemically characterized process, results in the stimulation of new mRNA molecules, new proteins, which then produce the biological response of the hormone in question. In the case of vitamin D, the biological response of course is an increased calcium absorption process, and it is known that one of the new proteins produced in response to vitamin D in the intestine is a vitamin D-dependent CaBP. The lower right-hand panel summarizes the chronology or sequence of events known to occur in the intestine of a rachitic or vitamin D-deficient chick after giving a physiological dose of $1,25(OH)_2D$. All the responses are plotted as percent maximum effect. Shown in closed circles is the time course of localization of radioactive $1,25(OH)_2D$ in its receptor and its translocation to the

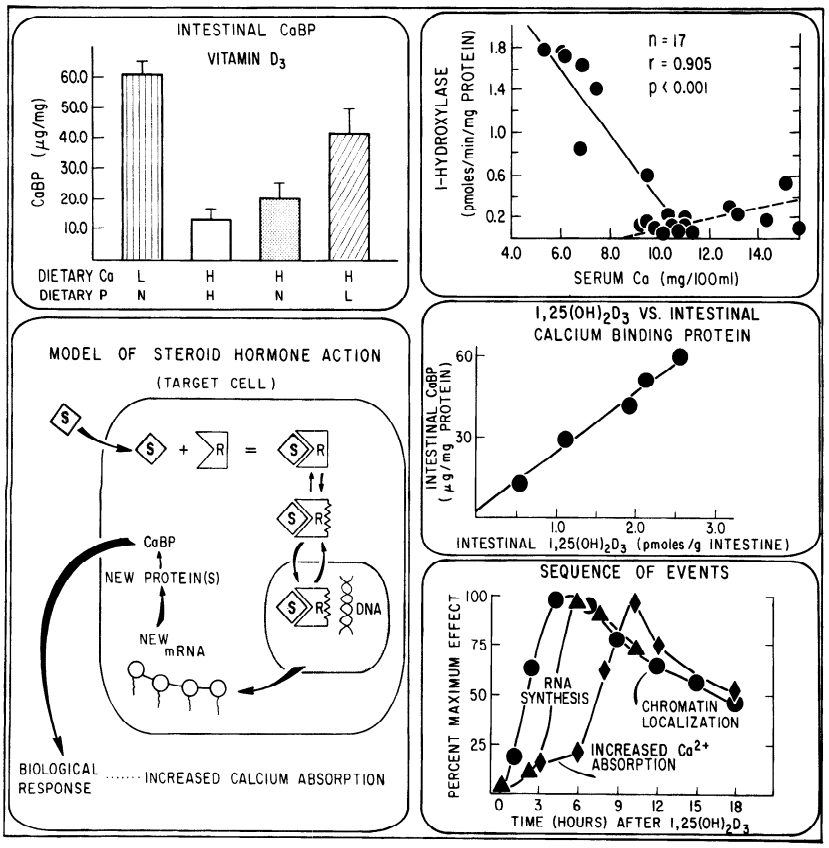


Figure 10
Summary of steroid hormone model of action of $1,25(OH)_2D_3$ in target intestine.

nuclear chromatin region of the cell. Shown in triangles is the time course of stimulation by $1,25(OH)_2D$ of intestinal mRNA synthesis, including that for the CaBP. Shown in diamonds is the time course of the biological response in the intestine to $1,25(OH)_2D$; this response is maximal by 8-10 h. Shown in the middle panel (on the right) is the steady-state relationship that exists in the intestine when chicks are dosed with varying doses of vitamin D for a 3-wk interval of time. The point that is being made here is that there is a linear correlation between the intestinal accumulation of $1,25(OH)_2D$ (shown on the abscissa) and the steady-state level of intestinal CaBP (shown on the ordinate). That is to say, the more $1,25(OH)_2D$ that localizes in the intestine, the greater is the steady-state production of CaBP.

The upper left-hand panel of Figure 10 emphasizes one of the complex yet intriguing aspects of the vitamin D endocrine system. This panel summarizes the changes in the steady-state level of intestinal CaBP that occur in the intestine as the dietary levels of calcium and phosphorus are changed. The left-hand histogram shows the adaptive increase that occurs in the steady-state level of intestinal CaBP when chicks are fed a diet deficient or devoid of calcium. Under these circumstances, the birds must work very efficiently to absorb what little calcium they are being given, and accordingly they produce a markedly increased level of CaBP. As shown in the two middle bars under circumstances of dietary excess of calcium, there is a "downregulation" in the amount of CaBP produced which reflects the fact that under these dietary circumstances the chicks do not have to be so careful about having a maximal level of intestinal calcium absorption. If it is kept in mind that the amount of CaBP produced is determined by the steady-state level of $1,25(OH)_2D$, then this suggests that dietary calcium and phosphorus must have some way of regulating the output of $1,25(OH)_2D$ by the kidney. This fact is confirmed by the data presented in the upper right-hand panel. This panel emphasizes the inverse relationship that exists between serum calcium and the specific activity of the renal 1-hydroxylase. As birds are fed a diet with very low calcium content, hypocalcemia ensues and there will be an increased secretion of PTH, which is believed to be the positive trophic stimulator of the 1-hydroxylase. Thus, as serum calcium falls, there will be an increase in the specific activity of the renal 1-hydroxylase, and there will be an increased output of $1,25(OH)_2D$. As normal calcemia is achieved, there would be a reduced secretion of PTH by the parathyroid gland, and there would be accordingly a concomitant fall in the renal 1-hydroxylase activity. Figure 11 summarizes the in-

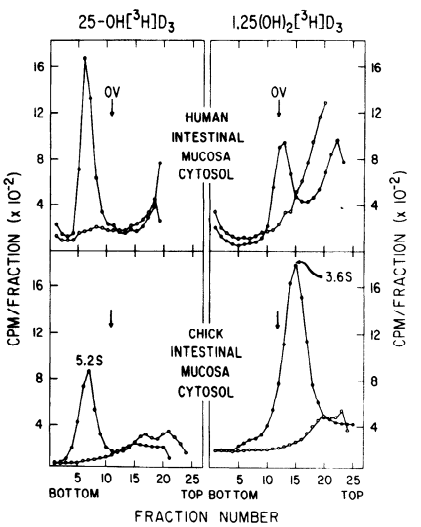


Figure 11
Intestinal receptors for $1\alpha,25(OH)_2D_3$.

Table 2
Biochemical Properties of Chick Intestinal Receptor for 1,α,25(OH)$_2$D$_3$

Molecular weight (agarose column)	67,000 ± 2,500
Stokes radius	34.1 ± 0.7 Å
5-20% Sucrose gradient mobility	3.5-3.7 S
Ligand specificity – optimal requirements	
Seco-steroid	3β,1α,25-OH groups
8-Carbon side chain	–SH group near ligand binding site
Receptor location	
Unoccupied	Principally nucleus
Occupied	Exclusively nucleus
Receptor-ligand	
K_{on}, $M^{-1}\cdot min^{-1} \times 10^{-7}$	0.92 ± 0.12
K_{off}, $M^{-1}\cdot min^{-1} \times 10^{-4}$	0.41 ± 0.15
$f_{1/2}$ (dissociation)	280 h
K_d	4×10^{-10} M

tegrated operation of the vitamin D endocrine system with respect to the relationships operative between the kidney and intestine.

Table 2 summarizes the biochemical properties of the chick intestinal receptor for 1,25(OH)$_2$D. The receptor is a protein of molecular weight of 67,000, and it has a Stoke's radius of 37 Å. In a 5-20% sucrose gradient, the receptor migrates with a mobility of 3.5-3.7 S. The receptor has a very high ligand specificity; in fact the 1,25(OH)$_2$D receptor appears to have a higher ligand specificity than that reported for any other classic steroid receptor, e.g., those for the estrogens or the glucocorticoids. In particular, the 1,25(OH)$_2$D receptor will only bind seco-steroids. Further, the ligand must have an intact 8-carbon side chain and of course it must have the critical 3β,1α,25,OH functional groups. In addition we have obtained evidence for a sulfhydryl group which must be reduced to permit ligand binding.

In terms of the subcellular localization of the receptor, we have recently obtained evidence that the unoccupied receptor appears to be largely localized in the nucleus. Certainly, however, the occupied receptor is found exclusively in the nucleus. Also in recent years we have studied from the persepective of the steroid receptor complex, its kinetics of ligand association as well as ligand dissociation. The dissociation constant (K_d) as determined by Scatchard analysis is 4×10^{10}M, indicating a high affinity of binding of the receptor for its ligand.

An important point has been to determine whether the biochemical properties of the receptor for 1,25(OH)$_2$D, which is present in the chick intestine, are unique for intestinal tissue or are unique and restricted to birds. As a consequence, we embarked on a comparison of the biochemical properties of 1,25(OH)$_2$D receptors present in a number of other tissues, and these results are summarized on Table 3. We have carefully examined the biochemical properties in the chick of the receptor present in the intestine, parthyroid gland, kidney, and pancreas. In addition we have studied in the rat the properties of the receptor present in the intestine and the placenta, and we have examined in the human the biochemical properties of the receptor present in the intestine and the parathyroid tissue. The results are most satisfying and encouraging. In general, all biochemical properties, including the molecular weight, the presence of the critical reduced sulfhydryl group, the mobility of the receptor on a 5-20% sucrose gradient, as well as the K_d, all seem to be quite similar. The principal conclusion, then, is that the biochemical properties of the 1,25(OH)$_2$D receptor in all these tissues are similar and that proteins are quite likely very homologous.

A particularly important point was to ascertain whether our studies on the chick intestinal receptor had relevance to humans. I present in Figure 11 some detailed data which bear on this point, namely the mobility in a 5-20% sucrose gradient. As can be seen in the right-hand panels, the receptor for 1,25(OH)$_2$D migrates at 3.6–3.7 S in both the chick intestinal mucosa as well as the human intestinal mucosa. These results are most assuring and suggest that our data obtained over the years in the chick are not unique to this species and indeed have relevance to humans and a variety of disease states.

A surprising development, from the viewpoint of many scientists, has been the wide distribution of receptors for 1,25(OH)$_2$D. Table 4 summarizes the tissue distribution of receptors of 1,25(OH)$_2$D in the chick, the rat, and the human, and it can be seen that good biochemical evidence has been obtained for the existence of this receptor not only in the intestine but also in the parathyroid, kidney, bone, eggshell gland, pancreas, placenta, cerebellum, pituitary, and parotid gland. To date some 25 different tissues have been shown to possess a receptor for 1,25(OH)$_2$D$_3$. As one measure of the possible significance of the receptor for 1,25(OH)$_2$D, we have developed a radioimmunoassay for the vitamin D-dependent CaBP and have studied in the chick its tissue distribution. Certainly, in the chick intestine we have obtained very good evidence that the initiating signal for the production of the CaBP is the formation of steroid receptor complex containing 1,25(OH)$_2$D. As can be seen in the chicken, we, as well as others, have obtained clear evidence for

Table 3
Biochemical Properties of 1α,25(OH)$_2$D$_3$ Receptors

		Mol Wt, $\times 10^{-3}$	–SH Ligand Binding	5-20% Sucrose, S	Scatchard K_d, M $\times 10^{10}$	$f_{1/2}$, h
Chick	Intestine	67.4	+	3.6	2.3	280
	Parathyroid	75.4	+	3.3	2.1	890
	Kidney	70	+	3.6	12.0	
	Pancreas	69	+	3.6	4.2	
Rat	Intestine	88.4	+	3.4	7.4	15
	Placenta		+	3.6		
Human	Intestine	73.8	+	3.5	1.8	16
	Parathyroid	76.4	+	3.6	5.4	218

Table 4
Diversity of Distribution of $1\alpha,25(OH)_2D_3$ Receptors and D-Dependent Calcium-Binding Proteins

Tissue	Receptor	D-Dependent CaBP
Intestine	C,R,H,	C,R,H,
Kidney	C,R,H	C,R,H
Parathyroids	C,R,H,B	C,B
Pancreas	C,R,	C
Pituitary	C,B,R	
Egg shell gland	C	C
Chorioallantoic membrane	C	C
Ovary	C,R	
Testes	R	
Epididymus	R	
Colon	R	R
Skin	R,H	
Placenta	R	R,H
Yolk sac	R	R
Parotid gland	R	
Uterus	R	
Bone	C,R,D	C,R,H
Thymus	B	
Monocytes	H	
Macrophages	H	
T-lymphocytes (activated)	R	
Leukocytes	H	
Mammary tissue	R,B,	
Cerebellum		R,H
Cancer cell lines (13)	R,H	

C, chick; R, rat or mouse; H, human; B, bovine; D, dog.

the presence of this vitamin D-dependent CaBP in the intestine, parathyroid, kidney, bone, eggshell gland, pancreas, and cerebellum.

Perhaps the most surprising entry on the list is the pancreas, since it had not been assumed that this tissue was indeed a legitimate target tissue for vitamin D action. Accordingly, we have pursued in some detail the presence

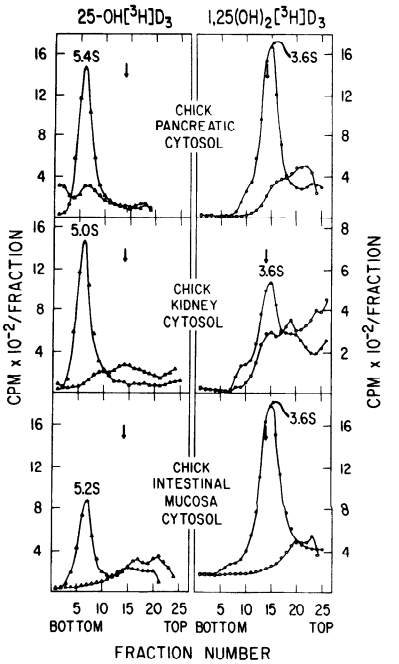


Figure 12
Sedimentation properties of 1,25-dihydroxyvitamin D receptors in chick intestine, kidney, and pancreas.

of the receptor for $1,25(OH)_2D$ as well as the CaBP in the pancreas, and our results in this regard are presented in the next several figures and tables.

Figure 12 summarizes the mobility of the receptor in the pancreas on 5-20% sucrose gradients. Shown in the bottom panel is the migration of the reference receptor for $1,25(OH)_2D_3$ present in the chick intestine; the top panel shows that the receptor has a mobility of 3.6 S.

Table 5 reports the tissue distribution of the vitamin D-dependent CaBP in the chick. The chief point I wish to make is that the pancreas has the third highest concentration of the CaBP after the intestine and the kidney. The concentration here can approach 3.1 μg/mg of protein.

As a consequence of the results presented in the last several figures and tables, we came to the conclusion that

Table 5
Tissue Distribution of Chick Intestinal Calcium-Binding Protein

	ng CaBP/mg Protein	*n*	ng CaBP/mg Protein	*n*	*P* Value
	−D		+D		
Jejunum	79±18	6	32,000±5,900	6	<0.001
Duodenum	36±14	6	25,000±4,400	6	<0.001
Ileum	31±5	6	10,000±1,400	6	<0.001
Kidney	477±13	4	3,100±370	5	<0.001
Pancreas	21±7	4	1,500±200	6	<0.001
Bone (tibia)	4±1	6	109±22	6	<0.001
Adrenal	18±1.3	3	46±3	4	<0.001
Lungs	0.75±0.7	5	21±8	6	<0.05
Esophagus	5.54±1	5	11±3	5	<0.05
Liver	1.2±0.3	5	3±0.6	5	<0.1
Skeletal muscle	2.0±0.2	4	4.3±1.5	5	<0.2
Myocardium	1.1±0.15	6	1.7±0.5	4	<0.2
Thyroid	17.6±5	3	11.3±2	3	<0.2
Hypothalamus	275±34	4	338±14	3	<0.2
Parathyroid glands	25±4	4	31.3±3	3	<0.4
Testes	16±3	3	15.4±4	5	<0.5
Cerebral cortex	248±18	4	261±22	2	<0.5
Serum	0	6	49±8 ng/ml (1.1 ng/mg protein)	6	<0.001

Values are means ± SE and are arranged in order of decreasing differences between +D and −D. *P* values: +D vs −D.

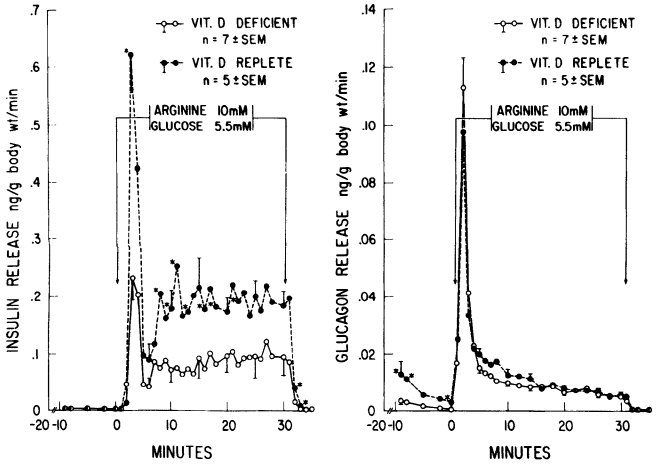


Figure 13
Effects of vitamin D status on the release of insulin and glucagon in isolated perfused rat pancreas.

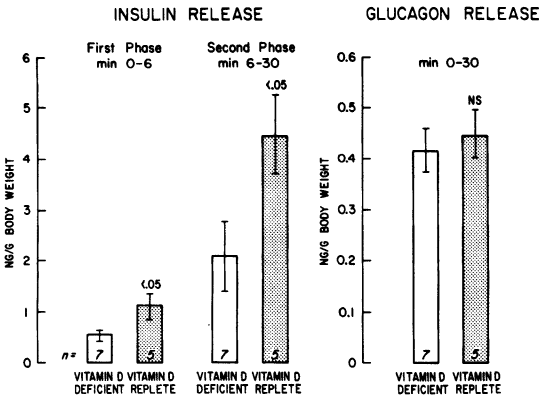


Figure 14
Summary of effects of vitamin D status on release of insulin and glucagon in isolated perfused rat pancreas.

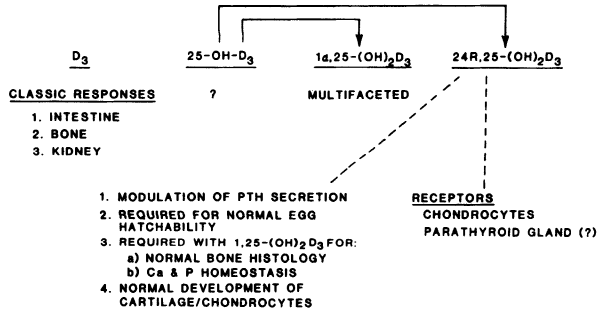


Figure 15
Proposed biological functions of $24,25(OH)_2D_3$.

the pancreas was indeed a legitimate target organ for $1,25(OH)_2D$ action. As a further test of this, we initiated a collaboration with Professor G. Grodsky in the Department of Biochemistry at the University of California, San Francisco. Dr. Grodsky is an expert at perfusing the pancreas and measuring the response of the pancreas to secretagogues for both insulin and glucagon. In the experiment reported on Figure 13, we raised vitamin D-deficient rats. Seventy-two, 48, and 24 h before perfusion of their pancreases, half of the rats were given a physiological dose of vitamin D. Both the pancreases were next removed from these animals and set up in the perfusion apparatus, and after a 20-min equilibration period, the secretagogues arginine and glucose were infused. As shown on the right-hand side of the figure, vitamin D deficiency had no effect on the response of the pancreas to secretagogues for glucagon. That is to say, there was no difference in the secretion rate of glucagon. As shown on the left-hand side of the figure, in marked contrast, the pancreases from the vitamin D-deficient rats displayed an ~50% inhibition of insulin secretion. This is present in both the "first phase," which is the first 10 min, as well as "second phase," which is the subsequent 20 min of study.

These results from the pancreas perfusion experiments are further summarized in Figure 14. As shown on the right, vitamin D deficiency had no effect on the secretion response of glucagon. In contrast, as shown on the left, vitamin D deficiency effected an ~50% inhibition of both first phase as well as second phase secretion of insulin. These appear to be the most exciting data and suggest that the involvement of vitamin D and particularly its metabolite $1,25(OH)_2D$ go much beyond preconceived notions of their action, namely, the intestine, bone, and kidney. As yet we have no knowledge of the detailed biochemistry in which vitamin D metabolites may be participating in the pancreas, but these are obviously areas of intensive investigation at the present time. This completes our consideration of $1,25(OH)_2D$.

Our attention will now focus on $24,25(OH)_2D$. Figure 15 summarizes what is known concerning the biological functions of $24,25(OH)_2D$. Evidence is available suggesting that this metabolite can modulate parathyroid hormone secretion, is required for normal egg hatchability in adult hens, and is required along with $1,25(OH)_2D$ for both normal bone histology and a normalization of calcium and phosphorus homeostasis.

By comparison with $1,25(OH)_2D$, our current understanding of $24,25(OH)_2D$ is not so well developed. In a large part, this appears to be due to the fact that most investigators have evaluated $24,25(OH)_2D$ actions in assay systems that are optimally "tuned" for $1,25(OH)_2D$, i.e., intestinal calcium absorption and bone calcium mobilization. It is not surprising that $24,25(OH)_2D$ does not give exciting results in these assays. Therefore, we devised a more general experiment designed to ask the question shown in Table 6, "Over a complete reproductive cycle, can doses of $1,25(OH)_2D$ and/or $24,25(OH)_2D$ alone or in combination achieve the response of the parent vitamin D?" In other words, we were asking the general question, can $1,25(OH)_2D$ alone produce all the effects of the parent vitamin D over a complete reproductive cycle, or somewhere in the growth and reproductive cycle would a requirement for $24,25(OH)_2D$ emerge?

The protocol of the experiment was such that we obtained 10 groups of chicks at hatching and raised them

Table 6
Experiment

Question:	Over a reproductive cycle, can doses of $1\alpha,25(OH)_2D_3$ and $24R,25(OH)_2D_3$ alone or in combination achieve the response of vitamin D_3?	
Protocol:	Ten groups of female chicks at hatching fed a rachitogenic diet and raised to sexual maturity 39 wk later: 22 variables measured in each bird	
Doses:	Vitamin D_3	Alone
	$1\alpha,25(OH)_2D_3$ $24R,25(OH)_2D_3$	Alone and in combination (low-high, low-low, high-low, etc.)

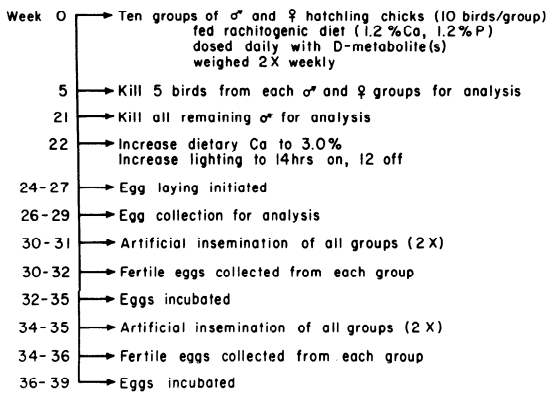


Figure 16
Experimental protocol to evaluate biological properties of combination doses of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$.

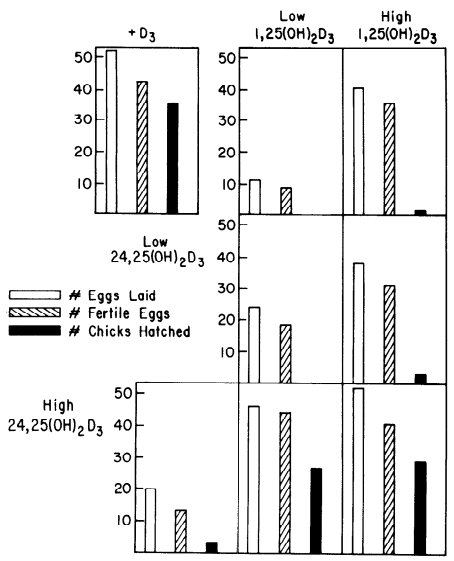


Figure 17
Effect of dihydroxylated vitamin D metabolites on egg production, fertility, and hatchability.

on a vitamin D-deficient or rachitogenic diet for some 39 wk. The birds were divided into 10 groups where one group received vitamin D alone, 2 groups received low and high physiological doses of $1,25(OH)_2D$ alone, another 2 groups received doses of low and high physiological $24,25(OH)_2D$ alone, and then finally 4 groups received combinations of low-low, low-high, high-low, and high-high physiological doses of $1,25(OH)_2D$ and $24,25(OH)_2D$.

The general outline of our experimental protocol is shown in Figure 16. Over the course of this experiment we measured *a total* of 22 separate variables in all of the birds, all of which were believed to be related in some way to effecting calcium and phosphorus homeostasis. After 24-27 wk when the birds' egg laying was initiated, and after stabilization of egg production, we then learned how to artificially inseminate the birds, and then over two separate intervals the fertile eggs were collected from each group and incubated. The most striking results are shown on the next figure.

Figure 17 summarizes the effects of the dihydroxylated vitamin D metabolites on egg production, fertility, and hatchability. Shown on the upper left-hand panel are the results obtained by the control birds, namely, the birds receiving vitamin D_3. As can be seen, these birds laid, as a group, ~50 eggs/wk, of which 90% were fertile and of which 90% ultimately hatched.

The other seven panels summarize the results obtained in terms of egg production, fertility, and egg hatchability for the two dihydroxylated metabolites. As can be seen, $1,25(OH)_2D$ at the high dose level could support normal egg production and egg fertility, but the most astonishing result was that these eggs were completely incapable of hatching. As shown in the lower left-hand panel, high doses of $24,25(OH)_2D$ alone supported only marginal egg productivity and marginal egg hatchability. The most exciting results are shown in the two bottom right-hand panels, where the birds received combinations of high $24,25(OH)_2D$ with either low or high $1,25(OH)_2D$. As can be seen, these birds laid an equivalent number of eggs that were fertile in comparison to the vitamin D-treated controls. And most importantly, the fertile eggs derived from these birds recovered the capability of hatching normally. In my view, these are the most dramatic results emphasizing the point that the seco-steroid $24,25(OH)_2D$ has an important function to offer in achieving a normalization of calcium and phosphorus homeostasis.

Thus our summary of the principal hypothesis derived from the experiment is as follows. For complete production of all vitamin D-dependent responses, i.e., a normal calcium and phosphorus homeostasis throughout a complete life cycle, both $1,25(OH)_2D$ and $24,25(OH)_2D$ must be provided. An important corollary that we have derived from this experiment is the circulating levels or ratio of $24,25(OH)_2D$ to $1,25(OH)_2D$ required to optimally generate all vitamin D-dependent responses may vary depending on *1*) dietary calcium and phosphorus availability; *2*) sex of the animal; *3*) the age of the animal; and *4*) the state of reproduction and lactation. Obviously we are continuing to further explore the significance of these results.

A discussion of some of the consequences of our newer thoughts concerning the scope of the vitamin D endocrine system follows. Figure 18 summarizes all the disease states in humans known to be related to vitamin D; this is a very long list. Clearly, as a consequence of the very complex vitamin D endocrine system, which involves metabolism in the liver, metabolism in the kidney modulated by parathyroid hormone, and subsequent systemic transport of the dihydroxylated metabolites $1,25(OH)_2D$ and $24,25(OH)_2D$ to the many target tissues including the intestine and bone, there is ample opportunity for problems to rise. A particularly important arena of dysfunction is the bone, as evidenced by disease states such as osteomalacia, osteoporosis, rickets, and renal osteodystrophy.

Among clinical disorders affecting calcium and phosphorus metabolism, chronic renal insufficiency is an important pathophysiological state that is believed to adversely affect the metabolism or actions of vitamin D. The regulation of calcium homeostasis involves maintenance of the skeleton structural integrity, as well as a precise control of Ca ion in the extracellular fluid (ECF). In pathological conditions, the homeostatic mechanism, involving vitamin D plus metabolites, PTH, and calcitonin, that regulates Ca in the ECF may do so at the expense of the skeleton, e.g., renal osteodystrophy. This can result in the generation of the bone disorders characterized by osteitis fibrosa, osteoporosis, osteosclerosis, or osteomalacia as well as hypocalcemia, hyperphosphatemia, and defective intestinal absorption of calcium and phosphorus. Although an association between renal disease, hyper-

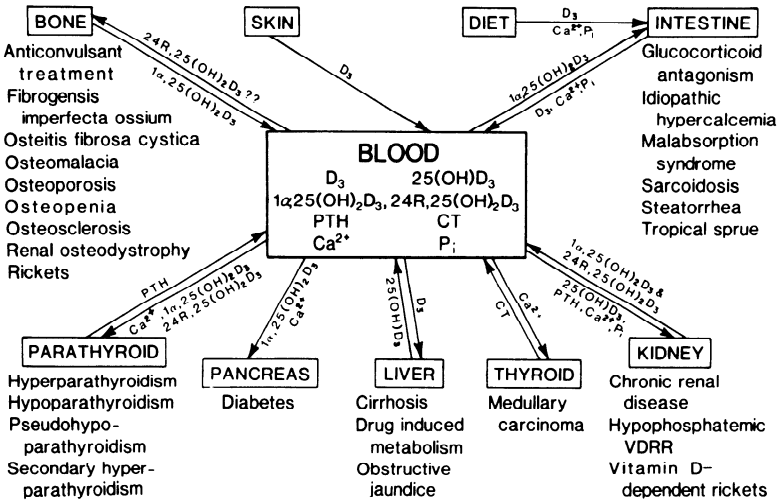


Figure 18
Disease states in humans related to vitamin D.

plasia of the parathyroid glands, and skeletal abnormalities have been known for several decades the incidence and clincal significance of abnormalities in divalent ion metabolism and its hormonal regulators, soft tissue calcification, and osseous pathology has only been more clearly recognized with the advent of renal transplantation, hemodialysis, and improved clinical therapy. An equally important development in the biochemical arena has been the discovery of metabolite $1,25(OH)_2D_3$ and its application to various of the bone disorders associated with renal osteodystrophy. $1,25(OH)_2D_3$ was recently approved in the fall of 1978 in the formulation of Rocaltrol, which is marketed by Hoffmann-La Roche.

The dominant role of the kidney for the production of the biologically active form of vitamin D considerably clarifies the pathogenesis of renal osteodystrophy. The abnormality in the metabolism of vitamin D as well as the increased levels of circulating PTH are presently considered as main causes for the development of the uremic bone disorder.

Table 7 reports the plasma concentrations of the vitamin D metabolites. There has recently emerged assay capability to determine the blood levels of these metabolites (see bottom of Table 7). The circulating concentration of $1,25(OH)_2D$ is unusually low in comparison to the other vitamin D metabolites as well as the classical steroid hormones shown at the top of Table 7. There are now a number of vitamin D assays available which permit the determination of these vitamin D metabolites. It is now possible to determine the plasma concentration of the three principal vitamin D metabolites in a 2.0-ml blood sample. Figure 19 shows the plasma levels of $1,25(OH)_2D$ measured in patients with chronic renal failure.

Table 7
Plasma Concentrations of Steroids

Steroid	Plasma Concentration*	
	M	pg/ml
Cortisol	3×10^{-7}	100,000
Progesterone	$2\text{-}20 \times 10^{-9}$	500-10,000
Testosterone	1×10^{-8}	4,000
Estradiol	$2\text{-}20 \times 10^{-10}$	50-500
Aldosterone	3×10^{-10}	100
D_3	8×10^{-8}	30,000
25(OH)D	7×10^{-8}	30,000
$24,25(OH)_2D$	5×10^{-9}	2,000
$1\alpha,25(OH)_2D$	8×10^{-11}	35

Recently developed radioreceptor assays were able to obtain clinical data indicating that the total circulating $1,25(OH)_2D_3$ was diminished in chronic renal failure. In additional studies, it could be demonstrated that nephrectomized patients had undetectable circulating concentrations of $1,25(OH)_2D_3$. The knowledge that a deficiency of the vitamin D hormone could be a major reason for the development of renal osteodystrophy initiated numerous therapeutic trials with this substance. Many groups were able to demonstrate an improvement of the uremic bone lesion as revealed by quantitative bone histology. Daily doses ranging between 0.5 and 3 μg of $1,25(OH)_2D_3$ have been found to heal not only the bone but also to increase the intestinal absorption of calcium and to suppress elevated PTH levels. Similar data were reported when analogues of $1,25(OH)_2D_3$ such as $1\alpha(OH)D_3$ $25(OH)D_3$ were used, the latter, however, only when applied in doses of 50-100 μg/day.

Despite so many promising therapeutic reports, it is apparent from the literature that not all patients with bone problems profit by $1,25(OH)_2D_3$ or its analogues. The renal bone disease will not improve in about 20-30% of the patients, suggesting that perhaps other metabolites such as $24,25(OH)_2D_3$ of other not yet identified factors might also be of importance in the pathogenesis of renal osteodystropy.

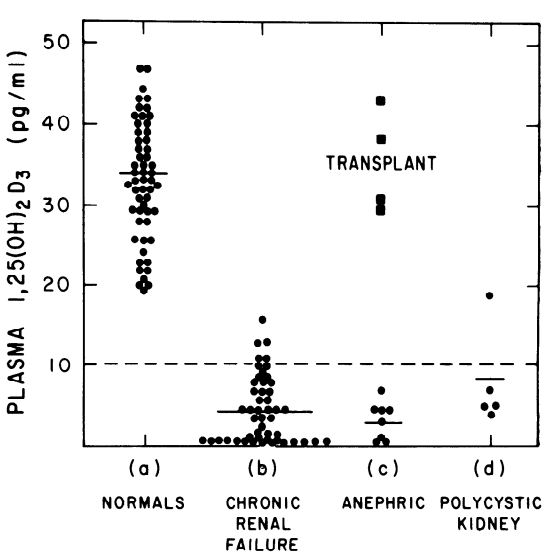


Figure 19
Plasma levels of $1,25(OH)_2D_3$ in renal disease. [From Ref. 5.]

Obviously, in terms of the many disease states summarized in Figure 18, we can anticipate many clinical developments in the future relating to the application of vitamin D metabolites to the many disorders of calcium metabolism.

Below is presented a summarized overview of vitamin D endocrine system, which emphasizes its evolution from that of a classic vitamin or a nutritionally important substance into two important steroid hormines.

Figure 20 emphasizes the early steps required for the conversion of the provitamin and the vitamin into the principal form of vitamin D known to be circulating in the blood, i.e., 25(OH)D. Under normal circumstances where an individual has some exposure to ultraviolet or sunlight, there is the capability of converting the provitamin present in the skin, i.e., 7-dehydrocholesterol, into vitamin D_3. Under circumstances where sunlight exposure is not available, the true "vitamin" nature of vitamin D emerges. In fact, many foods are supplemented with either vitamin D_3 or vitamin D_2. The only difference between these two vitamins is in the structure of the side chain.

It is important to emphasize that vitamin D_3 appears to have no intrinsic biological activity. That is to say, the molecule vitamin D_3 is inert. It only acquires "activity" by virtue of its subsequent activation and conversion into its daughter metabolites.

Over the past 15 years, there has been an evolution from the vitamin concept of D's action to a view that now emphasizes its hormone-like action. The first activation occurs in the liver, where the key 25-hydroxyl group is introduced onto the end of the side chain.

$25(OH)D_3$ is transported systemically throughout the body on a specific plasma binding protein known as the D-binding protein. This delivers this important substrate to the kidney, which is, in a nonpregnant animal, the principal site of production of both dihydroxylated metabolites, namely $1,25(OH)_2D$ and $24,25(OH)_2D$. Parathyroid hormone is believed to be the principal positive stimulator of the production of $1,25(OH)_2D$. However, there is evidence to support the involvement of a number of other endocrine modulators including estrogens, androgens, growth hormone, prolactin, and insulin. Also, as I indicated earlier, $1,25(OH)_2D$ induces the 24-hydroxylase, so that under normal physiological circumstances, both dihydroxylated metabolites are being simultaneously produced.

Figure 20 emphasizes the "feedback" relationships of the vitamin D seco-steroids on the parathyroid gland. The "long feedback loop" is certainly a rising serum calcium concentration. As serum calcium rises, there is a diminished secretion rate of PTH, and there will be in turn a diminished output of $1,25(OH)_2D$ by the kidney. I have, however, presented some evidence to support the existence of a "short feedback loop" wherein both $24,25(OH)_2D$ and $1,25(OH)_2D$ have receptors in the parathyroid gland and are capable of effecting or modulating PTH secretion. $1,25(OH)_2D$ and posibly $24,25(OH)_2D$ are known to interact in the classical target organs, namely the bone, intestine, and kidney, wherein they engage in actions that result in the elevation of serum calcium. As I have emphasized, certainly there is much data to support that the mode of action of $1,25(OH)_2D$ in the intestine is like that of a classical steroid hormone. Certainly one of the surprising developments in the past 5 years has been the realization that the vitamin D endocrine system's actions are not restricted to the three classical target organs, the intestine, bone, and kidney.

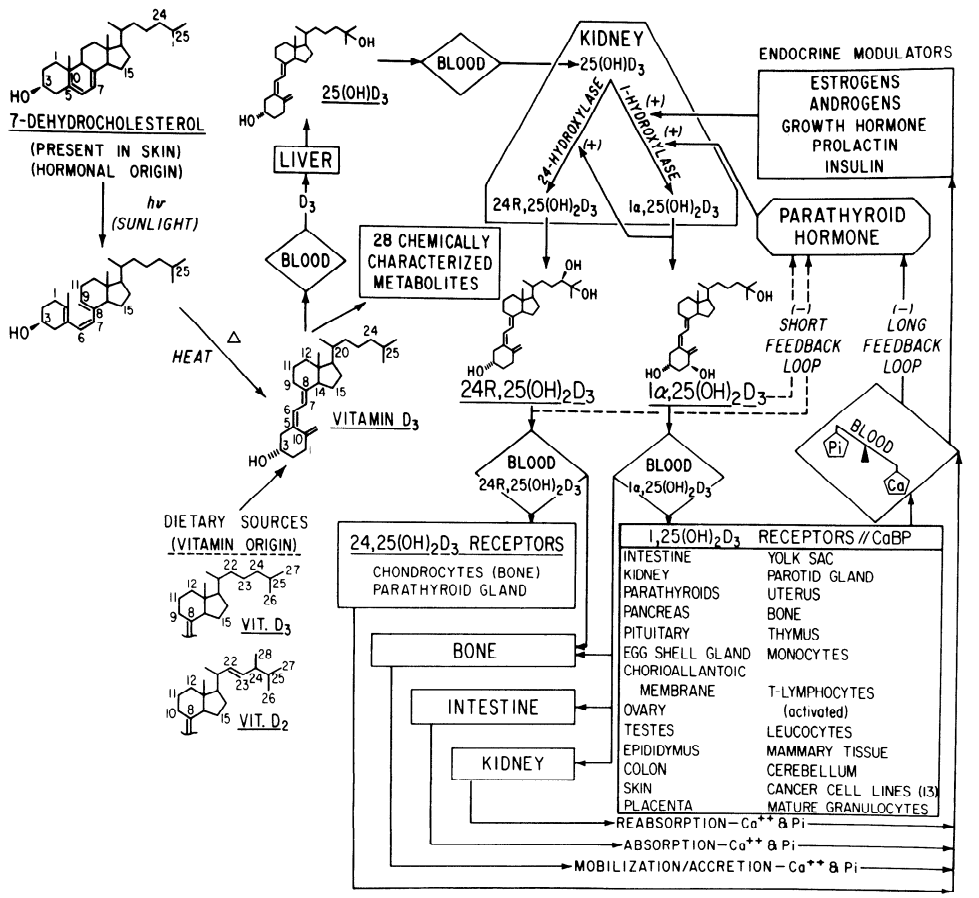


Figure 20
Vitamin D endocrine system.

Convincing biochemical data has been obtained in a number of laboratories to support the view that $1,25(OH)_2D$ has important responsibilities in a number of additional target tissues. These are summarized here and include the pancreas, the pituitary, the placenta, parotid gland, egg shell gland, cerebellum, parathyroid gland, mammary gland, and the skin. In many of these tissues, besides the presence of a receptor for $1,25(OH)_2D$, there is clear evidence to support the production of the vitamin D-dependent CaBP. It is clear that this vitamin D endocrine system is broad, comprehensive, and complicated to understand and that there will be many new developments with regard to the involvement of vitamin D and its metabolites in the future.

References

1. Barbour, G. L., J. W. Coburn, E. Slatopolsky, A. W. Norman, and R. L. Horst. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. *N. Engl. J. Med.* 305: 440–443, 1981.
2. Brickman, A. S., J. W. Coburn, G. R. Friedman, W. H. Okamura, S. G. Massry, and A. W. Norman. Comparison of effects of 1-hydroxyvitamin D_3 and 1,25-dihydroxy-vitamin D_3 in man. *J. Clin. Invest.* 57: 1540–1547, 1976.
3. Brickman, A. S., J. W. Coburn, D. J. Sherrard, E. G. C. Wong, A. W. Norman, and F. R. Singer. Clinical effects of 1,25-dihydroxyvitamin D_3 in uremic patients with overt osteodystrophy. *Contrib. Nephrol.* 18: 29–41, 1980.
4. Christakos, S., E. J. Friedlander, B. R. Frandsen, and A. W. Norman. Studies on the mode of action of calciferol. XVII. Development of a radioimmunoassay for vitamin D-dependent chick intestinal calcium binding protein and tissue distribution. *Endocrinology* 104: 1495–1503, 1979.
5. Haussler, M. R., and T. A. McCain. Review of vitamin D. *N. Engl. J. Med.* 297: 974–983, 1041–1050, 1977.
6. Henry, H. L., and A. W. Norman, Vitamin D: metabolism and biological actions. *Annu. Rev. Nutr.* 4: 493–520, 1984.
7. Hunziker, W., P. D. Siebert, M. W. King, P. Stucki, A. Dugaiczyk, and A. W. Norman. Molecular cloning of a vitamin D-dependent calcium-binding protein mRNA sequence from chick intestine. *Proc. Natl. Acad. Sci. USA* 80: 4228–4232, 1983.
8. Kadowaki, S., and A. W. Norman. Studies on the mode of action of vitamin D. XLIX. Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. *J. Clin. Invest.* 73: 750–766, 1984.
9. Klein, G. L., M. E. Ament, A. W. Norman, and J. W. Coburn. Rickets and deficiency of vitamin D metabolites. *Am. J. Dis. Child.* 136: 754–755, 1982.
10. Klein, G. L., R. L. Horst, A. W. Norman, M. E. Ament, E. Slatopolsky, and J. W. Coburn. Reduced serum levels of $1\alpha,25$-dihydroxyvitamin D with long-term total parenteral nutrition. *Ann. Intern. Med. 94: 638–643*, 1981.
11. Norman, A. W. *Vitamin D: The Calcimu Homeostatic Steroid Hormone.* New York: Academic, 1979.
12. Norman, A. W., Vitamin D metabolism and calcium absorption. *Am. J. Med.* 67: 989–998, 1979.
13. Norman, A. W., and H. L. Henry. Vitamin D to 1,25-dihydroxycholecalciferol: evolution of a steroid hormone. *Trends Biochem. Sci.* 4: 14–18, 1979.
14. Norman, A. W., V. L. Leathers, and J. E. Bishop. Studies on the mode of action of calciferol. XLVIII. Normal egg hatchability requires the simultaneous administration to the hen of $1\alpha,25$-dihydroxyvitamin D_3 and 24R,25-dihydroxyvitamin D_3. *J. Nutr.* 113: 2505–2515, 1983.
15. Norman, A. W., J. Roth and L. Orci. The vitamin D endocrine system: steroid metabolism, hormone receptors and biological response (calcium binding proteins). *Endocr. Rev.* 3, 331-*366, 1982.*
16. Reddy, G. S., A. W. Norman, D. M. Willis, D. Goltzman, H. Guyda, S. Solomon, D. R. Phillips, J. E. Bishop, and E. Mayer. Regulation of vitamin D metabolism in normal pregnancy. *J. Clin. Endocrinol. Metab.* 56: 363–370, 1983.
17. Roth, J., D. Brown, A. W. Norman, and L. Orci. Localization of the vitamin D-dependent calcium binding protein in mammalian kidney. *Am. J. Physiol. (Renal Fluid Electrolyte Physiol.* 12): F243–F252, 243 1982.
18. Schnoes, H., and H. F. DeLuca. Recent advances in our understanding of the vitamin D endocrine system. *J. Steroid Biochem.* 11: 35-*46, 1979.*

The Vitamin D Endocrine System Multiple Choice Questions

1. The three classical target organs where vitamin D and its metabolites produce biological responses include which of the following:
   a. intestine, muscle, bone
   b. intestine, liver, kidney
   c. bone, kidney, brain
   d. bone, kidney, intestine
   e. pancreas, parathyroid, brain

2. The metabolism of vitamin D_3 to its hormonally active daughter metabolites has been shown to occur in a number of tissues; in this regard the two preeminent vitamin D-metabolizing tissues are:
   a. kidney + muscle
   b. kidney + adrenals
   c. liver + spleen
   d. intestine + bone
   e. kidney + liver

3. Available evidence concerning the biochemical mode of action of $1,25(OH)_2D_3$ indicates that it functions analogously to which of the following:
   a. vitamin K (another fat soluble vitamin)
   b. estradiol (a steroid hormone)
   c. linoleic acid (an essential fatty acid)
   d. thiamine (a water soluble vitamin)
   e. isoleucine (an essential amino acid)

4. The circulating concentration of $1,25(OH)_2$-vitamin D_3 in the blood falls into what range of molarity:
   a. $1\text{-}10 \times 10^{-4}$ M
   b. $1\text{-}10 \times 10^{-7}$ M
   c. $1\text{-}10 \times 10^{-10}$ M
   d. $1\text{-}10 \times 10^{-13}$ M
   e. $1\text{-}10 \times 10^{-17}$ M

5. In which subcellular component of the cell would you expect to find the 25(OH)-vitamin D_3-1-hydroxylase activity:
   a. nuclei
   b. glomerulus
   c. ribosomes
   d. mitochondria
   e. lysosomes

6. The principal disease state in the adult resulting from an absence of regular access to vitamin D is:
   a. cirrhosis of the liver
   b. osteomalacia
   c. sarcoidosis
   d. hypoparathyroidism
   e. Wilson's disease

7. $1,25(OH)_2$-vitamin D_3 has, in the past 7-10 years, been available as a "drug form" of the hormonally active form of vitamin D: its use is currently approved by the Federal Drug Administration (FDA) for which of the following:
   a. diabetes
   b. renal osteodystrophy
   c. hyperparathyroidism
   d. sarcoidosis
   e. rickets

8. There is substantial biochemical, endocrinological and clinical evidence available which support the view that more than one daughter metabolite of vitamin D is responsible for production of all the biological responses attributable to vitamin D; in this regard the biologically important vitamin D metabolites are:
   a. $1,25(OH)_2D_3$ and $25,26(OH)_2D_3$
   b. $1,25(OH)_2D_3$ and $25(OH)D_3$
   c. $24,25(OH)_2D_3$ and $25(OH)D_3$
   d. $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$
   e. $24,25(OH)_2D_3$ and 25, $26(OH)_2D_3$

9. In recent years a number of new target organs [based on the presence of receptors for $1,25(OH)_2D_3$] for vitamin D have been identified; two of these include:
   a. parathyroid + muscle
   b. liver + lung
   c. pancreas + brain
   d. spleen + liver
   e. lymphocytes + lung

10. In the absence of access to dietary vitamin D, most individuals can satisfy their daily D-requirement by:
   a. exercise
   b. dieting
   c. increased essential fatty acid intake
   d. sunlight exposure
   e. increased dietary calcium intake

ANSWER FORM FOR CME TEST CREDITS

Return to: Dr. Martin Frank
American Physiological Society
9650 Rockville Pike
Bethesda, MD 20814

"Regulation of Energy Balance"

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____

Name _____
Address _____
_____
City _____ State _____ Zip _____

Announcements

Calcium and Cellular Regulation

"Calcium and Cellular Regulation" at Cornell University (Ithaca) presented on 3-4 October, 1985, by the Department of Pharmacology (New York State College of Veterinary Medicine). Invited lectures (B. Bean, P. Hess, R. Janis, G. A. Weiland, J. Putney, M. Prentki, R. Sha'afi, C. M. S. Fewtrell) will cover ion permeation through calcium channels, calcium agonists and antagonists, and the role of calcium, G proteins, and phosphoinositides in cellular regulation. *Information*: R. E. Oswald, Dept. of Pharmacology, NYSCVM, Cornell Univ., Ithaca, NY 14853.

Pathophysiology and Treatment of Cystic Fibrosis

A CME course on pathophysiology and treatment of cystic fibrosis is being sponsored by the Department of Pediatrics, State University of New York at Buffalo, Children's Hospital of Buffalo and Continuing Medical Education and the Cystic Fibrosis Foundation. The course is accredited through the American Medical Association and the American Academy of Family Physicians. It will be held on 11-12 October, 1985, at the Sheraton Brock Hotel, Niagara Falls, Ontario, Canada. *Information*: Rayna Saville, Coordinator, Pediatric Continuing Medical Education, Children's Hospital of Buffalo, 219 Bryant St., Buffalo, NY 14222 (716/878-7630).

National Student Research Forum

The 27th Annual National Student Research Forum will be held on 23-25 April, 1986, at the University of Texas Medical Branch campus in Galveston, TX. The Forum is intended for the competitive presentation of clinical and basic research by medical students, graduate students, interns, and residents. It is the sole national meeting of its kind, attended by participants from throughout the United States and Canada. *Deadline for abstracts*: 1 December, 1985. Supported by Grants from American Medical Association-Education & Research Foundation, Mead Johnson Nutritional Division, Roche Laboratories, and American Academy of Family Physicians. *Information*: National Student Research Forum, P.O. Box 54, Station 1, The University of Texas Medical Branch, Galveston, TX 77550 (409/761-3762).

International Society for Neuroethology

The inauguration of an International Society for Neuroethology (ISN) is announced. A proposed set of bylaws have been drafted and a newsletter initiated. The aim of the society is to serve ethologists interested in neural bases of behavior and neurobiologists interested in ethological relevance. *Information*: T. H. Bullock, President ISN, Dept. of Neuroscience A-001, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

CELLULAR SECRETORY MECHANISMS OF THE ADRENAL MEDULLA

A symposium sponsored by the Detroit Physiological Society, Feb. 6, 1985 at Wayne State University, Detroit, MI 48221

Douglas R. Yingst and Paul C. Churchill, Editors

The Detroit Physiological Society is one of the oldest continuously active physiological societies in the United States. On February 6, 1985 it sponsored a minisymposium entitled "Cellular Secretory Mechanisms of the Adrenal Medulla". The speakers were Drs. David Njus of Wayne State University, Ronald Holz of the University of Michigan, and Harvey Pollard of the National Institutes of Health. The Society thanks these scientists for their stimulating lectures, and is pleased to present a summary of their presentations. The subject of this minisymposium is of interest to physiologists and biochemists in both the basic and clinical sciences. Such a interdisciplinary topic is very much in the tradition of the Detroit Physiological Society. Preceeding these three papers we present a short history of the Society.

The Society would like to thank the Neuroscience Program at Wayne State University and its president, Dr. Thomas V. Getchell, for helping to fund the minisymposium. We also thank Dr. David R. Evans and Miss Katie Benoit for their assistance in preparing these manuscripts.

Douglas R. Yingst, Ph.D.
President

HISTORY AND FUTURE PLANS OF THE DETROIT PHYSIOLOGICAL SOCIETY

Conrad R. Lam* and Douglas R. Yingst+

*Department of Surgery, Henry Ford Hospital, and +Department of Physiology, Wayne State University, Detroit, MI, 48221

The Detroit Physiological Society owes its existence to the arrival in Detroit in 1936 of a unique person. Dr. Charles G. Johnston came from Philadelphia to be the first full-time Professor of Surgery at Wayne University College of Medicine at the age of 37. His training was unorthodox for a surgeon. He received his M.D. degree (1926) from Washington University in St. Louis, where there were many research-oriented faculty members. In 1926 he also became a Fellow in Medical Science of the National Research Council. Studies in biochemistry at the University of Pennsylvania then led to his receiving a Master's degree in that field in 1928. Next came a year as an intern in medicine at the Presbyterian Hospital in New York. With the idea of eventually choosing internal medicine as his specialty, he returned to the University of Pennsylvania as a Harriet M. Frazier Fellow in Surgery and there began his years of association with the respected Professor of Surgery, Dr. I.S. Ravdin. He was inspired to do basic investigation in the field of surgery, and a practical outcome of this was the application of the Miller-Abbott tube for decompression of the gastrointestinal tract. During this period, he had a year's leave of absence to study patholgy in Freiburg, Germany.

Address requests for reprints to Dr. Yingst.

Charles George Johnston

As the new head of the Department of Surgery at Wayne University, he made drastic changes in the staff, incurring the wrath of some of the clinical surgeons on the attending staff. In the words of his biographer Rudolph Noer (1), "With vision possessed by few, he became one of those most responsible for the development of the new era at Wayne, for he saw not only the immediate but the long range consequences of every action which bore upon the development of the school." One of his "visions" or dreams which came to fruition before he had been in Detroit a year was that there should be a "Detroit Physiological Society". He remembered the stimulating meetings of the Philadelphia Physiological Society.

At the time of the organizational meeting of the Detroit Physiological Society on April 29, 1937, there were 30 founding members. The composition of

this group reflected the broad interests that Johnston had in the basic sciences and also some biases he evidently held. Dr. Johnston apparently felt that surgeons should be applied physiologists and therefore needed to know much more than just how and where to cut (2). The only general surgeon invited to be in the founding group of the Detroit Physiological Society was Dr. Roy D. McClure, Surgeon-in Chief of the Henry Ford Hospital. It cannot be stated with certainty that no other general surgeons were present. There were three surgical specialists, a thoracic surgeon, an ophthalmologist and a gynecologist. Pathology was well represented, with four pathologists from the leading hospitals of the city. Pharmacology or at least pharmaceutical science was also well represented with five founding members, four of whom were from the firm of Parke, Davis & Company. One of these was J.J. Pfiffner, who later became a member of the Department of Physiology and Pharmacolgy at Wayne State University, and who developed an adrenal extract that was marketed by Parke Davis (2). There were two pediatricians, one of whom was Thomas B. Cooley, who described "Cooley's" anemia in children. One founder was from the Department of Physiology of Wayne University. Of the four internists, two were in private practice. One notable founder-member was Raymond B. Allen, M.D., Dean of Wayne University College of Medicine, 1936-39; later Dean of the University of Illinois College of Medicine, President of the University of Washington (Seattle) and Chancellor of UCLA. Dr. Icie Macy-Hoobler, Research Director of the Children's Fund of Michigan was also an active founding member of the society.

Naturally, the organization had its Constitution and By-Laws. "The object and purpose of the Society is the encouragement of research in normal and pathological physiology and in subjects relating directly or indirectly thereto." Membership: "The Society shall consist of Active and Honorary Members. Active Members shall be those actively engaged or interested in scientific research having a direct or indirect bearing upon physiology." Nominations for membership to the Society may be made in writing at any time to the Secretary by two active members. The Council then passes upon the eligibility of such nominations. The names of those considered eligible by the Council shall be submitted by the Secretary to the Society at any regular business meeting and the Society shall vote on the nominations. In order to be elected, a candidate must receive votes of at least two-thirds of the members present at the meeting." Dues: "Annual dues of one dollar from each active member shall be due and payable before January 1." Meetings: "The regular time for holding the meetings shall be the evening of the third Thursday of each month throughout the College year, but at its discretion the Program Committee may change the time of any particular meeting."

Appropriately, Charles G. Johnston was elected the first president. The organization was evidently off to a healthy start, because at the time of the tenth anniversary meeting, there were 135 members. Active members during the early years included Warren Nelson (Chairman of Anatomy), Hans Haterius (Chairman of Physiology), Arthur Smith and James Orten (Professors of Biochemistry), and Fritz Yonkman (Chairman of Pharmacology) of the Medical School and Conrad Lam of the Department of Surgery at Henry Ford Hospital. As prescribed by the constitution, monthly meetings were held regularly during the academic year. Typically, a meeting began at 8:00 pm, sometimes preceded by light refreshments. On other evenings there was a pre-meeting dinner at a nearby restaurant. The scientific sessions were held in a suitable facility in the Wayne State University School of Medicine, except on special occasions. At a typical meeting, there were three short papers, usually on non-related subjects, generally presented either by the members or their associates. Abstracts of some of these papers were published in the Journal of the Michigan State Medical Society. During the first ten years, there was a combined meeting with the Society of the Sigma Xi, with dinner and a prominent speaker. Notable among these were Anton J. Carlson (1942), C.J. Wiggers (1943), Irvin H. Page (1945), and Charles H. Best (1947).

During the 1950's, 1960's, and early 1970's the organization of the Society and the meeting format remained much the same. The composition of the Society, however, began to change as earlier members retired and new members joined. Many of the newer members were associated with the new basic science departments at Wayne State University School of Medicine which was in a period of growth. By the early 1960's members of the Departments of Physiology and Biochemistry were a significant factor in the organization. Individuals in the Department of Medicine and Surgery also continued to play an important role. One of the active members during the 1960's was Dr. Walter Seegers, Chairman of the Department of Physiology, and the first individual to purify thrombin and prothrombin which helped to establish the field of blood coagulation biochemistry (3). Other active members included Drs. Marion Barnhart, Raymond Henry, and Alan Silbergleit of the Department of Physiology.

In the mid 1970's the monthly meeting time was switched to the daytime to better accomodate students and faculty at Wayne State University. This change reflected the more active participation and interests of the newer members and a significant departure from the original format. The tradition of the evening

dinner meetings was maintained in the form of an annual banquet and scientific session held at various attractive locations throughout the Detroit metropolitan area. At least one meeting per year was devoted to shorter student presentations, with an award given each year to the best presentation.

This year the Society is offering a minisymposium in lieu of the monthly meetings. Both the annual dinner meeting and the student presentations are popular events we plan to continue. We hope that these events can serve as a catalyst for future research and discussion among our members in both the clinical and basic sciences. Such a goal was close to the heart of Dr. Charles Johnston.

1. Noer, R.J. Charles George Johnston (1899-1960) Memoir, Transactions of the American Surgical Association 79: 446-449, 1960.

2. Silbergleit, A. (personal communication).

3. Walz, D. (personal communication).

THE CHROMAFFIN VESICLE: A MODEL SECRETORY ORGANELLE

David Njus, Patrick M. Kelley, and Gordon J. Harnadek

Department of Biological Sciences, Wayne State University, Detroit MI

Summary

Catecholamines in the adrenal medulla are stored in intracellular vesicles. These chromaffin vesicles are an excellent model for a variety of intracellular organelles, particularly with regard to the energetics of the organelle membrane. The chromaffin-vesicle membrane has an inwardly directed H^+-translocating ATPase. Catecholamine accumulation is driven by this ATPase because amines are taken up in exchange for H^+. We believe that the H^+-ATPase also drives ascorbic acid regeneration by promoting the transfer of electrons from cytosolic electron donors to intravesicular semidehydroascorbate. These two H^+-linked phenomena may illustrate general mechanisms of transport and electron transfer in synaptic vesicles, secretory vesicles, lysosomes and other Golgi-derived organelles.

Introduction

For nearly a century, the phenomenon of secretion has been associated with studies of the adrenal medulla. Because the gland is dedicated to secretion, it has been the key to a number of significant discoveries. Epinephrine, the first hormone identified, was isolated following the finding by Oliver and Schafer (37) that adrenal extracts raise the systemic blood pressure in dogs. Subsequently, the isolation of the catecholamine-storing chromaffin vesicles (5,21) and the observation that the vesicle contents are all released during secretion (12,48) established the concept that secretion occurs by exocytosis. Furthermore, the well known requirement for extracellular calcium was discovered by Douglas and Rubin (13) in studies of perfused cat adrenals.

Two recent developments have stimulated new advances in the study of secretion from the adrenal medulla. First, it has become possible to bring isolated chromaffin cells into primary culture (15). This has permitted many new approaches including cloning, patch clamping, and working with permeabilized cells. Second, the biochemistry of the chromaffin vesicle has come to be well understood. It is our role in this symposium to describe recent studies on the chromaffin vesicles and the implications that these hold for secretory vesicles of other types.

The Role of Proton Gradients

In view of their function as secretory vesicles, it is not surprising that chromaffin vesicles contain extremely high concentrations of catecholamine. These vesicles accumulate catecholamines to a concentration (0.55 M) some 10,000 times higher than the cytosolic concentration. In 1962, Kirshner (28) and Carlsson et al. (6) independently discovered that ATP stimulates catecholamine uptake into the vesicles. The mechanism of this uptake was not apparent, however, until more than a decade later. Harvey Pollard, while visiting Oxford University, introduced the chromaffin vesicle to the laboratory of George Radda. There it was discovered that ATP-dependent epinephrine uptake is inhibited by uncouplers, which dissipate H^+ gradients (3). Interestingly, a similar observation had been reported by Von Euler and Lishajko (51) in 1969, but the role of H^+ gradients in oxidative phosphorylation was not then widely accepted and the implications for catecholamine transport were not recognized. Since uncouplers of oxidative phosphorylation dissipate transmembrane H^+ gradients, inhibition of ATP-dependent catecholamine transport implies that an adenosine triphosphatase (ATPase) generates an H^+ gradient and that this gradient in turn supplies the energy for catecholamine transport. The Oxford group (10) and and an independent group including Robert Johnson and Antonio Scarpa at the University of Pennsylvania (26) quickly showed that the chromaffin vesicle has an inwardly directed proton pump. This pump hydrolyzes ATP on the external (cytosolic) side of the vesicle membrane and can generate a membrane potential (inside positive) or a pH gradient

Table I. H^+-translocating ATPases on organelles

Organelle	Tissue	Reference
Chromaffin vesicle	Adrenal medulla	See text
Synaptic vesicle		
Adrenergic	Adrenergic neurons	50
Cholinergic	Torpedo electric organ	1
Glutamate	Cerebral cortex	34
Secretory vesicles	Neurohypophysis	42, 43, 46
	Adenohypophysis	8
	Intermediate hypophysis	31
	Parotid gland	36
Serotonin-containing granule	Platelet	7, 41, 52
Insulin granule	Islet cell tumor	23
Exocytotic vesicle	Turtle bladder epithelia	19
Lysosome	Rat liver	40, 47
Golgi	Rat liver	53

(inside acidic).

Although the H^+-ATPase was originally discovered because of its involvement in catecholamine transport, similar ATPases were soon found in many other secretory vesicles and related Golgi-derived organelles (Table I). Consequently, the question arose as to what the function or functions of these ATPases might be. In the following article, Ronald Holz will review his studies testing the involvement of this system in secretion. Our view is that the H^+-ATPase is responsible for supplying energy for all membrane-associated phenomena in chromaffin vesicles and in other Golgi-derived organelles.

Considering how many different ions and molecules must be transported into and out of cells and between intracellular compartments, the number of enzymes catalyzing active transport is quite small (30). Instead of having a separate ATPase for each compound to be transported, biological membranes seem to use a master pump to establish a transmembrane ion gradient. Other transport processes then draw upon the energy stored in this ion gradient and occur either by cotransport or exchange diffusion.For example, the plasma membrane of animal cells has a Na^+/K^+ ATPase which establishes transmembrane gradients in Na^+ and K^+. Other compounds are transported across the plasma membrane by coupling their transport to the inward movement of Na^+. Mitochondrial membranes use an electron transfer chain to establish H^+ gradients which are then used to transport ADP into and ATP out of the mitochondrion, as well as to drive the transport of many other compounds. We believe that most membranes derived from the Golgi contain an inwardly directed H^+-translocating ATPase and that transport across these membranes is coupled to the H^+ gradient. We are studying the chromaffin-vesicle membrane as a model for this third class of membranes.

H^+-Translocating ATPase

The first evidence that the chromaffin-vesicle membrane has an H^+ pump came from studies using fluorescent probes. In 1975 Radda and his colleagues (4) discovered that, when ATP is added to a chromaffin-vesicle suspension, the fluorescence of 1-anilinonaphthalene-8-sulfonate (ANS) is enhanced in a manner reminiscent of the response in energized submitochondrial particles. Moreover, this so-called "ANS response" was abolished when the uncoupler S-13 was added. This and the subsequent observation that S-13 abolished ATP-dependent epinephrine uptake into the vesicles (3) suggested that the vesicles have an inwardly directed proton pump and that catecholamine transport is coupled to this pump. To show definitively that the vesicles have a proton pump, we had to understand the anion permeability of the chromaffin-vesicle membrane. At this point, Harvey Pollard called our

attention to a phenomenon reported some years earlier by Poisner and Trifaro (39). When suspended in a Cl^- medium with ATP, chromaffin vesicles and many other secretory organelles release their contents. This happens because Cl^- is a permeant anion. In the presence of ATP, the H^+-ATPase pumps protons into the vesicles. Cl^- follows to neutralize the charge on the protons and the consequent HCl influx causes the vesicles to lyse osmotically (9). If the osmolarity of the suspension medium is increased, the lysis can be prevented and the pH within the vesicles drops. This drop in internal pH can be measured (10). If the suspension medium does not contain a permeant anion (osmotic support is provided by sucrose or K_2SO_4), ATP addition creates a membrane potential instead of a pH gradient. This, too, can be measured as shown by Ronald Holz (22). These experiments clearly demonstrated ATP-dependent changes in pH and membrane potential consistent with the operation of an inwardly directed H^+-translocating ATPase (Figure 1).

When we reached this point in about 1978, Ronald Holz and Harvey Pollard advanced to the more complex level of the isolated chromaffin cell. Our group retreated to a simpler preparation better suited to the study of proton- linked transport and other membrane phenomena. We employ a membrane preparation which we call chromaffin- vesicle "ghosts". Ghosts are made by suspending the chromaffin vesicles in an appropriate medium and then lysing them to release catecholamine and other soluble contents. The membranes then spontaneously reseal trapping the desired solution internally. Using this preparation, we have been investigating two H^+-linked processes which we will describe below. The first is catecholamine transport about which we now know a considerable amount. The second is ascorbic acid regeneration which involves electron transfer. We are now beginning to understand this process as well.

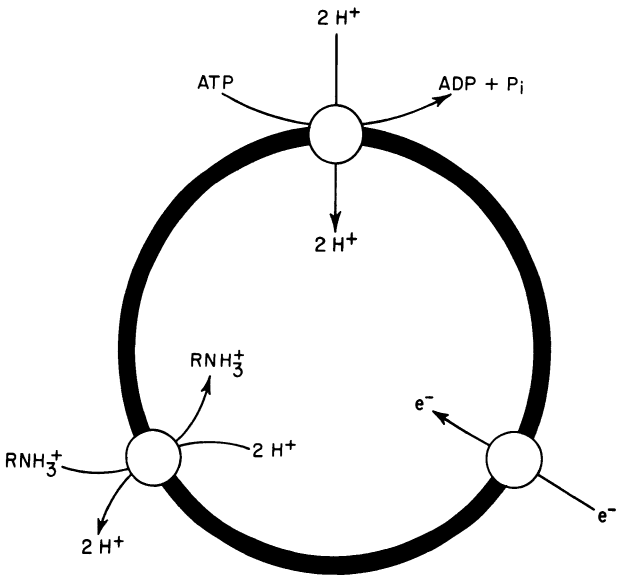


Figure 1. Summary of chromaffin vesicle membrane energetics. As described in the text, an ATPase transports protons (H^+) into the vesicle, cytochrome b-561 imports electrons (e^-) to regenerate ascorbic acid, and an amine translocator exchanges biogenic amines (RNH_3^+) for protons.

Catecholamine Transport

In chromaffin-vesicle ghosts, as in the intact vesicles, the H^+ pump can create either a membrane potential or a pH gradient depending on the suspension medium. If the suspension medium includes chloride, ATP addition causes a drop in internal pH but has very little effect on membrane potential. In the absence of chloride, the pH change is buffered out and ATP creates a membrane potential (positive inside) instead. Therefore, we can look at the dependence of catecholamine transport on either the membrane potential or the pH gradient. Using this approach, we (29) and others (25,38) have concluded that catecholamine transport occurs via an exchange of two H^+ per catecholamine cation. Two H^+ are needed to supply enough energy to transport a catecholamine molecule against the massive concentration gradient existing across the chromaffin-vesicle membrane in vivo.

The catecholamine/proton exchange is mediated by a specific translocator in the chromaffin-vesicle membrane (Figure 1). This activity can be solubilized using detergents and functionally reconstituted into phospholipid vesicles (24,32). Although a catecholamine-translocating protein has not yet been identified, several laboratories, including ours, are pursuing it. Gabizon et al. (18) synthesized a photoaffinity label, a derivative of serotonin, which blocks catecholamine transport and labels a 45,000 dalton peptide in the membrane. Scherman and Henry (45) have been working with derivatives of the inhibitor tetrabenazine. Reserpine, the classic inhibitor of vesicular catecholamine transport, also binds specifically to the chromaffin-vesicle membrane (11) but the protein to which it binds has not yet been identified. We have been working with an impermeant derivative of reserpine, reserpic acid, which blocks the translocator by binding to the external but not the internal surface of the membrane. Because H^+-linked transport is a widespread phenomenon, it will be interesting to understand more fully the mechanism of exchange mediated by the catecholamine translocator. This work is currently underway in several laboratories.

Ascorbic Acid Regeneration

Besides driving catecholamine transport, the H^+ pump may be coupled to ascorbate regeneration. Dopamine (β-hydroxylase, which converts dopamine to norepinephrine, is found within the chromaffin vesicle. This enzyme uses ascorbic acid as a one-electron donor and releases semidehydroascorbate as a product. Since ascorbate does not seem to be transported across the chromaffin-vesicle membrane at a detectable rate, we believe that ascorbate is regenerated from semidehydroascorbate simply by importing electrons from the cytosol (Figure 1). According to this scheme, the membrane potential generated by the proton pump would drive electrons into the vesicles. To demonstrate that an electron transfer pathway exists, we have adopted the following strategy. We trap ascorbic acid inside the ghosts and add an electron acceptor such as ferricyanide or ferricytochrome c on the outside. Then, we look for reduction of the external acceptor by transfer of electrons across the membrane from internal ascorbate. Indeed, both ferricyanide and cytochrome c can be reduced by internal ascorbate (20,35) so transmembrane electron transfer does occur.

The chromaffin-vesicle membrane contains a cytochrome b-561 which has the appropriate reduction potential to mediate this electron transfer, and there is now good evidence that it does. Using the assay described above, Patrick Fleming and his colleagues (49) have recently reported evidence of electron transfer across phospholipid membranes containing purified cytochrome b-561. Furthermore, in collaboration with James Russell at the National Institutes of Health, they found an immunologically identical cytochrome b-561 in secretory vesicles isolated from the neurohypophysis (14). Subsequently, we collaborated with James Russell to show that these neurohypophyseal secretory vesicles also have a transmembrane electron-transfer system (44). We found that the relative rates of electron transfer in chromaffin-vesicle ghosts and in neurohypophyseal secretory vesicles correlate with the relative amounts of cytochrome b-561 present in the two membranes. The neurohypophyseal vesicles do not contain dopamine (β-hydroxylase) but, instead, possess another ascorbate-requiring enzyme, peptide amidating monooxygenase. This enzyme, responsible for amidating the carboxyl termini of peptide hormones, may be found in many secretory organelles. Consequently, the cytochrome b-561-mediated ascorbate-regenerating system may also be widespread.

Conclusion

In summary, the chromaffin vesicle is a good model for secretory vesicle membranes. The H^+-translocating ATPase is widely distributed among Golgi-derived organelles and in the Golgi and endoplasmic reticulum membranes themselves (Table I). The amine transport system is found in catecholamine and serotonin storing vesicles (Table II). Its importance goes beyond that, however, since it is a specific example of the proton-linked transport of ions and molecules that undoubtedly occurs in many secretory vesicles as well as in mitochondria.

Table II. Reserpine-sensitive H^+-linked amine transport

Organelle	Tissue	Reference
Chromaffin vesicle	Adrenal medulla	25, 29, 38
Synaptic vesicle	Adrenergic neurons	2, 33, 50
Serotonin-containing granule	Platelet	7, 41, 52

Table III. Cytochrome b-561 or electron transfer

Organelle	Tissue	Reference
Chromaffin vesicle	Adrenal medulla	20, 35
Synaptic vesicle	Adrenergic neurons	16, 17
Secretory vesicles	Neurohypophysis	14, 44
Serotonin-containing granule	Platelet	27

Finally, the electron transfer system, responsible for regenerating ascorbate, has only begun to be investigated (Table III). Nevertheless, it is quite possible that ascorbate is commonly used as an electron donor for intraorganellar enzymes and that it is regenerated in all organelles by a cytochrome b-561 system. The past decade has been an exciting one for those interested in the energetics of the chromaffin-vesicle membrane. Much excitement remains, however, for we still have a lot to learn about the generalization of these ideas to other secretory vesicles and about the role of H^+ pumps and H^+ gradients in more complex phenomena such as sorting and packaging in the Golgi, maturation of secretory vesicles, exocytosis, and retrieval and recycling of the membrane.

This work was supported by NIH grants GM-30500 and GM-33849. David Njus is an Established Investigator of the American Heart Association.

References

1. Anderson, D.C., S.C. King, and S.M.Parsons. Proton gradient linkage to active uptake of (^{3}H)acetylcholine by Torpedo electric organ synaptic vesicles, Biochemistry 21: 3037-3043, 1982.

2. Angelides, K.J. Transport of catecholamines by native and reconstituted rat heart synaptic vesicles. J. Neurochem. 35: 949-962, 1980.

3. Bashford, C.L., R.P. Casey, G.K. Radda, and G.A. Ritchie. The effect of uncouplers on catecholamine incorporation by vesicles of chromaffin granules. Biochem. J. 148: 153-155, 1975.

4. Bashford, C.L., G.K. Radda, and G.A. Ritchie. Energy-linked activities of the chromaffin granule membrane. F. E. B. S. Lett. 50: 21-24, 1975.

5. Blaschko, H. and A.D. Welch. Localization of adrenaline in cytoplasmic particles of the bovine adrenal medulla, Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol. 219: 17-22, 1953.

6. Carlsson, A., N.A. Hillarp, and B. Waldeck. Analysis of the Mg^{++}-ATP dependent storage mechanism in the amine granules of the adrenal medulla. Acta Physiol. Scand. 59, Suppl. 215: 3-38, 1963.

7. Carty, S.E., R.G. Johnson, and A. Scarpa. Serotonin transport in isolated platelet granules. Coupling to the electrochemical proton gradient. J. Biol. Chem. 256: 11244-11250, 1981.

8. Carty, S.E., R.G. Johnson, and A. Scarpa. Electrochemical proton gradient in dense granules isolated from anterior pituitary. J. Biol. Chem. 257: 7269-7273, 1982.

9. Casey, R.P., D. Njus, G.K. Radda, and P. A. Sehr. Adenosine triphosphate-evoked catecholamine release in chromaffin granules. Osmotic lysis as a consequence of proton translocation. Biochem. J. 158: 583-588, 1976.

10. Casey, R.P., D. Njus, G.K. Radda, and P.A. Sehr. Active proton uptake by chromaffin granules: Observation by amine distribution and phosphorus-31 nuclear magnetic resonance techniques. Biochemistry 16: 972-977, 1977.

11. Deupree, J.D., and J.A. Weaver. Identification and characterization of the catecholamine transporter in bovine chromaffin granules using (^{3}H)reserpine. J. Biol. Chem. 259: 10907-10912, 1984.

12. Douglas, W.W., and A.M. Poisner. Evidence that the secreting adrenal chromaffin cell releases catecholamines directly from ATP-rich granules. J. Physiol. (London) 183: 236-248, 1966.

13. Douglas, W.W., and R.P. Rubin. The role of calcium in the secretory response of the adrenal medulla to acetylcholine. J. Physiol. (London) 159: 40-57, 1961.

14. Duong, L.T., P.J. Fleming, and J.T. Russell. An identical cytochrome b 561 is present in bovine adrenal chromaffin vesicles and posterior pituitary neurosecretory vesicles. J. Biol. Chem. 259: 4885-4889, 1984.

15. Fenwick, E.M., P.B. Fajdiga, N.B.S. Howe, and B.G. Livett. Functional and morphological characterization of isolated bovine adrenal medullary cells. J. Cell Biol. 76: 12-30, 1978.

16. Flatmark, T., H. Lagercrantz, O. Terland, K.B. Helle, and L. Stjarne. Electron carriers of the noradrenaline storage vesicles from bovine spenic nerves. Biochim. Biophys. Acta 245: 249-252, 1971.

17. Fried, G. Cytochrome b-561 in sympathetic nerve terminal vesicles from rat vas deferens. Biochim. Biophys. Acta 507: 175-177, 1978.

18. Gabizon, R., T. Yetinson, and S. Schuldiner. Photoinactivation and identification of the biogenic amine transporter in chromaffin granules from bovine adrenal medulla. J. Biol. Chem. 257: 15145-15150, 1982.

19. Gluck, S., C. Cannon, and Q. Al-Awqati. Exocytosis regulates urinary acidification in turtle bladder by rapid insertion of H^+ pumps into the luminal membrane. Proc. Natl. Acad. Sci. USA 79: 4327-4331, 1982.

20. Harnadek. G.J., E.A. Ries, and D.

Njus. Rate of transmembrane electron transfer in chromaffin vesicle ghosts. Biochemistry: in press, 1985.

21. Hillarp, N.A., S. Lagerstedt, and B. Nilson. The isolation of a granular fraction from the suprarenal medulla, containing the sympathetic catecholamines. Acta Physiol. Scand. 29: 251-263, 1953.

22. Holz. R.W. Evidence that catecholamine transport into chromaffin vesicles is coupled to vesicle membrane potential. Proc. Natl. Acad. Sci. USA 75: 5190-5194, 1978.

23. Hutton. J.C., and M. Peshavaria. Proton-translocating Mg^{2+}-dependent ATPase activity in insulin-secretory granules. Biochem. J. 204: 161-170, 1982.

24. Isambert, M.F., and J.P. Henry. Solubilization and reconstitution of the adenosine 5'-triphosphate dependent noradrenaline uptake system of bovine chromaffin granule membrane. Biochimie 63: 211-219, 1981.

25. Johnson, R.G., S.E. Carty, and A. Scarpa. Proton: substrate stoichiometries during active transport of biogenic amines in chromaffin ghosts. J. Biol. Chem. 256: 5773-5780, 1981.

26. Johnson, R.G., and A. Scarpa. Protonmotive force and catecholamine transport in isolated chromaffin granules. J. Biol. Chem. 254: 3750-3760, 1979.

27. Johnson, R.G., and A. Scarpa. The electron transport chain of serotonin-dense granules of platelets. J. Biol. Chem. 256: 11966-11969, 1981.

28. Kirshner, N. Uptake of catecholamines by a particulate fraction of the adrenal medulla. J. Biol. Chem. 237: 2311-2317, 1962.

29. Knoth, J., M. Zallakian, and D. Njus. Stoichiometry of H^+-linked dopamine transport in chromaffin granule ghosts. Biochemistry 20: 6625-6629, 1981.

30. Kyte, J. Molecular considerations relevant to the mechanism of active transport. Nature 292: 201-204, 1981.

31. Loh, Y.P., W.W.H. Tam, and J.T.Russell. Measurement of Δ pH and membrane potential in secretory vesicles isolated from bovine pituitary intermediate lobe. J. Biol. Chem. 259: 8238-8245, 1984.

32. Maron, R., H. Fishkes, B.I. Kanner, and S. Schuldiner. Solubilization and reconstitution of the catecholamine transporter from bovine chromaffin granules. Biochemistry 18: 4781-4785, 1979.

33. Maron, R., B.I. Kanner, and S. Schuldiner. The role of a transmembrane pH gradient in 5-hydroxytryptamine uptake by synaptic vesicles from rat brain. F. E. B. S. Lett. 98: 237-240, 1979.

34. Naito, S., and T. Ueda. Characterization of glutamate uptake into synaptic vesicles. J. Neurochem. 44: 99-109, 1985.

35. Njus, D., J. Knoth, C. Cook, and P.M. Kelley. Electron transfer across the chromaffin granule membrane. J. Biol. Chem. 258: 27-30, 1983.

36. Oberg, S.G., and M.R. Robinovitch. ATP-induced lysis of rat parotid secretory granules: Possible role of ATP in exocytotic release. J. Supramol. Struct. 13: 295-304, 1980.

37. Oliver, G., and E.A. Schafer. The physiological effects of extracts from the suprarenal capsules. J. Physiol. (London) 18: 230-276, 1895.

38. Phillips, J.H., and D.K. Apps. Stoichiometry of catecholamine/proton exchange across the chromaffin-granule membrane. Biochem. J. 192: 273-278, 1980.

39. Poisner, A.M., and J.M. Trifaro. The role of ATP and ATPase in the release of catecholamines from the adrenal medulla. I. Mol. Pharmacol. 3: 561-571, 1967.

40. Reeves, J.P., and T. Reames. ATP stimulates amino acid accumulation by lysosomes incubated with *amino* acid methyl esters. J. Biol. Chem. 256: 6047-6053, 1981.

41. Rudnick, G., H. Fishkes, P.J. Nelson, and S. Schuldiner. Evidence for two distinct serotonin transport systems in platelets. J. Biol. Chem. 255: 3638-3641, 1980.

42. Russell, J.T. Δ pH, H^+ diffusion potentials, and Mg^{2+} ATPase in neurosecretory vesicles isolated from bovine neurohypophyses. J. Biol. Chem. 259: 9496-9507, 1984.

43. Russell, J.T., and R.W. Holz. Measurement of ΔpH and membrane potential in isolated neurosecretory vesicles from bovine neurohypophysis. J. Biol. Chem. 256: 5950-5953, 1981.

44. Russell, J.T., M. Levine, and D. Njus. Electron transfer across posterior pituitary neurosecretory vesicle membranes. J. Biol. Chem. 260: 226-231, 1985.

45. Scherman, D., and J.P. Henry. Reserpine binding to chromaffin granule membranes. Characterization and comparison with dihydrotetrabenazine binding. Mol. Pharmacol. 25: 113-122, 1984.

46. Scherman, D., J. Nordmann, and J.P. Henry. Existence of an adenosine 5'-triphosphate dependent proton translocase in bovine neurosecretory granule membrane. Biochemistry 21: 687-697, 1982.

47. Schneider, D.L. ATP-dependent acidification of intact and disrupted lysosomes. Evidence for an ATP-driven proton pump. J. Biol. Chem. 256: 3858-3864, 1981.

48. Schneider, F.M., A.D. Smith, and H. Winkler. Secretion from the adrenal medulla. Biochemical evidence for exocytosis. Br. J. Pharmacol. Chemother. 31: 94-104, 1967.

49. Srivastava, M., L.T. Duong, and P.J. Fleming. Cytochrome b 561 catalyzes transmembrane electron transfer. J. Biol. Chem. 259: 8072-8075, 1984.

50. Toll, L., and B.D. Howard. Role of Mg^{2+} ATPase and a pH gradient in the storage of catecholamines in synaptic vesicles. Biochemistry 17: 2517-2523, 1978.

51. Von Euler, U.S., and F. Lishajko. Effects of some metabolic co-factors and inhibitors on transmitter release and uptake in isolated adrenergic nerve granules. Acta Physiol. Scand. 77: 298-307, 1969.

52. Wilkins, J.A., and L. Salganicoff. Participation of a transmembrane proton gradient in 5-hydroxytryptamine transport by platelet dense granules and dense-granule ghosts. Biochem. J. 198: 113-123, 1981.

53. Zhang, F., and D.L. Schneider. The bioenergetics of Golgi apparatus function: Evidence for an ATP-dependent proton pump. Biochem. Biophys. Res. Commun. 114: 620-625, 1983.

CONTROL OF EXOCYTOSIS FROM ADRENAL CHROMAFFIN CELLS

Ronald W. Holz

Department of Pharmacolgy, University of Michigan, Ann Arbor, MI

Summary

Calcium-dependent exocytosis of catecholamine from intact and digitonin-permeabilized bovine adrenal chromaffin cells was investigated. $^{45}Ca^{2+}$ uptake and secretion induced by nicotinic stimulation or depolarization in intact cells were closely correlated. The results provide strong support for Ca^{2+} entry being the trigger for exocytosis. Experiments in which the H^+ electrochemical gradient across the intracellular secretory granule (chromaffin granule) membrane was altered indicted that the gradient does not play an important role in exocytosis. The plasma membrane of chromaffin cells was rendered permeable to Ca^{2+}, ATP, carbohydrates and protein by the detergent digitonin without disruption of the intracellular secretory granules. In this system in which the intracellular milieu can be controlled, micromolar Ca^{2+} directly stimulated catecholamine secretion. Treatment of cells with phorbol esters which activate protein kinase C enhanced phosphorylation and subsequent Ca^{2+}-dependent secretion in digitonin-treated cells. The experiments suggest that protein phosphorylation can modulate Ca^{2+}-dependent secretion in adrenal chromaffin cells.

Introduction

Secretion of prepackaged hormones and neurotransmitters generally occurs by exocytosis. Although exocytosis has been extensively studied, little is known about the physiological and biochemical mechanisms underlying it. Studies on the perfused adrenal medulla and adrenal medullary slices helped define the process and demonstrate that in this tissue Ca^+ influx initiates secretion (3,21). Acetylcholine released from cholinergic nerves interacts with receptors on chromaffin cells to cause Ca^+ entry. In the bovine adrenal medulla nicotinic but not muscarinic receptor activation is responsible for these events. Although studies with the perfused gland and adrenal medullary slices led to a greatly increased understanding of secretion and served as a model for studies in other secretory systems, they were often limited in time resolution and the ability to investigate biochemical events associated with secretion. These limitations have been overcome by the development of techniques to dissociate large numbers of bovine adrenal chromaffin cells and to study secretions either from suspended cells or from monolayer cultures. The following

is a brief description of some of the studies we have undertaken in monolyer cultures and with suspended chromaffin cells to further elucidate those factors which control and perhaps underlie exocytosis.

Methods

Primary dissociated cells from bovine adrenal medulla were prepared and maintained as monlayer cultures or as suspended cells as previously described (7,8). Experiments with intact cells were generally performed at 25°C in a physiological salt solution (PSS) containing 142 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 2.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 15 mM Hepes (pH 7.4), 5.6 mM glucose and 0.5 mM ascorbate. Catecholamine secretion and $^{45}Ca^{2+}$ uptake were measured as previoulsy described (7). Experiments with digitonin-treated cells were usually performed in potassium glutamate solution containing 145 mM. Potassium glutamate, 5 mM EGTA, various concentrations of calcium, 1-5 mM MgATP, and 20 mM Pipes (pH 6.60) (4). The free Ca^{2+} concentration was calculated according to Portzehl et al. (19). Catecholamine secretion was measured by determining the fractional release of endogenous catecholamine or of [^{3}H]norepinephrine from cells which had been preincubated with [^{3}H]norepinephrine to label intracellular catecholamine stores.

Results and Discussion

Relationship between $^{45}Ca^{2+}$ uptake and catecholamine secretion. Douglas and co-workers demonstrated that extracellular Ca^{2+} is required for secretion from perfused adrenal medulla and that $^{45}Ca^{2+}$ uptake into the gland was associated with secretion (3). In monolayer cultures the relationship between calcium uptake and secretion could be determined more precisely. The rate of catecholamine secretion induced by the mixed nicotinic-muscarinic agonist carbachol was maximal during the initial 2 min and slowed thereafter (Fig. 1). Significant carbachol-stimulated Ca^{2+} uptake could be detected at 15 s before the occurrence of significant catecholamine secretion. The rate of carbachol-induced Ca^{2+} uptake was maximal between 0 and 1 min and significantly slowed after 5 min. Both catecholamine secretion and $^{45}Ca^{2+}$ uptake were stimulated by selective nicotinic agonists (nicotine, 1,1-dimethyl-4-phenylpeperazinium), by mixed nicotinic-muscarinic agonists (acetylcholine and carbachol) but not by selective muscarinic agonist (muscarine, methacholine) (7). Similarly, nicotinic but not muscarinic antagonists blocked carbachol-induced secretion and $^{45}Ca^{2+}$ uptake (7). Thus, nicotinic receptor activation is responsible for both increased calcium permeability and increased catecholamine secretion.

Secretion and calcium uptake were also stimulated by depolarization induced by elevated K^+ (Fig.2). Calcium probably entered the cell through voltage-sensitive calcium channels. Again, there was a close temporal relationship between calcium uptake and catecholamine secretion.

The studies strongly support the conclusion from the now classical work of Douglas and co-workers (3) that Ca^{2+} entry initiates and maintains secretion from adrenal medullary cells.

Absence of a role for the H^+ electrochemical gradient across the secretory granule membrane in exocytosis. A common feature of secretory granules is their low internal pH and a membrane-bound electrogenic H^+ pump ATPase (1,5,6,9,10, 12,18,20). The most extensively studied secretory granule, the chromaffin granule from adrenal chromaffin cells, has a pH of approximately 5.6 *in vitro* and an electrogenic H^+ pump ATPase that generates a H^+ electrochemical gradient across the granule membrane of over 120 mV (6). Catecholamine uptake into chromaffin granules is coupled to the H^+ electrochemical gradient. The common occurrence of H^+ electrochemical gradients across membranes of a wide variety of secretory granules raised the possibility that the H^+ electrochemical potential of granules may, in addition, be important for exocytosis. However studies using a variety of different manipulations indicated that the H^+ electrochemical gradient across the granule membrane is probably not involved in exocytosis (8). An important aspect of thse studies was the use of techniques to assess whether various manipulations altered the H^+ electrochemical gradient of chromaffin granules within cells. High concentrations of weak bases such as methylamine (11) or ammonium or the ionophore nigericin (10) which exchanges protons and alkali metal ions across membranes partially collapsed the pH gradient across chromaffin granule membranes *in vitro*. We determined using ^{31}P nmr of ATP within intracellular chromaffin granules (see accompanying paper by D. Njus in the symposium) and radiometric techniques based upon the uptake of radioactive methylamine that 20-30 mM methylamine or ammonium or 1 uM nigericin shifted the pH within intracellular chromaffin granules from 5.3 to 6.3 (8). Under those conditions cathecholamine secretion caused by veratridine-induced deploraization was not inhibited. For example, Fig. 3 demonstrates that concentrations of methylamine or ammonium as high as 20 mM had no significant effect on secretion.

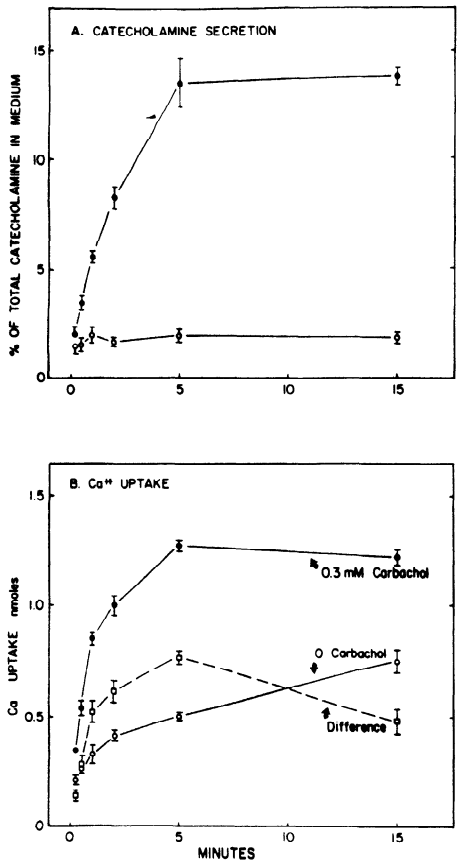


FIG. 1. Time course of carbachol-induced catecholamine secretion (A) and Ca^{2+} uptake (B). Cells were incubated in solution containing $^{45}Ca^{2+}$ in the presence (●) or absence (○) of 0.3 mM carbachol. The solution was aspirated at various times and saved for measurement of catecholamine released from the cells. The dashed line in B represents the difference between Ca^{2+} uptake in the presence and absence of carbachol. There were four wells per group. (From reference 7.)

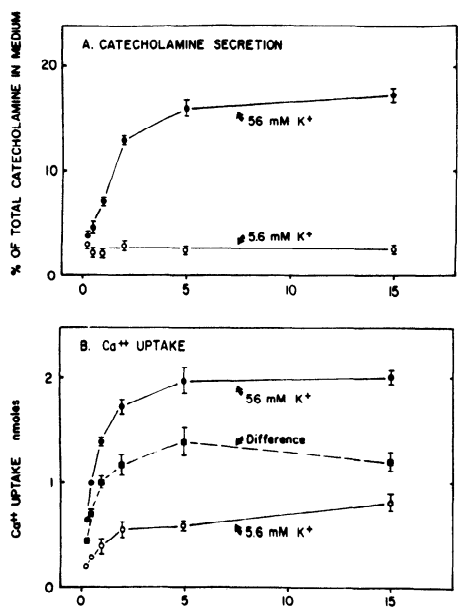


FIG. 2. Time course of depolarization-induced catecholamine secretion (A) and Ca^{2+} uptake (B). Cells were incubated in a solution containing $^{45}Ca^{2+}$ and either 5.6 mM or 56 mM K^+. Catecholamine secretion and Ca^{2+} uptake into cells were determined after various incubation times. The dashed line in B represents the difference between Ca^{2+} uptake in 56 and 5.6 mM K^+ (the depolarization-induced Ca^{2+} uptake). There were four wells per group. (From reference 7.)

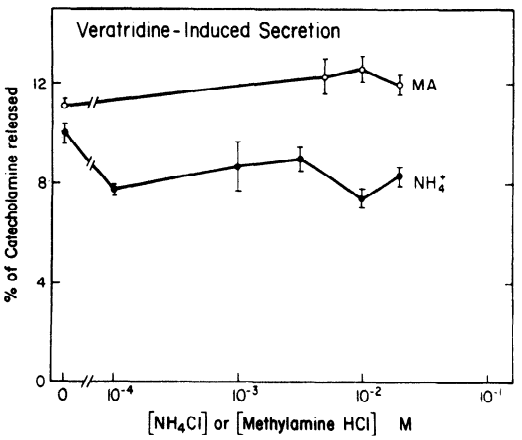


FIG. 3. The effect of NH_4Cl or methylamine HCl on veratridine-induced secretion from chromaffin cells. Cells were incubated in various concentrations of NH_4Cl or methylamine HCl. After 15 min the medium was changed to one containing the same NH_4Cl or methylamine HCl concentration with or without 0.1 mM veratridine. Cathecholamine release was 3-4% of the total in the absence of veratridine. The effects of NH_4Cl and methylamine HCl on secretion were determined on different cell preparations. There were four wells per group. (From reference 8.)

Another approach was the use of dicyclohexylcarbodiimide (DCCD), an irreversible inhibitor of a number of H^+ pump ATPases. Under conditions in which the H^+ pump ATPase of chromaffin granules within cells was inhibited, there was no effect on elevated K^+-induced secretion. Similarly, FCCP, a H^+ ionophore, which reduces the granule membrane potential to the H^+ equilibrium potential in isolated chromaffin granules (and thereby reduces to zero the H^+ electrochemical gradient) had no effect or caused a small enhancement of secretion induced by nicotinic cholinergic stimulation at concentrations which maximally uncoupled mitochodria within chromaffin cells (8).

These experiments indicate that secretion does not require 1) the complete H^+ gradient across the granule membrane, 2) a normal H^+ electrochemical gradient across the granule membrane, 3) an inside positive granule electrical potential, or 4) an active granule membrane H^+ pump ATPase.

Secretion from chromaffin cells with plasma membranes rendered leaky by

digitonin. One of the major limitations in the study of a complex cellular process such as exocytosis which requires cell structure is the inability to contol the intracellulr milieu. Recently Knight and Baker (15) permeabilized the plasma membrane of freshly suspended adrenal medullary cells by exposing cell suspensions to high voltage discharges which caused localized dielectric breakdown of the plasma membrane. They found that micromolar Ca^{2+} without secretagogue induced exocytosis. We have permeabilized monolayer chromaffin cells with the detergent digitonin which interacts with cholesterol in membranes (4). Although digitonin in high concentrations can completely disrupt cells, we found that exposure of cells to low concentrations of digitonin (10 uM to 20 uM) for short times (less than 15 minutes) caused the cells to become permeable to low molecular weight ions such as Ca^{2+} and ATP, to carbohydrates as large as the tetrasaccaride stachyose and to lactate dehydrogenase, a cytosolic protein marker, which exited from the cells with a half-time of 10-15 min. Intracellular chromaffin granules, however, remain intact and did not leak catecholamines. Most importantly, at these low concentrations of digitonin, micromolar Ca^{2+} stimulated catecholamine release (Fig. 4). Similar results were obtained by Wilson and Kirshner (22). Figure 5 demonstrates that the cells were responsive to calcium from below 1 to 10 uM. The Ca^{2+} response was specific since the 1 mM free Mg^{2+} that was present in the solutions did not support catecholamine release. The Ca^{2+} concentrations are within the physiological range which stimulates intracellular events in intact cells and are approximately 1/500th of that necessary to support secretagogue-induced secretion from intact cells (Fig. 5).

If cells are permeabilized for 10 min in the absence of ATP to deplete the cytosol of ATP, subsequent addition of ATP in the presence and absence of Ca^{2+} indicates that at least 75% of the Ca^{2+}-dependent secretion is also ATP dependent. The small amount of Ca^{2+}-dependent secretion that occurs in the absence of ATP may reflect residual intracellular ATP. The results suggest that ATP plays a direct role in the secretory process. Baker and Knight (15) using cells permeabilized by intense electric fields arrived at the same conclusion. Phorbol esters stimulate protein phosphorylation and secretion. Protein kinase C is Ca^{2+}-dependent and requires acidic phospholipids for maximal activity (13,23). Diglyceride in the presence of phosphatidylserine increased the calcium sensitivity of the enzyme to micromolar (14). Tumor promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA) substitute for diglyceride *in vitro* to increase the activity and calcium sensitivity of the enzyme (2). Most importantly, PMA activiates protein kinase C and protein phosphorylation in intact platelets and enhances serotonin secretion (2,24).

We investigated the effects of PMA on Ca^{2+}-dependent secretion in digitonin-treated cells (17). Pretreatment of intact cells with PMA for 15-30 min enhanced Ca^{2+}-dependent secretion from cells subsequently permeabilized with digitonin (Fig 6.). Secretion induced by 1 uM Ca^{2+} was approximately doubled by PMA. PMA both increased the sensitivity of the secretory response to low concentrations of calcium and increased the maximal response to calcium (Fig. 7). PMA also enhanced Ca^{2+}-dependent secretion from suspended chromaffin cells permeabilized by intense electric fields (16).

Another phorbol ester activator of protein kinase C, 4β-phorbol-12, 13-dibutyrate also enhanced Ca^{2+}-dependent secretion from digitonin-treated cells. 4β-Phorbol which does not activate protein kinase C did not stimulate secretion from digitonin-treated cells (17).

In order to obtain reproducible effects on secretion from digitonin-treated cells, it was necessary to preincubate the intact cells with phorbol ester for 15-30 min. Two-dimensional polyacrylamide gel electrophoresis of proteins from cells preincubated in ^{32}P- phosphate demonstrated that over 15 proteins were phosphorylated upon incubation in PMA (17). Thus, PMA probably increased protein kinase activity in the cells, possibly by activation of protein kinase C.

PMA also had effects on cells not treated with digitonin. For example, PMA induced a dose-dependent increase in catecholamine release from intact chromaffin cells which increased gradually over a period of at least 90 min (17). The release at 90 min was approximatley 10% of the total catecholamine and contrasts with the rapid release induced by nicotinic agonists or by depolarization of 15-20% of the total catecholamine in 5-10 min. PMA did not have reproducible effects on either nicotinic agonist or depolarization-induced secretion.

These experiments suggest that protein phosphorylation can modulate the secretory response in chromaffin cells. They are important because they identify for the first time a specific reaction that is able to modulate secretion in this system. There are many issues which the experiments raise. We do not know whether protein kinase C is activated during cholinergic-induced secretion from intact cells. We do not know whether protein kinase C modulates the normal Ca^{2+}-dependent pathway or initiates a separate one in digitonin-treated cells. We do not know the protein substrates of protein kinase C which may be responsible for the enhanced secretion.

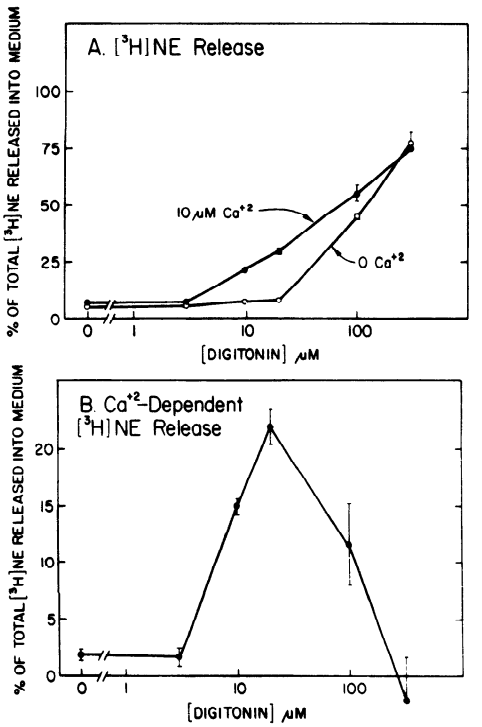


FIG. 4. Effect of various concentrations of digitonin on norepinephrine release from chromaffin cells. Cells containing [^{3}H]norepinephrine (NE) were incubated in potassium glutamate solution containing 5 mM MgATP and various concentrations of digitonin in the presence or absence of 10 uM Ca^{2+}. After 15 min, the solution was removed, and [^{3}H]norepinephrine released in the [^{3}H]norepinephrine solution and that remaining in the cells was measured. A, [^{3}H]norepinephrine released into the medium in the presence and absence of 10 uM Ca^{2+}; B, difference between release of [^{3}H]norepinephrine in the presence and absence of Ca^{2+}. There were three wells per group. (From reference 4.)

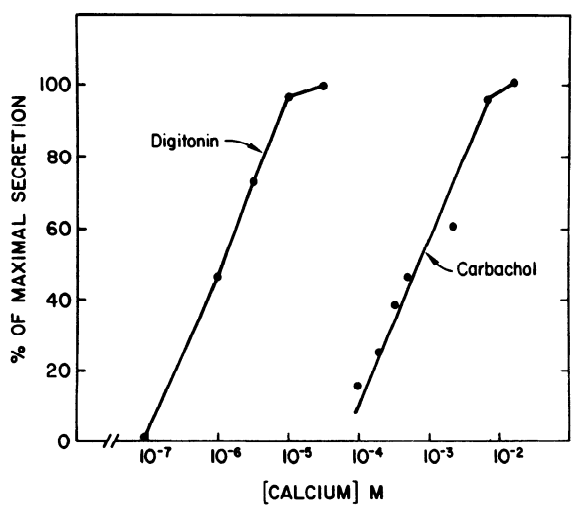


FIG. 5. Effect of calcium concentration on secretion in the presence of digitonin or carbachol. Ca^{2+}-dependent [^{3}H] norepinephrine release into the medium by digitonin-treated cells was measured in 139 mM potassium glutamate, 20 mM PIPES (pH 6.6), 5 mM MgATP, 5 mM glucose, 0.5 mM ascorbic acid, and 20 uM digitonin after 15 min. Calcium concentrations were buffered with 5 mM EGTA. Carbachol-dependent secretion of endogenous catecholamine was measured in 142 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 0.5 mM $MgCl_2$, 15 mM Na-HEPES (pH 7.4), 5.6 mM glucose, and 0.5 mM ascorbic acid with various amounts of calcium (in the absence of EGTA) after 2 min. Carbachol concentration was 0.3 mM. Maximal calcium-dependent secretion in the presence of digitonin was 28.5% of the total [^{3}H]norepinephrine. Maximal calcium-dependent secretion in the presence of carbachol was 9.6% of the total endogenous catecholamine. There were three samples/groups. (From reference 4.)

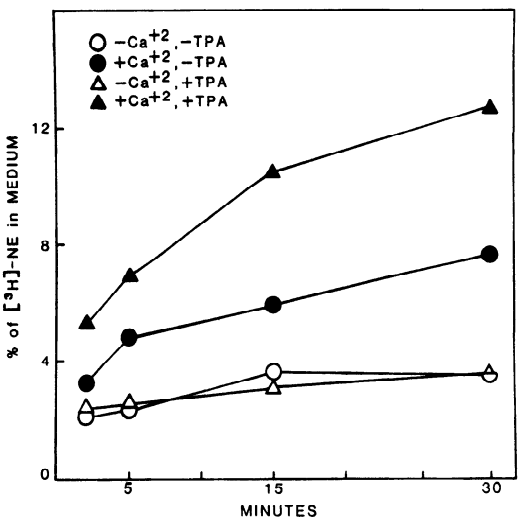


FIG. 6. The effect of PMA on the time course of secretion from digitonin-treated chromaffin cells. Chromaffin cells prelabeled with [^{3}H] norepinephrine were incubated in physiological salt solution in the presence (△, ▲) or absence (○, ●) of 100 nM PMA for 15 min. The solutions were then replaced with Ca^{2+}- free potassium glutamate solution containing 20 uM digitonin/1 mM MgATP in the presence or absence of 100 nM PMA for 5 min, after which cells were incubated in potassium glutamate solution without digitonin in the absence or presence of 100 nM PMA, with 1 mM MgATP, and 0 free Ca^{2+}(△, ○) or ; 1 uM free Ca^{2+}(▲ , ●). The percentage of [^{3}H]norepinephrine released into the medium was determined at various times. Each group contained four wells. (From reference 17.)

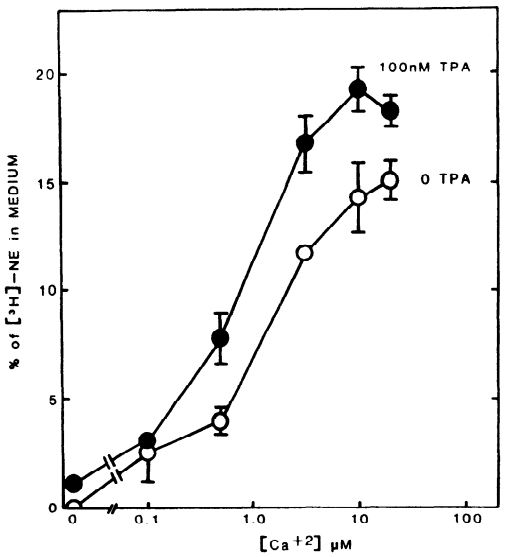


FIG. 7. Ca^{2+}-dose response for secretion from digitonin-treated chromaffin cells in the presence and absence of PMA. Chromaffin cells prelabeled with [^{3}H]norepinephrine were incubated in physiological salt solution in the presence (●) or absence (○) of 100 nM PMA. After 15 min, the solutions were replaced with potassium glutamate solution containing various concentrations of free Ca^{2+} in the continuing presence or absence of 100 nM PMA. The percentage of [^{3}H]-norepinephrine released into the medium was determined after 10 min. The percentage of [^{3}H] norepinephrine released in the absence of PMA and Ca^{2+}(8.3%) was subtracted from the data. Each group contained four wells. (From reference 17.)

We are just beginning to illuminate the intracellular processes involved in secretion. We are hopeful that experiments with permeabilized cell preparations will lead to a more profound understanding of exocytosis.

This work was supported by PHS grant RO1AM27959 and NSF grant BNS 82-11493.

REFERENCES

1. Casey, R.P., D. Njus, G.K. Radda, and P.A. Sehr. Active Proton Uptake by Chromaffin Granules: Observation by Amine Distribution and Phosphorus-31 Nuclear Magnetic Resonance Techniques. Biochemistry. 16:972-977, 1977.

2. Castagna, M., Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, and Y. Nishizuka. Direct Activation of Calcium-Activated, Phospholipid-dependent Protein Kinase by Tumor-Promoting Phorbol Esters. J. Biol. Chem. 257: 7847-7851, 1982.

3. Douglas, W.W. Secretomotor Control of Adrenal Medullary Secretion: Synaptic, Membrane and Ionic Events in Stimulus-Secretion Coupling. In: Handbook of Physiology Endocrinology, edited by H. Blashko, G. Sayer and, and A.D. Smith. American Physiological Society, Washington, D.C.: 1975, p. 367-388.

4. Dunn, L.A., and R.W. Holz. Catecholamine Secretion from Digitonin-treated Adrenal Medullary Chromaffin Cells. J. Biol. Chem. 258: 4989-4993, 1983.

5. Fishkes, H., and G. Rudnick. Bioenergetics of Serotonin Transport by Membrane Vesicles Derived from Platelet Dense Granules. J. Biol. Chem. 257: 5671-5677, 1982.

6. Holz, R.W. Evidence that Catecholamine Transport into Chromaffin Vesicles is Coupled to Vesicle Membrane Potential. Proc. Natl. Acad. Sci. U.S.A.. 75: 5190-5194, 1978.

7. Holz, R.W., R.A. Senter and R.A. Frye. Relationship between Ca^{++} Uptake and Catecholamine Secretion in Primary Dissociated Cultures of Adrenal Medulla. J.Neurochem. 39: 635-646, 1982.

8. Holz, R.W., R.A. Senter and R.R. Sharp. Evidence that the H^+ Electrochemical Gradient Across Membranes of Chromaffin Granules is not Involved in Exocytosis. J. Biol. Chem. 258: 7506-7513, 1983.

9. Hutton, J.C. The Internal pH and Membrane potential of the insulin secretory granule. Biochem. J. 204: 171-178, 1982.

10. Johnson, R.G., and A. Scarpa. Internal pH of Isolated Chromaffin Vesicles. J. Biol. Chem. 251: 2189-2191, 1976.

11. Johnson, R.G. and A. Scarpa. Protonmotive Force and Catecholamine Transport in Isolated Chromaffin Granules. J. Biol. Chem. 254: 3750-3760, 1979.

12. Johnson, R. G., A. Scarpa, and L. Salganicoff. The Internal pH of Isolated Serotonin Containing Granules of Pig Platelets. J. Biol. Chem. 253: 7061-7068, 1978.

13. Kikkawa, U., Y. Takai, R. Minakuchi, S. Inohara, and Y. Nishizuka. Calcium-Activated, Phospholipid-dependent Protein Kinase from Rat Brain. J. Biol. Chem. 257: 13341-13348, 1982.

14. Kishimoto, A., Y. Takai, T. Mori, U. Kikkawa, and Y. Nishizuka. Activation of Calcium and Phospholipid-dependent Protein Kinase by Diacylglycerol, Its Possible Relation to Phosphotidylinositol Turnover. J. Biol. Chem. 255: 2273-2276, 1980.

15. Knight, D.E. and P.F. Baker. Calcium dependence of Catecholamine Release from Bovine Adrenal Medullary Cells after

Exposure to Intense Electric Fields. J. Membr. Biol. 68: 107-140, 1982.

16. Knight, D.E. and P.F. Baker. The Phorbol Ester TPA increases the Affinity of Exocytosis for Calcium in "Leaky" Adrenal Medullary Cells. F. E. B. S. Lett. 160: 98-100, 1983.

17. Pocotte, S.L., R.A. Frye, R.A. Senter, D.R. Terbush, S.A. Lee and R.W. Holz. Effects of Phorbol Ester on Catecholamine Secretion and Protein Phosphorylation in Adrenal Medullary Cell Cultures. Proc. Natl. Acad. Sci.(USA). 82: 930-934, 1985.

18. Pollard, H.B., O. Zinder, P.G. Hoffman, and O. Nikodejevic. Regulation of the Transmembrane Potential of Isolated Chromaffin Granules by ATP, ATP Analogs and External pH. J. Biol. Chem. 251: 4544-4550, 1976.

19. Portzehl, H., P.C. Caldwell, and J.C. Ruegg. The Dependence of Contraction and Relaxation of Muscle Fibers from the Crab Maia squinado on the Internal Concentration of Free Calcium Ions. Biochim. Biophys. Acta. 79: 581-591, 1964.

20. Russell, J.T., and R.W. Holz. Measurement of ΔpH and Membrane Potential in Isolated Neurosecretory Vesicles from Bovine Neurohypophyses. J. Biol. Chem. 256: 5950-5953, 1981.

21. Viveros, O.H. Mechanism of Secretion of Catecholamines from Adrenal Medulla. In: Handbook of Physiology Endocrinology, edited by H. Blashko, G. Sayer, and A.D. Smith. Washington,D.C.: American Physiological Society, 1975, 389-426.

22. Wilson, S.P. and N. Kirshner. Calcium-Evoked Secretion from Digitonin-permeabilized Adrenal Medullary Chromaffin Cells. J. Biol. Chem. 258: 4994-5000, 1983.

23. Wise, B.C., R.L. Raynor, and J.F. Kuo. Phospholipid-Sensitive Ca^{++}-dependent Protein Kinase from Heart. J. Biol. Chem. 257: 8481-8488, 1982.

24. Yamanishi, J., Y. Takai, K. Kaibuchi, K. Sano, M. Castagna, and Y. Nishizuka. Synergistic Functions of Phorbol Ester and Calcium in Serotonin Release from Human Platelets. Biochem. Biophys. Res. Commun. 112: 778-786, 1983.

REGULATION OF SECRETION FROM ADRENAL CHROMAFFIN CELLS

Harvey B. Pollard, Richard Ornberg, Mark Levine, Keith Brocklehurst, Eric Forsberg, Peter I. Lelkes, and Kyoji Morita

Laboratory of Cell Biology and Genetics
National Institute of Arthritis, Diabetes, and Kidney Diseases
National Institutes of Health
Bethesda, Maryland 20205

Summary

Calcium-dependent secretion from cells occurs by both exocytotic and non-exocytotic mechanisms, and examples of each type are available in chromaffin cells. The ultra- structural basis of exocytosis is now becoming better understood with better fixation techniques. One emerging idea is that the initial contact and fusion events occur over a very small region of juxtaposed secretory vesicle and plasma membranes. A second primary idea is that exocytosis indeed involves initial membrane contact between the secretory vesicles and plasma membranes; subsequently the membranes undergo fusion. It is becoming increasingly apparent that these processes are probably regulated by calcium, and a variety of calcium mediators have been proposed and studied. These mediators include actin, as part of the cytoskeleton, as well as cytosolic proteins such as calmodulin, synexin, chromobindins, and the protein kinase C system. Other fusion factors directly associated with granule membranes have also been proposed. It is our purpose in this paper to review each of these concepts as it specifically relates to the chromaffin cell.

Introduction

Chromaffin cells appear to secrete catecholamines and other chromaffin granule components by exocytosis in response to calcium entry into the cell. Indeed, it was Douglas (21) who first showed that acetylcholine and high potassium stimulated secretion of catecholamines from chromaffin cells only if calcium were also present in the perfusion medium. Later studies on activation of exocytosis in cultured chromaffin cells showed that exocytosis was blocked if calcium entry were inhibited either by D600 (13) or by divalent cations such as magnesium or nickel (51). More directly, studies by Kilpatrick (35) and by Holz, Senter and Frye (31) showed that specific secretagogues in chromaffin cells induced a dramatic increase in radioactive calcium influx. Finally, using the fluorescent probe Quin 2, Knight and Kesteren (38) demonstrated that when chromaffin cells were stimulated by

either high potassium or acetylcholine the intracellular calcium concentration rose. In all these cases, calcium accumulation occurred only when there was calcium on the outside of the cell available for entry.

The problem we would therefore like to address here then is what calcium does to evoke secretory events once it enters the cell. If we concern ourselves exclusively with the process of exocytosis in chromaffin cells we are led to the following questions: How do secretory vesicles find their way to the plasma membrane? Are any of these processes *calcium-dependent?* Might movement involve the interaction of chromaffin granules with the rather widely distributed cytoskeletal element actin? Once the secretory vesicle has reached the plasma membrane, how does it interact and fuse with the plasma membrane? Finally, we will touch on the fact that in chromaffin cells, secretion can occur in a calcium-dependent manner which is not by exocytosis: ascorbate seems to be released directly from the cytosol. Is it thus possible that chromaffin cells harbor a variety of calcium-dependent processes which can act in parallel and lead to different kinds of secretion?

Ultrastructural Aspects of Exocytosis

The secretion process in chromaffin cells can be followed from early time intervals by application of new fast freezing methods. The questions that can be specifically asked involve: How does the secretory vesicle get to the plasma membrane, and secondly, what is the nature of the contact between secretory vesicles and plasma membranes? One result of this study is the appreciation that the large secretory granules do not necessarily have to move very far in the cytosol to reach the plasma membrane or an extension of the plasma membrane. In fact, compound or "piggy-back" exocytosis, in which one vesicle fuses with the membrane residue of a previous exocytotic event, is a very dominant event. Therefore, contacts are observed not only between chromaffin granules and plasma membranes, but between chromaffin granule membranes and those granule membranes that are already forming exocytotic structures with the plasma membranes. One interesting fact to be gleaned from these studies is that the region of contact between membranes engaged in exocytosis is not wide but rather punctate. By punctate, we mean that the region of contact extends over perhaps a few tens of lipid molecules in any one site. As a byproduct of the fast freeze study, we have discovered the existence of small, membrane-bound organelles within chromaffin granules. These intra- granular vesicles, to which we have attached the proprietary name "ornosomes", after their discoverer, Richard Ornberg, are also found in granules in the process of assembly in the Golgi, in the cytoplasm and in the region containing exocytosed material outside of chromaffin cells. At present, we do not know their contents: hence the proprietary name.

Calcium-Dependent Interaction of Chromaffin Granules with Cytoplasm

The cytoplasm of chromaffin cells is composed of a three-dimensional meshwork of cytoskeletal elements, and direct interactions between chromaffin granules and cytoskeletal elements, including actin, have been observed by stereoelectronmicroscopy of cells embedded in water soluble media (40). It has also been appreciated for over ten years (12) that actin is able to interact with chromaffin granule membranes when mixed in the test tube. More recently, Fowler and Pollard (26,27,) found that F-actin could interact with highly purified granular membranes. The technique they used was low shear falling ball viscometry, in which the chromaffin granule membranes become cross-linked to F-actin. This cross-linking reaction causes the viscosity of the solution to rise. The changes in the viscosity of the solution were inhibited by calcium, 50% inhibition occurring in 0.2 umolar free calcium concentration. The phenothiazine drugs were without effect on this calcium-dependent process. Treatment of the granular membrane with trypsin blocked the cross-linking activity indicating that the actin-binding site might be protein in nature. Aunis and Pemin (3) have suggested that the protein component to which actin binds may be a spectrin-like protein called fodrin.

Our interpretation of these data is that at least one action of calcium upon entry into the cell might be to break the natural interactions occurring between chromaffin granules and cytoskeletal elements such as actin at low calcium concentrations. This would allow the granules to move on to another stage of exocytosis, possibly that of contact and fusion with the plasma membrane.

Membrane Fusion and Calcium

Membrane fusion processes occurring during exocytosis can be divided into three distinct stages. The first events involve a close approach of the fusing faces of the membranes involved. To achieve this end there is a requirement for compensation of mutual repulsive forces between the two membranes by charge neutralization or dehydration (49). The second step involves a destabilization of the membrane architecture probably involving the transient formation of non-bilayer structures or lipidic particles (49,57). Finally, the two membranes merge and reorganize into a new bilayer structure. Needless to say, we are particularly uninformed about how these processes occur. However, certain models of these processes can be studied which

are calcium-dependent and are hoped to give insight to how calcium might act in processes such as these in cells. It was initially thought that the simple introduction of calcium alone could cause the specific membranes involved in exocytosis to make contact and undergo fusion. Indeed, if one simply mixes together liposomes composed of fusigenic negatively charged phospholipids, some contact and fusion results. However, these liposomes can be induced to fuse more profoundly by adding calcium in the concentration range of 1-2 mM (24,60).

However, it is a hallmark of secreting cells that membrane fusion does not occur in a very noticeable way spontaneously. Furthermore, the levels of calcium needed to activate these model systems seem relatively high compared with the levels which were thought to occur in the cytosol of secreting chromaffin cells. Therefore, as in other parts of the field of cell biology, interest has swung in the direction of searching for factors which would mediate the action of calcium, perhaps at much lower concentrations than those necessary for the *in vitro* experiments with liposomes. We will now direct our attention to examples of these putative mediating factors.

Calmodulin and Secretion

Mediators of calcium action in biological systems have been widely appreciated for some time. An example in the case of muscle is the molecule troponin-C. An analog of troponin-C that could be involved in exocytosis from cells is calmodulin. The adrenal medulla, in fact, was one of the first sources of calmodulin and its concentration in chromaffin cells has been estimated to be 10^{-6} molar (41). Consistent with the notion of calmodulin being involved in secretion has been the observation that a non- specific anti-calmodulin drug trifluoperazine (TFP) blocks secretion from these cells (5, 36,47,). However, TFP also interferes with calcium fluxes in chromaffin cells, and has other actions, to be discussed later.

In 1981, Burgoyne and Geisow (11) showed that radiolabelled calmodulin could specifically bind to the chromaffin granule membrane, and could induce the phosphorylation of certain proteins in that membrane. Furthermore, Creutz and his colleagues (16,19) have shown that a variety of other proteins in the cytosol of chromaffin cells would bind to chromaffin granule membranes in the presence of calcium or other factors. Geisow and Burgoyne (29) have also shown that certain cytosolic proteins from chromaffin cells can bind to secretory vesicle membranes in a manner dependent on both calcium and calmodulin.

However, none of the calmodulin dependent or related processes, putatively involved in exocytosis, could be blocked by trifluoperazine. Furthermore, Pollard, Scott and Creutz (47) have found that promethazine, a phenothiazine drug which interacts very poorly with calmodulin, is as effective as TFP in blocking exocytosis from chromaffin cells. Thus, the argument implicating calmodulin in a specific event involved in the secretion process is considerably weakened. On the other hand, Trifaro and colleagues (39) have found that introduction of anticalmodulin antibodies into secreting chromaffin cells causes a reduction in exocytosis. Whether this effect is due to a specific action of the antibodies on a secretion process, or merely on an important housekeeping event occurring in the cell during secretion, is not clear. In sum then, it is our opinion that the evidence linking an action of calmodulin with the specific process of exocytosis does not seem very compelling at present, but that the probable importance of calmodulin in chromaffin cells ought not be underestimated.

Synexin and Synexin-Related Proteins

A protein of special interest to us is synexin, a widely distributed 47,000 dalton calcium- binding protein, that causes isolated chromaffin granules to aggregate to one another by formation of pentalaminar membrane contacts (14). The process depends absolutely on calcium, being unaffected by other divalent cations. It is our opinion that such processes may indeed prove to be of physiological relevance since, as mentioned above, secretion from chromaffin cells proceeds by both simple and compound exocytosis.

In addition to forming specific contacts between chromaffin granules, the synexin aggregates of granules can be fused by the subsequent addition of small quantities of arachidonic acid (17,18). Large vacuolar structures are formed upon the addition of arachidonic acid to aggregated granules, and such structures are often found in secreting chromaffin cells. This has led us to conclude that synexin might indeed be performing some event related to exocytosis in chromaffin cells. Interestingly, chromaffin cells undergoing exocytosis release measurable quantities of arachidonic acid (28,34). Thus secretory granules, in the presence of substances we know to be present in secreting chromaffin cells such as calcium, arachidonic acid and synexin, do form structures which are very reminiscent of the secretion process in the intact cell.

The only pharmacological evidence for involvement of synexin with exocytosis in chromaffin cells come from a study of the phenothiazine drugs (47). In these studies it was found that trifluoperazine and promethazine inhibit exocytosis from chromaffin cells at roughly one micromolar concentrations. We discussed

these data earlier in reference to calmodulin when we noted that promethazine was quite effective at suppressing exocytosis while being quite ineffective in blocking various calmodulin-related events. However, in the case of synexin, promethazine and trifluoperazine are quite effective in the same concentration range at blocking synexin activity in vitro. Thus, if any conclusion can be drawn from the phenothiazine pharmacology, it is that we can not exclude synexin from a role in mediating exocytosis.

The mechanism of synexin action on isolated secretory granules is now becoming better appreciated. When calcium interacts with the synexin molecules, it does cause the synexin molecules to polymerize into large structures (15). It is these large polymers of synexin, rather than individual synxein monomers, that indeed cause the aggregation of isolated secretory granules. The receptors for synexin on secretory granule membranes and on plasma membranes are now also becoming more appreciated. Hong, Duzgunes, and Papahadjopoulos (33) have shown that synexin will cause aggregation and fusion of specific liposomes. Liposomes composed of phosphatidylserine were the best substrates, although other acidic phospholipids would also work. It would therefore appear that phosopholipids of this sort might be included in the receptor sites for synexin on secretory vesicles or plasma membranes (52).

Since the initial discovery of synexin in 1978, a number of related proteins have been discovered. One of these is a protein of about 67,000 daltons which inhibits synexin. Pollard and Scott (46) called this protein synhibin, because it was a synexin-inhibiting protein. The inhibition seemed to be competitive with synexin and later studies revealed that monoclonal and polyclonal antibodies to synexin also interacted with synhibin (Scott and Pollard, unpublished). Synhibin is found in liver, for example, but it is much more prevalent in the adrenal medulla. The existence of synhibin indicates that the action of synexin in cells is too important for the cell to leave it up to the presence or absence of calcium to regulate activity, and that other factors have been developed in order to control synexin activity. The study of the interaction of these two substances in the cell will hopefully lead to a more profound understanding of how membrane contact events, as well as perhaps the process of exocytosis, is regulated.

Another set of proteins with synexin-like activity have been reported from Whittaker's laboratory in studies on the electric organ of Torpedo. The proteins have been called "calelectrins" on this basis. The initial finding was of a protein of 35,000 daltons molecular weight (59), which was able to cause chromaffin granules to aggregate in a calcium-dependent manner similar to that of synexin. Sudhoff and colleagues have subsequently reported (53,54) that calelectrins could also be found in mammalian tissue including bovine liver and adrenal medulla, and that they were similar in certain of their properties to the original protein found in Torpedo. In mammalian tissues, the calelectrins were associated with molecular species of 32,000 and 67,000 daltons. Antibodies to each calelectrin species reacted with each of the other two proteins regardless of origin. In addition, like synexin, the calelectrins also caused phosphatidylserine liposomes to aggregate. This indicates a possible common mechanisim of action for calelectrins and synexins. It is therefore our feeling that the synexin-like family of proteins will prove to be of importance for membrane contact and fusion processes, not only in exocytosis but also in other aspects of cell biology.

Role of ATP in Exocytosis

It has been appreciated since the significant review by Viveros in 1975 (58) that ATP is required for secretion from chromaffin cells. Similar studies were reported by Pollard and his colleagues in 1984 using acutely prepared intact cells (48), and evidence for the importance of ATP in a model of exocytosis measured in permeabilized cells have been demonstrated (4,23,61). However, the action of ATP in the secretion process is at present very poorly understood.

A variety of theories on how ATP could work in the exocytosis process have been presented; however, all of them have profound flaws when examined in the context of the secreting chromaffin cell. In 1978, Pollard and his colleagues (45) proposed a chemiosmotic hypothesis for exocytosis, based on the action of the proton pumping ATPase then recently discovered to be in the chromaffin granular membrane. However, a variety of detailed studies have shown that the chemiosmotic hypothesis does not lead to an exact understanding of how secretion from these cells occurs (48, 32).

Another function of ATP in many cells is to provide phosphate groups for protein kinases and other kinds of kinase activities. It is possible that ATP could be supplying such energy-rich groups in secreting chromaffin cells to a specific protein or lipid. Amy and Kirshner in 1981 (2) showed that secreting chromaffin cells specifically phosphorylated proteins of 60,000 and 95,000 daltons. However, the 60,000 molecular weight protein was found to be tyrosine hydroxylase, and so is considered unlikely to be involved in the regulation of exocytosis (30). Brooks et

al. (10) also showed that cells permeablized with saponin would stop secreting if the ATP were replaced by ATP-γ-S. ATP-γ-S is a substrate for kinases but phosphoprotein phosphatase can not act on proteins phosphorylated with this product.

As previously iterated, the chromaffin granule membrane has a variety of cAMP calcium, and calmodulin dependent kinase activities. In addition there is a phosphatidylinositol kinase, exclusively located on the chromaffin granular membrane (44). Another separate protein kinase of increasing interest in modern cell biology is an enzyme called protein kinase C. Protein kinase C is dependent on both calcium and phosphatidylserine for its activity, and the affinity of the enzyme for these activators is increased by diacylglycerol and phorbol esters. Protein kinase C has been proposed by Nishizuka (43) to be important in the secretion event occurring in platelets. In 1983, Creutz and his collaborators (55) showed that protein kinase C could bind to chromaffin granule membranes in a calcium- sensitive manner. More recently, protein kinase C has been partially purified from chromaffin cells, where it has been found to resemble the enzyme purified from other tissues in that it is activated by diacylglycerol and by phorbol esters (8). Phorbol esters cause a modest increase in secretion from cultured intact chromaffin cells, and this effect is synergistic with that of the calcium ionophore, A23187. Also chromaffin cells permeabilized by high voltage discharge secrete catecholamines at a slightly lower concentration of calcium than in the absence of phorbol esters (37). This has also been found to be true for digitonin- permeabilized cells (9). The activation of protein kinase C by diacylglycerol implies the existence of a specific phospholipase C to generate the product.

It is widely appreciated that this specific phospholipase C may act on phosphatidylinositol and the various phosphorylated derivatives of this phospholipid (22). Of course, when phospholipase C acts on these phosphoinositides it also releases the charged inositol phosphates from lipids. Thus, concentrations of inositol phosphate, inositol bisphosphate and inositol trisphosphate are found to increase rapidly in some secreting cells (50). A large amount of investigation has lead to the conclusion that one or more of these inositol phosphates, in particular inositol 1,4,5 trisphospate, may be the true second messenger which releases the third messenger, calcium, from intracellular storage sites (7).

However, the relevance of this concept to chromaffin cells is unclear because the pharmacology seems to be incorrect. In bovine adrenal chromaffin cells, Trifaro (56), Adanan and Hawthorne (1) and Fisher et al. (25) found that the stimulation of phosphoinositide metabolism was mediated by muscarinic receptors, while nicotinic cholinergic receptors are known to be necessary for the secretion event to be induced. Recent studies in our own laboratory indicate that changes in phosphoinositide metabolism do indeed occur in chromaffin cells but only after the secretion event has ceased.

We are, therefore, left to conclude that the role of ATP in regulation of exocytosis from chromafffin cells is yet to be understood. While there are many hypotheses for how ATP might work, summarized in this section of our paper, it is clear that there is no special reason at present for believing any of them.

Calcium-Dependent, Non-Exocytotic Secretion of Ascorbate

Up to this point we have emphasized mechanistic regulation of exocytosis by calcium. However, calcium also appears to orchestrate nonexocytotic release of ascorbic acid from chromaffin cells. Ascorbate secretion can be induced in a concentration dependent manner by acetylcholine, carbachol, nicotine, high potassium, or veratridine (20,42), just as for catecholamine secretion. However, secreted ascorbic acid does not appear to originate from chromaffin granules, as supported by several observations. Most compelling is the fact that more ascorbic acid is secreted than is found in particulate fractions (including chromaffin granules) of chromaffin cells. Indeed, most ascorbate in disrupted cells is not associated with chromaffin granules. Further evidence for this proposal is obtained from studies on digitonin-treated chromaffin cells showing that newly transported ascorbate is almost entirely localized to the cytosol of these cells. Finally, the same digitonin-permeabilized cells release ascorbate in a calcium independent manner. By contrast, catecholamine secretion requires calcium under these conditions. Thus, these experiments suggest that the nonvesicular compartment from which ascorbate is secreted is the cytosol itself.

Catecholamine secretion and ascorbate secretion thus appear to occur by distinctly separate mechanisms; yet both are calcium dependent. Perhaps calcium entry regulates different processes for ascorbate secretion, which remain to be characterized. One good reason for supposing this to be the case is that the kinetics of ascorbic acid release are distinctly slower than those for cathecholamine secretion.

Conclusions

There are a variety of specific molecular processes where calcium can act and where its action can be studied in a

biochemically relevant manner. Examples such as these include the action of calcium on the interaction of actin and secretory vesicles, on various models of membrane fusion and on the action of various cytosolic protein modulators of calcium action such as calmodulin and synexin. Even the possible kinase mediator of exocytosis, protein kinase C, depends on calcium. It is therefore our feeling that there is not just one site of calcium action regulating the process of exocytosis but many, perhaps acting in parallel. Indeed, the existence of a non- exocytotic, but nonetheless calcium-dependent, secretion process also warns us to be careful about too readily assigning exclusive chemical responsibility for secretion to specific biochemical processes.

Finally, the new demonstration of non-calcium dependent fusion factors in chromaffin granule membranes can alert us to non-calcium related mediators that are pre-set in the membranes that will eventually fuse. Bental et al. (6) recently reported that chromaffin granule membrane vesicles fuse to specific liposomes by a process that can be blocked by treating the granule membrane with trypsin. It is thus clear that even an intellectual concentration on effects of calcium, as in this paper, to the exclusion of other effects, may be counter-productive.

References

1. Adnan, N.A., and J.N. Hawthorne. Phosphatidylinositol labelling in response to activation of muscarinic receptors in bovine adrenal medulla. J. Neurochem. 36: 1858-1860, 1981.

2. Amy, C.M., and N. Kirshner. Phosphorylation of adrenal medulla cell proteins in conjunction with stimulation of catecholamine secretion. J. Neurochem. 36: 847-854, 1981.

3. Aunis, D., and D. Perrin. Chromaffin granule membranes-F-actin interaction and spectrin-like protein of subcellular granules: A possible relationship. J. Neurochem. 42: 1558-1569, 1984.

4. Baker, P.F., and D.E. Knight. Calcium-dependent exocytosis in bovine adrenal medullary cells with leaky plasma membranes. Nature. 276: 620-622, 1978.

5. Baker, P.F., and D.E. Knight. Calcium control of exocytosis and endocytosis in bovine adrenal medullary cells. Phil. Trans. R. Soc. Lond. (B)296: 83-103, 1981.

6. Bental, M., P.I. Lelkes, J. Scholma, D. Hoeskstra, and J. Wilschut. Ca^{2+}-independent protein-mediated fusion of Chromaffin granule ghosts with liposomes. Biochim. Biophys. Acta. 774: 296-300, 1984.

7. Berridge, M.J. Inositol trisphosphate and diacylglycerol as second messengers. Biochem. J. 220: 345-360, 1984.

8. Brocklehurst, K.W., K. Morita, and H.B. Pollard. Characterization of protein kinase C and its role in catecholamine secretion from bovine adrenal medullary cells. Biochem. J. (in press), 1985.

9. Brocklehurst, K.W., and H.B. Pollard. Enhancement of Ca^{2+}-induced catecholamine release by the phorbol ester TPA in digitonin-permeabilized cultured bovine adrenal chromaffin cells. F. E. B. S. Lett. (in press), 1985.

10. Brooks, J.C., S. Treml, and M. Brooks. Thiosphosphorylation prevents catecholamine secretion by chemically skinned chromaffin cells. Life Sci. 35: 569-574,

11. Burgoyne, R.D., and M.J. Geisow. Specific binding of ^{125}I-calmodulin to and protein phosphorylation in adrenal chromaffin granule membranes. F. E. B. S. Lett. 131: 127-131, 1981.

12. Burridge, K., and J.H. Phillips. Association of actin and myosin with secretory granule membranes. Nature. 254: 526-529, 1975.

13. Corcoran, J.J., and N. Kirshner. Inhibition of calcium uptake, sodium uptake and catecholamine secretion by methoxyverapamil (D600) in primary cultures of adrenal medulla cells. J. Neurochem. 40: 1106-1109, 1983.

14. Creutz, C.E., C.J. Pazoles, and H.B. Pollard. Identification and purification of an adrenal medullary protein (Synexin) that causes calcium-dependent aggregation of isolated chromaffin granules. J. Biol. Chem. 253: 2858-2866, 1978.

15. Creutz, C.E., C.J. Pazoles and H.B. Pollard. Self-association of synexin in the presence of calcium: Correlation with synexin-induced membrane fusion and examination of the structure of synexin aggregates. J. Biol. Chem. 254: 553-558, 1979.

16. Creutz, C.E. Secretory vesicle-cytosol interactions in exocytosis: Isolation by Ca^{2+}- dependent affinity chromatography of proteins that bind to the chromaffin granule membrane. Biochem. Biophys. Res. Comm. 103: 1395-1400, 1981.

17. Creutz, C.E. Cis-unsaturated fatty acids induce the fusion of chromaffin granules aggregated by synexin. J. Cell. Biol. 91: 247-256, 1981.

18. Creutz, C.E., and H.B. Pollard. A cell-free model for protein-lipid interactions in exocytosis. Biophys. J. 37: 119-120, 1981.

19. Creutz, C.E., L.G. Dowling, J.J. Sando, C. Villar-Palasi, J.H. Whipple,

and W.J. Zaks. Characterization of the chromobindins: Soluble proteins that bind to the chromaffin granule membrane in the presence of Ca^{2+}. J. Biol. Chem. 258: 14664-14674, 1983.

20. Daniels, A.J., G. Dean, O.H. Viveros, and E.J. Diliberto Jr. Secretion of newly taken up ascorbic acid by adrenomedullary chromaffin cells orginates from a compartment different from the catecholamine storage vesicle. Molec. Pharm. 23: 437-444, 1983.

21. Douglas, W.W. Secretomotor control of adrenal medullary secretion: synaptic membrane and ionic events in stimulus-secretion coupling. In: Handbook of Physiology, Vol. VL, Section 7, Endocrinology, the Adrenal Gland, edited by H. Blaschko, G. Sayers, and A.D. Smith. Washington D.C.: Am. Physiol. Soc., 1983, p. 367-388.

22. Downes, P., and R.H. Michell. Phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate: Lipids in search of a function. Cell Calcium 3: 467-502, 1982.

23. Dunn, L.A., and R.W. Holz. Catecholamine secretion from digitonin-treated adrenal medullary chromaffin cells. J. Biol. Chem. 258: 4989-4993, 1983.

24. Duzgunes, N.J., Wilschut, R. Fraley, and D. Papahadjopoulos. Studies on the mechanism of membrane fusion. Role of head-group composition in calcium-and magnesium-induced fusion of mixed phospholipid vesicles. Biochim. Biophys. Acta. 642: 182-195, 1981.

25. Fisher, S.K., R.W. Holz, and B.W. Agranoff. Muscarinic receptors in chromaffin cell cultures mediate enhanced phospholipid labeling but not catecholamine secretion. J. Neurochem. 37: 491-497, 1981.

26. Fowler, V.M., and H.B. Pollard. Chromaffin granule membrane-F-actin interactions are calcium sensitive. Nature. 295: 336-339, 1982.

27. Fowler, V.M., and H.B. Pollard. In vitro reconstitution of chromaffin granule-cytoskeleton interactions: Ionic factors influencing the association of F-actin with purified chromaffin granule membranes. J. Cell. Biochem. 18: 295-311, 1982.

28. Frye, R.A., and R.W. Holz. The relationship between arachidonic acid release and catecholamine secretion from cultured bovine adrenal chromaffin cells. J. Neurochem. 43: 146-150, 1984.

29. Geisow, M.J. and R.D. Burgoyne. Recruitment of cytosolic proteins to a secretory granule membrane depends on Ca^{2+}-calmodulin. Nature. 301: 432-435, 1983.

30. Haycock, J.W., J.A. Meligeni, W.F. Bennett, and J.C. Waymire. Phosphorylation and activation of tyrosine hydroxylase mediate the acetylcholine-induced increase in catecholamine biosynthesis in adrenal chromaffin cells. J. Biol. Chem. 257: 12641-12648, 1982.

31. Holz, R.W., R.A. Senter, and R.A. Frye. Relationship between Ca^{2+} uptake and catecholamine secretion in primary dissociated cultures of adrenal medulla. J. Neurochem. 39: 635-646, 1982.

32. Holz, R.W., R.A. Senter, and R.R. Sharp. Evidence that the H^+-electrochemical gradient across membranes of chromaffin granules is not involved in exocytosis. J. Biol. Chem. 258: 7506-7513, 1983.

33. Hong, K., and N. Duzgunes, and D. Papahadjopoulos. Modulation of membrane fusion by calcium binding proteins. Biophys. J. 37: 297-305, 1982.

34. Hotchkiss, A., H.B. Pollard, J. Scott, and J. Axelrod. Release of arachidonic acid from adrenal chromaffin cell cultures during secretion of epinephrine. Fed. Proc. 40: 256, 1981

35. Kilpatrick, D.L., R.J. Slepetis, J.J. Corcoran, and N. Kirshner. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. J. Neurochem. 38: 427-435, 1982.

36. Knight, D.E., and P.F. Baker. Calcium dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. J. Membr. Biol. 68: 107-140, 1982.

37. Knight, D.E., and P.F. Baker. The phorbol ester TPA increases the affinity of exocytosis for calcium in "leaky" adrenal medullary cells. F. E. B. S. Lett. 160: 98-100, 1983.

38. Knight, D.E., and N.T. Kesteven. Evoked transient intracellular free Ca^{++} changes and secretion in isolated bovine adrenal medullary cells. Proc. R. Soc. Lond. 218: 177-199, 1983.

39. Kengsberg, R.L., and J.M. Trifaro. Microinjection of calmodulin antibodies into cultured chromaffin cells blocks catecholamine release in response to stimulation. Neuroscience 14: 335-347, 1985.

40. Kondo, H., J.J. Wolosewick, and G.D. Pappas. The microtrabecular lattice of the adrenal medulla revealed by polyethylene glycol embedding and stereo electron microscopy. J. Neurosci. 2: 57-65, 1982.

41. Kuo, I.C.Y., and C.J. Coffee. Purification and characterization of a Troponin-C-like Protein from Bovine Adrenal Medulla. J. Biol. Chem. 251:

1603-1609, 1976.

42. Levine, M., A. Asher, H.B. Pollard, and O. Zinder. Ascorbic acid and catecholamine secretion in cultured chromaffin cells. J. Biol. Chem. 258: 13111-13115, 1983.

43. Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature. 308: 693-698, 1984.

44. Phillips, J.H. Phosphatidylinositol kinase. A component of the chromaffin-granule membrane. Biochem. J. 136: 579-587, 1973.

45. Pollard, H.B., C.J. Pazoles, C.E. Creutz, and O. Zinder. The chromaffin granule and possible mechanisms of exocytosis. Int. Rev. Cytol. 58: 159-197, 1979.

46. Pollard, H.B., and J.H. Scott. Synhibin: A new calcium dependent membrane-binding protein that inhibits synexin-induced chromaffin granule aggregation and fusion. F. E. B. S. Lett. 150: 201-206, 1982.

47. Pollard, H.B, J.H. Scott, and C.E. Creutz. Inhibition of synexin activity and exocytosis from chromaffin cells by phenothiazine drugs. Biochem. Biophys. Res. Comm. 113: 908-915, 1983.

48. Pollard, H.B., C.J. Pazoles, C.E. Creutz, J.H. Scott, O. Zinder, and A. Hotchkiss. An osmotic mechanism for exocytosis from dissociated chromaffin cells. J. Biol. Chem. 259: 1114-1121, 1984.

49. Rand, R.P.A. Interacting phospholipid bilayers: Measured forces and induced structural changes. Ann. Rev. Biophys. Bioeng. 10: 277-314, 1981.

50. Rebecchi, M.J. and M.C. Gershengorn. Thyroliberin stimulates rapid hydrolysis of phosphatidylinositol 4,5,-bisphosphate by a phosphodiesterase in rat mammotropic pituitary cells. Biochem. J. 216: 287-294, 1983.

51. Schneider, A.S., H.T. Cline, K. Rosenneck, and M. Sonenberg. Stimulus-secretion coupling in isolated adrenal chromaffin cells: calcium channel activation and possible role of cytoskeletal elements. J. Neurochem. 37: 567-575, 1981.

52. Scott, J.H., C.E. Creutz, H.B. Pollard, and R. Ornberg. Synexin binds in a calcium-dependent fashion to oriented chromaffin cell plasma membranes. F. E. B. S. Lett. 180: 17-23, 1985.

53. Sudhof, T.C., J.H. Walker, and J. Obrocki. Calelectrin self-aggregates and promotes membrane aggregation in the presence of calcium. E. M. B. O. J. 1: 1167-1170, 1982.

54. Sudhof, T.C., M. Ebbecke, J.H. Walker, U. Fritsche, and C. Boustead. Isolation of mammalian calelectrins: A new class of ubiquitous Ca^{2+}-regulated proteins. Biochemistry 23: 1103-1109, 1984.

55. Summers, T.A., and C.E. Creutz. Phosphorylation of a chromaffin granule-binding protein by protein kinase C. J. Biol. Chem. 260: 2437-2443, 1985.

56. Trifaro, J.M. The effect of Ca^{++} omission on the secretion of catecholamines-^{32}P and the incorporation of orthophosphate-^{32}P into nucleotides and phospholipids of bovine adrenal medulla during acetylcholine stimulation. Mol. Pharmacol. 5: 424-427, 1969.

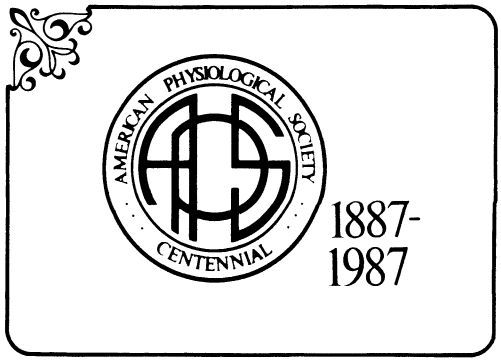
57. Verkleij, A.J. Lipidic intramembranous particles. Biochim. Biophys. Acta. 779: 43-63, 1984.

58. Viveros, O.H., L. Arqueros, R.J. Connett, and N. Kirshner. Mechanism of secretion from the adrenal medulla. Mol. Pharmacol. 5: 69-82, 1969.

59. Walker, J.H. Isolation from cholinergic synapses of a protein that binds to membranes in a calcium dependent manner. J. Neurochem. 39: 815-823, 1982.

60. Wilschut, J., N. Duzgunes, and D. Papahadjopoulos. Calcium/magnesium specificity in membrane fusion: kinetics of aggregation and fusion of phosphatidylserine vesicles and the role of bilayer curvature. Biochemistry 20: 3126-3133, 1981.

61. Wilson, S.P., and N. Kirshner. Calcium-evoked secretion from digitonin-permeablized adrenal medullary chromaffin cells. J. Biol. Chem. 258: 4994-5000, 1983.



INSTRUCTIONS FOR APPLYING FOR APS MEMBERSHIP

CURRENT APPLICATION FORMS

Most issues of The Physiologist routinely carry one copy of the current application form (following). This form will serve for all categories of membership. Any member desiring to sponsor more than one applicant may use a Xerox copy of this form. Any application submitted on an out-dated form will be redone on the acceptable form.

One application form serves all membership categories. There are, however, specific sets of instructions for each category. Therefore it is essential that sponsors and applicants carefully attend to those instructions specific to their desired category.

GENERAL INSTRUCTIONS

FOR ALL CATEGORIES:

Use only the current application form. Check the box indicating the category of membership for which you are applying. Use the SPECIAL INSTRUCTIONS for that category when filling out the form. Type the Application. Fill out all applicable spaces. Only completed applications will be reviewed.

Alien Residents. Alien residents of the U.S. must enter the Alien Registration Receipt Card number under the address block on the application. Canadian residents should furnish a copy of "Landed Immigrant Status" form. Mexican residents should furnish a copy of their form FM-2.

The Bibliography must be submitted in the form found in the Society's journals. An example of the current form is:

JONES, A. B., and C. D. Smith. Effect of organic ions on the neuromuscular junction in the frog. Am. J. Physiol. 220:110-115, 1974.

DO NOT INCLUDE A CURRICULUM VITAE

Send no reprints.

Deadline Dates: Completed applications received between February 1 and July 1 are considered for nomination by the Council at the Fall Meeting. Applications received between July 1 and February 1 are considered for nomination by the Council at the Spring Meeting. Applications are not complete until all materials, including sponsor's letters, are received.

QUALIFICATIONS (Except Students):

The Membership Advisory Committee uses the following five categories in evaluating an application:

1. Educational History. Academic degree and postdoctoral training are evaluated and assessed with regard to how closely the applicant's training has been tied to physiology.

2. Occupational History. Particular emphasis is given to those applicants who have a full time position in a department of physiology, or are responsible for physiology in another department. Relatively high ratings are given to people with positions in clinical departments and to people functioning as independent investigators in commercial or government laboratories.

3. Contributions to the Physiological Literature. This category is of major importance. The applicant's bibliography is evaluated on the basis of publications in major, refereed journals which are concerned with problems judged to be primarily physiological in nature. Emphasis is given to papers published as the result of independent research. Special note is taken of publications on which the applicant is sole author or first author.

4. Interest in and Commitment to Teaching Physiology. This evaluation is based on: (1) the fraction of the applicant's time devoted to teaching, (2) publications related to activities as a teacher including production of educational materials, and (3) special awards or other recognition the applicant has received for outstanding teaching effectiveness.

5. Special Considerations. This category permits the Membership Advisory Committee to acknowledge unique accomplishments of an applicant. These might be excellence in a specific area, or unusual contributions to Physiology resulting from talents, interest or a background substantially different from the average.

In general, persons who qualify for regular membership will have a doctoral degree in physiology or related area and will have published several papers in refereed journals. It should be clear that they have played a major role in some of this research. They should have a position in physiological research, teaching, administration or related area, other than a training position (Council, April 1984).

In general, applicants will be considered for associate membership if they have an advanced degree in physiology or related area and are doing research and/or teaching of physiology (Council, April 1984).

In April 1984, Council adopted: "any student who is actively engaged in physiological work which should lead to an advanced degree in physiology or related area, as attested by two regular members of the Society and who is a resident of North America, can qualify as a student member. No individual may remain in this category for more than five years, without reapplying."

SPONSORS:

Primary responsibility for membership rests with the two sponsors who must be regular members of the Society. Sponsors should discuss the appropriateness of the selected category of membership in this Society with prospective applicants.

Each sponsor should write an independent confidential letter about the candidate using the five categories listed above to evaluate the candidate. Furnish an original and seven copies to the Membership Secretary.

CHECK LIST:

1. Original copy of application signed by both sponsors.
2. Application on a current form, including the bibliography (1 original and 7 copies).
3. Mail the original, which has been signed by the two sponsors, plus 7 copies to:

   Membership Secretary
   American Physiological Society
   9650 Rockville Pike
   Bethesda, Maryland 20814

SPECIAL INFORMATION AND INSTRUCTIONS

FOR REGULAR MEMBERSHIP

Bylaws of the Society:

Article III, Section 2 - Regular Members. Any person who has conducted and published meritorious original research in physiology and who is a resident of North America shall be eligible for proposal for regular membership in the Society.

Duties and Privileges:

1. Hold Elective Office.
2. Vote at Society Meetings.
3. Serve on Committees, Boards and task forces.
4. Serve on Federation Boards and Committees.
5. Sponsor New Members.
6. Can present orally only one contributed paper, but, may co-author and/or sponsor more than one contributed paper by a non-member at the Spring (FASEB) and the Fall Meetings of the Society.
7. Receive The Physiologist.
8. Receive Federation Proceedings, Public Affairs Newsletters and annual Membership Directory.
9. Subscribe to handbooks and periodicals published by the Society at membership rates.
10. Register to attend scientific meetings of the Federation and the APS Fall meeting at membership rates.
11. Participate in FASEB Member's Life Insurance Program, Disability Program and in Hospital Protection Plan. (For Residents of the United States, its territories or possessions).
12. Eligible to receive the Daggs Award.
13. Eligible to be selected as Bowditch Lecturer (Members under 40 years of age).

FOR CORRESPONDING MEMBERSHIP

Bylaws of the Society:

Article III, Section 3 - Corresponding Members. Any person who has conducted and published meritorious research in physiology, who is presently engaged in physiological work and who resides outside of North America shall be eligible for proposal for corresponding membership in the Society.

Duties and Privileges:

1. Serve on Society Committees, Boards and Task Forces.
2. Serve as one sponsor of new Corresponding Members (One regular member must be the other sponsor of a new Corresponding Member).
3. Can present orally only one contributed paper, but, may co-author and/or sponsor more than one contributed paper by a non-member at the Spring (FASEB) and the Fall Meetings of the Society.
4. Receive The Physiologist.
5. Receive Federation Proceedings, and annual Membership Directory.
6. Subscribe to handbooks and periodicals published by the Society at membership rates.
7. Register to attend scientific meetings of the Federation and the APS Fall meeting at membership rates.

FOR ASSOCIATE MEMBERSHIP

Bylaws of the Society:

Article III, Section 5 - Associate Members. Persons who are engaged in research in physiology or related fields and/or teaching physiology shall be eligible for proposal for associate membership in the Society provided they are residents of North America. Associate members may later be proposed for regular membership.

Duties and Privileges:

Same as for Regular Members except for the privileges of:

1. Holding Executive Office, or membership on certain committees.
2. Voting at Society Meetings.
3. Sponsoring New Members.
4. Eligibility for receiving the Daggs Award.
5. Privilege of selection as Bowditch Lecturer.
6. May sponsor only those abstracts on which they are listed as first author or co-author.

FOR STUDENT MEMBERSHIP

Not all questions on the application form may be appropriate – Please place NA next to any such questions.

Bylaws of the Society:

Article III, Section 7 - Student Members. Any student who is actively engaged in physiological work as attested by two regular members of the Society and who is a resident of North America. No individual may remain in this category for more than five years, without reapplying.

Duties and Privileges:

1. Present one contributed paper at the Spring (FASEB) and the Fall scientific meeting with the endorsement of the student's advisor.
2. Receive The Physiologist.
3. Subscribe to handbooks and periodicals at member rates.
4. Register to attend scientific meetings of the Federation and the APS Fall meeting at student rates.

Submit original and 7 copies of application and supporting documents.

APPLICANT'S LAST NAME _____

Date _____

THE AMERICAN PHYSIOLOGICAL SOCIETY
9650 Rockville Pike, Bethesda, MD 20814

MEMBERSHIP APPLICATION FOR:

- REGULAR ☐
- CORRESPONDING ☐
- ASSOCIATE ☐
- STUDENT ☐

CURRENT MEMBERSHIP
CATEGORY; YEAR ELECTED _____

See Instructions

Name of Applicant: _____
First Middle Last

Mailing _____ Birth Date: _____

Address _____ Citizenship: _____

_____ Country of Permanent Residence:* _____

_____ Telephone No.: _____

* Alien residents of Canada and Mexico see General Instructions. Alien residents of U.S. enter Alien Registration Receipt Card number _____.

1. EDUCATIONAL HISTORY

Dates	Degree	Institution	Major Field	Advisor

Doctoral Dissertation Title:
(if any)

Postdoctoral Research Topic:

2. OCCUPATIONAL HISTORY

Present Position:

Prior Positions:

Dates	Title	Institution	Department	Supervisor

SPONSORS

#1. Name: _____ #2. Name: _____

Mailing Address: _____ Mailing Address: _____

_____ _____

_____ _____

_____ _____

Telephone No. Zip Code Telephone No. Zip Code

I have read the guidelines for applicants and sponsors and this application and attest that the applicant is qualified for membership.

#1 Signature _____ #2 Signature _____

Each sponsor must submit an original and 7 copies of a confidential letter of recommendation to the Society, under separate cover.

APPLICANT'S LAST NAME _____

3. **DESCRIBE YOUR PHYSIOLOGICAL TEACHING** – What percent of your time/effort is spent in teaching Physiology?_____

Describe in the space provided your teaching of physiology including course descriptions (content, format); supervision of pre-doctoral and post-doctoral students; special contributions (films, textbooks, etc.).

4. **INTEREST IN THE SOCIETY** – List any APS Meetings attended by date and check the appropriate box for any papers.

SPRING (FASEB)			FALL (APS)		
Date	Presented	Coauthor	Date	Presented	Coauthor
_____	☐	☐	_____	☐	☐
_____	☐	☐	_____	☐	☐
_____	☐	☐	_____	☐	☐
_____	☐	☐	_____	☐	☐
_____	☐	☐	_____	☐	☐

List other scientific societies of which candidate is a member:

In the space provided state your interest in wanting to join the Society:

5. **SPECIAL CONSIDERATION** – Include any other contributions (Administrative, university, national service, awards and honors) that may be important to physiology.

6. **DESCRIBE YOUR RESEARCH** – What percent of your time/effort is spent in research?_____

Describe the fundamental physiologic questions in your research and how you have answered these questions. Limit the paragraph to the space provided.

7. **BIBLIOGRAPHY** – Attach a list of your publications under the following categories:
   1. Complete physiological papers, published or accepted for publication.
   2. Physiological abstracts (limit to ½ page).
   3. Other papers not primarily physiological (limit to ½ page).

The entire bibliography should not exceed 2 pages. Give complete titles and journal references with inclusive pagination. Use the bibliographic form found in the Society's journals. List authors in the order in which they appear in the publication.

DO NOT INCLUDE A CURRICULUM VITAE

36th Annual Fall Meeting of the American Physiological Society

Niagara Hilton Hotel
Buffalo, New York

October 13-18, 1985

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.

Special Events

Satellite Symposium on Environmental Physiology in Honor of Hermann Rahn

Friday and Saturday, October 11 and 12
Hyatt Regency, Buffalo
Organizers: L. Farhi and C. Paganelli

American Society for Gravitational and Space Biology Business Meeting

Monday, October 14, 5:00 PM–8:00 PM
Niagara Hilton, Rainbow Ballroom North

APS Business Meeting

Wednesday, October 16, 11:00 AM–12:00 Noon
Convention Center, Ballroom 1

Bowditch Lecture

Wednesday, October 16, 4:45 PM
Convention Center, Ballroom 2
Topic: Physiology of the Circadian Timing System
Speaker: Martin C. Moore-Ede
Reception: Niagara Hilton, Rainbow Ballroom, 6:15 PM

Special Session

Use of Space Station for Biologic Research
Thursday, October 17, 7:30 PM–10:30 PM
Niagara Hilton, Rainbow Ballroom South

Evening Social Events

Opening Reception
Monday, October 14, 8:00 PM–10:00 PM
Niagara Hilton, Rainbow Ballroom

*Canadian Physiological Society Social**
Tuesday, October 15, 5:00 PM–6:30 PM
Niagara Hilton, Junior Suite 334
(*Open to members of CPS and their guests)

APS Past President's Address and Society Banquet
Tuesday, October 15, 6:45 PM
Niagara Hilton, Rainbow Ballroom
Topic: Physiology with Backpack
Speaker: John B. West

Refresher Course, Symposia, Tutorial and Workshop Sessions

Monday AM

Refresher Course
- Exercise physiology and its clinical applications. Session I

Workshop
- Comparison of animal models and true weightlessness

Monday PM

Refresher Course
- Exercise physiology and its clinical applications. Session II

Workshops
- Gravitational effects on animal development
- Vestibular problems in space

Tuesday AM

Tutorials
- The physiology of smooth muscle
- Development of the neuromuscular junction
- The adenohypophysis

Symposia
- Oxygen radical damage to lung tissue. Session I
- Functional activity and the plasticity of neurons
- Hormonal, physiologic, and clinical studies of factors affecting heat production during malignant hyperthermia (MH). Session I
- Current concepts in gravitational physiology

Tuesday PM

Tutorials
- Control system analysis of mechanisms of renal autoregulation
- Patch clamp and single channel recording
- Ultrastructural and functional relationships in transporting and secretory epithelia
- Frog tectum
- Physiologic efficiency

Symposia
- Oxygen radical damage to lung tissue. Session II
- Kinetics of muscular contraction
- Hormonal, physiologic, and clinical studies of factors affecting heat production during malignant hyperthermia (MH). Session II
- Fluid and electrolyte balance and cardiovascular function in response to weightlessness

Wednesday AM

Tutorial
- A tribute to Sid Robinson: Pioneer in environmental physiology

Wednesday PM

Symposium
- William Beaumont's World

Thursday AM

Tutorials
- Role of plasma sodium in the control of aldosterone secretion
- Importance of the angiotensin II receptor as a modulator of aldosterone secretion
- Functional significance of aldosterone
- Physiologic response to chronic centrifugation
- Comparative physiology of animal response to hyperbaria
- Circadian rhythms

Symposia
- Pleural space structure and function
- Neurobiology of mammalian magnocellular neurosecretory neurons
- Membrane signaling and the cytoskeleton

Thursday PM

Tutorials
- Synaptic transmission
- Limbic-motor integration
- Thermosensitive neurons

Symposia
- Renal functional derangements in hypertension
- Receptors: Their role in health and disease
- Gravireception in plants and animals

Thursday Evening

Special session:
- Use of space station for biological research

Friday AM

Tutorials
- Respiratory muscles: Functions and limits
- Exercise, respiratory heat loss, and bronchoconstriction
- Mechanisms for protection from hypoxia and hypothermia in animals

Symposia
- Renal reflexes
- Physiologic functions in conscious behaving animals
- Cellular and membrane function at high pressure

Sessions and Symposia with Associated Abstracts

4.1

EARLY MAMMALIAN DEVELOPMENT UNDER CONDITIONS OF REORIENTATION RELATIVE TO THE GRAVITY VECTOR. Debra J. Wolgemuth and George Grills. Columbia Univ. Coll. Phys. & Surg., NY, NY 10032.

The effects of altered gravitational states on critical stages of early mammalian development--meiosis, fertilization, and early embryogenesis--are being studied with in vitro cell culture under conditions of clinostat rotation. Initial studies focused on progression of mouse oocytes through meiotic maturation. Oocytes rotated perpendicular to the gravity vector at ¼,1,10, or 30 RPM underwent germinal vesicle breakdown and achieved metaphase II (MII) at a rate comparable to static and vertically rotated control cultures. At 100 RPM the experimentally rotated cultures exhibited a decreased progression to MII suggesting that chromosome movement is impaired under clinostat rotation conditions. A second stage of mammalian development, fertilization, was then investigated. Ova were placed with capacitated sperm and rotated at ¼ and 100 RPM. After culture, cells were observed for any gross morphological changes, fixed, stained and scored for normal pronucleus and polar body formation. Rates of normal fertilization were similar among control and experimental cultures. No increase in abnormalities of fertilization, such as polyspermy or parthenogenetic activation, was observed. Recently, a third stage of mammalian development, early embryogenesis, is under investigation. A modification of the culture system now permits immobilization of the embryos at the center of the axis of rotation. Two-cell embryos are being cultured at varying rotation rates and will be examined for timely embryonic progression and cell-cell orientation.

4.2

GROWTH AND DIFFERENTIATION OF MAMMALIAN EMBRYONIC TISSUES EXPOSED TO HYPERGRAVITY IN VIVO AND IN VITRO. Pauline Jackie Duke* and Liliana Janer* (SPON: Dr. John Simpson), Univ. of Texas Dental Branch, Houston, TX, 77225.

Suppression of in vitro morphogenesis in embryonic mouse limbs exposed to excess G was previously demonstrated using a morphogenetic scoring system (Teratol. 27, 427, 1983). Recently the cartilage area of these limbs was determined using a Zeiss Interactive Digital Analysis System (ZIDAS). Centrifuged (2.6G) limbs were shown to have significantly less cartilage than control (1G) limbs, especially in the paw region. Form factor analysis showed that the main effect was reduction in length of the element. Mouse palates exposed to excess G in vitro were analyzed by light and electron microscopy, and interactive image analysis and found to be in more advanced stages of fusion than control palates. In vivo studies were carried out using a small animal centrifuge. Four week old female mice exposed to excess gravities of 1.8 - 3.5G for 8 weeks weighed significantly less than controls. After a 4-5 week adaptation period, the mice were mated and then sacrificed on gestational day 12 or 18. Centrifuged mice had fewer pregnancies and crown rump lengths of centrifuged embryos were significantly less than those of controls. Centrifuged embryos also appeared to be of a younger gestational age than controls. These studies show that changes in gravity are able to alter development in vivo and in vitro and that some of gravity's teratogenicity may be due to effects at the cellular level.
Supported by NASA Grant NAG-2-332.

4.3

SIMULATED HYPOGRAVITY AND SYNAPTOGENESIS IN CULTURE. Raphael Gruener. Depts. of Physiology. Schools of Medicine, Universities of Arizona and Maryland (85724, 21201).

To survive in space, biosystem development must occur in microgravity. Since organ and cell functions are altered even during short flights, prolonged hypogravity may change cell development. Clinostat rotation was used to examine how simulated hypogravity affects Xenopus neurons and muscle cell development in culture. Cells were rotated at 1-50 rpm with the substratum turning through the horizontal plane. Vertical rotation had no effect and served as a motional and vibrational control. Cells were most sensitive to rotation at 1-10 RPM. Changes included: increases in myoctye surface area; decrease in yolk platelet disappearance and delay in appearance of striations; nuclear enlargement; and a reduction in neurite width and length with frequent appearance of varicosities. Spontaneous and nerve-induced aggregates of acetylcholine receptors appeared disorganized and reduced in size. Results indicate retardation of cell maturation by microgravity. If according to Bornens (Biol. Cell.35:115, 1979) the centriole is a gyroscopic oscillator, it could be the cellular gravisensor. As the centriole controls growth, movement and microtubular nucleation, it is possible that gravity would influence cell development which depends on these functions.
Generously supported by NASA. Special thanks to Dr. T. Halstead for continued concern and encouragement.

4.4

EFFECTS OF FETAL PERIOD NULL-GRAVITY EXPOSURE ON WISTAR RATS DURING THE FLIGHT OF COSMOS 1514. J.R.Keefe, I.B. Krasnov*, J.R.Alberts and L.V. Serova* Case Western Reserve University, Cleveland, OH,44106, Indiana University,Bloomington,IN 47405, Institute of Biomedical Problems, Moscow, U.S.S.R.

The first successful flight and recovery of pregnant Wistar rats, flown on the Soviet biosatellite COSMOS 1514 during the relatively stable mid-fetal period of E13-E18, revealed an effect of nearearth spaceflight on the normal developmental progression of neuronal maturation in three developmental aspects: (1) comparative overall systemic immaturity of all flighted specimens (24-36 hours delayed), evident in ocular, vestibular and cortical structures; (2) presence of abnormal, arrested mitotic figures in neuronal regions displaying high levels of neuroblast generation and migration along radial sustentacular elements; and (3) volumetric disturbances reflected in ocular, vestibular, cochlear and ventricular cavities. The deficits were no longer evident in newborns derived from the flighted dams that had undergone five days of readaptation to earth normal gravity prior to birthing.

4.5

EARLY POSTNATAL DEVELOPMENT OF RATS GESTATED DURING FLIGHT OF COSMOS 1514. J. Alberts*, L. Serova#, J. Keefe+, Z. Apanasenko# (SPON. T. Halstead) *Star Enterprises/Indiana Univ., Bloomington, IN 47405; #Inst. for Biomedical Problems, Moscow, USSR; +Case Western Reserve Univ.

Tactile, vestibular, olfactory, auditory, and visual function were evaluated postnatally in rat pups gestated in space (Embryonic Days 13-18) during the flight of Cosmos 1514. Maternal behavior of their dams was assessed to provide an interpretive context of the developmental consequences of exposure to microgravity. Parallel tests were conducted on rats from Vivarium and Synchronous control groups. Maternal behavior was adequate, but there were indices of maternal stress and altered energy metabolism in the dams. Sensory function was intact in each modality at the appropriate developmental stage, although Flight and Synchronous pups were smaller and grew slowly. Flighted pups showed a deficit in high frequency (40 kHz) auditory function. Male newborns in the Flight and Synchronous groups showed evidence of demasculinization, related to non-specific prenatal stress. Supported by NASA Contract NAS2-11533.

4.6

GROWTH AND DEVELOPMENT OF MICE AND RATS CONCEIVED DURING EXPOSURE TO CHRONIC CENTRIFUGATION UNDER GRADED HYPER-G INTENSITIES. J. Oyama, L. Solgaard*, J. Corrales*, and C.B. Monson*. Ames Research Center, NASA, Moffett Field, CA 94035.

Previous studies have shown that reproduction and growth are impaired by exposing animals to hyper-G intensities higher than 2G. In this study, prenatal and postnatal growth rates were measured in Swiss-Webster mice and Sprague-Dawley rats conceived under moderate G-intensities ranging from 1.27G up to 2.03G. Parent animals were fully adapted to each test G-level before being mated. Fetal growth of rats at 2.03G was not impaired although a significant decrease in lung/body mass ratios was found. Body masses at birth of hyper-G rats were generally smaller than 1G controls but showed no relationship to the G-intensity. Growth rates of rats during the suckling period and after weaning showed a substantial decrease at 2.03G while lower hyper-G intensities produced either small changes or no changes at all. Hyper-G effects on male rats were greater than on females. Growth effects of mice to hyper-G were generally similar to those of rats. Differences between the two species were found in their body size attained at maturity under hyper-G conditions vs 1G. Mice showed no significant differences in mature body size with different G-intensities while rats showed a G-effect. Results of the study demonstrate the scaling effects of body size on growth and development of animals to graded hyper-G exposures.

4.7

AN INVESTIGATION OF GRAVITATIONAL EFFECTS ON EARLY DEVELOPMENT IN A MARSUPIAL MODEL. William Jurgelski* (Spon: Jiro Oyama) NIH, Research Triangle Park, NC 27709

Sminthopsis sp. are mouse-like marsupials that can be maintained, bred and handled like the laboratory mouse. In these animals, embryogenesis is combined with precocious development of several organs and tissues within a 12 day gestation under support of a yolk sac placenta. Organogenesis is confined to the final four days of the gestation. At birth, marsupial mice are equivalent in specific aspects of development to the laboratory mouse at 12 days gestation. Fetal development is completed postnatally within the pouch where the nursing young are involuntarily attached to the teats for 40 days. For studies of gravitational effects, these adaptations provide advantages over rodent models in the number and magnitude of developmental processes that can be accommodated within the duration of a shuttle mission, the ability to distinguish between maternal and gravitational effects, and the assurance of maternal care to nursing young. In proposed experiments, marsupial mice at embryonic and fetal stages and at birth will be exposed to ground-based hyper-G and to micro-G in the middeck locker. Selected structural/functional comparisons between these animals and appropriate controls will test the hypothesis that major alterations in gravitational input during critical stages of development induce detectable morphologic and/or behavioral changes. Observation of birth in these experiments will reveal whether, as hypothesized, the newborn marsupial uses gravity sensing to locate the pouch.

4.8

MORPHOGENESIS OF THE OTOLITHS IN THE CHICK (Gallus domesticus) EMBRYO. C.D. Fermin* and M. Igarashi* (SPON: R.L. Vick). Dept. of Otorhinolaryngology and Communicative Sciences. Baylor College of Medicine. Houston, Tx. 77030

The origin and calcification of vertebrate otoconia is being investigated in the chick, because previous work on this species has shown the usefulness of the chick inner ear for this type of study[1-4].

Light and transmission electron microscopy (TEM) and histochemistry allowed us to identify two important aspects of otoconia formation that up to this point were unclear: (1) segmentation of the immature otolithic membrane into individual primitive uncalcified otoconia, and (2) incorporation of calcic components into the organic matrix by means of electron dense granules (20-150 nm diameter). To our knowledge, these findings constitute the first TEM demonstration of how each otoconium comes to exist, and how calcic components are incorporated into the organic matrix of each otoconium. These results will be presented and their relationship to previous work on otoconia morphogenesis in this and other species will be discussed. (Supported by NASA grant NAG 2-342; USPHS RR-05425 and NS 10940).

1. Fermin, C. & Igarashi, M. (1983) 41st Proc. EMSA, 572-73.
2. Fermin, C. & Cohen, G. (1984) Acta Otolaryngol.,97:39-51.
3. Fermin, C. & Cohen, G. (1984) Acta Otolaryngol.,98:42-52.
4. Fermin, C. & Igarashi, M. (1985) Acta Anatom., In Press.

4.9

CLINOSTAT STUDIES ON FERTILIZATION, DEVELOPMENT AND SPICULE FORMATION IN SEA URCHINS. Gerald Schatten, Christopher Stroud,* Calvin Simerly and Heide Schatten, Department of Biological Science, Florida State University, Tallahassee, FL 32306-3050.

Gravitational effects on fertilization and embryogenesis in sea urchins are being assessed by clinostat investigations. Freshly inseminated eggs are incubated in 40 ul or 100 ul droplets in sterile plastic dishes. Dishes are cultured in a stationary position or are rotated in either vertical or horizontal axes. Rotational speeds ranging from 1/4 rpm to 60 rpm are to be tested. Unlike amphibian eggs which are affixed to the substrate, sea urchin eggs are spawned into open water. It is predicted that fertilization and the first cleavages will not be under the influence of gravity since these eggs normally develop in suspension. However after hatching, the sea urchin larvae locomote by ciliary activity and might well orient themselves along a particular axis in relation to gravity. In particular the effects of gravity on the deposition of calcium in the sea urchin spicules will be investigated. Supported by NASA.

4.10

RESPONSE OF AMPHIBIAN EGG NON-YOLK CYTOPLASM TO GRAVITY ORIENTATION. A.W. Neff, R.C. Smith*, and G. M. Malacinski. Medical Sciences Program and Department of Biology, Indiana University, Bloomington, Indiana 47405.

The amphibian egg cytoplasm appears to be compartmentalized by the localization of yolk platelets. The movements of these cytoplasmic compartments in fertile eggs under 1 g and gravity orientation conditions can be correlated with early pattern specification. Although yolk platelets provide a convenient cytological marker they probably do not play a direct role in bilateral symmetrization. The following questions will be posed: Are there localized (compartmentalized) cytoplasmic non-yolk components? What is the response of these non-yolk cytoplasmic components to gravity orientation? Preliminary results on the compartmentalization of germ plasm and several vegetal-specific non-yolk cytoplasmic proteins at 1 g and in inverted eggs will be presented. Research Supported by NASA grants NAGW-60 and NAG 2 323.

4.11

AMPHIBIAN FERTILIZATION AND DEVELOPMENT IN MICROGRAVITY. Kenneth A. Souza* (Spon: J. Oyama) NASA-Ames Research Center, Moffett Field, CA 94035.

For more than a century embryologists have attempted to determine the influence and role of gravity in amphibian embryogenesis. Past efforts have always been hampered by the omnipresence of gravity. In the late sixties and early seventies, both US and Soviet scientists exposed fertilized amphibian eggs to microgravity and found that it did not seem to affect cell division and differentiation. Unfortunately, due to the constraints of those early unmanned missions, fertilization and the many cellular events preceding first cell division, occurred under normal terrestrial conditions. To extend the early spaceflight investigations, an experiment will fly aboard the Space Shuttle in 1987 or 1988. Four adult female frogs, Xenopus laevis, will be flown and ovulation will be induced with Human Chorionic Gonadotropin injected by crew members. Eggs will be fertilized and incubated at microgravity or artificial terrestrial gravity using a rotating centrifuge. Dorsal/ventral axis formation relative to sperm entry site will be correlated and various stages of development will be fixed. Scientific hypotheses will be discussed and the flight protocol and hardware described.

10.0

COMPARATIVE ANALYSIS OF THE EFFECTS OF HYPO- AND HYPERGRAVITY ON PRENATAL DEVELOPMENT IN THE RAT. L. Serova. Inst. of Biomedical Problems, Moscow.

No abstract submitted

10.1

SPACEFLIGHT AND BONE DYNAMICS. Emily Morey-Holton and B. Arnaud. NASA-Ames Research Center, Moffett Field, CA. 94035

Existing data in both humans and rats are insufficient to predict long term influence of spaceflight on bone dynamics. Concepts of spaceflight and bone dynamics are based on Skylab data, results from Salyut-6 crewmembers, and data from the Cosmos series. Bone loss in crewmen follows hypercalciuria and slight hypercalcemia. Salyut-6-Soyuz results suggest that bone loss may not be related to the duration of flight; individual variation in all flight studies is dramatic (1-20%). Intense conditioning of crews may have prevented predicted changes in bone dynamics during flight, but the changes could also be related to skeletal metabolism and/or responses to microgravity peculiar to each crewmember. Data from adolescent animals in the Cosmos series suggest that bone formation rather than bone resorption may be impaired in these rats; changes in strength, progenitor cell population, and growth have been found in almost every bone examined. However, factors other than microgravity may contribute to these bone changes. If disuse osteoporosis is a model for alterations in bone dynamics during spaceflight, then alterations in calcium homeostasis and bone are transient and occur in three phases (an acute phase, a transitional phase, and a chronic phase). However, if disuse osteoporosis is not a model for such changes during flight, then bone changes may not be transient. We can only learn the natural sequence of bone changes during spaceflight when data are obtained from humans or animals uncompromised by exercise or other factors.

10.2

RESPONSES OF SKELETAL MUSCLE TO UNLOADING-A REVIEW. Marc E. Tischler, Stephen R. Jaspers*, Erik J. Henriksen* and Stephan Jacob*. Univ. of Arizona, Tucson, AZ 85724

Use of suspension models have permitted the study of muscle response to reduced activity without the constraints of total limb immobilization. During 6 days of tail-casting, the soleus (SOL) atrophies while the extensor digitorum longus grows normally. After discounting those changes in both muscles due primarily to increased secretion of adrenal hormones, the following conclusions regarding the specific responses of the SOL could be drawn. 1) Atrophy is probably due primarily to increased protein degradation. 2) Decreased synthesis of glutamine may result from reduced availability of ammonia due to diminished use of ATP. 3) Greater muscle glycogen seems to reflect an increased response to insulin of glucose uptake which leads to greater glucose metabolism. 4) Faster catabolism of branched-chain amino acids can be attributed to enhanced flux through ketoacid dehydrogenase. Studies by others with tail-cast suspended rats showed in the SOL: 1) a gradual switch from type I to type II fibers, 2) increased acid protease activity and 3) altered muscle function and contractile duration. Using harness suspended rats, others showed in the SOL: 1) signficant atrophy, 2) increased numbers of glucocorticoid receptors and 3) no change in muscle fatigability. (Supported by NASA grant NAGW-227. M.E.Tischler is an Established Investigator of the American Heart Association.)

10.3

SKELETAL MASS CHANGE AS A FUNCTION OF GRAVITATIONAL LOADING. Nello Pace, Donald F. Rahlmann and Arthur H. Smith. Univ. of California, Berkeley CA 94720

It has long been recognized, in accordance with Wolff's Law, that the skeletal system is responsive to changes in the loading imposed on it. We have examined the elemental composition of the body after 6 weeks of chronic centrifugation at 2.0*g* in 4 species of metabolically mature animals, the hamster, rat, guinea pig and rabbit, which represent a body mass range of 0.15 to 3.5 kg. Six animals of each species were killed immediately after centrifugation, and 6 were killed 4 weeks after cessation of centrifugation to serve as a control. Body water and fat contents of each animal were measured by lyophilization and petroleum ether extraction, respectively, and the dried, defatted residue was homogeneously comminuted for elemental analysis. Body calcium, phosphorus, sodium and magnesium contents as per cent of fat-free body mass were significantly greater in the animals immediately after centrifugation than in the control animals 4 weeks after centrifugation. Based upon Neuman's empirical formula for the composition of bone mineral the results indicate an increase of about 15% in bone mineral mass after 6 weeks at 2.0*g*. This may be compared to the decrease of about 15% in bone mineral mass estimated by the same procedures in rats after 18.5 days in weightlessness in Cosmos 1129. It is postulated, therefore, that the body bone mineral mass responds linearly to gravitational loading in the range of 0 to 2*g*.

This work was supported under NASA grant NSG-7336.

10.4

RESPONSE OF RAPID DYNAMIC LOADING TO THE HUMAN HEAD. Werner Goldsmith*(SPON: N. Pace). Univ. Calif., Berkeley CA 94720

Whole-body accelerations produced by centrifugal loading, orbital motions or external vibrations induce effects in animals ranging from insignificant to fatal and in humans from none to traumatic. Similarly, direct impact or impulsive loading via torso and neck to the human head can also generate a complete spectrum ranging from no influence to death, with intermediate stages of performance deterioration and minor, major and catastrophically irreversible injury. For nonpenetrating head impact, a tolerance criterion and its offshoots are described based on cadaver drop tests in which skull fracture is equated to the onset of concussion, supplemented by animal and human volunteer experiments, and extrapolation to man. A second criterion based on a mechanical head model, cadaver head resonance, mechanical and physiological impact response of primates and scaling to living humans is also presented. Both criteria are based on linear acceleration levels and durations; the model also gives an injury level association.

A similar head injury criterion presented for rotational head motion primarily due to torso loading as in a whiplash. The linear acceleration criteria are applied to certain forms of sports where the permitted tolerance is greatly exceeded, as in boxing or possibly football. Finally, the head loading on a tank gunner due to gun recoil is presented as an example of a non-injurious condition where, however, the acceleration level and possibly its duration can result in serious performance deterioration, with potentially dire consequences.

10.5

IS SPACE SICKNESS A FORM OF MOTION SICKNESS? Herbert L. Borison. Inst. Brain Stem Studies, Dept. Pharmacol. Toxicol., Dartmouth Medical School, Hanover NH 03756, U.S.A.

My purpose in this review paper is to examine the characteristics of space sickness for similarities and differences by comparison with those of terrestrial motion sickness. Attention is given to (1) the adequate stimulus, (2) the response pattern, and (3) the reflex mechanism. Gaps in our knowledge are emphasized. Based on these considerations, spaceflight experiments are recommended to resolve the problem.

10.6

MALADAPTATION (SPACE SICKNESS) AND ADAPTATION TO MICROGRAVITY.
A.J. Benson. RAF Institute of Aviation Medicine, Farnborough, Hants. GU14 6SZ. UK.

The syndrome of space sickness has many features in common with that of terrestial motion sickness and it is now generally accepted that the malady is caused by the disparity of motion information signalled by the eyes, and the vestibular apparatus (and perhaps other mechanoreceptors) being at variance with the sensory information that is expected from past experience. The progressive decline in the incidence of space sickness during the first few days in the atypical force environment of space flight is a manifestation of the adaption that is a normal response to prolonged exposure to an unfamiliar motion environment. In microgravity, adaptive changes in the processing of otolithic signals are to be expected because the otoliths no longer carry information related to head position though they will be stimulated, albeit in an atypical manner, by voluntary head movements. Experiments conducted on Spacelab-1 astronauts have demonstrated that post-flight there was a reduction in ocular counter-rolling and impaired judgement of the vertical during body-tilt, and a greater deviation of the apparent vertical during optokinetic stimulation. However, post-flight responses to dynamic otolithic stimuli were found, in some tests, to be enhanced. These findings indicate that one feature of adaptation to microgravity is a decrement in the utilisation, or 'weighting', by the CNS of low frequency gravireceptor information.

MECHANICS OF BREATHING; STRUCTURE AND AIRWAY FUNCTION

11.1

EFFECT OF FLOW DIRECTION ON COLLATERAL VENTILATION IN EXCISED DOG LUNG LOBES.
L.E. Olson and P.A. Socha* Ohio State University, Columbus, OH 43210

Pressure-flow characteristics of a collaterally ventilating dog lung segment were evaluated in 5 cranial and 4 caudal excised dog lung lobes. A double lumen catheter was wedged in a subsegmental bronchus and pressure in the subtending lung segment (Ps) recorded as SF_6, He or N_2 was passed through the segment when the lobe contained air. Gas was then pulled through the segment by ventilating the lobe with the test gas and lowering Ps relative to airway opening pressure (Pao). Driving pressures (Ps-Pao = |0.25 - 2.0| cm H_2O) were evaluated at Pao = 5, 10 and 15 cm H_2O. Results were identical for cranial and caudal lobes. Flow was a strong function of Pao, Ps-Pao and test gas and the effects of Ps-Pao, test gas and flow direction were modified by Pao. Flow regime and alterations in segment geometry were assessed by plotting log normalized pressure against log Reynolds number. At Pao-15 and 10 cm H_2O, flow pattern and segment geometry appeared independent of flow direction or Ps-Pao. At Pao=5 cm H_2O flow pattern was unaffected by direction and segment dimensions appeared to increase as gas was pushed through the segment and decrease as gas was pulled from the segment, suggesting that only at a lower lung volume where both parenchyma and airways are more compliant, are segment dimensions increased by increases in transmural pressure and decreased by decreases in transmural pressure. (ALA of MN and BSRG RR05465-21)

11.2

THE EFFECT OF LUNG VOLUME AND AIRFLOW ON THE FREQUENCY SPECTRUM OF VESICULAR LUNG SOUNDS.
S.S. Kraman, V.A. Medical Center & the Univ. of Kentucky School of Med., Lexington. Kentucky. 40511

Despite recent interest in lung sound physiology, the effects of lung volume and airflow on the vesicular lung sound frequency spectrum have not been defined. It was the purpose of the present study to do so. 9 healthy subjects were studied. All were healthy young nonsmokers. The dependent variables were the points that divide the power spectrum of the vesicular lung sound into quarters (1st, 2nd and 3rd quartiles [Q1, Q2 & Q3]). Recording sites were the upper anterior (RUL) and lower posterior (RLL) right chest. To assess the effect of volume, lung sounds were recorded during an inspiratory vital capacity (VC) maneuver at near constant airflow rates. The frequency spectral parameters were determined at each sixth of the VC. To assess airflow, 5 of the subjects breathed from resting lung volume at inspiratory airflows of 1 to 2.5 L/s for a total of 12 breaths each and the frequency parameters of the lung sounds occurring during peak inspiratory airflow were determined. RESULTS: Volume, RLL.- Q1, Q2 & Q3 were independent of volume except near TLC. Volume, RUL.- There was a small but significant decrease in all three parameters with increasing lung volume. This was predominantly due to data acquired at the extremes of the VC. Airflow- All parameters were independent of airflow except for a weakly positive relationship ($r=0.285$, $P<0.05$) for Q3 at the RUL location. These results suggest that the frequency composition of the vesicular lung sound in healthy adults is minimally affected by changes in lung volume or airflow.

11.3

FUNCTIONAL VERSUS ANATOMIC DYSANAPSIS OF LUNGS AND AIRWAYS.
R. Castile, T. Wheeler*, J. Mead, H. Feldman*. Children's Hospital and Harvard School of Public Health, Boston, Ma. 02115.

Based on functional measurements of maximal flows and lung volumes, Green et al. (J. Appl. Physiol. 37:67,1974) suggested that increases in airway and lung parenchymal size during growth were unrelated (dysanapsis). To test this hypothesis anatomically, we made measurements of central airway and lung size in 36 normal adult males from chest x-rays taken at RV and TLC. Lung volumes, air and He_{O_2} flow-volume curves, static pressure-volume curves, and quiet breathing resistances were also obtained by standard plethysmographic techniques. On PA chest films, lung area (LA) was measured planimetrically. Mean intrathoracic tracheal diameter (TD) and bronchial diameters (BD) were also measured. LA correlated weakly with TD at TLC ($p<0.02$) but not at RV. BD at TLC showed a similar trend with LA ($p<0.06$). Correlations of LA with measured lung volumes (TLC, RV, VC) were excellent at TLC ($p<0.0001$) and RV ($p<0.005$). Among functional estimates of airway size including FEV1, FEF50, FEF75, specific and upstream conductance, only peak flow correlated consistently with TD and BD ($p<0.01$). As in Green et al's original data, FEF50 did not correlate with VC ($p<0.09$) or TLC ($p<0.06$). FEF75, however, correlated significantly with VC, TLC, and RV ($p<0.05$, 0.02, and 0.04 respectively). Measures of density dependence were unrelated to TD and BD. Airway size appears to be only very weakly dependent on lung size both anatomically and functionally.
(Supported by HL 19170).

11.4

TRACHEAL AREAS BY ACOUSTIC REFLECTANCE IN NORMAL YOUNG ADULTS.
T.R. Martin*, R.G. Castile, J.J. Fredberg, M.E.B. Wohl, and J. Mead. Children's Hospital, Boston, MA. 02115.

Mead (ARRD 121:339, 1980) suggested that adult males have larger airways relative to their lung size than do adult females. In 23 male and 28 female healthy nonsmokers, 20 to 35 years of age, tracheal area by acoustic reflectance (AAAR) and maximal expiratory flows (MEF) provided estimates of airway size. Total lung capacity (TLC) and vital capacity (VC) were measured as indices of lung size. The males were 5% taller than the females; their AAAR was 50% greater, their MEF at 50% VC was 30% greater, and their TLC was 33% larger than those of the females. For the males the ratio of AAAR to VC was 0.53± 0.14 cm^2/L (mean ± SD) and for the females it was 0.46±0.15 cm^2/L. The ratios of MEF_{50} to VC were 1.05±0.25 VC/sec for the males and 1.08±0.34 VC/sec for the females. The differences between males and females in these ratios were not significant. To eliminate the possible confounding influence of differences in height, a subset of 13 males and 12 females matched for height were analyzed separately. The following values (mean ± SD) were observed.

	Ht (cm)	AAAR (cm^2)	MEF_{50} (L/sec)	TLC (L)
M	176±4	3.14±0.70	5.73±1.3	6.84±0.76
F	176±4	2.09±0.55	4.09±0.75	5.97±0.59

Within this subset the differences between males and females in the above ratios were also not significant. In conclusion, we are unable to detect a sex related difference in the relationship of airway size to lung size. (Supported by HL 07010).

11.5

HISTAMINE TACHYPHYLAXIS IN THE INTACT DOG. P. Antol*, M. Fujita*, and R. E. Hyatt. Mayo Medical School, Rochester, MN 55905.

Histamine tachyphylaxis (HT) has been demonstrated in isolated canine airway smooth muscle but has not been reported in intact dog. Four male dogs (22-25 kg), anesthetized with thiamylal (25 mg/kg), were studied three times using repeated aerosol administrations of histamine diphosphate (H) dissolved in normal saline. Following a saline control, the H dose was increased logrithmically from 0.01-10.0 mg/kg and pulmonary resistance (R) was measured at each dose. H challenges were repeated twice in each dog with a 1 hour period between for R to return to near-baseline. The H dose to produce a 300% increase in R from control (ED300) increased from 0.70 mg/ml for challenge 1(C1) to 2.85 mg/ml for challenge 3(C3). To rule out an effect due to catacholamine release, studies were repeated during propranolol (P) administration (1 mg/kg + 10 ug/kg/min I.V.). ED300 increased from 0.81 mg/ml for C1 to 1.43 mg/ml for C3. Hence, P reduced but did not abolish HT. In vitro studies suggest HT is due to release of prostaglandin (PG) of the E series. Treatment with a PG inhibitor should result in challenges 2 and 3 being similar to C1. In 2 dogs treated with 4 mg/kg meclofenamic acid there was no change in C2 or C3 but C1 was shifted to the right and no HT was observed. The reason for this shift of the initial curve is unclear. (Supported by PHS grants HL 215844 and HL 07222).

11.6

INFLUENCE OF FREQUENCY (f) DEPENDENCE OF AIRWAY RESISTANCE (R_{aw}) ON INVERSE MODELING OF RESPIRATORY IMPEDANCE (Z_{rs}). A.C. Jackson and K.R. Lutchen*, Biomedical Engineering Department, Boston University. Boston, MA. 02215.

Mechanical parameters of the respiratory system are often estimated from Z_{rs} data using lumped-parameter models in which the values of the parameters do not vary with frequency. However, R_{aw} is known to be f-dependent due to velocity profile distortions and parallel inhomogeneities. We investigated the effects of using a f-dependent R_{aw} when fitting models to simulated Z_{rs} data of the dog respiratory system. Z_{rs} data was simulated using a model where the airways were represented by an asymmetrically branched network of tubes with a terminal impedance representative of known alveolar volume, and tissue resistance, inertance, and compliance (JAP.57:1222,1984). In the simulated data, R_{aw} varied from 0.26 $cmH_2O/l/s$ at 2 Hz to 0.60 at 128 Hz. Two 6-element models were fit to the simulated Z_{rs} data; one with f-independent R_{aw} and the other with f-dependent R_{aw} ($R_{aw} = \alpha + \beta f$). The model with the f-independent R_{aw} returned estimates for R_{aw} that ranged from 0.16 to 0.59 $cmH_2O/l/s$ depending on the frequency range of Z_{rs} data used (2-64 and 2-128 Hz, respectively). In addition, estimates of the other parameters were as much as 15% different from their expected values. The model with f-dependent R_{aw} returned estimates for all parameters that were within 4% of their expected values. We conclude that inverse models of the respiratory system that do not include f-dependent R_{aw} may result in significant errors in the estimates of R_{aw}, as well as in the other mechanical parameters, particularly when using Z_{rs} data for f<64 Hz.

Supported by NIH Grant HL-31248.

11.7

FREQUENCY AFFECTS ON RESPIRATORY MECHANICAL PARAMETERS OBTAINED FROM IMPEDANCE DATA. K.R. Lutchen* and A.C. Jackson Dept. of Biomed. Engineering, Boston Univ. Boston, MA 02215

Many previous studies have characterized respiratory mechanics by fitting lumped parameter models to impedance data obtained from forced oscillations. For frequency ranges higher than 4-32 Hz, a six parameter model is required with a central resistance and inertance in series with the parallel combination of a shunt compliance and a peripheral resistance, inertance and compliance in series. We have investigated the veracity of the parameter estimates one can expect to obtain with this approach; particularly the affects of frequency range. Using a known parameter set obtained from a previous study, impedance was simulated from 4-128 Hz. Impedance sensitivity to each parameter was also calculated over this range. These simulations indicated that a second resonance occurs above 64 Hz and that the impedance is highly sensitive to the parameters at frequencies surrounding this resonance. After adding random noise to the data, we attempted to extract the original parameters when fitting data over three frequency ranges; 4-32 Hz, 4-64 Hz, and 4-128 Hz. Parameter values were substantially incorrect when data from only 4-32 Hz or 4-64 Hz were fit, but near the original values when data from 4-128 Hz was fit. These reults indicate that in order to make physiological inferences from parameter estimates based on impedance data, the data must include the second resonant frequency.

Supported by NIH Grant 31248.

11.8

CUTANEOUS THERMAL REFLEXES AND AIRWAY FUNCTION IN NORMAL AND ASTHMATIC SUBJECTS. John L. Berk*, K.Lenner* and E.R.McFadden, Jr. Case Western Reserve University, Cleveland, Ohio 44106.

Cold air can theoretically produce bronchoconstriction through reflex effects arising from thermally sensitive receptors in the integument or from local temperature changes within the airways, however, the predominant mechanism is unknown. In addition, although the airway responsiveness of asthmatics to thermal stimuli is greater than that of normal subjects, is not known if this hyper-reactivity also extends to the skin. To provide data on these points, we applied ice to the face of 10 asthmatics and 12 normal subjects while we monitored specific conductance (sGAW). On another occasion, frigid air was inhaled during progressively increasing periods of eucapnic hyperventilation. Pulmonary mechanics were measured before and after each trial. Cooling the face reduced sGAW 14.5 ($p<0.05$) and 11.7% ($p<0.05$) from control in the asthmatics and normal subjects respectively, but there were no significant differences between groups. There were no changes in pulmonary mechanics with cold air breathing at rest in either group and bronchoconstriction only occurred when ventilation was elevated. As expected, the asthmatics were considerably more sensitive than the normals. These data demonstrate that cold induced airway obstruction derives primarily from local airway effects, and that the contribution of integumental reflexes is small. Further, asthmatics are no more responsive to cooling the skin of the face than are normals.

11.9

POTENTIATING EFFECT OF PRECONSTRICTION ON RESPONSES TO NERVE STIMULATION AND EXOGENOUS AGONISTS IN CANINE AIRWAY SMOOTH MUSCLE. S.J. Gunst and J.M. Pisoni*. Mayo Clinic and Fndn, Rochester, MN 55905.

The effects of preconstriction on responses to both cholinergic and α-adrenergic stimulation of tracheal and bronchial smooth muscle were investigated. Trachealis muscle strips were pretreated with indomethacin (Indo) ($5x10^{-6}$M), or with 5-hydroxytryptamine (5-HT) or histamine (His) to an initial tone of < 20% of maximum. Responses to electrical stimulation (16V, 0.5 msec, 3-20 Hz) of cholinergic nerves at frequencies which caused submaximal contraction were enhanced by pretreatment with each agent. Responses to 10^{-7}M acetylcholine (ACh) were also enhanced by pretreatment with each agent, whereas responses to 10^{-6}M ACh were enhanced only by Indo. In the presence of atropine (10^{-6}M) and propranolol (10^{-6}M), pretreatment of bronchial rings with either 5-HT, His, or Indo enhanced responses to electrical stimulation of α-adrenergic nerves at all frequencies. Responses to exogenous norepinephrine (10^{-7} to 10^{-4}M) were also enhanced. The results suggest that the potentiating effects of preconstriction may be due to direct sensitization of the smooth muscle tissue. Preconstriction could affect either the membrane or the contractile apparatus of the muscle to enhance its responsiveness to a subsequently imposed stimulus.

(Supported by HL29289, the Minnesota Heart Assoc., and the Puritan-Bennett Fndn).

11.10

EFFECT OF PHARYNGEAL MUSCLE ACTIVATION ON AIRWAY SIZE. J.M. Fouke, A.D. Wolin*, S. Sugg*, and K.P. Strohl. Case Western Reserve University, Cleveland, OH 44106.

Pharyngeal muscle activity may serve to modulate upper airway patency. We developed a model to define the results of muscle activation on the size of a cylindrical airway. The model allowed us to assign a vectorial position and length-tension characteristic to pairs of muscles along the cylinder. To test this model, in seven heavily anesthetized, artificially ventilated dogs, we isolated the upper airway by transecting the cervical trachea and sealing it from atmosphere and the lung. Pressure measured in this isolated upper airway was used as an index of forces acting on the airway. We mimicked activity with electrical stimulation of six pairs of pharyngeal muscles. Stimulation of separate muscle pairs using sub-maximal stimuli produced a fall in pressure (dilating force) in the isolated upper airway; as predicted by the model, the magnitude of the pressure change increased as the stimulus magnitude increased. Model predictions and experimental results demonstrate that skeletal muscles extrinsic to the airway can create a dilating force on the upper airway and that two muscle groups can act in concert to increase this force. (HL 01067 and 29726)

11.11

AIRWAY RESPONSIVENESS TO SUBSTANCE P (sub P) INCREASES WITH REPEATED sub P CHALLENGE IN GUINEA PIGS. S. Shore* and J. Drazen. Dept. of Environ. Sci. & Physiol., Harvard School of Public Health and Dept. of Medicine, Beth Israel Hospital, Boston, MA 02115

We examined the effect of intravenous substance P on pulmonary mechanics in anesthetized ventilated guinea pigs. In each animal, we obtained 3 dose response curves to sub P (i.v. bolus) with 40 minute recovery periods between curves. Changes in pulmonary conductance (G_L) and dynamic compliance (Cdyn) were used as indices of bronchoconstriction. Sub P produced dose-dependent and comparable decreases in G_L and Cdyn. Importantly, animals demonstrated increased sensitivity to sub P with consecutive challenges. The amount of sub P required to produce a 50% decrease in G_L($ED_{50}G_L$) was 2.8 fold ($p<0.02$) and 3.2 fold ($p<0.01$) less on the 2nd and 3rd trials than it was on the original trial (n=6). Sub P did not alter bronchoconstriction induced by i.v. histamine. Neither indomethacin (30 mg/kg i.p.), atropine (5 mg/kg i.v.) nor mepyramine (8 mg/kg i.v.) altered the initial response to sub P, yet indomethacin did prevent the enhanced responses to sub P which occurred with repeated challenge. After indomethacin, $ED_{50}G_L$ on the 1st, 2nd and 3rd dose response curves averaged 7.8, 5.5 and 7.6 Moles/kg x 10^{-9} (n=5, NS). We conclude that, in guinea pigs, responsiveness to sub P increases with repeated challenge. Cyclooxygenase products of arachidonic acid metabolism appear to be involved in the mediation of this enhanced response. (Supported by NIH HL17382 and MRC Canada.)

11.12

RESISTANCE (R), COMPLIANCE (C), AND TIME CONSTANT (TC) BY PASSIVE EXHALATION TECHNIQUES CORRELATE WITH ESOPHAGEAL BALLOON TECHNIQUES. J.C. Cunningham,* R.J. Lemen, W.J. Morgan,* M.L. Witten, J.L. Magarelli,* J.L. Stevenson,* and S.F. Quan.* Departments of Pediatrics, Physiology and Internal Medicine, Division of Respiratory Sciences, Arizona Health Sciences Center, Tucson, AZ.

Measurements of respiratory mechanics in small infants with esophageal balloon techniques are difficult to perform and have numerous technical problems. Passive exhalation techniques may overcome many of these problems but these methods must be validated. We performed histamine challenges in Beagle puppies (ages 82 ± 2 days) and compared measurements of R, C, and TC obtained with standard (S) esophageal balloon and passive exhalation (PE) techniques. These studies were performed on Days 0 and 3 of an acute canine parainfluenza 2 infection. Six dogs were anesthetized with Chloralose (80mg/kgIV) and mechanically ventilated (VT=15ml/kg). Data were obtained after aerosol challenge with 5 breaths of saline or 30 mg/ml histamine aerosol generated by an ultrasonic nebulizer. Computer assisted analysis of flow volume curves were used to determine TC by PE techniques. Respiratory System Resistance and Compliance were measured by the method of Mortola, et al (J. Appl. Physiol. 58 (2): 528-533, 1985). As expected resistance increased and compliance decreased significantly ($P < .05$) following histamine aerosol challenge by both techniques. TC did not change after aerosol challenge by either technique. Linear regressions indicate that S and PE techniques were significantly correlated for resistance ($R = 0.82$) and compliance ($R = 0.91$). We conclude that analysis of respiratory mechanics using passive exhalation techniques correlates well with data obtained by esophageal balloon techniques. Passive exhalation techniques are technically simpler than standard techniques and can detect changes in respiratory mechanics associated with histamine challenge or acute viral infections. (Supported by HL14136, HL07249, CFF Training Grant, and HL01377.)

RENAL PHYSIOLOGY I

12.1

Hindquarter Gln Contribution During Chronic Acidosis In Rat. T.C. Welbourne LSU, Shreveport, LA 71130

The contribution of hindquarter gln release to that consumed by the kidneys was studied during chronic metabolic acidosis in the rat. Chronic acidosis was induced by maintaining 6 rats on 1.2% NH_4Cl in 5% glucose; 6 controls received NH_4HCO_3 in glucose. After 4 days hindquarter gln release was determined from arteriovenous gln concentration differences and hindquarter blood flow measured by the PAH dilution principle. Briefly the rats were anesthesized with inactin, placed on a heated animal board and cannulas were placed in the carotid artery for arterial blood samples and jugular vein for maintenence infusion of saline, 15 ul/min/ 100g. A midline abdominal incision was made and a 30 gauge infusion needle was inserted and cemented into the abdominal aorta just above the iliac artery bifurcation; inferior vena cava blood was collected from an indwelling 24 guage needle cannula cemented in place at the same level. Labeled ^{14}C PAH was infused at the rate of 0.005 uC/min/100g into abdominal aorta while 3 serial samplings from the artery and vena cava were drawn at 15 minute intervals. Hindquarter flow was calculated from the dilution principle while plasma gln was measured by an enzymatic fluorometric method. Metabolic acidosis increased hindquarter blood flow from 1.76±0.20 to 2.69±0.48 ml/min/100g and A-V [gln] increased from -49±8 to -110±16 nmol/ml resulting in net gln release increasing from 86±26 to 296± 70 nmol/min/100g.

12.2

CONTACT ACTIVATION OF HUMAN PLASMA PRORENIN: FURTHER EVIDENCE LINKING THE COAGULATION AND RENIN-ANGIOTENSIN SYSTEMS. Carl F. Brown* and Daniel H. Osmond. Dept. of Physiology, Univ. of Toronto, Toronto, Canada, M5S 1A8.

Factor XII (FXII) and other coagulation factors participate in cryoactivation (cryo) of human plasma prorenin, implying an enzymic cascade of prorenin activation in vitro, which may also operate in vivo. Individual plasmas exhibit a positive correlation between rates of cryo of prorenin and prekallikrein ($r=0.816$, $P<0.025$). The natural activator of prekallikrein is FXII, so we examined whether glass-activation of FXII promotes cryo beyond the rate observed with siliconized glass or plastic. Glass speeded the cryo of plasma (A) but not plasma (B). Mixed 1:1, cryo of these normal plasmas proceeded much faster than with either alone, implying transfer of a FXII-activating enzyme from B to A as well as input of backed-up (non-activated) prorenin from A to B. Heating plasma B to 56°C for 15 min. destroyed the transferable enzyme and abolished cryo. Since this treatment does not destroy FXII or kallikrein, an enzyme other than FXII and kallikrein must also be involved in cryo, which is heat-labile and able to activate FXII in the absence of glass. Thus, glass contact promotes cryo of prorenin in some plasmas more than others due to differences in the level or activity of this unidentified heat labile enzyme. Prorenin activation is multifactorial, yet lack of one or more factors can be extremely rate-limiting.

Supported by the Ontario Heart Foundation.

12.3

ON THE MECHANISM OF PRORENIN ACTIVATION IN THE CIRCULATION. Gregory M.T.Hare* Arline Y. Loh*,& Daniel H. Osmond.Dept.of Physiology, Univ. of Toronto,Toronto, Canada, M5S 1A8.

Plasma prorenin can be activated to renin by trypsin whose action is probably mediated by endogenous proteases/inhibitors. In an attempt to define the trypsin-independent physiological mechanism of activation outside the kidney we induced 20 min. left arm venous occlusion (occl.) in 8 men, which is known to activate possible prorenin convertase enzymes. The unoccluded right arm provided control plasmas. where cont.1 and cont.2 represent samples taken after 0 and 17 min. of occlusion on the opposite side. Renin/prorenin are expressed as angiotensin I, ng.ml.hr.

	Active renin	Prorenin tryp. 1.5mg.ml	Prorenin tryp. 2mg ml	α -1 anti-tryp. units
Cont.1	3 6 ± 0.5	17.0 ± 1.5	9.4 ± 1.5	0.79 ± 0.01
Cont.2	3.1 ± 0.4	15.3 ± 1.5	8.8 ± 1.7	0.76 ± 0.03
Occl.	5 1 ± 0.6	17.3 ± 1.3	25.1 ± 1.9	1.18 ± 0.04

Enhanced prorenin convertase in the occl. arm is suggested by the higher renin value relative to cont.1 or 2, presumed to represent converted prorenin. Instead of falling reciprocally prorenin rose when determined with trypsin at 2 mg.ml. This implies release of prorenin from within the occluded arm. The increase in α -1-antitrypsin raises the requirement of trypsin to at least 2mg ml, which becomes excessive in controls, illustrating a dynamic relationship between protease and inhibitor activity on the outcome of prorenin activation. The precise origin and conversion mechanism of occl. arm prorenin is unknown.

Supported by the Ontario Heart Foundation.

12.4

A SIMPLE AND RAPID METHOD FOR THE INVESTIGATION OF RENIN RELEASE FROM PURIFIED RAT RENAL CORTICAL PLASMA MEMBRANES. Shirley M. Russo* and J.C.S. Fray U. Mass. Medical School, Worcester, MA. 01605

A method for a rapid, reproducible isolation of highly purified rat renal cortical plasma membrane vesicles (PMVs) using isotonic medium and Percoll self-forming gradient centrifugation is demonstrated. Vesicles were characterized by enzyme markers, conconavalin A affinity chromatography, and electron microscopy. Contamination by mitochondria, lysosomes, granules, and ER membranes was minimal as indicated by the low specific activities of the respective enzyme markers. Repeated extrusion of the membrane fraction through a 26G hypodermic syringe resulted in PMVs of >80% inside-out orientation as confirmed by using a Con A column to separate the mixed population of vesicles. Trypsin treatment (2mg/ml) increased the renin specific activity (RSA) of the isolated PMVs 5- to 8-fold over the untreated controls. If inactive renin is defined as the absolute difference in RSA between trypsin-treated PMVs (total renin) and the controls, no trypsin-treatment (endogenous active renin); then 80-85% of membrane-associated renin is in an inactive, but trypsin-activable form. Additional studies have shown renal renin content increased in sodium deprived and hypophysectomized (Hx) rats. However, renin released from Hx rat kidneys was substantially lower than controls and sodium deprived, suggesting that the plasma membrane may be defective in the Hx rats and may store a large amount of renin. Thus RSA in PMVs was compared in kidneys from control, sodium deprived, and Hx rats. Results showed that RSA was significantly greater in the sodium deprived and Hx rats which also had a high renal renin content. Advantages of this new method for isolating PMVs include the use of isotonic media throughout; a high proportion of inside-out polarity; and a relatively short isolation time. Based on both biochemical and morphological criteria, plasma membranes prepared by this method are of equal or greater purity compared to those prepared by widely used sucrose gradients and lengthy differential centrifugations; and the yields are comparable or several folds higher. This method may be useful in the study of renin secretion. Supported by grants from NIH (HHL 01021) and NSF (*PCM 8302798).

12.5

RENIN RELEASE FROM ISOLATED RENIN GRANULES. D.H. Sigmon* and J.C.S. Fray. U Mass. Med. School, Worcester, MA 01609

Renin granules were isolated from a homogenate of kidney cortex using a combination of differential and discontinuous sucrose-density gradient centrifugation technique developed by Sagnella and Peart (Biochem J., 182:301, 1979), with minor modification. The isolated granular fraction showed a 67-fold increase in renin specific activity compared to the homogenate. Renin secretion from the isolated granules was inversely related to osmolarity between the range of 150-900 mOsm. The granules released only 23.6% of its renin at 150 mOsm. Renin secretion increased when the pH was lowered from pH 7 to pH 5 and again when raised from pH 7 to pH 9. The pH effect was Cl^- dependent and was abolished when KCl was replaced with K-glutamate. Renin granular release was three-fold higher in KCl medium compared to $MgCl_2$. The protonophore FCCP had no effect on the secretory pattern. This suggests that the Cl^- effect was not simply a symport mechanism between H^+ and Cl^-. The anion blockers SITS and DIDS and the proton ATPase blocker oligomycin had no effect. These findings are consistent with the hypothesis that a transport system exists between H^+, K^+, and Cl^- in the renin secretory granule in which there is a transport of K^+ and Cl^- into the granule to H^+ out. The net effect is osmotically active ions moving into the granule, water following, granular swelling, and renin expulsion. Supported by grants from NIH (#HL01021) and NSF (#PCM 8302798).

12.6

NA-K-ATPASE ACTIVITY IN SPECIFIC NEPHRON SEGMENTS OF RATS DEVELOPING SPONTANEOUS HYPERTENSION (SHR) AND WISTAR-KYOTO (WKY) CONTROLS. Lal C. Garg and Neelam Narang*. University of Florida, Gainesville, FL 32610

In order to demonstrate the difference in renal tubular Na^+ transport, if any, we examined Na-K-ATPase activity in three specific nephron segments of 5 and 12 week old SHR and age-matched WKY. Na-K-ATPase activity was determined in individual nephron segments by a microfluorometric assay in which ATP hydrolysis is coupled to NADH oxidation. The nephron segment examined were: proximal convoluted tubule (PCT), medullary thick ascending limb (MTAL) and cortical collecting duct (CCD).

Rat strain and age	PCT	MTAL	CCD
SHR-5 week	107 ± 5*	74 ± 6*	35 ± 2
WKY-5 week	78 ± 4	97 ± 8	35 ± 2
SHR-12 week	98 ± 8	134 ± 6	30 ± 4
WKY-12 week	95 ± 5	116 ± 4	31 ± 3

Each value represents mean ± SE of 5 rats in $pmol \cdot min^{-1} \cdot mm^{-1}$. *$p<0.05$ vs WKY-5 week.

Na-K-ATPase activity was higher in PCT, lower in MTAL and not different in CCD in 5 week old SHR than in WKY. There was no difference in the enzyme activity in any of three segments in 12 week old SHR and WKY. The results suggest that the abnormal pattern of Na-K-ATPase activity in 5 week SHR may play a role in producing abnormal renal function in this strain.

12.7

RENAL FUNCTION OF INNERVATED AND DENERVATED KIDNEYS BEFORE AND AFTER HEXAMETHONIUM IN RATS UNDER INACTIN ANESTHESIA. J. Krayacich*, R.L. Kline and P.F. Mercer Dept. of Physiology, Univ. of Western Ont., London, Ontario, Canada N6A 5C1.

Renal function in the innervated right kidney and the chronically denervated left kidney were compared under Inactin anesthesia in volume expanded Wistar rats. Mean arterial pressure was 122 ± 5 mmHg before and 70 ± 2 mmHg after ganglionic blockade with hexamethonium (Hex).

	Right Kidney		Left Kidney	
	Control	Hex a	Control	Hex a
CPAH ml/min/g KW	2.82±0.30	5.37±0.60 a	3.50±0.60	5.72±0.90 a
CInulin ml/min./g KW	1.12±0.10	0.67±0.11 a	1.26±0.14	0.57±0.08 b
$\dot{V}$ ul/min./g KW	3.11±0.22	10.54±2.42 a	6.91±1.05	6.84±1.25 a
$U_{Na}\dot{V}$ uEq/min./g KW	0.10±0.02	0.34±0.05 a	0.77±0.15	0.27±0.10 b

Mean±SEM, n=9, a=P<0.05 compared to control
b=P<0.05 compared to Right Kidney

These results suggest that under Inactin anesthesia there is a significant degree of sympathetic activity which influences renal tubular function. (Supported by the Heart and Stroke Foundation of Ontario)

12.8

EFFECTS OF ACUTE RENAL DENERVATION ON SODIUM EXCRETION IN MINIATURE SWINE WITH CIRRHOSIS AND ASCITES. Edward J. Zambraski, Rutgers Univ., New Brunswick, N.J. 08903.

Recent studies have suggested that patients with cirrhosis may have elevated peripheral and renal sympathetic tone. To evaluate the possible neurogenic component to the sodium retention in this state, the effects of acute renal surgical denervation (RDX) were evaluated in 6 cirrhotic Yucatan miniature swine (YMS). Cirrhosis was induced by chronic ligation of the bile duct (4-6 wks). At the time of study all animals had pronounced ascites. In anesthetized YMS urine was collected bilaterally before and after unilateral left kidney RDX. Mean arterial pressure (107±6 mmHg) was not affected by RDX. UV-urine volume-(ml/min); UnaV-sodium excretion-(uEq/min); *P<0.05 versus control.

	Left Kidney		Right Kidney	
	UV	UnaV	UV	UnaV
Control	.42±.06*	10.8±3.2	.56±.07	18.0±7.7
RDX	.49±.08	19.4±7.9	.47±.09	17.4±7.5

The significant increase in left UV and increases in left UnaV for 4 of the 6 animals were not due to a change in filtered sodium load since neither left or right GFR were altered by left RDX (left GFR-81±22 control; 60±13 (ml/min) RDX). These data suggest that the renal nerves and increased neurogenic renal tubular sodium reabsorption may be contributing to the sodium retention seen in YMS with cirrhosis and ascites.

12.9

PROXIMAL TUBULE SODIUM REABSORPTION IN RESPONSE TO CHANGES IN RENAL PERFUSION PRESSURE (RPP) DURING PROSTAGLANDIN INHIBITION. J. A. Haas, J. P. Granger, F. G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester, MN 55905

Previous studies from our laboratory have shown that sodium reabsorption in deep, but not superficial, proximal tubules is inhibited in response to increases in RPP. The objective of the present study was to determine the role of prostaglandins in mediating the RPP-induced decreases in proximal sodium reabsorption and increases in fractional sodium excretion (FE_{Na}). Proximal sodium reabsorption at the whole kidney level, as estimated by fractional lithium reabsorption (FR_{Li}), was measured in 11 control (C) and 8 meclofenemate-treated (M) rats in response to acute changes in RPP by means of suprarenal aortic constriction.

RPP (mmHg)	FE_{Na} (%)		FR_{Li} (%)	
	C	M	C	M
90 ± 1	1.3±.2	0.6±.2	74±2	79±5
109 ± 1	2.1±.3*	0.8±.2	69±2*	77±3
128 ± 2	4.5±.4*	1.6±.2*	60±2*	72±3*

*$p<.05$, vs RPP of 90 or 109

These results demonstrate that prostaglandin inhibition attenuates the natriuretic response to increases in RPP. Differences in FR_{Li} between C and M groups suggest that RPP-induced decreases in proximal sodium reabsorption may be partially mediated by renal prostaglandins.

12.10

ANGIOTENSIN BLOCKAGE AT EIGHT VASCULAR LEVELS IN THE RAT MICROCIRCULATION. D. L. Wiegman, S. Weis*, R. Dussel* and M. Steinhausen*. Department of Physiology, University of Louisville, KY, and First Institute of Physiology, University of Heidelberg, FRG.

The hydronephrotic rat kidney with intact circulation and innervation was split and spread out as a thin sheet in a tissue bath. The microvasculature was observed in vivo via television microscopy. This preparation allows visualization of many vascular levels. We quantitated the effects of increasing concentrations (10-9 to 10-5 M) of saralasin (angiotensin II antagonist) applied locally in the tissue bath on microvascular diameters at eight vascular levels from the interlobar artery to the distal efferent arteriole. When saralasin produces a dilation, it implies that the vessel has been constricted by endogenous angiotensin. Saralasin produced an increase in preglomerular diameters which was largest (37±11%) in the interlobular artery (there was no dilation in the interlobar artery or the distal afferent arteriole) and an increase in postglomerular diameters which was largest (17±4%) in the proximal efferent arteriole. If these types of findings would hold for the normal kidney, it would suggest a role for angiotensin in the control of total renal blood flow, in the regional distribution of flow, and in the control of filtration fraction. (Supported by Deutsche Forschungsgemeinshaft, SFB 90.)

12.11

COUPLING OF GFR AND RENAL BLOOD FLOW AUTOREGULATION DURING ANGIOTENSIN CONVERTING ENZYME INHIBITION IN SODIUM DEPLETED DOGS. L.G. Navar and L. Rosivall* and W.J. Champion*. Univ. of Alabama at Birmingham, Birmingham, Alabama 35294

The role of the renin-angiotensin system in the autoregulation of glomerular filtration rate (GFR) remains controversial. To assess this issue the effects of angiotensin converting enzyme (ACE) inhibition on the coupling of GFR and renal blood flow (RBF) autoregulation were evaluated in 12 anesthetized dogs fed a low sodium diet for 7 days. The coupling ratio was calculated from the quotient of the GFR autoregulatory efficiency ratio and the RBF autoregulatory efficiency ratio. ACE inhibition with captopril decreased systemic blood pressure by 16±4%, increased RBF by 36±7% and increased GFR by 26±11%. In response to reductions in renal arterial pressure (RAP), RBF was efficiently autoregulated (less than a 10% decrease) to RAP values of 75-80 mmHg during control periods and to even lower values during ACE inhibition. Overall GFR autoregulatory efficiency was also well maintained during ACE inhibition; however evaluation of the coupled autoregulatory responses at RAP of 75 to 90 mmHg indicated that 5 experiments had coupling ratios below .9 (.68±.05) during ACE inhibition as compared to only 1 during control periods. These data indicate that blockade of the renin-angiotensin system does not consistently impair GFR autoregulatory capability. Nevertheless, a subgroup of experiments exhibited reduced coupling ratios in the lower autoregulatory range during ACE inhibition.

BLOOD PRESSURE AND HYPERTENSION I

13.1

CHRONIC INFUSION OF A VASOPRESSIN PRESSOR ANTAGONIST DOES NOT PREVENT DEVELOPMENT OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). Celia D. Sladek*, Martha L. Blair, and Michael Mangiapane. Univ. Rochester, Rochester, NY 14642.

The hypothesis that abnormalities in vasopressin release may contribute to increased peripheral resistance in SHR was tested by chronic infusion of a specific antagonist of the vascular effects of VP. From 4-13 wks of age, SHR and Wistar Kyoto rats (WKY) received either isotonic saline or 0.1 ug/hr of $d(CH_2)_5$ Tyr (Me) AVP by osmopump. Systolic blood pressure (sBP) was measured by tail cuff from 5-11 weeks of age. In SHR, the VP analogue did not alter the rate or degree of increase in sBP. In WKY, sBP in the VP analogue group was slightly reduced compared to the saline infusion until 10 weeks of age ($F=10.08$, $p=.008$). At 12-14 weeks of age, all animals were prepared with indwelling arterial and venous catheters. Resting MAP was not significantly altered by the VP analogue infusion in either strain, but the response to VP infusion (1, 3, or 10 ng/200 gm b.w.) was markedly attenuated by the analogue treatment indicating that plasma levels of the VP analogue were sufficient to block pressor effects of endogenous VP. A bolus injection of the angiotensin II converting enzyme inhibitor, teprotide, resulted in a decrease in MAP ($p<.05$) which was comparable in all groups. Thus, chronic blockade of the pressor effects of endogenous VP does not alter the course of hypertension in SHR, and there is no evidence for compensation by the renin-angiotensin system. Supported by R01-HL-28172 and RCDA to M.L.B. K01-HL-00966.

13.2

GENETIC ASSOCIATION OF HYPERTENSION AND VASCULAR CHANGES IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SHRSP). Cathy A. Bruner, J. Hurley Myers, Charles F. Sing*, Pentti T. Jokelainen,* R. Clinton Webb. Univ. of Mich., Ann Arbor, MI.

Isolated tail arteries from SHRSP exhibit oscillatory contractile activity in response to norepinephrine (NE), whereas those from Wistar-Kyoto rats (WKY) do not. To determine whether the NE-induced oscillations are related to high blood pressure or to separable genetic differences between strains, the response to NE was studied in adult SHRSP, WKY, and progeny of genetic crosses of SHRSP and WKY (F_1, F_2, F_1 x SHRSP, F_1 x WKY). Helical tail artery strips were mounted in a tissue bath for isometric force recording. Rats were classified as "responders" if the oscillatory contractions to 1.8×10^{-7} M NE exceeded 80 mg in amplitude. The blood pressures (mm Hg; tail cuff method) and percent of rats exhibiting NE-induced oscillations were: WKY: 109±3, 0%; F_1: 129±4, 0%; F_2: 150±4, 38%; F_1 x WKY: 137±3, 9%; F_1 x SHRSP: 188±7, 71%; SHRSP: 207±7, 100%. This distribution of the occurrence of oscillatory activity fits a one gene model for inheritance of this vascular trait, with the WKY allele being dominant. In the segregating F_2 progeny, the blood pressure of the responders was higher than that of nonresponders (161±7 vs. 144±4 mm Hg, $p<0.05$). We conclude that the genetic factor that controls the NE-induced oscillatory activity is associated with an increment in arterial pressure in SHRSP. (Supported by NIH, HL-06968, HL-27020, HL-00813, HL-18575)

13.3

THE EFFECTS OF INDOMETHACIN ON BLOOD PRESSURE RESPONSIVENESS IN SODIUM RESTRICTED SHR. C.B. Toal* E.A. Wilczynski* and F.H.H. Leenen* (SPON: A. Veress). Toronto Western Hospital, Toronto, Ontario, M5T 2S8.

Rats given a "low sodium diet" for 9-11 days have been shown to exhibit a decreased blood pressure response to bolus administration of noradrenaline and angiotensin II compared to animals on a "high sodium diet", possibly related to an enhanced synthesis of vasodilatory prostaglandins. To test this possibility in SHR and WKY rats raised from birth to 16 weeks of age on a sodium restricted diet (ie. 9 uMol/g food), dose-blood pressure response curves for these pressor agents were constructed before and after indomethacin (INDO, 5 mg/kg). INDO significantly decreased basal blood pressure in only the SHR and only on control diet. The blood pressure response to both pressor agents was not significantly altered by administration of INDO to either group of animals. Basal PRA was markedly increased in the sodium restricted animals vs the control diet animals. INDO administration significantly decreased the basal PRA in control diet animals but elevated PRA in the sodium restricted animals. This study suggests that the observed decrease in blood pressure response in severely sodium restricted SHR and WKY is not mediated by an enhanced prostaglandin release but may be due to a decreased smooth muscle responsiveness and/or a decrease in receptor affinity or number.

13.4

LYMPHOCYTES FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR) EXHIBIT CALCIUM-RELATED ABNORMALITIES. P.B. Furspan, P. Ross* and D.F. Bohr, Univ. Michigan, Ann Arbor, MI 48109

We examined the effect of varying external calcium concentration ([Ca]) on net potassium efflux from lymphocytes of SHR, SHR-stroke prone (SHRSP) and Wistar Kyoto (WKY) rats.

$[Ca]_{out}$ (mM)	Net K Efflux (mmol/kg dry wt/hr) WKY (n=5)	SHR (n=4)	SHRSP (n=3)
0.1	9.9 ± 1.0	15.7 ± 0.4	17.1 ± 1.0
0.3	7.7 ± 1.2	12.9 ± 0.2	13.6 ± 0.4
1.0	5.0 ± 0.8	10.7 ± 0.7	10.3 ± 0.4

Net potassium (K) efflux is greater in lymphocytes from SHR than in those from WKY but in both the efflux is depressed by increments in bath [Ca]. Using the Ca-sensitive indicator quin2, we determined that lymphocytes from the stroke prone SHR exhibited elevated intracellular free-[Ca] (176.2 ± 12.1 nM, n=5) when compared to lymphocytes from WKY (82.1 ± 10 nM, n=4) and Sprague-Dawley (117 ± 8.8 nM, n=5) rats. We postulate that the elevated K efflux in lymphocytes from SHR and SHRSP reflects the increased activity of a Ca-dependent K channel. The depression in K efflux by elevations in $[Ca]_{out}$ suggest that membrane permeability is decreased by increasing the amount of Ca bound to the membrane. These data support the hypothesis that in hypertension there is a generalized increase in cell membrane permeability to Ca which may result from a reduced number of Ca binding sites on the plasma membrane. Similar results have been obtained with vascular smooth muscle (Jones, Circ Res 34-35 Suppl 1:117, 1974).

13.5

DOSE DEPENDENT ANTIHYPERTENSIVE EFFECT OF ASPIRIN IN THE SHR. R. Tuttle, S. Patel* and N. Northrup*. Masonic Medical Research Laboratory, Utica, NY 13504.

Aspirin (100mg/Kg/day) per os has an antihypertensive effect in young spontaneous hypertensive rats (SHRs). SHRs receiving aspirin in their drinking water from 28 to 91 days of age remain normotensive with controls (WKY), while heart rates increase steadily. SHRs at 91 days of age receiving plain water experience pressures of 165-170mmHg, but these may be reduced to normotensive levels by 7 days exposure to aspirin. This dose of aspirin is an order of magnitude greater than used for analgesia or antiplatelet activity and brings into question the relevancy of antihypertensive effect. A dose dependency study of the antihypertensive effect of aspirin in the young SHR and WKY rat was undertaken using aspirin levels of 5, 10, 50 and 100mg/Kg/day per os. Total prostaglandin (PG) analysis were performed on renal tissues from rats after 35 days of treatment with aspirin at each dose level. The results show that the antihypertensive effect in the SHR is proportional to dose but the diminution of total renal PG by aspirin is not. We conclude that the antihypertensive effect in the young SHR is not a toxic manifestation of high dose levels.

Supported by Central NY Heart Chapter, Inc., New York Academy of Sciences and NIH Grant #1 S03 RR03367-01.

13.6

ENDOTHELIUM-DEPENDENT RESPONSES TO ADENOSINE DIPHOSPHATE AND SEROTONIN IN THE AORTA OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. Thomas F. Lüscher* and Paul M. Vanhoutte. Department of Physiology, Mayo Clinic, Rochester, MN 55905

Experiments were designed to study endothelium-dependent responses to adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT) in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto rats (WKY) (30-34 weeks). Rings of thoracic aorta, with and without endothelium, were suspended for isometric tension recording. ADP induced concentration-dependent relaxations in rings with, but not in those without endothelium in both SHR and WKY; at high concentrations of ADP the relaxations were significantly reduced in the SHR. In quiescent rings of both strains, 5-HT induced concentration-dependent contractions; rings without endothelium were more sensitive than those with endothelium. Rings (with and without endothelium) of SHR were significantly more responsive to 5-HT than those of WKY. In contracted rings with, but not in those without endothelium, lower concentrations of 5-HT induced relaxations. Higher concentrations of the monoamine induced contractions which, in SHR, but not in WKY, were larger in rings with than in those without endothelium. The decreased relaxations to ADP and the augmented contractions to 5-HT in the SHR suggest functional changes of the endothelium in this animal model of hypertension, possibly because these substances release endothelium-derived contracting factors. (Supported in part by NIH grant HL 31183.)

13.7

EFFECTS OF TWO ARTERIAL VASODILATORS ON BP AND CARDIAC HYPERTROPHY IN 2-K, 1-C HYPERTENSIVE RATS. James Tsoporis*, Sheryl Prowse* and Frans H.H. Leenen*. (SPON: A. Veress) Toronto Western Hospital, Toronto, Canada.

The time course of changes in LV and RV weight, BP, plasma volume, plasma catecholamines and the BP response to hexamethonium were evaluated during treatment for 2 days, or 1, 2, 3 and 5 weeks with either hydralazine 80 and 120 or minoxidil 40 and 120 mg/L drinking water in 2-K, 1-C hypertensive rats. Both vasodilators induced initially a clear antihypertensive effect (mean BP from 170-180 down to 135-145 mm Hg), subsequently tolerance developed. Plasma volume showed only small increases with treatment. Plasma catecholamines and the BP decrease caused by hexamethonium were not affected by treatment. Both LV and RV (dry and wet) weights and LV internal diameter (ID) showed significant increases by 5 weeks of treatment. Treatment had no effect on LV wall thickness. (p<0.05 treat. vs untreat.)

	Dry LV Weight (mg/100g BW)	Dry RV Weight (mg/100g BW)	LV ID (mm)
Sham	46±1	12±.1	3.8±.2
Clip, Untreated	69±2	13±.5	3.7±.2
Clip, Minoxidil (120 mg/L)	79±2*	16±.9*	5.1±.2*
Clip, Hydralazine (120 mg/L)	86±4*	15±.5*	4.8±.2*

Treatment with either vasodilator causes RVH and eccentric LVH possibly related to prolonged cardiac volume overload.

13.8

ROLE OF SYMPATHETIC NERVOUS AND RENIN-ANGIOTENSIN SYSTEMS IN THE CHRONIC PHASE OF TWO-KIDNEY, ONE CLIP HYPERTENSIVE RATS. Javier Salazar*, Marlano Ubeda*, Miguel G. Salom*, Luis Carbonell*, Joaquin Garcia-Estan*, and Tomas Quesada* (SPON: J.C. Romero). Depart. of Physiology, University of Murcia, Spain

The purpose of the present study was to examine the roles of the renin-angiotensin and the sympathetic nervous systems during the chronic phase (16 weeks) of two-kidney, one-clip hypertension in conscious unrestrained rats. At this stage mean arterial pressure (MAP) (p<0.001) and plasma angiotensin II (31.9±1.5 to 125.8±19.9 pg/ml, p<0.005) were significantly increased as compared to normotensive rats. The converting enzyme inhibitor Captopril produced a significant decrease of MAP (181.2±8.2 to 140.0±5.5 mmHg p<0.001). This hypotensive response was similar when Aprotinin (a Kallikrein inhibitor) and Captopril were simultaneously infused. Alpha$_1$-adrenergic receptor blockade by Phenoxybenzamine (POB) significantly decreased but did not normalize MAP (179.8±12.4 to 135.8±10.4 mmHg, p<0.001). However, when infused after POB, Captopril induced a further decrease of MAP to 86.7±9.4 mmHg (p<0.001). This MAP level was not different from that found in normotensive rats after infusion of the two drugs (83.2±5.3 mmHg).

These results suggest that both the renin-angiotensin system and the sympathetic nervous system by activating peripheral α_1-adrenergic receptors maintain high blood pressure during the chronic phase (16 weeks) of two-kidney, one-clip hypertension in conscious rats.

13.9

Coronary Vascular Reactivity in Deoxycorticosterone Acetate Hypertensive Pigs. David R. Bell* and David F. Bohr. U. of Mich., Ann Arbor, MI 48109

We examined constrictor and dilator responses in isolated, helically cut strips of large, 3mm and small 0.5-0.9mm (OD) coronary arteries from 6 deoxycorticosterone acetate (DOCA) hypertensive and 11 normotensive (NT) pigs. Strips were mounted in a vertical bath containing warm (37°C), aerated(95%O_2,5%CO_2) physiological salt solution and connected to Grass FT.03 force transducers. Large, but not small, coronary strips displayed spontaneous tonic contractions which were significantly greater in DOCA pigs (16.6±5.1 vs. 4.1±1.3, %maximum contractile force; $\bar{X}$±SEM; p<0.05). Small coronary arteries were more sensitive to relaxation by isoproterenol, norepinephrine (NE) and adenosine (AD) compared to large coronary arteries, with enhanced relaxation to AD in small DOCA vs. small NT(ED_{50}:DOCA= 4.5×10^{-8}M, NT=16×10^{-8}M; p<0.05). Relaxation to NE was attenuated in large DOCA strips (p<0.05, profile analysis). Only large coronary stips diplayed contractions to NE+propranolol. Large coronary alpha-adrenergic sensitivity was increased whereas beta adrenergic sensitivity was unchanged in DOCA pigs (ED_{20}alpha:DOCA=1.3×10^{-7}, NT=8.4×10^{-7}; p<0.05). Nitroglycerin and serotonin responses were similar in all strips. We conclude that coronary vessels from DOCA pigs are characterized by increased myogenic tone, and AD and alpha-adrenergic sensitivity. This latter factor is primarily responsible for attenuated NE relaxation of large DOCA coronary arteries. Supported by NIH grant HL18575; DRB is a recepient of NRSA-HL06973.

13.10

BLOOD PRESSURE CHANGES AND VASCULAR RESPONSE TO OUABAIN IN CENTRALLY INFUSED MINERALOCORTICOID HYPERTENSIVE RATS. L. Thompson*, F. Lamb*, A. Kaynard, and R.C. Webb. University of Michigan, Ann Arbor, MI 48109

This study tests the hypothesis that deoxycorticosterone acts centrally to mediate elevated blood pressure and increased vascular reactivity to ouabain in mineralocorticoid hypertensive rats. Adult male rats were made hypertensive by either subcutaneous implantation of deoxycorticosterone(SC-DOC 200 mg/kg) or by cerebroventricular infusion (IVT-DOC; 6×10^{-10} mol/day). Two weeks after administration of the steroid, the increase in systolic blood pressure from normotensive controls was similar for SC-DOC rats (33±9 mmHg) and IVT-DOC rats (26±5 mmHg). Aortic strips from SC-DOC(n=4), IVT-DOC(n=4), and normotensive controls were helically cut and suspended in muscle baths for measurement of isometric force generation. The magnitude of contraction to the maximal dose of ouabain (10^{-3}M) differed (p<.05) between SC-DOC rats (345±146 mg) and IVT-DOC rats (88±27 mg) but was not different between control groups (81±17 and 62±31 mg, respectively). The contractile response to 130 mM KCl was not different between the four groups. These results suggest that the increase in blood pressure in mineralocorticoid hypertensive rats is mediated by a central component. The increase in vascular reactivity to ouabain in aortae from SC-DOC rats is not attributed to the central action of the steroid. Supported by American Heart Association of Michigan, NIH HL-00813 and HL-18575.

13.11

THE EFFECT OF ISOMETRIC FOREARM TENSION ON THE HEMODYNAMIC RESPONSES TO LBNP. M. L. Smith*, D.L. Hudson*, and P.B. Raven
Dept. of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Six healthy male subjects underwent progressive LBNP to -50 torr. During LBNP each subject maintained forearm muscle tensions at relaxed (CR) and at contracted states of 75μV (C75) and 180μV (C180) as monitored by electromyographic activity (EMG). Abdominal and lower limb musculature was maintained relaxed (EMG<10μV) throughout each LBNP protocol. LBNP was terminated when pre-syncopal reactions occurred or at the completion of 5 minutes of -50 torr LBNP. Heart rates (HR) were monitored continuously and blood pressures (BP) were automatically recorded every minute. Changes in leg volume (LgV) were derived from calf circumference changes measured with a calibrated Whitney Strain guage. Metabolic measurements and cardiac outputs (CO2 rebreathe technique) were determined from respiratory gas measurements collected at each stage of LBNP. Mean changes ($\bar{x}$)±S.E.M. from 0 to -50 torr LBNP are summarized below:

	HR(bpm)	SBP(torr)	MBP(torr)	LgV(l)
CR	+26.4 ±6.4	-19.6 ± 2.2	-10.9 ± 1.1	+0.672 ± 11
C75	+29.2 ± 4.8	-14.3 ± 3.0	- 8.8 ± 1.4	+0.685 ± 0.2
C180	+38.6 ±12.4	- 4.4 ± 2.3*	- 0.9 ±2.4*	+0.659 ± 0.2

*Significantly different from CR, P<0.05

From this data it was concluded that maintenance of arterial blood pressure during LBNP was mediated by a somatic reflex effect.

(Sponsored in part by Brooks School of Aerospace Medicine, Dept. of the Air Force Contract #FY7624-85-25415).

13.12

FITNESS RELATED DIFFERENCES IN RESPONSIVENESS TO A PHENYLEPHRINE CHALLENGE. P.B. Raven, H. Graitzer, M.L. Smith*, D.L. Hudson*. Dept. of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

In previous studies we have observed differences in baroreflex sensitivity between low and high fit subjects when challenged with lower body negative pressure, The Physiologist 27;S59, 1984. In the present investigation phenylephrine (PE) was used as an α-receptor challenge with incremental infusion rates up to 120 μg/min in a group of endurance trained subjects (ET;$\dot{V}O2max$ = 67.4 ml/kg/min) and untrained subjects (UT;$\dot{V}O2max$ = 40.6ml/kg/min). At rest and during the hemodynamic steady state for each infusion rate, heart rate (HR) blood pressures, and forearm blood flow (BF) were determined. Mean changes (Δ) from rest at a calculated plasma volume dose of PE μg/l are summarized below:

	Dose (μg/l)	ΔSBP (torr)	ΔDBP (torr)	ΔBF (ml/100ml/min)	ΔHR/ΔMBP
UT	80.2	+30.0	+10.5	-2.44	1.92
ET	85.3	+32.0	+18.5	-2.76	1.08
P	NS	NS	<0.05	NS	<0.01

Comparing the means at any absolute infusion rate resulted in significant differences for several variables. However when the infusion dose was corrected for differences in plasma volume (ET=22% > UT), there was no significant difference in α-receptor sensitivity between UT and ET at equipotent doses. However, there was a significant difference in the baroreflex index (ΔHR/ΔBP) of responsivity. This finding supports our previous finding of a fitness related attenuation of the baroreflex response to uploading.

(Sponsored in part by Brooks School of Aerospace Medicine, Dept. of the Air Force Contract #FY7624-85-25415).

COMPARATIVE PHYSIOLOGY: TEMPERATURE ADAPTATION AND ENERGETICS

14.1

THE ROLE OF SATURATED AND UNSATURATED PHOSPHOLIPIDS IN ACTIVATING CYTOCHROME OXIDASE FROM THERMALLY ACCLIMATED CRAYFISH. Nancy P. Neas and Geoffrey L. Hodge*. Colgate Univ. Hamilton, New York 13346.

The fatty acid moieties of most membrane phospholipids from liver tissue of the crayfish Orconectes propinquus become more unsaturated following acclimation to 5°C (CA) compared to those acclimated to 20°C (WA) (Unsaturation/Saturation ratio of total phospholipids CA = 3.57; WA = 3.26). Cardiolipin (CL), a lipid confined to the inner mitochondrial membrane, exhibits the opposite trend becoming more saturated following cold acclimation (Unsaturation/Saturation ratio, CA = 1.03; WA = 1.89). The role of CL in activating cytochrome oxidase was investigated by assaying the enzyme both in a lipid-depleted form (acetone powder) and following addition of lipids isolated from CA and WA crayfish. In the lipid-depleted form cytochrome oxidase activity is low (CA = 0.026 μmoles [mg·min]$^{-1}$; WA = 0.070 μmoles [mg·min]$^{-1}$). Preliminary studies indicate that CL from WA crayfish enhances the rate of cytochrome C oxidation from both WA and CA animals nearly 10-fold; CL from CA crayfish enhances cytochrome oxidase activity from WA animals near 10-fold, and enhances the enzyme from CA animals near 10^2-fold. Thus the restructuring of CL with temperature may reflect its role as a cofactor in aerobic metabolism as opposed to a homeoviscous adaptation.

14.2

HIGH ENERGY ORGANOPHOSPHATE LEVELS IN THE MYOCARDIA OF COLD ACCLIMATED AND COLD-HYPOXIC FRESHWATER TURTLES, CHRYSEMYS PICTA. Albert J. Rotermund, Jr. and Vasilis Makris*. Loyola University of Chicago, Departments of Biology and Chemistry, Chicago, IL 60626

Energy levels, as reflected by ATP, ADP, AMP and creatine phosphate concentrations, were examined in the cardiac tissue of Chrysemys picta. Animals were divided into three groups: controls at room temperature, cold acclimated, and cold acclimated-hypoxic. Animals were allowed to acclimate for two and four weeks to experimental conditions, after which cardiac organophosphates were assayed. ATP levels declined by 38% in the cold and 52% in the cold-hypoxic group. Paralleling this decline was a significant increase in creatine phosphate levels in the cold group, while levels for the cold hypoxic group remained the same. ADP levels rose in the cold-hypoxic group by 33%, remaining steady for the cold group, while AMP rose by 20% in the cold group and 40% in the cold hypoxic group. No significant differences were observed between the organophosphate levels in the two week and the four week animals. These results suggest that, after an initial discharge during the first two weeks, in both experimental groups the energy levels of C. picta heart remained unchanged during two additional weeks of cold and cold-hypoxia.

14.3

CA-ATPASE IN THE BILLFISH HEATER TISSUE. Barbara A. Block. Duke University, Durham, NC 27706

Billfish have a heat producing tissue warming the brain and eyes. It is not clear how this tissue generates heat. Previous studies have shown that the heater tissue is derived from an eye muscle and contains cells which are packed with mitochondria. The mitochondria are able to couple oxidative energy to the production of ATP. The mitochondria-rich cells also possess an extensive cytoplasmic smooth endoplasmic reticulum. In this study, the smooth membranes were isolated from the heater tissue, to determine if the membranes house an ATP-dependent ion pump. The close morphological relationship between the heater tissue and muscle has suggested that the smooth membranes might be homologous to sarcoplasmic reticulum, and thus, rich in calcium-ATPase. The heater tissue was homogenized and separated by differential centrifugation into a heavy fraction which had many mitochondria and some membranes, and a light fraction which contained mostly membranes. The heater tissue has a large amount of calcium-stimulated ATPase activity in both fractions. The remaining portion of the eye muscle from which the heater tissue is derived, was also examined as a control. Oligomycin, which inhibits the mitochondrial ATPase did not reduce the calcium-stimulated ATPase activity in the heater fractions. Energy-dependent calcium uptake activity has also been characterized in homogenates from the heater tissue and eye muscle of these fish.

Supported by NIH grant 5-RO1HL02228-30.

14.4

MOLECULAR SPECIES COMPOSITION OF PHOSPHATIDYLCHOLINE IN HEPATOCYTE MEMBRANES OF THERMALLY ACCLIMATED TROUT. J. R. Hazel and Eileen Zerba*. Department of Zoology, Arizona State University, Tempe, AZ 85287

Mitochondria, plasma membranes and microsomes were isolated from liver of rainbow trout acclimated to 5 °C or 20 °C. Phosphatidylcholine(PC) was purified by HPLC and the molecular species composition determined by gas chromatography of TMS ethers of diacylglycerols prepared by digestion of PC with phospholipase C. Plasma membranes and mitochondria exhibited temperature dependent changes in species composition consistent with homeoviscous theory. In plasma membrane, cold acclimation resulted in a loss of short chain (≤C34) mono- (34[0,1]) and dienoic (30[0,2]) species and the accumulation of long chain (≥ C38) tetra- and hexaenoic (primarily 38[0,6], increasing from 27.8 to 37.6%) species, resulting in a significant increase in unsaturation index (UI) from 3.62 to 4.37. Mitochondria from warm-acclimated trout were unique in possessing large quantities (14%) of 34[1,1]; this species declined in abundance following cold acclimation (to 2%) and was replaced by long=chain penta- and hexaenoic species (primarily 36[0,5] and 38[0,6]. In contrast, microsomal PC of cold-acclimated fish exhibited greater amounts of 36[0,5] (7 vs 3%), but lower proportions of 38[0,6] (34 vs 46%)and higher proportions of saturated species. Consequently, UI did not vary with acclimation temperature. These data more precisely define the nature of homeoviscous membrane restructuring and underscore the membrane-specific nature of the acclimation response. (Supported by NSF grant DCB-8301757)

14.5

HEAT-SHOCK RESPONSE IN A HIGHLY EURYTHERMAL TELEOST (FUNDULUS HETEROCLITUS). Michael Koban and Dennis A. Powers. The Johns Hopkins University, Baltimore, MD 21218

Every organism responds to a heat-shock by a diminution of normal protein synthesis and the induction of a group of proteins (heat-shock proteins, hsps) with functions reported to confer protection against thermal injury and other environmental stresses. To gain more understanding of the effects of environment on gene expression, we investigated the heat-shock response of the eurythermal teleost Fundulus heteroclitus. Fish were acclimated to 10, 20, and 30°C. RNA was isolated from liver and skeletal muscle of fish heat-shocked for various times. The RNA was used to direct protein synthesis in a cell-free rabbit reticulocyte lysate with ^{35}S-methionine or ^{3}H-leucine as label. Labeled polypeptides were resolved by SDS-polyacrylamide gel electrophoresis and visualized by fluorography. Temperature increases from 10 to 20°C, 10 to 30°C, and 20 to 30°C did not induce hsps. An increase from 20 to 35°C induced hsps in both liver and muscle in a time-dependant manner. The hsps in these tissues differed in their molecular weights, suggesting a tissue-specific response in Fundulus. These results are in contrast to previous work on catfish hepatocytes, wherein we found a modulation of threshold temperature for hsp synthesis by acclimation temperature (Koban et al., Am. Zool. 24: 97A, 1984). Thus, there appears to be a complex interaction between natural history of species and acclimation status for the temperatures at which hsps are synthesized.
(Supported by NSF BRS-8207006 and Maryland Sea Grant)

14.6

CONSTANT TEMPERATURE AND THERMOPERIOD ACCLIMATION OF METABOLISM IN THE SALAMANDER DESMOGNATHUS QUADRAMACULATUS. Jack R. Layne, Jr. and Dennis L. Claussen. Miami University, Oxford, Ohio 45056

The acclimation responses of ectotherms to cyclic temperature regimes (thermoperiods) have received little attention despite the closer approximation to natural environmental conditions by thermoperiods as opposed to constant acclimation temperatures. We examined the oxygen consumption of the salamander D. quadramaculatus acclimated to a constant temperature (5, 15, or 25 C) or to a 5 to 25 C thermoperiod. These determinations were made on adult males collected in summer and fall. Metabolism was determined at various acute temperature (5, 10, 15, 20 and 25 C). The acute metabolic responses of salamanders acclimated to a constant temperature showed a strong thermal dependence. In contrast, the metabolism of thermoperiod-acclimated salamanders was markedly less temperature sensitive. These data suggest that exposure to a cyclic temperature regime induces a homeometabolic response, which may promote constant levels of organismal functions in spite of variability in the natural thermal environment.

14.7

METABOLISM AND THERMOREGULATION IN A FASTING LIZARD, TUPINAMBIS NIGROPUNCTATUS. M. J. Martinez*, R. K. Dupre*, and S. C. Wood. Univ. of New Mexico Sch. of Medicine, Albuquerque, NM 87131.

The influence of fasting on the standard metabolism and thermal preference was examined in the golden tegu, Tupinambis nigropunctatus. During feeding (one mouse on alternate days), tegus had a mean oxygen consumption at 35°C of 4.36±0.44 (SE) ml O_2 STPD/kg/min. In a thermal gradient of 27 to 44° C, the mean selected body temperature was 36.6±0.3 (SE)°C. After control data were collected, food was withheld until the animal reached a weight approximately 75% of initial body weight (ca. 4 mos.). Our hypothesis was that both standard metabolism and preferred temperature would decrease during periods of food deprivation as a means of conserving body energy stores thus preventing tissue digestion. However, during the fast period, standard metabolism at 35°C did not decrease significantly from pre-fast levels. Also, mean selected body temperature was higher than pre-fast levels. Post-mortem exam of one animal revealed remaining fat deposits and all animals appeared to preserve fat depots in the tail. The predatory habits of the tegu may dictate that the animal not compromise its state of "readiness" should prey be encountered. This research was supported by NSF Grant PCM 83-00472 and NIH Grant SO6RR-08139 (SCW).

14.8

THE EFFECT OF TEMPERATURE ON THE ENERGETICS OF VOCALIZATION BY AN ANURAN AMPHIBIAN. Theodore L. Taigen and Kentwood D. Wells*. Univ. of Connecticut, Storrs, CT 06268.

Male spring peepers (Hyla crucifer) emerge early in the spring and begin calling to attract females shortly after ponds are free of ice. Sustained vocal advertisement by males continues for 6-8 weeks. During this period, calling frogs experience body temperatures ranging from 5 to 25° C. We investigated the vocal behavior and energetics of sound production in these animals at 7, 10, 15, 19, and 23° C. With decreasing temperature, calling rate (calls/h) declines, while call duration (msec/call) increases. At each of the five experimental temperatures, metabolic rates during sustained calling were 15-20 times higher than resting metabolism. Also at each temperature, metabolic rates in calling frogs were considerably higher (38-100%) than metabolic rates during exhaustive locomotor exercise. A multiple regression analysis identified calling rate as the most significant determinant of oxygen consumption in calling frogs, accounting for 82% of the total variation in metabolism (n=59). Body mass accounted for an additional 7% of the total variance in calling metabolism, suggesting that large frogs have higher sound pressure levels than do small frogs. Temperature accounted for only 2% of the total variance in oxygen consumption during calling. Hence, the energetic cost of the muscle activity that creates the thoracic pressure necessary for sound production appears relatively constant, and independent of temperature.

14.9

VENTILATION AND BODY TEMPERATURE OF THE BAT PHYLLOSTOMUS HASTATUS DURING FLIGHT AT DIFFERENT AIR TEMPERATURES. Ann T. Farabaugh*, Dian B. Thomas* and Steven P. Thomas, Dept. Biol. Sci., Duquesne University, Pittsburgh, Pa. 15282

We have measured breathing frequencies (f, breaths/min) and inspired tidal volumes (V_t, cm^3 STPD) from two P. hastatus (mean body mass = 105.2 g) equipped with hot-thermistor flow probes as they undertook steady windtunnel flights at a constant speed over a range of air temperatures (T_a) from 18.1 to 30.5°C. Rectal temperatures (T_r) were also measured within 10 seconds from the end of each flight. Mean f was 493.8 at 18.1°C, and was independent of T_a. Mean V_t had a value of 1.62 cm^3 at 18.1°C, and showed only a 7% increase with temperature over the range of T_a's investigated. Consequently, mean minute ventilation rate showed only a very modest (7%) increase as T_a increased from 18.1 to 30.5°C. Mean T_r was linearly related to T_a, and varied from 38.8 to 41.9°C as T_a increased from 18.1 to 30.5°C. While flying birds can vary their ventilation, and thus their respiratory evaporative heat loss, over a wide range to help compensate for different levels of environmental heat stress, results from the present study indicate that this thermoregulatory adjustment is not used to any significant extent by flying P. hastatus.
(Supported by NSF grant PCM 8303050)

14.10

DIRECT MEASUREMENT OF TEMPERATURE GRADIENTS IN THE RETE OPHTHALMICUM OF THE DOUBLE-CRESTED CORMORANT (Phalacrocorax auritis). D.M. Hudson, P.M. Clair*, & M.H. Bernstein, Dept. of Biology, New Mexico State Univ., Las Cruces, NM 88003

Countercurrent heat exchange in the rete ophthalmicum (RO) has been implicated in avian brain and eye temperature regulation. However, until the recent discovery of a large (2.5 cm,L) surgically accessible RO in cormorants, confirmation of temperature gradients along the length of the intact RO was impossible. Using surgically implanted thermocouples, we have now simultaneously recorded temperatures at several RO sites (T_r), in brachiocephalic arterial blood (T_c), and in the pre-optic hypothalamus (T_b) of 5 awake birds. At an ambient temperature (T_a) of 23°C T_b decreased anteriorly along the RO; T_b and the most caudal T_r were within 0.4°C of T_a. Lowering T_b to 5°C decreased T_r, while T_c remained constant. Even though the RO temperature gradient increased to 4-5°C, T_b decreased by less than 1°C, which suggests that RO heat exchange is maintained at this T_a, but that cerebral blood is supplied primarily from non-RO sources. Raising T_a to 37°C caused a rise in all temperatures and a reduction in the RO gradient to less than 1°C. The onset of panting and gular flutter restored the RO gradient to 4°C between T_c and the anterior T_r, while T_b was maintained 2-3°C below T_c. This suggests that RO heat exchange is maintained during evaporative cooling at high T_a and that the RO may supply a significant share of cerebral blood flow during heat exposure. (Supported by NSF grant PCM-8402659)

14.11

SURFACE TEMPERATURE REGULATION IN GUANACOS DETERMINED BY THERMOGRAPHY. Daniel A. de Lamo* and James E. Heath. Univ. of Illinois, Urbana, IL 61801

Surface temperatures were obtained from fifteen unrestrained guanacos (Lama guanicoe Muller) raised in captivity. A portable thermal imaging camera was employed to measure the surface temperature of the animals outdoors at night, to avoid interference from solar radiation. Regions with intermediate and short pelage (1.5 cm) follow Ta passively above 0°C with a differential of 8°C. Active vascular mechanisms maintain Ts above freezing at Ta's of 0°C. Long furred areas (>3 cm) follow a similar pattern but at a smaller differential temperature range (3°C). Bare regions (0.5 cm or less) follow Ta at a larger differential (15°C) keeping the same pattern even at Ta's well below freezing. The exposure of these zones are modified by postural responses. The mean surface temperature (Ts) was determined by the surface temperature region in which the animal was divided and the relative area of each region. Ts varies with Ta and is dependent on behavioral responses and seasonal compensations.- D. A. de Lamo was a CONICET (Argentina) fellow, 1983-85.

14.12

THERMAL CONDUCTANCE OF BIRD NESTS - GENERALIZATIONS BASED ON BODY MASS AND AMBIENT TEMPERATURE. A. Ar AND Y. Yom-Tov*. Tel Aviv University, Tel Aviv, 69978, Israel.

A model was constructed for closed bird nests in order to test an assumption made commonly in literature, that relates nest to thermal insulation. The model utilizes known allometric relations for restig metabolic rate (RMR), heat transfer coefficient (CB), body temperature (TB) and lower critical point (TC), to calculate heat production rate at night in the nest (NMR), when ambient temperature (TA) is usually below TC, as a function of body mass (BM). This is done by assuming that the night temperatures of non-evaporating, non-metabolizing dummy eggs (TE) placed in the nest, represent by and large, nest temperature (TN). The model shows that nest conductance (CN) increases roughly with the 0.5 power of BM and is proportional to the ratio: (TB - TN)/(TN - TA). Since both TB and TE (=TN) are relatively constant among birds, CN must be directly related to the increase in TA. Solving the model for several species of birds shows that CN is of the same order of magnitude as CB, indicating that the nest acts as a second set of feathers around the incubating bird.

NEURAL CONTROL OF CIRCULATION I

15.1

INFLUENCE OF PULSE FREQUENCY ON SINGLE UNIT BARORECEPTOR DISCHARGE. Mark W. Chapleau, Laurie J. Fankhauser,* and Francois M. Abboud. The University of Iowa, Iowa City, IA 52242.

The contour of the arterial pressure pulse can affect baroreceptor discharge (BD). When utilizing sinewave pulsations the influence of pulse frequency (PF) is difficult to examine because of changes in dP/dt and duration of systole. We performed experiments in the anesthetized dog comparing the effects of sinewave frequency to the effects of the frequency of natural pressure pulses on BD. The isolated carotid sinus was connected to an electromagnetic pressure converter driven by a voltage generator connected to the arterial pressure channel. Pulse pressure was maintained constant and natural PF was decreased by vagal stimulation. Changes in sinewave frequency did not influence BD when BD was always continuous (Group 1) or phasic (diastolic silence, Group 2), but diastolic silence was eliminated by decreasing sinewave frequency at some pressures (Group 3) causing increased BD (imp/sec).

	11	20	83	155(pulses/min)
Group 1	54±6 imp/sec	--	53±5	--
Group 2	26±6	25±6	26±6	24±6
Group 3	46±5	42±4	38±4	36±4

In contrast, decreases in natural pulse frequency from 187 to 116 pulses/min decreased BD from 36±9 to 29±7 imp/sec because of fewer systoles. Thus, when PF is slowed during sinewave pulsation the direct effects of the decreased frequency on BD are offset by the influence of the increased duration of systole and decreased negative dP/dt. Supported by NIH HL07121.

15.2

INTERACTION OF PULMONARY ARTERY, PULMONARY VEIN, AND CAROTID SINUS BARORECEPTORS IN THE OPEN LOOP STEADY STATE CONTROL OF SYSTEMIC RESISTANCE IN THE DOG. Arthur W. Wallace* and Artin A. Shoukas. Johns Hopkins Medical School, Baltimore, MD 21205

Cardiopulmonary and carotid sinus baroreceptor control of systemic resistance were studied in the mongrel dog during total cardiac bypass. The steady state gain of the pulmonary artery, pulmonary vein and carotid sinus baroreceptors were measured by quantitative open loop analysis. Pulmonary artery, pulmonary vein, carotid sinus, and central venous pressure were independently controlled. The carotid sinuses were isolated. Aortic, coronary, left ventricular baroreceptors, and carotid chemoreceptors were denervated. A random sequence of pulmonary artery, pulmonary vein, and carotid sinus pressure was then used. Results were analyzed by multiple nonlinear regression with correction for time variance. Systemic resistance was controlled by carotid sinus, pulmonary artery, and pulmonary vein pressure. The gain of the pulmonary artery and pulmonary vein baroreceptors was affected by carotid sinus pressure. Systemic resistance can be modelled by the equation:

SAP = A + B*CSP + C*CSP^2 + D*CSP^3 + E*(PAP/CSP^2) + F*(PVP/CSP^2)

The nonlinear carotid sinus terms give sigmoidal response of systemic arterial pressure to carotid sinus pressure. The the response to pulmonary artery and venous baroreceptors is influenced by the carotid sinus pressure. The terms in the equation for SAP are: A=92.9, B=2.02, C=-0.0206, D=0.0000533, E=692, F=648. The gain of the carotid sinus baroreceptor reflex on systemic resistance is given by:

G(csp) = B + 2*C*CSP + 3*D*CSP^2 - 2*F**PAP/CSP^3 -2*G*PVP/CSP^3

The peak gain of the carotid sinus baroreceptor reflex on resistance was found to be -0.66. The gain of the pulmonary artery baroreceptor reflex was given by G(pap) = F/CSP^2. The maximal gain of the pulmonary artery reflex was 0.28. The gain of the pulmonary venous baroreceptor reflex was given by G(pvp) = G/CSP^2. The maximal gain of the pulmonary venous reflex was -0.2592. (NIH grant HL-19039).

15.3

REFLEX RESPONSES TO BILATERAL CAROTID OCCLUSION ARE ENHANCED IN CONSCIOUS HYPERTENSIVE DOGS

DA Kirby* and SF Vatner, Harvard Med Sch, Brig & Wom Hosp., NE Reg Primate Res Ctr, Southboro, MA.

Impaired baroreflex sensitivity in hypertension has been well documented in terms of heart rate (HR) slowing in response to phenylephrine. Fewer studies have examined the effects of bilateral carotid occlusion (BCO). We compared baroreflex sensitivity in response to phenylephrine (10μg/kg iv) with effects of BCO in 5 chronically instrumented conscious dogs during the development of perinephritic hypertension. Reflex HR slowing (pulse interval) was plotted against the rise in systolic arterial pressure (AP) in response to phenylephrine. The slope of this relationship prior to hypertension was 22±5 msec/mmHg and was significantly depressed ($p<0.05$) to 8±3 msec/mmHg after 3 wks of hypertension. Prior to hypertension, BCO increased mean AP by 39±4 from 102±2 mmHg and HR by 16±2 from 71±6 bpm, while cardiac output (electromagnetic flowmeter)(CO) did not change from 2.6±0.2 L/min. At 3 wks after hypertension, BCO increased mean AP (65±10 from 164±12 mmHg), CO (0.7±0.2 from 3.7±0.6 L/min) and HR (59±16 from 92±13 bpm). The increases in AP, HR and CO with BCO were significantly more ($p<0.05$) after hypertension. Thus, baroreflex cardiac slowing in response to phenylephrine was impaired in hypertension, but responses to BCO were actually enhanced.

15.4

THE EFFECT OF PEEP ON CARDIOVASCULAR AND RESPIRATORY BAROREFLEX RESPONSES. Martha J. Brunner, Stephen F. Hatem^A, Arthur Wallace* and Andrew S. Greene. University of Maryland at Baltimore, Baltimore, Maryland 21201.

Excitation of cardiopulmonary receptors by PEEP was hypothesized to influence cardiovascular and respiratory baroreceptor reflex responses. Experiments were performed in the spontaneously breathing, pentobarbital-anesthetized rabbit. The aortic nerves were cut, and the bilateral carotid sinus was isolated and perfused at controlled pressures. Arterial pressure (AP), heart rate (HR), respiratory frequency (f) and ventilation($\dot{V}_E$) were measured. Decreases in intrasinus pressure resulted in reflex increases in arterial pressure (39%), heart rate (6%), respiratory frequency (20%) and ventilation (25%). The addition of 5 cm H_2O PEEP did not significantly change the cardiovascular (AP and HR) responses to changes in carotid baroreceptor pressure. However; the addition of PEEP abolished the baroreceptor-induced reflex change in respiratory rate and ventilation. After bilateral vagotomy, the addition of PEEP had no effect on baroreceptor reflex responses in any of the variables measured. It is concluded that excitation of cardiopulmonary receptors by PEEP inhibits the respiratory (f and $\dot{V}_E$) but not cardiovascular (AP and HR) responses to changes in baroreceptor pressure.

(Supported by a grant from the American Lung Association)

15.5

INFLUENCE OF CENTRAL RESPIRATORY ACTIVITY ON THE CARDIAC AUTONOMIC NERVE RESPONSE TO BARORECEPTOR STIMULATION. M. Kollai* and K. Koizumi. SUNY Downstate Med. Ctr., Brooklyn, NY 11203

Stimulation of arterial baroreceptors is known to produce changes in both central autonomic and respiratory neuron activity. In chloralose anesthetized and artificially ventilated dogs responses of phrenic, cardiac sympathetic and vagal nerve activity to changes in "isolated" carotid sinus pressure were analyzed. We found: 1) When phrenic nerve activity was absent due to hyperventilation, a stepwise increase in the sinus pressure resulted in sustained reciprocal changes in cardiac autonomic nerve activity; that of vagal nerve increase, sympathetic decrease. Plotting relative changes in cardiac nerve activity against changes in the sinus pressure produced sigmoid curve, with the maximum gain at the same sinus pressure level for both nerves in a given animal. 2) When phrenic activity was present, sustained increase in the sinus presure strongly inhibited phrenic activity only initially, followed by a moderate inhibition. Similarly, the sympathetic activity was inhibited strongly only in the initial phase and it returned to control value during maintained stimulus. The vagal activity, however, remained elevated as long as sinus pressure was high. At high sinus pressure (above 200 mmHg) paradoxical increase in phrenic rhythm frequency altered responses of cardiac autonomic nerve activity. It was concluded that changes in central respiratory activity profoundly interfered with the autonomic nerve response to baroreceptor stimulation. (Supported by USPHS Grant NS00847.)

15.6

INTERACTION BETWEEN ANGIOTENSIN II AND VASOPRESSIN IN BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY. I. Abe,* D.B. Averill* and C.M. Ferrario. Research Division, Cleveland Clinic, Cleveland, OH 44106.

Intravenous (IV) infusion of angiotensin II (Ang II) raises mean arterial pressure (MAP) and reduces postganglionic sympathetic renal nerve activity (RNA). If vasopressin (VP) is released into the circulation by a central action of Ang II, it may modify the circulatory response to the increase in MAP. With this in mind, computer averaged beat-by-beat values of MAP, heart rate (HR) and RNA were obtained in two groups of dogs anesthetized with morphine-pentobarbital. In a first group of dogs (n = 6) we measured the effect of Ang II infused IV at 5, 10 and 20 ng/kg/min. In a second group of dogs (n = 7), a VP antagonist [d(CH_2)$_5$Tyr(Me)AVP] was given (20 µg/kg) prior to infusion of Ang II. Ang II produced increments in MAP and reflex decreases in RNA that were proportionally greater with larger doses of the peptide. On no occasion did HR change from baseline values. Pretreatment with the VP antagonist did not alter the time course or the magnitude of the pressor response and the reflex reduction of RNA at the two lower doses of Ang II. At the highest dose of Ang II, dogs pretreated with the VP antagonist showed a more rapid rise in MAP and a more prompt suppression of RNA, but at the plateau of the response, there were no statistical differences ($p > 0.05$). These data indicate that neither the magnitude nor the main characteristics of the cardiovascular response produced by small doses of Ang II given IV are influenced by blockade of the systemic V1 type VP receptors. (Supported in part by NHLBI grant, HL-6835).

15.7

CENTRAL ANGIOTENSIN II AND PROSTAGLANDIN E_2 ACT INDEPENDENTLY TO INCREASE BLOOD PRESSURE IN CONSCIOUS SHEEP. B.A. Breuhaus* and J.E. Chimoskey, Michigan State University, East Lansing, MI 48824-1101, and North Carolina State University, Raleigh, NC 27606.

Eight conscious sheep chronically prepared with catheters in their carotid arteries (IC), jugular veins (IV), and lateral cerebral ventricles (IVT) were used to test the hypothesis that centrally-administered prostaglandin E_2 (PGE_2) increases blood pressure (BP) by activating the brain renin-angiotensin system. IVT angiotensin II (AII) 50 ng/kg/min, IC PGE_2 10 ng/kg/min, and IVT PGE_2 300 ng/kg/min increased BP 23, 21, and 14 mmHg respectively ($p<.05$). Infusion of the AII receptor antagonist [Sar^1Thr^8]AII, 1000 ng/kg/min IVT, had no effect on BP and prevented the pressor response to IVT AII, but did not alter the pressor responses to IC PGE_2 (+17 mmHg, $p<.05$) or to IVT PGE_2 (+16 mmHg, $p<.05$). Subsequent experiments showed that IVT AII does not increase BP by increasing brain prostaglandin synthesis, since neither indomethacin (4 mg/kg subcutaneously) nor flunixin meglumine (3 mg/kg intravenously) prevented the IVT AII pressor response. We conclude that central PGE_2 and AII do not act in series to increase BP in conscious sheep (i.e. administration of one does not act through synthesis or release of the other). Supported by HL30239 and HL06840.

15.8

DIFFERENT EFFECTS OF LOWER BODY NEGATIVE PRESSURE ON FOREARM AND CALF BLOOD VESSELS

Louis K. Essandoh*, Donald S. Houston*, John T. Shepherd, Mayo Clinic, Rochester, MN 55905

Modest degrees of lower body negative pressure (LBNP) cause a reflex constriction of forearm blood vessels attributable to deactivation of the low-pressure baroreceptors. In the present study we sought to determine if the calf vessels respond similarly. Left forearm and right calf blood flows were measured simultaneously by strain-gauge plethysmography in six volunteers. To preclude artifacts in blood flow measurements due to engorgement of the right calf with LBNP, the right lower leg was excluded from LBNP by enclosing it in a sealed boot. Decreases in forearm flow at LBNP of 15 and 20 mm Hg were significantly greater than the corresponding decreases in calf flow; at 10 mm Hg forearm flow decreased by more than 34% whereas there was no significant change in calf flow. At 40 mm Hg LBNP and immediately following a Valsalva maneuver, both forearm and calf flows decreased markedly and to similar degrees. We conclude that, unlike forearm vessels, calf vessels are unresponsive to deactivation of low-pressure mechanoreceptors and speculate that the vasoconstriction in the calf at higher levels of LBNP is mediated by deactivation of arterial baroreceptors. (Supported by: NHLBI Grant HL-05883)

15.9

INTERACTION OF HEMORRHAGE AND HYPOXIA IN THE CONTROL OF BLOOD PRESSURE, RENIN, ACTH AND ADRENAL FUNCTION IN CONSCIOUS RATS. H. Raff, R. Sandri*, and T.P. Segerson*. Dept. of Medicine, Med. Coll. Wisconsin-St. Luke's Hospital, Milwaukee, WI 53215

We studied the effect of chronic hypoxia (HYP) on mean arterial pressure (MAP), renin (PRA), ACTH, aldosterone (ALDO), and corticosterone (CORT) responses to hemorrhage (HEM). 6 Long-Evans rats with femoral catheters were placed in chambers vented with normoxic (21% O2) or hypoxic (10% O2) gas. After 42 hrs, rats underwent HEM at 6 ml/kg over 1 min. 20 min later, another sample was drawn. HEM blood was used as the control sample (CTL). Samples were analyzed for blood gases, electrolytes, PRA, ACTH, ALDO and CORT. HYP (PaO2=43±1 torr) resulted in significant respiratory alkalosis (PaCO2=25±1 torr, pHa=7.52±.01) and hypokalemia (plasma K+=3.5±.2 mEq/l).

RESPONSES TO HEM AT 42 HRS OF NORMOXIA & HYPOXIA

	MAP	PRA	ALDO	ACTH	CORT
NORMOXIA	(mmHg)	(ng/ml/hr)	(ng/dl)	(pg/ml)	(ug/dl)
CTL	104±6	7.1±1.2	4±1	37±5	2±2
HEM	88±6*	9.4±1.2	24±6*	43±11	9±4
HYPOXIA					
CTL	100±6	6.4±2.1	2±1	41±5	4±2
HEM	79±9*	12.9±3.0*	28±3*	273±65*‡	34±3*‡

(*HEM≠CTL $p<0.05$; ‡ HYPOXIA≠NORMOXIA $p<0.05$)

Hypoxia augmented the PRA, ACTH and CORT but not the MAP or ALDO responses to hemorrhage. Hypoxia-induced hypokalemia may attenuate the ALDO response to increases in PRA and ACTH. HYP and HEM interact in endocrine control. (AHA, WISC 84-GA-01)

15.10

EFFECTS OF THE NEW SOVIET ANTIARRHYTHMIC ETHACIZIN UPON AUTONOMIC EFFERENT ACTIVITY TO THE CANINE HEART. G.R. Hageman, B.H. Neely, F. Urthaler and L.V. Rosenshtraukh*. University of Alabama at Birmingham and the All-Union Cardiology Center, Moscow, USSR.

This study examined the effects of ethacizin (the diethylamino analog of ethmozin) (1 mg/kg, i.v.) on the spontaneous and reflexly elicited efferent activity in discrete thoracic cardiac sympathetic and parasympathetic nerves. Nitroglycerin and phenylephrine (4 and 8 µg/kg, i.v.) were administered to 15 anesthetized mongrel dogs while monitoring blood pressure and heart rate. In each dog two nerves were isolated and efferent multifiber neurograms were simultaneously recorded and analyzed by microprocessor. Ethacizin significantly ($p < .05$) attenuated the spontaneous sympathetic activity in both left and right, preganglionic (n=8) and postganglionic (n=14) sympathetic nerves to the heart. In contrast, reflex changes in sympathetic activity elicited by baroreceptor challenges, were not affected by ethacizin. Also, ethacizin did not significantly affect either spontaneous or baroreceptor reflex induced parasympathetic efferent activities in 8 preganglionic nerves. Thus, this new phenothiazine derivative may exert part of its antiarrhythmic action through a reduction of the spontaneous sympathetic activity to the heart. The fact that ethacizin neither reduced the reflex-induced changes in sympathetic or parasympathetic activities nor influenced the tonic vagal activity suggests that ethacizin is not likely to interfere with reflexly mediated cardiovascular adaptive changes.

15.11

RECIPROCAL INHIBITION IN VOMITING. J. Zabara, J. Coleman* and S. Elfar*. NASA-Ames Research Center, Moffett Field, CA 94035.

Neuroinhibition is an important process to be considered in the analysis of motion sickness and vomiting. Since vomiting is inhibited during inspiration, apparently to prevent aspiration of vomitus, and inhibition of the respiratory centers occurs during vomiting, it is reasonable that ventilation and vomiting are related by a reciprocal inhibitory process. Evidence to support this hypothesis was obtained in chronic experiments with female cats. The cats weighed from 3 to 4kg., and were anesthetized with nembutal for surgical implantation of cuff electrodes to stimulate autonomic nerve bundles. After full recovery from surgery, animals were tested in weekly 45 min. sessions. Rectangular pulses were applied to the electrodes in a range of 1-10 ma., 1-150 Hz. and 0.1-1.0 msec. duration via a dual channel photon - coupled constant current source. Stimulation was carefully adjusted to activate each fiber group sequentially in the nerve bundle. In this way a respiratory response was produced by activation of a known nerve population. During the presence of a respiratory response to stimulation, no retching or vomiting could be elicited by stimulation. When the respiratory response was absent, retching or vomiting could be produced readily by activation of selected afferents. In general, respiratory responses occurred at a lower threshold than retching or vomiting, so that these effects could be clearly delineated. Thus neural circuits involved with vomiting may have a reciprocal inhibitory interaction with respiratory neurons in the brainstem.

15.12

BRAINSTEM EFFERENTS FROM A LATERAL HYPOTHALAMIC SITE WHICH PRODUCES A DECREASE IN CORONARY BLOOD FLOW. A.C. Bonham*, L.D. Wilkin*, J.M. Arthur*, D.D. Gutterman*, G.F. Gebhart, M.L. Marcus, M.J. Brody. Dept. of Pharmacology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242

We recently reported 1) a decrease in coronary blood flow (CBF) from electrical stimulation (ES) in cat lateral hypothalamus (LH), requiring ipsilateral cardiac nerves and 2) retrogradely-transported (RT) Fast Blue dye showed projections to the active site in LH from the paraventricular nucleus (PVN) and dorsal raphe nucleus (DRN). To study efferent projections we microinjected horseradish peroxidase-wheat germ agglutinin (HRP-WGA) into the active site in LH. RT HRP-WGA confirmed projections from PVN and DRN to the active site. Anterogradely-transported HRP-WGA labelled nerve terminals in ipsilateral periaqueductal gray (PAG) extending caudally to the level of the inferior colliculus. From LH injection site, fibers coursed posteriorly and laterally between red nucleus and substangia nigra, ultimately sweeping both dorsomedially and to lesser extent medially to terminate in ipsilateral PAG. Projections appeared to be in part reciprocal since some retrogradely labelled perikarya were observed in ventromedial PAG. The results indicate that coronary vasoconstrictor responses, activated from LH, depend on intermediate projections to PAG which presumably give rise to neural systems at brainstem and cord level that regulate sympathetic outflow to the coronary circulation. (Supported by HL-14388, HL-32295, HL-07001 and an AHA Medical Student Research Fellowship).

EXERCISE I

16.1

THE EFFECT OF HEAT STRESS ON EXERCISING MUSCLE BLOOD FLOW IN MAN. G.K.Savard, B.Nielsen, J.Laszczynska* B.Saltin, B.Larson*. August Krogh Institute, University of Copenhagen, DK-2100, Denmark.

The effect of heat stress on exercising muscle blood flow was determined using a seated bicycle exercise. Wearing a water-perfused suit, subjects exercised at 60-65%m$\dot{V}O_2$ for a total of 75 min (control 0-25min, hot (25-50min) and cold (50-75min) perfusion of the suit. During heating, $\bar{T}_{sk}$ increased to 38.5°C and core temperature reached 39.5°C. No significant changes in whole body $\dot{V}O_2$ (2.5l/min), mean arterial blood pressure (105-110mmHg) or cardiac output (20-22 l/min) measured using acetylene rebreathing, were observed between control, hot and cool periods, whereas during heating, HR increased an average of 30 bpm up to 170 bpm. Skin blood flow, measured by venous occlusion plethysmography, increased to 25ml/100ml/min during heating. Leg blood flow, measured in the femoral vein of the exercising leg using the thermodilution technique (T_{sk} of the leg kept at 25°C), was 5.87, 5.58 and 5.74 l/min in control, hot and cool periods, respectively, and no significant differences were observed between periods in $(a-v)O_2$. In conclusion, a heat stress which more than doubles skin circulation and increases core temperature to 39.5°C, does not seem to affect active muscle blood flow, which, under these conditions, is maintained.

16.2

NEGLECTED ROLE OF LOW TEMPERATURE AND HUMIDITY IN THE PERFORMANCE OF MAN AT EXTREME ALTITUDE. S. Lahiri. University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

The air at high altitude is thin, cold and dry. Ever since the work of Paul Bert the inevitable role of barometric pressure in the O_2 supply at extreme altitudes has been well recognized, but the role of air temperature as a determinant has not received enough attention. The purpose of this presentation is to make a theoretical assessment of this likely role of temperature. Man's successful ascent of Mt. Everest without supplemental O_2 in recent years has led to new measurements and reassessment of the factors which limit performance at great altitudes. The recently measured barometric pressure on the Everest summit on October 24, 1981, was 253 Torr instead of the predicted 236 Torr. At 37°C the calculated inspired PO_2 gain of 3.6 Torr was thought to have made the critical difference. However, the temperature equilibration may not occur when the subject breathes a very cold, dry air at these altitudes at a very high rate which seems to be a prerequisite for a lowlander to succeed. If so, a lower pulmonary air temperature than the core could significantly contribute to O_2 flow in the following ways: lowering PH_2O and consequently raising inspired PO_2; increasing alveolar O_2 fraction; and a greater O_2 loading of pulmonary capillary blood due to a greater affinity. Rewarming in the tissues would then facilitate unloading of O_2 from the blood. The benefit will depend on the magnitude of the initial cooling of air in the lungs.

16.3

EFFECT OF ENDURANCE TRAINING ON PUTATIVE DETERMINANTS OF $\dot{V}O_2$ DURING HEAVY EXERCISE R. Casaburi, T. Storer*, I. Ben-Dov*, B. Beekley*, Q. Sims*, K. Wasserman. Harbor-UCLA Medical Center, Torrance, CA 90509

When moderate exercise begins, oxygen uptake ($\dot{V}O_2$) reaches a steady-state within 3 min. However, with heavy exercise, $\dot{V}O_2$ continues to rise beyond 3 min. ($\dot{V}O_2$ drift). We sought to identify factors contributing to $\dot{V}O_2$ drift. Ten young, healthy subjects performed cycle ergometer tests of 15 min. duration at 4 constant work rates: 90% of the anaerobic threshold (AT) and 25, 50 and 75% of the difference between $\dot{V}O_2$max and AT. Time courses of $\dot{V}_E$, $\dot{V}O_2$ and rectal temperature (T_R) were recorded. Blood lactate was measured at the end of exercise. Eight weeks of daily cycle ergometer endurance training improved average $\dot{V}O_2$max by 15%. Subjects then performed 4 tests identical to pre-training studies. For above AT tests, training resulted in substantial reductions in end-exercise lactate and in $\dot{V}O_2$ drift; a moderate attenuation in T_R rise was also seen. For the 32 studies in which the subject tolerated 15 min. of exercise, the training-induced decrease in $\dot{V}O_2$ drift was well correlated with the decrease in end-exercise lactate (r=0.81), but not with the attenuation in T_R rise (r=0.26, NS). Training also decreased the drift in $\dot{V}_E$ observed for work rates above AT. This was also well correlated with the decrease in end-exercise lactate (r=0.78), but not with the attenuation in T_R rise (r=0.25, NS). Thus, the slow rise in $\dot{V}O_2$ during heavy exercise seems linked to lactate metabolism, though a component dictated by lactate-stimulated work of breathing cannot be ruled out.

16.4

CHANGES IN HEART RATE FLUCTUATIONS IN EXERCISE TRAINING K.C. Lee*, Y.H. Ahn*, R. Belza*, S. Elliot*, R. Howard*, E. Jarboe*, J. Krebs*, P. McGinnis*, E. Sames* (SPON: D.C. Randall). Dept of Phys. & Biophy., Uni. of KY, Lex. KY 40536

In studying the heart rate (HR) fluctuations with power spectrum analysis, Akselrod et al (Sci 213:220, 1981) showed that the sympathetic (Sym) and parasympathetic (PSym) systems modulate different frequency peaks (FP). The high FP (.3-.5Hz), the respiratory rate, and the mid FP (.1-.2Hz) are modulated by the PSym. The low FP (<.1Hz) is modulated by both the Sym and PSym with the FP at .04Hz strongly influenced by the renin-angiotensin system in dogs. We examined changes in fluctuations of HR recorded at rest due to exercise training by this method.

The training protocol was running 3-4 miles in 45-60 min for 14 days. The HR's of 3M and 1F, 24-27 years old, were recorded before and after this training protocol. The respiratory rate was paced with a metronome at .53Hz during HR recording. 128 sec of data were used to compute the frequency spectrum from .0078 up to 320Hz.

The results showed that the FP at .53 and .04Hz were significantly enhanced after training, suggesting the PSym and renin-angiotensin modulations of HR fluctuations were enhanced and suppressed, respectively. The results also showed that the lower the pretrained mean HR, the less the training bradycardia; the larger the training bradycardia, the greater the increase in HR fluctuations.

16.5

ELECTROPHYSIOLOGICAL ANALYSIS OF THE FASTIGIAL NUCLEUS (FN): A NUCLEUS INVOLVED IN THE CARDIOVASCULAR RESPONSE TO EXERCISE. **T.G. Bedford*, R.J. Person* and K.J. Dormer. Univ. of Oklahoma HSC, Oklahoma City, OK 73190.**

Arterial blood pressure moderately declines during treadmill exercise in the dog after lesions of FN in the cerebellum. The location(s) of the interaction of efferent FN projections with cardiovascular regions of the brainstem active in exercise are not known. We sought to first explore regions of the brain stem that might be active in cardiovascular regulation and to substantiate if polysynaptic projections from FN could be located electrophysiologically. Dogs were anesthetized with α-chlorolose. FN was located by a characteristic pressor response to electrical stimulation. Tungsten microelectrodes were utilized to search in the ventral portion of the lateral medulla-pons for cells that responded to FN stimulation primarily, and to various autonomic and somatic inputs. Results are listed as number of cells responding to FN stimulation that also responded to other inputs over the number of cells tested for each manuever. Cardiovascular 3/11, respiratory 2/10, somatic 3/3, spinal cord stimulation 13/20, vagal stimulation 8/17, spontaneity 18/29. Some of these cells responded to multiple inputs. No cells were antidromically fired from the spinal cord dorsal lateral funiculus but could have been monosynaptic from FN. These results indicate that FN projects to the ventral lateral medulla and pons and can interact with several modalities of inputs. Supported by NIH-HL24082.

16.6

FAT METABOLISM FOLLOWING PROLONGED WALKING: THE INFLUENCE OF PERCENT BODY FAT AND OXYGEN UPTAKE CAPACITY. L.A. Kaminsky*, R.G. Knowlton*, R.M. Perkins, III*, and R. K. Hetzler*. (Spon: M.N. Sawka). Northeastern Illinois Univ., Chicago, IL 60625 and Southern Illinois Univ., Carbondale, IL 62901.

Relationships of percent body fat and peak $\dot{V}O_2$ with serum free fatty acid (FFA) and glycerol (GOL) responses were investigated using multiple regression techniques in 14 males during one hour of passive recovery following a one hour walk (36% of peak $\dot{V}O_2$). The subjects' mean body fat was 17.9% (range 7.5-30.0) and mean peak $\dot{V}O_2$ was 47.7 ml·kg^{-1}·min^{-1} (range 35.1 - 66.3). FFA and GOL concentrations were corrected for plasma volume changes. During the walk no significant differences in serum FFA response could be attributed to either percent fat or peak $\dot{V}O_2$. However, there was a tendency ($p=0.074$) for individuals with higher peak $\dot{V}O_2$'s to have greater elevations in serum GOL during exercise. Immediately following exercise FFA concentrations increased transiently. Subjects with higher $\dot{V}O_2$'s had greater peak FFA concentrations and subsequently cleared the excess FFA from the blood more quickly ($p<0.05$). A similar relationship was found between peak $\dot{V}O_2$ and the recovery FFA·GOL^{-1} molar ratio response ($p<0.05$). It was concluded that the different post-exercise FFA responses, as related to peak $\dot{V}O_2$, were not due to different rates of lipolysis. It was also indicated that more FFA were utilized in the one hour recovery period in individuals with higher peak $\dot{V}O_2$'s. There was no evidence that percent body fat was related to the post-exercise fat metabolism.

16.7

REDUCED RUNNING ENDURANCE IN GLUCONEOGENESIS-INHIBITED RATS. Henry B. John-Alder, Richard McAllister*, and Ronald L. Terjung. SUNY-Upstate Medical Center, Syracuse, NY 13210.

This study investigated the functional significance of gluconeogenesis during submaximal activity requiring about 80% of aerobic capacity in untrained and endurance-trained rats. Gluconeogenesis was inhibited by 3-mercaptopicolinic acid (3-MPA). 3-MPA reduced endurance 26% ($p<0.001$) in trained and 32% ($p<0.01$) in untrained rats. At exhaustion, trained, 3-MPA-treated rats (Group I) were severely hypoglycemic and had only 3% of their resting liver glycogen. Trained, sham-injected rats (Group II) sacrificed at 130 min, the mean exhaustion time of Group I, were slightly hyperglycemic and had 23% of resting liver glycogen. Group I at exhaustion had depleted a significantly greater fraction of resting glycogen in fast-twitch white muscle fibers than had Group II at 130 min, while glycogen depletion in fast-twitch red fibers was not different between groups. In Group I, plasma free fatty acids (FFA) were significantly elevated at 90 min but not at exhaustion; in Group II, FFA's were elevated at 90 and 130 min. These data demonstrate that gluconeogenesis makes an essential contribution to activity metabolism not only in trained animals capable of prolonged activity but also in untrained animals capable of sustaining activity for only about 55 min. Supported by NIH Grants AM21617 and AM00681.

16.8

BODY COMPOSITION OF COLLEGIATE WRESTLERS. Roger G. Soule and Matt Orr*. Biola University, La Mirada, CA 90639

During the past four years body composition of the Biola wrestling team has been determined using hydrostatic weighing techniques. Each year the changes in body fat for these populations changed significantly from pre-season to peak-season. The 1984-85 team demonstrated a 2% change.

#	Wgt-kg	% Bdy Fat (10/84)	Wgt-kg	% Bdy Fat (2/85)
1	58.5	9	58.0	4
2	60.8	10	64.4	8
3	63.0	4	62.6	2
4	64.9	4	63.5	4
5	67.6	10	67.6	10
6	68.9	10	67.6	7
7	72.6	8	73.0	9
8	73.9	8	76.6	8
9	78.0	9	77.6	9
10	80.3	8	73.9	3
11	78.5	10	78.5	12
12	85.3	9	84.4	7
13	87.5	14	82.1	8
14	98.9	13	95.9	7

It was demonstrated that the better teams had lower % of body fat pre-season than the average teams. National level competitors maintained a lower % of body fat during the off season than other team members. The data show again that a competitive season of training is sufficient to lower significantly the % of body fat for collegiate wrestlers.

16.9

Weight Distribution and Muscle Utilization During Computer Controlled Walking in the Paralyzed

Jerrold S. Petrofsky, Chandler A. Phillips, Debra M. Hendershot, Roy Douglas and Paul Larson
Wright State University National Center for Rehabilitation Engineering and Louisiana State University School of Medicine, New Orleans.

Three paraplegic subjects (one male and two female) were examined during computer controlled walking at approximately one mile per hour to measure the weight distribution in the body (upper versus lower body) and electromyographic activity of some upper body muscles. Strain gauges were mounted on canes, walkers and in their shoes to measure the instantaneous and average weight. The electromyogram was measured on the triceps, latissimus dorsi and pectoralis major muscles with bipolar silver silver-chloride disk electrodes. Ambulation with walkers required about ten percent higher oxygen cost than voluntary walking at the same speed in three nonparalyzed volunteers. The average weight distribution on the arms during walking with either canes or walkers only averaged about two pounds in the best subject with impulses ranging as high as 15 pounds. There was variation in response between the subjects in the range of the response. In general, however, less than 10% of the body weight was carried through the upper part of the body when walking either with canes or walkers in any of the three subjects examined.

16.10

EFFECTIVE DOSE OF OZONE INHALED DURING EXERCISE AND LUNG INJURY IN RATS. William J. Mautz* (SPON: K. M. Baldwin). Univ. of California, Irvine, CA 92717.

Sprague-Dawley rats (n=10 per group) were exposed in a treadmill to ozone under varying conditions of concentration (range 0.2-0.8 ppm), exposure duration (1.0-3.75 h), and activity (rest, intermittant exercise, or continuous exercise up to 3 times resting metabolic rate). Oxygen consumption ($\dot{V}_{O_2}$) of rats was measured while the animals exercised in the treadmill, and an index of effective ozone dose was estimated as the product of concentration, duration and $\dot{V}_{O_2}$ assuming that $\dot{V}_{O_2}$ was proportional to ventilation at the moderate exercise levels studied. 48 h post-exposure, lungs were removed, fixed, sectioned, and stained. Lung injury was assessed as % area of parenchyma involved in focal lesions induced by ozone. Parenchymal lesion areas were linearly related to concentration and to exposure duration when other effective dose variables were held constant, however exercise had a much greater effect on lesion areas. Constant effective dose exposures at higher exercise intensities and shorter durations resulted in large increases in lesion areas with curvilinear relationships to average $\dot{V}_{O_2}$. In conclusion, exercise ventilation is a more important dose variable than concentration or exposure duration in affecting lung tissue injury from single exposures to low concentrations of ozone. Supported by California Air Resources Board A2-129-33 and Electric Power Research Institute RP-1962-1.

16.11

ENERGETICS OF LOCOMOTION OF CRAWLING CATERPILLERS. Clyde F. Herreid, II, D. A. Sperrazza*, R. B. Weinstein*, and R. J. Full*. SUNY, Buffalo, N.Y. 14260.

Tobacco hornworms, Manduca sexta, pass through their complete life cycle from egg to sphinx moth in about two months at 23°C. After hatching, larvae grow rapidly over three weeks until they reach 8-10 g; during this time their weight specific O_2 consumption ($\dot{V}_{O_2}$) drops rapidly. At their peak mass, the caterpillers stop eating and begin crawling for several hours. In the natural environment, this wandering occurs when the caterpillers search for a pupation site. We measured $\dot{V}_{O_2}$ during the wandering phase using an Applied Electrochemistry S3A O_2 Analyzer. Air was drawn through a polyethylene tunnel in which the caterpillers crawled. Their speed (up to 0.024 $km \cdot h^{-1}$) was a direct function of the locomotion waves along the body and the "stride length" per wave. $\dot{V}_{O_2}$ was directly related to their velocity. Their minimum cost of transport (ml $O_2 \cdot g^{-1} km^{-1}$) was about three times higher than that value predicted for a pedestrian vertebrate.

FLUID AND ELECTROLYTE BALANCE AND CARDIOVASCULAR FUNCTION IN RESPONSE TO WEIGHTLESSNESS

22.1

CARDIOVASCULAR ADAPTATION TO MICROGRAVITY: SIMULATION METHODS. C. Gunnar Blomqvist. University of Texas Health Science Center, Dallas, TX 75235

The opportunities to perform physiological research in space are steadily improving but in-flight studies will always be subject to severe limitations in terms of methodology and numbers of subjects. There is a definite need for methods by which crucial events at microgravity can be reproduced in ground-based laboratories. Brief periods of weightlessness can be achieved during parabolic flight but all simulation methods applicable to extended studies leave residual hydrostatic gradients. Nevertheless, comparisons between cardiovascular data obtained during and after space flight and observations during ground-based studies have established important similarities between space flight and (a) horizontal bed rest, (b) head-down tilt, and (c) upright water immersion. The common denominator of all conditions is a central or headward shift of body fluids. The post-intervention state is uniformly characterized by hypovolemia, orthostatic intolerance, and decreased exercise capacity in the upright position. Less information is available on the accuracy of each simulation method during the phase corresponding to weightlessness. Limited data indicate that head-down tilt and bed rest are more likely to mimic actual in-flight hemodynamic conditions than upright water immersion. The principal application of water immersion may be to induce rapidly a state of cardiovascular dysfunction similar to that present during post-flight readaptation to normal gravity.

22.2

MECHANISM OF HEMODYNAMICS AND WATER-SALT METABOLISM ADAPTATION TO SIMULATED WEIGHTLESSNESS IN MAN. V.E.Katkov*, B.V.Morukov*, V.Ju.Semjonov*. Institut biomedical problems, Moscow, USSR (Spon:H.Bjurstedt

Head-down hypokynesia (HK) and water immersion (WI) were used to simulate physiologic effects of weightlessness. In the first hours of HK pulmonary artery pressure (PAP) was increased while central venous pressure (CVP) remained unchanged. The most important cause of that was an increased pulmonary vessels resistance. The changes observed reveal increase of the left atrium transmural pressure during initial phase of adaptation to HK. At the same time the significant decrease of plasma renin activity (PRA) and concentration of aldosterone in blood were observed. PAP and CVP did not change during the first day of WI as well as the significant decrease of PRA and concentration of aldosterone in blood were not present. Both in HK and WI renal excretion of water and ions was increased and was more pronounced in WI. From the 3-d day of WI and HK CVP and PAP were lower comparing to initial values. Thus, the initial period of adaptation to WI and HK manifests in thoracic blood pool deposition, in negative water-salt balance due to various regulating mechanisms.

22.3

EFFECTS OF GRAVITY ON THE FLUID BALANCE AND DISTRIBUTION IN MAN. Dag Linnarsson, Bo Tedner and Ola Eiken. Karolinska Institutet, S-10401 Stockholm, Sweden.

Marked fluid shifts have been observed in man during space flight and simulated microgravity (head down tilt, HDT). Three different components can be identified: First a rapid cephalad blood shift which is almost instantaneous, second a shift of extravascular fluids from the legs to the blood and upper body which is manifested within hours, and third a net loss of water through the kidneys resulting in a substantial weight loss within days. We have investigated the second phase in eight healthy men during 7° HDT. Segmental fluid distribution and changes in total body fluids were monitored by means of a tetrapolar impedance technique. With this technique blood shifts are not detected, only changes in the interstitial and intracellular fluid volumes.

Leg fluid volume was reduced as an exponentially decaying function of time, with a time constant of 30-40 minutes. During the first two hours after the transition to HDT no simultaneous increase in the fluid volume of the trunk could be detected. This indicates that the fluid removed from the legs then is to be found in the blood. This is also supported by others who have found hemodilution and increased blood volume during similar experimental conditions. Our results also show the feasibility of the impedance technique to monitor fluid distribution and total body fluid changes during μG.

22.4

VOLUME REGULATING HORMONES DURING SIMULATED WEIGHTLESSNESS. Cl. Gharib, A. Güell, M. Cantin. GEMPS 8, avenue Rockefeller 69373 Lyon Cédex 08; CHU Rangueil 31054 Toulouse and Clinical Research Institute of Montreal Qué. H2W 1R7

The absence (or decrease) of the hydrostatic pressure during space flights (microgravity state) or simulations of weightlessness (by immersion, bed rest or head-down tilt) result in a body fluid shift and an engorgement of the central circulation where mechanoreceptors involved in plasma volume regulation are located. Their activation induce the initial (first hours) hormonal response with a decrease in plasma vasopressine, renin and aldosterone and probably an increase of natriuretic factor (Gauer reflex). The prolonged exposure to microgravity leads to more complex and often hypothetical responses : cardiovascular decontitioning, modifications of secretion and circadian rhythms of above cited hormones. After 24 years of studies our knowledge on hormonal and cardiovascular adaptation to space flight is still at the beginning.

22.5

FLUID AND ELECTROLYTE CONTROL IN SIMULATED AND ACTUAL SPACEFLIGHT. C. S. Leach and P. C. Johnson. NASA/Johnson Space Center, Houston, TX 75508

Effects of microgravity on an astronaut's body fluid distribution and electrolyte and hormonal levels have been studied since the early manned space missions. Inflight samples have been available from 1 Gemini, 1 Apollo, 1 Spacelab and 3 Skylab missions (18 astronauts). Bedrested subjects have been used as controls to separate effects of microgravity from those of hypokinesia. These investigations have led to documentation of the physiological perturbations during spaceflight and to a unified theory of causation and response. In microgravity crewmembers have a net loss of body water, sodium and potassium. This seems to be initiated by passive transfer of extracellular fluid resulting in increased central venous pressure (CVP). In response there is decreased thirst, negative water balance and a disproportionate loss of sodium with decreased osmolality. Potassium decreases as aldosterone increases in an attempt to increase serum sodium. CVP decreases below control levels and cardiac chamber size tardily decreases. This new equilibrium state is maintained during flight; it does not change in response to increasingly negative calcium and nitrogen balances. On reexposure to gravity, profound water and salt retention occurs to replete extracellular fluid. Attempts to replete water and salt before leaving microgravity have been partially successful with regard to postural hypotension but have had little effect on CVP, cardiac chamber size or electrolyte dynamics.

22.0

FLUID MOVEMENTS BETWEEN VASCULAR AND EXTRAVASCULAR COMPARTMENTS IN SPACE FLIGHT AND DURING READAPTATION. K. Kirsch. Free Univ. of Berlin.

No abstract submitted

22.6

MECHANISMS FOR NEGATIVE WATER BALANCE DURING WEIGHTLESSNESS: IMMERSION OR BED REST. John E. Greenleaf. NASA Ames Research Center, Moffett Field, CA 94035

The mechanism for the apparent decrease in body fluid volume in astronauts during spaceflight remains obscure. The widespread postulate that the hypohydration is the result of the Gauer-Henry reflex has not been established with measurements on astronauts. An hypothesis is proposed which accounts for fluid - electrolyte shifts during weightlessness. Upon entering orbit, a moderate but transient increase in central venous pressure occurs that is insufficient to activate the Gauer-Henry reflex, but sufficient to stimulate the release of atrial natriuretic peptides. Increased sodium excretion would facilitate some increased urinary water loss. The resulting relatively dilute plasma and interstitial fluids would cause fluid to shift into the cellular space resulting in edema in the head and trunk and inhibition of thirst and drinking. Thus, the negative water balance in astronauts would be caused by a gradual natriuresis and diuresis, coupled with reduced fluid intake, until the proper equilibrium level of total body water is reached. Responses during immersion and bed rest will be discussed as they relate to this hypothesis.

22.0

CARDIORESPIRATORY RESPONSES TO TILT AND LNBP TESTS AFTER EXPOSURE TO SIMULATED WEIGHTLESSNESS V. Katuntsev. Inst. of Biomedical Problems, Moscow.

No abstract submitted.

PULMONARY CIRCULATION: LUNG INJURY

23.1

IS THERE GRAVITY INDEPENDENT REGIONAL HETEROGENEITY OF EXTRAVASCULAR WATER WITH HYDROSTATIC AND PERMEABILITY LUNG EDEMA? R.P. Michel, Lyman Duff Labs, McGill Univ., Montréal, Canada.

In hydrostatic edema, there is gravity dependent lung water accumulation. The hypothesis that a gravity independent gradient also exists was tested in anesthetized dogs with: 1) hydrostatic edema (HE) induced by fluid overload (5 supine, 4 prone); 2) alpha-naphthylthiourea (ANTU)-induced edema (n=4); 3) nitrogen dioxide (NO_2)-induced edema (n=6). Wet/dry weight ratios (W/D) corrected for blood water were measured in frozen middle lobes (ML), and at the top and bottom, and center and periphery (gravity independent) of frozen lower lobes (LL). Results showed ML had smaller W/D ($p<0.05$) than LL in the supine HE group. Regional W/D within LL are tabulated as means ± SE. *$p<0.05$, paired Student t test.

GROUP	TOP	BOTTOM	PERIPHERY	CENTER
HE supine	5.95* ±0.29	7.20* ±0.31	6.43* ±0.27	6.91* ±0.30
HE prone	5.81 ±0.64	5.22 ±0.47	5.40 ±0.60	5.87 ±0.79
ANTU	7.73* ±0.83	8.43* ±1.18	7.92 ±1.21	8.03 ±0.94
NO_2	8.05 ±0.48	8.57 ±0.74	7.86 ±0.64	8.02 ±0.59

There was more edema in dependent areas with supine ANTU and HE, less in dependent areas in prone dogs. More water was present in central than peripheral parenchyma in HE. In conclusion, significant heterogeneity in edema distribution exists, some apparently gravity independent.

Supported by MRC of Canada Grant No. MT-7727

23.2

LUNG PERMEABILITY CHANGES IN NORMOBARIC HYPEROXIA: PROTECTIVE EFFECTS OF SOD AND CATALASE, VITAMIN E OR BUTYLATED HYDROXYANISOLE (BHA).

J.M. Jacobson, J.R. Michael, G.H. Gurtner, Johns Hopkins Medical Institutions, Baltimore, Maryland.

The effects of antioxidants in hyperoxia were studied using isolated whole blood perfused rabbit lung. O_2 exposures were 48 hrs of 100% O_2 with animals being pretreated with polyethylene glycol (PEG) conjugated Superoxide Dismutase and Catalase or BHA (100 mg/kg x 3 days) or Vitamin E (50 mg/kg x 2 days). Pulmonary artery pressure (PPA), lung weight gain (W) and alveolar-capillary permeability to aerosolized Tc-DTPA (diethylene-triamine penta acetate, MW=492) and FITC-Dextran (MW=7,000) were measured during the first 30 minutes of perfusion. After hyperoxia, PPA was unchanged from control levels of 8± 3 mmHG; W was increased 2.2 times over control levels of 0.05 ml/min ($p<.001$); alveolar-capillary permeability to DTPA was increased 8 fold ($p<.001$) and permeability to Dextran was increased 3.4 fold ($p<.01$). Pretreatment with PEG-SOD and PEG-Catalase during the 48 hour O_2 exposure prevented the lung weight gain ($p<.001$), increased transfer rate of Dextran ($p<.01$) and DTPA ($p<.05$). Pretreatment with either BHA or Vitamin E also prevented the lung weight gain ($p<.001$) and increased rate of transfer of Dextran ($p<.001$) and DTPA ($p<.001$) decreased. We conclude that the pretreatment with antioxidants was as effective as pretreatment with SOD and Catalase in preventing hyperoxia induced injury.

23.3

EFFECT OF DIETHYLCARBAMAZINE ON ENDOTOXIN-INDUCED RESPIRATORY FAILURE. N.C. Olson* and D.L. Anderson* (SPON: C.E. Stevens). North Carolina State University, Raleigh, NC 27606

We evaluated the effects of diethylcarbamazine citrate (DEC), a putative inhibitor of leukotriene biosynthesis, on the porcine cardiopulmonary response to E. coli endotoxin (E). E was infused into anesthetized and paralyzed 10-14 week old pigs at 5 μg/kg the first hr followed by 2 μg/kg/hr for 3.5 hrs. In a second group, DEC (200 mg/kg) was infused IV during the 30-45 min period prior to E + 44 mg/kg/hr during endotoxemia. DEC alone caused a transient increase in cardiac index (CI) and aortic pressure, but these values returned to baseline prior to E. In endotoxemic pigs, the phase 1 (i.e., 0-2 hrs) granulocytopenia, thrombocytopenia, decreased dynamic lung compliance (DLC) and increased mean pulmonary artery pressure, pulmonary vascular resistance (PVR), and alveolar-arterial oxygen gradient ($\Delta A\text{-}aO_2$) were not modified by DEC. During phase 2 (i.e., 2-4.5 hrs) of endotoxemia, the decreased CI, increased PVR, and granulocytopenia (at 4.5 hrs) were attenuated approximately 30% by DEC. However, DEC did not modify the phase 2 thrombocytopenia, decreased DLC, or the increased $\Delta A\text{-}aO_2$. The E induced-increase in postmortem gravimetric lung water and bronchoalveolar lavage albumin content were also not modified by DEC. We conclude that leukotrienes may contribute to the phase 2 decrease in CI, increased PVR and granulocytopenia, but the increases in alveolar-capillary permeability, lung water, and impaired gas exchange may be largely due to other mediators.

23.4

NEUTROPENIA DOES NOT DIMINISH THE HYPEROXIC INJURY TO THE ALVEOLAR EPITHELIUM. M.J. Laughlin*, L. Wild*, P. Nickerson* and S. Matalon. SUNY at Buffalo, Buffalo, NY 14214

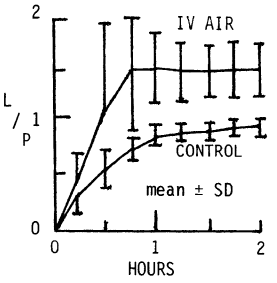
We sought to determine whether neutropenia would diminish the increase in alveolar permeability due to hyperoxia. Two groups of eight rabbits each received either a single injection of 2 mg/kg of nitrogen mustard (NM) or an equal volume of saline (SA) and were exposed to 100% O2 for 64 hrs. At the end of exposure the number of PMN in one ml of blood and 0.025 mm^2 of lung tissue were 30±13 and .37±.1 in the NM group vs. 6500±491 and 6.3±.5 in the SA group. We then instilled 15 ml of saline containing Co-57 cyanocobalamin (r=6.5 A) I-131 cytochrome c (r=17 A) and I-125 albumin (r=35 A) into the right lobe and measured their rate of disappearance from the alveolar space. The values of solute flux of cyan. and cyt.c (min^{-1}) were 33±5 and 7±2 for the NM and 29±5 and 6±1 for the SA group respectively. These variables were ninefold higher than their corresponding values in animals exposed to room air. We concluded that significant depression of PMN does not diminish the hyperoxic alveolar injury.

(Supported by NIH grant HL31197)

23.5

INTRAVENOUS (IV) AIR EMBOLI OPENS ADDITIONAL PATHWAYS FOR THE CLEARANCE OF SOLUTES FROM THE LUNGS OF SHEEP. B.T. Peterson Univ. of Texas Health Center at Tyler, Tyler, Texas 75710.

To determine if lung injury alters the clearance pathway of solutes from the air spaces of the lungs, 6 anesthetized sheep received aerosolized 99mTc-labeled diethylenetriamine pentaacetate (DTPA) for 2 minutes and the DTPA concentration in lymph and plasma was measured for 2 hours. In 3 control sheep the lymph to plasma concentration ratio (L/P) during the last 30 minutes was 0.98 ± 0.07. This supports the conclusion by Coates, et al that DTPA normally passes directly into the blood and then equilibrates with the interstitium (Am. Rev. Resp. Dis. 127:299). The 3 sheep that received IV air at 0.3 ml/kg/min for 2 hours before the DTPA deposition had an L/P ratio of 1.6 ± 0.3 after 45 minutes ($p<0.05$ compared to control). This probably was not due to any transient phenomena because the plasma concentration changed only 6% during the last 30 minutes of the study. The L/P ratio greater than 1 implys that lung injury caused by IV air opens additional pathways that give the DTPA direct access to the pulmonary interstitium.



23.6

CATALASE ATTENUATES AIR EMBOLI-INDUCED INCREASED LUNG VASCULAR PERMEABILITY IN UNANESTHETIZED SHEEP. Michael R. Flick, Shawn A. Milligan*, and John M. Hoeffel*. University of California, San Francisco, California 94143.

We tested the effect of scavenging hydrogen peroxide with the enzyme catalase (CAT) on the leukocyte-dependent lung vascular injury caused by intravenous air emboli. In 6 unanesthetized sheep we measured lung lymph flow ($\dot{L}$) and protein concentration [L], pulmonary arterial pressure (Ppa), plasma protein concentration [P], and arterial leukocytes, and calculated pulmonary vascular resistance (PVR). After a 2h baseline we infused air i.v. at a constant rate to increase PVR 3 fold. We repeated the experiment after giving bovine liver CAT 50 mg/kg i.p. over the 24h prior to air emboli. The mean data included:

Condition	Ppa (cmH_2O)	PVR ($dyn\text{-}s\text{-}cm^{-5}$)	$\dot{L}$ (ml/h)	[L]/[P]	Leukocytes (cells/nl)
Untreated					
baseline	17	159	8.3	0.59	8.3
emboli	35	492	28.6	0.58	7.2
CAT Treated					
baseline	17	175	6.8	0.64	9.9
emboli	36	539	18.8	0.60	7.5

CAT treatment resulted in attenuation of expected increased vascular permeability. We conclude that hydrogen peroxide or its products (such as the hydroxyl radical) may play a role in the mechanism of air emboli-induced acute lung injury in sheep. [Supported by HL-19155 (Pulmonary Vascular SCOR)]

23.7

STARLING-LYMPHATIC INTERACTION DURING PROTEIN RICH HYDROTHORAX REABSORPTION IN ANESTHETIZED RABBITS. D. Negrini* and G. Miserocchi*. Ist. Fisiologia Umana Univ., Milano, Italy. (SPON: A.E. Taylor, Univ. of South Alabama, Mobile, AL 36688)

The time course of volume and protein reabsorption from 2ml of homologous plasma injected into the right pleural cavity of 46 anesthetized spontaneously breathing supline rabbits were studied. Pleural liquid volumes and protein contents were determined after 5, 36, 90, and 150 minutes following the injection. Pleural liquid volume decreased according to:

$$V_{tot} = 0.84 + 0.5e^{-0.018t} \quad \ldots 1$$

The quantity of globulin is described as:

$$Q = 1.05 - 1.49e^{-0.04t} \quad \ldots 2$$

No significant changes in control volume and protein content were observed up to 90 minutes from anesthesia and induction. Assuming a major lymphatic globulin clearance and no filtration during the reabsorption period, the decay rate of pleural liquid volume will equal that of the globulin quantity. The decay rate of the globulin quantity is given by the exponential in equation 2. Accordingly, a reasonable estimate of lymphatic flow $(dV/dt)_L$ will be: $(dV/dt)_L = -0.04(V_{tot}-0.84)$. The Starling filtration flow was calculated as: $(dV_{tot}/dt) - (dV/dt)_L$. The lymphatic drainage and the Starling filtration flow decreased 15-fold at 150 minutes, the former being twice the latter.

23.8

THE ROLE OF GRANULOCYTES IN THE FORMATION OF HYALINE MEMBRANES. Mori, S, Kawano, T, Cutz, E, Cybulsky, M, and Bryan, AC. Research Institute, Hospital for Sick Children, Toronto, Canada

Hyaline membranes are traditionally believed to be the result of pulmonary barotrauma, but our results suggest that the damage is done by degranulation of neutrophils. Four New Zealand white rabbits were depleted of neutrophils (<100/cu mm) by 1.75 mg/kg of Nitrogen Mustard IV and four served as controls. The animals were anesthetized, paralysed and tracheostomized. ^{125}I-Albumin (20μCi) was injected IV. The lungs were repeatedly lavaged with normal saline to deplete surfactant. They were ventilated with a Harvard pump: FIO_2 of 1.0, VT = 12 ml/kg, PEEP = 5 cm H_2O. Blood gases were measured every 30 minutes. The rabbits were sacrificed after 4 hours ventilation. The lungs were lavaged and the radioactivity measured. A separate series of animals were done for pathology.

*P<0.001	Final PaO_2 (mm Hg)	Final MAP (cm H_2O)	alveolar protein leak	Hyaline membrane
Control	83.0±19.6*	16.5±1.4*	5.20±1.58*	extensive
WBC depleted	402.0±99.1*	10.7±2.0*	0.63±0.46*	scanty

These results suggests that products of neutrophil degranulation such as oxygen free radicals and proteases are the ultimate vectors of lung damage in the surfactant deficiency lung.

23.9

EFFECT OF ALVEOLAR HYPOXIA ON THE DISTRIBUTION AND SITE OF PULMONARY VASCULAR RESISTANCE IN LAMBS. T.A. Hazinski* & K.A. Kennedy* (SPON: H. Sundell) Dept. Ped., Vanderbilt Univ., Nashville, TN.

Alveolar hypoxia increases pulmonary vascular resistance (PVR)in lambs and sheep but for unknown reasons increases lung lymph flow only in lambs (Biol Neonate 38: 1980). We tested the hypothesis that hypoxia increases PVR beyond fluid exchange vessels in lambs. In 5 awake lambs, we measured pressure in the aorta, pulmonary artery (PA), pulmonary wedge position (PW), left atrium (LA), and pleural space. We measured cardiac output (CO) and computed total PVR (PVRT) as well as the PVR of the segment lying between PW and LA, and termed this PVR of the distal segment (PVRDS). We measured the above variables while the lambs breathed air and after 8 minutes of breathing 12% O_2. Pressures were referenced to pleural. Data are shown below (mean ± 1 SD, * indicates $p<.05$):

	PA	PW	LA	(PW-LA)	PVRT	PVRDS
Air	18±3	3±2	2±2	1±1	5.40±1.2	.54±.23
12% O_2	31±3*	8±3*	1±2	7±2*	8.02±2.4*	2.02±.46*

The percent of PVRT attributable to the distal segment increased from 9.8 ± 5% to 24.8 ± 6%. In 2 lambs with lung lymph fistulae and left atrial balloons, we measured lymph flow and L/P ratio for 2 h of hypoxia: lymph flow increased and L/P ratio fell. Then LA pressure was increased to 1 torr less than PW: no further changes in lymph flow or L/P ratio occurred. We conclude that in lambs, alveolar hypoxia increases PVR distal to fluid exchange vessels probably by causing pulmonary venous constriction. (Newborn Lung SCOR & BRSG #RR054524)

23.10

CORRELATION OF CHANGES IN ARTERIAL OXYGENATION AFTER ACUTE LUNG INJURY WITH CHANGES IN REGIONAL PULMONARY BLOOD FLOW AND LUNG WATER MEASURED BY POSITRON EMISSION TOMOGRAPHY. D.P. Schuster* and G.F. Marklin* (SPON: A. Roos). Washington University, St. Louis, MO. 63110

We have recently reported methods for measuring regional pulmonary blood flow and extravascular water using positron emission tomography (PET) and oxygen-15 labelled radionuclides (ARRD 129:329, 348, 1984 and 131:400, 1985). Since the correlation of gas exchange abnormalities after acute experimental lung injury with global measurements of extravascular water has been poor, we investigated whether the correlation would be improved if regional measurements of lung water and blood flow were made with PET. In nine anesthetized dogs, a focal lung injury was induced by administering oleic acid into a lobar pulmonary artery. On average, blood flow in the injured area decreased 54% and extravascular water increased 117% from baseline values. Increases in the alveolar-arterial PO_2 (A-a) gradient correlated best with quantitative measurements of residual blood flow to the injured area (R^2=0.69). The correlation was significantly improved (R^2=0.82) by adding in the influence of changes in mixed venous PO_2. Regional changes in extravascular water by themselves did not correlate well with changes in the A-a gradient, and also did not add significantly to the results of the multiple regression analysis. The results suggest that PET techniques can be used to examine the influence of pulmonary blood flow and extravascular water on gas exchange after acute lung injury.

23.11

PULMONARY MICROVASCULAR RESPONSE TO FAT EMBOLISM IN AWAKE SHEEP. K.E. Burhop*, W.M. Selig*, and A.B. Malik. Dept. of Physiology, Albany Medical College, Albany, New York 12208.

We examined the alterations in pulmonary transvascular fluid and protein exchange following i.v. injection of fat emboli (bone marrow suspension, BMS) in awake sheep prepared with lung lymph fistulas. BMS injection (0.2 ml/kg/15 min) caused a rapid increase in mean pulmonary artery pressure (P_{pa}) from 18.0±1.0 to 25.0±2.6 mmHg. Pulmonary lymph flow (Q_{lym}) increased steadily from 6.70±0.96 to 21.62±3.09 ml/hr (120 min post-BMS) with no change in the lymph-to-plasma protein conc. ratio (L/P). Changes in lung vascular permeability were evaluated by elevating pulmonary microvascular pressure (left atrial balloon catheter inflation) at 120 min post-BMS. Q_{lym} increased to a steady state mean value of 42.46±2.99 ml/hr and the L/P decreased to a steady state value of 0.48±.03. The protein reflection coefficient (σ=1-L/P) decreased from a control mean of 0.70±0.01 to 0.52±0.03. Heparin pretreatment (700 U/kg) enhanced the BMS-induced increases in P_{pa} and Q_{lym} and caused a greater decrease in the reflection coefficient. Therefore, BMS increases lung vascular permeability and pretreatment with heparin may potentiate the hemodynamic and permeability changes. (Supported by HL-17355; HL-26551; and HL-07529).

23.12

ENDOTOXIN REDUCES LUNG OXYGEN TOXICITY IN LAMBS. K.A. Kennedy,* T.A. Hazinski,* & T.N. Hansen (SPON: H. Sundell) Dept. Ped., Vanderbilt Univ., Nashville, TN; & Dept. Ped., Baylor Col. Med., Houston, TX.

In rats, a large dose of endotoxin reduces pulmonary oxygen toxicity by augmenting the synthesis of lung antioxidant enzymes. To determine the effect of endotoxin on oxygen-induced lung injury in lambs, we administered 0.75 µg/kg of E. coli endotoxin intravenously to 4 lambs prior to exposing them continuously to >95% O_2. Vascular and pleural pressures, cardiac output, and blood gases were measured before and every 24 h in O_2; 2 lambs had lung lymph fistulae. Four other lambs, 2 with fistulae, served as controls. Hemodynamic and gas exchange variables at 72 h are shown below (mean ± 1 SD, *$p<.05$).

	AO	PA	LA	CO	pH	pCO_2	pO_2
Endotoxin (n=4)	84±8	15±3	2±1	318±37	7.38±.10	45±4	443±25
Control (n=4)	80±5	16±3	3±3	350±40	7.19±.14*	77±23*	335±109

During the 4th day in O_2, lymph protein clearance (lymph flow x L/P ratio) increased in control lambs by 334% above baseline values but increased by 162% in the endotoxin-treated lambs. The control lambs died of respiratory failure at 87 ± 11 h, while the endotoxin-treated lambs died at 140 ± 7 h ($p<.05$). Lung antioxident enzymes were not different in the 2 groups, but the endotoxin-treated lambs had a higher ratio of reduced glutathione to oxidized glutathione (200:1 vs 32:1). We conclude that a single small dose of endotoxin reduces oxygen-induced lung injury in lambs but does not augment antioxident enzyme synthesis. We speculate that endotoxin may reduce free radical production. (Newborn Lung SCOR & BRSG #RR054524)

NEUROBIOLOGY: FROM INVERTEBRATES TO HUMAN

24.1

EXCITATION AND INHIBITION OF MUSCLE CONTRACTION IN Hymenolepis diminuta (CESTODA). C.S. Thompson and D.F. Mettrick. Dept. Zoology, University of Toronto, Toronto, Ontario, M5S 1A1.

Spontaneously occurring and evoked contractions of the longtudinal musculature of Hymenolepis diminuta were measured in whole worms and in small sections of worms. In sections of worms, but not in intact worms, acetylcholine (Ach) and Ach agonists (nicotine, muscarine, carbachol) inhibited contraction while Ach antagonists (atropine, scopolamine, α-bungarotoxin, d-tubocurarine chloride) stimulated contraction. Acetylcholinesterase stimulated contraction while a cholinesterase inhibitor , eserine, inhibited contraction. The neurotransmitters epinepherine, norepinepherine, glycine, gamma-aminobutyric acid (GABA), octopamine, and dopamine had no effect on contraction. 5-Hydroxytryptamine (serotonin) produced variable effects on contraction, most commonly increasing the frequency, but not amplitude, of contraction. Glutamate produced sustained contractures in high concentration and rhythmic contractions resembling those recorded from the intact animal in lower doses. Aspartate slightly stimulated contraction at high concentrations. These data suggest that Ach is an inhibitory neurotransmitter and glutamate is an excitatory neurotransmitter in cestode longitudinal musculature.

24.2

THE ROLE OF ACETYLCHOLINE IN Fasciola hepatica. S.C. Sukhdeo and D.F. Mettrick. Dept. Zoology, University of Toronto, Toronto, Ontario, M5S 1A1.

Acetylcholine is a putative inhibitory neurotransmitter in Fasciola hepatica, a trematode parasite of vertebrate bile ducts. Acetylcholine and other cholinergic agonists, including carbachol and nicotine, specifically decrease the amplitude and frequency of contractions and baseline tension. The cholinesterase inhibitor, eserine, also significantly inhibits contractions. Atropine, a cholinergic antagonist, stimulates contractions and reverses the inhibitory effects of the agonists. Acetylcholine and its associated enzymes were measures with radiochemical extraction assays in the parasite and acetylcholinesterase activity was localized at the light and electron microscope level. The results indicate that acetylcholine satisfies most of the criteria for neurotransmitters in this parasite. This study was supported by NSERC grant to A4667 to DFM.

24.3

HORMONAL CONTROL OF ECDYSIS IN RHODNIUS PROLIXUS. E.J. Ampleford. York University, Dept. of Biology, Downsview, Ontario CANADA.

A circadian system times ecdysis in the insect Rhodnius prolixus. The daily oscillation in the level of the steroid moulting hormone (ecdysteroid) is also modulated by a circadian system. Both these rhythms can be abolished by prolonged (2 week) exposure to constant light (LL). 20-Hydroxyecdysone (HE) injected into animals reared in LL is capable of inducing an ecdysis rhythm in addition to influencing the phase of the free-running rhythm generated by L/D to DD transfers. Therefore HE can act on the circadian system timing ecdysis. The timing system is located in the brain since gated ecdysis is abolished by brain removal and restored by brain implantation. Therefore ecdysis would appear to be elicited by hormonal means. Bursting patterns of electrical activity, recorded from the neurohaemal area of the brain, are correlated with the final decline in ecdysteroid. Head extracts prepared from pharate adults are capable of stimulating premature ecdysis. Therefore the control of ecdysis in Rhodnius would appear to be accomplished by ecdysteroids influencing the timing of the release of some factor from the head.

24.4

UNMASKING OF ACTION POTENTIALS IN INSECT OOCYTES. M.J. O'Donnell* (SPON: R.G. Chiang), Department of Biology, York University, Downsview, Ontario, Canada, M3J 1P3.

I have previously shown that calcium action potentials (AP's) can be elicited in response to depolarizing current injection in the developing oocytes of a blood-feeding hemipteran, Rhodnius prolixus. In oocytes from three other species of insect (Locusta migratoria, Periplaneta americana, Hyalophora cecropia) AP's cannot be elicited under similar conditions, although small anode-break responses have previously been found for Locusta oocytes. Injection of depolarizing current after application of potassium channel blocking agents (barium, 4-amino pyridine, tetraethylammonium chloride) to oocytes of these three species resulted in the production of AP's lasting as long as 40 s. These AP's were inhibited by agents such as lanthanum (1 mM) which block voltage-dependent calcium channels in other systems. In conjunction with previous studies on Rhodnius oocytes, these results suggest that calcium channels are common to insects with different types of ovarioles from several orders. However, in Locusta, Periplaneta and Hyalophora, the opening of calcium channels in response to depolarization appears to be masked by a high conductance to potassium that tends to restore membrane potential to the resting level. The results will be discussed in relation to events during fertilization and early development in insect embryos. Supported by NSERC (Canada) grants to M.J. O'Donnell.

24.5

CHANGES IN ELECTRICAL ACTIVITY OF ISOPOD SINUS GLANDS DURING THE MOULT CYCLE. R.G. Chiang and C.G.H. Steel. Department of Biology, York University, Downsview, Ont., Canada, M3J 1P3.

The on-going electrical activity of the sinus gland (SG) was recorded extracellularly from almost intact animals in order to delineate the major times of neurohormone release during moulting. During intermoult, action potentials (APs) occurred in long (20-80s) bursts at high frequency (30-50Hz) suggesting sustained high levels of release. At the initiation of premoult, this activity decreased markedly (burst duration, less than 15s; frequency of APs, less than 10Hz) suggestive of the arrest of release of a moult-inhibiting hormone. Activity of the SG increased again during premoult, in parallel with increases in ecdysteroid titres (measured by RIA) and calcium storage. SG activity decreased in late premoult, increased prior to ecdysis, then dropped to very low levels at ecdysis. SG activity increased as calcification occurred after ecdysis. These results suggest the occurrence of major release times in each moult cycle. Each may involve the release of several hormones, for several types of AP can be identified and the SG contains at least 5 types of terminal. This is the first demonstration of a relationship between neurohormone release from the SG in situ and physiological events dependent on SG hormones.
Supported by a NSERC operating grant to C.G.H.S..

24.6

PHOTOPERIODIC MANIPULATION OF RELEASE OF PROTHORACICOTROPIC AND MOULTING HORMONES IN MALE AND FEMALE RHODNIUS PROLIXUS. Carl A. Knobloch and Colin G. H. Steel. York University, Toronto, Ontario, Canada, M3H 1P7.

The timing of cessation of release of prothoracicotropic hormone (PTTH) from the brain was determined by decapitation at 4 hr intervals after feeding in 5th instar R. prolixus maintained in 12L:12D or LL light regimes. PTTH release was deemed complete in an individual at the time of decapitation only if new cuticle was secreted. In 12L:12D PTTH release was completed over the 24hrs of day 5 or day 6 after feeding in females and males respectively. In LL PTTH release was completed over a broad period of 5-8 days after feeding in both sexes. Decapitation prior to these times resulted in a rapid, permanent decrease in haemolymph moulting hormone (MH) titer (ecdysteroids measured by RIA) in both sexes. Decapitation later did not prevent increases in MH normally associated with cuticle formation. These results demonstrate a relationship between timing of release of PTTH and increases in MH titers, and indicate sex differences in the timing of hormone release. They also show that light influences time of completion of PTTH release suggesting the involvement of a biological clock in hormone release.

24.7

REGULATION OF ACETYLCHOLINE RECEPTOR PATCH SURVIVAL ON NERVE-CONTACTED MUSCLE CELLS IN CULTURE. E.M. Wilson* and M.W. Cohen. McGill Univ., Montreal, Quebec, H3G 1Y6.

One of the cardinal events in the formation of the neuromuscular junction is the accumulation of acetylcholine receptors (AChRs) at the synaptic site. Previous studies in cell culture have shown that the process of AChR localization along nerve contact is accompanied by the disappearance of the high density aggregates or "patches" present elsewhere on the muscle cell surface. The mechanisms of the maintenance and disappearance of these patches remain unclear.

In the present experiments muscle cell cultures prepared from Xenopus embryos were pulse-labelled with rhodamine-α-bungarotoxin (R-αBT), rinsed and maintained in a high concentration of carbachol. Following the addition of dissociated spinal cord cells, survival of AChR patches was observed using fluorescence microscopy. One day later 62-76% of previously labelled and identified patches on non-contacted cells had survived while the corresponding value for contacted cells was only 8-15%. In the presence of carbachol only those AChRs previously labelled by R-αBT could be visualized, thereby excluding any newly synthesized and inserted AChRs. These results therefore indicate that the nerve-induced patch disappearance cannot be explained simply by an interuption of this supply. Further experiments should distinguish between two other possibilities, namely an unanchoring process or increased degradation. (Supported by MRC)

24.8

HOW COLD-BLOODED ANIMALS COMPENSATE FOR CHANGES IN MUSCLE TEMPERATURE. Lawrence Rome. Zoology, Univ. of Tenn. Knoxville, TN

Animals face a difficult challenge: they must generate the same mechanical power to locomote at cold temps as they do at warm temps even though the maximum mechanical power output of their muscles decreases with a Q_{10} of 2-3. They perform this feat by using more muscle fibers and faster fiber types while locomoting at a given speed at low temps.[1] This "compression of the recruitment order"over a narrower range of speeds at low temps enables animals to compensate over a moderate range of speeds. There is an unavoidable reduction at low temps in: 1) maximum sustainable performance[1] (all aerobic fibers are recruited at low speeds, and fast fatiguing anaerobic fibers must be recruited);and 2)maximum performance[2] (the total musculature is recruited at relatively low speeds). To exceed these limitations, animals must increase the mechanical power output of their muscle by adding on more muscle or modifying the muscle proteins to enable each fiber to generate greater power. Carp do both during long term acclimation to the cold and can increase the mechanical power output of their aerobic muscle 2.4-fold, thereby increasing their sustainable swimming speed by 50%.[3] Cold-blooded animals are therefore able to reduce the influence of muscle temp changes on locomotory performance by an acute motor control mechanism and a long-term modification of their musculature.

1. Rome, Loughna, & Goldspink (1984) AJP 247:R272-R279
2. Hirano & Rome (1984) J. Exp. Biol 108: 429-439
3. Rome, Loughna, & Goldspink (1985) Science 228: 194-196

24.9

M WAVE DISTRIBUTION SHIFT AND CONCOMITANT H REFLEX INCREASE WITH CONTRACTION EFFERENCE J.D. Brooke and W.E. McIlroy*. Univ. of Guelph, Guelph,Ont. N1G 2W1

It has been reported that maximal stimulation of autogenous group I afferents and alpha efferents can reflexly-evoke H amplitudes equalling M waves (direct motor activation) when the muscle is contracted. We hypothesized that this was due to both change in the distribution curve of M amplitudes and to H potentiation. Twenty reflex EMG responses were evoked by max. stimulation in the popliteal fossa with soleus contracted at 50% and 75% isometric max, and relaxed, in four subjects. With contraction the M distribution widened and in two subjects shifted to the left, some M amplitudes being <H and reduced by 90% or more compared to relaxed muscle ($p<.05$). H increased ($p<.05$) as M reduced below ca. 40% of relaxed Mmax. Largest H amplitudes matched Hmax in relaxed muscle. Potentiated H and M responses occurred also in tibialis anterior but no short latency heteronymous reflexes were seen in vastus medialis. The altered M distribution may be an indirect tool reflecting motoneuron firing synchrony, if M diminishes due to stimulus-efference collision. Supported by NSERC (Canada).

24.10

TRIGEMINAL INHIBITION OF OCULOMOTOR NUCLEUS CELLS. Paul J. May* and Robert Baker. N. Y. U. Med. Ctr., N.Y., N.Y. 10016

Previous anatomical investigations demonstrated a trigemino-oculomotor pathway originating in cells along the ventral and rostral border of the sensory trigeminal nucleus (n.) and terminating in the caudal oculomotor n. Intracellular recording and staining techniques were used in the cat to study the synaptic potentials elicited by this pathway and the morphology of its target cells. Of the antidromically identified oculomotoneurons displaying bilateral vestibular IPSPs, a portion also showed long latency (3-7msec) bilateral IPSPs from orbital stimulation. Following bipolar stimulation of the main sensory n. of the trigeminal, IPSPs measuring <3mV were elicited with a latency of ≈1.6msec in these motoneurons, whose position along the midline in the caudal third of the nucleus suggests they supply the levator palpebrae. Their somata (D≈25um) give rise to 7-11 thick primary dendrites that branch sparsely, but radiate out past the border of the nucleus. The prominence of this dendritic field bilaterally, above the oculomotor n., may explain the bilaterality of the vestibular and trigeminal inputs. A second class of neurons that could not be antidromically driven from the orbit, but displayed similar trigeminal and vestibular IPSPs was found in the same region. These neurons had smaller somata (D≈20um) and thinner, more sparsely branched dendrites. We suggest that the trigemino-oculomotor pathway is part of a fast defensive reflex inhibiting levator palpebrae motoneurons and interneurons during blinking.

24.11

LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) AND RAPID EYE MOVEMENT SLEEP (REM) IN THE FETAL LAMB. Robert M. Abrams and Alastair A. Hutchison*. Depts. of Ob/Gyn and Peds., University of Florida Medical Center, Gainesville, FL 32610

Augmented cerebral metabolism during REM has been postulated on the basis of rises in cerebral blood flow during this behavioral state. Since REM occupies a large fraction of total sleep time in the late gestation sheep fetus, it was possible to test this hypothesis by employing the [^{14}C] Deoxyglucose ([^{14}C] DG) method to measure LCGU (RM Abrams et al, Am J Physiol 246:R608, 1984). 12 fetal lambs with chronic brachiocephalic and superior vena cava catheters, cerebral cortical and orbital screw electrodes, and neck EMG electrodes were studied 3-8 days postoperatively at gestational ages of 133-141 days. Fetal behavioral state was recorded continuously for 1.5-3 hrs prior to infusion of 300 μCi [^{14}C] DG. The total time the fetuses spent in REM after infusion was determined as a percentage of the time during which LCGU was measured (23-35 min). Fetal pH, pO_2, pCO_2 and hematocrit were within normal limits. A positive relation between LCGU and % REM was found in all 42 cerebral and spinal cord structures measured. The effect was most apparent in the cerebral cortex where LCGU, during high % REM, was 4-5 times that seen when % REM was low. Autoradiographs from animals with high % REM showed prominent dark bands near the surface of the cerebral cortex. These observations demonstrate clear increases in LCGU with REM and emphasize the necessity of monitoring behavioral state during studies of cerebral metabolism.

24.12

SENSORY STIMULATION IN HEAD-INJURED PERSONS. Kathy M. Kater* and Marilyn B. Rubin. St. Louis University, St. Louis, MO., 63103

An experimental study demonstrated the relationship between the presence or absence of a structured sensory stimulation program and the reacquisition of previous abilities in head-injured individuals. The relationship between the level of functional return, and the type of pre-injury experiential background the person had engaged in was also evaluated. The samples were taken from two settings; each consisting of 15 head-injured persons. Subjects in both groups were divided according to their Glasgow Coma Scale score. The data indicated a significantly higher ($p=.05$) mean functional score for recipients of the sensory stimulation program. There was also a significant ($p=.01$) treatment by severity effect, indicating that the effect of the stimulation varied according to the subject's coma severity score. Persons in the control group, however, who came from an enriched pre-injury environment, achieved significantly higher ($p=.05$) functional level scores. These results emphasize the importance of incorporating sensory stimulation into the care of head-injured persons, as well as help one to gain insight into the nature of brain recovery in response to injury.

COMPARATIVE PHYSIOLOGY: RESPIRATION AND ACID-BASE BALANCE

25.1

THE INFLUENCE OF BLOOD GAS PROPERTIES ON GAS TENSIONS AND pH OF VENTRAL AND DORSAL AORTIC BLOOD IN FREE SWIMMING TUNA (Euthynnus affinis). David R. Jones and R.W. Brill*. Zoology Dept., Univ. of British Columbia, Vancouver, Canada & NMFS, Honolulu Laboratory, Honolulu, HI 96182, U.S.A.

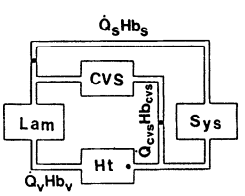
We have developed a technique for capture, anaesthetisation, instrumentation and release of tuna and have made the first determinations of blood gas values in dorsal and ventral aortae of free swimming tuna. Dorsal aortic P_{O_2} varied from 34.5 to 91.7 mmHg, while P_{CO_2} was always high (5.05 ± 0.4 mmHg; mean ±SE; n=9). Dorsal aortic bood (pH_a = 7.77 ± 0.04; n=8) was more alkaline than ventral aortic blood (pH_v = 7.65 ± 0.02; n=7). Warming dorsal aortic blood from 25 to 35°C in a closed system caused P_{O_2} and P_{CO_2} to rise and pH to fall. Oxygen combining curves for whole blood were sigmoid (mean Hill's number = 1.72 ± 0.05; n=11; range 1.57 to 2.0) and P_{50} over the pH range found in free swimming animals was 21 ± 1.75 mmHg (n=8). The CO_2-induced Bohr coefficient ($\Delta \log P_{50}/\Delta pH$) was -0.59 ± 0.046 (n=30). Unusual features of CO_2 combining curves are attributed to a significant Root effect. Although these in vitro properties of tuna whole blood are at variance with other published data they nevertheless substantiate our determinations made in vivo.

Supported by an NSERC International Collaborative Award to D.R.J.

25.2

IN VIVO CHARACTERIZATION OF THE LAMELLAR AND CVS COMPARTMENTS IN THE GILL OF RAINBOW TROUT. George K. Iwama, Atsushi Ishimatsu* and Norbert Heisler. Department of Physiology, Max-Planck-Institut f. experimentelle Medizin. D-3400 Gottingen/FRG

After cannulation vessels draining the secondary lamellae (Lam) and the central venous sinus (CVS), the partioning of cardiac output ($\dot{Q}_v$) between systemic ($\dot{Q}_s/\dot{Q}_v$) and CVS ($\dot{Q}_{cvs}/\dot{Q}_v$) flows was calculated using the formulae:



$$\dot{Q}_s/\dot{Q}_v + \dot{Q}_{cvs}/\dot{Q}_v = 1 \quad \text{, and:} \quad [Hb]_s \cdot \dot{Q}_s/\dot{Q}_v + [Hb]_{cvs} \cdot \dot{Q}_{cvs}/\dot{Q}_v = [Hb]_v$$

Mean values were 6.55 g/dl for $[Hb]_s$, 6.15 for $[Hb]_v$ and 1.07 for $[Hb]_{cvs}$. Accordingly, 93% of $\dot{Q}_v$ was directed to the systemic beds (Sys) and the rest to the CVS. pH_{cvs} values were lower than pH_s values by 0.13 units. Total CO_2 and Pco_2 values in CVS blood were higher than arterial levels by 0.86 mM and 1.3 mmHg, respectively. The results of this in vivo investigation indicate considerably lower flow through the CVS than all previous in vitro perfusion studies.

25.3

PERFUSION-O2 MATCHING IN AMPHIBIAN SKIN. G.M. Malvin and M.P. Hlastala. Univ. of Washington, Seattle, WA 98195.

In mammalian lungs, an important mechanism matching local blood flow to regional O_2 availability is the hypoxic vasoconstrictor response of the pulmonary vasculature. This study tested whether a direct vascular response to O_2 can mediate regional perfusion-O_2 matching within another vertebrate respiratory organ: amphibian skin. A halothane (H) anesthetized frog, R. pipiens, was equilibrated with 9.9% Freon-22 (F), 1% H and air in a box. A gas mixture from a separate source was passed through a small test chamber placed on the belly. Under these conditions, the partial pressure gradients for F and H across the skin under the sample chamber was constant. Thus changes in F or H excretion into the sample chamber reflected changes in skin perfusion. [F], [H] and $[CO_2]$ in the sample chamber was measured with a mass spectrometer in response to independent changes of the $[O_2]$ in the sample chamber or box. Raising sample chamber $[O_2]$ produced concentration-dependent increases in the excretion of all gases measured. Lowering chamber $[O_2]$ decreased gas excretion. Increasing box $[O_2]$ while keeping sample chamber $[O_2]$ constant, decreased gas excretion into the chamber. The results indicate that blood flow and gas exchange to a region of skin is a direct function of the $[O_2]$ directly above that portion of skin, and inversely related to the $[O_2]$ over the adjacent skin. This regional perfusion-O_2 matching may act to optimize total cutaneous gas exchange in the frog. Supported by NIH grants HL 06770 and NIH HL 12174.

25.4

CUTANEOUS DIFFUSING CAPACITY INCREASES DURING HYPOXIA IN COLD, SUBMERGED RANA CATESBEIANA. Alan W. Pinder. Univ. of Massachusetts, Amherst, MA 01003.

Gas exchange through amphibian skin is primarily diffusion limited. In one model of cutaneous gas exchange, cutaneous diffusing capacity (D,O_2) is anatomically fixed; in another model, D,O_2 can be varied by perfusing more or less of the available cutaneous capillaries (capillary recruitment). To distinguish between these two models, curarized, cannulated bullfrogs were submerged and exposed to progressive aquatic hypoxia at 5°C, conditions under which all O_2 uptake (M,O_2) is cutaneous, and regulation of cutaneous gas exchange can be expected to be most evident. In normoxic water, arterial P,O_2 is low (26 mmHg) and the P,O2 gradient across the skin is high (114 mmHg). M,O_2 was constant as ambient P,O_2 decreased from 140 to 80 mmHg. The P,O_2 gradient across the skin decreased 40% (from 114 to 68 mmHg) and D,O_2 increased 40% (from 36 to 49 pmol/g·min·mmHg) over this range of ambient P,O_2. D,O_2 almost doubled in severe hypoxia (PO_2 30 mmHg) compared to normoxia. When bullfrogs were allowed access to air, there was little evidence of regulation of cutaneous gas exchange. It is concluded that D,O2 of the skin is physiologically variable, and that cutaneous gas exchange is regulated, although it may not be regulated under all conditions.

25.5

DIVING ADAPTATIONS IN THE AQUATIC FILE SNAKE, ACROCHORDUS GRANULATUS. H.B. Lillywhite and A.W. Smits, Univ. of Florida, Gainesville 32611 and Univ. of Massachusetts, Amherst 01003.

Diving capabilities of Acrochordus granulatus are facilitated by blood volumes (mean 12.5% body mass) and hematocrits (mean 50.1%) that are twice the values of most other reptiles. Blood oxygen capacity can exceed 20 vol%. Average O_2 uptake is low (24 µl/g·h at 25 °C), and 21-34% occurs through the skin. Snakes kept in hypoxic water increase pulmonary oxygen uptake by increasing the frequency and volume of ventilation. Pulmonary CO_2 losses average 4.5 µl/g·h and are unrelated to ambient oxygen tensions. Decreases in lung volume during submergence, greater inspiratory than expiratory volumes, and low pulmonary RQ (0.25) indicate that CO_2 is eliminated primarily cutaneously. Voluntary submergence times are typically 2-3 h and correlate with volumes of inspired air. Arterial oxygen is reduced about 80% during submergence. Concomitantly, resting heart rates are reduced to 4 or 5 bpm during late stages of apnea, but accelerate to 20-30 bpm during short breathing episodes when blood oxygen is restored. Effective oxygenation of blood is related to reflexive coupling of ventilation (1-3 breaths) with perfusion of the vascular lung which extends the entire body length. Prolonged dive times enable snakes to remain submerged inside burrows of mangrove swamps where they are probably protected from high temperatures and predation. (Supported by National Institutes of Health grant HL 33821-01 to H.B.L.)

25.6

ACID-BASE STATUS AND ELECTROLYTES IN PLASMA AND RED CELLS OF TURTLES SUBMERGED AT 3°C: CONSEQUENCES FOR BLOOD - O_2 TRANSPORT DURING WINTER HIBERNATION. Leigh A. Maginniss and Bernard M. Hitzig*. Div. of Biol. and Med., Brown Univ., Providence, RI 02912 and Pulmonary Unit, Harvard Med. Sch., Boston, MA 02114.

Two groups of adult Chrysemys picta were studied; control turtles were maintained at 24°C with free access to air and diving animals were submerged for 8.5 to 9.5 wk in aerated water ($P_{O_2} \simeq 150$ Torr) at 3°C. Prolonged cold submergence elicited a substantial lactic acidosis ($pH_a = 7.70$; plasma $[lactate^-] = 42.5$ mEq/l H_2O). This acid load was largely balanced by changes in plasma strong ions: $[Cl^-]$ decreased; $[Ca^{++}]$ and $[Mg^{++}]$ increased. ^{31}P - NMR spectroscopy and chloride ratio ($[Cl^-]_i/[Cl^-]_o$) measurements revealed a lower pH gradient between plasma and RBC for diving turtles than control animals. This relative alkalosis for submerged turtle red cells is not explained by compensatory adjustments of strong inorganic ions. However, ATP, the major organic phosphate in control turtles (9.4 mmol/l RBC H_2O), was reduced by 78% in submerged animals. Furthermore, RBC volume in diving turtles increased 39% above control levels, decreasing the effective concentration of impermeant protein anion. The combined effects of reduced Hb allosteric modifier (ATP) and the relative alkalosis of the intracellular environment account for the high blood - O_2 affinity previously observed in submerged turtles at 3°C (Respir. Physiol. 53:15-29, 1983). Supported by NSF PCM8202702.

25.7

IONIC COMPENSATION TO CHRONIC HYPERCAPNIA IN TURTLES: NO RENAL CONTRIBUTION. Randi B. Silver* and Donald C. Jackson. Div. Biol. & Med., Brown Univ., Providence, RI 02912.

The ionic compensatory response of freshwater turtles, Chrysemys picta bellii, to prolonged respiratory acidosis consists of an increase in plasma S.I.D. as indicated by an increase in $[HCO_3^-]$. This investigation evaluated the specific ionic changes associated with chronic hypercapnia and assessed the contribution of the kidneys and urinary bladder to this compensatory response. Arterial blood gases, pH and ionized Ca^{++} were measured periodically during CO_2 breathing. Plasma was analyzed for ions (Na^+, K^+, Cl^-, total Ca and total Mg) and osmolality. Ureteral urine was collected and analyzed for pH, ions, NH_3, total CO_2, osmolality, and titratable acid. When 6% CO_2 was breathed at 20 C, plasma $[HCO_3^-]$ increased from 41.4 to 51.5 mM within 48 hr. The only significant associated strong ion changes observed were increases in total and ionized Ca and in total Mg. No significant changes occurred in plasma Na^+, K^+, or Cl^-. These results were unaffected by surgical removal of the urinary bladder. Urine collected from cystectomized turtles showed no significant changes in acid excretion during hypercapnia. These data argue against compensatory roles for the kidneys and urinary bladder in the ionic compensation to respiratory acidosis in this species and point to internal ionic exchanges involving bone and shell.

Supported by NSF Grant PCM82-02419

25.8

PULMONARY GAS TRANSPORT IN THE LOGGERHEAD SEA TURTLE. M. Lutcavage*, H.Baier, P.L. Lutz*. Rosenstiel School of Marine and Atmospheric Science and Pulmonary Division, Univ. of Miami, Miami, Fl 33101

Pulmonary carbon monoxide diffusing capacity (DL_{CO}), lung volume (V_L), oxygen uptake ($\dot{V}O_2$) and pulmonary blood flow ($\dot{Q}_{pul}$) were measured simultaneously in 5 loggerhead sea turtles Caretta caretta (mean weight 10.2 kg, range 8-11 kg) using non-invasive techniques. Mean (± S.D.) minimum lung volume (open glottis) determined from helium dilution was 38.0 ± 14.1 ml/kg. Calculated DL_{CO} during rebreathing was 0.10 ± 0.02 ml/min/mmHg per kg body weight at 25°C. This is twice the value encountered in non-varanid reptiles and about 33% of that of resting mammals. Mean $\dot{V}O_2$ calculated from the fractional concentration change in oxygen was 0.158 ± 0.050 l/kg/hr. $\dot{Q}_{pul}$ was calculated using the slope of the disappearance curve of acetylene in the rebreathing gas mixture corrected for tissue volume. The mean $\dot{Q}_{pul}$ was 86.0 ± 29.7 ml/min/kg. Pulmonary arterial to venous O_2 difference was 3.4 ± 1.7 vol % resulting in a mean blood convection requirement $\dot{Q}_{pul}/\dot{V}O_2$ of 34.6. While lung volume in the loggerhead sea turtle is not large compared to other reptiles, pulmonary gas transport is markedly higher than in most other reptiles and equal to varanid lizards. Efficient coupling of pulmonary diffusion and perfusion, high arterial saturation and compartmentalized lungs in the sea turtle suggest an aerobic capacity typically associated only with endotherms.

25.9

ENVIRONMENTAL pH AND GILL MEMBRANE PHOSPHOLIPIDS. A.A. Benson*, J.C. Nevenzel*, and A.G. Gibbs* (SPON: H.T. Hammel).

Fatty acyl ester linkages of gill membrane phospholipids of aquatic animals are relatively stable in seawater (pH 8.1) and most freshwater systems. In high pH environments (Mono Lake, Eagle Lake, pH 9-10) hydrolysis of their ester linkages could occur. Gill phospholipids include plasmalogens, in which a fatty ester is replaced by a long chain aldehyde in a 1-alkenyl ether linkage. Algae and higher plants have no plasmalogens. Such acid-labile lipids comprise up to 98% of certain crustacean gill membrane phospholipids and reflect the stability of such membranes during their oceanic evolution. Analyses of gill phospholipids of fish, crustacea, and molluscs exposed to low pH indicate adaptations in their gill membrane compositions. Plasmalogen content of fish gill phospholipids were highest, 22% for catfish and ranged from 3.6% of total gill lipid in Salmo gairdneri to 12% in leopard shark. Mussel, oyster, and rock scallop gills contained 19-22% of plasmalogen. Salmo trutta, exposed to pH 4.5 for five days, lost 30% of their gill phosphatidylethanolamine plasmalogen. Animals with higher gill plasmalogen content, on the other hand, were not observed to lose plasmalogen upon exposure to low pH. Freshwater clams, Unio sp. and crayfish at pH 4.0 for seven days maintained or increased their gill plasmalogen content. Acid-stimulated plasmalogen turnover will be investigated. (Supported by Office of Health and Environmental Research, Department of Energy.)

25.10

ACID-BASE AND ELECTROLYTE BALANCE DURING EXERCISE-INDUCED METABOLIC ACIDOSIS IN LARVAL AMBYSTOMA TIRGRINUM. J.W. Rohrbach* and D.F. Stiffler, Calif. State Polytechnic Univ., Pomona, CA 91768

Larval A. tigrinum were forced to swim to exhaustion in order to induce a metabolic acidosis. This acidosis was characterised by an over 6-fold increase in blood lactic acid concentration from 1 to 6.5 mM and a 50% decrease in $[HCO_3^-]$ (from 12 to 6 mM) causing pH to drop over 0.6 units. Extracellular electrolyte concentrations also shifted as indicated by a marked decrease in the strong ion difference (SID). Recovery from the acidosis was complete after 8 h and changes in both influxes and effluxes of Na^+ and Cl^- suggest that ion transport mechanisms are involved in restoring extracellular pH. Increased Na^+ influx during the 8 h recovery period suggests that cutaneous ion transport is involved. This possibility was supported by the fact that when larvae are forced to recover from similar metabolic acidoses in flowing distilled water their recovery is prolonged. Increased Na^+ and Cl^- efflux suggests that there may also be a renal component to the recovery. (Supported by a grant from Sigma Xi to J.W.R.)

25.11

LIMITATIONS OF H^+ EFFLUX FROM MUSCLE CELLS DURING LACTACIDOSIS

N. Heisler and H. Weitz*. Department of Physiology, Max-Plank-Institut f. experimentelle Medizin, D-3400 Gottingen/FRG

The mechanisms of elimination of the dissociation products of lactic acid, H^+ and lactate ions, from muscle cells are still not completely understood. Lactate efflux is a relatively time consuming process, whereas the efflux time constants determined for H^+ ions are comparatively large. However, the ratio of the absolute amounts of H^+ ions/lactate ions transferred to the extracellular space may vary considerably between values close to 1, and 40, depending on the type of experimental approach.

In order to study this phenomenon, the efflux of H^+ and lactate from isolated blood perfused dog gastrocnemius muscle was studied as a function of the blood perfusion rate. The ratio of H^+/lactate efflux was extremely perfusion dependent and rose with increasing muscle blood flow from values of about 1 at 15 mL/(min · 100g) to about 8 at 150 mL/(min · 100g).

Model calculations on the basis of the effective buffer values of the perfusate (buffer system closed for CO_2), and the actual rate of perfusion revealed that the efflux of H^+ ions from muscle cells is largely equilibrium- and perfusion-limited. Efflux of H^+ ions from muscle tissues according to the transmembrane transfer time constant could only be expected at blood perfusion rates of much higher than 150 mL/(min · 100g), flow rates never encountered in vivo. It is concluded that the extreme variability in the apparent efflux time constants reported in the literature is mainly attributable to equilibrium- and perfusion-limitation of variable extent.

25.12

DECREASED CRITICAL $P_{\bar{v}O_2}$ AND CRITICAL OXYGEN TRANSPORT DURING INDUCED HYPOTHERMIA. David C. Willford, Esther P. Hill, Francis C. White and William Y. Moores*. UC San Diego and San Diego VA Medical Center, La Jolla, CA 92093.

In order to evaluate critical venous P_{O2} and critical O_2 transport, we surgically instrumented 8 normothermic (36.8°C) and 10 hypothermia (29.3°C) immature pigs under halothane anesthesia. Inspired O_2 tensions were decreased in steps, and O_2 consumption ($\dot{V}_{O2}$) was determined from cardiac output ($\dot{Q}$) and arterial-venous O_2 contents ($C_{aO2}-C_{\bar{v}O2}$) using the Fick equation. Blood lactate was also measured. Plots of $\dot{V}_{O2}$ and lactate vs. venous P_{O2} ($P_{\bar{v}O2}$) or total oxygen transport ($TOT=\dot{Q}\cdot C_{aO2}$) revealed dependent and independent regions which could each be fit by straight lines. We defined the critical value as the intersection of the lines. Critical $P_{\bar{v}O2}$ (±S.E.) decreased from 22.0±1.4 torr at 36.8°C to 15.5±1.0 torr at 29.3°C. Similarly, critical TOT decreased from 7.9±0.7 ml/min/kg at 36.8°C to 5.2±0.4 ml/min/kg at 29.3°C. Although the critical $P_{\bar{v}O2}$ decreased during hypothermia, the difference between normal $P_{\bar{v}O2}$ and the critical $P_{\bar{v}O2}$ also decreased. TOT showed a similar trend. We conclude that although tissues are more tolerant of low P_{O2} during hypothermia, the margin of reserve in the oxygen transport system is also decreased at the lower temperatures. Supported by NIH grant HL-17731 and the Veterans Administration.

RENAL-CARDIOVASCULAR INTEGRATION

26.1

EFFECT OF HYDROGEN ION CONCENTRATION ON CORTICOSTEROID SECRETION. K.J. Kadke, E.G. Schneider, R.E. Taylor, Jr., and R.E. Kramer*. Dept. of Physiol. and Bioph. and Dept. of Pharmacol., Univ. TN. Ctr. Hlth. Sci., Memphis, TN 38163.

We have previously reported that changes in extracellular hydrogen ion concentration ($[H^+]$) have a direct, modulatory effect on angiotensin II- and potassium-stimulated aldosterone (aldo) secretion by isolated, perfused canine adrenal glands. Acidosis enhanced, whereas alkalosis inhibited, the aldo secretory responses to both primary regulators. In the present study, the direct effects of acid-base disturbances on corticosteroid secretion by isolated, perfused canine adrenal glands were examined under basal and ACTH-stimulated conditions. Acidosis or alkalosis, produced by altering either PCO_2 or $[HCO_3^-]$ of the Krebs-bicarbonate perfusate, had no significant effect on basal or ACTH-stimulated cortisol secretion. In contrast, alkalosis markedly inhibited ACTH-stimulated aldo secretion. Moreover, within the range of pH from 7.19 to 7.85, there was a positive correlation between perfusate $[H^+]$ and aldo secretion but negative correlations between perfusate $[H^+]$ and the secretion of both corticosterone and 18-hydroxy-corticosterone in response to ACTH. These data suggest that (1) changes in $[H^+]$ modulate the stimulatory effect of ACTH on aldo secretion by a direct action on the adrenal cortex, (2) the effect is specific to the zona glomerulosa, and (3) changes in $[H^+]$ may influence events occurring late in the pathway for aldosterone biosynthesis. (Supported by USPHS Gr. HL-27749, HL-06984, and HL-07339).

26.2

ESSENTIAL ROLE OF ANGIOTENSIN II (ANG II) IN THE DRINKING RESPONSE OF HYPOTENSIVE RATS. M. Evered* and P. Lee* (SPON: J. Thornhill). Univ. of Saskatchewan, Saskatoon, Canada S7N OWO

We investigated the effects of captopril (CAPT, 100 mg/kg SC), an inhibitor of ANG II synthesis, on the relationship between thirst and the hypotension caused by a vasodilator, diazoxide (DIAZ). To circumvent the problem that ANG II-blockade enhances the depressor action of DIAZ, we selected a lower but overlapping range of 5 DIAZ doses for CAPT-treated rats (2-25 mg/kg, IV) than for rats given DIAZ alone (5 doses, 5-50 mg/kg, IV). These two sets of doses were confirmed, in conscious rats with femoral arterial cannulas, to produce similar ranges of reductions of blood pressure (5-60 mmHg) under the two conditions. But whereas drinking by DIAZ ALONE rats was proportional to the hypotensive action of the drug, ranging from 3.5 ± 0.9 to 17.0 ± 2.1 ml/2 hr (mean + SEM), that of CAPT + DIAZ rats never increased above baseline levels at any dose. The latter did drink, however, when ANG II was replaced. As a measure of the amount needed, ANG II was infused IV at rates just sufficient to return the blood pressure of CAPT + DIAZ rats to that of rats given the same dose of DIAZ alone. ANG II replaced at these rates caused CAPT + DIAZ rats to drink amounts similar to those of DIAZ ALONE rats. The complete blockade of drinking by captopril, independent of the arterial pressure, and the simple restorative effect of ANG II suggest to us that ANG II is the most important if not exclusive controller of thirst caused by vasodilator-induced hypotension. (Supported by MRC).

26.3

Voluntary dehydration in young men. P.C. Szlyk, R.W. Hubbard, R.P. Francesconi, W.T. Matthew, I.V. Sils, D.B. Engell, L.E. Armstrong, and C. Matthew. USARIEM, Natick, MA, 01760-5007.

During exercise in the heat, body wt (BW) deficits can occur even when water is plentiful. This phenomenon, although not planned behavior, has been named voluntary dehydration (DV). This study was designed to investigate the effects of water temperature and flavoring on DV in two populations: drinkers (D) that maintained BW loss < 2% during 15°C water trial and nondrinkers (ND) that became dehydrated. Fourteen males (21-33 yrs) were studied during 6hr of intermittent treadmill exercise (1.34 m/sec, 5% grade) in a climatic chamber (40.6°C d.b./28.5°C w.b.). Subjects consumed each of 4 iodinated beverages on 4 nonconsecutive days: 15°C water, 40°C water, 15°C flavored water, and 40°C flavored water. ND consumed 24% less cool water (2.51 ± .15 kg) than D (3.28 ± .22 kg), and this resulted in markedly greater BW loss in ND (2.53 ± .12%) relative to D (.80 ± .10%). In comparison, warm water resulted in significant reductions in consumption in D (2.07 ± .30 kg) and ND (1.04 ± .30 kg) and decreases in BW (2.38 ± .40% and 3.90 ± .41%, respectively). The only significant effect of flavoring was to enhance consumption of warm water (1.86 ± .31kg) and to reduce BW loss (3.30 ± .28%) in the ND group. Rectal temperature, heart rate and Posm were not different between ND and D, but significantly increased when drinking warm rather than cool drinks. Data indicate that cooling or flavoring warm iodinated water enhances fluid consumption and reduces dehydration in persons reluctant to drink.

26.4

HEMORRHAGE AS A COUNTERMEASURE TO FLUID SHIFTS IN WATER IMMERSION. **Karl E. Simanonok* and Edmund M. Bernauer. Human Performance Laboratory, Univ. of California, Davis, CA 95616**

We hypothesized that a hemorrhage prior to a fluid shift by the quantity that expands central blood volume during a fluid shift would reduce or negate the sequelae of the fluid shift. Water immersion was used as a zero-g model to induce fluid shifts in human subjects. The applied countermeasure was a total blood volume reduction of 15% just prior to immersion to the sternal notch in a seated position in water at a temperature of 34.5 degrees C. (WE = wet experimental). Subjects twice served as their own controls one and two weeks prior to the hemorrhage experiment, once while seated in air (DC = dry control) and once while in water (WC = wet control). The characteristic stimulation of diuresis by water immersion observed in the WC was reduced to near DC levels by the blood withdrawal. During the WE hematocrit and hemoglobin fell below both control values but remained stable after the second hour of immersion, whereas during the WC hemoconcentration occurs as seen by a gradual increase in hematocrit and hemoglobin. These results indicate that hemorrhage is a successful countermeasure to the dehydration and hemoconcentration which are consequences of water immersion. We believe that hemorrhage merits further investigation as a countermeasure to the fluid shifts experienced by space travelers.

26.5

SEADRAGON VI: ENHANCED RENIN BUT ELIMINATED ADH RESPONSES TO TILT AT 31 ATA. J.R. Claybaugh, N. Matsui, K. Shiraki, H. Nakayama, and S.K. Hong. Tripler Army Medical Center, Hawaii; Univ. of Nagoya, Nagoya, Japan; Univ. of Occup. & Envir. Health, Kitakyushu, Japan; JAMSTEC, Yokosuka, Japan, and SUNY at Buffalo, N.Y.

A previous report from these studies (Lin et al., Undersea Med Soc, June 1985) provided evidence that cardiovascular deconditioning occurred at 31 ATA. Three subjects were tilted from supine to upright for 15 min while bearing their weight. Three tilts were conducted on each of 2 days at predive 1 ATA, 2 days at 31 ATA, and 1 day postdive 1 ATA. Blood samples were obtained from 2 of the 3 tilts, at 8 AM and 8 PM. Plasma was analyzed for ADH (PADH) and renin activity (PRA). At predive 1 ATA the mean increase in PADH in response to tilt was 0.35 μU/ml (P<0.025). At 31 ATA there was no response. PADH levels at 1 ATA were 2 times higher than 31 ATA (P<0.005). PRA responses were qualitatively opposite, tilt increased PRA about 2½ times more at 31 ATA than 1 ATA (P<0.005). The PRA response to tilt was about 2 times greater in the AM (P<0.005). Basal levels did not vary. We conclude that cardiovascular deconditioning decreased ADH, but increased PRA responses to postural change. This altered response contributes to the chronically increased aldosterone and decreased ADH at hyperbaria. Supported by US Army Health Services Command, NOAA Contract 41-USC-252-C-3, JAMSTEC, and NIH grant HL-28542.

26.6

CENTRAL ATRIAL NATRIURETIC FACTOR (ANF) INCREASES URINARY WATER EXCRETION IN CONSCIOUS, SODIUM DEPLETE SHEEP. J. Lee*, B.-S. Huang*, R.J. Grekin, and R.L. Malvin. Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

Although the renal and hemodynamic effects of ANF have been intensively studied, its action in the CNS has not been investigated. The purpose of this work was to determine if ANF has centrally mediated effects on renal function. Chronically instrumented, sodium deplete sheep with an indwelling lateral cerebroventricular cannula and an isolated vascular loop in the neck were studied. Following a 1 hr basal clearance period, ANF (atriopeptin III, Peninsula), 100 pmol/min, was administered into the cerebral cannula for 2 hr with a 1 hr recovery period (n=7). GFR (inulin clearance), RPF (PAH clearance), BP, HR, and plasma Na and K were constant throughout the experiment.

	Urine Volume (ml/min)	U_{osm} (mosm/l)	C_{H_2O} (ml/min)
Basal	0.89±0.24	1082±180	-1.48±0.26
90 min	3.04±0.87*	365±106*	0.83±0.61*
Recovery	1.07±0.24	768±166	-0.61±0.30

*p<0.05 compared to basal

In control studies (n=5), animals received the same dose of ANF intravenously, and showed no significant change in any of the variables studied. Central ANF induces increased free water excretion and diuresis, presumably due to inhibition of ADH secretion. (Supported by NIH Grant HL18575).

26.7

SIMULATED HYPERVOLAEMIA DECREASES THE NATRIURETIC POTENCY OF ATRIAL EXTRACT DETERMINED BY IN VIVO BIOASSAY. ADS Meikle*, and Susan Kaufman. Univ of Alberta, Edmonton, Alta T6G 2G3.

Male Wistar rats (250-280g) were chronically implanted with small inflatable balloons at the right/SVC/atrial junction. The balloon was inflated for 60min in 8 rats, the remainder serving as non-inflated controls. The rats were sacrificed by stunning, the atria were dissected and rinsed in 0.9% NaCl in 10mM phosphate buffer (PBS) at pH 7.20, frozen in dry ice and ethanol and stored at -40°C. Crude extract was prepared as follows: a 1:10 (w/v) homogenate in PBS was boiled, centrifuged at 26,000g for 30min and subjected to ultrafiltration through a YM30 (Amicon) membrane (MW cutoff 30,000d). The extract was bioassayed in male Wistars (250-270g) anaesthetized with Inactin and infused with saline. Control extract (130 μl.250g^{-1}) was made up to 400 μl in saline and infused IV over 2 min. 40 min later a second injection of either control or experimental extract was given in the same manner followed by 20min recovery. The increase in Na^+ excretion between the 1st and 2nd injections was significantly greater in the control/control group (1.59±0.15 μEq.min^{-1}, n=13) than in the control/experimental group (1.15±0.09 μEq.min^{-1}, n=14) ($p \leq 0.01$, Student's t-test). We conclude that inflation of a balloon in the right SVC/atrial junction in the rat causes a reduction in potency of atrial natriuretic factor in the right atrial tissue.

26.8

ATRIAL NATRIURETIC FACTOR POTENTIATES THE PRESSURE DIURETIC NATRIURETIC RESPONSE. K. Takezawa*, A.W. Cowley, Jr. and R.J. Roman. Med. Coll. Wisc., Milwaukee, WI 53226

The present investigation examined whether atrial natriuretic factor (ANF) alters the pressure-natriuretic response to elevations in renal perfusion pressure (RPP). Studies were performed in four groups of anesthetized, adrenalectomized, uninephrectomized rats. Neural influences on the kidney were eliminated by acute denervation, and plasma levels of angiotensin II, vasopressin, aldosterone, norepinephrine and corticosterone were maintained constant by i.v. infusion. The rats received an i.v. infusion of rat atriopeptin III (Peninsula Lab) at a dose of either 30, 100 or 500 ng/kg/min or vehicle alone. In control rats, increasing RPP from 100 to 125 to 150 mmHg in three 20 min steps produced progressive 4-fold and 6-fold increases in urine flow ($\dot{V}$) and Na excretion. RBF and GFR were not significantly altered and averaged 5.6±0.5 and 1.1±0.1 ml/min/g kwt, respectively. Infusion of ANF produced dose-dependent increases in the pressure diuretic and natriuretic responses. The slopes of the relationships relating $\dot{V}$ and Na excretion to RPP were significantly increased by approximately 35%, 46% and 67%, respectively, in rats treated with 30, 100 and 500 ng/kg/min dose of ANF. In contrast, RBF and GFR were similar in the vehicle and ANF infused animals at all levels of RPP. These findings indicate that ANF can modulate, at least acutely, the pressure diuresis and natriuresis relationship independent of its actions on whole kidney hemodynamics. (NIH 1-PO1-HL29587 and AHA 83-853)

26.9

RENAL HEMODYNAMIC EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) ACCOUNT FOR ITS NATRIURETIC ACTION. M.J.F. Camargo* and T. Maack. Department of Physiology, Cornell University Medical College, New York, New York 10021

One of the major renal hemodynamic effects of ANF is to increase GFR. To assess the role of this effect on ANF-induced diuresis (V) and natriuresis ($U_{Na}V$), we performed delayed clamp experiments in rats in which the GFR was returned to control levels during continuous infusion of ANF. After control periods (C), synthetic ANF (auriculin A) was infused i.v. (2 µg/Kg BW•min^{-1}) throughout the experiment (150 min). After pre-clamp periods, the left kidney (LK) was clamped to reduce perfusion pressure to about 75 mmHg. The right kidney (RK) served as a time-control. In the RK, ANF increased GFR from 1.5±0.1 to 1.8±0.1* ml/min (mean±SE, *p<0.01, n=6), V from 14.1±2.4 to 48.4±4* µl/min and $U_{Na}V$ from 1.8±0.4 to 9.3±0.9* µEq/min. In the LK, during pre-clamp, ANF increased GFR from 1.5±0.1 to 1.8±0.1* ml/min, V from 16.7±4.5 to 52.6±5.0* µl/min and $U_{Na}V$ from 2.1±0.6 to 10.0±0.9* µEq/min. The clamp returned all parameters in LK to C values: GFR to 1.4±0.1 ml/min, V to 6.3±1.2 µl/min and $U_{Na}V$ to 1.0±0.3 µEq/min (p>0.05 vs. C), while in RK, GFR (1.8±0.1 ml/min), V (38.3±3.9 µl/min) and $U_{Na}V$ (7.8±0.8 µEq/min) remained elevated (p<0.01 vs. C). These data demonstrate that upon return of GFR to control levels, the ANF-induced diuresis and natriuresis is abolished. The results support the view that the increase in GFR is central to the natriuretic action of ANF.

GRAVITY SENSITIVE SYSTEMS IN ANIMALS

27.1

VERTEBRATE GRAVITY SENSORS AS DYNAMIC SYSTEMS. *Muriel D. Ross. Univ. of Michigan, Ann Arbor, MI 48109*

Ciliary tuft configurations and orientations in a model mammalian species (rat) were compared with those described previsouly in frog (Lewis and Li, 1975, Brain Res. 83:35-50). Results showed that three of six tuft types present in rat were morphologically comparable to those characterized as tonic, phasic-tonic, and phasic in frog, but no tufts described as "auditory" were present. Rat tufts were not oriented in parallel; the kinocilia were fixed in the recovery stroke position; and type II cells had shorter and more slender stereocilia. A hypothesis growing out of this and related work is that gravity sensing requires a dynamic receptor. Fundamental tenets are that kinocilia are motile, to keep the system responsive to gravity, a constant stimulus to which the sensor would otherwise readily adapt; and that comparators of acceleratory force add discriminative ability. A trend toward more organic material and less mineral in otoliths/otoconia may have been critical in increasing sensitivity to translational linear acceleratory force with evolution of terrestrial species. Changes in rate, strength and/or direction of ciliary beating, amplified by the stereociliary tuft and striated infrastructure, would drive the hair cell responses to linear accelerations. A corollary hypothesis is that maculas evolved toward pure accelerometers as functional mosaics that only partly reflect the linear acceleratory environment of the host.

Supported by NASA Grant# 9073G and Contract #NAS2-10535.

27.2

CHRONIC ACCELERATION AND EGG PRODUCTION IN DOMESTIC FOWL. Arthur H. Smith, Emerson L. Besch and Russell R. Burton. Univ. of California, Davis, California 95616.

Egg laying in domestic fowl requires the establishment of specific endocrine relationships, increased synthesis of organic materials and an increased energy metabolism. Research in several laboratories has demonstrated that chronic exposure to hyperdynamic environments ($+G_z$) significantly affects these physiological processes, potentially limiting egg production in chronically accelerated birds.

Groups of chickens were chronically accelerated at 1.5-2.3 G, with hatch-mate gravity controls, and the effect on egg production examined. The principal effect was a limitation on the number of hens attaining a laying condition. The effects of this treatment upon egg size and composition will also be reported.

In groups not highly selected for chronic acceleration tolerance, egg production in 2 G fields was terminated after 3-4 eggs by oviduct prolapse. However, when the experiments were repeated with hens from an acceleration selected line (S_{22}), oviduct prolapse was encountered only rarely.

27.3

HORIZONTAL AND VERTICAL COMPENSATORY EYE MOVEMENTS IN WEIGHTLESSNESS. Søren Vesterhauge, Arne Månsson and Torben Staehr Johansen. Otoneurological Laboratory, Rigshospitalet, DK-2100 Copenhagen and Royal Danish Air Force, Denmark.

Space motion sickness has become an increasing problem during space missions. Astronauts state that head motions in pitch are particularly provocative. Previously our group has demonstrated significant gain variations of compensatory eye movements elicited by head movements in yaw caused by gravity alterations during parabolic flight and 2-G turns in a small aircraft. Access to a larger and more powerfull aircraft, the Gulfstream III, made it possible to repeat the experiments during a much longer period of weightlessness, 25-30 sec, and to compare eye movements elicited by horizontal and vertical head rotations. Gain variations are still present during the relatively long periods of weightlessness and with vertical head movements. The significance of otolith stimulation for this reflex under normal gravitation is confirmed by our results and they emphasize the need for otolith reinterpretation during weightlessness. Our results indicate that this reinterpretation might play an important part in the development of space motion sickness.

Supported by grants from the Danish Space Board.

27.4

ANIMAL MODELS OF MOTION SICKNESS: ARE NONEMETIC SPECIES AN APPROPRIATE CHOICE ? K.-P. Ossenkopp* and M. D. Ossenkopp* (SPON: M. Kavaliers). Univ. Western Ontario, London, Ontario Canada, N6A 5C2

Motion sickness is a phenomenon observed in a variety of animal species and because of its debilitating effect on humans of special clinical interest. The indicator normally used to quantify motion sickness is the occurrence of an emetic response and based on this index clear evidence exists for motion sickness in nonhuman primates and cats and dogs. However, a number of species which do not seem to possess an emetic reflex nevertheless exhibit other signs and symptoms related to motion sickness when subjected to the appropriate vestibular stimulation. In the present study we conditioned rats and guinea pigs in a conditioned taste aversion paradigm. These animals were given a novel taste (sodium saccharin solution) followed by exposure to intermittent body rotation (vestibular stimulation) for one hour. When subsequently presented with the saccharin solution both these species showed strong avoidance of this taste. It is suggested that the pairing of the taste stimulus with the body rotation-induced motion sickness resulted in the conditioned taste aversion. These results suggest that nonemetic species do experience motion sickness and that formation of a conditioned taste aversion is a useful index of this phenomenon in non-emetic species.

27.5

Effects of Non Weight Bearing on Callus Formation. JR Sweeney*, HE Gruber*, ME Kirchen* and GJ Marshall* (SPON JP Meehan), Los Angeles Orthopaedic Hospital/USC, Los Angeles, CA 90007.
The relationship of osteopenia to weight bearing has been established and the development of an inverted suspension cage by Morey has provided a way to investigate fracture healing in suspended rats. Long Evans rats, 9-11 months old, were divided into suspension (S) and weight bearing (WB) groups. Rats from each group had fibular osteotomies and were sacrificed under humane conditions at 9,18 and 36 days. The fibulae from the fractured and non-fractured animals were processed for non-decalcified stained sections. 9-Day: The callus of WB rats contained cartilage, calcified cartilage and minimal osteoid. Periosteal bone formation was active at the fracture site and diminished toward the epiphyses. Absorption and remodelling were minimal. Fibulae from the S rats had smaller calluses with minimal cartilage and calcification. New bone and osteoid were deficient. Periosteal bone formation was diminished. 18-Day: WB calluses were well formed. Collagen in the osteoid reflected woven and mature bone. S rats showed a lag in callus formation and diminished periosteal bone. 36-Day: In WB fractures the callus approached complete bridging. There was extensive remodelling of the fractured cortical ends. The S rats were still in a lag phase with all aspects of fracture healing delayed. Summary: In S rats all aspects of healing were delayed and additional time may be essential for completion of the process when weight bearing is disassociated from the process.

27.6

CORRELATED LIGHT AND ELECTRON MICROSCOPY OF THE VASCULATURE OF CORTICAL BONE IN RAT FEMORA AND TIBIAE. Richard M. Dillaman and Robert D. Roer. Univ. of N.C. at Wilmington, Wilmington, N.C. 28403.

The macro- and micro-anatomical description of vessels within the cortex of mammalian bone is necessary in order to assess the role of the vasculature in the growth and homeostasis of bone. The dense, mineralized nature of bone precludes the direct observation of the vasculature. Furthermore, fixation of bone for histological and ultrastructural examination usually entails either immersion in or perfusion with fixative, neither of which provides uniform fixation throughout the cortex of bone. To overcome these handicaps, freshly dissected bones are frozen in liquid nitrogen and are then either stored in liquid nitrogen or transferred to a -20°C cryostat where bones are fractured. The pieces of frozen bone are then placed in room temperature glutaraldehyde-paraformaldehyde fixative and photographed for later reconstruction. Contiguous pieces are then stained and cleared for determination of the vascular pattern using a dissecting scope, critical point dehydrated for scanning electron microscopy or processed for transmission electron microscopy. Application of this protocol has indicated no freezing artifacts and has permitted an overall characterization of bone vascular patterns as well as fine structural analysis of individual vessels.

27.7

THE EFFECTS OF SPACEFLIGHT ON BONE TURNOVER ARE DISTINGUISHABLE FROM THE EFFECTS OF IMMOBILIZATION. R. T. Turner and B. Szukalski. V.A. Hospital, Loma Linda, CA 92354

Mechanical loading of the skeleton consists of two components: 1) gravitational, and 2) muscular. The gravitational component is always directed toward the earth's center, whereas muscular loading is aligned along the axis of muscle connections and is only applied during muscle contraction. To determine the effect of each component on bone turnover we compared the effects of orbital spaceflight, which reduces gravitational loading to essentially zero, and nerve section, which greatly reduces muscle loading, on the rat tibia diaphysis. Spaceflight had no significant effect on endosteal bone formation, whereas denervation resulted in a 25% decrease ($P<0.01$). Spaceflight resulted in a 45% decrease ($P<0.01$) in the periosteal bone formation rate compared to denervation which resulted in a 25% decrease ($P<0.001$). Further, when bone apposition rates were determined at specific anatomical sites the extent of inhibition was site specific and differed between spaceflight and immobilization. These data are consistent with the hypothesis that the inhibition of bone formation during spaceflight is due to the reduced gravitational loading and not due to immobilization.

27.8

ARE THERE CONDITIONS IN WHICH ADRENALECTOMY IMPEDES THE ATROPHYING EFFECTS OF DENERVATION? Richard R. Almon and Debra C. DuBois*. State University of New York at Buffalo, Buffalo, New York 14260

The results of previous work from this and other laboratories suggest the possibility that the atrophy of skeletal muscle which occurs following denervation or immobilization may be caused by the muscle becoming hypersensitive to glycocorticoids. If this hypothesis is correct then a possible test would be to remove the source of the hormone and such atrophy should not occur. Initially we observed that adrenalectomy by itself does not greatly impede the atrophying effect of denervation. However adrenalectomy has a substantial effect on many aspects of body metabolism. These include effects on both digestion and insulin release from the pancreas. The objective of this study was to evaluate the effects of diet on denervation atrophy in normal and adrenalectomized animals. One leg of adult male Sprague-Dawley rats was denervated. Six days following denervation, the animal was sacrificed, the muscles were removed and the wet and dry weight of the several individual muscles were recorded. All adrenalectomized animals were provided with saline throughout the experiment. The variations in diet ranged from a high caloric liquid diet to starvation. Denervation atrophy was defined as the ratio of the denervated muscle to its contralateral control. The results clearly demonstrate that diet has a significant effect on the above ratio in adrenalectomized animals but not in normal intact animals.

27.9

G-TOLERANCE AND MUSCLE STRENGTH TRAINING. Ulf I. Balldin. Department of Aerospace Medicine, Karolinska Institute and National Research Institute, S-104 01 Stockholm, Sweden.

A good G-tolerance is crucial in pilots flying high performance aircraft, and is desirable to counteract the circulatory deconditioning in astronauts on the return to normal earth gravity after a long space flight. At our laboratory a muscle strength training program lasting 3 months has been shown to increase G-tolerance in 11 pilots by more than 30%. The G-tolerance was measured as time to exhaustion when executing respiratory and muscle straining maneuvers during varying G-loads in a human centrifuge. The training program consisted of exercises comprising slow dynamic contractions, mainly in the leg muscles but also involving muscles of the whole body. In collaboration with RNoAF Institute of Aviation Medicine a muscle training program involving only abdominal muscles in 10 pilots did, however, not improve the G-tolerance. In collaboration with Finnish AF another muscle strength training program combined with aerobic conditioning lasting one year, increased the G-tolerance by more than 20% in 20 pilots after 6 months of training. The gain in G-tolerance was maintained but not further increased after 12 months of training.

The strength training programs, now used to increase G-tolerance by high performance aircraft pilots, should, when applied in space flight, also benefit an elsewise circulatory deconditioned astronaut during reentry. The exercises are easy to execute with the proper equipment in a spacecraft and require only a limited time.

27.10

MODIFICATION OF THE RESTING OXYGEN CONSUMPTION LEVEL OF BIOLOGICAL BODY AND ITS TISSUES, DURING PROLONGED HYPODYNAMICS EXPOSURE. Hisashi Saiki, M. Nakaya*, M. Sudoh* and S. Ikawa*. St. Marianna Univ., 213 Japan. Jikei Univ., 105 Japan

Using voluntary human subjects, 6 days water immersion conditioning, with head out supine position was performed. The day time resting metabolic rates with other metabolic parameters were analyzed by method of least squares, Gauss, to estimate the circadian rhythm. For the animal experiments, albino rats were exposed to the another hypodynamics codition, through horizontal suspension technique, and the adaptive changes of energy metabolism of whole body and several organ tissues were studied. Results: 1) The day time resting metabolic rates were expressed as highly significant curvilineal regression lines of second degree. The deformation of the circadian rhythm curve, was observed systematically with the lapse of the time of hypokinetics exposure. 2). The average value of the resting metabolic rate during the day time was almost same through all the periods. 3). The whole body resting metabolic rate of the test animals slightly increased at the beginning of the hypokinetics exposure period, transiently, and then levels off on the control level. Conclusion: Induced hypodynamic conditioning, through water immersion or suspension procedure, under normal terrestrial gravity, seems to be difficult to produce the decreased basal metabolic rate in biological body, during such duration of the conditioning.

28.1

CLAUDE BERNARD AND THE NATURE OF GASTRIC ACID. Thomas J. Sernka. Wright State University, Dayton, OH 45435.

In his thesis for the doctoral degree in 1843, Claude Bernard inferred incorrectly that gastric acid was lactic rather than hydrochloric acid. Not only was he mistaken in this issue, but he also contradicted prior research by Prout and Beaumont leading to the correct conclusion. Careful reading of his published article with Barreswil provides some clues as to how Bernard may have been misled on such a basic problem. Although Bernard tested gastric juice for hydrochloric acid by the silver nitrate method, he detected *almost no silver* chloride precipitate upon distillation. It is possible, however, that the gastric juice obtained from his "...very productive dogs..." contained so much hydrochloric acid and other chlorides that soluble chloro complexes of silver formed. His negative results regarding hydrochloric acid led Bernard to investigate lactic acid. He found several similarities in the reactions of lactic acid and gastric acid, but none that we would regard as specific or critical nowadays. Conceivably, the lactic acid that he detected in gastric juice was a contaminant introduced by his use of wooden cannulas. In conclusion, Claude Bernard in his investigations of gastric acid exemplifies a case of reverse serendipity: great intellect, thorough testing and inductive reasoning led to the wrong conclusion.

28.2

THE MEASUREMENT OF RESPIRATORY VOLUMES IN "DE MOTU ANIMALIUM" BY G. A. BORELLI. Giuseppe Sant'Ambrogio and Roberta Forattini Bolchini*. Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Giovanni Alfonso Borelli (1608-1679) discusses several aspects of respiratory physiology in two chapters (VII & VIII) of the second part of "De Motu Animalium". Two of the "Propositiones" of chapter VII are almost entirely dedicated to respiratory volumes. "Propositio" 81 describes how he measured the volume of inspired air by means of a glass tube of known dimensions. He inspired through the glass tube and noted the vertical displacement of a bubble that was produced at its far end by dipping it in soapy water. The value obtained was of 14 cubic digiti that, when the digitus is translated into its metri equivalent, would correspond to 88.6 ml. A very small tidal inspiration. Possibly the digitus used by Borelli differed from the Roman unit, as implied by J. Jurin (Phil.Trans.Roy.Soc. London, 30:748-766, 1718) in his reference to Borelli's measurement. Additional difficulties originate when the dimensions of the glass cannula, as stated in the text, are considered. "Propositio" 94 examines the amount of air that remains in the lungs at the end of a normal expiration and discusses the difference in volume between inspired and expired air, attributed to the change in temperature. In the same "Propositio" Borelli seems to imply the existence of a residual volume.

NEURAL CONTROL OF CIRCULATION II

29.1

EFFECT OF INCREASED HEART RATE AND SYSTEMIC BLOOD PRESSURE ON GLUCOSE AND FATTY ACID UTILIZATION BY THE RAT HEART: A NEW AUTORADIOGRAPHIC TECHNIQUE David R. Kostreva and David Roerig*. Med. Col. Wis. and VA Medical Center, Milwaukee, WI, 53193

Eight sodium pentobarbital anesthestized (35 mg/kg i.p.) and ventilated rats were divided into two groups. One group of 4 animals was injected with [^{14}C]2-deoxyglucose 100 uCi/kg i.v. in a single bolus. The second group of 4 animals was injected i.v. with a single bolus of [^{14}C] erucic acid that was bound to FA free serum albumin in a molar ratio of two at a pH of 7.4. Prior to injection, two animals from each group were given an i.v. isoproterenol and neosynephrine drip to increase the heart rate and systemic blood pressure, thereby increasing the work of the heart. The substrates were then injected and after 45 minutes the animals were sacrificed, the hearts frozen and sectioned at 20 um, covered with film, exposed for 12 days, then developed and scanned. All of the images were converted to relative values of utilization using the Sokoloff equation and lumped constant for rat brain. The results demonstrate for the first time that this new autoradiographic cardiac metabolic imaging technique can demonstrate that increased cardiac work results in increases in both fatty acid and glucose utilization by the heart as expected.

29.2

"Cardiopulmonary" or left ventricular mechanoreceptors (LVM)? Evidence against inhibitory impulses to the vasomotor centers arising from the lungs. J.J. Leonard*, C.R. Swayze*, M.E. Anderson*, A.M. Booth* and I.J. Fox, University of Minnesota, Minneapolis, MN 55455

Seven dogs, carotid sinuses isolated and perfused at constant pressure (50 mmHg), were placed on cardiopulmonary bypass at constant-rate systemic perfusion and their lungs ventilated (<20 mmH_2O) at 14/min with air+O_2. Control mean arterial pressure was 99 ± 11 (SEM) mmHg with lungs ventilated and heart beating. Successive excision of the lungs did not alter mean arterial pressure and 10 min after dual pneumonectomy it was unchanged, 91 ± 9 mmHg, (P>0.05), indicating inhibition of vasomotor centers does not arise from the lungs. However, after cross-clamping the root of the aorta, the right ventricle, left atrium and pericardium were excised, termination of retrograde coronary perfusion resulting in global cardiac infarction and asystole, followed by a progressive rise in arterial pressure, indicating tonic inhibition from the beating LV. Ten min after aortic clamping, arterial pressure had risen 19 ± 9% to 118 ± 12 mmHg (P<0.01). Since nervous tissue survives >1 hour after global cardiac infarction, as shown by an intact LVM reflex, reflexes from unperfused lungs (bronchial circulation perfused) would also be expected to remain intact. Thus, in presence of a beating heart, the ventilated lungs do not produce detectable inhibition of vasomotor centers, whereas such inhibitory impulses originate in the beating LV, rendering the concept of "cardiopulmonary" receptors misleading.

29.3

Effect of bilateral cervical vagotomy (Vx) on initial hemodynamic response to exercise with and without aortic (Ao) resistance maintained constant. A.M. Booth*, M.L. Partington*, D.A. Gerasch*, C.R. Swayze* and I.J. Fox, University of Minnesota, Minneapolis, MN 55455.

To study effect of maintaining Ao resistance constant, before and after Vx, on initial response to exercise, a servo-controlled Ao occlusion cuff was used in 5 instrumented dogs (Ao flow probe, s-state pressure gauges in LV, Ao) to maintain Ao resistance constant at pre-exercise level (Occ) during 15 sec treadmill exercise, 9 kph, 0% grade. At 5 sec of exercise, the following results were obtained (% change from control):

	Before Vagotomy		After Vagotomy†	
	Without Occ	With Occ	Without Occ	With Occ
Ao Resistance	-34±2	-6±1	-29±1NS	-6±1NS
Ao Pressure	--	13±1	-14±1**	8±1**
Ao Flow	47±3	20±2	22±1**	15±2*
Heart Rate	51±3	23±3	8±1**	2±1**
Stroke Volume	--	--	13±1**	13±3**
LV peak dP/dt	9±1	8±2	-5±1**	--**

†Resting (control): resistance was 1/5 higher, heart rate was 1/3 higher and stroke volume was 1/3 lower after Vx. --=no change. For difference from before Vx, NS=not significant, **=P<0.01, *=P<0.05.

Thus, abolition of the LV mechanoreceptor reflex (LVMR) by Vx interfered with contractility-peripheral resistance matching, a major function of the LVMR, as well as with other aspects of the initial exercise response.

29.4

LOSS OF MUSCARINIC CHOLINERGIC RECEPTORS IN HEART FAILURE AND CARDIAC DENERVATION. Dorothy E. Vatner*, Charles J. Homcy*, Alan M. Fujii*, Michel Lavallee*, Jun Amano*, and Stephen F. Vatner. Dept. of Medicine and Children's Service, Mass. Gen. Hosp., and Harvard Med. School, Boston, and the New Eng. Primate Res. Ctr., Southboro, MA 01772.

It is possible that loss of myocardial sympathetic innervation, which is observed in some models of heart failure, may induce loss of muscarinic receptors on pre-junctional sympathetic nerves. We compared muscarinic receptor density in hearts from 6 dogs with chronic pressure overload induced left ventricular (LV) failure and 8 normal dogs. LV failure was associated with increased LV end diastolic pressure (32.6 ± 4.5 vs. normal of 7.6 ± 0.7 mmHg), increased LV weight/body weight ratio (6.9 ± 0.4 vs. normal of 3.4 ± 0.2 g/kg), and reduced LV tissue norepinephrine (221 ± 77 vs. normal of 575 ± 87 pg/mg). In heart failure, LV muscarinic receptor density was reduced (115 ± 15 vs. normal of 252 ± 14 fmol/mg protein). We also examined the extent to which total chronic surgical cardiac denervation altered muscarinic receptor density. In 6 denervated hearts, which exhibited normal hemodynamics and morphology, LV norepinephrine was reduced (2.2 ± 1.2 pg/mg) as was muscarinic receptor density (193 ± 14 fmol/mg protein). Thus, the reduced muscarinic receptor density observed in heart failure may be attributed only partially to sympathetic denervation and concomitant loss of muscarinic receptors on pre-junctional sympathetic nerves.

29.5

FOURIER ANALYSIS OF THE COMPONENTS OF BLOOD PRESSURE REGULATION IN NORMAL AND CARDIAC DENERVATED DOGS DURING SINUSOIDAL ACCELERATION. D. Randall, C. Knapp*, J. Evans*, and K. Lee*, UNIV OF KENTUCKY, LEXINGTON, KY 40506.

We previously reported (Physiologist 27:215, 1984) that cardiac denervated (D) dogs were less able to minimize acceleration-induced excursions in arterial blood pressure (ΔAP) than were normally innervated (N) dogs. We now explain this observation based on results of a fast Fourier transform analysis of AP, cardiac output (CO), stroke volume (SV), heart rate (HR) and peripheral resistance (PR). Ten chronically instrumented dogs (5D and 5 N) were subject to $+2G_z$ acceleration extending from 0.005 Hz to 0.25 Hz (periods of 200 to 4 sec) using a large animal centrifuge (Am. J. Physiol 243:H998, 1982). When compared to N dogs, D dogs had a larger ΔAP for all tests up to 0.1 Hz: at 0.04 Hz the amplitude peaked at 3X larger. The PR responses (mean, 1/2 amplitude, phase) were not different between the two groups: up to 0.01 Hz, PR responses were large and were optimally phased with respect to acceleration input to minimize ΔAP; however between 0.01 and 0.04 Hz, PR responses were large but increasingly lagged acceleration, reaching 180° phase lag by 0.05 Hz thereby contributing to the pressure excursions in both N and D dogs. At higher frequencies the amplitude of PR responses diminished rapidly. CO, SV and HR responses did differ markedly between the two groups. Denervation totally eliminated compensatory HR fluctuations which were an effective component of AP regulation in N dogs. Denervation more than doubled the amplitude of SV excursions but did not change the passive phase relationship (180°) between SV and acceleration. The loss of effective magnitude and phase regulation of both SV and HR to counteract the out-of-phase PR response appears to explain the large AP oscillations seen in D as compared to N dogs. (Supported by AFOSR #49620-83-K-0002 and HL 19343)

29.6

EFFECTS OF EPINEPHRINE ON CAROTID SINUS BARORECEPTOR ACTIVITY. J.L. Seagard, F.A. Hopp*, and J.P. Kampine. Depts. of Anesthesiology and Physiology, Med. Col. of Wisconsin and VA Medical Center, Milwaukee, WI. 53193

In some previous studies, catecholamines and sympathetic efferent nerve activity have been shown to sensitize arterial baroreceptors. Other studies, however, have not shown any baroreceptor response to either stimulus. In this study, the effects of epinephrine (10^{-8}M to 10^{-6}M) on carotid sinus baroreceptor activity were studied utilizing an isolated, perfused, in situ carotid sinus preparation in anesthetized mongrel dogs (Sodium thiopental, 25 mg/kg plus 5 mg/kg/hr). Few-fiber and whole nerve recordings were made of afferent carotid sinus baroreceptor activity from the carotid sinus nerve during constant and ramp changes in carotid sinus pressure. Sinus perfusion of 10^{-7}M epinephrine produced increases in ongoing (baseline) activity at a sinus pressure of 125 mmHg in whole nerve recordings and increases in rate of change of firing in few fiber preparations during ramp pressure changes. Perfusion of 10^{-6}M epinephrine evoked activity in previously silent fibers, but also decreased firing in some active fibers. Addition of phentolamine returned carotid sinus nerve activity to control levels. The biphasic response suggests that epinephrine may have multiple effects on baroreceptors, including a direct sensitizing effect and an indirect "unloading" effect due to actions on sinus wall muscle. Supported by VA 7793-02P.

29.7

FASTIGIAL STIMULATION INHIBITS PARASYMPATHETIC ACTIVATION INDUCED BY SUDDEN ELEVATION OF BLOOD PRESSURE IN CATS. C. H. Chen and L. O. Lutherer, Depts. of Physiol. and Int. Med., Texas Tech Univ. Health Sci. Ctr., Lubbock, Texas 79430.

It is believed generally that stimulation of the cerebellar fastigial nucleus (FN) inhibits baroreflex bradycardia mainly via a generalized augmentation of the sympathetic nervous system outputs. In the present experiment, propranolol (300 μg/kg) was given to six anesthetized cats (chloralose-urethane) to eliminate cardiac beta-adrenergic responses, and adequate blockade was validated with isoproterenol (1 μg/kg). Prior to blockade, reflex bradycardia induced by intravenous bolus injection of phenylephrine (PE, 20 μg/kg) was diminished from -0.45 ± 0.11 to -0.03 ± 0.03 beats/mmHg/min ($p < 0.01$) by a simultaneous FN stimulation (50 Hz, 0.1 ms, 50 μA). During beta-adrenergic blockade, simultaneous FN stimulations reduced also the PE-induced bradycardia from -0.43 ± 0.15 to -0.06 ± 0.03 beats/mmHg/min ($p < 0.05$). The normal bradycardia response to PE under beta-adrenergic blockade appeared immediately after the FN stimulation was stopped (-0.25 ± 0.04 beats/mmHg/min, $p < 0.05$). PE failed to induce bradycardia in two cats pretreated with atropine only (100 μg/kg), indicating that this reflex is mediated primarily by parasympathetic activation in cats. These findings suggest that in cats inhibition of parasympathetic activation by FN stimulation plays a significant role in blocking baroreflex bradycardia. (Supported by AHA Grant-in-Aid 82-1235 and Tarbox Institute TTUHSC).

29.8

MYOCARDIAL AND VASCULAR INTERACTIONS AMONG OPIATE AND ADRENERGIC SYSTEMS. James L. Caffrey and John F. Gaugl Dept. of Physiology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Opiate peptides depress and opiate receptor blockade enhances cardiovascular function in anesthetized dogs. The fall in blood pressure after IV methionine-enkephalin (ME) increases during carotid baroreceptor occlusions (BCOs). Isolated heart-lung and hindlimb models were employed to identify cardiac and peripheral vascular participation in this response. Naloxone enhanced cardiac contractility after isoproterenol 50-100% , shifted the dose effect curve to the left and extended significantly the duration of the response. Blockade of neuronal reuptake (imipramine) extended inotropic effects slightly without altering peak effects. Furthermore, inotropic responses to tyramine remained demonstrable after naloxone. Saturation of non-neuronal uptake with excess normetanephrine produced effects similar to naloxone suggesting that naloxone may modify non-neuronal uptake mechanisms. Systemic injection of ME produced transient increases in hindlimb blood flow coincident with acute declines in systemic blood pressure. This opiate mediated decline in femoral vascular resistence and systemic blood pressure both enlarged when sympathetic outflow is increased via BCO. Opiate actions within the circulation may be mediated through cardiac and peripheral vascular means. The magnitude of these effects depends in part upon the current level of sympathetic tone. Supported by the American Heart Association, its Texas Affiliate, and the National Osteopathic Foundation.

29.9

AN ANALYSIS OF BETA ADRENERGIC BLOCKADE IN UNTREATED AND GUANETHIDINE PRETREATED DUCKS DURING THE DIVE REFLEX. G. Friedrichs*, L. Wittmers*, R. Pozos*, and L. Beck* (SPON: P. Royce). Univ. of Minnesota, Duluth; Duluth, MN 55812.

The dive reflex was elicited in unanesthetized mallard male ducks by immersing the head into a beaker of water. B-adrenergic blockade was produced by intravenous injection of 10 mg/kg of propranolol. Guanethidine was given intramuscularly or subcutaneously in three divided doses of 10-15 mg/kg per dose over a week interval to deplete noradrenergic nerves. The diving time was maintained for 30 seconds unless the animal became agitated. Comparisons of heart rate (HR) were made in the pre-dive state; in the last 10 seconds of the dive (immersion); and in the first 10 sec. of removal of the head from water (emersion). In untreated controls, HR rate fell very significantly during immersion and rose significantly above control rate during emersion. Guanethidine pretreatment did not significantly affect pre-dive HR; but resulted in less significant lowering of HR during immersion; and resulted in a higher emersion heart rate. Propranolol significantly decreased pre-dive HR in control ducks; decreased dive HR on average (from 80 to 56); and lessened the HR increase upon emersion (256 in untreated vs 220 in guanethidine pretreated ducks). Following guanethidine treatment propranolol also lowered predive, immersion and emersion heart rates; however, propranolol lowered HR more in untreated ducks compared to guanethidine treated ducks. (Supp. by Sea Grant No. 0652-5618.)

29.10

AUTOREGULATORY ESCAPE FROM NEURAL CONTROL OF INTESTINAL CIRCULATION IN DEVELOPING SWINE. N.M.Buckley, M.Jarenwattananon*, P. M.Gootman and I.D.Frasier*. Albert Einstein College of Medicine, New York, NY 10461.

We examined the capability of the intestinal circulation to maintain a vasoconstrictor response (VC) during postganglionic adrenergic nerve stimulation in 32 swine aged 2 days- 2 months. All were anesthetized with pentobarbital and ventilated to sustain blood gas composition. Aortic and portal venous pressures, ECG, and superior mesenteric artery flow (F) were recorded. Intestinal vascular resistance(R) was calculated as mean pressure difference/ mean F. Section of the splanchnic nerve and postganglionic fibers decreased R in all animals. A fiber bundle arising from the celiac ganglion and coursing around the artery was tied, sectioned and stimulated. Stimulus-locked F decreases were obtained to mesenteric nerve stimulation (MNS) for 20 sec at 5-17 Hz, 1.5-2.5 mA and 0.5 msec pd. Latencies of VC shortened with age and with increasing MNS frequency at any age. R increased with increasing MNS frequency in all animals. This VC was greater as the animals matured: e.g.,MNS at 17 Hz increased R 35% at ≤ 1 week, 46% at 2 weeks, and 63% at 4 weeks after birth. Prolonging MNS for 60 sec led to sustained high R in 2-7 day olds at 10 and 12 Hz, and in 2 week olds at 12 Hz. In older swine, R decreased toward control before the end of the 60-sec MNS period. This finding suggests that the least mature intestinal circulation was incapable of autoregulatory escape during VC and provides more evidence of functional immaturity in that circulation. (Supported by NIH grant HL-21865).

29.11

THE EFFECT OF PYRIDOSTIGMINE ON CIRCULATORY FUNCTIONS OF AWAKE DOGS AT REST AND DURING EXERCISE. W. Ehrlich, A.R. Jayaweera*, T. Guilarte*, and H. Abbey*. The Johns Hopkins Univ. Med. Inst. Baltimore, MD 21205.

The effect of cholinesterase inhibition on the circulation of resting and exercising (1.5 mph, 9° inclination) dogs was studied in 40 experiments with 9 instrumented awake dogs. One mg·kg^{-1} pyridostigmine i.m. (pyr) lowered plasma-cholinesterase to 40% of control values, caused salivation, mucus formation, urination and defecation. Pyr did not change cardiac output, left ventricular work or aortic pressure in resting or in exercising dogs from the respective control values. It elevated transmural (tm) pulmonary artery pressure by 2.8 mmHg (resting) and 2.65 mmHg (exercising). Pyr did not change heart rate and mean left ventricular pressure in resting dogs, lowered heart rate by 17 beats·min^{-1} and mean left ventricular pressure by 5.11 mmHg in exercising dogs. Pyr elevated tm left atrial pressure by 2.07 mmHg and tm right atrial pressure by 1.79 mmHg in resting dogs. These functions remained unchanged in exercising dogs. We suppose that the effect of enhanced muscarinic activity caused by pyr on the heart is counteracted by enhanced sympathetic activity originating in the reflexly stimulated respiratory center as is indicated in these dogs by greatly increased respiratory frequency and minute volume. Pyr, therefore, does not compromise the enhancement of cardiac functions during exercise. The muscarinic activity increases the tone of pulmonary artery in the resting state and during exercise.

Supported by USAMRDC contract DAMD 17-83-C-3182

BLOOD PRESSURE AND HYPERTENSION II

30.1

ABNORMALITIES IN CORONARY CIRCULATION AND MYOCARDIAL OXYGEN CONSUMPTION IN ISOLATED HEARTS FROM SPONTANEOUSLY HYPERTENSIVE RATS. Y. Edoute*, T.F. Luscher* and P.M. Vanhoutte. Dept. Physiol. and Biophys., Mayo Clinic, Rochester, MN 55905

Hypertension causes structural and functional changes in the coronary circulation which may impair adaptation to different levels of perfusion pressure (P). Experiments were designed to study the effects of different levels of P on coronary flow (CF), coronary vasodilator reserve, myocardial tension development and myocardial oxygen consumption (MVO_2) in spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY). Isolated hearts were paced at constant rate and perfused by the Langendorff technique at varying P (50-150 cm H_2O). Transient coronary occlusion (60 sec) was used to determine vasodilator reserve. In SHR CF, vasodilator reserve and myocardial tension development were lower than WKY at all levels of P. At low P (50, 75 cmH_2O) CF was markedly depressed and no coronary vasodilator reserve was present in SHR. The oxygen consumption per amount of work was independent of P in both SHR and WKY. These results suggest that functional and structural alterations of the coronary circulation in hypertrophied hearts of SHR impair CF at any level of P. The functional significance of these findings is supported by an increased MVO_2 and a decreased myocardial tension development at low P in SHR. This may cause an imbalance between O_2-supply and demand under conditions of increased metabolic activity or when coronary P is inadequately low. (Supported in part by NIH grant HL 31547.)

30.2

DEPENDENCE OF VESSEL CROSS SECTIONAL AREA ON CIRCUMFERENCE OF EXCISED RING SEGMENTS FROM NORMOTENSIVE AND HYPERTENSIVE ANIMALS. Joel M. Price. University of South Florida, College of Medicine, Department of Physiology, Tampa, FL. 33612

Intact vessels are restrained longitudinally such that length remains constant with circumferential distention. As a result cross sectional area (A) is independent of circumference (C) whereas thickness (h) is not. Accordingly, studies of perfused vessels in hypertension consider cross sectional area to be a more accurate index of wall mass than thickness. When excised rings are studied the cut edges of the ring are free of longitudinal forces and cross sectional area as well as thickness may depend on circumference. In this study a theoretical analysis and experimental measurements show that cross sectional area and thickness of chemically fixed aortic rings from WKY rats and SHRs depend on circumference . For an anisotropic vessel wall the relationship between thickness and circumference is $h = \alpha + (\alpha^2 + \beta/C)^{1/2}$. Where α and β are constants. The relationship between cross sectional area and circumference has a similar form and the same constants. Best fit curves to the experimental data show that at each ring circumference cross sectional area and thickness of the aorta is greater in the SHR than in the WKY rat. It may be concluded that circumference must be accounted for when cross sectional area or thickness is compared in normal and hypertensive animals and that both are equally accurate indices of wall mass in excised rings. Supported by NIH grant #21103 and American Heart Association, Florida Affiliate.

30.3

RED CELL MEMBRANE TRANSPORT AND CLINICAL CHARACTERISTICS OF NORMOTENSIVE AND HYPERTENSIVE SUBJECTS. N.C. Adragna, J.L. Chang*, M.C. Morey*, and R.S. Williams*. Depts. of Physiology & Medicine, Duke Univ. Med. Ctr. Durham, N.C. 27710.

To test the hypothesis that factors other than blood pressure associated with cation transport properties differ in subsets of an heterogeneous population, we studied 15 clinical and 18 membrane transport characteristics and their relationships in a total population of 78 Caucasian males consisting of 30 normotensives, 17 labile and 31 fixed hypertensive patients. Some of the strongest correlations were: 1)In the total population: red cell Na in Na-loaded cells with age and diastolic blood pressure at maximum exercise capacity; 2) In the normotensive population: the red cell K concentration in Na-loaded cells with plasma cholesterol and triglycerides; 3) In the labile hypertensive subgroup: the rubidium leak with maximum exercise capacity; 4) In the fixed hypertensive population: pump velocity in fresh cells with systolic blood pressure at maximum work capacity ($p=0,003$). In addition, a different pattern of correlations was found for each subgroup. We conclude that past and future studies of cation transport abnormalities in the pathophysiology of hypertension may be complicated by the confounding effects of several clinical variables such as body weight or plasma lipoprotein levels, and that such effects may affect normotensive and hypertensive populations in a different manner In addition,within the hypertensive population, blood pressure seems to be the main factor associated with red cell membrane transport parameters (Supp.by NIH grant H125146 & Pepsico Fndt).

30.4

EFFECTS OF NORADRENERGIC AND CHOLINERGIC BLOCKADE UPON PRE-ECLAMPTIC PLACENTAL EXTRACT INDUCED HYPERTENSION IN RATS. Dean V. Papoutsis* and John J. Curry. Dept. of Physiology, Ohio State University, Columbus, Ohio 43210

Preeclampsia, also termed toxemia of pregnancy, is a disease afflicting human pregnant women. The major symptoms, hypertension and proteinuria, develop after the 24th week of gestation. In our laboratory we have subjected cesarean delivered preeclamptic and normotensive placentas to an acetic acid extraction procedure, developing extracts from each. Preeclamptic Placental Extract (PPE) contains a putative vasoactive substance that induces hypertension upon infusion into virgin female and pregnant rats. In addition, PPE causes an increase in protein excretion. Normotensive Placental Extract (NPE) is without such effects. Prior to infusion of PPE, phenoxybenzamine (an α-noradrenergic blocker), propranolol (a β-noradrenergic blocker), or atropine (a cholinergic blocker) were administered by intraperitoneal injection to three groups of virgin female rats and three groups of pregnant rats. It was found that propranolol blocked PPE-induced hypertension and protein excretion in both virgin females and pregnant rats. Phenoxybenzamine and atropine failed to block such effects. These findings suggest that β-noradrenergic receptors are necessary for the expression of PPE-induced hypertension in virgin females and pregnant rats.

30.5

PLASMA AND AORTIC RENIN ACTIVITY IN THE DOCA HYPERTENSIVE PIG. J.M. Terris and T.M. Martin*. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20814-4799

Aortic segments (Thoracic(T), upper abdominal(UD), lower abdominal (LD)) were collected in ice cold saline from pigs 24 hrs to 6 wks post-DOCA (100 mg DOCA/Kg) implantation. Blood and connective tissue were removed. Two grams of diced tissue were freeze-thawed 3 times in a dry ice/acetone slurry and 37° C water bath and homogenized in 10 ml of cold distilled water and 15 mg Na_2EDTA. Mixtures were centrifuged, supernatants (S) taken to pH 2.8 with 2N H_2SO_4, 1.18 ml cold 95% ethanol added, and left undisturbed for 1 hr on ice. Following centrifugation S were taken to pH 4.5 with 2N KOH and dialyzed for 18 hrs against 2L of cold distilled water. Dialysates were adjusted to pH 2.8 with 2N H_2SO_4 and NaCl added to produce a 0.8M solution. Following centrifugation, $(NH_4)_2SO_4$ was added to the S to produce a 1.0M solution, centrifuged and $(NH_4)_2SO_4$ added to S to produce a final 2.3M solution and centrifuged. Pellets from the 2 $(NH_4)_2SO_4$ steps were combined and stored at -20°C. For plasma renin activity (PRA) blood was collected on ice in Na_2EDTA, centrifuged, and plasma stored at -20°C. Renin activities were determined by RIA. There was a significant positive correlation between PRA and aortic renin activity (VRA) for all 3 segments (T;Y=6.5X + 56.2, r=0.83: UD;Y=4.0X + 126, r=0.63: LD;Y=8.1X + 118.7, r=0.79). VRA did not persist when PRA decreased to undetectable levels. We conclude that VRA does not contribute to the maintenance of the elevated blood pressure in this model.

30.6

Increased Renal Clearance of Lysine Vasopressin in the DOCA-Hypertensive Pig. William D. Ling*, David P. Brooks, Joan T. Crofton, Leonard Share, and David F. Bohr. U. of MI, Ann Arbor, MI 48109, and U. of TN, Memphis, TN 38163

We have observed an elevated urinary excretion of lysine vasopressin(LVP) but no increase in plasma LVP(Plvp) in the DOCA-hypertensive pig. The present study determines whether this paradox could be explained by an altered renal clearance of LVP. Clearance of LVP(Clvp) was measured in 5 DOCA-hypertensive(DOCA) and 5 normotensive(NT) conscious female pigs during a continuous infusion of 5% dextrose and during infusion of a 5% dextrose solution containing LVP. The infusion rate for the entire experiment was maintained at a constant rate of .2ml/kg·min. The LVP concentration of the LVP infusate was adjusted to maintain a constant infusion rate of 400 uU/kg·min. Clearance levls of the DOCA pigs during the control infusion were higher than those of the NT pigs(2.00± .40 vs 0.50± .15 ml/kg·min). This difference also occurred with infusion of exogenous LVP (DOCA= 2.81± .66, NT= 1.39± .31 ml/kg·min). Urine minute volume was greater in the DOCA than in the NT animals (Control infusion: DOCA= 3.10± .65, NT= .83±.09 LVP infusion: DOCA= 3.92± .58, NT= 1.37± .21 ml/kg·min). There was no difference in Plvp between DOCA and NT pigs during the control infusion (DOCA= .75± .34, NT= 1.51± .27 uU/ml) and during the LVP infusion (DOCA= 17.54± 4.02, NT= 18.51± 1.93 uU/ml) These findings suggest that the polyuria of the DOCA hypertensive animal may be responsible for the increased Clvp. Supported by NIH grant HL18575.

30.7

MONOSODIUM GLUTAMATE OBESITY SYNDROME: EFFECTS ON BLOOD PRESSURE AND VASOPRESSIN. Richard W. Clough*, Paul F. Aravich* and Celia D. Sladek* (Spon.: R.J. Connett). Univ. of Rochester Sch. Med. Dent., Rochester, NY 14642.

Monosodium glutmate (MSG), a neurotoxin to developing circumventricular organs, hypothalamic arcuate tissue and portions of the medulla oblongata, produces several endocrinopathies following neonatal exposure. Few studies have investigated the effects of MSG on neurohypohysial function and systolic blood pressure (SBP) in male and female rats treated with MSG as neonates (4 mg/g; days 1,3,5,7&9). MSG produced a sex-selective systolic hypotension in females at 6-12 weeks of age (tail cuff; $p<.05$). VP content of several brain areas was not altered in MSG rats with the exceptioin of reduced male supraoptic nucleus VP. All groups were able to concentrate their urine during 48-hrs. of dehydration. MSG rats were obese at sacrifice and had reduced adenohypophysial and gonadal weights. These studies indicate that in contrast to the adenohyphysis, the neurohyphysial VP system is not adversly affected by neonatal MSG, and that the effect of MSG on SBP is sex dependent. Supported by NIH Grant AM 1976.

30.8

A CHRONICALLY INSTRUMENTED RAT MODEL FOR SYSTEMIC AND PULMONARY CIRCULATORY STUDIES. G.L. Sardella* and L.C. Ou, Department of Physiology, Dartmouth Medical School, Hanover, NH 03756.

A rat model with chronically implanted electromagnetic flow probes, femoral and pulmonary arterial catheters has been developed. Chronic probes from Carolina Medical Electronics were placed around the ascending aorta in rats weighing from 300 to 350g. After initial weight loss of about 30g following surgery, the animals gained weight steadily and appeared healthy. Stable resting cardiac output (CO), systemic (P_{SA}) and pulmonary (P_{PA}) arterial pressures were maintained for a period of two weeks. The mean values of CO, P_{SA} and P_{PA} were 453.8 ± 54.4 (SE), 111.2 ± 14.0, and 15.6 ± 1.0, respectively. On autopsy, the thoracic cavity, heart and lungs were clear and free of apparent infection. The left and right ventricular to body weight ratios did not differ significantly from those of the non-operated controls. Hypoxia caused systemic vasodilation and pulmonary vasoconstriction. Angiotensin II (AII) elevated the vascular resistance of both systemic and pulmonary circulations. Changes in CO were inversely related to changes in P_{SA}. The sensitivities of the pulmonary pressor responses to hypoxia and AII were much greater in the intact awake rats than in isolated-perfused lungs. The data indicate that normal hemodynamic parameters and responses to vasoactive agents of both circulations were maintained in chronically instrumented rats. This model may prove to be ideal for long-term hemodynamic and pharmacological studies of both circulations. (Supported by NIH grant HL 21159.)

30.9

ARTERIAL PRESSURE CONTROL IN CONSCIOUS SINO-AORTIC DENERVATED SHEEP†. M. Miki*, K. Miki*, J.A. Krasney, D. Curran-Everett* and K. McAndrews*. Dept. of Physiology, SUNYAB, Buffalo, NY 14214.

The role of the arterial baroreceptors in the control of blood pressure over time was studied in 5 SAD sheep after chronic section of the carotid sinus and aortic depressor nerves. The sheep showed no change in heart rate (HR), arterial pressure (Pa) or ventilation in response to intravenous injections of NaCN. Also, there were no changes in HR in response to injections of phenylephrine or nitroprusside. Pa, HR, and vertical position were recorded continuously over 16 hours in 6 normal and 5 SAD ewes. Calculated systemic vascular resistance was 22% less and Pa 15% less in the SAD group ($p<0.05$). HR tended to increase by 33% in the SAD group, but SV decreased by 23% so that cardiac output (thermodilution) was not different between the two groups. Chronic SAD leads to vasodilatation and a decrease in SV with insufficient compensation by increased HR to maintain Pa at the control level. Frequency distribution curves showed no significant difference in the variability of Pa and HR between normal and SAD sheep. (Supported by PHS Grant HL-27683)

†Sino-aortic denervated = SAD

30.10

SUSTAINED INCREASES IN ARTERIAL PRESSURE CAUSED BY CENTRAL NERVOUS SYSTEM ISCHEMIA. D.J. Dzielak and R.A. Norman, Jr. University of Mississippi Medical Center, Jackson, MS 39216

Ischemia of the central nervous system (CNS) causes an intense activation of the sympathetic vasoconstrictor system that can often raise mean arterial pressure (MAP) to a level as high as 250 mmHg. The intense pressor response functions to protect the CNS from hypoperfusion during a circulatory crisis in an effort to maintain neuronal integrity. Past investigations into the nature of the CNS ischemic response have been concerned mainly with the acute elevations in arterial pressure observed when the blood supply to the brain is completely stopped. The duration of the ischemic reflex produced under these conditions is limited to the amount of time the vasomotor center can maintain its function without a nutritional supply. We have developed a servo-control technique for precise control of cerebral arterial pressure levels (Fed. Proc. 43(3): 694,1984). The time course for MAP changes produced by CNS ischemia was examined. Mongrel dogs of either sex weighing 30-50 lbs. were studied. In separate experiments, cerebral arterial pressure (CAP) was lowered to and maintained at 50 and 35 mmHg respectively for a period of 8 hours. The average increase in MAP over the 8 hour period when CAP was 50 mmHg was 35.8±1.8. The average increase in MAP when CAP was at 35 mmHg was 24.0± 1.8. It is concluded that increases in MAP caused by CNS ischemia can be sustained for prolonged periods.

30.11

THE ROLE OF CARDIAC NERVES IN THE REGIONAL CIRCULATORY RESPONSES TO HEAD-OUT WATER IMMERSION IN CONSCIOUS DOGS. G. Hajduczok*, K. Miki*, and J.A. Krasney. Dept. of Physiology, SUNYAB, Buffalo, NY 14214

Previous studies in conscious dogs indicate that head-out water immersion (WI) leads to a sustained elevation of cardiac output (CO) accompanied by relative increases of blood flows to several gastrointestinal tissues during early WI, but then these flows are redistributed to skeletal muscles later in WI (Fed. Proc. 44:1200). The degree of involvement of reflex mechanisms in these responses is unknown. In this study, conscious cardiac denervated (CD) and sham denervated (S) dogs were studied in the quadruped position during 100 min in air, followed by 100 min of WI. Regional blood flow responses were measured with 15 m radiolabelled microspheres. CO increased significantly (p 0.05) by 38.7% in S dogs and 39.2% in CD dogs. Flows in S and CD groups increased significantly to the heart (40%, 38%), skin (93%, 96%), fat (79%, 83%), diaphragm (44%, 48%), and intercostals (58%, 55%), while there were no changes in brain and kidney flows during WI. Gastrointestinal tissue flows increased only during early WI in both S (45%) and CD (47%) groups. However, flows to skeletal muscles increased only during late WI in S (53%) and CD (47%) groups. There were no significant differences between the two groups. These data indicate that the regional circulatory responses to WI are similar in both S and CD animals and that cardiac nerves are not important in mediating these responses.

30.12

RIGHT ATRIAL VOLUME DURING HEMORRHAGE IN THE DOG. D.E. Carlson, K.W. Burchard*, and D.S. Gann. Brown Univ./R.I. Hospital, Prov., RI 02902.

Atrial B receptors elicit reflex changes after hemorrhage(H) and respond to changes in atrial volume, but the latter have not been measured in H. The impedance catheter devised by Baan et al(Cardiovascular Res. 15, 328, 1981) to measure changes in ventricular volume was advanced via the femoral vein into the right atrium to measure changes in its volume during H. Ten splenectomized dogs anesthetized with choralose underwent H at 2% of blood volume(BV, estimated as 75ml/kg) per min. H was stopped for 10 min intervals for measurements when the blood removed equaled 5, 10, 20, and 30% of BV. Cardiac output(CO) was measured with indocyanine dye. We examined the following variables: 1) Vmax, the maximal atrial volume at the end of ventricular systole, 2) Vs, the amount of filling during ventricular systole while the tricuspid valve was closed, 3) $\dot{V}s$, the rate of change of Vs, and 4) Ve, the volume ejected during the atrial beat. Vmax and Vs decreased significantly($P<0.05$) after removal of 10% BV. $\dot{V}s$ decreased significantly($P<0.05$) after removal of 20% BV. Ve decreased significantly($P<0.05$) only after removal of 30% BV. Atrial output(AO) as determined by multiplying Ve by heart rate(HR) did not change with H. However, the ratio of the AO to CO increased in direct proportion to the HR($P<0.01$) so that at 30% H, for which HR was high, the decreases in CO from baseline in individual dogs correlated significantly with the changes in AO($P<0.01$). We conclude that Vmax, Vs, and $\dot{V}s$ are the atrial variables that are most sensitive to H. In contrast, the ability to maintain Ve and AO during large H appears to be important in minimizing the fall in CO. Supported in part by NIH Grant #GM27946.

30.13

REGIONAL ORGAN PERFUSION WITH VASODILATOR THERAPY IN ACUTE HEART FAILURE. E.M. Farrell*, G.C. Taichman*, W.J. Keon*, (SPON: J.S. Cowan). University of Ottawa Heart Institute, Ottawa, Ontario, K1Y 4E9.

Vasodilator therapy has emerged as an important adjunct in the management of acute postoperative heart failure. We have begun a study to compare regional organ perfusion (OP) responses to various vasodilator agents under similar hypotensive conditions. Accordingly, anaesthetized dogs were subjected to a reproducible level of acute cardiac failure with induced pericardial tamponade (mean aortic pressure [mAP] of 60-70 mmHg). Systemic OP was measured with radiolabelled microspheres (15 μ). Measurements were made before and after tamponade and 30 min following initiation of vasodilator therapy. Hydralazine(Hd), nitroprusside(Np) and nitroglycerin(Ng) were independently titrated to reduce mAP from its post-failure level by no more than 5 mmHg. Hd therapy (n=9) re-established control OP levels to the heart, brain, and liver as well as improving flow in the lung, small intestine, thyroid and bladder. Np treatment (n=9) improved flow within the liver, small intestine, pancreas, and skeletal muscle to values below control. Thus, despite a common mechanism of action, Np and Ng, evoked significantly different responses. Hd, improved OP more than either Np and Ng. These data have extremely important implications for the management of postoperative cardiac patient.

30.14

LACK OF PRESSOR RESPONSE TO AMMONIUM CHLORIDE INJECTIONS IN CAT HINDLIMB. S.F. Lewis and M.P. Kaufman. Univ. Tx. Hlth. Sci. Center, Dallas, Tx. 75235

The precise stimulus to the metabolically sensitive type III and IV muscle afferents involved in the cardiovascular response to exercise is unknown. Patients with myophosphorylase deficiency (McArdle's syndrome) have excessive mean arterial pressure (MAP) and heart rate (HR) responses to exercise and a marked excess of ammonia (NH_3) in working muscle venous effluent. We injected 1 ml ammonium chloride (NH_4Cl) in doses of 80, 160, 320, 640, 1200, 2400, and 4400 μg/ml into a gracilis artery catheter with tip placed at the gracilis and femoral artery juncture in 7 chloralose-anesthetized cats. No MAP or HR responses to NH_4Cl were observed in 4 cats but in 3 others, modest MAP (5-10 mmHg) and HR (10 beats/min) increases were observed beginning at doses of 640 or 1200 μg/ml. Injection of 640 μg/ml NH_4CL gave femoral venous effluent NH_3 levels similar to muscle venous effluent NH_3 of McArdle patients in heavy exercise. In 2 of 3 cats responding to NH_4Cl, elevations in MAP and HR increased as the effects of anesthesia lessened. No MAP or HR responses to the same injected doses of NH_4Cl were observed in 3 decerebrate cats suggesting that a depression of the cardiovascular control centers by general anesthesia was not the cause of the pressor response in the 3 positively responding cats. The data suggest that NH_4Cl injected into the hindlimbs of cats may not be a critical stimulus for increases in MAP and HR.

CELL MEMBRANE BIOCHEMISTRY

31.1

β-ADRENERGIC RECEPTOR IN IN VITRO CULTURED RABBIT TRACHEAL EPITHELIAL CELLS. Carole M. Liedtke. Case Western Reserve University, Cleveland, OH 44106.

β-adrenergic receptor activity was compared in tracheal mucosa-submucosa (Liedtke, et.al., Biochim. Biophys. Acta 719: 169-177, 1982), in freshly isolated tracheal epithelial cells (Liedtke and Tandler, Am. J. Physiol. 247 (Cell Physiol. 16): C441-C449, 1984) and in cells grown 8-9 days in culture. Cells were isolated under sterile conditions and cultured in enriched medium at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2. After cells reached confluence (7-9 days), β-adrenergic receptor activity was assessed. (-)-Epinephrine caused a dose-dependent increase in cAMP levels and in PGE_2 release that was similar to that found for freshly isolated epithelial cells.

Days in Culture	EC50 (μM) cAMP Levels	PGE_2 Release
0	0.22	1.10
8	0.15	0.82

(-)-Isoproterenol and (-)-norepinephrine also caused a dose-dependent increase in the release of PGE_2. The effects of the β-adrenergic agonists were blocked by (+)-propranolol indicating the presence of β-adrenergic receptors in the cultured rabbit tracheal epithelial cells. The results demonstrate that β-adrenergic receptors are expressed in tracheal epithelial cells grown in in vitro culture. Supported by a gift from the Cystic Fibrosis Foundation Sports Challenge.

31.2

USE OF FORSKOLIN IN PREPARATION OF RAT LIVER ADENYLATE CYCLASE AND RECONSTITUTION OF GTP-SENSITIVITY.
J.A. Ruiz, Q.H. Shi and R.J. Ho
Dept. of Biochem., Univ. of Miami Sch. of Med., Miami, FL

Rat liver membrane with high glucagon-sensitive adenylate cyclase (650-1200 pmol/mg/min) have been prepared. Forskolin (Fo) stablizes adenylate cyclase (AC) in all steps during preparation of AC including solubilization, column chromatography and storage. Four types of column have been used in preparation of rat liver AC: Sephacryl S-300 (1.5 x 150 cm), sephadex G-75, DEAE Biogel A (0.7 x 2.5 cm) and DEAE Affigel Blue (0.7 x 7.5 cm). Ommition of Fo from any of these column systems resulted in inactivation of AC (greater than 90%). The inactivated enzyme lose its ability to response to Fo activation. With Sephacryl S-300 column, the catalytic component (C) can be separated from the regulatory proteins (N). C is identified by AC assay, Ns by GTP stimulation and by cholera toxin stimulated ADP-ribosylation, and Ni by ADP-ribosylation stimulated by pertussis toxin. C was not stimulated by Gpp(NH)p but by Fo. GTP-sensitivity can be restored by adding Ns fraction to C. A potentiation of the stimulatory effect on AC was observed when both Gpp(NH)p and Fo were present in the reconstitution assay system. Isolated C is stable at least for 3 month in liquid nitrogen temperature in the presence of 15 uM Fo, and completely inactivated in 7 days without Fo. It is concluded that Fo is useful in preparation and purification of membrane adenylate cyclase. (NSF grant number DCB 84-17472.)

31.3

DIFFERENCE IN ELUTION PROFILE FROM FORSKOLIN-AFFINITY COLUMN OF FORSKOLIN-SENSITIVE AND INSENSITIVE ADENYLATE CYCLASES.
Q.H. Shi, J.A. Ruiz and R.J. Ho
Dept. of Biochm., Univ. of Miami Sch. of Med., Miami, FL

Forskolin-affinity column (Fo-Af Col) has been used in comparative studies of membrane bound Fo-sensitive adenylate cyclase (MAC) and cyctosolic Fo-insensitive adenylate cyclase (CAC). The former was prepared from rat adipocyte membrane and the latter from rat testis. AC from both sources was loaded on Fo-Af Col (0.7 x 2 cm) and eluted stepwise with a buffer with varying concentrations of NaCl, with or without forskolin. NaCl, 200 mM, eluted more than 70% of the retained CAC from column; less than 5% of MAC was eluted with the same buffer system. MAC was eluted with buffer containing 300-500 mM NaCl and Fo, 100 uM. Fo has no activation effect on CAC, it activates MAC regardless of whether the assay was done before or after Fo-Af Col. The guanine regulatory protein (N) of MAC can be separated from the catalytic unit (C) on Fo-Af Col. As a result, the fractions lose their response to GTP, but still can be stimulated by Fo. This GTP-sensitivity can be restored by combining the N and C fractions in the assay. CAC, on the other hand, has no response to GTP. The results are interpreted to mean that the difference in response to Fo between CAC and MAC may be related to the binding of Fo to the enzymes. A linear correlation of Fo binding and MAC activation shown 1984 support this view. (NSF grant number DCB 84-17472).

31.4

Effects of Hyperthermia and Membrane Fluidity on the Distribution of Spectrin in Lymphocytes. Christine S. Hughes*, Elizabeth A. Repasky* & John R. Subjeck*. (spon. by James Goldinger) Roswell Park Memorial Institute, Buffalo, New York 14263.

We have recently observed that spectrin occurs in a polar submembranous aggregate or PSA (similar in appearence to a "cap") in populations of isolated lymphocytes and in some lymphocyte cell lines. Here we report that following moderate hyperthermia (40.5°) for 60 minutes there is a dramatic reduction of the PSAs found in lymphocytes isolated from Balb/c mice spleen, thymus and lymph nodes and in PSA-positive cell lines. Similar results are seen following incubtion with linoleic acid (Repasky & Bankert, 1984, Fed. Proc. 44, 3106a). These two treatments are similar in that they increase membrane fluidity. Agents such as stearic acid may insert into the gel phase of the membrane (Klausner et al., 1980, J. Biol. Chem. 258, 4715). When stearic acid and hyperthermia treatments are combined, the integrity of PSA remains intact. Similarly, treatment with hyperthermia and cholesterol the PSAs are preserved. When a PSA-negative cell line (Molt-4) is examined, no changes were observed by treatment with free fatty acids and hyperthermia or cholesterol and hyperthermia in the distribution of spectrin. In summary, the changes in spectrin distribution caused by hyperthermia can be modulated by free fatty acids and cholesterol addition; therefore these results suggest that spectrin's distribution at the plasma membrane is influenced by the state of membrane fluidity.

31.5

COMPLEMENTARY-cDNA MODULATING LYMPHOCYTE FUNCTIONS IN VIRAL INFECTION. Anwar A. Hakim. Loyola University Medical Center. Maywood. Illinois 60153.

The study of cell surface glycoprotein synthesis in a cell-free system is one of the most promising approaches for analysis of the mechanism of cytotoxic gene expression. Suppressor cells from HBsAg vaccine recipients(SSC.Im) interact, whereas similar subsets from patients with chronic active hepatitis (SSC-CAHB) defect the interaction with Con A, fail to produce suppressor-cytotoxic factor(SCF) and produce an impaired Sheep erythrocyte Rosette (SER). In the present study, peripheral blood lymphocytes (PBL) from HBsAb-sero positive before and after vaccination with HBsAg vaccine and from patients with CAH-B were cloned and cultured in a serum-free media supplemented with Interleukin-2(IL-2). The membrane-boundpolysomes were prepared and mRNA extracted using 1%SDS and a mixture of Phenol Chloroform, followed by sucrose density gradient centrifugation and Oligo(dT) cellulose affinity chromatography.Complementary (cDNA) was prepared in a system containing the mRNA, Poly A, $Oligo(dT)_{12-18}$, dithiothreitol, dATP, dCTP, dTTP, 10 uCi ^{32}P-dCTP, bovine serum albumin, RNase Inhibitor, and reverse transcriptase in Tris.HCl-$MgCl_2$ and pyrophosphate buffer. The reaction was terminated with EDTA-SDS. Following Sephadex-50 chromatography and RNA hydrolysis various species of DNA were obtained. When these DNA preparations were incubated with a mixture of the 24 amino acids and material for energy followed by electrophoresis on SDS-PAGE, intensely labeled proteins were resolved. One was similar to HBsAg, and the others were detected by interaction with Con A and suppressing mitogenic response.

31.6

BIOCHEMISTRY AND BIOPHYSICS OF VIRAL SURFACE PROTEINS:DIFFERENTIATION BETWEEN HEPATITIS A, B AND NON A-NON B VIRUSES. Charles M. Siraki* and Anwar A. Hakim. Loyola University Medical Center Maywood. Illinois 60153.

At present, up to 90% of the cases of posttransfusion hepatitis are not related to hepatitis A or B and are designated as "Non A-Non B hepatitis,i.e. HNANB".Although this type of viral infection was recognized a decade ago, no specific test for the agent(s) has yet been identified. Several assays including agar diffusion, Counter electrophoresis, immunofluorescence, radioimmunoassay (RIA) and Enzyme-Linked-Immunosorbent assay (ELISA) were designed to detect antigens at the viral coat correlating with acute or chronic HNANB in serum. All these assays showed only a low efficiency of detecting infectious sera. The present studies report on an enzyme"Reverse Transcriptase" i.e. RT, at the surface of the virus that copies the viral RNA genome into DNA which can then become integrated as a "Pro-virus" into the DNA genome of infected cells. Viral particles were sedimented by ultracentrifugation of sera from patients with hepatitis A, B, and HNANB virus infections. The DNA preparations copied by the RNA from these viruses differed in their electrophoretic patterns and produced specific diagnostic probes to differentiate between these infective viruses. Sito, et al(Lancet i, 941,1984) reported the potential use of RT to test for the agent that cause HNANB, but the test system described failed in the hands of several investigators. The present studies showed differences between the products of the RT enzyme from these related viruses.

31.7

LECTINS FROM PLASMA AND HEMOCYTE MEMBRANE OF THE OYSTER CRASSOSTREA VIRGINICA. Gerardo R. Vasta and John J. Marchalonis, Medical University of South Carolina, Charleston, USA

Previous work showed that oyster hemocytes could bind specifically certain radiolabelled glycoproteins and that a hemocyte microsomal fraction could agglutinate specifically untreated and enzyme-treated vertebrate erythrocytes (RBC)(Vasta et al., J. Invertebr. Pathol. 40: 367, 1982).

Rabbit antibodies raised against oyster whole serum (RbαOS) and rat (Rt) antibodies raised against oyster serum lectins (RtαOL) which bind rat RBC, showed multiple and two precipitation lines respectively, against oyster whole serum in immunodifusion tests. Analyzed by immunofluorescence microscopy both antisera RbαOS and RtαOL bound to 90% of the oyster hemocytes in a double layer technique at 4°C using FITC-conjugated GoatαRb and GoatαRt antisera. Patching- and capping-like patterns of the surface bound material were observed.

Both antisera RbαOS and RtαOL blocked the agglutination of pronase-treated horse RBC by the hemocyte microsomal fraction in microhemagglutination assays. These results represent additional evidence for the presence of lectins on the oyster hemocyte membrane and suggest that those lectins are serologically related to the serum lectins which bind to rat and horse RBC (Vasta et al., Cell. Immunol. 88: 475, 1984).

(Supported by Fogarty International Fellowship (NIH) No. 1FO5TW03155-01, NSF grants PCM 8208016 (NSF), GM 30672 (NIH) and the State of South Carolina appropriations for Biomedical Research.)

31.8

HEMOLYTIC ACTIVITY FROM THE PELLET FRACTION OF ADULT SCHISTOSOMA MANSONI. M.R. Kasschau and M.H. Dresden*. University of Houston-Clear Lake, Houston, TX 77058 and Baylor College of Medicine, Houston, TX 77030.

We have identified an active hemolytic component in homogenates from the adult blood parasite, Schistosoma mansoni. The role of such an agent in schistosome nutrition is important in releasing hemoglobin which is necessary for parasite growth. This hemolytic agent appears to be unique since it has maximum hemolytic activity at acid pH (5.0) and is found in the pellet fraction of homogenates. Most hemolysins are neutrally active and soluble. In order to further characterize this agent we have solubilized this hemolytic agent using a sulfobetaine detergent Zwittergent 3-12. At least 75% of the activity is found in the supernatant fraction after overnight extraction at 4°C followed by 24 h dialysis against citrate buffer (pH 5.1); further characterization is being undertaken by HPLC. Using light microscopy we have observed the presence of membrane blebs protruding from the red blood cells (RBC's) during the lag phase prior to hemolysis. Following lysis, spherocytic ghosts are formed. Under SEM the RBC's first appear to produce extensive membrane projections which then merge into a few larger blebs immediately prior to hemolysis. The existence of a lag phase prior to hemolysis, in addition to the presence of the ghosts and observed morphological changes suggests that hemolysis is due to colloidal osmotic effects. Supported by NIH AI15864 and UH-CL ORF.

32.1

NON-COMPETITIVE PARTIAL INHIBITION OF HUMAN RED CELL CHLORIDE EXCHANGE BY EOSIN (E) AND EOSIN MALEIMIDE (EM). Philip A. Knauf, Nancy A. Mann*, and Jeffrey Penikas*. Univ. of Rochester, Rochester, N.Y. 14642

E and EM have been used as fluorescent probes for the red cell anion exchange protein, known as band 3 or capnophorin. Because disulfonic stilbenes, which are competitive inhibitors of anion exchange, interfere with binding of E and EM, it appeared that E and EM might inhibit chloride and sulfate transport by binding to the anion transport site. To examine this question, we measured the effects of changes in chloride concentration, with $Cl_i = Cl_o$, on the ability of E or EM to inhibit chloride exchange at 0°C. E partially inhibits chloride exchange (up to about 75%), with half-maximal inhibition at ≈45 μM E (at 150 mM chloride). Cl^- has a very small effect on the inhibitory potency, corresponding to a dissociation constant for chloride at the E inhibitory site of about 450 mM, far higher than the dissociation constant of the transport site for chloride (≈65 mM). Under conditions where the covalent reaction of EM is slow (0°C), EM is also a partial inhibitor, with a maximum inhibition of between 80 and 90%. Over a range of Cl^- from 10 mM to 600 mM, there is no significant effect of chloride on the inhibitory potency of EM, indicating noncompetitive inhibition. Thus, E and EM may both be useful fluorescent probes for conformational changes of the anion exchange system, since they do not completely inhibit transport, but they cannot be used as probes for the transport site itself. (Supported by NIH grant AM27495.)

32.2

Na^+K^+ PUMP ACTIVITY OF HIGH K^+ SHEEP RED CELLS WITH INTERNAL Mg^{2+} and Ca^{2+} LEVELS ALTERED BY A 23187. H. Fujise*& P.K.Lauf, Dept.Physiol. Duke Univ.,Durham,N.C. and Dept. Physiol.&Biophysics, Wright State Univ. School of Medicine, Dayton, Ohio.

High K^+(HK) and low K^+(LK) sheep red cells have kinetically (P.G.Hoffman & D.C. Tosteson, J.Gen. Physiol. 58: 1971,438)and quantitatively (C.H.Joiner & P.K.Lauf, J.Physiol. 283:1978,155) different Na^+K^+pumps. We investigated the effects of Mg^{2+} and Ca^{2+} on K^+ pump flux, measured as ouabain-sensitive Rb^+influx in buffered $NaNO_3$ or glucamine-NO_3 media, in both cell types. Here we report data on HK red cells. Treatment with the divalent Me^{2+} (Me^{2+}) ionophore A23187 reduced K^+ pump flux by 30%, an effect due to lowering of intracellular Mg^{2+} concentrations, $(Mg^{2+})_c$, as cell ATP and volume were unaltered. However, with A23187 and 10^{-4}M external Mg^{2+}, V_{max} of K^+ pump flux was close to that of controls while $K_{0.5}$ for external Rb^+ increased only slightly from 0.202 to 0.269 mM (external Na^+replaced with glucamine). Total $(Mg^{2+})_c$ was around 2 mM/L.cells in both controls and treated cells. K^+ pump flux was a hyperbolically saturating function of $(Mg^{2+})_c$ with a $K_{0.5}$ of 0.51 mM/L.cells, equivalent to 5×10^{-6}M ionized Mg^{2+} calculated/L.cells, hence ca 4 fold lower than reported for human red cells (P.Flatman& V.Lew,J.Physiol.315:1981,421)required for pump phosphorylation by ATP. At optimal $(Mg^{2+})_c$, Ca^{2+} in presence of A23187 inhibited K^+pump flux to 50% at 6.6×10^{-4}M $(Ca^{2+})_c$,equivalent to 2.3×10^{-4}M ionized Ca^{2+} calculated/L.cells. Kinetically the Ca^{2+} effects on K^+ pump flux are complex and in part due to competition with Mg^{2+} at the cytoplasmic side of the Na^+K^+pump.(Supp.by NIH AM 28236)

32.3

EVIDENCES AGAINST THE K^+-pNPPase BEING A PARTIAL REACTION OF THE GASTRIC H^+,K^+-ATPase SYSTEM. Tushar K. Ray and Jyotirmoy Nandi. Department of Surgery, The State University of New York Upstate Medical Center, Syracuse, New York 13210.

Studies with intact and lysed gastric microsomal vesicles demonstrate that there are two pNPP and one ATP hydrolytic sites within the gastric H^+,K^+-ATPase complex. While the ATPase site is located exclusively on the vesicle exterior,the pNPPase sites are distributed equally on both sides of the bilayer. Competition by ATP for the pNPPase reaction on the vesicle exterior suggests that both ATP and pNPP are hydrolysed at the same catalytic site present at the outside surface of the intact vesicles. However, a biphasic inhibition of the K^+-pNPPase by ATP in the lysed vesicles suggest the pNPPase site of the vesicle interior to have very low affinity (Ki ≈ 1.2 mM) for ATP compared to the vesicle exterior (Ki ≈ 0.2 mM). Differential effects of the ATP and pNPP hydrolytic activities towards ADP,ITP and CTP were also observed. Studies with spermine and Na^+,which compete with K^+ for the K^+-pNPPase reaction without inhibiting the H^+,K^+-ATPase,suggest there are two separate K^+ sites for the pNPPase reaction and another distinct K^+ site for the ATPase reaction. Contrary to the K^+ site for the ATPase which is located opposite to the catalytic site across the bilayer,both the K^+ and the catalytic site for the pNPPase are located on the same side. The data clearly demonstrate that the pNPPase is not a manifestation of the phosphatase step of the total H^+,K^+-ATPase reaction.

32.4

PIG CORONARY ARTERY SMOOTH MUSCLE: CA PUMP AND ACIDOSIS S.E. Samson* and A.K. Grover. Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

The properties of the pig coronary artery smooth muscle Ca-transport mechanisms and the effects of pH and adenosine were examined. The Ca-pump in the plasma membrane requires 45-60 μM MgATP for half maximal activity but the pump in the endoplasmic reticulum requires 720-900 μM MgATP. The Ca-uptake by the various subcellular organelles occurs with the following affinity characteristics towards Ca ion: plasma membrane - K_m = 0.91 μM and Hill coefficient = 1.31; endoplasmic reticulum - K_m = 0.58 μM and Hill coefficient = 2.48; and mitochondria K_m = 7.1 and Hill coefficient = 1. The active Ca-transport systems showed similar pH-dependence in that Ca-uptake at pH 6.8 was greater than at 7.6. However, the pH effects were much larger on the Ca-uptake by the endoplasmic reticulum and mitochondrial membranes than by the plasma membrane. Adenosine had no effect on the Ca-uptake by the plasma membrane or the endoplasmic reticulum. The plasma membrane also showed a small amount of pH-dependent high affinity Ca-binding. Ca-efflux from passively loaded plasma membrane vesicles was similar at pH 6.4 and 7.4. From these data, it is concluded that the endoplasmic reticulum - Ca-pump may play a larger role in the vasodilation of large coronary artery, but the role of the plasma membrane may be more as a steady state mechanism. Supported by Heart and Stroke Foundation of Ontario.

INTRACELLULAR ORGANELLES

33.1

QUIN-2 MEASUREMENTS OF CALCIUM LEVELS IN MAST CELLS: LOSS OF QUIN-2 DURING DEGRANULATION. P.C.Bibb* and D.E.Cochrane, Tufts University, Medford, MA 02155.

The indicator of Ca, quin-2(Q-2), has been used in a variety of cells to follow changes in free intracellular Ca, $[Ca^{2+}]_i$. We report here that in rat mast cells, a significant portion of the incorporated Q-2 is externalized during degranulation. The interaction of this released Q-2 with extracellular Ca appears to account for much of the increase in fluorescence that occurs during degranulation. Purified mast cells were loaded with 10 μM Q-2/AM for 90 min at 37°C. Aliquots containing 5×10^5 cells were removed, washed and resuspended in buffer containing 1 mM Ca. Q-2 fluorescence was measured before and after stimulation with compound 48/80, somatostatin or A23187. Two minutes after stimulation(during which an increase in fluorescence was noted)the cells were separated and the fluorescence of the supernatant measured before and after the addition of Mn^{2+}. Compared to unstimulated cells, stimulated cells released 4 X's as much Q-2. No increase in fluorescence was noted when Mn^{2+} (0.1mM) was present during stimulation. Metabolic poisoning or Ca deprivation prevented degranulation and the loss of Q-2. In other experiments, we loaded mast cells with ^{3}H-Q-2 and found that stimulated cells released up to 23% of the total label. Isolation of intact secretory granules from unstimulated cells revealed that they are a significant site of Q-2 localization(33% of the total incorporated ^{3}H-Q-2). Supported by NIH Grant AI-19436.

33.2

MITOCHONDRIAL TRANSPORT AND METABOLISM OF ASCORBATE. Richard C. Rose and Kathryn LaNoue. Departments of Physiology and Surgery, The Pennsylvania State University, College of Medicine, Hershey, PA 17033

Rat liver mitochondria were incubated at 37°C in buffer consisting of 135 mM KCl, 7% Dextran, 20 mM MOPS, 5 mM succinate, 0.015 mM rotenone and 1 mM thiourea (pH 7.0). ^{14}C-DHA was freshly produced from commercial ^{14}C-ascorbic acid (AA;17 uCi/uM) by bromine oxidation. Mitochondria were exposed to ^{14}C-labeled DHA or AA at 10 uM. The identity of the ^{14}C-label in the bath or in extracts of mitochondria was determined by HPLC. The uptake of DHA proceeds at a much higher rate than uptake of AA. Following a 3 minute exposure to ^{14}C-DHA, radiolabel accumulated against a 3-fold gradient within the mitochondria; this label was present primarily in the form of AA. Samples of the bathing media following three-minute incubations with ^{14}C-DHA indicate that approximately 50% of recently reduced AA had left the mitochondria. The uptake of ^{14}C-DHA was 43% lower if 200 uM nonlabeled DHA (but not AA) was present in the bathing medium. An attempt was made to assess whether DHA uptake proceeds by any of the known mitochondrial transport systems. The uptake of ^{14}C-DHA was uninfluenced by the presence of leucine, pyruvate, glutamine, ornithine, malate or succinate at 2-5 mM. We suggest that mitochondria are an avid site of DHA uptake and reduction. Support: NIH AM19119.

34.1

THE CONTRACTILITY OF RAT GASTROINTESTINAL TISSUE IN RESPONSE TO BOMBESIN AND CHOLECYSTOKININ. Russell L. Margolis*, Timothy H. Moran*, Paul R. McHugh. The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Adult male rats were sacrificed, and gastrointestinal tissue samples (intact sections of gastric fundus, antrum, and pylorus, proximal duodenum, and distal ileum) were rapidly removed, placed in Tyrodes buffer, and attached to a pressure transducer. Changes in tissue tension produced by bombesin and cholecystokinin (CCK) were recorded. Bombesin increased gastric antral and fundic tension, slightly decreased duodenal tension, and did not affect pyloric or ileal tension. CCK increased pyloric tension, but did not affect the other tissues. This pattern of response is consistent with the distribution of bombesin and CCK receptors in the rat gastrointestinal tract. (Supported by NIH grant AM19302).

34.2

EFFECT OF VIP AND SUBSTANCE P ON THE NEURONS OF CAT PANCREATIC GANGLIA. J. A. Love* and J. H. Szurszewski. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905

Immunohistochemical studies of the feline pancreas have revealed a network of nerves containing a variety of peptides. These peptidergic fibers appear to innervate the cell bodies of the intrinsic ganglia of the pancreas but the role of the peptides in transmission in the ganglia is unknown. We have begun to study the effects of two of these peptides, substance P (SP) and vasoactive intestinal polypeptide (VIP), on the postganglionic neurons. Pancreata were removed from male cats and intrinsic ganglia were dissected free from the surrounding parenchyma and perfused with warm (37°C) oxygenated Krebs solution. Intracellular recordings were made using standard techniques. The peptides were applied by either superfusion or by pressure ejection from a glass micropipet positioned close to the recording electrode. Both SP (5×10^{-5} M) and VIP (10^{-6} - 5×10^{-5} M) applied by either superfusion or pressure ejection caused a slowly developing depolarization of the postganglionic neurons. These depolarizations characteristically lasted several minutes and resembled the slow depolarization observed following repetitive preganglionic nerve stimulation. These results suggest that SP and VIP may play a role in synaptic transmission and integration in pancreatic ganglia and thus affect the endocrine and exocrine tissue innervated by these ganglia. Supported by AM 17632 and HLO-7111.

34.3

EFFECT OF CCK ON THE INTRACELLULAR ELECTRICAL AND MECHANICAL ACTIVITIES OF THE OPOSSUM SPHINCTER OF ODDI AND DUODENUM. A.J. Bauer* and J.H. Szurszewski. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905

Factors regulating the flow of biliary and pancreatic secretions into the duodenum have not been clearly defined, but in vivo studies have demonstrated the significance of CCK in this process. The aim of this study was to define the intracellular electrical and mechanical activities of the sphincter of Oddi (SO) and contiguous duodenal musculature, and to determine the effects of CCK. Intracellular electrical activities of these muscles were distinct. SO cells had an average RMP of -51 mV and 15-30 mV slow waves with bursts of spike potentials which often overshot zero potential. These slow waves occurred at a frequency of 1-4 per min. In contrast, duodenal cells had an average RMP of -64 mV and 10-20 mV slow waves usually with no spike potentials. Duodenal slow waves occurred at a frequency of 10-14 per min. When SO was attached to duodenal muscle, SO slow waves could be seen to propagate into the contiguous duodenal muscle. Surprisingly, CCK had no effect on the SO but did have a direct effect on duodenal musculature. This effect was typified by a depolarization in membrane potential and an increase in spike potentials on the slow wave. Mechanically this effect was manifested by an increase in tonic and phasic activities. The in vivo effect of CCK on the opossum SO, therefore, does not appear to be due to a direct effect on the SO muscle. Supported by AM 17238 and HLO 7111.

34.4

RESPONSE OF GASTRIC VAGAL MECHANORECEPTORS TO CIRCULATING EXOGENOUS CCK OCTAPEPTIDE IN THE RAT. J.S. Davison, Medical Physiology, University of Calgary, Calgary, Alberta, Canada.

Cholecystokin (CCK) is believed to be an endogenous satiety factor in several species including the rat. The effect of CCK is dependent upon the integrity of gastric vagal afferent fibres suggesting that the site of action of CCK is on sensory nerve endings. The present study was undertaken to establish whether the discharge in gastric, vagal mechanoreceptors could be modulated by circulating CCK. Rats were anaesthetized with Urethane (I.P.) and the stomach cannulated. Filaments were dissected from the peripheral end of the left vagus nerve and lifted onto bipolar silver electrodes until one was found which contained a single, spontaneously active afferent fibre which increased its discharge upon gastric distension. All fibres, thus isolated, also increased discharge during spontaneous contractions of the stomach. Because of this and the characteristic dynamic and static features of their response to inflation they were identified as gastric tension receptors. CCK octapeptide was injected via an indwelling jugular cannula in doses of 20-40nmol kg^{-1}. After a latency of about 10-15 sec. the discharge frequency rose rapidly to a higher frequency which within 30 sec. proceeded to decline slowly over a period of three min. This increase in discharge was not secondary to contraction of the stomach, since CCK caused gastric relaxation. Hence it appears that CCK can directly activate these gastric, vagal mechanoreceptors and this may provide the basis for the satiety effect of systemic CCK. (MRC supported)

34.5

ATTEMPTS AT QUANTIFICATION OF GASTRIC DILATATION. Naila Kayani *, Mozafareddin K. Karimeddini*, Richard P. Spencer. Dept. Nuclear Med. Univ. Connecticut Health Ctr.Farmington,CT 06032

Radiopharmaceutical studies can observe gastric dilatation by: 1) Agents secreted by the gastric wall (iodide, pertechnetate); 2)Compounds entering nearby tissues but not the stomach ("negative outline"); 3) Radiolabeled food or drink (gastric emptying tests). Quantification of dilatation is of interest since the phenomenon is also found in nonhuman primates (potential comparisons across species). From radionuclide images, a circumference (C) of the circular presentation ($C = 2\pi r R$) of the gastric sphere is measured, or a radius averaged over several diameters. Retained volume of gas/fluid is calculated by $V = (4\pi r/3)R^3$. In addition, if the stomach weight is approximately known as from age/height/weight tables, a functional thickness of the normal wall can be calculated. It is the difference between the outer stomach wall and the sphere defining the gastric contents. Let W be the weight of the stomach wall (density = 1). Then the description is $W = (4\pi r/3)(F^3 - R^3)$, where F is the radius to the far stomach wall. For example, in a teen age male the gastric contents had a radius of 6 cm. The calculated volume was 907 cm^3. Estimated weight of the stomach at puberty is 80 grams. Calculation of F (from W and R) yields 6.17 cm. The difference between F and R is a functional wall thickness of 0.17 cm. Efforts are needed to further define functional gastric wall thickness and contained volume. (Supported by USPHS CA 17802, National Cancer Institute).

34.6

CHLORHYDRO-PEPTIC ACTIVITY FOLLOWING H 2 BLOCANTS AND STIMULANTS IN THE ADRENALECTOMISED RATS.

Basil Zamphyr and Laurian Mohay.Dep.of Physiol. Faculty of Medicine.Bucharest.Romania.

The experiences was undertaken on white rats divided in 8 groups.Gastric juice (GJ) was reaped by Schay method gthe dosage of acidity(A) was accomplished by micrctitration and the pepsine (P) by Mirsky-Bucher method.
In the adrenalectomised rats the A and P decreased significantly.
Histamine(0.05 mg/kg) increased especially the A but decreased P.
Cimetidine(20 mg/kg) decreased slightly the A and deeply the P.
The adrenals glands have a determinants role in the secretory mechanism of the gastric juice.

34.7

PROTON PUMP INHIBITION AS EVIDENCE FOR SEPARATE INTRACELLULAR MECHANISMS OF ACID AND MACROMOLECULAR SECRETION. Michael Zdon MD*, Irvin Modlin MD, Garth Ballantyne MD*, Richard Gambria MD*, Gary Fratesi MD*, David Schafer PhD. Yale Univ. Dept of Surgery, VA Med. Ctr., West Haven, CT 06516.

Parietal cell macromolecular (intrinsic factor-IF) secretion is currently postulated to be secondary to acid secretion or via a washout phenomenon. If this hypothesis is correct then specific inhibition of the proton pump would inhibit IF secretion. This study was designed to elucidate whether the acid secretory pathway is discrete from the IF secretory mechanism. Rabbit isolated gastric glands (IGG) were prepared and histamine (H) (10^{-5}M) and 8Bromo-cAMP (8B) (10^{-5}M) used to stimulate acid and IF secretion. A specific H^+/K^+ ATPase inhibitor (H 168/68) (O) (10^{-5}M) was used to block the proton pump. Acid was measured by ^{14}C-aminopyrine (AP) accumulation and IF by binding of ^{57}Co-cyanocobalamin at 15 minute intervals. Results are expressed as % change relative to unstimulated glands MEAN ± SEM. (Significance = $p < 0.05$ by t-test) Results are expressed at 45 min. The data were identical to 5, 15 and 30 min results.

	H(10^{-5})	H(10^{-5})+O(10^{-5})	8B(10^{-5})	8B(10^{-5})+O(10^{-5})
^{14}C-AP	195±22	-91±1	121±5	-78±1
IF	478±42	462±25	29±1	40±2

H significantly increased ^{14}C-AP accumulation ($p < 0.05$). O significantly inhibited ^{14}C-AP accumulation ($p < 0.05$). H and 8B significantly stimulated IF secretion, ($p < 0.05$). Addition of O failed to alter IF secretion, ($p > 0.05$). These results suggest that specific inhibition of the proton pump does not affect IF secretion and are consistent with the hypothesis that despite having a common membrane activator site, acid and intrinsic factor are secreted by separate intracellular mechanisms.

34.8

MECHANISM OF THE GASTRIC ANTISECRETORY EFFECT OF THIOCYANATE: SCN^- IMPEDEMENT OF THE H^+,K^+-ATPase MEDIATED VECTORIAL TRANSLOCATION OF PROTONS. Sandip Bandopadhya*, Jyotirmoy Nandi and Tushar K. Ray. Department of Surgery, The State University of New York, Upstate Medical Center, Syracuse, New York 13210.

Previous studies from our laboratory (Arch. Biochem.Biophys. 216:259-271, 1982) suggested that SCN^- binds to a luminal low affinity K^+ site and there by causes impedement or interference of the vectorial translocation of H^+ without inhibiting the H^+,K^+-ATPase activity. This idea is in sharp contrast to the alternate hypothesis which considers SCN^- in the acid form, HSCN to be acting as a protonophore. As predicted from the former concept, the present study demonstrated that SCN^- has a specific and characteristic binding site within the H^+,K^+-ATPase complex. Thus pure and highly active H^+,K^+-ATPase molecules bind SCN^- to the extent of about 15 n moles/mg protein which could be displaced by K^+ and congeners. Similar to the SCN^- binding studies showing a competition between K^+ and SCN^-, a K^+/SCN^- antagonism was also observed in the gastric K^+-pNPPase reaction. This observation is consistent with our recent report suggesting the K^+-pNPPase to monitor the ion channel activity rather than representing the phosphatase step of the H^+,K^+-ATPase reaction. In addition, contrary to the protonophore hypothesis, SCN^- was unable to dissipate an artificial pH gradient across the microsomal vesicles. The data strongly suggest that the proton impedement effect of SCN^- best explains the SCN^- inhibition of gastric H^+ transport.

34.9

EFFECTS OF ALPHA AND BETA ADRENERGIC BLOCKADE ON CENTRALLY INDUCED GASTRIC ULCERATION, D.L. Innes, Dept. of Basic Med. Sci., Mercer University School of Medicine, Macon, GA 31207.

In an attempt to elucidate the adrenergic fiber involvement in the gastric ulceration (GU) process induced by amygdaloid stimulation (AS), the actions of α and β adrenergic receptor blockers were investigated. Rats, fasted for 36 h, were anesthetized and administered either 5 mg/kg phentolamine or 1 mg/kg propranolol ip 30 m prior to the onset of unilateral AS. Following 4 h of AS the brains were removed and prepared for histological verification of electrode placement. The stomachs were opened, examined for the presence and severity (S) of GU and the pH was recorded. The results indicate that neither drug prevented nor significantly reduced the S of GU. In fact, even though it is difficult to determine in this model, phentolamine may have augmented the incidence of GU inasmuch as 60% of the unstimulated controls demonstrated some degree of GU. These results suggest that the pathologic gastric activity resulting from AS is not transmitted peripherially by fibers blocked by these agents. However, blocking the α adrenergic receptors may compromise a protective mechanism.

	N	GU	avg pH	S(0-5 max)
AS	12	12	1.0	3.5
Phentolamine + AS	22	22	2.0	2.3
Phentolamine	14	8	2.4	1.4
Propranolol + AS	24	24	1.4	2.5
Propranolol	18	0	2.4	0
Sham AS	12	0	2.5	0

34.10

STRUCTURAL CHANGES IN GLYCOPROTEINS FROM RAT GASTRIC CORPAL MUCOSA DURING ULCEROGENESIS. Bernt Walther, Hans K.Bakke* and Terje Christensen*,Depts. of Biochem., Univ. of Bergen and Oslo, NORWAY.

Rat gastric corpal mucosa constitutes 3-5%(w/w)of the corpal stomach, and may be isolated free of the submucosal tissues by scraping the epithelial stomach lining with a blunt spatula. In rats subjected to immobilization-stress, the glycoproteins of the mucosa exhibit multiple depletions of molecular species judged by PAGE-SDS separation and silver-stain visualization of periodate-oxidized glycoproteins. We have analyzed the carbohydrate composition of the glycoproteins by methanolysis followed by Gas Chromatography of Trimethylsilyl-derivatized sugars.After 23 hours of immobilization we find a marked depletion of certain sugars from the glycoproteins.Normalized to galactose we find a 36% reduction in galactosamine, a 19%reduction in glucosamine, and little alteration in sialic acid contents. Also the mucosal glycoproteins appear to contain more galactosamine than the glycoproteins of the remaining stomach wall. - Our data are consistent with a reduction in mucin-type glycoproteins of the ulcerating stomachs. Such absence of terminal galactosamine in short sugar-chains would possibly leave the mucins more exposed to proteolysis, and less able to block ulcerogenesis.

34.11

HISTOCHEMISTRY OF THE ENDOSTYLAR CELLS OF STYELA PLICATA. Frank Hohenleitner and Louis V. Caso*. Temple University Health Sciences Center, Philadelphia, PA 19140.

Tunicates trap plankton in a sieve-like pharynx by secreting a sticky mucus from a longitudinal groove called the endostyle. We have reported that non-aqueous cyanuration fixation can be useful for demonstrating positive responses to stains for acidic glycoproteins (sulfomucins and sialomucins) in endostyles of Ciona, which are usually negative after aqueous fixation. We extended this method to Styela plicata (n=30) by staining with Alcian blue (AB) for polyanions at pH 2.5 and 1.0; high-iron diamine (HID) for sulfomucins; hematoxylin and eosin (H & E); and periodic-acid Schiff. We also tested for sensitivity to acid hydrolysis (AH+) and saponification (SAP+). The large secretory cells of zones 2, 4 and 6 had a mid-cellular band which was negative for AB 2.5 and 1.0 as well as H & E. Zone 6 was almost invariably AB+. Zone 2 was AB+ (n=16) whereas zone 4 was weakly AB+ (red-blue)(n=10). Other responses were: AB 1.0-; HID- (occ. slightly +, zone 2); AH+; and SAP+ in all 3 secretory zones. Zones 3 and 5 were usually AB 2.5+, AB 1.0-, HID-, AH+ and SAP-. We conclude that most of the AB in zones 3 and 5 is not mucus but probably DNA. In secretory zones 2, 4 and 6 histological responses suggest the almost complete absence of sulfomucins and the presence of sialomucins. (This project was supported in part by BRSG-05339-12 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, NIH and Temple U Grant-in-Aid).

34.12

THE IMPORTANCE OF GASTROINTESTINAL AND INTRAPERITONEAL SECRETIONS TO THE COMMON LIVER FLUKE, Fasciola hepatica. M.V.K. Sukhdeo and D.F. Mettrick. Dept. Zoology, University of Toronto, Toronto, Ontario, M5S 1A1.

The trematode Fasciola hepatica parasitizes the bile ducts of a wide range of vertebrate hosts. The objective of this study was to determine the physiological conditions of the vertebrate host that control activation and eclosion of the infective stage and that guide the mobile juveniles to the liver. The effects of gastrointestinal secretions were assayed in vitro by cinematic analysis of parasite behavioural responses. Specific behaviour patterns that result in eclosion were stimulated by the sequential effects of CO_2 and glycine-conjugated cholic acid (a component of herbivore bile). Penetration of the gut and migration to the liver are controlled by cues originating in the tissues of the duodenum and liver. The orientation mechanism is a chemo-orthokinesis. This study was supported by NSERC grant A4667 to DFM.

36.1

DIRECT TURGOR PRESSURE MEASUREMENTS DURING PLANT GRAVITROPISM. Daniel J. Cosgrove* Penn State University, University Park, PA 16802

The aim of this work is to identify and quantitate the changes in the biophysical properties of growing stem tissues which induce asymmetric growth and consequently curvature in response to gravitropic stimulation. Marker and transducer studies of gravitropism in dark-grown cucumber (Cucumis sativus L.) hypocotyls show a latent period of about 10 min before bending starts. Thereafter bending proceeds very rapidly, with a 90 degree curve established in about 45 min from the start of stimulation. The growth rate of the lower surface accelerates by more than two-fold whereas the upper surface of the hypocotyl undergoes a small and transient contraction. To examine the basis of cessation of growth on the upper surface, direct turgor pressure measurements were made of the cortical cells, using the pressure microprobe. The average turgor pressure of vertical seedlings was about 0.4 megapascals (MPa). This corresponded with a water potential of about -0.05 MPa. During gravitropic bending, turgor of the upper side stayed nearly constant, dropping by about 0.01 MPa. These results show that the loss of growth and transient contraction on the upper side are not due to loss of turgor pressure.

36.2

Gravity Perception, Transduction, and Response in Cereal Grass Shoot Pulvini

Peter B. Kaufman, Il Song*, and Najati. S. Ghoseh*
Dept. of Cellular and Molecular Biology. Div. of Biol. Sciences, Univ. of Michigan, Ann Arbor, MI 48109

Graviperception is temperature-dependent, reaching a maximum at 30 C and is inhibited reversibly at 4 C. Gravity transduction causes asymmetric distribution of indole-3-acetic acid (IAA) and gibberellins (GAs), as shown directly with hormone analyses and indirectly when pulvinus segments and first gravistimulated for 24 h, then treated with 30 μM gibberellic acid (GA_3) + 0.1 M sucrose (S) in an upright position. Striking curvature develops in S + GA_3 treated segments during the next 24 h. During asymmetric growth phase at 3 and 6 h. after gravistimulation, five proteins (32, 39, 57, 105, 110 kd) increase in the bottom halves and two (41, 81 kd) increase in the top halves. One (81 kd) decreases dramatically in the bottom halves. Hydrolytic enzymes also change during gravitropic curvature: invertase activity increases within 3 h. and reaches much higher levels in the bottom halves as compared with top halves: cellulase activity increases in the top and bottom halves by 3 h, and increases greatly in top halves later. Results will be discussed in terms of a model for differential wall synthesis/wall loosening in the graviresponding pulvinus system. (Sponsored by NASA Grant NAGW-34).

36.3

HOW FAR CAN CLINOSTATS BE TRUSTED? A. H. Brown and D. K. Chapman. University of Pennsylvania, Philadelphia, PA. 19104

Clinostat technology in plant physiology becomes increasingly sophisticated but theoretical bases for considering the clinostat environment a valid simulator for any particular g level in the hypogravity range, zero to unit g, remain mostly intuitive. Possible difficulties (vibration, sag, and flopping) have been mentioned repeatedly. Two physiological phenomena, hyponasty and circumnutation, have been tested (at zero g only) during NASA spaceflights. On two issues confirmation of clinostat validity was urgently needed: (A) Can we use clinostat-simulated weightlessness to deduce circumnutational behavior in true weightlessness or micro-g? (important for testing whether gravitropism drives nutation) and (B) Can we use effects of simulated hypogravity to calculate changes of sensitivity of test plants to g stimuli in different parts of the real hypogravity range? (to test the theory that nutational displacement has evolved because it enhances the sensitivity of plants' perception of the g vector direction). In the Shuttle Spacelab (SL-1) HEFLEX Experiment it was demonstrated that results from simulated weightlessness and from true micro-g were widely different. Thus, results from simulations were invalid.

36.4

EVIDENCES FOR CHANGES IN SENSITIVITY TO AUXIN AND IN CELL-WALL PROPERTIES DURING GRAVITROPIC BENDING OF DICOT STEMS. Frank B. Salisbury, Patricia A. Rorabaugh, and Rosemary White. Plant Science Dept. UMC 48, Utah State University, Logan, Utah 84321

Horizontal soybean hypocotyls (young stems below the cotyledons) immersed in auxin solutions of various concentrations do not bend when auxin levels are high. This is because the top of the hypocotyl normally does not grow, but increasing auxin in the solutions stimulates growth of the top while slightly inhibiting the normally rapid growth of the bottom of the hypocotyl. This appears clearly when measured growth of the top or the bottom is plotted as a function of the amount of ^{14}C-IAA (auxin) that penetrates the tissues. Thus, turning a hypocotyl on its side allows bottom cells to remain maximally sensitive to the auxin that is present, and added auxin only inhibits their growth. Sensitivity of top cells is greatly reduced, however, since they grow only in response to high concentrations of added auxin.

Halting of growth on top of a horizontal stem could be due to cellulose microfibril reorientation or to "tightening" of the wall so microfibrils can't slip past each other as easily as during rapid growth. TEM or polarized-light investigations show no reorientation of microfibrils, so extensibility changes during gravistimulation seem likely. Walls of top cells become slightly thicker than those of bottom cells, indicating slight cell compression during bending.

36.5

EFFECTS OF SIMULATED GRAVITY NULLIFICATION ON SHOOT-INVERSION RELEASE OF APICAL DOMINANCE IN PHARBITIS NIL. M.G. Cline and I.K. Prasad*. Botany Dept, Ohio St. Univ., Columbus, 43210

Inversion of the upper shoot of Pharbitis nil induces the outgrowth of the highest lateral bud adjacent to the bend in the stem within 24-36 hr (Ann. Bot. 52:217; 53:897). This release of apical dominance is thought to be caused by ethylene restriction of growth of the inverted shoot (Plant Sci. Lett. 38:163). The objective of this study is to test this hypothesis. Gravity nullification is simulated by rotating the plant (with its upper shoot inverted) in a vertical plane perpendicular to the axis of a horizontal clinostat. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity is measured by the method of Kang et al. (Plant & Cell Physiol. 25:249) and ethylene production is determined with a gas chromatograph equipped with a flame ionization detector. The results indicate that simulated gravity nullification (1) prevents the release of apical dominance, (2) significantly reduces ACC synthase activity and ethylene production in the inverted shoot and (3) largely eliminates growth restriction of the inverted shoot. These data are consistent with the hypothesis that shoot-inversion release of apical dominance is caused by ethylene-induced restriction of growth in the inverted shoot.

36.6

GRAVISTIMULATION-INDUCED CHANGES IN CURRENT PATTERNS AROUND CORN ROOT CAPS. Thomas Björkman*, A. Carl Leopold, Carl Scheffey* and Lionel F. Jaffe*. Boyce Thompson Institute, Cornell University, Ithaca, NY 14853 and Marine Biology Laboratory, Woods Hole, MA 02543

In an effort to characterize processes in the sensing of gravity preceding differential growth, changes in the current patterns around the root cap of corn following gravistimulation were determined using a vibrating probe. Current out of the root cap in the region lateral to the statocytes (500 μm from the tip) shows a transient increase on the upper side beginning 1 to 4 minutes after the onset of gravistimulation and lasting approximately 10 minutes. The average increase is from 0.5 to 1 μA cm^{-2}. The time of this event suggests that it is associated with the initial establishment of asymmetry in the gravity-sensing region of the root. Current out of the root in the elongation zone (2500 μm from the tip) is not affected by gravistimulation, remaining constant at 1 μA cm^{-2} for at least 20 minutes, suggesting that the altered current pattern is not a product of differential growth per se. In killed roots, no currents were detected, either before or during gravistimulation. The current changes began during the presentation time of 4 minutes, but had passed well before curvature began. The current changes were observed only around the root cap, the site of the gravity sensor in roots. This observed current pattern change may reflect a physiological asymmetry in the root cap which is then transmitted to the elongating zone, and there results in differential growth. (NASA Grant NSG-NAG-W3)

36.7

EFFECT OF CALMODULIN AND AUXIN TRANSPORT INHIBITORS ON CALCIUM NET UPTAKE ALONG APICAL CORN ROOTS. Kathryn L. Edwards. Kenyon College, Gambier, OH 43022

The roles of calcium and auxin in root gravitropism are unknown in either the root cap (RC), site of gravity perception, and the elongation zone (EZ), the site of differential gravity-induced growth. Calcium in the RC and auxin have been implicated in directing gravity-induced growth. Furthermore, the movement of calcium and auxin have been linked in some shoot systems, and gravity has been shown to produce an asymmetry in calcium and possibly auxin transport. The effect of inhibitors of calmodulin, a calcium transport protein, and auxin transport inhibitors on calcium net uptake and efflux from various regions along the root was investigated. Three day old intact corn roots were incubated vertically for 3hr in aerated test solutions containing 0.15uM or less 45Ca in 10mM buffer (pH 6.0) following which the RCs were surgically removed and the root excised into 1mm slices. Caps or slices taken from the 1, 3, 5, and 9mm of root were briefly rinsed, and radiolabel content determined or effluxed into buffer test solutions. Calcium is most readily taken up by those tissues most active in gravitropism, the RC and the EZ. Calcium uptake is greatest in the RC. Uptake is primarily by diffusion. Acid affects uptake differentially in the RC and the EZ. Specific calmodulin inhibitors enhance uptake in the same regions. No link between auxin transport and calcium uptake or calmodulin was discerned, but calmodulin is implicated in calcium efflux from RC and EZ cells. Supported by NASA NAGW-368.

36.8

RED LIGHT SHIFTS THE LOCUS AND RATE OF GRAVITROPIC CURVATURE IN ETIOLATED PEA EPICOTYLS. M.A. Harrison and B.G. Pickard. Biology Department, Washington University, St. Louis MO 63130

Gravitropic curvature of seedling shoots may either begin exclusively at a sharply defined locus or begin more or less uniformly over a considerable length of axis. Such variations have often been attributed to differences between species; but probably for any given species the part of the stem capable of initiating gravitropism may be regulated by the regime of irradiation prior to gravitropic stimulation. Similarly, the extent of counterreaction is probably environmentally controlled. As groundwork for mechanistic studies, we are examining the effects of selected light regimes on 1) kinetics and axial distribution of gravitropic curvature, 2) axial distribution of elongation during response, and 3) production of ethylene. Increasing doses of red light cause the zone of curvature to condense from about 20-25 to 5 mm. Moreover, the increase causes rates of early net curving to decrease, but causes rates of late net curving to increase. Counterreaction is an important component of the late phase of response. Red light shifts the axial distribution of elongation, but this is not fully responsible for the shift in locus of curvature. Rather, the shift must reflect to some extent a redistribution of the gravitropic receptor system. As already known, red light diminishes ethylene production; the influence of exogenous ethylene on the rates and loci of curvature and elongation of dark-grown and red-treated plants is being assessed.

Funded by NASA Space Biology Grants NAGW-70 and NAGW-420.

36.9

EFFECT OF ASYMMETIC EXOGENOUS AUXIN GRADIENTS ON HELIANTHUS HYPOCOTLL CURVATURE. David Rayle and Fernando Migliaccio*. San Diego State University, San Diego, CA 92182

We tested the capacity of small (1.5 to 3 fold) asymmetric exogenous auxin (IAA) gradients to initiate asymmetric cell elongation (curvature) in clinostated sunflower seedlings. Such gradients applied over a wide concentration range produced substantial (25° to 75°) curvature responses. Similar asymmetric gradients produced much less curvature (10° to 25°) in vertical nonclinostated seedlings. The symmetrical application of auxin at these concentration ranges produced no net curvature in vertical or clinostated seedlings. Over similar time intervals (up to 5 h) gravistimulated controls (horizontally oriented non-auxin treated seedlings) exhibited more rapid curvature and became completely reoriented (90° curvature). While we were not successful in precisely simulating gravicurvature with exogenous auxin gradients, we argue our results when combined with the kinetics of 3H-IAA lateral redistribution, strongly support the notion that auxin plays a major role in shoot gravitropism. Nevertheless, our own data do not preclude the possibility that other agents (e.g., calcium) may also participate in gravistimulated asymmetric growth. Supported by NASA grant NAGW-230.

36.10

THE STRUCTURE AND DEVELOPMENT OF THE STARCH SHEATH IN PEA EPICOTYLS. Fred D. Sack. Dept. Botany, Ohio State Univ., Columbus, OH 43210

Gravity perception in plant stems is thought to occur in the cells of the starch sheath, the statocytes. Pea epicotyls were studied because techniques exist for the isolation of these statocytes (Gaynor and Galston, Physiologist, 25:232) and because presumptive auxin transport carriers have been localized by immunofluorescence in these cells using monoclonal antibodies (Jacobs, Pl. Phys. Suppl., 77(4):2). Statocyte ultrastructure and development were examined to: (1) provide structural background for immunolocalization studies of the presumed auxin transport antigen, (2) determine whether the cells are symplastically or apoplastically isolated from other cells, and (3) analyze the stage of cell maturation found at the level of the stem where amyloplast sedimentation and epicotyl gravicurvature are distinguishable. In the 3rd internode of 7-8 day old, etiolated plants of Pisum sativum (cv. Alaska), the endodermis is developed as a starch sheath which surrounds the vascular tissue (including leaf traces). In the same location in older plants, prominent, multigranular amyloplasts are absent and the radial walls contain a casparian strip. The results are discussed in the context of implications for plant graviperception and transport between the stele and the cortex. (Supported by the National Aeronautics and Space Administration).

36.11

DOES PHOSPHORYLATION PARTICIPATE IN GRAVITROPIC ACTIVATION OF LATERAL AUXIN TRANSPORT? J. Henry Slone* and Barbara G. Pickard. Washington University, St. Louis, Mo. 63130

According to a recently developed model (Ann. Rev. Plant Physiol. 36:55-75), gravitropic, lateral transport of auxin occurs when an auxin carrier, presumably associated with membrane-bound calmodulin and kinase, is activated by phosphorylation that results from locally increased Ca^{2+}. In vitro preparations of crude and purified plasma membrane vesicles obtained from pea stems will be used to test this model of gravity reception. Baseline auxin transport activity of vesicles will be characterized. This activity presumably represents the action of the molecular components of the axial transport system of the intact plant, since axial transport is relatively stable in the absence of external stimuli. Ca^{2+} and ATP will be added to the vesicles to test whether an additional component of auxin transport activity can be elicited. Resolution of membrane proteins phosphorylated by addition of Ca^{2+} and [^{32}P]ATP will be attempted by gel electrophoresis and autoradiography. If enhancement of transport is manifested and if it can be correlated with phosphorylation of membrane proteins, it will tentatively be interpreted as the consequence of activating the gravitropic auxin transport system present in the vesicles. If successful, these experiments should contribute information permitting identification and isolation of the proteins responsible for gravitropism.

36.12

PLANT GROWTH RESPONSES TO ATMOSPHERE AND OTHER ENVIRONMENTAL VARIABLES IN THE SPACE SHUTTLE PLANT GROWTH UNIT. Melanie D. Cuellar and Cary A. Mitchell. Purdue University, West Lafayette, IN 47907

Studies using a ground-based version of the Plant Growth Unit (PGU) were conducted with mung bean (Phaseolus aureus Rosb. cv. Berken). Composition and turnover rate of the atmosphere within four Plant Growth Chambers (PGCs) mounted in the PGU were investigated. Environmental conditions attainable within the PGCs included continuous photosynthetically-active radiation (PAR) of 105-115 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and ambient temperature of 26±0.5°C. Greater uniformity of plant growth occurred using agar/vermiculite or pressed rockwool as a substrate compared to an agar/Miracloth wicking method. Addition of Hoagland's No. 1 nutrient solution to the medium resulted in superior growth performance over that using only water. Flowing atmospheres at 1 or 2 $liters \cdot h^{-1}$ through the PGCs for 7 days had no significant effect on plant growth relative to that in static atmosphere within the PGU. It has been shown that imbibed seeds are unable to regain their initial air dry weight before 9 days. Humidification of the airstream and/or use of 1000 $\mu liter \cdot l^{-1} CO_2$ vs. 350 $\mu liter \cdot l^{-1}$ had no significant modifying effects on plant growth under the prevailing environmental conditions of the PGU. However, preliminary experiments have shown a positive growth response to flowing atmospheres and CO_2 elevation when plants were grown at 190 $\mu mol \cdot s^{-1} \cdot m^{-2}$ of PAR.

Sponsored by NASA grant NSG 7278.

36.13

EFFECTS OF MECHANICAL STRESSES AND/OR OUTDOOR EXPOSURE ON GROWTH AND ABSCISIC ACID CONTENT OF EGGPLANT. Joyce G. Latimer and Cary A. Mitchell. Purdue University, West Lafayette, IN 47907

Periodic seismic (shaking) or thigmic (flexing) stresses reduced the growth and productivity, but strengthened the stems, of tender eggplant (Solanum melongena L. 'Black Beauty') seedlings grown in a greenhouse. Relative growth rate (RGR) was retarded by either mechanical treatment or by transfer to an outdoor, summer environment. Outdoor exposure caused, and sustained, a significant increase in leaf diffusive resistance of well-watered plants relative to undisturbed control plants retained in the greenhouse. Neither mechanical stress had a significant effect on diffusive resistance 1.5 h after treatment, although exogenous abscisic acid (ABA) tended to increase leaf resistance in the greenhouse. Levels of endogenous ABA in leaf and stem tissues are being correlated with changes in RGR, leaf diffusive resistance, and leaf water potential as a function of mechanical treatment and/or outdoor exposure.

Sponsored in part by NASA grant NSG 7278.

36.14

AN ATTEMPT TO LOCALIZE AND IDENTIFY THE GRAVITY SENSING MECHANISM OF PLANTS. Robert S. Bandurski*, Aga Schulze*, and Dennis M. Reinecke*. (Spon. ASGSB). Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

A gravitational stimulus induces a change in the amount of the growth hormone, indole-3-acetic acid (IAA), in the mesocotyl cortex of Zea mays, in 3 minutes (The Physiologist 27:S-123(1984) and un-published). The amount of IAA in the tissue is determined by 1) the metabolism of IAA, that is, the "inputs-to", and "outputs-from", the IAA pool, and, 2) by transport of IAA to the site. We report progress in understanding both metabolism and transport of IAA. A new route for the catabolism of IAA involving oxidation to oxindole-3-acetic acid has been found (Plant Physiol. 71:211(1983) and we wish to report on the properties of an enzyme catalyzing that oxidation. The enzyme is soluble, requires 0_2, and is stimulated by addition of a lipoidal factor. Since this oxidation is the first step in the catabolism of IAA, it is important that its mechanism and control be understood. We also wish to report progress in understanding how IAA moves from the kernel, through the stele, and into the cortical cells. This movement appears to be under metabolic control and may be a target for the gravitational stimulus. (Supported by Space Biology, NASA-NAGW-97,ORD 33355, and by Metabolic Biology, NSF-PCM-8204017,ORD 30668)

36.15

CYTOCHEMICAL LOCALIZATION OF CALCIUM IN CAP CELLS OF PRIMARY ROOTS OF ZEA MAYS L. Randy Moore, Department of Biology, Baylor University, Waco, Texas 76798

I monitored the distribution of calcium (Ca) in caps of vertically- and horizontally-oriented primary roots of Zea mays to determine its possible role in root gravisensitiveness. A modification of the antimonate precipitation procedure was used to localize Ca in situ. In vertically-oriented roots, the putative graviperceptive (i.e., columella) cells were characterized by minimal and symmetric staining of the plasmalemma. No precipitate was present in cell walls or plasmodesmata. Within 5 min after horizontal reorientation, staining was associated with the portion of the call wall adjacent to the distal end of the cell. This asymmetric staining of columella cells persisted throughout the onset of gravicurvature. I did not observe any staining of lateral cell walls of columella cells at any stage of gravicurvature, suggesting that a lateral flow of Ca through the columella tissue of horizontally-oriented roots does not occur. The outermost peripheral cells of roots oriented vertically and horizontally secrete Ca through plasmodesmata-like channels in their cell walls. These results will be discussed relative to a novel model for the involvement of Ca in root gravicurvature. This work was supported by Grant No. NAGW-734 from the National Aeronautics and Space Administration.

36.16

IMMUNOCYTOCHEMICAL LOCALIZATION OF CALMODULIN IN PEA ROOT CAPS AND PLUMULES AND ITS RELEVANCE TO HYPOTHESES ON GRAVITROPISM. Stanley J. Roux and Marianne Dauwalder*. The University of Texas at Austin, Austin, TX 78713

Calcium activation of the regulator protein, calmodulin, may be an important step in the control of gravitropism. To clarify what cellular functions could be regulated by calmodulin, we investigated its intracellular and tissue distribution in pea root caps and plumules. Samples were fixed in formaldehyde, and ethylene glycol-embedded materials were stained by standard procedures, using rhodamine-labeled second antibody to visualize the locale of calmodulin. We found high concentrations of calmodulin in root cap cells, the site of stimulus perception for root gravitropism. In these cells, the highest concentration of calmodulin was present in the columella cells, which are thought to be the key gravity-sensing cells in the cap. Within each columella cell, two major organellar locales for calmodulin were the nucleus and the amyloplasts. Amyloplast calmodulin was primarily within the stroma, with virtually none associated with the starch. No staining was evident in the nucleolus or the cell walls of cap cells. In the plumule, most cell types showed a fairly general staining in the cytoplasm and the nucleoplasm, but, as in the root caps, cell walls and nucleoli were not stained. Some stem and leaf epidermal cells showed a strong staining reaction in the vacuoles. The staining pattern of calmodulin, especially in pea root caps, is consistent with the postulated role of this regulator in mediating gravitropism. (Supported by NASA Grant NSG 7480)

36.17

EFFECTS OF NORFLURAZON ON THE LEVELS OF ABSCISIC ACID AND XANTHOXIN IN CAPS OF GRAVISTIMULATED ROOTS OF MAIZE. Lewis J. Feldman, Dept. of Botany, Univ. of Calif., Berkeley, CA 94720

In many cultivars of maize if seed is germinated in darkness the root fails to respond to gravity and grows in a direction determined by the orientation of the seed. Brief illumination with white light initiates gravitropic bending. We have previously demonstrated that two growth substances,abscisic acid (ABA) and xanthoxin (Xa) are redistributed from the cap to the elongation zone as a result of illumination. Using the carotenoid biosynthesis inhibitor norflurazon (NF) (Sandoz 9789) we show a drop in the level of ABA, from 152 ng/gm FW for caps from control, dark roots, to 4 ng/gm FW for caps from norflurazon-treated roots. The effects of NF on Xa levels are more complex and are summarized below:

	CAP		SUBJACENT 1.5 MM	
	light	dark	light	dark
Control	96*	32	76	32
+ NF	126	66	76	25

*ng/gm FW

Both ABA and Xa are hypothesized to have a role in root gravitropism. Our results with NF confirm previous reports of Moore (Planta, in press) showing that roots can bend even when ABA levels are artifically lowered. However since NF does not similarly depress Xa levels we cannot not exclude a role for this substance in root gravitropism.

36.18

SPACE FLIGHT, CHROMOSOMES, AND ORGANIZED DEVELOPMENT IN ASEPTICALLY CULTURED PLANT CELLS. A.D. Krikorian, Stefania A. O'Connor and R.P. Kann. State University of New York at Stony Brook, Stony Brook, NY 11794

Experiments on plants in space indicate that there can be significant chromosomal derangement. A system has been developed to test whether specimens of aseptically cultured plant cells can undergo normal cell divisions and subsequent organized growth in microgravity at a level equivalent to that which occurs at 1g. It can test (1) whether normal rate, frequency and patterning of cell division in morphogenetically competent cultures can be obtained on exposure to micro g and (2), whether the fidelity of the partitioning of the chromosomes can be maintained during and after exposure to space flight. Criteria for comparison include cell number, weight and quantitative morphological and histological examination. Chromosome karyotype analysis of cells predominantly arrested in metaphase but other stages in the mitotic cycle can be studied as well. Samples could be prepared on Earth, cooled to eliminate cell divisions, allowed to express their normal behavior in space at permissive temperatures, and then could be automatically fixed in space to permit later analysis. The range of analysis possible would definitively evaluate behavior in space of the plant cells in question from the perspective of cell composition, cell division and chromosome integrity. Supported by NASA NSG-7270.

36.19

THE CALCIUM DEPENDENCE OF AUXIN ACTION IN MAIZE ROOTS. Michael L. Evans and Karl-Heinz Hasenstein*. Ohio State Univ., Columbus, OH 43210

We investigated the interaction of Ca++ and auxin on root elongation in Zea mays L. Seedlings were raised to contain high levels of Ca++ (HC=imbibed and raised in 10 mM $CaCl_2$), low levels of Ca++ (LC=imbibed and raised in distilled water), or very low levels of Ca++ (VLC=imbibed and raised in distilled water and roots subsequently treated with 1 mM EGTA). The growth rates of roots of VLC, LC and HC seedlings were similar during the time course of the experiments. Exposure of roots of HC or LC seedlings to 10 uM IAA resulted in strong prolonged inhibition of elongation. In roots of VLC seedlings 10 uM auxin had only a slight inhibitory effect. Adding Ca++ (0.5 mM) to IAA-treated VLC roots allowed normal expression of the inhibitory action of the hormone. Inhibition of elongation in LC roots by IAA was reversible by 1 mM EGTA. The inhibitory action of auxin could then be reestablished by supplying Ca++ (0.5 mM). The data indicate that Ca++ may be necessary to the growth-regulating action of auxin. This finding if of potential significance to our understanding of the mechanism of gravitropic curvature. There is increasing evidence for gravistimulated redistribution of Ca++ in plant organs. If Ca++ levels are important to the action of auxin, gravi-induced gradients of Ca++ could initiate asymmetric growth patterns without establishment of gradients in hormone concentration.

36.20

CORRELATIONS BETWEEN IN VIVO CALMODULIN ACTIVITY AND GRAVITROPIC SENSITIVITY IN ROOTS OF MAIZE Charles L. Stinemetz and Michael L. Evans. Ohio State Univ., Columbus, OH 43210

There is limited indirect evidence that calmodulin plays a role in gravitropism. Calmodulin inhibitors interfere with gravitropism and, in both shoots and roots, calcium redistribution precedes gravitropism. Previous reports suggest that some of the calmodulin in maize roots may be extracellular. Using two calmodulin-specific assay systems (phosphodiesterase and NAD kinase activation) we examined calmodulin activity in intact roots (Zea mays, L. cv. Merit and B73 x Missouri 17), in crude extracts of root sections, and in partially purified root plasmalemma preparations. We found calmodulin activity in all cases. In intact roots calmodulin activity per g fresh weight was four times greater in the apical 1 mm than in more basal sections. Furthermore, in cultivars of maize which require light for gravitropism we found calmodulin activity in apical segments of dark-grown roots to be only 25% as great as in light-grown roots. Upon illumination of dark-grown roots there was a parallel increase in calmodulin activity and gravitropic competence. The results are consistent with the possibility that calmodulin plays a role in the action of calcium in the mechanism of gravitropism.

EXERCISE II

37.1

THE CARDIOVASCULAR RESPONSE TO ISOKINETIC EXERICSE (IE). R. Haennel*, K.K. Teo*, M. Hetherington*, P. Greenwood*, G. Snydmiller*, A. Quinney* and C.T. Kappagoda. Depts. of Phys. Ed. and Med., Univ. of Alberta, Edmonton, Alta. Can., T5P2G9.

The acute cardiovascular response to maximal IE was studied in 5 males (21-28 yrs) who performed 1 minute of maximal knee extension-flexion exercise on a Cybex II Dynamometer at 3 separate speeds. Heart rate (HR) stroke volume (SV) and cardiac output (CO) were measured by impedance cardiography (IC) immediately before and (within 3 secs) after each exercise bout. Systolic (SBP) and diastolic (DBP) blood pressures were monitored continuously via an intra-arterial cannula. A repeated-measure ANOVA revealed significant differences for all parameters ($p<0.01$) as shown below:

	Pre Ex.	30°/sec	90°/sec	150°/sec	*indicates no
HR(b/min)	79	125	135	138	significant
SV(ml/b)	121	103*	105*	108*	difference
CO(l/min)	10.1	12.7	13.7	14.5	between
SBP(mmHg)	147	175	194	187	subsets
DBP(mmHg)	78	108	101*	102*	p>0.05

Thus during IE there is a significant increase in HR and peripheral vascular resistance. A second study in which 6 males (24-34 yrs) performed maximal IE (150°/sec) with simultaneous measurements of IC and cardiac dimensions, using M-Mode echocardiography showed a reduced SV and no change in left ventricular end-diastolic diameter with IE. It is suggested that IE produces a large increase in sympathetic drive with little change in venous return.

37.2

IMMERSION ALTERS THE KINETICS OF EXERCISE OXYGEN CONSUMPTION. E.T. Flynn*, T.J. Doubt, and M.P. Mullen*. Hyperbaric Medicine Program Center, Naval Medical Research Institute, Bethesda, Maryland 20814-5055.

Oxygen consumption ($\dot{V}O2$) was measured during exercise to determine if $\dot{V}O2$ responses were altered by head-out immersion or gas density. Fifteen subjects did 6 min of bicycle exercise in the dry at 1 and 2 W/kg while breathing air or normoxic helium. Workloads during immersion (water temp = 32°C) were lowered to approximate dry exercise $\dot{V}O2$. Resting $\dot{V}O2$ was less during immersion breathing air (4.0 ± 0.1 ml/min/kg dry, 3.2 ± 0.1 wet), and breathing heliox (4.3 ± 0.2 dry, 3.2 ± 0.1 wet). During the last min of exercise at 1 W/kg there was no difference in $\dot{V}O2$ between dry and immersed tests with air (16.9 ± 0.7 dry, 16.4 ± 1.6 wet) or heliox (15.8 ± 0.4 dry, 14.0 ± 1.0 wet); but at 2 W/kg $\dot{V}O2$ was underestimated during immersion with air (28.0 ± 0.8 dry, 23.2 ± 1.1 wet) and heliox (29.5 ± 0.8 dry, 22.9 ± 1.1 wet). Immersion increased the time constant for the single exponential rise in exercise $\dot{V}O2$ at 1 W/kg on air (0.68 ± 0.04 min dry, 1.03 ± 0.11 wet) and on heliox (0.65 ± 0.12 dry, 1.08 ± 0.12 wet); and also increased the time constant at 2 W/kg with air (0.93 ± 0.11 dry, 1.24 ± 0.11) and with heliox (0.96 ± 0.11 dry, 1.27 ± 0.07 wet). These findings indicate that head-out immersion slows the rate of $\dot{V}O2$ rise with exercise. Reduction in gas density does not alter the kinetics of the $\dot{V}O2$ response in either dry or immersed conditions. (Supported by NMRDC Work Unit M0099.01.007).

37.3

COLD WATER EXPOSURE LESSENS TOTAL WORK CAPACITY OF LEG MUSCLES DURING HIGH INTENSITY/SHORT DURATION EXERCISE. T.J. Doubt and E.T. Flynn*. Hyperbaric Medicine Program Center, Naval Medical Research Institute, Bethesda, Maryland 20814-5055

Power and fatigue were measured by a modified Wingate power test consisting of 30 sec at a maximal bicycle workload (kp = 0.075 x wt) followed by 30 sec at kp = 50% of maximum. Five divers were tested after a 2 hr immersion in cold water (2°C) which involved minimal leg movement. Control data were obtained on a non-diving day. The decline in power over 1-15 sec was significantly faster after cold exposure (control slope = -470 ± 79 kpm/m/sec, cold = -617 ± 86, $p<0.05$). However, there was no difference in power decline at 15-30 sec (controls = -45 ± 10, cold = -48 ± 6). Total work achieved after 30 sec was 9% lower following cold exposure (controls = 1404 ± 142 kpm, cold = 1279 ± 68, $p<0.05$). At 50% maximum workload there was a faster decline in power at 30-60 sec after cold exposure (control slope = -7 ± 7 kpm/m/sec, cold = -15 ± 3, $p<0.05$). Total work done over 60 sec was reduced 15% after cold exposure (control = 2247 ± 219 kpm, cold = 1907 ± 100, $p<0.05$). These results indicate that cold water exposure increases the rate of fatigue of the fast component of the Wingate power curve, with no change in the slow component. Further, power could not be subsequently maintained at 50% maximum following cold immersion. (Supported by NMRDC Work Unit M0099PN.01.0007).

37.4

EFFECTS OF TRAINING ON THE CARDIOPULMONARY RESPONSES TO A STANDARD EXERCISE TEST IN THE PONY. W.L. Sexton*, and H.H. Erickson. Kansas State University, Manhattan, KS 66506.

We previously developed a standard exercise test (SET) to evaluate the cardiopulmonary responses of ponies during graded treadmill exercise (Fed. Proc. 27:251, 1984). Seven ponies, 175±28 kg, performed the SET before and after an 8 week combined endurance and interval training program. The ponies were trained indoors on a treadmill at 7° incline, 4 days/week for 20 min/day. After training, resting values for heart rate (44±2 b/min), right ventricular dP/dt (371±35 mmHg/sec), blood lactate (10±2 mg/dl) and blood temperature (37.9±0.1°C) were unchanged. The max heart rate achieved during the SET decreased significantly from 204±6 to 189±4 b/min, as did the max dP/dt, 2296±98 to 1882±82 mmHg/sec. Peak lactate and blood temperature were significantly reduced, 91±6 to 44±7 mg/dl and 40.7±0.1 to 39.7±0.1°C, respectively. Training had no effect on the responses of cardiac output, hematocrit, and arterial, right ventricular and pulmonary artery pressures to the SET. After training PaO_2 was reduced from 115±6 to 97±5 torr at the highest work level, while pHa was increased from 7.32±.02 to 7.43±.02. Training had no effect on the progressive hypocapnia observed during the SET. The rate of recovery for all variables was enhanced following training. These results indicate that in the pony training significantly improves cardiac efficiency and temperature regulation and decreases blood lactate accumulation during exercise. (Supported by the American Heart Association, Kansas Affiliate and the USDA).

37.5

EXERCISE LIMITATION IN ASBESTOS WORKERS. PG Agostoni*, Dorsett Smith*, R Schoene, T Robertson, J Butler. University of Washington, Seattle, Washington 98195.

Dyspnea is difficult to quantify and prove. Yet compensation for ventilatory impairment in asbestos-exposed workers (wks) is a frequent cause of litigation. We exercised 120 asbestos-exposed wks referred because they were seeking compensation for dyspnea. They were 59.8 (± 9.5 SD) yrs old with 23.3 (± 11) yrs of exposure and were 34.4 yrs (± 8) from first exposure. 22 wks were non-smokers, 75 were smokers and 23 ex-smokers (>10 yrs). X-rays, normal in 5, showed pleural disease in 42 and interstitial disease (= ILO ≥ 1/0) in 73. Limitations: Ventilatory = PCO_2 rising above 45 torr; Max Exercise Vent. > 80% of low (< 80% pred) Max Volunt Vent.. Cardiac = O_2 pulse < predicted -1 SD (not on β blockers); significant ECG abnormality or angina. Pulmonary vascular = dead space % tidal vol rising > 60%; A-a DO_2 rising to > 35 torr. Periph circ = claudication.

LIMITATION :	NONE IDENTIFIED	NON-VENTILATORY	VENTILATORY
NON SMOKERS 22	13 (59%)	7 (32%)	2 (9%)
EX-SMOKERS 23	11 (48%)	7 (30%)	5 (22%)
SMOKERS 75	35 (47%)	16 (21%)	24 (32%)

In the 24 smokers with ventilatory limitation, the pulmonary function test showed a restrictive abnormality in 7 and obstructive abnormality in 20. We conclude that in these ex-asbestos wks with dyspnea, only 1/4 had ventilatory limitation, and of these it was usually due to obstructive, rather than restrictive, disease. Supported by #T32HL07287.

37.6

EFFECT OF VARIED INSPIRED OXYGEN FRACTIONS ON KINETICS OF OXYGEN CONSUMPTION ADJUSTMENT IN SUPINE LEG EXERCISE. D. Sheehan*, D. Pendergast, A. Olszowka, D. Wilson*, M.L. Wilson*. SUNYAB, Buffalo, NY 14214.

The effect of varied inspired oxygen fractions ($F_{I_{O2}}$=.21,.15,.10 in N_2) on the rate constant, k, for $\dot{V}O_2$ adjustment to square wave exercise was investigated in 10 healthy males during supine leg pedaling. The protocol was: rest to 75W (5min), a 5 min recovery, 100W (3min), and a work load progression (25W) up to peak $\dot{V}O_2$. Breath by breath $\dot{V}O_2$ data was collected, from which peak $\dot{V}O_2$ and 75-on and 75-off $\dot{V}O_2$ k values were determined. No significant difference was found between each corresponding k value for 21% and 15% O_2. The 75-on k value of .015 s^{-1} at 10% O_2 was found to be significantly less than the corresponding value at 21% (k=.022 s^{-1}) and 15% O_2 (k=.022 s^{-1}). Also, the 75-off k value of .018 s^{-1} at 10% O_2 was significantly less than the corresponding k value of .031 s^{-1} at 21% O_2. Peak $\dot{V}O_2$ and peak work load were not significantly different between 21% (3.4 l/min, 278W) and 15% O_2 (3.0 l/min, 265W). However, these variables were significantly diminished at 10% O_2 (2.2 l/min, 195W) with respect to both 21% and 15% O_2. These observations imply a slower rate of adjustment for $\dot{V}O_2$ and a decreased maximal $\dot{V}O_2$ in supine leg exercise for $F_{I_{O2}}$ less than .15. Previous studies from this lab have shown that O_2 transport is not limiting $\dot{V}O_2$ kinetics at 21% O_2, however, these results suggest O_2 transport does become the limiting factor at extreme $F_{I_{O2}}$; i.e. $F_{I_{O2}}$<.15. [PHS Grant HL28542].

37.7

Measurement of Energy Expenditure During a Simulated Triatholon Using the $D_2{}^{18}O$ Method.

T.P. Stein,[1] R.W. Hoyt,[2] M. O'Toole[3] and D.W. Hiller[4]
[1]Dept. of Surgery, UMDNJ-SOM, Camden, NJ 08103 and
[2]Dept. of Pediatrics and [3]Institute of Environmental Medicine, University of Pennsylvania, Philadelphia, PA 19106

Three competition level triatholon atheletes performed a simulated triatholon consisting of 3.0 hrs. of cycling and 5.0 hrs. of uphill treadmill running in the laboratory. Mean percent of VO_2 max. for the exercise period was 55%. Prior to the study, 3 subjects drank water enriched with $D_2{}^{18}O$. Urine and blood samples were taken at various times before and after exercise and on subsequent days. From the isotopic enrichment in D and O of the various urines collected, we calculated the energy expenditure for the 8 hours of the simulated triatholon. During the triatholon, energy expenditure was also measured by indirect calorimetry at 30 minute intervals. Preliminary analysis of the isotopic data showed that the doubly labeled water values for energy expenditure were within 20% of the values determined by indirect calorimetry.

37.8

THE EFFECTS OF ACETAZOLAMIDE ON ACID-BASE STATE, PLASMA VOLUME AND IONS DURING WORK OF LOW AND MODERATE INTENSITY.

R.S. McKelvie*, M.I. Lindinger*, G.F.J. Heigenhauser and J.R. Sutton. Dept. of Medicine, McMaster University, Hamilton, Ont., Canada. L8N 3Z5.

Following saline (control) or acetazolamide (ACZ) infusion, 6 healthy males exercised on a cycle ergometer for 6 min at 300 kpm followed immediately by 6 min at 75-80% $\dot{V}O_2$ max. Arterial blood was taken at rest and during exercise. At 300 kpm, an 8-10% reduction in plasma volume and ionized calcium (iCa^{++}) occurred while potassium (K^+) and plasma protein increased 10% and sodium (Na^+), chloride (Cl^-) and acid-base state did not change significantly. There was no significant difference between controls and ACZ. At the end of the high workload in the control study, a purely metabolic acidosis (pH = 7.31 ± 0.02; PCO_2 = 38.7 ± 1.7 Torr) occurred whereas ACZ produced a combined respiratory/metabolic acidosis (pH = 7.27 ± 0.02; PCO_2 = 47.0 ± 1.8 Torr). Paradoxically, plasma lactate and non-volatile acid were reduced by ACZ: (6.49 ± 1.0 vs 8.88 ± 1.2 mmol/l) and (5.44 ± 1.0 vs 6.32 ± 1.4 mmol H^+/l) respectively. Plasma volume decreased by 19% of resting values, with no significant difference between treatments. Plasma Na^+ and Cl^- increased slightly, iCa^{++} remained unchanged, K^+ increased 43% and protein increased 19%. Thus, ACZ produces significant effects on acid-base state and metabolism during exercise; with no additional influence on plasma volume, protein or ionic concentrations.

37.9

EFFECTS OF COMPENSATED RESPIRATORY ALKALOSIS DURING RESISTIVE UNLOADING OF THE LUNG IN EXERCISE.

D.A.Bayliss*, B.A.Wilson, S.P.McKay*, A.Robinson*, J.Hoare*, J.P.Van Dijk*, and T.E.Graham, University of Guelph, Guelph, Ont., Canada N1G 2W1.

Metabolic and respiratory alkalosis (alk) have been associated with a higher blood lactate (La) which is usually attributed to greater La release. However, studies on exercising man breathing helium-oxygen mixtures (heliox), with significantly greater ventilations than air breathing, do not show any lactate differences despite alk. We studied six competitive cyclists (20-25 yrs., VO_2max 58-68 ml/kg) over three rides at 110% VO_2max to exhaustion: breathing either air (A), heliox (H) or isocapnic heliox (C). Arterialized-venous blood samples were drawn during the maximal ride and after 5 mins. of recovery for blood gas and La analysis. No differences were found in La between the three trials. However a significant negative correlation between PCO_2 and La was found over all three trials. The potential alk effect on La release may be partially obscured by an increased arterial saturation (Dempsey, J.Phys.,1984). A trend toward lower La on C and significantly higher VO_2 on H and C lend additional support to this hypothesis.

Supported in part by NSERC A7167

37.10

CALCIUM ANTAGONISTS: EFFECT ON SKELETAL MUSCLE STRENGTH AND ENDURANCE IN MAN. R.A. Lehnhard*, T.E. Kirby*, H.J. Richardson-Lehnhard* (SPON: D.R. Lamb). The Ohio State University, Columbus, Ohio 43210

In vivo research in man involving calcium antagonist agents (CAA) has confined itself to central and peripheral changes in the cardiovascular system caused by the effect these agents produce on cardiac and smooth muscle tissue. This investigation was undertaken to determine if three CAA: verapamil, nifedipine and diltiazem, have any in vivo effect on skeletal muscle strength and endurance function in humans.

Eight healthy males, aged 19-26, were given eight separate treatments of CAA and a placebo in a double blind protocol. Treatments included a low and a high dose of verapamil (80, 120 mg qid), nifedipine (10, 20 mg qid) and diltiazem (90, 120 mg qid) and two placebos. Following each treatment the subjects were tested for knee extension/flexion strength and endurance on the Cybex II dynamometer, and for anaerobic power on the Fitron stationary bicycle. A one-way ANOVA was used to analyze the data. Strength was tested at 3 speeds: 60°/sec, 180°/sec, 300°/sec. There was no significant difference (P<.05) between treatments at any of these speeds. There was no significant difference (P<.05) between treatments in the maximal amount of work performed in 45 sec. on the Fitron. Pedal speed was set at 150 rpm, and mean work values ranged from 20.4 ± 2.6 to 22.3 ± 2.8 kgm/kg/min. The results of this investigation confirm the specific cardiac and smooth muscle effects of this class of drugs in man.

37.11

PLASMA LEVELS OF BETA-ENDORPHIN IN AEROBIC EXERCISE. Enzo Molina, Ugo Zuliani*, Antonio Bonetti*, Paolo Zeppilli* and Maurizio Cecchettin*. Univ. of Parma, Cathol. Univ. of Rome, USL 10 Treviso, Italy.

We collected serial blood samples to measure plasma levels of beta-endorphin in nine well trained skiers engaged in a unique long cross country skiing race: the athletes have to ski, non stop, for 24 hrs. Our subjects started at 2:00 PM on saturday and ended the race at 2:00 PM on sunday. They skied distances from 70 to 200 miles. Blood samples were drawn on saturday morning, 6 hrs before the race (FASTING), after 12 hrs from the start (MID RACE), at the end (END) and after 6 hrs of resting (RECOVERY). Plasma values of endogeous beta-endorphin expressed as MEAN + S.E. are reported in the following table:

	FASTING	MID RACE	END	RECOVERY
BETA-ENDORPHIN pg/ml	6.5+.7	29.2+4 **	41.5+4 *	16.6+1 **

* p<.03 ** p<.001

Conclusions: fasting values of athletes were in the normal range; during the race an highly significant progressive increase of endogenous beta-endorphin was observed; recovery after 6 hrs was significant but statistically higher than fasting value.

37.12

ANTIOXIDANT STRESS ENZYME LEVELS IN RABBIT TISSUE AFTER A CHRONIC AEROBIC EXERCISE PROGRAM. B. Girten*, B. Doerr*, E.W. Kanabus and A.J. Merola*. Depts. of Exer. Physiol., Physiol. Chem. & Physiol., The Ohio State Univ., Columbus, OH 43210.

Enzyme activity levels of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GP) of blood, liver and three hindlimb skeletal muscles were measured in eight male New Zealand White rabbits to determine what effect an aerobic exercise program would have on these antioxidant stress enzymes. Rabbits were age and weight-matched and randomly assigned to either an exercise (EXER) or a sedentary (SED) group. EXER animals underwent a ten month training program that involved exercising on a treadmill twice a day for five days per week at a maximum time, speed and grade of 20 min, 32 m/min and 8%, respectively. The level of training achieved was documented by significant differences ($p < .05$) in heart rates and muscle oxygen consumption values between EXER and SED. Student's t-tests indicated a significant difference ($p < .05$) in levels of CAT, SOD and GP between the EXER and SED animals in each of the tissues examined, with the EXER values being greater in all cases. These findings are of particular importance in view of recent reports of increased free radical damage with acute bouts of intense exercise. The greater levels of antioxidant stress enzymes that resulted from this long term moderate exercise program provide evidence for compensatory changes in scavenging systems that point to overall positive effects of aerobic training. (Supported in part by the Central Ohio Heart Chapter)

37.13

PRELIMINARY REPORT ON BALANCE/STANCE IN PARAPLEGIA WITH AND WITHOUT A COGNITIVE FEEDBACK SYSTEM. Chandler A. Phillips and Jerrold S. Petrofsky. National Center for Rehabilitation Engineering, Wright State University, Dayton, OH 45435.

A cognitive feedback system (CFS) has been developed to assist spinal cord injured (SCI) individuals who have sensory anesthesia below the level of injury. The CFS consists of foot-load transducers mounted below the calcaneus and distal lateral metatarsal on each of both feet. Other components are a carrier-oscillator, balanced-modulator and vibrocutaneous interface (VCI) for each of the four channels. A paraplegic subject (T-4 level, complete) stood in a long leg orthosis with the four VCI's positioned over the upper chest wall. An inclinometer was mounted at hip level to measure continuous forward/backward angular displacement. The subject initially stabilized themself in a free-standing attitude with eyes open and the CFS activated. The subject was then blindfolded. There was an initial period of 30±15 seconds during which the subject continuously adjusted their posture (and inclination varied up to ±5 degree peaks). This was followed by a five minute (average) period of prolonged standing (during which inclination varied up to ±1 degree peaks). The CFS was then deactivated while the subject was still blindfolded. Within an average 5±2 second period the subject lost their balance (at which time inclination exceeded ±10 degrees). It is concluded that the CFS operates as a successful adjunct for the SCI individual in order to maintain balance without the use of visual cues.

37.14

SUPPLEMENTAL FORCE OUTPUT DURING COMBINED VOLUNTARY AND ELECTRICAL STIMULATION INDUCED CONTRACTIONS. F.J. Servedio*, R.L. Pohlman, A. Servedio* and R.M. Glaser. Wright State University Sch. of Med., Miami Valley Hospital and VA Medical Center, Dayton, OH 45435

This investigation was to determine the effects of electrical stimulation (ES) as a means of supplementing biceps force output during voluntary (VOL) isometric contractions. Subjects (age=25.8±4.5 yr, $\bar{X}$±SD) performed VOL and ES contractions against a wrist-attached strain guage with the elbow fixed at 90° of flexion. Maximum VOL contractions (MVC=18.9±6.7 kg) and max tolerable ES contractions (5.6±2.7 kg), which were found to be 30±12% MVC (range=12-55%), were initially performed. Max ES voltage (68.2±2.0 V) was determined by increasing intensity (biphasic pulses, 300 µsec duration at 35 Hz) across 2 surface electrodes placed over motor points of the biceps. Combinations of VOL and ES contractions were then performed. Subjects attempted to hold VOL contractions of 30% (n=14) to match max ES force, 40% (n=8), 60% (n=8) and 100% (n=6) MVC for 5 sec. During the 3rd sec of VOL contractions, max ES voltage was applied to the muscles to determine additional force generated. At 30% MVC, there was a significant increase ($p<0.001$) in force output ($\bar{X}$=1.85±0.9 kg; +32%). When ES was applied during 40, 60 and 100% MVC, the supplemental force output was +10%, +4%, and 0%, respectively. These data suggest that the technique of combining VOL and ES exercise could permit a higher level of training at lower VOL effort. (Supported in part by VA Rehab. R&D Service)

37.15

BREATHING PATTERNS AND THORACOABDOMINAL MOTION DURING EXERCISE IN MILD CHRONIC OBSTRUCTIVE LUNG DISEASE. R. Viggiano*, B. Hurley*, J. Rodarte, R. Hyatt, C. Heise*, and B. Staats. Mayo Clinic & Mayo Foundation, Rochester, MN 55905.

Exercise response in patients with mild COPD has not been well studied. Graded cycle ergometry was performed in 13 male patients (P) (55 ± 8.1 yrs) FEV_1/FVC 80 ± 9.2% pred. and compared to 9 normal (N) volunteers (49 ± 7.0 yrs) FEV_1/FVC 108 ± 7.4% pred. Vital capacity (VC) and maximal voluntary ventilation (MVV) were measured prior to exercise. An inductive plethysmograph (Respitrace) was used to estimate the relative contribution of the rib cage (RC) and abdomen (Abd) to breathing. Maximal workload (Wmax) of P was 75% of N ($p < .05$). The following parameters measured at Wmax were not different ($p > .05$), heart rate (HR), respiratory rate, tidal volume (VT) absolute or as a fraction of VC, timing (Ti/Ttot) and fractional contribution of RC and Abd. There was no paradox. Significant ($p < .05$) parameters are shown below.

	VO_2max % pred.	$V_{E_{max}}$ L/min	$V_{E}max/MVV$	$V_{E_{max}}/VO_2max$	Wmax
N	99.8	112	.60	42	227 W
P	75.5	80	.71	47	171

The identical HRmax suggests that P like N are limited by cardiovascular factors. While $V_E$max was less, it was greater relative to VO_2max and MVV consistent with inefficient ventilation and limited reserve. The ventilatory pattern was intermediate to that observed in N and in severe COPD.

37.16

REPRODUCTIVE HORMONE STUDIES ON RUNNERS IN THE FIRST WOMEN'S OLYMPIC MARATHON TRIALS. J.A. Yu-Yahiro*, S.B. Wigutoff*, and E.B. Schoomaker* (SPON: J.E. McKenzie). Department of Physiology, Uniformed Services Univ., Bethesda, MD 20814-4799

Menstrual irregularities are common among elite woman distance runners. We examined the effects of a marathon race upon levels of key reproductive hormones in 63 runners. By menstrual history, 41 were eumenorrheic (EU), 9 oligomenorrheic (OL), and 13 amenorrheic (AM). Blood samples were drawn before and immediately after the marathon and assayed for estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (P), and cortisol (F). No differences were found in percent body fat by skinfold (12.5±0.3, 12.7±0.9, 11.2±0.5), miles run per week (70±2, 67±5, 74±4), or best marathon times (2 hrs 47±1.6 min) (mean ± SEM, all three groups). AM were significantly younger than EU (24.8±1.2 vs. 30.8±0.8; $p < 0.05$). All AM were nulliparous while 17 EU had at least one pregnancy. E2 levels were significantly lower in AM than in EU who were in the follicular phase of their cycles (22.5±5.1 vs. 33.7±5.4 pg/ml; $p < 0.05$). For all groups post-run F and P were significantly higher than pre-run levels ($p < 0.05$). However, no differences were observed among the groups in the magnitude of the F and P changes induced by the race. These data suggest that AM are hypoestrogenemic and that the menstrual irregularities observed are not related to previously suspected predisposing factors such as body fat, performance times, miles run per week, or to exercise-induced hyperprolactinemia. Supported by USUHS grant #C076AD.

37.17

THE EFFECTS OF EXERCISE TRAINING ON SELECTED STEROID HORMONES IN POSTMENOPAUSAL WOMEN. H.J. Richardson-Lehnhard*, T.E.Kirby*, R.A. Lehnhard* (SPON: D.R. Lamb), The Ohio State University, Columbus, Ohio 43210

The purpose of this study was to assess the effects of aerobic training on physiologic and hormonal parameters in older women. Nine untrained postmenopausal women (1 to 10 yrs) engaged in a walk/jog exercise training regime at 70% of maximal oxygen consumption ($\dot{V}O_2max$), 3 x week, 30 min/session for 10 weeks. Pre- and posttraining evaluation of $\dot{V}O_2max$, body composition (BC) and concentration of estradiol (E_2), estrone (E_1), testosterone (T) and dehydroepiandrosterone sulfate (DHEA-S). $\dot{V}O_2max$ was determined by open circuit spirometry during work on a motorized treadmill. BC was assessed using skinfold calipers to estimate fat percentage (% fat), fat weight (FW) and lean body weight (LBW). Concentrations of the circulating steroids were determined using RIA. Comparison of the pre- and posttraining data indicated a significant increase in $\dot{V}O_2max$ (25.7 ± 1.5 to 28.4 ± 1.4 $mL \cdot kg^{-1} \cdot min^{-1}$) $P<.05$. Body composition showed no significant difference in % fat (28.6 vs 29.2%) or LBW (43.3 vs 42.09 kg) and FW (17.6 vs 17.9 kg) following training. Concentrations of E_2 (25.0 ± 1.7 to 20.3 ± 1.5 pg/mL were significantly decreased ($P<.05$). Concentrations of T and DHEA-S showed no significant changes with training. Present data suggest that specific sex steroids important in the physiology of the postmenopausal woman do exhibit changes after an aerobic exercise training regime.

37.18

THE EFFECT OF PRECEEDING LACTIC ACID IN BLOOD ON SUBSEQUENT ACCUMULATION DURING EXERCISE. C.M. Wilson*, D.A. Bascom*, D.W. Wilson*, M.L. Wilson* and D.R. Pendergast. Department of Physiology, SUNYAB, Buffalo, NY 14214.

In previous studies it has been shown that the rate of accumulation of net lactic acid in blood ($\dot{L}a$) is constant at a given super maximal workload and increases with increasing workloads. The purpose of the present study was to determine the effect the presence of La in blood prior to two levels of super maximal bicycle exercise. After $\dot{V}O_2$ peak was determined (3.71±.45 $l \cdot min^{-1}$) a workload representing 110 and 125% of this value was selected for each of ten subjects. The subject then pedalled to exhaustion and at 2 time intervals and the La was determined. In a second series of experiments the subjects pedalled for 1/3 or 2/3 of the total time at each workload, rested for 7 min and after which they pedalled to exhaustion. Venous La was determined 7 min post exercise in all conditions and $\dot{V}O_2$ was determined during the last 30 sec of each exercise bout. The $\dot{L}a$ was 3.01±.7mM and 5.02±.9mM for the 110 and 125% workloads respectively during both protocols. $\dot{V}O_2$ reached one half its maximum in of 45±5 and 49±8 sec for the 110 and 125% workloads respectively. The total La was not significantly different if the La prior to exercise was resting (.8±.2mM) or elevated (2.5±.5 and 5.3±.7mM) by exercise. The apparent net La when high La was present prior to exercise was reduced by the amount of the preceeding La indicating La consumption during the subsequent exercise. [PHS Grant HL28542].

CONTROL OF BREATHING: CENTRAL AND PERIPHERAL MECHANISMS

38.1

REFLEX TRACHEAL CONTRACTION IN RESPONSE TO PARTIAL OBSTRUCTION OF MITRAL VALVE (MVO). K.K. Teo*, G.C.W. Man*, C.T. Kappagoda. Dept. of Medicine & SMRI, Univ. of Alberta, Edmonton, Alta., Canada.

This study was undertaken to determine the effect of MVO on tracheal tone in dogs anesthetized with chloralose. The dogs were ventilated artificially and MVO was achieved by inflating a balloon in the lumen of the left atrium. The latter increased the mean left atrial pressure (LAP) by 10.9 ±0.3 mm Hg. During MVO the mean arterial pressure was maintained by inflating a balloon in the descending aorta. Tracheal tone was measured isometrically in-vivo using the method of Brown et al (J. Appl. Physiol. 49:84-94, 1980). In 13 dogs MVO was applied a total of 44 times, on each occasion for 5 mins. The heart rate increased from 146±5 to 172±4 beats/min. The mean arterial pressure changed from 119±2 to 114±3 mm Hg. In each instance the tracheal tone increased 27±3 sec after the MVO and returned to control values 26±4 sec after the stimulus was removed. The tracheal tone increased by 6.6±0.7g from a resting tone of 91.9±2.4g. This response was abolished when both cervical vagi were cooled to 7-8°C. Obstruction of the tricuspid valve produced a comparable increases in heart rate but failed to produce an increase in tracheal tone. It is suggested that this increase in tracheal tone is a reflex response secondary to stimulation of receptors in the cardiopulmonary region, discharging into afferent myelinated nerve fibers in the vagi.

38.2

Effects of chloral hydrate on passive respiratory mechanics in infants. D.A. Schaeffer*, F.J. Cerny. SUNY/Buffalo, Dept. Pediatrics and Children's Hospital, Buffalo, NY 14222.

Passive respiratory mechanics in infants can be measured during a single relaxed expiration. The infants must be asleep when a mask is placed over the nose and mouth. Some infants may need to be sedated with chloral hydrate (CH) during these tests. The effects of CH on the measured values of lung compliance (C) and resistance (R) are not known. To determine if CH affects C & R measurements, we tested 4 infants (4-9 months of age) during natural (NS) and CH sleep (CS). Mouth pressure (Pm) was measured after airway occlusion at end-inspiration. The passive time constant (τ) was calculated from the slope of the subsequent expiratory flow-volume ($\dot{V}$-V) curve. Total expiratory volume (V_E) and flow after release of occlusion ($\dot{V}_o$) were determined by extrapolation of the linear portion of the $\dot{V}$-V curve. $R = Pm/\dot{V}_o$ and $C = V_E/Pm$.

	C ($ml \cdot cm\ H_2O^{-1}$)		R ($cmH_2O \cdot ml^{-1} \cdot sec$)		τ	
	NS	CS	NS	CS	NS	CS
$\bar{X}$	10.2	11.0	0.025	0.028	0.25	0.30
SD	1.75	1.39	0.004	0.011	0.03	0.09

There were no differences between the two conditions. Thus, CH has no effect on measurements of passive lung mechanics in infants up to 9 months of age.
(DAS was supported by Cystic Fibrosis Foundation.)

38.3

Hering-Breuer Reflex in infants: effects of chloral hydrate. F.J. Cerny, D.A. Schaeffer*. SUNY/Buffalo, Dept. Pediatrics and Children's Hospital, Buffalo, New York 14222.

The measurement of passive respiratory mechanics in infants from a single expiration is based on the presence of the Hering-Breuer reflex (HB) and it is unclear whether HB is present beyond the neonatal period. Since the measurement requires a sleeping infant and this state is often difficult to achieve, sedation may be required. To evaluate the presence of HB in older infants and the effect of chloral hydrate on HB, we tested 4 infants (4-9 mo.) before (B) and after (A) chloral hydrate sedation (50 mg/kg). Presence of HB was determined by placing a mask over the infant's nose and mouth and occluding the airway at end inspiration and expiration. Inspiratory and expiratory duration during the occlusion were determined by measuring mouth pressure. Prolongation of the duration of inspiration or expiration during occlusion indicated the presence of HB.

	Prolongation of:			
	Inspiration		Expiration	
	B	A	B	A
$\bar{X}$	49%	40%	51%	49%
SD	11.4	15.4	15.3	17.1

There were no significant differences.
We conclude that HB was present in all infants and was not affected by chloral hydrate.
(DAS was supported by Cystic Fibrosis Foundation).

38.4

CEREBELLECTOMY (CX) OR FASTIGIAL NUCLEUS LESION (FNL) ALTERS SPONTANEOUS RESPIRATION. J. L. Williams and L. O. Lutherer. Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Although several studies have reported altered respiration after cerebellar depression or lesion, the precise nature of these changes has not been defined clearly. Recently, we have demonstrated that simulation of the rostral fastigial nucleus (FN) can excite or inhibit respiration significantly (Physiologist 27:218,247). In 15 anesthetized (chloralose-urethane), spontaneously breathing cats, respiration 1-3 hours after CX was compared to that in sham-operated (SO) cats. Significant changes ($P<0.05$) were:

	$\dot{V}_E$ (cc)	Frequency	T_I (sec)	V_T/T_I (cc/sec)
SO	509 ± 33	18.5 ± 1.2	1.6 ± 0.1	18.7 ± 1.2
CX	373 ± 26	12.2 ± 0.8	2.1 ± 0.2	16.3 ± 1.4
	T_I/T_{TOT}	pH	$PaCO_2$	PaO_2
SO	0.46 ± 0.02	7.38 ± 0.01	29.6 ± 1.0	71.2 ± 2.4
CX	0.40 ± 0.02	7.32 ± 0.01	33.7 + 1.0	65.7 ± 2.3

Despite large changes in tidal volume, the difference between groups was not significant. Similar changes occurred in 3 cats after bilateral FNL. The cerebellum appears to excite respiratory centers tonically in the spontaneously breathing cat. This excitatory influence may be mediated by FN.
(Supported by AHA grant 82-1234 and Tarbox Institute TTUHSC.)

38.5

PHRENIC NERVE AFFERENT PROJECTIONS TO THE DORSAL RESPIRATORY GROUP. D.F. Speck and W.R. Revelette, Dept. of Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536.

The present study extends our previous report (Fed. Proc. 44: 1586, 1985) concerning the projections of phrenic nerve afferents to neurons in the dorsal respiratory group (DRG). Cats were anesthetized with chloralose-urethane, paralyzed with gallamine, artificially ventilated and vagotomized. Right extrathoracic phrenic nerve stimulation (PNS) during inspiration elicited a short latency reduction in the motor discharge of the contralateral phrenic nerve activity in all 9 animals studied. In contrast, neither right thoracic PNS just above the diaphragm nor left extrathoracic PNS produced the contralateral "inhibition" in 4 of 5 cats. In 6 cats, a total of 50 spontaneously discharging inspiratory units were recorded in the right DRG. For each of these units the responses to both ipsilateral vagal stimulation (VS) and extrathoracic PNS were examined. Of the 50 inspiratory modulated neurons, 19 were affected by VS alone, 7 by PNS alone, 10 by both PNS and VS, and 14 by neither. Ten non-respiratory modulated units in the DRG were excited by PNS but were not affected by VS. These results suggest that: 1) phrenic nerve afferents can affect DRG neuronal discharge patterns, 2) quiescent or tonically active neurons in the DRG may be recruited by PNS, and 3) phrenic afferent projections may arise from non-diaphragmatic sources. (Supported in part by the PSP Research Fund and the UKMC Research Fund).

38.6

POWER SPECTRAL PEAKS IN THE PHRENIC NEUROGRAM: STABILITY UNDER PENTOBARBITAL TITRATION. C.L. Webber, Jr. Department of Physiology, Loyola University of Chicago, Maywood, IL 60153.

In the frequency domain, the cat phrenic neurogram exhibits two strong power spectral peaks around 37 and 88 Hz (superior-collicular decerebrate) or 44 and 99 Hz (mid-collicular decerebrate). It has been reported that the higher spectral peak is exceptionally sensitive to anesthetics in the decerebrate state. For example, this peak is reduced, flattened or eliminated by ketamine or chloralose-urethane (Brain Res 233: 317, 1982). In the present study frequency spectral analysis was performed on the phrenic neurogram recorded from pentobarbital anesthetized cats (30-35 mg PB/kg) with intact neuroaxes. Carbon dioxide was held at hypercapnic levels. It was postulated that the high frequency peak might be greatly attenuated or even absent in such preparations. The results demonstrated that not only could dual peaks (33, 102 Hz) be recorded at low levels of PB (35 mg/kg), but that the positions of the peaks remained constant (37, 105 Hz) at increased PB levels (50 mg/kg). The peaks were decreased in amplitude and flattened (increased bandwidth), however. Still higher levels of PB (80 mg/kg) had more of an effect on the lower frequency peak than the higher one (24 Hz, 98 Hz). These results suggest that both the low and high frequency peaks are resistant to anesthetic depression, somewhat different from that in decerebrate cats. The neural source of the dual phrenic peaks still remains speculative. (Supported by 1 R01 HL 32888).

38.7

CRICOTHYROID MUSCLE RESPONSE TO RESPIRATORY STIMULI, G.E. Woodson, F.B. Sant'Ambrogio, O.P. Mathew and G. Sant'Ambrogio, Depts. of Physiology and Pediatrics, U.T.M.B., Galveston and Dept. of Otorhinolaryngology, Baylor College of Medicine, Houston, Texas 77030.

The cricothyroid (CT) muscle is believed to be an accessory muscle of respiration. To assess the effects of respiratory afferents on CT function, CT activity and PCA activity were recorded during spontaneous breathing through the larynx (UAB) or tracheotomy (TB) and during breathing efforts against an occluded trachea (TO) or upper airway (UAO) in anesthetized dogs. This protocol was repeated following cold blockade of the recurrent laryngeal nerve (RLN) and in some dogs following section of the sensory branch of the superior laryngeal nerve (SSLN). Activity in both muscles was lowest during TB, activity approximately doubled during UAB. During TO activity also increased, activity was maximally increased with UAO. The CT was especially sensitive with an increase in activity of 1500% of TB compared with 400% for the PCA. CT responses to UAO were not affected by RLN blockade but were markedly diminished by SSLN section. We conclude that CT activity is extremely sensitive to upper airway pressure changes and that this effect is probably mediated by fibers in the SSLN.

Supported by NIH grants HL20122 and HL01156.

38.8

HUMAN INSPIRATORY MUSCLE RESPONSES TO OCCLUSION. Brenda L. Plassman* and Robert W. Lansing. U. of Arizona, Tucson, AZ 85721.

Inspiratory muscle responses to airway occlusion were studied in five males. Surface EMGs were recorded from neck, parasternal intercostal, and diaphragm muscles. Subjects inspired from FRC to near vital capacity at a constant flow rate (.4 L/sec). During these trained inspiratory efforts, the airway was unpredictably occluded for .5 sec. The amplitude and latency of EMG responses to the occlusion were obtained from rectified, averaged, and smoothed computer recordings. The stimulus onset was indicated by the pressure rise in the mouth. When subjects were trained to relax in response to the occlusion, neck muscles and diaphragm showed an inhibitory response (IR), mean latency: 28 msec, followed by an excitatory response (ER), mean latency: 69 msec. We could not distinguish these response patterns in the low amplitude parasternal recordings. When subjects were trained to react as rapidly as possible to the occlusion (reaction time or RT task), group mean RTs were 70 msec (±7) for neck muscles, 91 msec (±17) for parasternal muscles, 86 msec (±15) for the diaphragm, and 119 msec (±8) for the mouth pressure responses. Many RTs were as short as 50-60 msec. IRs (mean latency: 28 msec) were also found in the RT task. Excitatory and inhibitory reflexes and very short RTs have been shown for human inspiratory muscles, including the diaphragm, in response to occlusion.

38.9

SYNCHRONIZATION OF RESPIRATION AND STEP-CYCLE DURING RUNNING. R.D. Wurster, A. Lawdermilk*, P. Attenborough* and C.L. Webber, Jr.

To determine the synchronization of respiration to step cycle, 20 subjects (14 females and 6 males) ran on a treadmill at different speeds (2, 3, 4, 5, 6 mph at 10% elevation) and different grades (0, 5, 10, 15, 20 % elevation at 4 mph). Step cycle and respiratory timing were determined by an accelerometer placed on one leg and by a pneumotachometer attached to a face mask. K values (ratio of peak to mean amplitude) were determined from cross-correlograms and used as an index of synchronization.

For all subjects at all speeds and elevations, K values exceeded 1.8. Subjects with a K value of 3 or greater during any one of the exercise levels were considered strong synchronizers (n=9). With increases in speed but not in grade, these subjects had mean K values which increased from 1.8 to 3.7. With increases in grade but not in speed, the K values did not increase. The other subjects (n=11) showed no trends to increase K values with changes either in speed or grade. No consistent pattern existed between K values determined using inspiration versus expiration as the timing reference.

It was concluded that nearly half of all subjects possessed strong synchronization of respiration with the step-cycle particularly at higher running speeds. These results suggest that the step-cycle provides a cue to determine the timing of ventilation. A model for this synchronization is proposed.

38.10

VENTILATORY EFFECTS OF STIMULATION OF PHRENIC AFFERENTS. J. Road*, B.N. Van-Vliet*, N.H. West* and J. Mink* (Spon: D.J. Cotton), Department of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N 0X0.

Afferent fibers in the phrenic nerve are known to be varied in type. Afferent neural activity from peripheral skeletal muscles generally increases $\dot{V}_E$. We studied the effect of activation of phrenic afferents on overall $\dot{V}_E$ in 7 chloralose anesthetized dogs. The left phrenic nerve was transected and the proximal end electrically stimulated. No change in $\dot{V}_E$ occured at low intensities of stimulation. No change in the contralateral costal integrated EMG was noted. At high intensities of stimulation, 40-60 times the twitch threshold, $\dot{V}_E$ increased by 40 ± 14% (± SD, $p < .001$), frequency by 15 ± 6% ($p < .001$) and tidal volume by 23 ± 11% ($p < .001$). $ETCO_2$ decreased by .23 ± .1%. Mean arterial pressure (MAP) increased by 10 ± 10%. ($p < .001$). We applied similar stimulation parameters of high intensity to the proximal end of the transected left gastrocnemius nerve and produced similar increases in $\dot{V}_E$ and MAP. We conclude that activation of phrenic afferents at high intensities increases $\dot{V}_E$ and has a pressor effect similar to other skeletal muscle afferents. In view of the high stimulation thresholds, we conclude that thin myelinated and/or non-myelinated fibers probably mediate this response.

Supported by MRC and SHRB.

38.11

EFFECTS OF LUNG COMPLIANCE ON RAPIDLY ADAPTING RECEPTOR ACTIVITY. A. Jonzon*, T.E. Pisarri*, J.C.G. Coleridge, and H.M. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Although rapidly adapting receptors (RARs) are known to be sensitive to a variety of mechanical stimuli, their response to sustained changes in dynamic lung compliance has not been examined systematically. We recorded activity of 34 RARs in anesthetized open-chest dogs whose lungs were ventilated at constant rate and tidal volume, with a positive end-exiratory pressure (PEEP) of 3 cm H_2O. After a standard hyperinflation (3 V_T), discharge averaged 1.5 ± 0.5 impulses/s. When we reduced dynamic compliance in steps by removing PEEP for 5, 10 and 20 ventilator cycles, activity increased significantly. Activity averaged 5.4 ± 0.8 impulses/s when compliance was reduced by 50%. Increases in activity at low compliance were well sustained, but the response adapted by about 33% over the first minute. Activity was restored to control by hyperinflation. When PEEP was applied, activity was confined to inflation, but when PEEP was removed most RARs fired in deflation and activity increased. Activity also increased, and peak tracheal pressure increased, when we reduced inflation time, keeping tidal volume and cycle length constant. We conclude that baseline activity of RARs is related inversely to dynamic lung compliance. (Supported by HL-24136 and HL-07192.)

38.12

GRADED CHANGES IN AIRWAY SMOOTH MUSCLE TONE EVOKED BY THE CAROTID BAROREFLEX IN DOGS. H.D. Schultz, T.E. Pisarri*, H.M. Coleridge, and J.C.G. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Large changes in blood pressure in the carotid sinus have been shown to evoke reflex changes in airway smooth muscle tone, but the influence of carotid sinus baroreceptors on the airways has not been investigated systematically. We sought to determine whether carotid sinus baroreceptors evoke graded changes in airway smooth muscle tone. In chloralose anesthetized dogs, we isolated and distended the carotid sinuses at controlled pressures and measured isometric tension in an innervated segment of the upper cervical trachea. The influence of aortic baroreceptors was eliminated by cutting the aortic nerves. Decreasing mean sinus pressure below a control level of 100-110 mmHg evoked increases in tracheal tension, heart rate, and aortic pressure which were proportional to the decrease in sinus pressure. A decrease in sinus pressure of as little as 10-15 mmHg evoked tracheal contraction in some dogs. Increasing sinus pressure above the control level had the opposite effect. Cutting or cooling the sinus nerves to 0°C, or cutting the vagal supply to the cervical trachea, eliminated the tracheal responses to changes in sinus pressure. These results indicate that carotid sinus baroreceptors reflexly evoke graded changes in airway smooth muscle tone. (Supported by HL-33797, HL-07192, and HL-24136.)

38.13

PULMONARY VAGAL AFFERENTS STIMULATED BY PULMONARY ARTERIAL INJECTION OF HYPERTONIC SALINE. T.E. Pisarri*, A. Jonzon*, H.M. Coleridge, and J.C.G. Coleridge. Cardiovascular Research Institute, UCSF, San Francisco, CA 94143.

Pulmonary arterial injection of hypertonic NaCl solutions in dogs evokes vagally-mediated bradycardia, hypotension, tracheal contraction, and rapid shallow breathing, effects which are attributed, at least in part, to stimulation of lung C-fibers (Schertel et al., Fed. Proc. 44, 835, 1985). We have examined the effects of injecting hypertonic NaCl solutions (7.2-20%; 0.5-1.5 ml/kg) into the right atrium on pulmonary afferent activity in the cervical vagus nerves in anesthetized dogs. Injection of saline stimulated 20 of 23 pulmonary C-fibers, firing increasing from 0.4 ± 0.1 (SE) to 7.5 ± 2.3 impulses/s (latency 2-5 s; duration of discharge, 3-148 s). Bronchial C-fibers were not stimulated. Saline sensitized 14 of 16 rapidly adapting receptors (RARs) to inflation, activity increasing from 1.6 ± 0.4 to 4.8 ± 0.8 impulses/s (latency 6.5 ± 3.8 s). With small volumes of saline, RAR stimulation was brief (3-9 s); larger volumes produced more sustained firing accompanied by a decrease in lung compliance. Slowly adapting stretch receptors did not respond consistently to saline. Our results suggest that pulmonary C-fibers play a major part in initiating the reflex cardiovascular and respiratory responses to pulmonary arterial injection of hypertonic saline; RARs may be involved in evoking the reflex bronchoconstriction. (Supported by HL-24136 and HL-07192).

38.14

A technique for recording activity from intact phrenic nerve dorsal root filaments. W.R. Revelette and D.T. Frazier. Dept. of Physiol. and Biophys., Univ. of Ky., Lex., Ky. 40536

The purpose of this study was to develop a technique to record activity from uncut spinal cord dorsal root filaments subserving diaphragmatic receptors. Six anesthetized cats (thiopental) were tracheostomized and placed in a spinal suspension frame. A bipolar hook electrode was placed on the diaphragm to monitor respiratory timing and for direct electrical stimulation. The right extrathoracic phrenic nerve was isolated and positioned across a bipolar silver wire stimulating electrode for measurement of conduction velocities of isolated dorsal root filaments. The vertebrae from C_3 to C_7 were removed and the dura mater cut to expose the dorsal root filaments. Temperature of the cord was maintained by continuous perfusion of warm mineral oil. A bundle of filaments was placed on a very fine monopolar tungsten electrode and presence of phasic activity verified. The filaments were then split under a dissecting microscope and individually tested. Dissecting tools consisted of metal wire etched to a fine point. Single unit activity was recorded from the C_5 and C_6 roots during eupenic breathing. Classification of the receptor was accomplished by calculating its conduction velocity (extrathoracic phrenic nerve stimulation) and functionally by electrical stimulation of diaphragm. It is concluded that phrenic afferents can be recorded in continuity by this technique.

MIRCRO- AND PERIPHERAL CIRCULATION

39.1

MECHANISMS OF INTESTINAL O_2 UPTAKE RESPONSES DURING AND AFTER HYPOXIC HYPOXIA. S.L. Dodd, C.E. King, and S.M. Cain, University of Alabama at Birmingham, 35294

Because hypoxia evokes sympathetic output which may affect intestinal O_2 delivery, we tested the hypothesis that the accumulated O_2 deficit and subsequent excess oxygen uptake ($\dot{V}O_2$) during recovery are different between the intact and denervated gut. Anesthetized, paralyzed dogs were ventilated with 9% O_2-91% N_2 for 30 min of hypoxia followed by room air for 30 min of recovery. Serial arterial and venous blood samples were drawn and venous outflow monitored from exteriorized, isolated segments of intact or denervated intestine. $\dot{V}O_2$ was calculated using the Fick principle. The O_2 deficit incurred during hypoxia and the excess $\dot{V}O_2$ during recovery were compared to pre-hypoxic and post-recovery $\dot{V}O_2$ levels. In the intact intestine, the excess recovery $\dot{V}O_2$ was less than 50% of the deficit incurred during hypoxia. Denervation did not alter this imbalance, nor did it alter responses of blood flow or arterio-venous O_2 difference. These data suggest that gut innervation does not alter the intestinal O_2 deficit/excess relationship during and after hypoxia and does not play a major role in intestinal responses to severe hypoxia. (Supported by NHLBI Grant #HL-26927)

39.2

BLOOD FLOW AND O_2 UPTAKE IN WORKING CANINE MUSCLE DURING SEVERE HYPOXIA. C.E. King, S.L. Dodd, W.N. Stainsby, and S.M. Cain. University of Alabama at Birmingham, 35294.

We wished to test the hypothesis that the excess O_2 used by muscle in recovery from hypoxia corresponds with the level of energy stores depletion. This was accomplished by imposing severe hypoxia on 2 levels of O_2 demand in skeletal muscle. Dogs were anesthetized, paralyzed and ventilated. The left gastrocnemius muscle and its venous outflow were isolated. The knee joint was fixed and the tendon was freed and attached to a strain gauge. Isometric contractions were induced by sciatic nerve stimulation (4 or 2 twitches/sec (tw/s)). After 20 min, the animals were ventilated with 9% O_2 in N_2 for 30 min of hypoxia and then returned to room air breathing for another 30 min. During hypoxia, developed tension, an index of energy requirement, decreased to a similar extent at 4 and 2 tw/s. In contrast, muscle O_2 uptake ($\dot{V}O_2$) fell more during hypoxia at 4 tw/s because O_2 extraction was relatively impaired. During the first 5 min of recovery, excess muscle $\dot{V}O_2$ in the 4 tw/s group was 10-fold greater than in the 2 tw/s group. This excess O_2 was most likely used to replenish energy stores which had been more greatly depleted. At 4 tw/s, the lower O_2 extraction values suggest that muscle blood flow distribution was less homogeneous and this may have contributed to the more severe O_2 uptake limitation observed during hypoxia. (Supported by NIH Grant HL-26927 and Cdn. Heart Foundation)

39.3

FACTORS AFFECTING REGIONAL BLOOD FLOW IN THE DOG GASTROCNEMIUS MUSCLE. C.Marconi*,N.Heisler*,M.Meyer*,H.Weitz*,D.R. Pendergast, J.Piiper and P.Cerretelli. (Depts of Physiology Max Planck Inst. Göttingen (FRG), Univ.of Geneva (CH) and C.S.Fisiologia Lavoro Muscolare, Milano (I)).

Previous measurements of regional blood flow ($\dot{q}_R$) in the dog isolated-perfused gastrocnemius by 15 μm diameter radioactive microsphere trapping (*) showed a wide heterogeneity both at rest and during stimulation. The aim of the present study was to assess whether: a) at rest and during graded stimulation (0.2s tetani followed by either 2.8 or 3.8s intervals over 30 min) $\dot{q}_R$ as determined by 10,15 and 25 μm microspheres changes with time; b) for a given mean blood flow ($\bar{\dot{q}}$) the contraction affects the distribution of $\dot{q}_R$ within the muscle. The results show that: 1) 10 and 15 but not 25 μm microspheres yield identical $\dot{q}_R$ distribution patterns; 2) $\dot{q}_R$ undergoes large fluctuations with time, particularly in the highly perfused regions of the muscle; 3) the degree of $\dot{q}_R$ inhomogeneity is not affected by the stimulation. It is concluded that: 1) 15μm microspheres may be used for assessing blood flow in the muscle; 2) besides a spatial inhomogeneity quantitated by the degree of the relative dispersion of $\dot{q}_R$ (RD=$\sigma/\bar{X}$) which amounts to 0.52±1.17, a less pronounced temporal inhomogeneity (0.28± 0.21) may affect regional gas exchange and metabolism within the muscle; 3) the $\dot{q}_R$ inhomogeneity is not originated or enhanced by the contraction.

(*) Piiper et al., J. Appl.Physiol.(1985, in press).

39.4

THE LONG TERM EFFECTS OF HYPERPROTEINEMIA ON INTERSTITIAL FLUID PROTEIN CONCENTRATION, BLOOD VOLUME, AND ARTERIAL PRESSURE. R.D. Manning, Jr., Univ. Miss. Med. Ctr., Jackson, MS 39216

Hyperproteinemia was produced in 6 conscious dogs over a 10 day period by daily intravenous infusion of 300 ml of autologous plasma to determine the effects of increases in plasma protein concentration (PPC) on fluid volume regulation, interstitial protein concentration, and arterial pressure. The dogs were splenectomized and were equipped with chronic indwelling arterial and venous catheters. Interstitial fluid protein concentration was determined by collecting prenodal lymph from a lymphatic medial to the cephalic vein while the forepaw was massaged 50 times a minute. Control values of interstitial fluid protein concentration, plasma colloid osmotic pressure, PPC, blood volume, sulfate space, and mean arterial pressure were 1.62 g/dl, 19.8 mm Hg, 6.9 g/dl, 65.2 ml/kg, 237.6 ml/kg, and 83.6 mm Hg, respectively. By day 10 of plasma infusion, plasma colloid osmotic pressure had increased to 29.8 mm Hg; PPC had increased to 9.3 g/dl; interstitial fluid protein concentration was 5.0 g/dl; blood volume was 95.7% of control; sulfate space was 111.7% of control; and mean arterial pressure was 112.3% of control. In conclusion, the maintenance of blood volume during marked hyperproteinemia can be attributed an increase in the interstitial protein concentration, which causes the transcapillary colloid osmotic pressure gradient to remain at its control value. (Supported by NIH grants HL11678 and HL01222).

39.5

THE RESPONSE OF LYMPHATICS TO DOPAMINE INFUSED INTO THE BRACHIAL ARTERY OF THE DOG. J.M. Dabney, M.J. Buehn*, and D.E. Dobbins. Dept. Physiology, USUHS, Bethesda, MD 20814-4779

We infused dopamine HCl into the brachial artery of the forelimb of the anesthetized dog while measuring lymphatic perfusion pressure (N=7). A lymphatic in the dorsum of the paw was cannulated (PE 10) and pump perfused at .034 ml/min with control lymph which was the supernatant of a 1:1 mixture of arterial blood and heparinized Krebs solution. The osmolarity of this lymph averaged 306 mOs/l and protein concentration 2.48 g %. Dopamine was delivered to the forelimb at a blood concentration of 6.45×10^{-9}, 6.45×10^{-8}, 6.45×10^{-7}, and 6.45×10^{-6} molar of base. Forelimb perfusion pressure and small artery pressure were slightly raised by the lowest dopamine infusion but an increase in lymphatic pressure did not occur until the dosage reached 6.45×10^{-7} molar. A further increase occurred at 6.45×10^{-6} molar. This dosage also increased skin small vein pressure. The large doses of dopamine further increased both large and small artery pressures in the forelimb. Systemic pressure was not changed nor was central venous pressure at any dosage tested. These data show that dopamine causes constriction of forelimb lymphatics when delivered in the blood, but the concentration needed is significantly more than is required to affect arterial smooth muscle. Comparison with previous work in which norepinephrine was infused into the brachial artery shows that dopamine is about 1/100th as potent in causing lymphatic constriction.

39.6

ENPROFYLLINE DOES NOT BLOCK HISTAMINE-MEDIATED EDEMA IN THE CANINE FORELIMB IN THE PRESENCE OF A β_2-RECEPTOR ANTAGONIST. D.E. Dobbins, M.J. Buehn*, and J.M. Dabney. Dept. Physiol., Uniformed Services University, Bethesda, MD 20814-4799.

The local intra-arterial infusion of Enprofylline (3-propylxanthine) significantly attenuates histamine edema formation. Enprofylline also significantly decreases systemic arterial pressure allowing for the possibility that Enprofylline's attenuation of histamine's effects are mediated through increased circulating levels of catecholamines. To test this hypothesis, we have infused Enprofylline and histamine in the presence of a β_2-receptor blockade established by the intra-arterial infusion of the β_2-receptor antagonist ICI 118551. ICI 118551 completely blocks the vasodilation produced by the intra-arterial injection of 500 ng of the β_2-receptor agonist terbutaline without affecting forelimb vascular pressures or lymph parameters. Subsequent infusion of Enprofylline results in changes in forelimb pressures qualitatively similar to that seen in the absence of β_2-receptor blockade. Lymph parameters were unaffected. Infusion of histamine during the continued infusion of Enprofylline and ICI 118551 resulted in significant increases in lymph flow, protein concentration and protein transport similar to that produced by histamine alone. These data suggest that Enprofylline attenuates histamine-mediated edema formation in the canine forelimb indirectly as a result of increased circulating levels of catecholamines subsequent to systemic hypotension.

39.7

PHYSICAL AND HYDRODYNAMIC FACTORS AFFECTING CELL ADHESION. G. Chang*, A.B. Strong*, W. Zingg and D.R. Absolom*. Research Institute, The Hospital for Sick Children, Toronto.

In an attempt to develop a better understanding of thrombus formation following exposure of blood to a synthetic material we have investigated the roles which substrate hydrophobicity and various hydrodynamic flow conditions play in determining the extent of cell adhesion with a novel flow chamber which gives rise to laminarescent flow. We have examined the effect on erythrocyte-surface interactions of flow rate, shear force, substrate hydrophobicity and hematocrit level as well as the kinetics of cell adhesion to polymer surfaces. The results indicate: (1) the most important parameter is the hydrophobicity of the substrate material. At a flow rate of 1 ml/min, the extent of cell adhesion increases with increasing hydrophobicity i.e. with decreasing surface tension; (2) the extent of cell adhesion is independent of the flow rate over a ten-fold increase in the flow rate (0.5 ml/min to 5 ml/min); (3) the rate of cell attachment is considerably more rapid on hydrophobic polymers. On both hydrophobic and hydrophilic surfaces a Langmuir type of cell attachment profile is noted. Maximum adhesion occurs after approximately 10 minutes exposure to all surfaces; (4) substrate surface defects, such as surface roughness, have a dramatic effect on the pattern of cell adhesion but does not alter the overall number of cells adhering per unit surface area when compared to defect free substrates.

Supported by the MRC of Canada.

39.8

PREGNANCY-INDUCED CHANGES IN THE MECHANICAL PROPERTIES OF RAT ARTERIAL RESISTANCE VESSELS. M.K.McLaughlin* and T.M.Keve* (SPON: E.D. Hendley) Univ. of Vermont, Burlington, VT 05405

During pregnancy, blood volume and cardiac output is increased while total peripheral resistance is reduced such that mean arterial pressure is decreased. We determined the result of these changes upon some of the mechanical properties of mesenteric arterial resistance vessels. Cylindrical vessel segments (800 microns length, 160 microns diameter) were removed from late gestation Sprague-Dawley rats and age-matched virgin females. These were mounted in a myograph system which measured circumferential force generation and vessel dimensions. The resting circumference-tension relationship was determined by stretching the vessels in a physiologic salt solution (PSS) over a range of circumferences of .7 to 1.2 L_1, where L_1 is defined as .8L_{100}, L_{100} being the circumference that a relaxed vessel would have at a transmural pressure of 100 mmHg. This relationship was shifted in the pregnant rats to the right; these vessels generated less tension at circumferences >L_1 than those from the nonpregnant rats ($P<.05$). Active circumference-tension curves were generated by changing the circumference over the same range of L_1 and activating with 124 mM K^+ - PSS. The pregnant rat vessels produced 50% less active tension and active media stress ($P<.025$). Pregnancy would appear to be associated with a change in passive wall elements and a marked reduction in the K^+ generated contractile ability of the vessels which is not due to a change in media thickness. PHS #AD18162-03

39.9

CAPILLARY CONTENT AND RELEASE OF CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE. EFFECT OF ADENYLCYCLASE ACTIVATION. Eugenio A. Rasio. Notre-Dame Hospital, University of Montreal School of Medicine, Montreal, Que. H2L 4K8, Canada.

The rete mirabile (average weight 113 ± 14 mg) of the eel swimbladder was isolated and its blood capillaries were countercurrent perfused with Krebs-Ringer bicarbonate buffer containing glucose 5 mM and albumin 4 g/L. The perfusions were carried out at 20 °C through the arterial and venous inputs of the rete with constant flows of 0.52 ± 0.01 ml/min and stable head pressures of 45 cm H_2O. The levels of cyclic 3',5'-adenosine monophosphate (cAMP) were measured in the arterial and venous effluents before and after the addition of the adenylcyclase activator forskolin (7β-acetoxy-8,13,-epoxy-1α,6β,9α-trihydroxy-labd-14ene-11-one) to the input media, at a concentration of 10^{-4}M. Forskolin immediately raised the outflows of cAMP from undetectable control values to steady average values of 51.1 ± 15.0 and 91.4 ± 14.6 pmoles/ml, in the arterial and venous effluents, respectively (N=6). The effect was significant ($P < 0.001$) and lasted throughout the hour of forskolin infusion. The cAMP content of the forskolin treated rete averaged 6.87 ± 2.15 μmoles/g DNA as opposed to 0.38 ± 0.06 μmoles/g DNA ($P < 0.001$) in the controlateral blood-free rete removed at the beginning of the experiment. The results indicate that the capillaries of the rete contain measurable amounts of cAMP, sensitive to adenylcyclase stimulation. (Supported by grant MA 4222 of the Medical Research Council of Canada).

39.10

A POTENTIAL ROLE OF GLUTATHIONE IN THE REGULATION OF HEPATIC PERFUSION. Walter G. Bottje[1]*, Asslam S. Hassan[1], and Ken R. Holmes[1,2]. Department of Veterinary Biosciences[1] and Bioengineering Faculty[2], University of Illinois, Urbana 61801

Diethylmaleate (DE) has been shown to rapidly deplete hepatic glutathione (GSH) concentration while L-cysteine (CY), the rate limiting amino acid in GSH synthesis, produces an increase in hepatic GSH concentration in rats previously depleted of GSH. In the present study, we examined the effect of intraperitoneal injections of DE and CY on hepatic blood perfusion (HP) in Na-pentobarbital anesthetized rats. HP was measured using a recently developed thermal pulse decay method (KR Holmes and MM Chen, 1983. In, Measurement of blood flow and local tissue energy production, W. Miller-Schaumberg et al. ed, Thieme-Stratton, NY, p50-56.). In comparison to control and CY-treated rats, DE increased HP by 43 and 25%, respectively. CY had no affect on HP when administered to control rats but reduced HP by 23% in rats previously injected with DE. The data suggest that tissue GSH may play a role in the regulation of HP. (Research supported in part by a New Investigator Research Award, HL-30934 to AS Hassan, and NIH-NHLBI grant HL27011.)

39.11

INTRAHEPATIC RESISTANCE TO PORTAL BLOOD FLOW IS POSTSINUSOIDAL W. Wayne Lautt, Clive V. Greenway, and Dallas J. Legare*. Dept. of Pharmacology and Therapeutics, Faculty of Medicine, Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3E OW3.

It is frequently assumed that portal resistance is presinusiodal with sinusoidal pressure being close to central venous pressure (CVP) and significantly below portal venous pressure (PVP). By placement of a catheter in the hepatic lobar veins we find a postsinusoidal, lobar venous pressure (LVP) less than 1mmHg below PVP. The resistance site is a narrow segment of vein prior to entry into the large hepatic veins. Changes in central venous pressure (passive occlusion) and changes in portal pressure (superior mesenteric and celiac artery occlusions) caused identical changes in both LVP and PVP. Thus altering either inflow or outflow perfusion resulted in equal responses from both pressure sites indicating that the LVP and PVP are measuring the same pressures. Occlusion of the hepatic artery decreased both LVP and PVP but subsequent effects of altered portal inflow and hepatic venous outflow produced the same qualitative responses as described above. We thus conclude that almost all the basal resistance to portal flow occurs at a postsinusoidal site and that portal pressure is a reasonable index of sinusoidal pressure, imposing an error of less than 1/2mmHg by such an estimate. Sympathetic nerve stimulation produced identical changes in LVP and PVP showing that all portal constriction occurred postsinusoidally. Norepinephrine and angiotensin caused some presinusoidal constriction. Supported by MRC of Canada and Manitoba Heart Found.

39.12

THE EFFECT OF PLATELETS ON CORONARY MICROVASCULAR PERMEABILITY TO MACROMOLECULES. Paul F. McDonagh. Texas Tech Health Sciences Center, Lubbock, TX 79430

Several studies in organs other than the heart suggest that platelets play a role in maintenance of microvascular integrity. In an earlier study, we reported that blood cells reduce coronary microvascular permeability to macromolecules and called this phenomenon the "cell effect." At that time, we could not differentiate the contributions made by red cells and platelets. To examine the specific role of platelets in the cell effect, isolated rat hearts were perfused with a mixture of Krebs, albumin (2g% BSA), and rat plasma that was either rich in platelets (PRP) or poor in platelets (PPP). For the nine PRP hearts studied, the perfusate contained an average of 10^4 platelets/μl. For the nine PPP hearts, the perfusate contained $5x10^1$ platelets/μl. The left ventricular epicardial microcirculation was observed directly using intravital fluorescence microscopy, and coronary microvascular permeability to macromolecules was assessed by monitoring the transcoronary extravasation of fluorescent albumin (FITC-BSA). We found that the measure of transcoronary FITC-BSA extravasation, the (O/I) ratio, was 0.57 ± 0.02 (n=70 fields) for the PPP perfused hearts and 0.47 ± 0.02 (n=70) for the PRP hearts ($P < 0.05$). This finding suggests that, when prepared carefully, platelets do play a role in maintenance of the normal semipermeable membrane characteristics of the coronary exchange vessels. (Supported by NIH HL 32330 and The Charles A. Lindbergh Fund.)

39.13

HISTAMINE'S EFFECT ON LANTHANUM (La^{3+}) BINDING TO VASCULAR ENDOTHELIUM IN RABBIT MYOCARDIUM. Scott A. Barman*, Jack T. Saari and Mark D. Olson*. Departments of Physiology and Anatomy, University of North Dakota, School of Medicine, Grand Forks, ND 58202

A major effect of histamine is to increase permeability of vascular endothelium. A possible mechanism of action of histamine may be due to endothelial contraction causing submicroscopic gaps between endothelial cells (Majno et al, J. Cell Biol. 42, 647-672, 1969). It was therefore postulated that a calcium (Ca^{2+}) mechanism may be essential, as in other contractile processes to induce an endothelial contractile response causing an increase in permeability. We have previously shown that the calcium channel blocker verapamil inhibits lanthanum (La^{3+}) binding on vascular endothelium, La^{3+} being used as an electron opaque marker for Ca^{2+} binding sites. Therefore it was postulated that if histamine enhances Ca^{2+} binding to vascular endothelium it should cause the re-appearance of La^{3+} binding in the presence of verapamil. Isolated rabbit hearts were first perfused with 1.1×10^{-4} M histamine for 3 min. as a pre-treatment, secondly with histamine and verapamil (10^{-5} M) for 30 min. and subsequently with histamine in the presence of La^{3+} (10 mM) and verapamil for 30 min. Transmission electron microscopy revealed the presence of bound La^{3+} on vascular endothelium in rabbit myocardium. This evidence supports the hypothesis that histamine may increase vascular permeability by causing an enhancement of Ca^{2+} binding to vascular endothelial membranes. Supported by NIH Grant HL28217.

39.14

ARTERIOLAR DIMENSIONS FROM UNANESTHETIZED RABBITS. H. Hashimoto* and R.L. Prewitt Department of Physiology and Biophysics, LSU Medical Center, Shreveport, LA 71130

Arteriolar dimensions were determined from unanesthetized rabbits using the ear chamber technique. 16 New Zealand white rabbits were used. 8 weeks after the chamber implantation, inside (ID) and outside diameters (OD) of arterioles were measured by closed circuit TV microscopy using a Vista 308 image splitter. Wall thickness (WT), wall-to-lumen ratio (W/L) and cross-sectional wall area (CSWA) were calculated. Arteriolar dimensions during the vasodilated state of natural vasomotion are reported as mean ± SD.

ID(μm)	n	ID(μm)	OD(μm)	WT(μm)	W/L	CSWA(μm²)
5-15	82	9.8±2.9	20.3±3.8	5.3±1.1	0.59±0.23	253±96
15-25	63	20.2±2.8	33.8±4.9	6.8±1.7	0.34±0.08	591±206
25-35	65	30.6±2.6	46.2±4.2	7.8±1.5	0.26±0.05	951±234
35-45	55	39.7±3.1	58.0±5.8	9.2±1.8	0.23±0.04	1424±387
45-55	48	49.7±2.5	71.3±5.1	10.8±1.7	0.22±0.03	2073±442
55-65	46	60.7±3.0	85.7±4.9	12.5±1.6	0.21±0.03	2894±490
65-75	24	69.7±3.2	97.3±5.0	13.8±1.7	0.20±0.02	3640±554

The precapillary arteriolar walls were relatively thicker than those of larger arterioles; this structural property of resistance vessels is important in regulating peripheral resistance, blood flow and downstream capillary density. (Supported by NIH Grants HL32865 and HL28989)

39.15

CAPILLARY ANISOTROPY IN BIRD AND MAMMALIAN HEART. O. Mathieu-Costello and C.M. Durand.* Dept. of Med., UCSD, La Jolla, CA 92093

Using capillary counts on transverse sections only, often used to assess heart capillarity, assumes high capillary orientation (anisotropy). Comparing those counts within and between animals assumes similar anisotropy in the samples. We measured by morphometry capillary anisotropy in perfusion fixed myocardium (lateral portion of ventricular free wall; right and left) from Sprague-Dawley rats and Show Racer pigeons; heart/body weight ratio, 4-5 times larger in pigeon than rat. Ratios between capillary counts/fiber mm^{-2} in transverse and longitudinal sections were used to estimate: 1) capillary anisotropy (concentration parameter K), and 2) the coefficient *c(K,0)* relating capillary counts on transverse sections and capillary length density. At sarcomere length 2.2 to 2.3 μm, we found K=2 and 4 (and c(K,0) = 1.20 and 1.10) in rat left and right midmyocardium, respectively. In pigeon, the value of K was 3.5 and 3 (superficial and deep portion of left ventricular wall; c(K,0) = 1.10 and 1.12, respectively), as compared to K=5 (and c(K,0) = 1.06) in right midmyocardium, indicating higher capillary anisotropy in pigeon heart, and differences between right and left ventricular wall in both species. Supported by NIH grant HL-17731 and by the Puritan-Bennett Foundation.

39.16

HETEROGENEITY OF CAPILLARY SPACING IN DEVELOPING AND HYPERTROPHIC HEART-COMPARISON OF TWO METHODS. Zdenek Turek and Karel Rakusan, Catholic University, Nijmegen, The Netherlands, and Univ. of Ottawa, Ottawa, Ontario, Canada.

The method of capillary domains and the closest-individual method were used for evaluation of capillary spacing in the rat myocardium. Three experimental situations were studied: maturing rats, rats with cardiac hypertrophy due to aortic constriction and rats with spontaneous hypertension. For comparison, the results from the two methods were converted into the mean radius of the Krogh tissue cylinder (R) and its logarithmic standard deviation (log SD), which served as the heterogeneity index. Both methods yielded similar values for mean R but the heterogeneity index obtained with the domain method was the lower. Increased R as well as increased log SD represent less favorable conditions for O_2 supply. They were both larger in the two types of hypertrophy. However, in growing animals, a lower log SD mitigates the deleterious effect of an enlarging R. Both methods exhibited similar sensitivity in detecting the above results. Thus, for practical purposes these two methods may be used as alternatives for estimating the heterogeneity of capillary spacing but the domain method is more direct and less time-consuming. (Supported by the MRC of Canada).

39.17

DENERVATION AUGMENTS ALPHA$_2$- BUT NOT ALPHA$_1$-ADRENERGIC RESPONSES IN CANINE SAPHENOUS VEINS. Nicholas A. Flavahan*, Virginia M. Miller and Paul M. Vanhoutte. Dept. Physiol. and Biophys., Mayo Clinic, Rochester, MN 55905

In the canine saphenous vein, contractile responses evoked by sympathetic nerve stimulation or by tyramine are more sensitive to alpha$_2$- than to alpha$_1$-adrenergic blockade. This suggests that the innervated receptors are predominantly alpha$_2$-adrenoceptors. The present study examined the effect of sympathetic denervation on postjunctional alpha$_1$- and alpha$_2$-adrenergic receptors. In female dogs anesthetized with pentobarbital, the left lumbar sympathetic chain was excised from L1-L7. After 3-5 week recovery period, the left (denervated) and right (control) saphenous veins were removed, cut into rings and suspended for isometric tension recording. Denervation abolished contractions evoked by the indirect sympathomimetic amine tyramine, and shifted the concentration-effect curve to norepinephrine to the left under control conditions (30-fold shift) and also following blockade of neuronal and extraneuronal uptake (3-fold shift). The contractile responses evoked by the alpha$_2$-adrenoceptor agonist, UK 14,304, were augmented significantly by denervation (10-fold shift in curve) whereas those produced by phenylephrine, an alpha$_1$-adrenoceptor agonist, were unaffected. The selective augmentation of alpha$_2$-adrenergic responsiveness by denervation confirms that these receptors are innervated preferentially by the sympathetic nerves. (Supported in part by NIH grant HL 05883.)

39.18

IMPORTANCE OF BLOOD FOR CATECHOLAMINE CONJUGATION. M. Amies*, N.H. West* and J.X. Wilson. University of Saskatchewan, Saskatoon; The University of Western Ontario, London, Canada.

The reappearance of circulating catecholamines after i.v. transfusion with perfluorocarbon blood-substitute (FC-43) and the importance of blood for catecholamine conjugation were determined in adult, male rats anesthetized with urethane. Rats were respired with 100% oxygen. In controls, plasma noradrenaline (NA) was conjugated with both sulfate and glucuronide. Plasma concentration of free dopamine (DA) was similar to that of sulfoconjugated DA, but was about ten-fold less than glucuronoconjugated DA levels. The concentrations of free adrenaline (Ad) and Ad glucuronide in the controls' plasma each exceeded that of Ad sulfate. There were no significant differences between initial and final concentrations of any circulating catecholamine in controls during 180 min. Replacement of blood in the FC-43 transfused rats lowered hematocrit to 1.4%, caused both tachycardia and hypotension, and decreased free and glucuronoconjugated Ad concentrations. Free Ad returned to control levels by 30 min post-transfusion, but approximately 180 min were required to restore Ad glucuronide. Free NA concentration rapidly increased following FC-43 transfusion, followed by a delayed rise in conjugated NA concentrations. Free DA also increased after transfusion. DA glucuronide concentration was decreased by the transfusion, but no significant changes were detected in DA sulfate. The data indicate the importance of blood in catecholamine metabolism. (Supported by MRC Canada).

39.19

DYSBARIC OSTEONECROSIS IN SHEEP: A MODEL OF ASEPTIC BONE NECROSIS. W.M.C. Adams*, C.E. Lehner*, R.R. Dubielzig*, J.W. Wilson*, M. Palta*, and E.H. Lanphier. School of Veterinary Medicine, Medical School, and The Biotron, University of Wisconsin, Madison, WI 53706.

Decompression during caisson/tunnel work or diving can lead to ischemic necrosis of long bones that may permanently disable the victim. Lack of a satisfactory animal model has hindered understanding of this condition. Five Suffolk sheep (75-95 kg) were exposed to compressed air (2.5-2.9 ATA), then decompressed, during 12-13 exposures of 24 h over a 2-month period. Clinical signs of decompression sickness were common, at times occurring sequentially in the same limb after "dives." Radiography indicated the presence of bone lesions, and these were confirmed histopathologically. Most lesions involved endosteal thickening with new-bone formation in the diaphysis and frank necrosis of marrow. No articular surfaces collapsed. Dysbaric osteonecrosis probably involves interruption of blood flow by bubbles formed during decompression. The most likely mechanisms are: 1) extravascular bubbles may elevate intramedullary pressure sufficiently to collapse vessels by a Starling resistor effect, or 2) intravascular bubbles may obstruct venous outflow. With either or both of these developments, ischemia with hemostasis, local blood coagulation, and necrosis can be expected to follow. Other studies have shown a relationship between acute "limb bends" and elevated intramedullary pressure. (Supported by the University of Wisconsin Sea Grant College Program.)

40.1

FRACTIONAL RESISTANCE OF THE BASOLATERAL MEMBRANE OF MALPIGHIAN TUBULES DECREASES IN THE PRESENCE OF ISOLATED MOSQUITO HEAD FRACTION I AND DIBUTYRYL-cAMP. D.H. Petzel, C.P. Prosper*, and K.W. Beyenbach. Cornell University, Ithaca, NY, 14853.

Peritubular addition of a saline extract of mosquito heads and dibutyryl-cAMP (cAMP) changes the transepithelial voltage (V_T) and transepithelial resistance (R_T) of isolated perfused Malpighian tubules (MT) of the mosquito Aedes aegypti. We now report the effects of Fraction I isolated from head extracts by HPLC (Am. J. Physiol.,in press) and cAMP on the fractional resistance of the basolateral membrane (f_{Rbl}) of the MT. Both Fraction I and cAMP decrease R_T and f_{Rbl} but do so by different mechanisms as evidenced by their effects on voltage. Fraction I depolarizes V_T and hyperpolarizes the basolateral membrane voltage (V_{bl}). In contrast cAMP hyperpolarizes V_T and depolarizes V_{bl}.

BATH	R_T(KΩ.cm)	f_{Rbl}	V_T(mV)	V_{bl}(mV)
control,n=13	16.1±1.6	0.66±.07	31.3±7.1	64.4±4.4
FRACTION I,n=6	13.1±1.0*	0.37±.07*	11.2±6.6*	78.4±5.1*
cAMP,n=7	9.9±1.9*	0.33±.08*	64.1±13.3*	23.8±2.0*

(mean±SE, n=number of MT,*p<0.01, paired t-test)

Furthermore, a ten fold reduction in Cl concentration of the bathing media eliminated the resistance and voltage effects of Fraction I. These effects are consistent with Fraction I induced increase in Cl conductance and cAMP induced increase in the Na conductance of the basolateral membrane. Supported by NSF PCM-8503305(KWB) and National Kidney Foundation(DHP).

40.2

OUABAIN BINDING IN THE VENTRAL SKIN OF THE LARVAL BULLFROG. Douglas H. Robinson* and John W. Mills*. (SPON:H. VALTIN) Dartmouth Medical School, Hanover, N.H. 03756.

Recently it was demonstrated that the ventral skin of the larval bullfrog is able to transport Na^+ actively, the transepithelial potential (TEP) and short-circuit current (SCC) are inhibited by only 50% with 10^{-2}M ouabain applied to the serosal surface for 1.5 to 2 hours (Am. J. Physiol. 237:R74, 1979). We investigated if the residual TEP and SCC are due to the inability of ouabain to penetrate the tadpole skin adequately to inhibit all of the Na/K ATPase or if this is a result of a low affinity ouabain receptor on Na/K ATPase. We found that in intact skin treated with 10^{-6}M ouabain in 0mM K^+ Ringers, equilibrium of binding is achieved by 30 minutes. The rate of uptake is decreased with incubation in 45mM K^+ Ringers indicating that the binding is specifically to Na/K ATPase. Autoradiographic localization of ^{3}H-ouabain (8×10^{-7}M) binding sites shows that ouabain is evenly distributed throughout the epithelium at 1.5 hours of incubation. Scatchard analysis of binding in tadpole skin yields a high affinity dissociation constant (K_D) of 1.08×10^{-7}M which is not significantly different from the adult frog skin K_D of 1.68×10^{-7}M. We conclude that the failure of 10^{-2}M ouabain to completely inhibit TEP and SCC in the tadpole cannot be explained by the presence of inaccessible binding sites or a low affinity ouabain receptor.

(Supported by NIH AM32077 and NIH AM07031. D.H.R. is a fellow of the A.J. Ryan Foundation.)

40.3

Na^+-H^+ AND ANION EXCHANGE IN BROOK TROUT URINARY BLADDER William S. Marshall, Dept. Biology, St. Francis Xavier Univ., Antigonish, Nova Scotia, Canada B2G 1C0.

Electrically-silent Cl uptake by the isolated brook trout urinary bladder was not inhibited by the replacement of mucosal Na with either tetraethyl ammonium or choline, indicating independence of Cl uptake from Na coupling. The Cl uptake was saturable both in the presence of Na and in Na-free mucosal solutions; in both cases the apparent Kt was approximately 36 mM, with a maximum uptake rate of 3.2 $\mu eq.cm^{-2}.h^{-1}$. Unidirectional Na uptake was unaffected by complete bilateral substitution of Cl with gluconate, indicating independence of Na uptake from coupling with Cl. Na uptake in Cl-free, but not in Cl-replete solutions, was accompanied by the secretion of titratable acidity, indicating the presence of Na-H exchange. Na uptake was inhibited partially by amiloride (10^{-4} M), but not by 10^{-4} M bumetanide, supporting pharmacologically the presence of Na-H exchange rather than coupled neutral transport. To account for the lack of net acid or base secretion in Cl-replete solutions and the electroneutrality of Cl uptake, Cl-OH and Cl-HCO_3 exchanges are possible. Voltage-clamping experiments indicated that passive serosa-to-mucosa Cl flux was mostly paracellular, while that for Na was transcellular. The ability of this species to secrete acid into the urine may be of advantage in coping with low pH stress. (supported by NSERC and by Cdn. Nat. Sportsmen's fund).

40.4

MEASUREMENT USING QUIN2 OF CYTOSOLIC CALCIUM LEVELS IN AN ION-TRANSPORTING EPITHELIUM. Christopher A. Loretz and John A. Assad*. State University of New York, Buffalo NY 14260.

Cytosolic Ca^{2+} is a regulator of Na^+ and Cl^- transport in a number of epithelial tissues, including the posterior intestine of the goby, Gillichthys mirabilis. Serosal application of the Ca^{2+} ionophore A23187 (10^{-6}M) reduced mucosal-to-serosal and net $^{36}Cl^-$ transport; $^{36}Cl^-$ transport was stimulated by trifluoperazine (10^{-4}M) and R24571 (10^{-5}M, Janssen Pharmaceutica) suggesting mediation by calcium-calmodulin. Despite the pharmacological evidence for Ca^{2+} involvement, the direct measurement of cytosolic Ca^{2+} in epithelial cells and correlation with alterations in ion transport have not been achieved. We have applied the quin2 technique of Tsien et al. (J. Cell Biol. 94:325, 1982) to the goby intestine. Epithelial cell suspensions were prepared by mucosal scraping and mild collagenase treatment (Type IV, 0.1 mg/ml); cell viability exceeded 95% based on trypan blue exclusion. Cells were loaded with the acetoxymethyl ester of quin2 and following washing were placed in a cuvet (10^6 cells/ml in Ringer solution containing 0.1 mM Ca^{2+}; Cl^- transport is not affected by Ca^{2+} reduction to this level) to measure relative fluorescence intensity (excitation 339 nm, emission 492 nm) in a spectrofluorometer. For calibration, maximum fluorescence signal was obtained by treating cells with digitonin (10 uM) or Triton X-100 (0.025%); minimum fluoresense was achieved by final addition of EGTA (1 mM) and Tris (25 mM). The calculated cytosolic Ca^{2+} concentration was 158±15 nM (12 tissues). Aided by National Institutes of Health grant AM-31709 to C.A.L.

40.5

ONTOGENY OF ALDOSTERONE STIMULATION OF SHORT-CIRCUIT CURRENT IN THE SKIN OF LARVAL BULLFROGS. S.D. Hillyard and W. Van Driessche. Dept. Biol., Univ. of Nev., Las Vegas, 89154 and Lab. voor Fysiol., K.U. Leuven, Belgium.

In developmental stages I-XX (Taylor and Kollros, Anat. Rec. 94:7-23,1946) the apical membrane of the larval bullfrog skin contains a poorly-specific cation channel. When any of the alkalai metals are placed in the solution bathing the apical surface of the isolated skin and NaCl Ringer's bathe the serosa a small (1-2 $\mu A/cm^2$), inward-directed, short-circuit current (Isc) can be measured. Furthermore, amiloride produces a transient stimulation of this Isc as opposed to its blocking effect in the adult skin. Fluctuation analysis of the Isc in these preparations reveals characteristic modifications in the gating kinetics of this channel. Between stages XXI and XXII the larval skin begins to resemble the adult skin; specifically, an inward-directed Isc is seen only with NaCl in the apical solution and that Isc is blocked by amiloride. Isolated skin from early stage XXI larvae frequently had the larval-type cation channel in the apical membrane and did not change during incubation periods up to 24 hours. If skin from these larvae was treated with 1 µM aldosterone, the Isc increased to 15-20 $\mu A/cm^2$ over a 9-12 hour period and the stimulated Isc was blocked by amiloride. Thus, it is possible to study the events associated with the development of an amiloride-sensitive Na+ transport system and the role of aldosterone in this process.

S.D.H was supported by N.S.F. Grant # INT. 83 111 67

40.6

APICAL MEMBRANE SODIUM PERMEABILITY (P^a_{Na}) IN SPLIT TOAD SKIN DURING HIGH POTASSIUM DEPOLARIZATION. D. J. Wilkinson*, S.K. Hong and M. E. Duffey, Dept. of Physiology, Sch. of Medicine, SUNY Buffalo, Buffalo, NY., 14214.

P^a_{Na} of a tight epithelium can be determined by transepithelial current-voltage (IV) analysis during elevation of the serosal bathing solution K concentration. To examine the accuracy of this method in toad skin, the transporting epithelium was split from underlying tissue (after exposure of the serosal surface to collagenase), mounted in an Ussing chamber, bathed in SO_4 Ringer and continuously short-circuited. The short-circuit current(Isc) was 8 $\mu A/cm^2$, transepithelial resistance was 5900 ohm cm^2, basolateral membrane potential (ψ_b, determined by microelectrode) was -62 mV and apical membrane fractional resistance (f_R) was .90. Exposure of the apical membrane to amiloride reduced Isc to near zero, while PCMBS increased Isc to 25 $\mu A/cm^2$. When the tissue was exposed to 110 mM K in the serosal bath ψ_b depolarized to -16 mV and f_R increased to .96. Under these conditions, the tissue was voltage-clamped through a series of 100 ms transepithelial voltage steps (±150 mV), while transepithelial and transapical membrane IV values were simultaneously recorded, in the absence and presence of amiloride. P^a_{Na}, determined by transepithelial and transapical measurements, was 6.26 and 6.79×10^{-7} cm/s, respectively. These data show that elevation of the serosal K concentration does not completely depolarize this tissue, but suggest that transepithelial IV analysis can be used to make reasonable estimates of P^a_{Na}. (Supported by NIH HL28542).

40.7

EFFECTS OF ISOPROTERENOL ON SODIUM TRANSPORT AND PUMP COUPLING RATIO IN FROG SKIN. Thomas C. Cox, *Martin Grieme, and *Rebekah Woods, Dept. of Physiology, Southern Illinois University, Carbondale, IL 62901.

The specific beta agonist, isoproterenol (ISO) was used to stimulate sodium transport across the isolated epithelium of frog skin (R. pipiens). In a chloride free (sulfate) Ringer, control short circuit current (Isc) and transepithelial Na flux (INa) were 12.2±2.3 and 11.9±2.6 $\mu A/cm^2$(7), respectively. ISO ($9x10^{-6}M$) caused Isc and INa to increase to 28.5±3.3 and 25.4±2.6 $\mu A/cm^2$, respectively, after 30 minutes. Similar experiments were done to measure the effect of ISO on ^{42}K uptake on paired pieces of skin taken from the same frog. After 20 minutes, uptakes measured under control and ISO conditions were 8.2±0.8 and 7.9±1.6(5) $\mu A/cm^2$, respectively. Therefore, no change in K uptake occurred while Na transport was probably increased 2-3 fold. K uptakes were also measured in tissues mounted in flux chambers allowing simulatneous measurement of Isc. Tissues were selected so that there was a range in Isc that overlapped between control and ISO treated skins. ISO did not affect the pattern of K uptake vs. Isc when compared with control tissues. These studies suggest that K uptake does not vary much in spite of large changes in Na transport rate. Therefore, K uptake is not a good measure of changes in pump activity in this tissue. It also appears that there may be significant increases in the coupling ratio of the pumps as Na transport rate increases.

40.8

EFFECTS OF TRICHLORMETHIAZIDE (TCTH) ON CHLORIDE TRANSPORT AND INTRACELLULAR pH (pHi) IN RABBIT COLON. P. C. Ferriola*, M. A. Acara* and M. E. Duffey. Depts. of Physiology, and Pharmacology and Therapeutics, Sch. of Medicine, SUNY Buffalo, Buffalo, N. Y., 14214.

The cellular mechanisms of action of thiazide diuretics on ion transport by the kidney and other epithelial tissues are not well understood. The effects of a diuretic and non-diuretic thiazide compound on Cl^- absorption and pHi in rabbit distal colon were assessed in tissues mounted in Ussing chambers. This epithelium absorbs Cl^- by an active transport process that includes a Cl^--HCO_3 exchange mechanism in the apical cell membrane. Net $^{36}Cl^-$ absorption (1.5±.3 $\mu Eq/cm^2 hr$) across short-circuited tissues was decreased 53% following addition of the diuretic thiazide, TCTH ($10^{-4}M$, kindly supplied by Schering Corp.), to the mucosal bathing solution. This effect resulted from a decrease ($P<0.02$) in the mucosa-to-serosa unidirectional Cl^- flux, while neither the serosa-to-mucosa Cl^- flux nor short-circuit current were affected. The non-diuretic thiazide, diazoxide, had no effect on Cl^- transport. pHi was also determined in short-circuited tissues using liquid ion exchanger microelectrodes. TCTH caused pHi to rise ($P<0.01$) from 6.90±.04 to 7.10±.03, while the apical cell membrane potential did not change (-57±2 mV). Diazoxide exposure did not affect pHi. These results suggest that the effect of TCTH on Cl^- transport and pHi in rabbit distal colon is inhibition of the apical membrane Cl^--HCO_3 exchange mechanism. (Supported by NIH AM29703 and a PMAF Fellowship)

40.9

ELECTROPHYSIOLOGIC CORRELATES OF TRANSAPICAL L-LYSINE (LYS) ENTRY IN NECTURUS INTESTINE. M. Acevedo* and W. McD. Armstrong. Dept. of Physiology & Biophysics, Indiana University School of Medicine, Indianapolis, IN 46223.

Microelectrode measurements in stripped intestinal segments mounted between oxygenated (100% O_2) phosphate-buffered media (23°C, pH 7.3) containing in mM, 100 Na^+, 5.4 K^+, 1.8 Ca^{++}, 100 Cl^-, 3.6 gluconate (with mannitol to maintain constant osmolality throughout) showed that Lys (added to the mucosal medium) induced rapid depolarization (followed by partial repolarization) of absorptive cell apical membrane potentials (V_a). Depolarization appeared to be saturable with respect to Lys concentration. When Tris replaced external Na^+: (i) 10mM Lys depolarized V_a, (ii) this depolarization was abolished by 10 mM Leucine (Leu), which alone had a negligible effect on V_a, (iii) with 30 mM Lys depolarization was essentially zero. These results are consistent with the presence in the brush-border membrane of an amino acid (AA) transporter with two binding sites, a Na^+ site and a AA site. Other AAs (e.g. Leu) compete with Lys for the AA site. Lys can also occupy the Na^+ site. When the AA site is occupied by a Lys molecule, occupation of the Na^+ site by Na^+ or its "screening" by the ε-amino group of the lysine molecule (low mucosal Lys, no external Na^+) permits Lys entry into the cell. If the Na^+ site is empty (Leu, no external Na^+) or occupied by a Lys molecule (Leu + Lys, or high Lys, no external Na^+) AA entry is inhibited.

Supported by USPHS AM12715.

40.10

A SIMPLIFIED QUANTITATIVE DESCRIPTION OF BICARBONATE EFFLUX COUPLED TO PROTON EFFLUX IN APICAL EPITHELIAL MEMBRANES. D.E. Schafer, A.K. Thakur*, and I.M. Modlin, Surgery Dept., Yale Univ. School of Medicine, VA Med. Ctr., West Haven, CT 06516, Hazleton Laboratories America, Vienna VA 22180.

Apical symport of Na^+ and Cl^- has been reported in renal, gallbladder, and ileal epithelia. Several recent studies suggest that such symport may result from coupling of two antiport mechanisms, one a Na^+-H^+ exchange and the other a Cl^--anion (e.g., HCO_3^-) exchange. We have therefore examined some quantitative implications of this coupling hypothesis. To facilitate practical analysis we propose a mathematical description incorporating essential features of the coupling hypothesis: dissociation of carbonic acid, reassociation of H^+ and HCO_3^-, and efflux of HCO_3^- by a saturable mechanism. Intracellular or intravesicular concentrations of H^+ and HCO_3^- are given as functions of rate constants for H^+-HCO_3^- association and H_2CO_3 dissociation, the Michaelis-Menten constants for bicarbonate (HCO_3^-) efflux, the rate of H^+ efflux and the surface-to-volume ratio. The system has been investigated for a realistic range of relevant parameters, based on data from whole-cell and vesicle studies in renal, gallbladder, and ileal epithelia. The results reveal the effects of system parameters on the time course of Cl^--HCO_3^- exchange in response to changes in Na^+-H^+ exchange. The surface-to-volume ratio emerges as a major determinant of coupling, suggesting that coupling efficiency may be significantly enhanced in microvillus structures.

40.11

ASPARTATE UPTAKE BY ISOLATED RAT VENTRAL PROSTATE CELLS. Renty B. Franklin, L.C. Costello, V.G. Akuffo*, and B. I. Kukoyi*, Dental School, University of Maryland at Baltimore, Baltimore, Maryland 21201

Citrate is a secretory end-product of metabolism in prostate epithelial cells. Therefore, citrate oxidation is limited, and consequently citrate is not recycled as a source of oxalacetate (OAA). Aspartate which is present in extraordinarily high levels in rat ventral prostate (RVP) provides the principal 4-carbon source of OAA for citrate production. We have shown that aspartate in RVP is contained primarily in the cellular compartment. Therefore, we studied the uptake of aspartate by isolated RVP cells to determine whether a special cell transport mechanism might exist for uptake of exogenous aspartate. Suspensions of epithelial cells isolated from RVP were prepared by collagenase treatment and differential sedimentation. Cell viability was determined by permeability to trypan blue. Transport of aspartate was measured by determining the accumulation of ^{3}[H] aspartate by isolated cells in suspension. Results indicated that the uptake of aspartate was linear with time over a period of 8 min. and was dependent upon the aspartate concentration. Lineweaver-Burk plots of the data gave an apparent Km of 0.5-1.0 mM. In addition, the uptake rate was dependent upon the intracellular concentration of citrate. These results suggested that aspartate was actively transported by RVP cells, and that uptake of aspartate may be linked to citrate efflux.

(Supported in part by NIH grant HD16193 and AM28015).

40.12

WATER-ION COUPLING IN THE RECTUM OF AN INSECT: ANALYSIS BY COMPUTER SIMULATION. David B. Brown and John Machin. University of Toronto, Toronto, Ontario M5S 1A1.

The mealworm rectal complex, one of the most powerful reabsorbing systems known, is capable of extracting liquid water or vapor down to activities of 0.88. The driving force for this transport apparently comes from standing osmotic gradients established in tubules surrounding the rectum. The coupling of water flow to ion transport was modelled by a numerically solved partial differential equation using data derived from water vapor absorption by the complex *in vivo*. Blind end osmotic pressures which routinely reach 6.7 osmols. kg^{-1} are achieved through low water permeabilities (8×10^{-7} $cm.s^{-1}.osmols^{-1}$); without counter-current multiplication. Since longitudinal diffusion in the relatively long (0.8 cm) tubules is negligible, the observed decline in osmotic pressure must be caused by a longitudinal decrease in ion transport or increased water permeability. The resulting match of ion transport to water flow, which may protect the tubules from risk of electrolyte precipitation, is not found in other tubules transporting water iso-osmotically.

(Supported by Natural Sciences and Engineering Research Council Canada, Operating Grant A1717).

41.1

BONE LOSS DURING SIMULATED WEIGHTLESSNESS: IS IT GLUCOCORTICOID MEDIATED? D.D. Bikle, B.P. Halloran, C.M. Cone, E. Morey-Holton. V.A. Medical Center, San Francisco, CA 94121

Using a model of simulated weightlessness in which the hindlimbs of the rat are unweighted by tail suspension, we have demonstrated that skeletal unloading results in a temporary cessation of bone growth by 5 to 7 d. In this study, we evaluated whether adrenalectomy would prevent the cessation of bone growth in the suspended animals. Animals were adrenalectomized (Adx) or sham operated (sham), then tail suspended (S) or pair fed (PF) to the suspended group beginning 1 d after Adx or sham. After 7 d the tibia was obtained for fat free weight (FFW) and total calcium content (Ca). These results were normalized to body weight (BW). The results (table) show that Adx did not prevent the decrement in bone mass and calcium content in suspended animals. However, the bones of the Adx animals were larger than the sham animals.

	Mg / FFW	mg FFW / g BW	Mg / Ca	Mg Ca / g BW
Sham-PF	225 ± 6	1.22 ± .03	48.0 ± .6	.260 ± .007
S	200 ± 5*	1.11 ± .02*	42.6 ± .7*	.239 ± .002*
Adx- PF	236 ± 3	1.39 ± .03+	50.3 ± 1.4	.297 ± .005+
S	209 ± 6*	1.16 ± .04*	45.9 ± 1.5*+	.264 ± .01*+

*P<0.05 vs. pair fed; +P<0.05 vs. sham

We conclude that the temporary cessation of bone growth following skeletal unloading is not due to a stress-induced increase in adrenal glucocorticoid production.

41.2

LYSATES OF PURE OSTEOCLAST POPULATIONS DEGRADE BONE COLLAGEN AT pH 4.8. H.C. Blair, A.J. Kahn*, J.J. Jeffrey*, and S.L. Teitelbaum*. Washington University-Medical Center, St. Louis, Missouri 63110.

A growing body of evidence suggests that bone mineral is solubilized by a low pH compartment at the osteoclast-bone matrix interface, but the mechanism of organic matrix removal is unknown. Bone matrix degradation by >98% pure populations of isolated chicken osteoclasts (OC) was studied by 1) analyzing the degradation of devitalized rat bone labeled with ^{45}Ca or L-(5-^{3}H) proline and 2) measuring degradation of cross-linked type I collagen by explanted OC enzymes. Mineral mobilization begins within two hours of culture, while organic matrix degradation by OCs appears 12 hours later. Resorption of the organic and inorganic phases proceeds at parallel rates thereafter until substrate exhaustion occurs. Each OC resorbs 0.15 ng of bone/day/nucleus. 34% of ^{3}H is released as 4-OH-proline, indicating >90% of substrate is collagen. However, gel filtration chromatography shows that >99% of degradation products are irregular fragments <10,000 M_r, 70% <1,000 M_r. Moreover, no TC^A or TC^B fragments, characteristic of neutral collagenase activity, could be seen by SDS-PAGE and exogenously added TC^A/TC^B fragments are not degraded. Enzyme activity from OC lysates on bone collagen reveals high activity, far in excess of 100 µg/ml pepsin control, at pH 4.8 but not at neutral pH. Hence, OCs can degrade the collagenous component of bone by what appears to be a collagenase-independent process.

41.3

SPECIFIC MIGRATION OF PRECURSOR CELLS DURING OSTEOBLAST DIFFERENTIATION. W. E. Roberts, H.B. Wood and E.R. Morey. UOP School of Dentistry, CA 94115 and NASA-Ames Research Center, CA 94035

Maxillary 1st molars of five 6-8 week old male rats were serially sectioned at 3µm. Periodontal ligament (PDL) cells were classified as to nuclear volume: A(40-79), B(80-119), C(120-169), or D cells (≥170µm3). Proximity to the nearest blood vessel (NBV) was specified as within 10, 11-20, 21-30, 31-40, or 40µm paravascular zones. Total cells/1000µm^2 for the five zones was 7.9±0.2, 5.2±0.2, 3.8±0.1, 4.0±0.2, and 6.5±0.1 respectively. Number of A cells was 8.6±1.4, 9.2±1.6, 2.6±1.0, 3.2±0.7, and 2.8±0.9µm respectively, $p < .01$ within 20µm of NBV. Number of preosteoblasts (C&D cells combined) for the five zones was 4.2±0.5, 3.8±1.1, 5.6±1.8, 9.0±1.5, and 12.4±2.1 respectively, $p < .001$ in zones 30µm away from NBV. A' cells are committed osteoprogenitors, and the osteoblast differentiation sequence is A→A'→C→D→Osteoblast. The high concentration of A/A' cells <20µm from NBV, while C/D cells are primarily >30µm from NBV, indicates A' cells migrate away from blood vessels prior to increasing in nuclear size (differentiating) to become preosteoblasts. Osteoblast histogenesis involves a specific pattern of cell migration: perivascular A' cells move away from blood vessels to areas of low cell density, differentiating to preosteoblasts, proliferate and migrate toward the bone surface. Loss of extracellular fluid during weightlessness may inhibit osteoblast differentiation by disrupting cell density and migration patterns. NASA Grant NCC2-224.

41.4

ELECTROPHYSIOLOGY OF BONE AND THE OSTEOPOROSIS OF DISUSE. Mark S. Cooper. Department of Zoology, Univ. of California, Berkeley, CA 97420.

During the past decade, electrical current has been increasingly used to stimulate the healing of recalcitrant non-union bone fractures. In addition, evidence has been obtained indicating that pulsed electric current may be able to counteract the osteoporosis of disuse, which occurs when the normal mechanical stress to the skeleton is removed. This clinical problem is prevelent in paraplegics, individuals with immobilized bone fractures, and also in weightless astronauts. The electrophysiological responses of osteogenic cells and tissues to external electric fields can be analyzed in terms of their core conductor and cable properties. From cable theory, it can be shown that electrotonic coupling via gap junctions increases the sensitivity of tissues to field-induced perturbations of membrane potential by a factor of 10-100 over single, uncoupled cells. Electrotonic coupling also changes the temporal responses of tissues to externally applied fields. Estimations of pulse parameters necessary to maximally stimulate osteogenic tissues with transcutaneous current at a given electric field strength are provided. Preliminary results on the extent of electrotonic coupling in the periosteum of rat limbs will also be discussed.

41.5

LOCALIZATION OF CALCIUM STIMULATED ADENOSINE TRIPHOSPHATASE ACTIVITY IN BLOOD VESSELS OF THE SKELETON. Stephen B. Doty, Columbia University NY, NY, 10032.

Maintenance of the skeleton is highly dependent on its vascular system and the blood flow within calcified tissues. Fluid shifts and vascular changes can occur in weightless environments or in the orthostatic models of non-weight bearing. The adenosine triphosphatase (ATPase) activity in blood vessels has been localized by histochemistry and electron microscopy. The ATPase activity of blood vessels in bone is (1) calcium sensitive, (2) not inhibited by ouabain, and (3) not similar to other phosphatases found in bone cells. The reduction in skeletal alkaline phosphatase previously documented in non-weight bearing animals, is sensitive to extracellular calcium and phosphate content. By regulation of these ions, the blood vessels could play a role in bone cell activity. This suggests a possible regulator function by the vascular system in the skeletal response to non-weight bearing. (Supported by NASA grant NCC 2-325.)

41.6

EFFECT OF SIMULATED WEIGHTLESSNESS AND CHRONIC 1,25(OH)$_2$D ADMINISTRATION ON BONE METABOLISM. B.P. Halloran, D.D. Bikel, T.J. Wronski*, R. Globus, M.J. Levens and E. Morey-Holton. VA Medical Center, San Francisco, CA 94121, and *University of Florida, Gainsville, FL 32610

Using the tail suspension model to simulate weightlessness, we have previously demonstrated that acute skeletal unloading in the young growing rat results in a transitory reduction in the serum concentration of 1,25(OH)$_2$D, a temporary inhibition of bone formation and a reduction in total bone mass. To determine if the fall in serum 1,25(OH)$_2$D was responsible for the inhibition of bone formation and reduction in bone mass, we chronically infused suspended and pair-fed rats with 1,25(OH)$_2$D (75 pmoles/d) for 2 weeks. Chronic 1,25(OH)$_2$D administration prevented the fall in serum 1,25(OH)$_2$D, but did not prevent the reduction in bone mass induced by unloading. However, when compared to vehicle infused rats, both control and suspended rats infused with 1,25(OH)$_2$D, had greater overall skeletal (tibial) mass. Furthermore, osteoblast numbers were increased while osteoclast numbers were decreased in animals infused with 1,25(OH)$_2$D.

Infusate	BW(g)	mgCa/ gBW	OB%	OC%
vehicle	251 ± 9	.226 ± .005	21.5 ± .6	36.1 ± 2.1
1,25(OH)$_2$D	247 ± 9	.251 ± .011*	30.8 ± 1.3*	10.5 ± 2.1*

*p<.001

These results suggest that 1,25(OH)$_2$D is not likely to be directly involved in the bone changes induced by skeletal unloading but can, when chronically administered, increase bone mass.

41.7

COMPARISON OF BACK HARNESS AND TAIL SUSPENSION TECHNIQUES ON BONE PARAMETERS IN GROWING RATS. Emily R. Morey-Holton, Christopher A. Maese, and Thomas J. Wronski. NASA-Ames Research Center, Moffett Field, CA 94305 and University of Gainesville, FL 32610.

Previously published data (Metab. Bone Dis. & Rel. Res. 4:69, 1982) using the back harness, suggested that bone changes in both the proximal tibia and tibial diaphysis lasted longer than 2 weeks and that the rats on the back harness, like rats on the Cosmos biosatellites, gained less weight per gram of food consumed. Using the same strain of animal (Munich-Wistar rats approximately 40 days old) in which the hindquarters were elevated by tail traction rather than back harness, the elevated animals were found to gain weight at the same rate as the control group and the bone changes in the proximal tibia were normal within 2 weeks of experimentation. However, bone changes in the tibial shaft continued to show suppression of bone formation (approximately 45% change from control values) throughout the 2 week experiment similar to animals on the back harness. Thus, the transient nature of proximal tibial changes may depend on the ability of the animal to gain weight normally.

41.8

RESPONSE OF RAT HINDLIMB MUSCLES TO 12 HOURS RECOVERY FROM TAIL-CAST SUSPENSION. Marc E. Tischler, Erik J. Henriksen,* Stephan Jacob,* Paul Cook* and Stephen Jaspers*. Univ. of Arizona, Tucson, AZ 85724

Female Sprague-Dawley rats were subjected to 6 days tail-cast hindlimb suspension followed by 12 hours of weight bearing. The soleus muscle (SOL), which atrophied with suspension, showed increased mass in recovery but without greater protein content. The extensor digitorum longus (EDL) and gastrocnemius (GAS) showed reduced growth in suspension with no change of mass or protein in recovery. In contrast, the plantaris (PLN) recovered mass and protein after showing reduced growth in suspension. In the SOL during recovery, tyrosine decreased, malate and aspartate plus asparagine increased, and the ratio of glutamine/glutamate increased. These changes were opposite to those occurring in suspension. The rise in glutamine/glutamate indicates restoration of the muscle's ability to produce glutamine. In the PLN during recovery, tyrosine also decreased but no other significant changes occurred. The fall of tyrosine in SOL and PLN is probably indicative of improved protein balance. In the SOL, GAS and EDL during recovery, muscle alanine increased. None of the other measured parameters changed in the GAS and EDL. These results show that the SOL is most responsive to resumption of weight bearing presumably because of its atrophy during unloading. (Supported by NASA grant NAGW-227 and an Established Investigatorship from the American Heart Association to M.E. Tischler.)

41.9

POSSIBLE MECHANISM FOR CHANGES IN GLYCOGEN METABOLISM IN UNLOADED SOLEUS MUSCLE. Erik J. Henriksen* and Marc E. Tischler. University of Arizona, Tucson, AZ 85724

Six days of tail-cast suspension caused marked alterations in glycogen metabolism in the soleus muscle from female Sprague-Dawley rats. The in vivo glycogen content in the soleus from suspended rats (TCS) was significantly higher than in the soleus from tail-casted weight-bearing control animals (TCWB). Using muscles initially glycogen-depleted by incubation with isoproterenol, glucose metabolism in the soleus from TCS animals was found to be significantly more responsive to insulin (10^{-4} U/ml) than the loaded soleus. Measured parameters included net glycogen turnover, glucose oxidation, and release of lactate and pyruvate. No differential effect due to insulin was observed for pyruvate oxidation or for pyruvate incorporation into glycogen. In non-glycogen-depleted muscles, the uptake of 2-deoxyglucose in the presence of insulin was much greater in the unloaded soleus than in the loaded muscle. These results suggest that the accumulation of glycogen in the soleus muscle of TCS rats may be related to the increased glucose uptake in the presence of insulin. Altered levels of glucose-6-phosphate and glycogen synthase activity may be important in this response. (Supported by NASA grant NAGW-227 and by an Established Investigatorship from the American Heart Association to M.E. Tischler.)

41.10

CHANGES IN SKELETAL MUSCLE PROPERTIES FOLLOWING HINDLIMB SUSPENSION. W-D. Dettbarn, G.T. Patterson*, R.C. Gupta*, C. Petrone* and K.E. Misulis*. Vanderbilt Univ., Nashville, TN

This laboratory has studied the effects of hindlimb suspension on a variety of skeletal muscle properties. We report here our findings to date on the effects of this non-neurogenic disuse on the extensor digitorum longus (EDL) and soleus muscles from rats subjected to hindlimb suspension for 1 to 4 weeks. In the parameters studied, we have found disuse to affect the EDL very little. However, the soleus is markedly atrophied after 2 weeks. Acceleration in the contractile speed of the soleus and a doubling of Type 2 (fast) fibers in its fiber population occurred after 3 weeks. Total muscle acetylcholinesterase (AChE) activity increased more than 200% of control values by the second week. No changes in the contribution of activity of the AChE molecular forms to the total AChE activity of the soleus was found at any time point studied. The calcium (Ca^{+2}) loading rates of sarcoplasmic reticulum from soleus muscles after 2 weeks of hindlimb suspension increased (45%) to a level intermediate of that found for control soleus and EDL. Similarly, Ca^{+2}-stimulated ATPase activity of the experimental soleus increased. These results indicate that soleus muscles undergo some degree of conversion towards the characteristics of a functionally fast muscle. It appears that the weightbearing activity is intimately involved in the maintenance of normal soleus functional characteristics. Supported by NASA Grant #NAG 2-301.

41.11

CONTROL OF ARACHIDONIC ACID RELEASE IN CHICK MUSCLE CULTURES. Gordon H. Templeton, Mark Padalino,* and Woodring Wright. Univ of Tex Hlth Sci Ctr, Dallas, Tx. 75235

Previous studies indicate the relationship between muscular inactivity and atrophy may involve a sequence consisting of cytosolic calcium elevation, arachidonic acid release from muscular membranes, prostaglandin biosynthesis and changes in protein degradation and synthesis. Since arachidonic acid is the rate limiting step in prostaglandin biosynthesis, intracellular and extracellular parameters which control arachidonic acid release are being tested in chick muscle cell cultures. Initial studies indicate that the majority of ^{3}H-arachidonic acid is incorporated into phosphatidylethanolamine followed by phosphatidylcholine. After preincubation for 24 hr (at which time equilibrium was established), release of ^{3}H-arachidonic acid into the medium was stimulated by a sublytic concentration (2 μg/ml) of melittin, a phospholipase activator. This melittin-induced increase in arachidonic acid release was modified by variations in Na+ and K+ in the medium and by elevation of cytosolic Ca^{2+} with ionomycin (1 μM). Total protein synthesis, as indicated by tyrosine uptake into trichloroacetic acid precipitable counts was not influenced by epidermal, growth factor (50 ng/ml) or phorbol myristic acetate (124 ng/ml). (Supported in part by NASA Grant NAGW-140).

41.12

CAGE-SIZE AND GENDER EFFECTS ON FATIGUE IN RATS. **R.M. Enoka and D.G. Stuart.** Depts. of Physiology and Exercise & Sport Sciences, Univ. of Arizona, Tucson, AZ 85724.

Male (M) and female (F) SD weanling rats were raised separately (n=6-7/group) for 100-135 days in either a small cage (SC; 3-4/cage) or one 25ßx larger (LC; 7/cage). Two test muscles (soleus, SOL; extensor digitorum longus, EDL) were subjected in vivo to a 6-min fatigue test (Burke et al., J. Physiol. 234: 723, 1973).

Cage size did not affect body weight or normalized muscle weight. However, there were significant differences ($p < .05$) due to gender in both body weight (SC+LC: M=4.3±0.3, F=2.7±0.1 N) and normalized muscle weight (M-SOL=0.52±0.07, F-SOL=0.62±0.09; M-EDL=0.56±0.06, F-EDL=0.63±0.05 mN/N). There was a cage-size effect on normalized peak force (40 Hz) exerted during the fatigue test for M-SOL (SC=0.6±0.2, LC=0.8±0.1 N/mN) but not for M-EDL (SC=0.8+0.1, LC=0.9+0.1 N/mN) or for the females (SC+LC: SOL=0.8±0.1, EDL=0.8±0.2 N/mN). Neither cage size nor gender affected EDL fatigability or EDL and SOL relaxation times. However, M-SOL fatigability was affected by cage size (SC=89±21, LC=112±13%; force at 6 min). Average measurements of area and normalized amplitude for the 13 muscle action potentials (APs) within each train revealed no cage size or gender effect on AP amplitude for either muscle. However, AP area for SC-EDL (50±19%; value at 6 min) in males was greater than that for LC-M (24±18%) and both female groups (SC=20±14, LC=23±10%). No effect was seen in SOL. Since the cage-size effects were only present in the male rats, these observations suggest caution in the interpretation of results based upon this animal model.

Supported by grants from NASA (NAGW 338) and NIH (HL07249).

41.13

ALTERED CARBOHYDRATE METABOLISM IN THE WHOLE BODY SUSPENDED RAT. J. M. Steffen, R. D. Fell and X. J. Musacchia. Dept. Physiol. Biophys. and Exer. Physiol. Lab., Univ. of Louisville, Louisville, Ky. 40292

Studies of metabolic adjustments to 7 days of hypokinesia/hypodynamia (H/H) using the whole body suspended rat showed increased muscle fatigability and decreased oxidative capacity. The present studies investigated alterations in carbohydrate metabolism which could regulate energy supply for muscle contractile activity. Male Sprague Dawley rats (@ 200 gm) were suspended for 7 days and the following determined: serum insulin, response to oral glucose loading, hindlimb muscle sensitivity to insulin (rate of glucose uptake using a perfusion technique) and muscle glycogen at rest and following electrically induced contractile activity. Serum insulin was comparable in control and H/H rats. H/H rats exhibited significantly greater hyperglycemia in response to an oral glucose load. This suggested loss of insulin sensitivity which was confirmed by perfusion studies of hindlimb muscle glucose uptake. This insulin insensitivity may be related to increased glycogen in atrophied hindlimb muscles e.g., red gastrocnemius, control vs H/H, $7.88 \pm .91$ vs $9.27 \pm .50$ mg/g, respectively. Glycogen was depleted to a greater extent in muscles from H/H rats (60% vs 52%) during moderate intensity stimulation. These results suggest alterations in carbohydrate metabolism with whole body suspension and increased dependence on anaerobic metabolism during contractile activity. (Supported by NASA: NSG 2325)

41.14

ROLE OF THE HYPOTHALAMUS IN THE REGULATION OF RENIN SECRETION IN RATS. E. Gotoh, T.D. Bahnson, R.H. Alper and W.F. Ganong. Univ. of California, San Francisco, CA 94143

Previous studies demonstrated that serotonergic neurons from the dorsal raphe nucleus (DR) to the hypothalamus mediated the increase in renin secretion produced by the serotonin-releasing drug p-chloroampheta-mine (PCA). To determine which of the nuclei in the hypothalamus are involved, bilateral electrolytic lesions were produced in the paraventricular nuclei (PV) and the dorsomedial nuclei (DM) in male Sprague-Dawley rats. Compared to saline-injected controls, plasma renin activity (PRA) 60 minutes after PCA (10 mg/kg ip) was significantly increased in sham-operated rats and rats with DM lesions but not in rats with PV lesions. To examine if vasopressin-secreting neurons from PV to the hindbrain mediated this response, we tested the effect of PCA on PRA in Brattleboro rats. In these rats, resting PRA was elevated and there was a greater renin response to PCA than in Long-Evans controls. We also studied the PRA response to immobilization for 10 minutes in rats with PV lesions, Brattleboro rats, and rats with electrolytic DR lesions. The increase in PRA observed in sham lesioned rats was significantly reduced in rats with PV lesions whereas the increase in PRA was potentiated in Brattleboro rats and rats with DR lesions. In preliminary experiments, bilateral knife cuts behind the PV reduced the renin response to immobilization and the gravitational stress of head-up tilting. These findings suggest that 1) the PV are involved in the renin response to PCA, immobilization and head-up tilt; 2) vasopressin-secreting projections from the PV do not mediate these responses; and 3) the renin response to immobilization does not require the serotonergic pathway from DR. (Supported by NASA, Am. Heart Association, and USPHS Grant HL29714).

41.15

OXYGEN CONSUMPTION DURING COLD EXPOSURE AT 2.1G IN RATS ADAPTED TO HYPERGRAVIC FIELDS. J. Horowitz., S. Patterson* and C. Monson. Univ. of Calif., Davis, CA 95616 and NASA Ames Research Center, Moffett Field, CA 94035.

In rats raised at earth gravity, 1G, the activation of thermogenic mechanisms is impaired at low temperatures in gravitational fields of 2G (J. Appl. Physiol. 55(3):990-995, 1983). To determine the effect of hypergravity adaptation on the activation of thermogenic mechanisms, oxygen consumption was measured in rats exposed to 2.1G. Two groups of rats were studied. One group of 7 rats (338 ± 8 g, mean $\pm$ S. E. body mass) was adapted to 1G prior to centrifugation. A second group was adapted to 2.1G (322 ± 12 g) -- that is the rats were reared on a centrifuge that was rotated to produce a 2.1G field. During the experimental test period, ambient temperature was 22°C for the first hour and 9°C for the second and third hour. The field was 1G for the first two hours, and 2.1G for the third. The oxygen consumption in rats adapted to 1G did not significantly differ from that of rats adapted to 2.1G during the first two hours. However, over the third hour, the oxygen consumption of the rats adapted to 1G fell markedly (from 14.9 to 6.6 ml/min) while that of the rats adapted to 2.1G remained relatively constant (from 13.7 to 14 ml/min). Thus, adaptation to a 2.1G field enabled the rats to activate thermogenic mechanisms when cold exposed in a hypergravic field and to maintain their core temperatures in the range of 35.5°C to 37.4°C. In contrast rats acclimated at 1G did not do so, and their core temperatures fell to the range of 27.9°C to 32.4°C. (Supported by NASA grant NSG-2234).

41.16

CHANGES IN RAT BRAIN METABOLISM FOLLOWING EXPOSURE TO HYPER-DYNAMIC ENVIRONMENTS. D. M. Murakami* and C. A. Fuller. Univ. of California, Davis, CA 95616.

Chronic exposure to hyperdynamic fields via centrifugation, leads to new steady-state levels of various physiological systems (i.e., bone, muscle, body temperature, etc.). The central nervous system mechanisms that regulate these physiological systems have not been examined under hyperdynamic conditions. This study examines the changes in metabolic activity in the brain regions of Wistar rats exposed to a 2 G acceleration field for 17 days. The relative metabolic activity of various brain regions was revealed using the cytochrome oxidase (CyOX) method. Coronal 40 µm sections through the brain were cut on a freezing microtome. One series of sections was stained with thionin in order to reveal the various nuclear boundaries. Another series of sections was stained for CyOX in order to reveal the pattern of metabolic activity. Preliminary analysis has revealed small changes in metabolic activity in the suprachiasmatic nucleus, substantia nigra reticulata, mammillary nuclei, and anteroventral thalamic nucleus. These neural changes may reflect the physiological adaptation of those systems known to be affected by hyperdynamic environments. Further studies examining metabolic changes in the brain over extended exposure to hyperdynamic environments and during neonatal development are necessary in order to understand these possible relationships. [Supported by NASA Grant NASG 2-349 to CAF and NASA Space Biology Research Fellowship to DMM.]

41.17

INITIAL EXPERIENCE WITH A NEW PLETHYSMOGRAPH FOR ZERO-G USE. J.C. Buckey, D.E. Watenpaugh, L.T. Kim*, M.L. Smith*, F.A. Gaffney* and C.G. Blomqvist*. University of Texas Health Science Center, Dallas, TX 75235

Venous occlusion plethysmography (VOP) is a useful technique for studying limb flow and compliance changes in zero-G. Current devices (mercury-in-silastic (MIS) strain gauges and latex cuffs) suffer from either toxic materials unsuitable for Spacelab use or lengthy set-up and calibration procedures. We have developed and evaluated a new system for venous occlusion plethysmography (SVOP), which uses an optical shaft encoder connected to a band encircling the leg. Circumference changes are converted to digital pulses (one pulse equals .02 mm) and displayed. This digital approach means the unit is calibrated when manufactured and does not need recalibration. Five subjects had VOP flow measurements simultaneously on both calves with the MIS and SVOP at rest and after 3 min of arterial occlusion. Measurements were repeated after switching the devices to the alternate leg. Flow was expressed in ml/min/100 ml. Results showed:

	Slope	Inter	Corr	SEE	# Meas	MIS	SVOP
Overall	0.90	0.44	0.95	3.5	120	10.4	9.7
Rest	0.62	0.75	0.89	0.4	70	2.3	2.2

Overall the results are excellent. The rest flow range was narrow (0.8-5.6 ml/min/100 ml), overall the flow range was 0.8-49 ml/min/100 ml. We conclude the SVOP shaft encoder approach produces good flow data, needs no calibration and is simple to use making it useful for zero-G applications.

41.18

CAT VESTIBULAR NUCLEAR NEURON RESPONSES TO ACTIVE HEAD ROTATIONS. F. Robinson, J. Hollerman* and D. Tomko*. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

This study was undertaken to determine how neurons in the vestibular nuclei of alert cats respond during active voluntary head rotations. Each cat was confined in a net bag and placed on a turntable so that the table's axis of rotation, which was oriented vertically, passed directly through the cat's C_1-C_2 joint. A head holder previously mounted to each cat's skull allowed the head to be fixed to the turntable or, when the head was free to move, made it possible to monitor the movements with a precision potentiometer attached to the head holder. Single unit activity was recorded with etched tungsten microelectrodes driven through the cerebellum to the vestibular nuclei with a miniature microdrive which attached to a chamber mounted over a craniotomy on the dorsal surface of the skull.

During voluntary movements the activity of most of the recorded neurons in the vestibular nuclei that responded to yaw rotation was predictable from their activity during externally applied rotation. A few vestibular nuclear neurons, however, showed little or no response to passive rotations, but fired vigorously during active head rotations. The activity of these cells seemed related to velocity of the head rotation in one direction. Activity sometimes led the associated movement and sometimes followed it. To date, these cells have been histologically confirmed in only the posterior region of the medial vestibular nucleus. Studies are continuing to determine the origin of the response of these neurons during active movement.

Supported by NASA grant NaG2-155, NIH grant NS17585 and a NASA Research Associate Award.

41.19

GRAVITY RECEPTION AND ORIENTATION IN A LOWER ORDER CRUSTACEAN: RHEOCEPTIVE ANTENNAL-SOCKET SETAE OF DAPHNIA MAGNA. Dewey G. Meyers. The Biomedical Office, NASA, and The Bionetics Corporation, BIO-1, Kennedy Space Center, Florida 32899

Higher Order Crustaceans have internal gravity-detection organs known as statocysts. No similar mechanisms are reported for Lower Order Crustacea. Continuously swimming, Lower Order Daphnia magna are known to orient to light during the day, but at night in the absence of visual cues, are suspected of maintaining orientation through gravity perception. Erratic swimming behavior was revealed when D. magna were exposed to a neutrally buoyant medium in the dark, and, thus, indicated an apparent lack of internal gravity sensing organs. Removal of basal setae on the swimming antennae also elicited a disoriented behavior in an unilluminated water. Setae appear essential to night orientation, apparently functioning as gravity-induced current detectors, or rheoceptors, stimulated by the rush of water past the daphnid as it sinks between upward swimming strokes. Test of the sensitivity of this mechanism revealed a gradual threshold near a density difference between the animal and its environment of less that 0.25%. Anatomical studies suggest that the setae contain dendritic connections through to the distal ends of their shafts. Informal observations during zero-"g" of parabolic jet flight appear to confirm the findings of ground-based simulations.

41.20

MOTION SICKNESS: VESTIBULAR PATHWAYS TO THE GUT. Yasuhiro Torigoe. Univ. of Calif., Irvine, CA. 92717

Both peripheral and central neuronal pathways involved in vestibularly-induced motion sickness have been investigated in the cat and rabbit using horseradish peroxidase (HRP) and tritiated amino acids (AA). Peripherally, the sympathetic innervations of the gut via the greater splanchnic nerve originate in the ipsilateral intermediolateral column, lateral funiculus, intercalated region and central autonomic area; the distribution between the four regions is dependent on the level of the thoracic spinal cord segment. The parasympathetic preganglionic innervation of the gut from bilateral dorsal motor nucleus of vagus (DMV) is vicerotopically organized. The rostral DMV innervates the proximal portion (stomach) and the caudal DMV innervates the distal portions of the gastrointestinal tract (ileum). Centrally, the posterior vermis of the cerebellum and the vestibular nuclei have been implicated in motion sickness. Data from AA injections into the fastigial nucleus show strong projections to several brainstem autonomic nuclei including the "vomitting center," lateral solitary tract nucleus and several catecholaminer gic areas. Among the vestibular projections, there is a direct pathway to the caudal-most level of the DMV. These anatomical studies have determined some of the pathways by which the brainstem and spinal autonomic centers receive vestibular information.
Supported by NASA Research Associate Award (MAGW-70).

41.21

MAMMALIAN GRAVITY RECEPTORS: STRUCTURE AND METABOLISM. *Muriel D. Ross. Univ. of Michigan, Ann Arbor, MI 48109.*

Type I and type II hair cells of rat gravity receptors are integrated into common neural circuitry, indicating that information processing begins at the periphery in the vestibular system. Such processing depends partly upon ciliary tuft orientations; hair cells are dynamically polarized by kinociliary postion. Kinocilia of integrated hair cells were not oriented identically; and ciliary tufts of type II cells were more slender and shorter than those of type I cells. Kinocilia were fixed in recovery stroke, strongly suggesting that they are motile. This suggests that calcium metabolism must be strictly controlled and that otoconia, which load the maculas, possibly play a role. We have begun to pursue the issues of the meaning of differing hair cell ciliary tuft configurations, of their otoconial loading, and of neural linkage of hair cells with specific tuft types. Six kinds of ciliary tufts were localized to various parts of the receptors. Findings were compared with those in frog, in which 6 kinds of tufts exist in the inner ear, with 3 types associated with auditory or seismic functions. Only 3 kinds of rat vestibular hair cells were comparable; all were of the sorts defined as tonic, phasic-tonic, or phasic to linear acceleration in frog. Comparative work with other species is underway to learn if ciliary tuft organiztion can provide clues to the functions of macular regions and, coincidentally, indications of the linear acceleratory environment of the species.
Supported by NASA Grant #NAS2-325 and Contract #NAS2-10535.

41.22

EFFECTS OF CLINOSTAT ROTATION ON AURELIA STATOLITH SYNTHESIS. Dorothy Spangenberg, Eastern VA. Medical School, Norfolk, VA.

Aurelia polyps develop eight graviceptors (rhopalia) during their metamorphosis from polyps to ephyrae. These structures are composed of statoliths, epidermal cells, hair cells and neurons. Statocytes form one statolith per cell.

The effects of clinostat rotation on the synthesis of statoliths was studied. For each experiment, groups of 6 polyps were rotated in the horizontal or vertical plane and controls were kept stationary in the horizontal or vertical plane. Ten ephyrae from each group were collected after 5 or 6 days at 27o - 28°C and the numbers of statoliths per rhopalium were counted. Three experiments each were done using the rotational speeds of ¼ rpm or 24 rpm. Statistical analyses revealed that horizontal clinostat rotation at ¼ rpm caused the synthesis of statistically fewer statoliths in the rhopalia than were found in controls. Ephyrae which had developed during 24 rpm horizontal rotation did not show reduced statolith numbers.

In Aurelia, the graviceptor structures are utilized for positional orientation with respect to gravity during swimming. The finding that ¼ rpm horizontal clinostat rotation reduces statolith numbers suggests that the developing ephyrae received modified stimuli (as compared to stationary controls and those rotated vertically) which caused fewer statocytes to mineralize. A comparison of statolith number in graviceptor structures in ephyrae developing at rotational speeds between ¼ rpm and 24 rpm are in progress to help to elucidate the mechanisms involved.

41.23

PROTEIN CONCENTRATION ELEVATIONS IN LUNGS OF MICE FOLLOWING SUDDEN, TRANSIENT CEPHALAD ($+G_z$) ACCELERATION. Charles J. Gutierrez*, Daniel J. Crittenden*, J. Stephen Lytle*, Daniel R. Dukesherer*, and Carmen I. Delmoral*, (SPON: D.L. Beckman) University of Central Florida, Respiratory Therapy Program, Orlando, FL, 32816.

Laboratory and feral lineages of mice were subjected to $+G_z$ accelerations of 6.22 ± .47 (SD) G for 1.8 seconds aboard a solid fuel rocket. Spectrophotometric analysis of bronchoalveolar lavages retrieved post launch revealed protein concentrations of 1.53 ± 0.8 (SD) mg/g wet lung for controls (animals not placed in rocket and not launched), 5.73 ± 4.05 (SD) mg/g wet lung for sham controls (animals placed in rocket but not launched), and 13.09 ± 8.31 (SD) mg/g wet lung for experimentals (animals placed in rocket and launched). Protein was elevated in experimentals compared to controls ($p < .001$). Lung wet weight/body weight ratios, .008 ± .002 (SD) for controls and .008 ± .001 (SD) for sham controls and experimentals, were not elevated. Sudden transient imposition of $+G_z$ acceleration at lift-off may have induced hypervolemia of basilar pulmonary microvasculature with concomitant migration of protein from intravascular to juxtaalveolar perivascular compartments. Exudates may have entered bronchiolar airways subsequently gravitating toward alveoli. Lavage proteins may have been recovered from both bronchiolar and alveolar regions.

41.24

TRANSIENT DEHYDRATION OF LUNGS IN TAIL-SUSPENDED RATS. John Steskal, Alan R. Hargens, and Emily R. Morey. Div. of Ortho. (V-151), Univ. of Calif., La Jolla, Ca 92093 and Ames Research Center, Moffett Field, CA 94035

The tail suspension model (head-down tilt) simulates hypogravity in terms of musculoskeletal loss in the rat. However, little is known of possible alterations of lung water content as a result of this posture. Six male Munich-Wistar rats and 10 male Sprague-Dawley rats (140-280gm) were suspended using methods of E.R. Morey (Bioscience 29: 168-172, 1979) for 2 days and 14 days, respectively. The animals were killed and the lungs were removed and weighed immediately. The wet lungs were placed in an oven at 130° C and dried to constant weight. Water content (as % of total lung weight) of the suspended rat lungs were calculated and compared to similar data of control rats (N=6 siblings for 2 days and N=7 non-siblings for 14 days) which maintained normal horizontal posture. Compared to controls, 2 day suspended rats had dehydrated lungs but lung water content returned to normal after 14 days of suspension.

Lung Water Content (%)	2 Day[1]	14 Day[2]
Suspended	74.7±0.78	78.5±0.19
Control	77.7±0.45	80.3±0.33

[1] $p < 0.001$ [2] $p = 0.151$ $\bar{X} \pm$ S.E.

These results indicate that rats in this suspension model experience lung dehydration after 2 days but not after 14 days of head-down tilt. **(Supported by NASA and the Veterans Administration).**

41.25

THE INFLUENCE OF EXPOSURE TO A PROLONGED HYPERDYNAMIC FIELD ON BODY TEMPERATURE IN THE SQUIRREL MONKEY. Charles A. Fuller. Univ. of California, Davis, CA 95616.

Squirrel monkeys exhibit an inability to regulate body temperature (T_{co}) during an acute (60 min) daytime exposure to a 2 G hyperdynamic field via centrifugation. However, T_{co} can be regulated at control levels during a similar 2 G exposure during the night. The current study was designed to examine the influence of extended exposure (48 hr) to 2 G on the regulation of T_{co} in the squirrel monkey. The experimental protocol consisted of monitoring T_{co} of 6 restrained animals housed on an 18 ft diameter centrifuge at 1 G for 48 hrs followed by 48 hrs of 2 G acceleration. The animals were exposed to the protocol twice: 1) with 2 G onset during the middle of the light period and, 2) 2-weeks later, during the middle of the dark period. In both protocols, the animals at 2 G showed 0.5-1.0°C falls in T_{co} during the lights-on period. T_{co} showed no sign of recovery precentrifugation levels during the 2 G period. At night, however, T_{co} remained at control levels. Thus, the amplitude of the T_{co} 24-hr rhythm was reduced by approximately 1°C, as was the 24-hr mean. These data support previous observations that hyperdynamic fields have a direct influence on the homeostatic regulation of both body temperature and the circadian timekeeping system. [This research was supported by NASA Grant NAG 2-349.]

41.26

CHANGES IN ORTHOSTATIC HEART RATE AND HEART SIZE IN HUMANS AS A FUNCTION OF SPACE FLIGHT DURATION. J.B. Charles* and M.W. Bungo* (SPON: C.L. Huntoon). NASA Johnson Space Center, Houston, TX 77058

Space flight has long been known to influence the functioning of the cardiovascular system. Between 1962 and 1975, these effects were routinely documented before and after U.S. manned space flights by standard PA radiographs and orthostatic stress testing. The ratio of the dimensions of the heart to the thorax (cardiothoracic ratio, C/T) was maximally decreased (-0.039 ± 0.008 SEM, $p<0.01$) after flights of about 3 days duration, but was not different from preflight values after flights of 9 and 11-14 days duration. The increase in orthostatically stressed heart rate was maximal (+65.8% ± 12.1% SEM, $p<0.001$) after flights of about 8 days, but was less elevated (+38.0% ± 11.3% SEM, $p<0.05$) following flights of 11-14 days duration. There was a negative correlation between the percent change in orthostatic heart rate and the absolute change in C/T. These results suggest that space flight durations of about a week produce larger changes in cardiac dimensions and orthostatic heart rate than shorter and longer durations. The reason for the "inflight recovery" on the longer flights is not known.

41.27

HINDLIMB SUSPENSION EFFECTS ON INTEGRATED ELECTROMYOGRAPHIC ACTIVITY IN SELECTED RAT HINDLIMB MUSCLES. E.K. Alford*, R.R.Roy, P.C.Chiang*, & V.R.Edgerton, BRI UCLA, Los Angeles, CA 90024

Hindlimb suspension (S) is frequently utilized to induce hypokinesia in the hindlimb musculature and is effective in producing significant alterations in the contractile properties, protein degradation rates, and fiber-type distributions in predominately slow-twitch muscle. These alterations generally have been assumed to be due to a reduction of activity during suspension. We tested the hypothesis that S results in reduced muscle activity by monitoring the mean integrated electromyographic activity (MIEMG) in the soleus (SOL), medial gastrocnemius (MG), and tibialis anterior (TA) in four rats via chronically implanted fine wire bipolar electrodes. MIEMG was obtained for 15-min periods each hour for 24 consecutive hours seven and three days prior to S, on the day of S, and three and seven days after S. SOL MIEMG activity was reduced by 60% during the seven day suspension, while MIEMG for the MG and TA were increased by 19% and 89%, respectively. These data indicate that S results in hypokinesia in predominately slow-twitch postural muscle, but apparently does not affect the MIEMG activity level of mixed, fast agonists and antagonists. These data are in accord with the pronounced contractile alterations observed in slow-twitch muscle following S, and the relative absence of such changes in fast-twitch muscle. (Supported by NASA NCA2-1R390-502)

41.28

HINDLIMB SUSPENSION EFFECTS ON THE MORPHOLOGIC AND METABOLIC PROPERTIES OF RAT MEDIAL GASTROCNEMIUS. M.A. Bello*, R.R. Roy, and V.R. Edgerton. UCLA, L.A., CA 90024.

Following 28 days of hindlimb suspension, the medial gastrocnemius (MG) was removed, weighed and frozen for quantitative histochemical analysis. Serial cross-sections (10um thick) were stained for myosin ATPase (base preincubation), and 150-250 fibers were typed as dark or light staining and fiber cross-sectional area (CSA) was determined. Succinate dehydrogenase (SDH, an oxidative marker) and alpha glycerophosphate dehydrogenase (GPD, a glycolytic marker) activities of 35-50 typed fibers were determined using a computer image processing system. Fibers were sampled from superficial (sup) and deep regions. Muscle mass of suspended rats (S, n=10) was 28% lower than that of age-matched controls (C, n=8). No differences were found in fiber type distributions. The CSA of dark stained fibers in sup and deep regions were 16 and 21% smaller in S vs C rats, respectively. The light stained fibers in the deep region of S was 36% smaller than that of C. Enzymatic activity changes observed after suspension included: 1)a 33 and 24% decrease in SDH activity of dark fibers in sup and deep regions, respectively, 2)a 16% decrease in SDH activity of light fibers within the deep region, and 3)an 80% (light fibers) and a 120% (dark fibers) increase in GPD activity within the deep region. Thus, suspension results in metabolic and atrophic changes that are region specific (greatest in the deep region), but consistent within both identified fiber types. (Supported by NASA-NCA2-1R390-502).

41.29

HINDLIMB SUSPENSION EFFECTS ON THE SIZE AND ENZYMATIC PROFILE OF RAT TIBIALIS ANTERIOR MUSCLE FIBERS. P. Bouissou*, R.R. Roy, and V.R. Edgerton. Brain Research Institute, UCLA, L.A., CA 90024.

Quantitative histochemical measurements of succinate dehydrogenase (SDH) and alpha glycerophosphate dehydrogenase (GPD) activities were determined for a sample of ~100 fibers from the tibialis anterior (TA) of 6 post-pubertal, female rats (Sprague Dawley) hindlimb suspended (S) for 28 days, and 6 non-suspended age-matched control (C) rats. Wet muscle weights were 621±65 and 598±55mg for C and S, respectively. The distribution of fiber types (identified as dark or light staining by myosin ATPase activity, alkaline preincubation) was similar in both groups, approximating 90% dark and 10% light staining fibers. Suspension resulted in a decreased mean SDH and no change in mean GPD staining activities for both identified fiber types. Fiber size of either type was not affected by suspension. These results suggest that four weeks of suspension may reduce the oxidative potential without altering the glycolytic capacity of the rat TA. In conjunction with the findings in a collaborative study, it also appears that a reduction in oxidative enzyme content may occur without a decrement in muscle fatigability as assessed by a commonly used fatigue test. In contrast to the effects of hindlimb suspension on a slow extensor and to a lesser extent, a fast extensor, the flexor muscle appears to be minimally affected by the absence of weight support. (Supported by NASA grant NCA2-1R390-502).

41.30

HINDLIMB SUSPENSION EFFECTS ON THE MORPHOLOGIC AND METABOLIC PROPERTIES OF THE RAT SOLEUS. E. Hauschka*, R.R. Roy, and V.R. Edgerton. UCLA, L.A., CA 90024.

Hindlimb suspension, a model of hypokinesia, results in alterations to the contractile and morphologic properties of soleus (SOL) muscle. Our intent in this study was to determine at the muscle fiber level, size and metabolic adaptations resulting from 28 days suspension of post-pubertal, female Sprague Dawley rats. Approximately 125-250 fibers from frozen 10 um thick sections were typed as light or dark staining for myosin ATPase, base preincubation. Using a computerized image processing system, quantitative microdensiometric analsis of succinate dehydrogenase (SDH-an oxidative marker) and alpha glycerophosphate dehydrogenase (GPD-a glycolytic marker) activity and fiber cross-sectional areas (CSA) were determined for approximately 50 typed fibers from serial cross-sections of suspended (S) and age-matched controls (C). Following suspension there was a 47% decrease in SOL wet weights. Mean CSA of light and dark staining fibers was decreased 72 and 53%, respectively. This differential fiber atrophy resulted in similar CSA's for the two fiber types in S, whereas in C, the dark staining fibers were 47-79% smaller than the light staining fibers. Suspension resulted in a 20% increase of dark staining fibers. Mean SDH activity was minimally affected, while mean GPD activity was increased 125 and 185% in light and dark staining fibers, respectively. These results support physiological data suggesting that there is a conversion from slow to fast fibers with suspension.

41.31

EVIDENCE FOR BONE MODELLING DURING PROLONGED HINDLIMB SUSPENSION. A.C. Vailas*, D. DeLuna*, V.R. Edgerton and R.R. Roy. University of California, Los Angeles, CA 90024.

The purpose of this study was to investigate the morphological and biochemical changes of mid diaphyseal femur bone in post pubertal female rats (Sprague-Dawley), that were suspended for 28 days. No differences in body weight, femur length and wet weight, mid shaft cortical density, periosteal and endosteal circumferences, endosteal and cortical areas, mid shaft calcium and phosphorus concentrations, DNA concentration, hydroxyproline and uronic acid concentration were observed between suspended and age-matched non suspended rats. However, there was a change in the geometric configuration of the diaphysis. This altered shape was primarily characterized by a 9.8% increase in the anterior-posterior diameter. These data indicate that although no biochemical and mass changes were evident, suspension induced a change in femur bone modelling. Bone modelling associated with prolonged hindlimb suspension may reflect changes in mechanical forces during suspension. (Supported by NASA grants #NCA2-1R390-501 and NCA2-1R390-502).

41.32

HINDLIMB SUSPENSION EFFECTS ON MECHANICAL PROPERTIES OF RAT SKELETAL MUSCLE. A.M. Winiarski*, R.R. Roy, E.K. Alford*, P. Chiang*, and V.R. Edgerton. UCLA, L.A., CA 90024.

In situ isometric properties were determined in five age-matched control (C) and ten 28-day suspended (S) post-pubertal, female Sprague-Dawley rats for the tibialis anterior (TA, fast dorsiflexor), medial gastrocnemius (MG, fast plantarflexor), and soleus (SOL, slow plantarflexor). SOL wet weight was 43% lower in S vs C. Maximum isometric twitch (Pt) and tetanic (Po) tensions were 61 and 70% lower in S vs C, respectively. The MG in S had an 18% lower wet weight and 17 and 24% lower Po and Pt, respectively, when compared to MG in C. No differences in wet weight, Po, or Pt were observed in TA. Contraction times were 20% shorter in SOL and unchanged in MG and TA following suspension. Similarly, the percent of Po attained during a 330 msec tetanus at 20 Hz (P20/Po) was 13% lower in SOL and unchanged in MG and TA. A fatigue index, calculated as the ratio of tension after two min. of stimulation at 40 Hz for 330 msec once per sec to the maximum tension developed during the test, was used as a measure of muscle fatigability. For all three muscles, the fatigue resistance was minimally affected by suspension. These results indicate that hindlimb suspension selectively affects the mass and force-generating capabilities of the plantarflexors, particularly the predominantly slow SOL. (Supported by NASA grant NCA2-1R390-502).

41.33

CHANGES IN MUSCLE PROTEIN COMPOSITION INDUCED BY DISUSE ATROPHY: ANALYSIS BY TWO-DIMENSIONAL ELECTROPHORESIS. Stanley Ellis, Ames Research Center, Moffett Field, CA; C.S. Giometti*, Argonne National Laboratory[1], Argonne, IL; and Dan A. Riley*, Medical College of Wisconsin, Milwaukee, WI.

Disuse atrophy of skeletal muscle results in marked ultrastructural changes in myofibrillar morphology, one of which is a more or less random deletion of myofilaments to yield a "moth-eaten" appearance. In order to relate the morphological changes to protein composition, two dimensional electrophoresis (2-D) was performed. Proteins from whole homogenates of rat soleus (S) and extensor digitorum longus (EDL) muscle from control limbs, and those held in suspension for 10 days were separated by 2-D. Proteins were detected by silver staining and the patterns were analyzed with computerized image processing. Four sets of marker proteins were identified: those predominant in control S, those predominant in EDL, those showing a measurable increase or decrease in atrophied S, and those showing an increase or decrease in atrophied EDL. More protein changes were found in the atrophied S muscle than the atrophied EDL. A significant number of the proteins that showed quantitative changes in both the atrophied S and EDL samples were markers of the opposite muscle type in the controls, i.e., atrophied S expressed proteins normally found in EDL and atrophied EDL expressed proteins normally seen in S.

[1] Contract No. P-8445c

41.34

CONSIDERATIONS IN THE DESIGN OF LIFE SCIENCES RESEARCH FACILITIES FOR THE SPACE STATION. Milton Heinrich* and C. E. Rudiger* (SPON: C. M. Winget). Lockheed Missiles & Space Co., Sunnyvale, CA 94088.

When a permanent Space Station is placed in orbit in the early 1990s, it will include a Science Laboratory Module (SLM) devoted largely to the study of Life Sciences in the space environment. Human research, with crew members as subjects, will address the physiological changes associated with the microgravity of spaceflight. Animal research can explain the mechanisms of some of the basic gravity sensing and response systems. Animal studies can also provide information about the mechanisms of the changes observed in the crew, because animal experiments can provide tissues and use techniques not possible with human subjects. Plant studies are important to define their very sensitive gravity detection and response systems, and as a basis for efficient food plant production in the Closed Ecological Life Support System. It is important to design the SLM so that it can accommodate all these activities. The approach is to provide a "generic" laboratory with basic facilities, adaptable to a variety of studies, and easily modified to accommodate new experiments and techniques. In designing the SLM, input from the scientific community will be useful in several areas, including: species of animals and plants desired for the first and succeeding missions; numbers of specimens needed; sampling intervals; controls needed; bioisolation required; instrumentation.

41.35

HYPOKINESIA AND SPACE FLIGHT REFLECTED ON RATS STOMACH. P.Groza*, I.Lazăr*, E.Dragomirescu*, S.Ionescu*, V.Zamfir*, A.Bordeianu* (SPON: H.Bjursted). Inst. of Physiology, Bucharest, Romania, B-dul 1 Mai 11, 78159

We found in rats that mimiked weightlessness, by hypokinesia (HK) obtained by including rats in small cages, and space flight (on Soviet biosatelites Cosmos 936, 1129 and 1514) decreased glicoproteins and increased the content of some enzymes of digestive tract. At stomach level there are a diminished content of neutral glicoproteins and an accumulation of eosinophiles in the submucosa. As result from researches only after HK, these reactions do not change after adrenalectomy, despite to the great diminution of corticosterone plasma concentration. HK increased gastric acid output, effect that is maximal after two weeks. After that it decreased graduaky becoming normal after two months. This hypersecretion is present too after adrenalectomy. Also we investigated gastric secretion (g.s.) in pregnant rats after 6 days of HK. Pregnancy as such increased considerably gastric acid output, that may be in relation with the decreased pH of blood founded by us in these condition. Pregnant rats do not present augmented acid output after HK. Rats born from hypokinetic rats presented augmentated g.s. after 30 day of life.

41.36

VIVARIA REQUIREMENTS FOR ANIMAL LIFE CYCLE STUDIES ON SPACE STATION. Richard C. Mains* and Jeffrey R. Alberts* (SPON: C. M. Winget). Mains Associates, Berkeley, CA 94704 and Star Enterprises, Bloomington, ID 47405.

Vivaria design concepts, species descriptions and science requirements are proposed for conducting amphibian and rodent life cycle studies in the Life Science Research Facility (LSRF) for the Space Station (SS). The SS LSRF will allow conduct of life cycle and trans-generational studies in the space environment for the first time. In-flight and ground controls are described which can clarify the effects of spaceflight on breeding, parturition, nesting, development, and aging. Animals held in these automated vivaria during development would allow conduct of long-term metabolic balance studies. A proposed on-board centrifuge would support studies of animal development under conditions from 0 to 1 g on the LSRF. Preliminary results are described for rodent vivaria models under development for test in the Shuttle Orbiter mid-deck lockers. Due to special requirements of the SS LSRF for high experiment autonomy, life cycle and trans-generational studies will require the integrated development of animal preparations, experiment protocols and vivaria.

41.37

TISSUE CULTURE APPARATUS FOR FLIGHT EXPERIMENTATION. H.W. Scheld, A.D. Krikorian, and J.W. Magnuson*. PhytoResource Research, Inc., College Station, TX 77840 and Biochemistry Department, State University of New York at Stony Brook, Stony Brook, NY 11794.

A significant portion of the complex biological research aimed at probing biological responses on shuttle/spacelab or space station will involve as its prime experimental goal, or include as an important support procedure, the in-flight treatment of cells, tissues or small organisms for microscopic and chemical analysis. R&D efforts to develop modular flight hardware which will accommodate a broad range of plant and animal cell experiments are in progress. Sets of design criteria have been established at several levels of complexity. These are being evaluated for use in derivative form utilizing functional bread-board models to test and demonstrate approaches. Results of development tests carried out thus far are being incorporated into development hardware and the design/fabrication of the final hardware will be based on these. Supported by NASA's SBIR Program.

CHRONOBIOLOGY

42.1

CIRCADIAN VARIATION OF VOLUME AND CONCENTRATION OF ORTHOSTATICALLY SHIFTED FLUID. M.Moser*, F.Vauti*, H. Pinter* and T. Kenner. Dept.Physiology, Univ.Graz, A-8010 Austria

The amount and time course of orthostatically induced microvascular fluid filtration was investigated in 6 male volunteers during day and night. Blood density and plasma density, hematocrit (Hct), hemoglobin content, erythrocyte count, leucocyte count, arterial blood pressure and heart rate were measured every 3 hours during the day in reclined (0°) position and after tilting to upright (70°) position.

From the change of blood and plasma density and Hct, volume and concentration of orthostatically filtered fluid were computed.

The amount of filtered fluid was largest at 6 am (8.3+/- 1.6 % of blood volume (BV)) and smallest at 6 pm (5.8 +/- 1.4 % of BV, N= 18).

The density of the filtered fluid varied from 1007.6+/- 2.9 g/l (0 am) to 1004.5 +/- 3.9 g/l (6 pm, N=18). This correspondence to a protein concentration of 24.6 g/l in the morning and 14.2. g/l in the late afternoon.

It can be concluded from our data, that orthostatic lability is most pronounced in the morning, when large amounts of fluid of a high protein concentration is filtered and is smallest at 6 pm.

Moreover we could observe a circadian variation of the lower body capillary filtration coefficient.

42.2

DAY COURSE OF BLOOD AND PLASMA DENSITY IN RELATION TO OTHER HEMATOLOGICAL PARAMETERS. F. Vauti*, M. Moser*, H. Pinter* and T. Kenner. Dept.Physiology, Univ.Graz, A-8010 Austria

Since there are no data on the circadian variation of blood density available, hematological parameters were measured in reclined and standing position in 144 experiments at different times of day.

The measured minima and maxima of the parameters during 24 hours were:

	for reclined position		for standing position		
Blood density:	1049.6	1051.2	1053.3	1054.3	(g/l)
Plasma density:	1017.8	1018.7	1019.6	1020.3	(g/l)
Hematocrit:	44.1	45.4	48.2	49.8	(%)
Hemoglobin:	150.5	156.3	164.2	171.6	(g/l)
Erythrocytes:	4.52	4.69	4.92	5.05	(Mio/µl)
Leucocytes:	5552.0	7289.0	6074.0	7921.0	(N/µl)

In these parameters minima were usually observed during the night between 0 and 6 am, whereas double-peak maxima were seen in the afternoon (12 am to 9 pm).

Leucocytes show a significantly time-shifted day course (Min. at 9 am, Max. between 9 pm and 3 am). There is a high correlation between change of the different parameters due to orthostasis but no correlation between change of leucocyte count and the amount of orthostatically filtered fluid.

42.3

REDUCTION OF CIRCADIAN DISRUPTION FOLLOWING SURGERY BY INGESTION OF CAFFEINE IN THE LABORATORY RAT. L.A. Farr, C. Campbell-Grossman*, S.L. Langenberg-Panzer*, J.M. Mack*, M.J. Petersen* & T.J. Smith*. College of Nursing, University of Nebraska Medical Center, Omaha, Nebraska.

The purpose of this study was to test the effects of caffeine ingestion, prior to surgery, on disruption of circadian rhythms following surgery. We measured 20 male Sprague-Dawley rats' locomotor activity every 15 minutes, using an infrared-monitoring system and temperature, every hour by radio/telemetry for two weeks. Eight rats were subjected to surgical laparotomy using general anestheia and then monitored until their activity had returned to pre-surgical levels. Two groups of six rats each received either caffeine in distilled water or distilled water, orally for five days. On day six, animals were subjected to surgical laparotomy and data were recorded as before. Both groups shifted their activity peaks during the treatment period. Shifts were more pronounced with caffeine. Temperature cycles shifted during treatment only with caffeine. Following surgery, unmanipulated rats shifted their activity and temperature peaks to inappropriate times of day. Neither treatment group experienced as much disruption. Rats which received caffeine returned to their pre-surgical levels faster than did either unmanipulated rats or animals which received water alone. We conclude that caffeine provides a cue to circadian timing following surgery above that of handling the animals. Supported in part by DHHS Grant #RO1 NU001098-01.

44.1

RENAL NERVES AND THE INITIAL EXCRETORY RESPONSES TO RECUMBENCY IN MONKEYS AND DOGS. Thomas V. Peterson, Nancy L. Hurst* and Jamie A. Richardson*. Dept. of Medical Physiology, Texas A&M Univ. College of Medicine, College Station, TX 77843.

Experiments were performed in anesthetized Macaca fascicularis monkeys and mongrel dogs to determine if there are species differences concerning the involvement of the renal nerves in mediating the initial renal effects occurring with the assumption of recumbency. All animals underwent acute unilateral renal denervation with the contralateral kidney serving as the innervated control. Renal perfusion pressure was controlled throughout each experiment. Control measurements were made with all animals tilted head-up 45° and recumbent measurements 5-15 mins. after the animals were lowered to 0°. In the monkeys, the responses to recumbency were similar in both kidneys in that urine flow and absolute sodium excretion increased 26% and 18% respectively in the innervated kidneys and 26% and 17% in the denervated kidneys. Increases in fractional sodium excretion and creatinine and para-aminohippurate (PAH) clearances were also similar in both kidneys. In the dogs, recumbency caused urine flow and absolute sodium excretion to increase 81% and 120% respectively along with increases in fractional sodium excretion and creatinine and PAH clearances but these effects occurred only in the innervated kidneys. These results demonstrate that the initial diuresis and natriuresis of recumbency are dependent on the presence of the renal nerves in the dog but not in the monkey. (Supported by NIH Grants HL-31987 and HL-01383).

44.2

EFFECT OF RAT ATRIAL NATRIURETIC FACTOR (ANF) AND CHICKEN HEART EXTRACT ON BLOOD PRESSURE AND RENAL FUNCTION IN CHICKENS. C. M. Gregg and R. F. Wideman, Jr. Depts. of Bio. and Poultry Sci., Pennsylvania State Univ., University Park, PA 16802

Natriuretic factor(s) are found in hearts of many species. However, chicken heart extract is not natriuretic in rats. We studied the renal and B/P effects of synthetic rat ANF and chicken heart extract (CHE) in anesthetized pullets (n=20). Clearances of inulin, PAH, osmoles, and free water were measured, as well as Na and K handling. After a 30 min. control period, one of the following was infused for 30 min. into an anterior tibial vein: extraction vehicle (0.1 N acetic acid), ANF (27 pmol/kg-min.), or CHE (0.004 heart-equiv./kg-min). Compared to vehicle, both ANF and CHE decreased B/P similarly (-25±8 and -22±7 mmHg, respectively). Compared to the control period, rat ANF increased ($p<0.01$): urine flow (148%±10%), fractional Na excretion (385%±93%), and osmolal clearance (130%±9%), but had no measurable effect on inulin and PAH clearances. In contrast, neither CHE or vehicle caused a measurable effect on any parameters of renal function.

44.3

EXTRACELLULAR FLUID (ECF) AND PLASMA VOLUME (PV) RESPONSES TO HEAD-OUT WATER IMMERSION (WI) IN ANESTHETIZED NEPHRECTOMIZED DOGS. K. Miki*, G. Hajduczok*, S.K. Hong, and J.A. Krasney. Dept. of Physiology, SUNYAB, Buffalo, NY 14214

ECF (^{125}I-iothalamate space), blood volume (^{51}Cr-labeled erythrocyte space), and hematocrit (Hct) were measured continuously to study the kinetics of fluid movements between intracellular, interstitial, and plasma compartments during 60 min in air and 120 min of WI at 38°C in 7 splenectomized, and acutely nephrectomized dogs. PV, calculated from blood volume and Hct, started to increase immediately after the onset of WI. PV rose in an almost linear fashion and was increased by 12±2 (SE) ml/kg (33% of initial PV, $p<0.05$) above the control level by 120 min of WI. No change in red blood cell volume was observed throughout the experiment. Hct and plasma protein concentration decreased significantly ($p<0.05$) by -5.5% and -0.8 g/100 ml by 120 min WI respectively. ECF increased linearly during WI to 10±2 ml/kg (4% of initial ECF, $p<0.05$) above the control level by 120 min of WI. The results of this study indicate that WI leads to a sustained fluid shift in the direction of the plasma compartment from the intracellular compartment. We estimate that about 80% of the fluid entering the intravascular compartment is derived from the intracellular space. We conclude therefore that WI causes acute hypervolemia and cellular dehydration in the nephrectomized dog. (Supported by PHS Grant HL18542)

44.4

BODY WATER EXCESS, EXTRACELLULAR VOLUME AND "DRY" WEIGHT IN ANURIC HYPERGLYCEMIA. Antonios H. Tzamaloukas* (SPON: K. D. Gardner, Jr.) VA Medical Center and University of New Mexico, Albuquerque, NM 87108.

In anuric hyperglycemia, the magnitude of the changes in effective osmolality and in serum glucose and sodium concentrations is determined by the change in body glucose, the initial ICF:ECF volume ratio and the initial osmolality (Tzamaloukas, Physiologist 22:126, 1979). Based on this relation, a mathematical method computing body water excess, extracellular volume and "dry" weight was developed. This method requires body weight and initial and final serum glucose and sodium concentrations of anuric hyperglycemic patients treated with insulin alone. In 15 hyperglycemic episodes in dialysis patients, the above parameters were computed and were compared to estimates from a method based on clinically determined dry weight. Results (x = clinical dry weight method): Body water excess; $y = -0.7 + 0.99x$ 1/1, $r = 0.99$ ($p<0.01$). ECF volume at normoglycemia; $y = -0.6 + 1.01x$ 1/1, $r = 0.98$ ($p<0.01$). Dry weight; $y = 2.3 + 0.97x$ kg/kg, $r = 0.97$ ($p<0.01$). In 14 of the 15 episodes clinical dry weight method and new method agreed in the direction of the change in body water and weight from dry weight conditions. Conclusion: If osmotic equilibrium is present at the beginning and end of correction of anuric hyperglycemia with insulin, characterization of the state of body fluids from initial and final serum glucose and sodium concentration and from body weight at presentation is feasible.

44.5

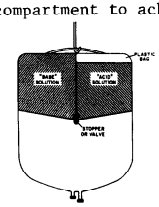
TREATMENT WITH IV INFUSION OF LACTATED RINGER'S OR HUMAN ALBUMIN SOLUTION IN PICHINDE VIRUS-INFECTED GUINEA PIGS. C.T. Liu, R.P. Sanders*, R.S. Dixon*, and C.J. Peters*. U.S. Army Med. Res. Inst. Infect. Dis., Fort Detrick, MD 21701.

Dehydration, electrolyte loss, cardiac depression, and pulmonary edema are common findings in Pichinde virus (PV)-infected strain 13 guinea pigs (GP). This study evaluated the potential use of IV infusion of isotonic lactated Ringer's (LR) or human albumin (25%) solution for supplementing lost body fluids and electrolytes. Blood pressure, heart rate, and cardiac output were measured at various levels of fluid loading (FL) in control and PV-infected GP on days 7, 9, and 11 post-inoculation. Infused fluid volume (g/100 g body weight) ranged from 0.5 to 20%. LR failed to improve cardiovascular functions in PV-infected GP. These GP also could not tolerate the same level of FL as compared with controls. Further, the ability for producing diuresis following volume expansion was lost with PV infection. In contrast, albumin solution infused to expand volume by 0.5 to 1.5% of body weight increased cardiac output and decreased total peripheral resistance. Infused albumin appeared to be confined to the circulation, resulting in an effective increase of plasma or blood volume. The low tolerance to FL and severe terminal organ edema in GP after PV infection suggest an overall increased capillary permeability to water and electrolytes, especially in the lung. Thus, we conclude that in this model of animal hemorrhagic fever, IV infusion of colloid solution (albumin) is superior to crystalloid fluid in PV-infected GP.

45.1

PREPARATION OF PLASMA-RESEMBLING, BICARBONATE-BUFFERED, PHYSIOLOGICAL SOLUTION FOR PARENTERAL USE. T.S. Ing*, J.T. Daugirdas*, Z. Nawab*. (SPON: M. Sayeed), VA Hosp., Hines, IL 60141).

Sterile parenteral solutions containing HCO_3, Ca, and Mg, are difficult to prepare, sterilize, and store, because Ca and Mg form insoluble salts in a bicarbonate solution unless pH is kept below 7.6. We have devised a method by which such solutions can be prepared. A three-compartment plastic bag consists of a pair of vertical, upper compartments on top of a third, lower compartment (Fig). One upper compartment houses a "base" solution containing $NaHCO_3$ (26 mmoles); the other upper compartment harbors an "acid" solution containing acetic acid (2 mmoles) and appropriate amounts of Ca, Mg, Na, K and Cl. A stopper or valve is located at a point where the 3 compartments meet. When the stopper is detached (a maneuver performed from the outside, through the wall of the lower compartment), the solutions from the upper compartments will rush into the lower compartment to achieve thorough mixing. Upon mixing, the acetic acid will titrate an equivalent amount of HCO_3 to form carbon dioxide and water. The high PCO_2 so generated (60 torr), when the HCO_3 level is 24 mM, will result in a pH of 7.25, a pH that will maintain Ca and Mg in solution. The final solution resembles plasma in composition. Because it supplies HCO_3, our solution is inherently superior to Ringer's lactate or Ringer's acetate in the treatment of shock or related conditions.



45.2

ZINC EXCRETION IN COMBINED UREMIA AND ZINC DEFICIENCY (ZND). P.L. Kimmel*, D.W.Watkins, E.B. Teller*, T.M.Phillips and S. Dosa*. George Washington Univ. Medical Center, Washington,DC 20037

Patients with chronic renal insufficiency (CRI) often have hypozincemia and symptoms consistent with ZND. Since increased Zn excretion could lead to ZND and hypozincemia, we studied the metabolism of Zn in 30 Lewis rats under conditions of ZND and/or CRI. One group of animals was fed a Zn deficient (-Zn) diet (~2 ppm), the other group was pair fed an identical diet except for Zn content (100 ppm). Half of the animals in each group underwent partial nephrectomy (N); the others were sham operated (S). Animals were housed in plastic metabolic cages, with access to double distilled H_2O. Fecal and urinary Zn were monitored during 3-day collection periods for four to six weeks post N. Urinary Zn excretion was higher in N compared to S groups both for -Zn (0.023 ± 0.004 vs 0.014 ± 0.006 μEq/100g/d, $P<0.01$) and +Zn animals (0.138 ± 0.033 vs 0.088 ± 0.018 μEq/100g/d, $P<0.01$) during the first post-operative week. Thereafter, the -Zn N group maintained a higher urinary Zn excretion rate than S controls while there was no difference in the +Zn animals. Fecal Zn excretion was higher for the -Zn N group than for the S controls for the first week only (0.215 ± 0.054 vs. 0.124 ± 0.020, μEq/100g/d, $P<0.01$). The data suggest that ZND may occur on low Zn diets early in the course of CRI. Both the gut and the kidney may be sources of Zn loss. Support: NKF, Capital Area, and NIH, # 507 RR5359-23.

45.3

TRANSPORT OF PAH IN NECTURUS RENAL BASOLATERAL(BLM) AND BRUSH-BORDER MEMBRANE VESICLES (BLM). H.M. Kwon*, S.K. Hong and J.M. Goldinger. SUNY at Buffalo, New York 14214

In contrast to the mammalian kidney, the Necturus kidney actively transports organic anions across both brush border membranes and basolateral membranes, while it does not transport phenolsulfonphthalein (PSP). To investigate further the mechanism of these phenomena, PAH transport in BLM and BBM derived from Necturus kidney was studied. BLM and BBM vesicles were prepared by Percoll density gradient centrifugation. Marker enzyme activities were enhanced 13 times in BLM (Na-K-ATPase) and 23 times in BBM (alkaline phosphatase) compared to the homogenate. The BBM exhibited an overshoot of Na-dependent glucose uptake while the BLM did not. Probenecid, 5 mM, inhibited vesicle uptake of PAH both in BLM (40%) and BBM (70%). The rate of PAH uptake into BBM vesicles was greater (48%) than that of BLM. Inhibition of PAH uptake by NAP-taurine (1-5 mM) and probenecid (.5-5 mM) was nearly the same. 1 mM octanoate did not affect PAH uptake in BLM but PAH uptake by BBM was significantly inhibited (35%). PSP up to 10 mM did not inhibit PAH uptake by BLM vesicles, while it inhibited PAH uptake by BBM vesicles (40% inhibition at 10 mM PSP). These results indicate that a probenecid and NAP-taurine sensitive PAH transport system appears to be present in both BLM and BBM. However, PSP- and octanoate-inhibitable PAH transport was demonstrated only in the BBM. (Supported by USPHS grant AM-18918)

45.4

LOCALIZATION OF INSULIN RECEPTORS IN THE RENAL PROXIMAL TUBULAR CELL. J.B. Angel*, Y. Kwok*, C.C. Yip*, M. Silverman, Dept. of Medicine and Banting and Best Dept. of Medical Research, University of Toronto, Toronto, Ontario, Canada.

The role of insulin (Ins) in regulating epithelial cell function, in particular that of renal proximal tubules, is largely unknown. Ins binds to and stimulates autophosphorylation of a specific receptor at the antiluminal membrane (ALM) of renal proximal tubule cells (AJP:247:408:1984). The evidence, however, argues against the presence of receptor activity at the brush border membrane (BBM). The present study was undertaken to reassess the interaction of Ins with the BBM. Ins binding to purified BBM was measured using a millipore filtration technique. ^{125}I-Ins could be displaced by excess (10μM) cold Ins; equilibrium binding occurs by 60 minutes at 22°C; binding increases proportionally with increasing amounts of protein; Scatchard analysis of specific binding revealed the presence of a high affinity binding site with a K_d of 1 to 3 nM and an N of 150-300 fmoles/mg. Photoaffinity labelling with ^{125}I labelled azido-benzoyl Ins revealed the presence of a specific Ins receptor with similar subunit structure (90 K and 130 K subunits) to that observed in liver cells and adipocytes. Specific Ins dependent autophosphorylation of the β subunit was observed. This was accomplished using a method in which Ins receptors are bound to immobilized Ins and then incubated with [γ-^{32}P]ATP This eliminates the effect of endogenous phosphatase activity. It is concluded that the Ins receptor is present at the BBM as well as the ALM of the renal proximal tubule. The function of each of these receptors remains to be determined.

45.5

ACTIVE AND INACTIVE MULTIPLE RENIN FORMS IN RAT PLASMA AND ADRENAL GLAND. S.H. Kim*, F.M. Sessler* and R.L. Malvin. Dept. of Physiol., Univ. of Mich., Ann Arbor, MI 48109.

Although active (AR) and inactive renin (IR) have been described in extrarenal tissues, their physiological significance remain unclear. In this study, we characterized AR and IR forms, and their relationship in rat plasma and adrenal gland. Control, low and high-Na, and nephrectomized Sprague-Dawley rats were decapitated. Plasma and adrenal homogenates were subjected to isoelectric focusing gel in triplicate: 1-control; 2-after activation of IR with trypsin (5mg/ml); 3-before activation, followed by activation of each gel slice. Results showed that AR and IR levels were inversely correlated in plasma but not in adrenal gland. The ratio of inactive/total renin concentration was higher in adrenal gland than in plasma. After nephrectomy, adrenal renin concentration did not change. Adrenal AR focused into 8 peaks: 6 of them at pH 5.9, 5.7, 5.4, 5.2, 5.0, 4.8 were similar to those found in plasma; the 2 other were at pH 6.3 and 6.1. With trypsin activation either before or after isoelectric focusing, only the renin activity of these latter 2 peaks was significantly increased, showing that adrenal IR focused at the more basic pH. In plasma, IR focused into 2 peaks at pH 5.9 and 4.8 which were the same as peaks 1 and 6 of AR. Our results suggest that adrenal renin is relatively independent from plasma renin and could be under the control of different mechanisms. (Supported by NIH grant HL 31946)

45.6

ANGIOTENSIN II, ATRIAL NATRIURETIC FACTOR AND SUBSTANCE P REGULATE CATECHOLAMINE AND SALT METABOLISM IN DUCKS. John X. Wilson. University of Saskatchewan, Saskatoon, The University of Western Ontario, London, N6A 5C1 Canada.

Angiotensin II (AII), atrial natriuretic factor (ANF) and substance P (SP) were administered i.v. to conscious, salt-loaded ducks to study the regulation of catecholaminergic, renal and extrarenal excretory responses to osmotic stress. Hypertonic saline (0.5M NaCl) infusion elicited nasal salt excretion which could be inhibited by coadministration of AII. Coinfusion of the converting enzyme antagonists, enalapril and captopril, or of the adrenergic antagonists, prazosin and propranolol, did not alter the salt-retaining effect of AII. AII, ANF and SP each stimulated urine production. SP inhibited nasal salt excretion by decreasing the solute concentration of the nasal fluid, without altering nasal fluid volume or systemic arterial pressure. However, SP reversed the inhibitory effect of AII on nasal salt excretion. In AII-infused ducks, concomittant administration of ANF or SP increased arterial plasma noradrenaline and adrenaline concentrations. The combination of AII and ANF also elevated circulating glucose levels. In conclusion, the data indicate that AII, ANF and SP may regulate catecholamine metabolism and salt excretion in ducks. (Supported by NSERC (Canada) and MRC (Canada) grants).

45.7

ANGIOTENSIN II BINDING TO RAT RENAL MICROVILLI AFTER UNILATERAL NEPHRECTOMY. Mary J. Hinzman* and Jean K. Paddock. Thomas Jefferson Univ., Philadelphia, PA 19107

Binding properties of ^{125}I-Angiotensin II (AII) and AII analogs were studied using microvillar membrane (mvm) isolated from rat kidney 48 hours following unilateral nephrectomy or sham operation. Binding of ^{125}I-AII to compensating mvm was increased compared to sham. Equilibrium was reached after 15 minutes and maintained throughout a 60 minute incubation at 24°C. ^{125}I-AII could be displaced by competition with unlabelled hormone and by AII analogs. Scatchard analyses revealed curvilinear plots with two or more classes of binding sites. The compensating mvm showed an increased affinity at site one, Kd 0.64±0.03x10^{-9}M (±SE) compared to sham, Kd 1.26±0.12x10^{-9}M ($p<0.05$). A decrease in affinity was observed in compensating mvm for site two, Kd 2.6±0.31x10^{-8}M compared to sham, Kd 1.8±0.21x10^{-8}M ($p<0.05$) but an increase in the number of receptor sites in compensating, 1640±327 fmol/mg vs. sham 462±100 fmol/mg ($p<0.01$) mvm within this second class of sites. A low affinity site, Kd 2.3±0.18x10^{-7}M containing 11,254±938 fmol/mg was observed in the compensating but absent in sham mvm. MVM marker enzymes were increased 1.5 fold in specific activity ($p<0.05$); this suggests an enrichment of AII receptors and transport related enzymes in the mvm as a compensating response.

45.8

EFFECTS OF CONVERTING ENZYME INHIBITION ON KIDNEY FUNCTION OF ACUTELY DENERVATED RATS. Ricardo Rademacher* and David W. Ploth. University of AL at B'ham, Birmingham, AL 35294.

Blockade of the renin angiotensin system (RAS) in normal rats with the converting enzyme inhibitor (CEI) SQ20881 produces significant increases in glomerular filtration rate (GFR), renal blood flow (RBF), urine flow rate (V), and fractional (FeNa) and absolute (UNaV) sodium excretion. We examined the effects of blockade of the RAS on renal function during conditions of acute surgical denervation (DNX). Experiments were done in 10 normal rats to assess the responses of each kidney during control periods (CONT); after DNX of the left renal artery; and during systemic infusion of CEI following DNX. Blood pressure decreased from 122 to 108 with DNX and to 100 mmHg with addition of CEI. There were no changes in GFR, RBF, or filtration fraction throughout the experiment. Excretory function was as follows:

	Right Kidney			Left Kidney (Denervated)		
	CONT	DNX	DNX-CEI	CONT	DNX	DNX-CEI
UNaV, nEq/min	89 ±27	59 ±27	231 ±63	51 ±23	176 ±46	516 ±125
FeNa, %	.07 ±.02	.04 ±.02	.17 ±.05	.04 ±.02	.10 ±.03	.42 ±.12
V, µl/min	4.5 ±.6	4.2 ±.4	4.5 ±.5	3.6 ±.5	5.2 ±.4	5.8 ±.5

The results of this study suggest that intact renal nerves allow more complete expression of the effects of blockade of the RAS on renal function.

SKELETAL MUSCLE

46.1

EFFECT OF FATIGUE ON THE CONTRACTION KINETICS OF INTACT, FAST-TWITCH MAMMALIAN MUSCLE FIBERS. B. Calancie and R.B. Stein. Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Small bundles of muscle fibers were dissected from the mouse extensor digitorum longus (EDL) muscle and placed in a bath containing Kreb's. One end of a bundle was attached to a force transducer, and the other end was fastened to a length servo for application of small (<0.3% L_o) random binary-switched length perturbations. The effect of fatigue on the stiffness of the bundle to various frequencies of stretch was determined while alternately tetanizing and resting the bundle for ≈ 8 second periods.

A non-fatigued EDL bundle exhibited a distinct frequency dependence for both the magnitude and phase of muscle stiffness. Nyquist plots of these data indicated that 3 exponential processes contributed to the observed frequency response, with frequencies of approximately 4, 24, and 80 Hz (processes A, B and C respectively). Fatigue led to a decline by 50% or more in the observed frequencies of processes B and C, with no change in the frequency of process A. These results can be incorporated into a model of contraction kinetics to suggest which muscle rate constants are affected by fatigue.

46.2

IS THE CHANGE IN INTRACELLULAR pH DURING FATIGUE LARGE ENOUGH TO BE THE MAIN CAUSE OF FATIGUE? Jean Marc Renaud, Yvon Allard and Graham W. Mainwood. University of Ottawa, Physiology Department, Ottawa, Canada K1H 8M5

The intracellular pH of frog sartorius muscles was measured with pH microelectrodes according to the technique of Amman et al. (1981). The intracellular pH of sartorius muscles exposed to an extracellular pH 8.0 (25 mM HCO_3^-, 1% CO_2) was 6.9 - 7.1. Following a fatiguing stimulation period (one tetanic contraction per sec for 3 min), the intracellular pH was 6.5 - 6.7. When similar experiments are repeated with frog sartorius muscles exposed to pH 6.4 (2 mM HCO_3^-, 1% CO_2), the intracellular pH was 6.8 - 6.9 at rest, and 6.3 - 6.4 following fatigue. So, in both experiments the intracellular pH decreased by 0.4 - 0.5 pH unit during fatigue. When the CO_2 concentration of the bathing solution was increased from 1% to 30%, the intracellular pH of resting muscles decreased from 7.0 to 6.2 - 6.3. Although the effect of CO_2 on the intracellular pH is greater than the fatigue effect, the decrease in tetanic force with CO_2 was less than 40% while during fatigue the tetanic force decreased by at least 70%. Therefore in frog sartorius muscle the decrease in tetanic force during fatigue exceeds the decrease that is expected from just a change in intracellular pH.

Amman et al. 1981. Anal. Chem. 53:2267-2269.

Supported by an MRC grant no. MT-0625.

46.3

THE EFFECTS OF ACIDOSIS ON SKELETAL MUSCLE LACTATE AND NON-CARBON DIOXIDE HYDROGEN ION EFFLUX IN THE STIMULATED RAT HINDLIMB. George J.F. Heigenhauser, Lawrence L. Spriet and Norman L. Jones. Dept. of Medicine, McMaster University, Hamilton, Ont., Canada. L8N 3Z5.

The rate of lactate (La^-) efflux and non-CO_2 hydrogen ion (H^+) efflux from an isolated perfused rat hindlimb preparation was examined during heavy exercise in 3 conditions: control (C), metabolic acidosis (MA) and respiratory acidosis (MA). A one-pass system was used to perfuse the hindlimb for 20 min of rest and for 20 min of tetanic stimulation via the sciatic nerve. During the 20 min stimulation, H^+ efflux exceeded La^- efflux in all conditions. La^- efflux was greater in C (86.9 µM) than RA (60.5 µM) and MA (54.0 µM/min). H^+ efflux was also greatest in C (1492 µM), intermediate in RA (1281 µM) and lowest in MA (875 µM/min). Total La^- produced, calculated as muscle La^- plus La^- efflux, was significantly greater in C (141.0 µM) than in RA (92.3 µM) or MA (91.0 µM). The decreased La^- and H^+ efflux rates during acidotic conditions were associated with a 32% decrease in Cl^- influx compared to C. The rate of Na^+ influx during C was decreased by 30% and 60% as compared with RA and MA respectively. During all conditions, there was no net K^+ flux. We concluded that the rate of efflux of La^- and H^+ is dependent on both extramuscular pH and bicarbonate concentration. The mechanism for decreased H^+ and La^- efflux in acidosis is due in part to a decreased glycolytic rate and reduction in both Cl^- and Na^+ influx.

46.4

INTRACELLULAR OXYGENATION AND NEUROMUSCULAR CONDUCTION ASSOCIATED WITH METABOLIC ALKALEMIA. Richard M. Millis and Columbus K. Anonye*. Animal Physiology Laboratory, Department of Zoology, Howard University, Washington, D.C. 20059.

Previous studies have shown increased affinity of hemoglobin for oxygen during metabolic alkalemia and dependence of intramitochondrial cytochrome oxidase activity upon arterial oxyhemoglobin saturation. The present studies were designed to test the hypothesis that metabolic alkalemia produces tissue hypoxia independent of arterial oxygen desaturation. Neuromuscular conduction latency was used as an indicator of functional impairment, and was measured following electrostimulation of the sciatic nerve (1-5 V, 0.5 msec duration, 1-40 Hz) and recording of the electromyogram from the gastrocnemius muscle of Sprague-Dawley rats (240-260 g) anesthetized with pentobarbital sodium (25 mg/kg, i.p.). To increase the affinity of hemoglobin for oxygen, sodium bicarbonate (1.3 mg/kg, i.v.) was administered in graded doses every 15 min. Statistical significance of changes was guaranteed at the $P<0.01$ level by the paried Student t-test. Arterial bicarbonate ion concentration increased from 25 ± 1.3 mM to 39.0 ± 3.0 mM while arterial pH increased from 7.30 ± 0.02 to 7.50 ± 0.03 ($P<0.01$). Neuromuscular conduction latency increased from 1.9 ± 0.13 msec to 2.7 ± 0.18 msec ($P<0.01$). These changes were accompanied by decreased uptake of 3,3'-diaminobenzidine by muscle mitochondria suggesting hypoxia.

46.5

EXTRACELLULAR Ca^{2+} AS A SOURCE FOR THE REGULATION OF SKELETAL MUSCLE PHOSPHORYLASE KINASE (PK). L.P. Garetto, R.C. Carlsen and D.A. Walsh*. Univ. of California, Davis, CA 95616.

The allosteric regulation of skeletal muscle PK by Ca^{2+} is a well documented phenomenom leading to activation of phosphorylase (Ph). The primary source of this Ca^{2+} is the sarcoplasmic recticulum (SR), however, it is unclear whether other sources of Ca^{2+} contribute to its activation. To investigate if extracellular Ca^{2+} can regulate PK activity, incubated rat flexor digitorum brevis muscles (FDB) were stimulated to contract under various conditions. FDB was chosen for these studies since it exhibits a robust inward Ca^{2+} current which is manifest by an increase in baseline tension during electrical stimulation. Control FDB showed a rapid increase in Ph activity which plateaued following 30 sec of stimulation. However, when FDB was incubated with an agent which blocks sarcolemmal Ca^{2+} channels (Verapamil, 10^{-4}M), the increase in the baseline tension was depressed and Ph activity was biphasic, initially increasing, then, during the second minute of stimulation decreasing sharply. This biphasic nature in the presence of Verapamil suggests that initially the SR contributes nearly all the Ca^{2+} necessary to activate PK, but subsequently, an inward current of Ca^{2+} becomes responsible for maintaining PK activation and thus Ph activity. These studies indicate that the enhanced activation of Ph during electrical stimulation is the result of an allosteric activation of PK by Ca^{2+} contributed not only from the SR, but also via an inward Ca^{2+} current from extracellular sources.

46.6

PREFERENTIAL REINNERVATION IN SOLEUS MUSCLE OF THE MOUSE. George Desypris*, and David J. Parry. University of Ottawa, Ottawa, Canada K1H 8M5.

The right soleus (SOL) nerve of 3 week old C57BL/6J mice was sectioned close to its point of entry in the muscle. One month later, the number of motor neurons (mn's) supplying both reinnervated (R) and contralateral control (C) SOL was determined by HRP retrograde labelling of the SOL motor nucleus. No difference was seen between R and C sides (R:22±1; C:21±1). The muscles were fully innervated, since tetanic tension (Po) elicited via nerve stimulation did not differ significantly from that elicited directly. Myofibrillar ATPase staining revealed a significant increase in the proportion of type I fibers in R SOL (C:41%; R:73%). Using the ventral root splitting technique, no difference was seen between the number of motor units (mu's) of R and C solei 4 months post denervation (R:24±3; C:22±2). Motor unit sizes ranged from 2.2% to 8.6% of Po in C SOL, and 0.5% to 12.3% of Po in R SOL. In R SOL all the smaller Units (Po<1.6%) had times to peak tension (TTP) < 13 msec, while the largest units (Po>8.6%) were always slow contracting (TTP > 15 msec). No correlation between TTP and unit size was seen in C SOL. It thus appears that type II mn's are capable of reinnervating the SOL, but are less adept at collateralising and thus expanding their field of innervation. The alternate possibility, namely that type I mn's regrow at a faster rate, thus leaving less available territory for the type II mn's is not excluded.
Supported by MDAC.

46.7

SHORTENING VELOCITY OF SKELETAL MUSCLES DURING RECOVERY FROM INJURY. K.K. McCully*, D.R. Claflin* and J.A. Faulkner. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109

During recovery from injury induced by lengthening contractions, twitch contraction times (CT) of extensor digitorum longus (EDL) muscles from mice are significantly increased at 3 and 7 days but return to control values at 14 days. Our purpose was to determine if a decrease in shortening velocity is associated with the increase in CT. Injury was induced by lengthening contractions performed in situ on EDL muscles of anesthetized mice. Muscles were maintained at 37°C and stimulated at 150Hz for 500ms every 4s for 10min. During each 500ms contraction, muscles were lengthened 20% of optimal fiber length (L_f) at a velocity of 0.5 L_f/s. Five days after lengthening contractions, CT, maximum isometric force (P_o), and shortening velocity at 11 different afterloads (10-50% of P_o) were measured in vitro at 25°C. The P_o of the injured muscles was 29 ± 5% (mean ± SE, n=4) of the contralateral control value. The CT increased by 48 ± 6% of the control value. Shortening velocity at a given afterload decreased by 40 ± 2 to 48 ± 2% of the control value. Histological analysis indicated that few muscle fibers degenerated as a result of the injury. At 5 days these fibers had not regenerated sufficiently to contribute to force development. We conclude that injury induced by lengthening contractions results in slowing of the contractile characteristics of skeletal muscles. (USPHS NS17017 and HL34164)

46.8

NONLINEAR MECHANICAL BEHAVIOR IN STRIATED MUSCLE AND ITS RELATIONSHIP TO UNDERLYING CROSSBRIDGE ACTIVITY. Gyan C. Agarwal*, Dennis D. Vaccaro*, and Gerald L. Gottlieb*. (SPON: Allen Rovick), Univ. of Illinois at Chicago, Chicago, IL 60680

Small muscles dissected from the frog (Rana Pipien) toe were submersed in a cooled (0°C) Ringers solution and attached at opposite ends to a tension transducer and length regulating servodevice. Muscle stiffness was measured by imposing a length disturbance (bandlimited (0 to 500 Hz) noise or single frequency sinusoid) on an initially isometric muscle preparation and recording this disturbance and the concomitant tension response on a digital computer. Estimates of the muscle stiffness as a function of frequency were obtained using spectral density methods. The nonlinearity was characterized by forming the sinusoidal, RMS, and the random describing functions. A third estimate of muscle stiffness was obtained by analyzing the sinusoidal time records of both length disturbance and concomitant tension response. Passive muscle was characterized as a highly compliant elasticity. The linear behavior of active muscle (electrically stimulated fused tentanus) was modeled as a linear spring in parallel with a linear dampener. The asymmetric nature of the tension response of active muscle to stretch and release was shown to be the cause of the observed nonlinearity. Active muscle appears to 'soften' in response to release and 'harden' in response to stretch. The describing function results could not adequately take this into account.
(Supported by NSF grant IESE-82-12067 and NIN grant NS 12877)

46.9

THE EFFECT OF VERAPAMIL ON HAMSTER DIAPHRAGM TENSION AND RESTING MEMBRANE POTENTIAL. S.A. Esau* and D.F. Rochester. Univ. of Virginia, Charlottesville, VA 22908

Verapamil is a calcium channel blocker. Some patients feel weak while taking verapamil, and Aubier et al. reported a loss of the potentiation of diaphragm contractility produced by theophylline when these two drugs were given together. Theophylline hyperpolarizes hamster diaphragm cell membrane. We postulated that verapamil acts to lower the resting membrane potential (E_m) of the diaphragm. Diaphragm muscle strips were obtained from 5 adult golden hamsters. The strips were fixed at the rib end to a stationery rod and the tendon end was attached to a force transducer. The muscles were suprafused with Krebs solution (KS), Krebs + verapamil 5 μM (KV) and Krebs + verapamil + theophylline 1 mM (KVT) in that order with 15 minutes equilabration in each solution. E_m and maximum tension (Tmax) produced by 0.2 msec pulses at 100 Hz for 0.4 sec were measured. E_m in KS was 76 ± 5mV (mean ± SE); in KV, E_m was 71 ± 3 mV (p=<.05; paired t-test); in KVT, E_m was 74 ± 3 mV (p=NS). Tmax in KS was 100%; in KV, Tmax = 84 ± 4% (p<.05) in KVT Tmax = 89 ± 3% (p=NS). Verapamil caused a decrease in both E_m and Tmax compared to control. When both verapamil and theophylline were present, there were no significant changes in E_m or Tmax. These drugs appear to have opposite effects on Tmax and E_m. The precise role of calcium channel block in mediating these changes is not clear. (Supported by NHLBI K08-HL01183)

46.10

COMPARISON OF MUSCLE FUNCTION AND MUSCLE FIBER CHARACTERISTICS WITH AGING. P. Arabadjis*, D.R. Pendergast, B. Edmonds*, R. Heffner*, and N. Fisher*. SUNYAB, Buffalo, NY 14214. ECMC, Buffalo, NY 14215.

In our previous work, the expected increase in force of contraction with increasing muscle length is severely limited in the rat plantaris(P) and gastrocnemius(G) when old rats (O) are compared to young(Y). However, the mean muscle weights of O(0.26±.02 P;1.09±.01 G) were not different from Y(.22±.02 P;1.08±.09 G). P and G were analyzed histochemically to determine alteration in the number of fibers; the distribution of type I, IIa, or IIb fibers; and muscle fiber size. Fischer 344 rats 6 months and 2 years of age (n=10) were compared. The muscles were quick frozen. A distal 2mm slice was cross-sectioned and differentiated using the "reverse" ATPase. Photographs were taken at 135X and digitized for determination of numbers and size of type I, IIa and IIb fibers. The number of fibers in YP was 1654±228 compared to OP with 1736±217. The % of the fiber types I, IIa, and IIb in YP were 8.0±0.1; 13.3±1.5; and 78.7±1.3 respectively. OP had % of types I, IIa, and IIb of 7.4±2.5; 9.9±0.6; and 82.7 ±1.9. The diameters of the type I, IIa, and IIb fibers in YP (μ) were 36.5±3.3, 39.6±2.9, and 67.7±3.9 and in OP were 31.8±3.7, 34.5±4.2, and 58.1±13.3. P and G from Y and O did not show significant differences in the number nor distribution of muscle fibers. Therefore, the decline in muscle function previously reported cannot be explained by alterations in number, size or fiber type.

46.11

MULTIPLE STIMULI PLUS DANTROLENE AMPLIFIES DIFFERENCES IN CONTRACTILE PROPERTIES OF NORMAL VERSUS MALIGNANT HYPERTHERMIA SUSCEPTIBLE PORCINE SKELETAL MUSCLE. P.A. Iaizzo*, J.G. Quinlan*, G.A. Gronert*, and S.R. Taylor. Mayo Fdn., Rochester, MN 55905.

Twitch characteristics of tibialis anterior muscles in situ were examined in malignant hyperthermia susceptible (MHS) and normal swine. Three groups of animals were studied: (i) purebred Pietrain MHS, (ii) purebred Yorkshire normal, and (iii) a mixed breed (Pietrain/Yorkshire) litter containing both MHS and normal animals. Both purebred and mixed breed animals gave qualitatively similar results. Single stimuli produced greater peak tensions and faster rates of tension development in MHS animals. Multiple stimuli (2-6 pulses at 5 msec intervals) increased peak tensions and rates of tension development, but did not augment differences between normal and MHS animals. Intravenous administration of dantrolene reduced peak tensions and rates of tension development for all stimuli. However, the reduction was significantly less ($P < 0.01$) for MHS animals. Multiple stimuli (4-6 pulses) plus dantrolene amplified differences in contractile properties between normal and MHS skeletal muscles; with MHS muscles obtaining larger peak tensions and faster rates of tension development. Normal and MHS animals can, therefore, be distinguished by these procedures. Latent periods between action potentials and the onset of force, or stimuli and the onset of force were the same ($P > 0.2$). (Supported by Dept. Neurology, Mayo Clinic; NSF INT-8116076; NS 14268; & NIH GM 21729.)

46.12

MAST CELL POPULATION IN THE DIAPHRAGMATIC MUSCLE OF THE GUINEA-PIG. G. Sánchez-Mejorada* y F. Alonso-deFlorida. Depto. Biofísica y Biomatemáticas. Inst. Invest. Biomed. U.N.A.M., Ciudad Universitaria 04510, México, D.F., México.

Recorded intracellular potential changes in allergized denervated guinea-pig diaphragm (GPD), when antigen was locally applied to microareas (about 10μm micropipette tip) suggested to us that the antigen-antibody interaction has a direct action in muscle (J. Gen. Physiol. 51,677;1968). This interpretation is now re-evaluated on the grounds of the mast cell population ascertained in the GPD. The average mast cell density found in muscle strips of denervated GPD, obtained from three different animals and considering together all tissue compartments, except the tendon, was of 206 cells/mm^3. The distribution of all cells counted in the three strips was as follows:

Experimental Conditions	Compartments of tissue				
	Endo.	Peri.	Epi.	Tendon	Total
Innervated	11	49	48	689	807
Denervated	37	260	237	112	646
Total	48	309	295	801	1453

The null hypothesis of independence is refused: $\chi^2=672$; $P<0.001$.

Denervation induced an increased mast cell population in the muscular tissue proper, correlative to a decreased mast cell population in the tendon. The mastocyte population found makes doubtful the direct action hypothesis and favours the mediator hypothesis.

46.13

THE MUSCLE SLICE - A NEW PREPARATION FOR THE CHARACTERIZATION OF β-ADRENERGIC BINDING IN FAST AND SLOW TWITCH SKELETAL MUSCLE. W.M. Watson-Wright* and M. Wilkinson. Dalhousie University, Halifax, N.S. B3H 4H7.

A new procedure is presented which characterizes the specific binding of the β-adrenergic antagonist, [^{3}H]-CGP-12177, to thick (1 mm) slices from fast-twitch (extensor digitorum longus (EDL)) and slow-twitch (soleus) mouse skeletal muscle. Binding is reversible, saturable, stereospecific, of high affinity and subject to agonist-induced desensitization, indicating that it is to β-adrenoreceptors and not to other sites. In both muscles the majority of specific binding is to the β_2 receptor subtype. B_{max} is approximately twice as high in the soleus (5.64±.52 fmol/mg wet wt) as in the EDL (2.66±.29 fmol/mg wet wt)($p<0.05$) whereas affinity is higher in the fast- (K_d = 0.30±.08 nM) than the slow-twitch muscle (K_d=0.45±.08 nM). The minimal tissue disruption associated with this procedure as well as its speed, simplicity and relatively low cost suggest that the slice preparation may prove invaluable for the future study of β-adrenergic receptor binding and associated responses in skeletal muscle.

Supported by the Medical Research Council of Canada

46.14

CHLORPROMAZINE AND QUINACRINE INHIBIT FLAVIN ADENINE DINUCLEOTIDE BIOSYNTHESIS IN SKELETAL MUSCLE. G. Raiczyk, P. Dutta*, and J. Pinto*. Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, N.Y. 10021

Chlorpromazine is a tricyclic antidepressant while quinacrine is a tricyclic compound utilized for the treatment of malaria and helminthiasis. Despite their divergent therapeutic uses, these drugs structurally resemble and form complexes with the tricyclic isoalloxazine ring of riboflavin (vitamin B_2) and its coenzyme, flavin adenine dinucleotide (FAD). Therefore, the present investigation determined whether chlorpromazine and quinacrine inhibit FAD biosynthesis in skeletal muscle. Groups of adult Holtzman rats of both sexes were given daily intraperitoneal injections of either chlorpromazine or quinacrine (20 mg/kg body weight) for three days. Age-matched, pair-fed control animals were given saline. One hour prior to sacrifice, all rats received a single subcutaneous injection of (^{14}C)riboflavin, 25 μCi/kg body weight. Skeletal muscle surrounding the femur was excised, and aliquots of tissue were analyzed for the formation of (^{14}C)FAD using techniques of reverse isotope dilution and anion exchange column chromatography. Compared to results in control animals, rats treated with either chlorpromazine or quinacrine exhibit diminished formation of FAD in skeletal muscle: for chlorpromazine, 750 ± 61 vs 1361 ± 92 dpm/100 mg tissue, mean ± SEM, $p < 0.001$; for quinacrine, 1158 ± 134 vs 1331 ± 176 dpm/100 mg tissue, $p < 0.05$. These data suggest that both chlorpromazine and quinacrine inhibit the formation of FAD which is required for energy production, oxidative phosphorylation, and for the activity of flavin-containing enzymes in general.

46.15

ELECTRICAL STIMULATION OF NORMAL AND DYSTROPHIC CHICKEN PECTORALIS MUSCLE. COMPARISON WITH LATERAL UNSTIMULATED CONTROL SIDE. Michael S.Hudecki*, Stephen P.Povoski*, Catherine M.Pollina*,and Carol C.Gregorio* (SPON: Carmelo A. Privitera).State Univ.of N.Y.at Buffalo, Amherst, N.Y. 14260

Transcutaneous high-frequency electrical stimulation (ES) used in some muscle rehabilitation studies has only recently been applied to atrophying muscle accompanying neuromuscular disease. In an experiment where the contralateral muscle of an animal serves as the control, the left pectoralis major muscle (PMM) of both normal and genetically dystrophic chickens (Lines 412 and 413, Dept.Avian Sci.,U.Calif.,Davis) was treated with transcutaneous ES three times per week beginning 10 days ex ovo. ES was administered (at 2400 Hz) by the Electrostim 180 (Nu-Med Surgical Co., Joliet, Ill.). Each session was composed of 5 cycles of 15 sec "on" followed by 50 sec "off". After the current was adjusted to elicit PMM maximum contraction, wing adduction was checked visually and quantitated by a push-pull strain gauge (Chatillon Co., N.Y.). Compared to the right unstimulated control PMM of both genotypes, ES caused increases in muscle mass, protein, and fiber diameter. In the dystrophic PMM, ES reduced the abnormal levels of total calcium, acetylcholinesterase activities, and the frequency of necrotic and vacuolated fibers. These results support the use of ES in rehabilitating normal as well as diseased muscle. (This work was supported by grants from the Task Force on Drug Development of the MDA, NIH (NS16219), and RCDA (NS00517) to M.S.H.)

47.1

AGE RELATED POTENTIATION OF ANGIOTENSIN-II INDUCED VENTRICULAR HYPERTROPHY BY BETA-BLOCKADE. Eric L. Yancey, Maurice S. Holder, Laval Cothran, and K.Y. Clayton, Florida A&M University, College of Pharmacy, Tallahassee, FL 32307 and College of Medicine, Howard University, Washington, D.C.

A small component (6-10%) of Angiotensin-II (AII) induced ventricular hypertrophy (VH) has been attributed to NE release (Daniels, Holder, 1980). Recently we have also seen some enhancement of AII VH with Metroprolol (M), Propanolol (P), and Oxprenolol (O) in the rat (Yancey, et.al., 1985). Age related differences in receptor sensitivity to AII and NE in smooth muscle have also been reported. The present study was done to determine whether the age-related differences in the extent of AII induced VH could be attributed to NE release in myocardial tissue. Male Sprague-Dawley rats were grouped according to age, young were approximately 6 weeks, old were 12 months and medium age were 3-6 months. Animals were challenged with M + AII, P + AII, and AII alone via mini-osmotic pumps implanted s.c. After 14 days, animals were sacrificed, hearts excised, ventricles separated from atria. Presence and degree of VH was determined by calculating ventricular weight/body weight ratios and comparing them to that of matched untreated controls. Younger animals exhibited the greatest degree of VH when compared to control in each treatment group. Results for youngest groups were: control = 2.53, M + AII = 2.98, P + AII = 2.94, and AII alone = 2.73. Older animals presented a lower degree of VH in each group: control = 2.07, M + AII = 2.42, P + AII = 2.36, and AII alone = 2.33. Medium age animal results were: control = 2.46, M + AII = 2.53, P + AII = 2.65, and AII alone = 2.60. Younger animals produced a greater hypertrophy with respect to control than did older animals in both M + AII and P + AII groups. It has been shown that older rats have a decreased response to NE, possibly due to increased NE uptake at neurons (Kreider, et.al., 1984). The present results suggest some contribution of beta-receptors to the degree of hypertrophy induced by AII in that when the receptors were blocked continously for 14 days the hypertrophic effect was greater than with a lesser time of blockade. The effect of AII and AII + P in the M group was different from that of Daniels and Holder, however, in the present experiments, animals were infused for a longer period of time. Since the largest enhancement occurred in the younger animals, the overall results confirm the role of NE activity in the hypertrophic response but also suggest a possible modulation of the myocardial AII receptors by beta-blockade, especially in younger animals. (Supported by NIH/MBRS RR 08111 and NASA Grant NAG 2-81).

47.2

MYOCARDIAL DOSE-RESPONSES TO GENTAMICIN (G), TOBRAMYCIN (T), AND CEPHALOTHIN (C) STUDIED IN MAN, P.J. Hendry*, G.C. Taichman*, S.J. Taichman*, W.J. Keon*, (SPON: J.S. Cowan) Ottawa Heart Institute, Ottawa, Canada, K1Y 4E9.

The antimicrobial agents G,T, and C are believed to not affect cardiac performance; however, direct verification has yet to be made and may be of significance to the cardiac patient recovering from corrective surgery. Accordingly, the potential inotropic properties of the aminoglycosides G (n=8), and T (n=8), and the cephalosporin C (n=8) were evaluated by measuring their cumulative dose-response relationship using human right atrial trabeculae contracting isometrically in vitro. Trabeculae were immersed in an aerated buffered Tyrode's solution maintained at 34°C and stimulated at 1.0 pulse/sec. Once maximum developed force (DF) had stabilized, each antibiotic was continuously added to a constant volume (80 ml) bath at various rates; G-6.4, T-5.6, and C-170 mg/min. In all cases a sigmoidal cardiotoxic dose-response relationship could be measured with the following dosages (mean ± SE) necessary to produce a 5 and 50% reduction in DF; G 0.7 ± 0.1 & 1.9 ± 0.1, T 0.4 ± 0.3 & 1.7 ± 0.1, and C 13.6 ± 6.6 & 75.3 ± 5.4 mg/ml, respectively. However, the dosages at which these cardiotoxic responses could be elicited were beyond the recommended clinical range for their therapeutic use (G 4-8, T 5-8, and C 20-80 mcg/ml plasma and thus will not adversely affect cardiac performance when used judiciously.
Supported by MRC & OHF.

47.3

THE DEVELOPING MYOCARDIUM: RESPONSE TO A CALCIUM ANTAGONIST. In-Sook Park* and Lloyd H. Michael. Department of Medicine, Baylor College of Medicine, Houston, Texas 77030.

Sarcolemmal calcium channel maturation may be an important factor in regulating calcium movement as the myocardium develops; blockage of these calcium channels results in decreased maximum force in adult muscle. The antagonist, nifedipine (NIF), was chosen to determine if there were differences in its effect on 3 ages of dog myocardium: 1-5 day, 3-5 week and adult. Isometric contractions of isolated ventricular muscles were analyzed in the presence of NIF ($1x10^{-8}$ - $1x10^{-5}$ M), NIF plus added calcium, and NIF plus post-extrastimulus potentiation (PESP). The negative inotropic response to increasing doses of NIF was similar in all age groups at $1x10^{-5}$ M NIF; at this dose of NIF, calcium was added to the bath in increments to 20 mM. Maximum force was restored as follows: 200±26% in 1-5 day; 116±19% in 3-5 wk; and 62±7% in adult (Values are mean ± SEM compared to control state = 100%). PESP increased maximum force to the same extent in all age groups in presence of NIF and 20 mM calcium. In these studies the young muscles respond significantly more to calcium increases than do the adult muscles; NIF suppresses this response although the young muscles retain greater % increase in tension. Maximum absolute tension is always less in young compared to adult. These studies support the hypothesis that calcium entry through the sarcolemma is an important regulator of intracellular calcium in the newborn. Supported by HL 28665.

47.4

RNA POLYMERASE ACTIVITY AND CHROMATIN FUNCTION DURING POSTNATAL RAT HEART GROWTH. Paul B. Taylor and Q. Tang*. Univ. of Windsor, Dept. of Biology, Windsor, Ont. N9B 3P4.

During early postnatal growth (21-50 days of age) the rat heart weight increases about 3.5 fold, while ventricular RNA concentration ($mg.g^{-1}$) significantly decreases during the same age range. To determine if nuclear function was drastically changed, RNA polymerase activity was measured in isolated myocyte and nonmyocyte nuclei. At 21 days of age both nuclear fractions had higher enzyme activity than 50 day old hearts. However, most of the reduction in RNA polymerase activity was associated with the nonmyocyte fraction. The capacity of chromatin from total ventricular nuclei to serve as a template for RNA polymerase was significantly higher at 21 days of age. Fractionation of myocyte and nonmyocyte nuclei clearly showed that nonmyocyte chromatin decreased significantly during ventricular growth while the myocyte fraction remained unchanged. The ability of chromatin to bind RNA polymerase and form rifampin-resistant sites were similar for myocyte and nonmyocyte chromatin at 21 days of age. With increasing growth (50 days) myocyte chromatin could bind about 30% more RNA polymerase. These data suggest that during the early ventricular growth phase when RNA synthesis is reduced, general nuclear function appears to decrease. However, there is a disproportionate shift between the myocyte and nonmyocyte nuclear fractions.

Supported by the Ontario Heart Foundation of Canada.

47.5

ALTERATIONS IN MEMBRANE CURRENTS OF CARDIAC MYOCYTES AFTER PRESSURE OVERLOAD. T.W. Lategan*, N.J. Lodge* and A.L. Bassett. Univ. of Miami, Miami, FL 33101.

Increases in action potential duration paralleled with altered contractile parameters have been reported in fibres of pressure overloaded and hypertrophied mammalian ventricle. Alterations in the slow inward Ca^{++} and outward K^{+} currents have been postulated as possible mechanisms for these effects and evidence supporting each has been offered. Whole cell patch-clamping with 2-6MΩ electrodes containing (mM):KCL 140; $CaCl_2$ 1; $MgCl_2$ 2; EGTA-KOH 11; HEPES-KOH 10; was used to monitor current-voltage relationships in cells isolated from normal and overloaded rat left ventricles (aortic banding for 4 weeks), maintained in standard Tyrode's solution. Cells were clamped at -40 mV and alternating depolarizing and hyperpolarizing pulses were applied. Depolarizing steps to membrane potentials of > -30 mV produced a net outward current in the overloaded cells, but not in the normal cells. This outward current was inhibited by Tetraethylammonium (20 mM). Isoproterenol in the bath solution induced outward current flow in normal cells after steps to membrane potentials > -30 mV, but did not enhance those measured in overloaded cells. These results imply that K currents may change in response to pressure-overload. (NIH 190944, Broward AHA Investigatorship.)

47.6

CORRELATED CHANGES IN α- and β-MYOSIN HEAVY CHAIN (HC) SYNTHESIS AND mRNA PROPORTIONS IN PRESSURE OVERLOADED (PO) RABBIT HEARTS. R. Nagai*, N. Pritzl*, R. Zak, R.B. Low*, N.R. Alpert and R.Z. Litten. Univ. of Vermont, Burlington, VT 05405 and Univ. of Chicago, Chicago, IL 60637.

Pulmonary artery banding (PO) leads to rapid increases in right ventricular (RV) weight (RV:total ventricular weight ratio increase 36% by day 2). HCβ (V_3) and HCα (V_1) synthesis was measured by constant infusion of ^{3}H-leucine (with correction for ^{3}H-leucyl-tRNA specific activity) by measuring ^{3}H-incorporation into myosin HC isolated by SDS PAGE of pyrophosphate gel purified isozymes. RV HCβ synthesis was 0.76±0.13 (SEM) mg/day in normal rabbits, 2.22±0.12 in 2-day PO (P<0.001), and 1.94±0.42 in 4-day PO (P<0.01). In contrast, the synthesis rate of HCα was not significantly increased. Relative levels of α- and β-HC mRNA were determined by nuclease S1 mapping using cloned cDNA probes specific for α- and β-HC. A linear correlation was obtained between the relative mRNA levels and relative synthesis rates of α and β-HC in control (r=0.95) and PO (r=0.99) rabbits. Our results indicate that during the early stages of PO hypertrophy there is a large increase in synthesis rate of HCβ with little change observed for HCα and that this is primarily due to altered levels of myosin isozyme mRNA. (Supported by NIH HL28001)

47.7

STUDIES ON SUBFRAGMENTS OF NORMAL ATRIAL AND THYROTOXIC VENTRICULAR MYOSIN. Surath K. Banerjee*, Sandra Livernois* and George Kaldor. Clinical Laboratory Service, VA Medical Center, Allen Park, MI 48101 and Department of Pathology, Wayne State University, School of Medicine, Detroit, MI 48202.

Expression of atrial myosin, unlike ventricular myosin, is not modulated by thyroid hormone. In order to explore the mechanism for the differential effects of thyroid hormone we chose to study subfragment of normal atrial myosin and compare it to that of V_1-isomyosin which is expressed in thyrotoxic ventricle. Subfragment-1 of atrial and V_1 ventricular myosin were prepared and purified in a DEAE-cellulose column chromatography. Although atrial muscle is known to contain equimolar amounts of A_1 and A_3 isomyosin, only a single component of atrial subfragment was obtained. Pyrophosphate-polyacrylamide gel electrophoretic patterns and column chromatographic profile of this atrial subfragment differ from those of V_1-isomyosin. On the other hand, Ca^{2+} and actin activated ATPase activities of these subfragments are identical. Comparison of peptide mapping after limited proteolysis of the heavy and the light chain, reveals identical patterns for the heavy chain peptides of these subfragments but the light chain patterns differ. These results suggest that the heavy chain of atrial subfragment may have identical structure and function as that of V_1-isomyosin. The differences between these subfragments are due to the differences in the nature of light chains associated with them.

Supported by VA Medical Research Fund and MHA.

47.8

IN VIVO ^{31}P-NMR MEASUREMENT OF METABOLIC PHOSPHATES AND pH IN HUMAN MUSCLE DURING WORK WITH CIRCULATION OCCLUDED. R. L. Coulson, P. A. Molé, and J. R. Caton. Southern Illinois University, Carbondale, IL 62901 and University of California, Davis, CA 95616.

With circulation to the right forearm occluded by a tourniquet, [PCr], [Pi], [ATP], and pH were measured following successive contractions of the flexor digitorum superficialis muscle. Human subjects performed work of 1.5 J on a hand-grip ergometer where contractions were of 1 s duration and repeated every 2 min until voluntary contractions could no longer be elicited. Eighteen contractions were performed in 36 min. Sixteen NMR scans, using an Oxford TMR-32 spectrometer were accumulated immediately after each contraction and again 1 min later. There was no change in any of the quantities measured during the minutes in which no contraction occurred. There was no change in [ATP] at any time or with any contraction. During the first 6 contractions at this work load, [PCr], [Pi], and pH were maintained. Thereafter, [PCr] declined and [Pi] rose by similar amounts with each contraction. pH did not fall suggesting that this amount of work does not tax the buffer capacity of the muscle groups involved.

Support of American Heart Association, California Affiliate, is acknowledged.

47.9

IN VIVO ^{31}P-NMR IN HUMAN MUSCLE: GATING OF METABOLIC PHOSPHATES AND pH OVER THE CONTRACTION-RELAXATION CYCLE OF STEADY STATE WORK. P. A. Molé, R. L. Coulson, and J. R. Caton. University of California, Davis, CA 95616 and Southern Illinois University, Carbondale, IL 62901

Using ^{31}P-NMR in vivo, concentrations of metabolic phosphates and pH were measured at 15 discrete instances over the contraction-relaxation cycle of human flexor digitorum superficialis muscle performing rhythmic steady state work. A handgrip ergometer adjusted to a work load of 1.5 J was used, and contractions were of 1 sec duration and repeated every 2 sec. After exercise had been maintained for 5 mins, ^{31}P-NMR resonance signals representing 16 scans were averaged at each of the 15 time intervals using an Oxford TMR-32 spectrometer and a gating trigger which matched average ^{31}P resonances to specific times during the contraction-relaxation cycle. Preliminary observations indicate there were significant variations in [ATP], [PCr], [Pi], and [H^+], with work within the contraction cycle. Two important patterns of variation were noted; one proportional to work done for PCr and Pi, and the other proportional to the rate of change in work for PCr, Pi, ATP, and H^+. Thus, enzymatic control of energetic reactions of muscle contraction and relaxation appear to contain both load-sensitive and rate-sensitive control elements.

Support of American Heart Association, California Affiliate, is acknowledged.

47.10

ELEVATED NUCLEAR MAGNETIC RESONANCE RELAXATION TIMES IN CARDIOMYOPATHY. L.K. Misra*, T.F. Egan*, D.W. Bearden*, D. Roy*, S.H. Kim*, and C.F. Hazlewood. Departments of Physiology and Pathology, Baylor College of Medicine, Houston, Tx 77030, and Physics Department, Rice University, Houston, Tx.

Cardiomyopathy in the Bio 14.6 strain of Syrian hamsters progresses in four well-characterized phases. Our objective was to test the hypothesis that various clinical and histologic phases of cardiomyopathy cause specific alterations in the proton nuclear magnetic relaxation (NMR) times of cardiac tissue. Cardiomyopathic and control hamsters were sacrificed at 190 days of age and left ventricles were excised. Sections were stained with hematoxylin-eosin and trichrome for light microscopy. The spin lattice relaxation times, T_1, were measured at 30 MHz and 80 MHz. The spin-spin relaxation times, T_2, were measured at 80 MHz.

Multifocal scanning of varying cellularity and collagen accumulation were observed in the left ventricular free wall and septum of cardiomyopathic hamsters. Admixed were also foci of dystrophic calcifications. A significant elevation in T_1 values was seen in the ventricles of the cardiomyopathic hamsters. The T_1 values of cardiomyopathic and control hamsters were 990±29 ms and 944±11 ms, respectively, at 80 MHz, and 667±16 ms and 629±17 ms, respectively, at 30 MHz. The resolution power of T_1 at 30 MHz (5.58) was higher than that at 80 MHz (4.71). In T_2, a significant difference in the long-fraction was found between the control (58±9 ms) and cardiomyopathic (98±16 ms) hamsters. These results show that changes in the NMR parameters are useful in detection of pathologic events of heart. (Supported by RO1-AM35479.)

SMOOTH MUSCLE

48.1

ESTROGENS AND ENDOTHELIUM-DEPENDENT RESPONSES IN THE FEMORAL ARTERY OF THE RABBIT. Veronique Gisclard* and Paul M. Vanhoutte. Dept. Physiol., Mayo Clinic, Rochester, MN 55905

Experiments were designed to determine the chronic effect of estrogens on vascular responsiveness to alpha-adrenergic activation and endothelium-dependent relaxations to acetylcholine. Female rabbits were oopherectomized and given either 17-β-estradiol (100 μg for 4 days) or solvent. One day after the last injection, the animals were sacrificed, and the femoral arteries excised. Rings with and without endothelium were suspended for isometric tension recording in organ chambers filled with physiological salt solution. In the control group, removal of the endothelium shifted the concentration-response curve to norepinephrine to the left. Treatment with 17-β-estradiol caused a shift to the right of the concentration response curve to the catecholamine, and abolished the effect of endothelium-removal. Inhibition of neuronal and extraneuronal uptake and of beta-adrenergic receptors, reduced responses to lower concentrations of norepinephrine in the treated, but not in the control arteries with endothelium. Treatment with 17-β-estradiol potentiated the relaxations evoked by acetylcholine in femoral arteries with endothelium. These experiments suggest that chronic treatment with estrogens enhance endothelium-dependent inhibitory responses to alpha-adrenergic and cholinergic activation in arteries of the rabbit. (Supported in part by NIH grant HL 31183.)

48.2

VASCULAR BETA-ADRENORECEPTOR ACTIVITY IS ALTERED IN HYPERTHYROID AND HYPOTHROID RATS. D. B. STRATTON. Dept of Biology, Drake University, Des Moines, IA 50311

Young male rats were treated daily for two weeks either with 200 μg injections of L-thyroxine (TRX) or 0.1% propylthiouracil (PTU) in their drinking water. Rings of thoracic aorta (2 mm) were mounted in muscle baths and isometrically contracted with 10^{-12} to 10^{-4} M norepinephrine (NE). NE concentrations above 5 x 10^{-8} M generated significantly less ring tension from TRX rats than from euthyroid control rats. Rings from PTU rats generated more tension than rings from controls at the highest concentrations of NE but not significantly so. All rings were subsequently precontracted to 70% maximum tension with NE and then sequentially relaxed with 10^{-10} to 10^{-7} M sodium nitroprusside, a nonspecific vasodilator, or 10^{-8} to 10^{-5} M isoproterenol, a beta-adrenoreceptor agonist. No difference was observed in the relaxation curves to sodium nitroprusside but significantly greater ring relaxation was observed from TRX rats and significantly less relaxation was seen from PTU rats when compared to controls. These results suggest that thyroxine treatment increases vascular beta-adrenoreceptor activity while propylthiouracil treatment decreases beta-adrenoreceptor activity in rat thoracic aorta.

48.3

INFLUENCE OF CHOLESTEROL FEEDING ON ENDOTHELIUM DEPENDANT RELAXATION (EDR) OF RABBIT AORTA TO ACETYLCHOLINE (ACH).
Raveendra L. Jayakody*, Manohara P.J. Senaratne*, Alan B.R. Thomson and C. Tissa Kappagoda. Dept. of Medicine, Univ of Alberta, Edmonton, Alta. T6G 2G3.

The effects of cholesterol feeding on the EDR of the aorta to ACH was examined in age-matched New Zealand rabbits. 8 week (wk) old males were randomized into control (C) and experimental groups (E). C was fed a standard laboratory diet and E were fed the same diet supplemented with 2% cholesterol. The concentration of cholesterol in blood in E after 4 and 8 wks were 2016±152 and 1360±210 mg% respectively. Six animals from each group were sacrificed after 4 and 8 wk and aortic rings from these animals were examined isometrically in tissue baths. The rings were precontracted with noradrenaline (-6.0 log mol/l) and ACH (-9.0 to -5.5. log mol/l) added to demonstrate EDR. Effects of removal of endothelium, incubation with atropine (-8.0 log mol/l) and indomethacin (-6.0 log mol/l) on EDR were assessed also. After 4 wk, the EDR to ACH (% of the contraction to noradrenaline) in C and E were 45.4±3.6% and 23.2±5.8% (n=6, $p<0.05$) respectively. After 8 wk, the corresponding data were 44.5±5.1% and 20.1±8.7% (n=6, $p<0.05$). Indomethacin had no effect on the relaxation to ACH (n=6, $p>0.05$). Removal of endothelium and atropine abolished EDR (n=6, $p<0.05$). There was no difference in relaxation to sodium nitrite in rings with and without endothelium in both groups (n=4, $p>0.05$). It is concluded that cholesterol feeding impairs EDR of rabbit aorta to ACH.

48.4

ENDOTHELIUM-DERIVED RELAXING FACTOR(S) HYPERPOLARIZES CORONARY VASCULAR SMOOTH MUSCLE.
Michel Feletou* and Paul M. Vanhoutte. Department of Physiology, Mayo Clinic, Rochester, MN 55905

To determine the electrophysiological effect of endothelium-derived relaxing factor(s) on vascular smooth muscle, the membrane potential was recorded in small coronary arteries (without endothelium; external diameter $\leq$ 250 µm) of the dog by means of intracellular microelectrodes. The organ bath also contained a strip of left descending coronary artery without endothelium, in which isometric force was measured, as well as a large fragment of femoral artery with endothelium, which served as the source of endothelium-derived relaxing factor(s); control experiments were performed in the presence of a femoral artery without endothelium. Under control conditions, the membrane potential averaged 55 mV. Acetylcholine (10^{-5}M) induced endothelium-dependent, transient hyperpolarizations (average: 11 mV) and relaxations which were not affected by indomethacin (10^{-5}M); ouabain (5×10^{-6}M) or potassium-free solution partially inhibited the relaxation but prevented the hyperpolarization. Return to control solution (6 mM K^+) or solution containing 12 mM K^+ after incubation in K^+-free solution caused hyperpolarization (average: 17 mM). These results suggest that in the canine coronary artery endothelium-derived relaxing factor(s) induces hyperpolarization of vascular smooth muscle by activating the Na^+,K^+-pump, but that this effect on the cell membrane only partially explains the endothelium-dependent relaxation to acetylcholine.
(Supported in part by NIH grant HL 31183.)

48.5

MUSCARINIC RECEPTOR SUBTYPES MEDIATING THE RELEASE OF ENDOTHELIUM-DERIVED RELAXING FACTOR(S) IN CANINE FEMORAL ARTERIES.
Gabor M. Rubanyi and Paul M. Vanhoutte. Dept. Physiol. and Biophys., Mayo Clinic, Rochester, MN 55905

To determine the properties of the receptors mediating endothelium-dependent responses to acetylcholine (ACh), canine femoral arteries with endothelium were perfused (2 ml/min) with physiological salt solution; the perfusate was bioassayed for endothelium-derived relaxing factor(s) by means of a coronary artery without endothelium. When infused upstream, but not downstream, of the femoral artery, small concentrations of ACh (10^{-8}-5×10^{-8}M) caused transient relaxations of the bioassay ring; higher concentrations caused sustained decreases in tension. Compound McN-A-343-11 induced only transient responses, while carbachol caused sustained relaxations. Pirenzepine inhibited the transient response with high (pA_2 = 8.2), and the sustained relaxation with low (pA_2 = 6.4) affinity. Atropine affected both in a similar fashion (pA_2 = 9.8 and 9.4, respectively). In rings of femoral arteries, suspended in organ chambers for isometric tension recording, ACh induced only sustained relaxations; pirenzepine antagonized those with low affinity (pA_2 = 6.5). These experiments indicate that in perfused blood vessels, ACh stimulates the release of endothelium-derived relaxing factor(s) by acting on subtypes of muscarinic receptors with high and low affinity; in the absence of flow, only the subtype with low affinity is involved in the responses.
(Supported in part by NIH grants HL 31183 and 31547.)

48.6

OXYGEN FREE RADICALS ATTENUATE VASODILATOR EFFECTS OF ACETYLCHOLINE (Ach).
Fred S. Lamb*, Karyn Herrel*, William Burkel* and R. Clinton Webb. Univ. of Michigan, Ann Arbor, MI 48109.

The vascular endothelium plays an important role in mediating vasodilator effects of several agents (Ach, thrombin). Thus, damage to the endothelium may alter vascular function by an indirect action. The goal of this study was to determine the action of oxygen radicals to alter endothelial function in an isolated tissue system. Helical strips of rat tail artery were suspended in muscle baths for measurement of isometric force generation. Following contraction induced by 5.9×10^{-7} M norepinephrine, the strips (n=4) relaxed in response to 10^{-7} M Ach (-29±9% change from contraction). The strips were then exposed to free radical metabolites generated by electrical field stimulation (9V, 1.0 msec, 4 Hz, three 5 min periods). Subsequent to this electrical stimulation, the relaxation response to Ach was abolished (-5±2% change). Treatment with 10^{-4} M ascorbate during the electrical stimulation periods protected the endothelium from the damaging effect of the free radicals (relaxation to Ach= -21±3%). Morphological examination (scanning electron microscopy) of the intimal surface indicated that the electrical stimulation procedure did not cause removal of the endothelium from the vascular segments. These results indicate that oxygen metabolites can inhibit the dilator activity of the vascular endothelium.
(Supported by NIH grants HL-00813, HL-27020, and HL-18575).

48.7

MYO-INOSITOL, LITHIUM AND SEROTONIN (5HT)-INDUCED CONTRACTILE RESPONSES IN RAT TAIL ARTERY.
Mila Turla* and R. Clinton Webb. Univ. of Michigan, Ann Arbor, MI 48109

Studies with non-vascular tissues suggest that activation of 5HT receptors results in the breakdown of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate, a product that mobilizes intracellular Ca^{2+}. Li^+ blocks the recycling of inositol phosphates into membrane phospholipids and was used to test the hypothesis that vascular smooth muscle contractions to 5HT are mediated via a phosphoinositide (PI) pathway. Helical strips of rat tail arteries were mounted in muscle baths for measurement of isometric force generation. The strips were contracted 4 times with 1 uM 5HT for 7 min periods with washout intervals of 5 min between each contraction. The following table summarizes the observations at 5 min into the fourth contraction as percentages of the initial response to 5HT:

Untreated (n=6)	85 ± 2%	
10mM Li^+ (n=7)	43 ± 3%	$P < 0.05$
10mM Li^+ + 10mM myo-inositol (n=10)	56 ± 5%	
10mM Li^+ + 10mM epi-inositol (n=2)	41 ± 9%	

Li^+ attenuated contractions to 5HT. Myo-inositol partially reversed this effect. Epi-inositol, a sugar that cannot substitute for myo-inositol in PI metabolism, did not reverse the inhibitory effect of Li^+. These results indicate that the PI pathway may be involved in 5HT-induced contractile responses in the rat tail artery.

48.8

RELAXATION OF K^+ CONTRACTED RABBIT AORTIC STRIPS IMPLIES CALCIUM CHANNEL BLOCKADE.
Russell J. Brittain* and Suzanne Moreland. The Squibb Institute for Medical Research, Department of Pharmacology, Princeton, NJ 08540

A fairly specific, large throughput, rapid screening method is necessary for drug development. This study was designed to determine the pharmacological specificity of a screen for calcium channel blockers. Circumferential strips of rabbit thoracic aortae were suspended between stationary supports and force transducers. The strips were bathed in physiological salt solution at 37°C and stretched to 4 g resting force during a 1-2 hr equilibration period. Contractions were elicited by depolarization with 100 mM KCl (equimolar substitution for NaCl). When force reached a steady state value, the solution was changed to one containing the experimental drug (1 µM) to be studied and the strips were allowed to relax to a new steady state force. It was found that, at this concentration, only calcium channel blockers, adenyl cyclase activators, and guanyl cyclase activators were capable of appreciably relaxing the K^+ depolarized aortic strips. Other classes of vasodilators and antihypertensive agents did not markedly affect force at 1 µM concentration. Therefore, this screening method is recommended for discovery of potential calcium channel blockers, however, compounds identified with this method should also be tested for their ability to activate adenyl cyclase and guanyl cyclase.

48.9

MECHANISM OF CALCIUM (Ca^{++})-INDUCED RELAXATION OF VASCULAR SMOOTH MUSCLE (VSM). E.E.Soltis and D.F. Bohr, University of Michigan, Ann Arbor, MI 48109.

This study investigated the mechanism(s) by which high concentrations of Ca^{++} result in the relaxation of VSM. Strips of rat femoral artery were contracted with norepinephrine(10^{-6} M) in 1.6 mM Ca^{++} physiological salt solution(PSS). Following plateau of the contraction a dose-response curve to Ca^{++} (4.1 to 21.6 mM) was obtained. A dose-dependent relaxation was observed in response to Ca^{++}. In the presence of ouabain(10^{-4} M) an increase in force generation was seen at 4.1 mM Ca^{++}. The relaxation at higher doses of Ca^{++} was significantly decreased when compared to the control response. Ca^{++}-induced relaxation was similarly attenuated in a potassium(K^+)-free PSS. A significant increase in the response to low doses of Ca^{++} was seen in strips treated with the sodium ionophore monensin(10^{-6} M). Maximal relaxation was not altered. Increasing the level of extracellular K^+ to 10.9 mM resulted in an enhanced relaxation response to Ca^{++}. Compounds which block K^+ efflux [tetraethylammonium(3 mM), barium(0.1 mM), and sparteine(1 mM)] significantly attenuated the response to low doses of Ca^{++} but had no effect on maximal relaxation. The results of this study suggest that there are at least 2 components to the relaxation induced by high levels of Ca^{++}: 1.) a hyperpolarizing effect due to a Ca^{++}-activated K^+ efflux seen at low doses of Ca^{++}, and 2.) a hyperpolarization attributed to the electrogenic sodium pump which occurs at all doses of Ca^{++}.

Supported by NIH grants HL-18575 and HL-27020

48.10

REDUCED TEMPERATURE AND CALCIUM UPTAKE IN VASCULAR SMOOTH MUSCLE CELLS. D.L. Davis and C.H. Baker. Univ. of South Florida Tampa, Fl. 33612

The influence of reduced temperature (1-2°C) on Ca uptake in vascular smooth muscle was studied in canine mesenteric arteries. Artery rings were excised under pentobarbital, placed in room-temperature PSS for removal of adventitial and sectioning into 1 mm rings. Rings were run in triplicate on stainless steel holders designed to maintain circumference near in situ values. Ca uptake rates were obtained from 5 sec ^{45}Ca uptake periods in a HEPES-buffered PSS. Uptake periods were terminated by transferring rings to a 0-Ca-PSS containing 5 mM EGTA at 1°C. Resting Ca uptake rates at 37°C averaged 0.2 mM/sec/g wet tissue. When rings were transferred to PSS at 1-2°C, Ca uptake rates decreased to 0.06, 0.04 and 0.02 nM/sec/g tissue during the first, second and third successive 5 sec uptake period, respectively. Ca uptake levels of 0.02 nM/sec/g tissue were obtained for the remainder of the low temperature exposure period. Ca uptake rates determined at 37°C, after exposure to 1-2°C for varying periods of time, showed rapid recovery to control rates. Uptake rates obtained during the first, second and third 5 sec uptake period averaged 0.22, 0.21 and 0.23 mM/sec/g tissue. These experiments indicate that during exposure to reduced temperature of 1-2°C Ca uptake is rapidly decreased to levels of approximately 10% of control values. When tissues are returned to 37°C control uptake rates are rapidly regained.

Supptd. in part by Sun Coast Chap. of the Fl. Affil. of the A. Heart Asso.

48.11

SIMULTANEOUS MEASUREMENT OF INTRACELLULAR K IONIC ACTIVITY AND MEMBRANE POTENTIAL DURING HIGH $[K]_o$ IN COLON SMOOTH MUSCLE. N.L. Shearin. Dept. of Surgery, Univ. Utah School of Medicine Salt Lake City, Utah 84112

Measurements of intracellular K ionic activity and membrane potential (E_m) were made using double-barrel microelectrodes. Whole muscle strips (5x10 mm) from proximal cat colon (mucosa removed) were mounted in a superfusion chamber. The smooth muscle strip was dissected from the longitudinal axis of the colon and circular muscle was mounted in the up position. Motion in the longitudinal muscle was recorded. Superfusion was done with modified Krebs solution (control, 5.9 mM K) and with a series of Krebs solutions with $[K]_o$ = 8-20 mM (Na ion substituted for K). Records were obtained for E_m, slow wave activity and intracellular K ionic activity. The following table of results were obtained:

	Control (5.9 mM $[K]_o$)	8-20 mM $[K]_o$
E_m	-60 + 15 mV	-35 + 12 mV
Slow wave activity (freq.)	3 + 1/min	7 + 4/min
E_k	-70 + 8 mV	-74 + 11 mV

Significant differences were obtained ($p<0.001$) between control $[K]_o$ and high $[K]_o$ for both E_m and slow wave frequency. E_k was not significantly different. There was no significant correlation between slow wave frequency and contractile activity in longitudinal muscle. The intracellular K ionic activity did not change significantly when $[K]_o$ was increased.

48.12

MEMBRANE POTENTIAL (E_m) AND OUABAIN (OU) BINDING IN BOVINE TRACHEALIS MUSCLE WITH AGE. Magdalena Souhrada, Karen G. Rothberg* and James S. Douglas, John B. Pierce Foundation Laboratory, New Haven, Ct. 06519.

Fresh trachealis muscle (T) was obtained from immature (I, approx 2 weeks old), developing (D, approx 5 months old) and mature (M, >5 years old) cows. E_m of airway smooth muscle was determined using a standard glass microelectrode technique.

E_m	IT	DT	MT
Baseline	-63.5±0.4	-62.2±0.8	-60.3±0.7
Ouabain (10^{-5}M)	-39.2±0.8	-43.2±0.6	-47.5±0.6
K^+ Free	-39.5±0.8	-43.2±0.6	-47.5±0.6

E_ms for IT, DT and MT were significantly different for each condition. Varying muscle tension did not eliminate the differences.

Saturation binding experiments with radiolabelled OU (2nM - 1uM) to 29,000g membrane fractions from IT, DT and MT were determined in Tris/ PO_4^{2-}/Mg^{2+} buffer (50mM/5mM/5mM). Non-specific binding was determined by incubating membranes in Tris buffer alone. OU bound with identical affinity in IT, DT and MT (Kd=35nM). Site density (fmoles/mg protein) decreased with age; IT, 4500: DT, 2800: MT, 1300.

Our data suggest that maturation alters the number of active Na^+/K^+ ATPase pump sites and the contribution of the Na^+-pump to E_m. These age-related changes in the membrane properties of airway smooth muscle cells may be related to changes in airway reactivity.

Supported, in part, by HL 28274 from N.H.L.B.I.

48.13

IN-VITRO EFFECTS OF ACETYLCHOLINE, OXYTOCIN, NOREPINEPHRINE, AND PROPRANOLOL ON UTERINE AND PROSTATIC TISSUES FROM Monodelphis domestica (MARSUPIAL MOUSE). WILLIAM P. VENTURA PACE UNIVERSITY. BIOLOGY DEPARTMENT. PLEASANTVILLE, N.Y. 10570

Uterine and prostatic tissues from M. domestica were used to record spontaneous motility and response to acetylcholine, oxytocin, norepinephrine, and propranolol. Each 4-5 hour experiment was divided into successive 20-minute control and treatment periods. Each tissue was mounted in a 25-ml organ bath at 39°C; bubbled with a 95% O_2- 5% CO_2 gas mixture; with constant perfusion of a balanced salt solution; and motility recorded on a Mark IV Physiograph. Each agent was tested in several dose ranges but only the range showing significance (>.005) are presented. All doses in micrograms/ml bath.

DRUG	DOSE RANGE	TISSUE	FORCE	FREQUENCY
NOREPINEPHRINE	(.00001-3.2)	uterus	down	up
	(.1 -3.2)	prostate	up	down
ACETYLCHOLINE	(.00001-1.0)	uterus	up	down
	(1.0 -32.)	prostate	up	down
OXYTOCIN	(.00001-1.0)	uterus	up	down
	(1.0 -32.)	prostate	up	down
PROPRANOLOL	(0.25 -2.0)	uterus	down	up
	(0.25 -2.0)	prostate	down	up
NOREPINEPHRINE(1) after PROPRANOLOL (2-4)		uterus	up	up

48.14

PRESSOR AND PARADOXICAL UTERINE EFFECTS OF OXYPRESSIN IN THE ANESTHETIZED RAT. Diana Gazis, Genevieve Gonzalez, and Milton Mendlowitz. Mt Sinai School of Medicine, CUNY, NY, NY 10029

Pressor and uterine responses to oxypressin, an equipotent analog of oxytocin and vasopressin, were studied simultaneously in vivo in urethane-anesthetized, indomethacin-pentolinium treated rats. These responses were compared to log plasma oxypressin concentration during a series of injections and infusions, for the correspondence of response to log plasma concentration is an indicator of how tightly response is linked to receptor occupation. During both injections and infusions, the blood pressure response seemed to be determined largely by plasma levels of oxypressin. The uterine response to oxypressin, however, behaved in a paradoxical way. The heights of individual uterine contractions seemed to be determined, throughout, by plasma oxypressin concentrations. The interval between contractions, however, seemed to be determined by plasma peptide concentrations during injections, but as peptide concentrations increased during infusions, contractions grew farther and farther apart, as they would if peptide concentrations fell. We conclude that blood pressure increase and uterine contraction height are relatively tightly linked to oxypressin receptor binding under all conditions. Uterine contraction interval is linked to binding during injections, but during infusions, it is determined by binding plus some additional factor. (Supported in part by NIAMDD Grant 10080.

48.15

THE EFFECT OF TUMOR DEVELOPMENT ON SMOOTH MUSCLE FUNCTION. Eugene A. Lentini, James G. Bassett* and Jalal Mobini*. The Medical College of Pennsylvania, Philadelphia, PA 19129.

A comparison of the dynamics of thoracic aortic preparations from normal and noncachectic tumor bearing rats (TH) was made. The experimental neoplasm was a mammary tumor induced by dimethylbenz (a)-anthracene. Experiments were performed in pairs so that aortic ring preparations from control and TH animals were run under identical conditions. Dose-response experiments were performed at 115, 130, and 160% of equilibrium length (EL) using K+ as the agonist. The isometric developed tension (F/cm^2) of TH rings responded in the expected manner in respect to stretch and increasing concentrations of K^+, however, the tension was less than that of rings from normal animals for each EL ($P<.05$). Histologic studies did not indicate any significant difference in wall thickness or elastin distribution. Tumors were epithelial and contained inflammatory cellular infiltrates. Passive length tension (stress-strain) studies did not reveal a significant difference from 100-150% EL. Rates of contraction differed only at the 10% level. The data suggest that a neoplasm is capable of affecting a blood vessel which is remote from the tumor. (Supported by R.J. Reynolds)

48.16

Mg^{++} REGULATION IN SMOOTH MUSCLE. P.F. Dillon. Depts. of Physiology and Radiology, Michigan St. Univ., E. Lansing, MI 48824

^{31}P-NMR spectra were obtained from the isolated, perfused rabbit bladder and porcine carotid arteries. We have previously reported the free Mg^{++} level to be less than 0.5 mM in the rabbit bladder and uterus. This concentration is below that required for full in vitro activation of the actin-activated, Mg-ATPase. Since the bladder has a 5X greater shortening velocity than the carotid media, Mg^{++} regulation of ATPase activity would require a different free Mg^{++} level in the carotid. Inferring the free Mg^{++} concentration of the carotid from the shift of the β-ATP peak, the average concentration is 0.4 mM. The similarity in Mg^{++} concentration indicates that Mg^{++} does not directly regulate the ATPase in smooth muscle. A constant free Mg^{++} in different smooth muscle implies that the free Mg^{++} concentration is highly regulated. Reduction in ATP levels liberates Mg^{++}, since the Mg^{++} association constants of the products of ATP hydrolysis are much smaller than those of ATP. During ischemia, the reduction in ATP-bound Mg^{++} is 0.63 μ/moles/g wet weight, but free Mg^{++} increases by only 0.12 μ/moles/g wet weight. The remainder is bound or extruded from the cells. When the total tissue Mg^{++} and number of binding sites are assumed to be constant during ischemia, the total number of sites is calculated to be 8.7 moles/g with a binding constant of 3.4 (pK_{Mg}). Partial maintenance of free Mg^{++} at submillimolar concentrations occurs in smooth muscle even under condition of severe metabolic impairment. Supported by The Whitaker Foundation and the Michigan Heart Association.

48.17

PHYSARUM MYOSIN BINDS CALCIUM: EQUILIBRIUM DIALYSIS RESULTS. Dietrich Kessler* and Beverly K. Dolberg* (Spon. N. Neas). Colgate University, Hamilton, New York 13346.

The plasmodium of the slime mold, Physarum polycephalum, exhibits rapid actomyosin-based protoplasmic streaming for which calcium is required. Physarum myosin is composed of a heavy polypeptide chain and two classes of light chains, LC-1 and LC-2. After electrophoresis on SDS polyacrylamide gels Physarum myosin LC-2 migrates faster when Ca^{2+} is present in the sample, changing from an apparent molecular weight of 16,900 in the presence of EGTA to 16,100 in Ca^{2+}. By the technique of equilibrium dialysis, calcium has been found to bind to Physarum actomyosin both in the presence and absence of 1mM magnesium. In the absence of magnesium, Scatchard plot analysis of the binding data indicates there are binding sites with two different affinities: $K_1 = 1.2 \times 10^7$ M^{-1} and $K_2 = 1.04 \times 10^6$ M^{-1}. Preliminary data indicates that the number of calcium binding sites/myosin molecule is 0.8 and 3.6 respectively. By autoradiography of nitrocellulose transfers of gels after SDS electrophoresis, calcium binding sites have been located only on Physarum myosin LC-2. In the presence of 1 mM magnesium one of the binding sites is eliminated, leaving only one set of sites detected by equilibrium dialysis. Here the binding constant is 4.8 x E6 (M-1) and 0.36 moles calcium/moles myosin. Until now calcium binding to myosin in physiological ionic conditions has been found only in mollusk myosin and in some other invertebrates, where it functions to regulate actomyosin contraction.

NEUROBIOLOGY: NEUROTRANSMITTERS, MOTOR CONTROL, BEHAVIOR

49.1

EFFECTS OF 5-HT ANTAGONIST ON THERMOREGULATION IN MATURING MICE. George Dobrea* and Cecilie Goodrich. Cleveland State Univ., Cleveland, OH 44115

Pirenperone (Janssen) is a selective antagonist of 5-HT_2 receptors in the CNS. Effects were studied 1 hr after an IP injection of drug or vehicle on littermate mice aged 1, 3, 5, 7 and 10 days postpartum. Body temperature (T-b) and temperature preference (T-pref) on a thermal gradient (18-37° C) were assessed at ~23° C ambient air temperature. A dose of 0.16 mg/kg was associated with significant decreases in T-pref at all ages, with the largest drug effect found in 5-day-old mice. No significant effects were found on T-b at any of these ages. When an increased dose (0.48 mg/kg) was used on 7-day animals, there was no greater effect on T-pref, and no significant effect on T-b. Alteration of the biochemical pathway for 5-HT through the use of elevated levels of precursors, or p-chlorophenylalanine inhibition of 5-HT production has previously produced opposite effects on T-b and T-pref at all maturational ages studied. However the 5-HT selective reuptake inhibitor citalopram has effects on T-b and T-pref which are separable on the basis of maturational age and drug exposure time. Others report high densities of 5-HT_2 receptors in frontal cortex and limbic structures, with smaller numbers in hypothalamus. Thus the current pirenperone results provide further support for a separation between T-b and T-pref responses in which T-pref is influenced by an antagonist of 5-HT_2 receptors.

49.2

THE EFFECT OF VIT. B_6 ON FOOD SELECTION BEHAVIOR OF RATS MAINTAINED ON DIETS VARYING IN PROTEIN AND CARBOHYDRATE CONTENT. R. R. Roth. Univ. of Western Ontario, London, Ont., Canada. N6A 5B7

Six groups of one-month old male Sprague-Dawley derived rats were maintained on nutritionally complete diets varying in their protein, carbohydrate and Pyridoxine (Px) content: gr.I- 82% prot., 0.04% Px.;gr.II- 82% prot.; gr.III- 15% prot., 67% carb., 0.04% Px.; gr,IV- 15% prot., 67% carb.; gr,V- 82% carb., 0.04% Px.; gr.VI- 82% carb. Groups V and VI were supplemented once weekly for 24 hours with the high protein diet of gr.II. After two months each rat was tested individually, for three days, for limited choice self-selection of the high protein diet of gr.II against the high carbohydrate diet of gr. VI. Results: With Pyridoxine supplementation, total food intake during the test period was inversely proportional to the protein intake levels during the maintenance period. Pyridoxine deficiency virtually reversed this relationship, i.e. total food intake was significantly reduced in the groups maintained on a balanced or protein deficient diet in comparison with the group fed a high protein diet. Pyridoxine deficiency also caused a marked protein avoidance behavior. Protein deficiency counteracted the anorexia, and had an attenuating effect on the protein avoidance behavior, induced by B_6 avitaminosis. Conclusion: Feeding behavior in rats appears to be determined by metabolic needs and nutrient interactions rather than olfactory-gustative stimuli.

49.3

POST-TRAIN FACILITATION KINETICS IN THE IN VIVO SUPERIOR CERVICAL GANGLION OF THE CAT. M.A. Morales*, S. Soza-Bulnes* y F. Alonso-deFlorida. Depto. Biofísica y Biomatemáticas. Inst. Invest. Bioméd. U.N.A.M. 04510, D.F., México.

Brown and McAfee (Science, 215: 1411, 1982) have found that the rabbit superior cervical ganglion in vitro shows both post tetanic potentiation (PTP) and long-term potentiation (LTP) with a mean decay time constant of 3 and 80 min respectively. We have studied the posttrain (supramaximal, 24 Hz 30 sec, train) effect in the cat superior cervical ganglion in vivo. We have found the classical PTP with a decay time constant of 35 sec, but we have not found the LTP. In ganglia subjected to constant partial (about 70%) hexamethonium blockade the development of facilitation (i.e. "recovery" from blockade) was much slower than in the unblockaded ganglia, reaching a peak (about 45% blockade) at very variables times, from 60 sec to more than 90 min. When a peak was reached, within the experimental time, the decay showed a time constant of about 45 min. The "recovery" induced neither reached the value found in the unblockaded ganglia, nor it returned to the control blocked value. We suggest that posttrain facilitation phenomena in general, including "decurarization" (i.e. recovery induced by a train), reflect a common synaptic mechanisms. It seems that in the ganglion in vivo, the LTP mechanism is normally inhibited and that it is disinhibited by partial hexamethonium action, presumably produced at the presynaptic endings. The LTP in the in vitro ganglion may have been due to defective oxigen supply of the deeper ganglion synapses.

49.4

INDEPENDENCE OF GANGLIONIC POSTTETANIC POTENTIATION DEVELOPMENT FROM CENTRAL NEURONS INFLUENCE. S. Soza-Bulnes*, M. A. Morales* y F. Alonso-deFlorida. Depto. Biofísica y Biomatemáticas. Inst. Invest. Bioméd. U.N.A.M. 04510, D.F. México.

One may conjecture whether the soma and axoplasmic transport could influence the posttetanic potentiation (PTP) effect in sympathetic ganglia. In order to approach this question the effect of acute severance (Decentralization, Factor A) upon PTP was ascertained in the in vivo superior cervical ganglion of the cat. In parallel experiments PTP was enhanced by partial hexametonium blockade, 5 Mg/Kg, (Factor B) (Christ., Br. J. Pharmac. 69, 249; 1980). The amplitude of the S_2 wave served to estimate the ganglion discharge volley induced by supramaximal, 0.2 msec. duration testing pulses administered at 0.1 Hz. A train applied for 30 sec at 24 Hz caused PTP. Potentiation, R_i/R_o, was measured as a function of time (R_i, amplitude of S_2 at the i-th time; R_o, control amplitude of S_2). The area under the plotted curve was measured for each individual experiment. The data did not show any significant difference between PTP in decentralized and non-decentralized ganglia, in spite of whether partial blockade has been induced or not. A two factor analysis of variance yielded:

$F_A = 3.74$	$F_{0.05}(1,16)=4.49$	not significant
$F_B = 58.55$	$F_{0.05}(1,16)=4.49$	significant
$F_{AB}= 0.18$	$F_{0.05}(1,16)=4.49$	not significant

It seems that PTP can be almost wholly sustained by the presynaptic endings, quite indepedently of the action of the body cells located centrally at the spinal cord.

49.5

LOCALIZATION OF OPIATE BINDING IN THE RAT FOREBRAIN: AUTORADIOGRAPHIC EXAMINATION OF SPECIFICITY WITH A PHOTOAFFINITY-LABELLED ANALOGUE. C.B. Lacey*, W.G. Tatton, C.W.T. Yeung*, J.N. Nobrega*. Playfair Neuroscience Unit, University of Toronto & Toronto Western Hospital, Toronto, Ontario M5T 2S8.

The recognition of opioid peptides as putative transmitters has prompted investigations of the regional and subcellular localizations of these substances and their receptors. The forebrain localizations of ^{125}I-DAGO(Tyr-D-Ala-Gly-NMe-Phe-Gly-ol) and ^{125}I-DADLE[D-Ala^2,D-Leu^5]-Enkephalin (considered to preferentially label mu and delta sites respectively) were examined using in vitro incubation of serial 20 micron coronal slices. In an attempt to determine regional differences in the competitive binding of the agents as compared to met-enkephalin, a photoaffinity labelled analogue (10^{-6}-10^{-9}M) was incubated with the two radioligands, with and without UV light activation. UV irradiation of the PAL-Enk induced significantly increased inhibition of ^{125}I-DADLE binding in the striatum, endopiriform nucleus, olfactory tubercle and deep layers of the neocortex. In contrast, ^{125}I-DAGO binding inhibition was not evident in regions with high levels of binding for this ligand (i.e. striasomal patches, discrete portions of the thalamus). The results suggest a comparative preference by PAL-Enk for delta binding sites. The mechanisms of UV irradiation-induced competitive binding of PAL-Enk as compared to two other opioid radioligands will be considered in the context of an evaluation of receptor specificity of the ligands. Supported by MRC (MT5218) and Parkinson Foundation grants.

49.6

NON-SELECTIVE DESTRUCTION OF CATECHOLAMINE CELLS IN THE MURINE BRAIN BY N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP). N.A. Seniuk* and W.G. Tatton. Playfair Neuroscience Unit, University of Toronto & Toronto Western Hospital, Toronto, Canada M5T 2S8. Supported by MRC (MT5218).

MPTP produces a Parkinsonian-like syndrome in human and non-human primates. Although the cumulative doses cited as necessary to produce behavioural, histopathological and biochemical changes differs greatly between primates (1.7 mg/kg) and rodents (300 mg/kg), MPTP reportedly causes selective destruction of neurons in the substantia nigra (SN) and reduces striatal dopamine(Burns et al.1983;Heikkila et al.1984). We examined the effect of varying total dosages of MPTP (40-300 mg/kg), administered in divided doses at intervals of 2-24h, on catecholaminergic neurons in C57BL/6J mice. Serial, 20µ,coronal sections were incubated with tyrosine hydroxylase (TH) antiserum and then reacted using avidin-biotin and a chromogen. Intense, reproducible TH staining of neuronal soma,processes and terminals occurred only in discrete,defined CNS nuclear regions. TH neuron numbers were quantitated by 3-D reconstruction and a Monte-Carlo cell-counting method. Almost complete destruction of TH cells in SN was observed together with significant reductions in the locus ceruleus. Smaller reductions of TH neurons were found in nucleus accumbens and midline cells in the brainstem. There was no detectable TH cell loss in the olfactory bulbs. These data suggest that MPTP does not only destroy neurons in the SN; and, that monoaminergic cells, particularly noradrenergic, can be destroyed by this agent.

49.7

AMINO ACID NEUROTRANSMITTER RELEASE DETECTED IN CORTEX OF RAT BY NEW DOUBLE-LUMEN PUSH-PULL PERFUSION SYSTEM. J.M. Peinado,* K.T. McManus*and R.D. Myers. Univ. of North Carolina School of Medicine, Chapel Hill, N.C. 27514

A new double-lumen polyethylene push-pull cannula system has been used to study the in vivo basal release from brain tissue of newly synthesized amino acids from ^{14}C-glucose precursor. Four different areas of the cerebral cortex, frontal, parietal, temporal and occipital, were examined in the freely moving rat. Aspartic acid, glutamic acid, glutamine, glycine and GABA were collected by perfusing selected cortical sites with an artificial CSF at a flow rate of 12 µl/min. The separation of ^{14}C-amino acids was performed by thin layer chromatography, with the radioactivity of each spot subsequently counted by scintillation spectrometry. The percent levels of newly synthesized amino acids were: 47% Glu, 22% Asp, 18% Gln and 8% Gly. The proportional recovery of GABA was very low or, in the temporal cortex, undetectable. A comparison between cortical areas revealed a remarkably high synthesis of 70% Glu in the frontal cortex but low synthesis of 3% Gln. In contrast, the occipital cortex exhibited a new synthesis of 31% Glu and of 34% Gln. No other significant differences were found for the rest of the amino acids studied. The double-lumen push-pull system is now being used to analyze the basal release of endogenous amino acids in different regions of the cerebral cortex, with quantitation by HPLC with electrochemical detection.

Supported in part by Grant BNS-84-10663 from the National Science Foundation.

49.8

SEROTONERGIC MEDIATION IN ANTICONVULSANT ACTIVITY OF DIPHENYLHYDANTOIN (DPH) AND 5-ETHYL,5-METHYLHYDANTOIN (EMH). L.M. Leadbetter*, S.J. Brumleve, G.G. Mayer* and S.S. Parmar. Department of Physiology and Energy Research Center, University of North Dakota, Grand Forks, North Dakota 58202

The possible mediation of serotonin on anticonvulsant activity of DPH and EMH was investigated using male ICR albino mice. Both compounds demonstrated dose-dependent protection against maximal electroshock (MES)-induced convulsions. Effective calculated doses of DPH and EMH providing 50% protection (ED_{50} values) were 4.67 mg/kg and 690.0 mg/kg, respectively. Pretreatment with tryptophan caused 4% and 33% increase in the ability of DPH or EMH, respectively, to provide protection against convulsions. Such an increase with 5-hydroxytryptophan was 13% for DPH and 8% for EMH. Biphasic activity was noted with p-chlorophenylalanine (p-CPA) prior to EMH administration; where a 2 hour pretreatment increased protection by 8% while a 48 hour pretreatment decreased protection by 15%. Administration of p-CPA prior to DPH did not alter the anticonvulsant activity of DPH. Methysergide administration prior to either DPH or EMH decreased anticonvulsant activity by 14% and 27%, respectively. These results have provided evidence towards possible mediation of serotonin during protection against MES-induced convulsions where increased levels of brain serotonin may presumably facilitate anticonvulsant activity of DPH and EMH. (Supported in part by the Dakota State Aerie Fraternal Order of Eagles and the Hoffmann-La Roche Foundation.)

49.9

EFFECT OF QUININE ON RELEASE OF NORADRENALINE AND ON CA-ACTIVATED K CHANNELS IN CHROMAFFIN CELLS. M.I. Glavinovic, A.P. Dagher*, J.M. Trifaro*. McGill University, Montreal, Quebec, Canada H3G 1Y6.

The [^{3}H] noradrenaline release was estimated in cultured bovine chromaffin cells in Locke solution and during stimulation with high potassium (30 and 56 mM), both in the absence and in the presence of quinine (20 and 200 μM). Quinine does not appear to affect the resting release significantly but does affect the release induced by high [K^+] in a dose dependent manner. While no effect of 20 μM quinine on the release induced by 30 mM [K^+] is observed, the release induced by 56 mM is marginally reduced. The effect of 200 μM quinine is much greater. It reduces the noradrenaline release induced by 30 and 56 mM [K^+] by approximately one third and one half respectively. The effect of quinine (10 μM to 1 mM) on Ca-activated potassium channels was examined using the patch-clamp technique. Quinine, when applied to the internal surface of the membrane, produced what appeared as "flickery" block. This block probably explains at least partly the changes in noradrenaline release induced by quinine.

Supported by MRC.

49.10

REGIONAL DISTRIBUTION OF ADENOSINE DEAMINASE ACTIVITY IN RAT BRAIN. T.H. Swanson,* C.L. Green,* and M.A. Moron.* (SPON: J.W. Phillis). Wayne State Univ., Detroit, MI 48201

Adenosine deaminase (ADA; EC 3.5.4.4) catalyzes the irreversible deamination of adenosine to inosine and ammonia. Adenosine, a purine nucleoside, exhibits diverse biological functions and has been described as having putative neurotransmitter properties in the central nervous system. Degradation of adenosine by ADA is an important mechanism for removal of extracellular adenosine which is present at concentrations rangeing from 10-7 to 10-6 molar, concentrations at which the A2 adenosine receptor is activated. ADA is present in at least four different molecular species, three of which are soluble, the smallest formed by subunit dissociation of the larger two. This raises the possibility that ADA kinetics may be influenced by the local microenvironment of the adenosine catabolic pathway, and be subject to feedback modification. Previous reports of ADA activity include the activity of 5'nucleosidase, which converts inosine to hypoxanthine and ribose. We inhibited 5'nucleosidase with para-chloro-mercuric benzoate to more accurately ascertain ADA activity. Kinetic analysis of ADA activity in our system revealed Km and Vmax values of 76.9μM and 96.3 pmole/min/mg protein. RESULTS: (expressed as pmole/min /mg protein of whole brain area homogenates) pons 43.8; thalamus 42.9; caudate 16.3; medulla 14.7; hippocampus 10.1; cerebellum 5.8; hypothalamus 3.0; and cortex 1.1. These values are different from values reported by other groups,possibly due to altered kinetics of ADA by feedback of hypoxanthine or ribose.

49.11

UNIT ACTIVITY IN AREA 5 OF THE MONKEY BEFORE AND AFTER A BILATERAL DENTATECTOMY. P. Burbaud*, C. Gross*, and B. Bioulac. (Spon) Lab. Neurophysiologie, Groupe Motricité, Université de Bordeaux II, 146 rue Léo Saignat, 33076 BORDEAUX CEDEX.

Previous studies (SEAL et al., 1982) have shown that after a total deafferentation of the trained forearm, early neuronal activity up to 300 ms before the onset of movement was still recorded in area 5 of the monkey. This activity was assumed to be purely central in origin. In this study, unit activity in area 5 of the posterior parietal cortex was recorded in a monkey trained to perform a flexion and an extension of the forearm about its elbow in response to an auditory cue. After a bilateral stereotaxic dentatectomy (electrolytic destruction according to the technique of Courville (1979) ; verification of the extent of lesion on a CT-Scan) the monkey exhibited a very pure chronic lateral cerebellar syndrom with clear kinetic alterations. Recordings in area 5 showed few changes in the pattern of neuronal discharge and early cells with changes before the onset of movement were still encountered. These preliminary results seem to indicate that cerebellar outputs (via the dentate nucleus) are not a major source of inputs accounting for early neuronal changes in area 5 and give an insight into the origin of this cortical activity.

49.12

REACTION TIME IN FAST UNIJOINT AND MULTIJOINT UPPER LIMB MOVEMENTS IN NORMAL AND PARKINSONIAN SUBJECTS. M.M. Thompson*, W.G. Tatton, M.C. Verrier*, A. Lang*. Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto, Ontario, M5T 2S8.

Prolonged reaction times (RTs) and movement times (MTs) to visual cues have been reported in parkinsonian patients (PKs) and N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)monkeys. Dual choice RTs together with MTs, maximum velocities and the patterns of extensor and flexor electromyographic (EMG) activity for "ballistic" wrist movements were measured in mildly-affected PKs and age matched normals. There was marked intertrial variability in the RTs and the movement parameters for the PKs. Individual values were often within the range found for the control subjects despite significant increases in mean values for the parameters compared to normals. There was no correlation between the RTs and the movement parameters. In order to determine how RTs and movement variables are altered for direct versus indirect multijoint upper limb movements to randomly-placed targets in 3-D space, kinematic variables were measured using an infrared system. PKs showed increased RTs, MTs and decreased maximal velocities compared to the normals for both the direct and indirect movements. RTs in normals increased by 40-85ms for indirect movements. PKs were unable to optimize the trajectories of the indirect movements. The results will be considered with regard to central "programming" of visually cued movements. Supported by MRC(MT5218), Parkinson Foundation and Conn Smythe Foundation Grants.

49.13

HUMAN OPTOKINETIC AFTER-NYSTAGMUS (OKAN): ROLES OF EYE MOVEMENT VERSUS RETINAL IMAGE SLIP. Stephen Liben* and Bernard N. Segal. Aerospace Med. Res. Unit., Dept. Physiol., McGill Univ., Montreal, Canada H3G 1Y6.

When a stationary subject is exposed to prolonged full-field optokinetic stimulation, and then all illumination is extinguished, eye movements may persist in darkness for minutes as OKAN. It is unclear whether OKAN is the product of motor (eye muscle) or sensory (visual afferent due to retinal image slip) activity, or both. To clarify this issue, we examined OKAN elicited subsequent to optokinetic stimulation during which the eyes had been held essentially stationary. We then compared such OKAN to previously described (Ref. 1) OKAN elicited subsequent to stimulation during which the eyes had been permitted to move. Three subjects fixated a stationary point, while an optokinetic drum rotated around them with sinusoidal profiles of .05-.01 Hz frequency and 60-120 deg/s peak angular velocity. Brief intervals of total darkness were used to periodically sample OKAN (lights on: 10s; darkness: 2s). Such OKAN, being primarily due to retinal slip, had approximately 1/5 the magnitude and 3 times the phase lag of OKAN previously observed (1) with eyes moving. Even larger magnitude differences were seen at slip velocities greater than 30 deg/s. We conclude that human OKAN results primarily from eye movement, and not visual afferent activity, even at low slip velocities.

(Ref. 1): Segal & Liben, Exp. Brain Res., in press, 1985.

49.14

UNILATERAL PATCHINC RESULTS IN A COMMON UNCALIBRATION OF THE SACCADIC SYSTEM AND THE VESTIBULAR-OCULAR REFLEX. Erik Viirre* and Tutis Vilis. Department of Physiology and Ophthalmology, University of Western Ontario, London, Canada N6A 5C1.

We previously reported that patching one eye for one week affects saccadic magnitude and causes post-saccadic drift selectively in the unseeing eye. Whether the VOR exhibited changes similar to those observed in the saccadic system was studied in three Macaca Fasicularis monkeys. Three common changes were noted. 1. Changes in VOR gain were correlated with changes in saccadic step magnitude. 2. During both horizontal saccades and horizontal VOR the patched eye developed a vertical component, each in the same direction. 3. In the patched eye horizontally directed saccades were followed by vertical post-saccadic drift. Surprizingly, since this could be observed in the absence of a vertical saccadic component, the drift was not related to a vertical component of the saccade. Horizontal saccades from position A to B and back to A exhibited a hysteresis loop in the patched eye when plotted in the horizontal-vertical plane. The VOR elicited by sinusoidal rotations of the head showed hysteresis loops in the patched eye in the same direction as those of the saccades. Thus the absence of visual input results in the uncalibration of common elements in both the VOR and saccadic systems and this occurs selectively in the unseeing eye. The presence of vertical drift resulting from both horizontal saccades and horizontal VOR suggests that integration from angular velocity to position is a vector process. (Supported by Medical Research Council).

49.15

THE OCULOMOTOR INTEGRATOR IN THREE DIMENSIONS. Douglas Tweed* and Tutis Vilis. Departments of Physiology and Ophthalmology, University of Western Ontario, London, Canada N6A 5C1.

Angular position is not the integral of angular velocity. Hence in a three dimensional extension of Skavenski and Robinson's model of the vestibulo-ocular reflex (J. Neurophysiol. 36, 1973), the eye velocity command $\vec{\omega}_v$ from the vestibular neurons cannot be integrated to yield the eye position signal required by ocular mechanics. Eye position is best represented by the vector $\beta\vec{n}$, which indicates that a rotation of magnitude β about the unit vector $\vec{n}$ would take the eye from primary position to its present orientation. The position command $\beta_v\vec{n}_v$ can be computed from $\vec{\omega}_v$ using a secondary representation of eye position as a rotation matrix A. Taking the cross product of $\vec{\omega}_v$ with each column of A gives $\dot{A}$; A is updated by integrating $\dot{A}$. The normalized cross product of any two nonzero columns in A-I equals $\vec{n}_v$. The component of $\vec{\omega}$ along $\vec{n}$ determines how fast the eye is spinning about the current $\vec{n}$, i.e. $\vec{\omega}_v \cdot \vec{n}_v = \dot{\beta}_v$; integration yields β_v. In this model, the six-component motoneuron activity vector $\vec{m}$ determines ocular motion by the matrix equation $\vec{m} = K\beta\vec{n} + R\vec{\omega}$. With matrices replacing the scalar coefficients of the one dimensional model, new and complex calibration errors may appear. Uncalibrated matrices not only may scale vectors incorrectly, causing dysmetria and drift, but may twist them so that, for example, a horizontal velocity command affects the vertical position command. Evidence for such uncalibration is presented in the accompanying abstract. (Supported by MRC).

49.16

EFFECTS OF GAMMA MOTONEURONS ON MUSCLE SPINDLE AFFERENTS DURING RESPIRATION IN THE CAT. J. Greer and R.B. Stein. Department of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7.

Evidence from recordings of gamma motoneurons and afferents to leg extensors of the cat during locomotion suggests that static gamma motoneurons fire tonically, while dynamic gamma motoneurons fire phasically, approximately in phase with alpha motoneurons (Taylor et al., J. Neurophysiol. 34: 341, 1985). Recordings during rhythmic jaw movements in the cat suggest an opposite pattern of firing, namely phasic static gamma activity with tonic dynamic activity (Gottlieb & Taylor, J. Physiol. 345: 423, 1983).

We have recorded the activity of muscle spindles and gamma motoneurons supplying external intercostal muscles during another cyclic activity, respiration. Recordings were made with the intercostal nerves lifted onto hook electrodes in paraffin, either in cats decerebrated at the intercollicular level or in anesthetized cats. A sinusoidal stretch was applied to the muscle containing the spindles of interest, before and after cutting the nerve proximally to eliminate fusimotor effects. Using methods described previously by Taylor et al. (1985) the mean rate and modulation in rate with stretch could be analyzed at various parts of the respiratory cycle. The mean rate was generally higher and the modulation to stretch lower at all phases of the breathing cycle when the efferent supply was intact. This suggests a tonic, static gamma drive to the muscle spindles in respiration.

49.17

Peripheral Neuropathy of Dietary Riboflavin Deficiency in Chickens. Neurophysiological Studies. C. Manetto,* L.G. Shell,* B.S. Jortner,* T.I. Lidsky.* (SPON:J.Lee) DVB, VPI, Blacksburg, VA.

Chickens fed from birth on a diet deficient of riboflavin (1.8 mg/kg) develop a variety of peripheral problems evident in anatomical, neurophysiological, and behavioral deficits. Light and transmission electron microscopic examination of sciatic nerve revealed demyelination and Schwann cell enlargement beginning at 6 days of age and marked demyelination at 21 days of age including Schwann cell hypertrophy associated with the neuropathy.

Neurophysiological measures revealed several functional concomitants of the morphological abnormalities. Compound action potentials recorded from the sciatic nerve showed a dimunition of firing of myelinated axons associated with increased fatigability. In addition, there were decreases in conduction velocity. Problems in alpha motoneuronal conduction were manifest as increased thresholds for activation of the gastrocnemius as well as decreased motor conduction and increased fatigability.

Clinical examination revealed a variety of neurological signs. Flexor and palpebral reflexes were attenuated. Postural abnormalities (e.g., sternal recumbency) were also apparent as were spacticity and disturbances of gait.

49.18

HYPOTHERMIA-INDUCED CHANGES IN INTERPEAK LATENCY OF BRAINSTEM AUDITORY EVOKED RESPONSES RECORDED FROM HAMSTERS AND RATS. L. Hoffman*, T. A. Jones, B. Fullerton*, and J. Horowitz. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

Changes in interpeak latency of the brainstem auditory evoked response (BAER) were measured in euthermic hamsters and rats following exposure to hypothermia. This study was designed to determine whether BAERs recorded from hamsters (animals that can hibernate) differ from BAERs recorded from rats (non-hibernators) as brain temperature is lowered. Four Syrian hamsters and six Long Evans Hooded rats were prepared under pentobarbital anesthesia for bone-conducted auditory stimulation and recording (J. Neurosci. Meth. 7:261, 1983). A calibrated thermistor was also implanted for recording brain temperature. BAERs were recorded from anesthetized animals in response to bone-conducted stimuli as brain temperature was lowered from 37 to 27°C in hamsters and from 39 to 32°C in rats. The bone-conducted stimuli (30 to 40 dBSL) originated by delivering square wave pulses (0.05 msec duration, 9.0 Hz) to a piezoelectric crystal. Each BAER was the average of 256 consecutive responses. Interpeak latencies were determined and plotted as linear regressions against brain temperature. The slopes from hamsters and rats were then grouped independently and compared by unpaired t-test. By this analysis it was determined that for each individual the correlation of interpeak latency with brain temperature was highly significant over the range of brain temperatures examined in this study. The mean slope ($\pm$ standard deviation) of the regressions of interpeak latency on brain temperatures from the hamsters and rats was -0.170 $\pm$ 0.053 and -0.134 $\pm$ 0.050, respectively. This indicates that interpeak latencies of BAERs recorded from euthermic hamsters and rats are not different for temperatures from 37 to 27°C. Supported by NASA Grant 2234.

49.19

AREA POSTREMA ABLATIONS IN CATS: EVIDENCE FOR SEPARATE NEURAL ROUTES FOR MOTION- AND XYLAZINE-INDUCED CTA AND EMESIS. M. Corcoran*, R. Fox*, K. Brizzee*, G. Crampton*, and N. Daunton* (SPON: J. Zabara). NASA Ames Research Center, Moffett Field, CA. 94035; San Jose State Univ., San Jose, CA. 95192; Delta Regional Primate Center, Covington, LA. 70433; Wright State Univ., Dayton, OH. 45435.

Previous studies on the role of the area postrema (AP) in vomiting induced in the cat by motion and drugs have shown that the AP is not essential for motion-induced vomiting, but is necessary for vomiting to apomorphine and xylazine. To confirm these findings and to determine the role of the AP in the formation of Conditioned Taste Aversion (CTA), the AP was ablated bilaterally in 10 adult female cats. With one exception, the ablated cats continued to vomit to the same motion that elicited emesis before the ablation. Doses of xylazine and apomorphine that elicit emesis in intact cats, failed to induce emesis in the ablated cats. Histological examination indicated that 8 cats had complete lesions and 2 had partial lesions. Investigations of effects of AP ablations on CTA revealed that cats with complete lesions did not form CTA to flavored milk paired with xylazine injections. However, cats with partial lesions developed xylazine-induced CTA. Seven of the 8 completely lesioned cats developed motion-induced CTA, even though emesis was not consistently elicited by motion. These results suggest that there are multiple routes for inducing CTA and the emetic reflex, that CTA can form without eliciting emesis, and that CTA may be a sensitive measure of sub-emetic motion sickness.

49.20

ADRENERGIC INVOLVEMENT IN STRESS-INDUCED INHIBITION OF THE JAW OPENING REFLEX. N.R. Myslinski*, R. Clarke* and B. Matthews*. (Spon: R. Franklin) Department of Physiology, School of Medicine, University of Bristol, Bristol, England, BS8 ITD.

Surgical trauma has been shown to increase the threshold (TH) of the jaw-opening reflex (JOR) elicited by tooth pulp stimulation. To determine if the adrenergic system is involved in the mechanism of this effect we administered adrenergic receptor blockers and noradrenalin (NA) systemically and centrally to cats traumatized with sterotaxic ear bars and surgery. Male and female cats anesthetized with Saffon were used. End tidal CO_2, blood pressure and core body temperature were monitored. The JOR was monitored electromyographically with copper bipolar electrodes inserted into the anterior digastric muscle. It was evoked by the stimulation of maxillary canine tooth-pulp with a bipolar stimulating device. All cats showed an increase in JOR TH after trauma. Subsequent administration of the alpha adrenergic blocker phentolamine (1.0 mg) (IC) decreased the TH in 66% of the animals (N=6) ($P < 0.001$). When microinjected into the spinal nucleus of V (10 µg), it also decreased the TH (N=2). The alpha blocker, RX781094:2-[1,4-imidazolinyl] - 1,4 - benzodioxane HCl (50 µg) (IC) decreased the TH in 71% of the animals (N=7). NA increased the TH in all animals when injected either IC (100 µl of 1:1000) (N=4) or directly into the spinal nucleus of V (1.0 µg) (N=2). These data support the involvement of the adrenergic system in the stress-induced inhibition of the JOR.
(Supported by the M.R.C.)

50.1

EFFECTS OF METHYLPREDNISOLONE UPON VASCULAR PERMEABILITY CHANGES IN ENDOTOXIN SHOCK. Joel D. Hubbard and H.F. Janssen. Depts. of Phys. & Ortho. Surg., TX Tech Uni. Hlth Sci. Ctr., Lubbock, Texas.

This study was designed to examine glucocorticoid effects upon vascular permeability increases caused by endotoxic shock in dogs anethetized with Pentobarbital Sodium (30mg/kg). Methylprednisolone Sodium Succinate (MP) was administered IV (30mg/kg) in two pretreatment doses before Escherichia coli endotoxin was administered (0.5mg/kg). Serum and left thoracic duct lymph samples were collected for measurement of total protein and separation by polyacrylamide gel electrophoresis. Four protein electrophoretic fractions with molecular weights (M.W.) ranging from 60,000 to 1,000,000 were consistently chosen for analysis. Endotoxin administration alone caused a significant increase in lymph flow, protein flux, lymph/plasma protein ratio (L/P), and permeability coefficient. Pretreatment with MP resulted in an attenuation of the early increase in total protein flux and L/P ratio, and significantly prevented the increase in permeability coefficient observed in the group given endotoxin alone. Endotoxin administration alone resulted in significant decreases in the reflection coefficient for all four electrophoretic fractions. Pretreatment with MP attenuated the decrease in reflection coefficient for only the lower M.W. fractions of 100,000 or less. These results suggest MP provides partial protection from endotoxin shock vascular permeability increases.
Supported in part by NIH training grant # T32 HL07289-08.

50.2

DO MONOKINES CONTRIBUTE TO INSULIN RESISTANCE IN SEPTIC SHOCK? James P. Filkins. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153

Biphasic alterations in insulin actions - - an initial increased responsiveness and a later phase of insulin resistance - - are key determinants of the metabolic dyshomeostasis of sepsis. The present study evaluated if monokines are potential factors in the altered insulin responsiveness of septic shock. The test paradigms involved caseinate-induced Holtzman rat peritoneal macrophages (PM) as a secretory source of monokines and insulin-induced ^{14}C-glucose oxidation in rat epididymal fat pads (EFP) as the index of insulin action. To evaluate insulin resistance, a maximal stimulus concentration of insulin determined as 1mU/ml was employed. EFP obtained from rats treated with cultured PM conditioned media exhibited increased basal glucose oxidation (68%) but had decreased responsiveness to insulin augmentation - - a 22% increase vs 140% with control media treatment. Incubation of EFP in PM conditioned media increased (35%) glucose use via the intrinsic insulin-like activity of monokines, but it also diminished insulin responsiveness (42% vs 160% increments). Co-culture of PM with EFP also increased EFP glucose oxidation (58%) but blunted the response to insulin stimulation from an increment of 93% without PM to 15% with PM. These preliminary data support a role for monokines in the insulin resistance of septic shock. (Supported by GM 29619)

50.3

SCALING OF PHYSIOLOGICAL RESPONSES DURING HEMORRHAGIC SHOCK. Richard Connett, Frederick Pearce and William Drucker. Departments of Physiology and Surgery, University of Rochester, Rochester, NY 14642

Standard protocols used to study hemorrhagic shock involve sampling at fixed time intervals and generating a time-based composite curve from each experiment. While each animal shows the same sequence of responses, the time, rate and size of the response varies from animal to animal. Thus, sampling times may be inappropriate to identify sharp transitions in the parameters and the composite curves don't reflect the size and shape of the individual responses. Hematocrit, blood lactate and glucose concentration data from a constant pressure shock model were examined using a number of non-dimensional scales. Scaling for fractional blood loss on the x-axis and maximal response on the y-axis resulted in convergence of the results from individual animals. Grouped results from fed, fasted-dehydrated as well as data from alternate hemorrhage protocols also converged after scaling. Preliminary studies on ~10 animals allows definition of blood loss in terms of body weight and permits prospective scaling for each animal and experimental condition. This permits reproducible sampling of each animal at appropriate frequencies and phases of shock which results in a better definition of the relative sequence of events during shock. Application of this method has permitted separation of a number of events during shock previously thought to be simultaneous. Supported by NIH Grant #GM 30095.

50.4

REDUCED HEMORRHAGE TOLERANCE IN HYPERTHERMIC CONSCIOUS PIGS B.F. Williams*, C.A. Bossone*, C.E. Wade, and J.P. Hannon Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800.

We evaluated the cardiovascular response to hemorrhage (38.5 ml/kg over 60 min) in conscious normothermic (n=8) and hyperthermic (40.4 ± 0.4; mean ± SEM, n=5) Duroc swine (18-25 kg); hyperthermia was defined as a rectal temperature exceeding the normal value, 39.0 ± 0.1; n=35. Animals were surgically prepared with a carotid artery catheter and splenectomized seven days prior to experiments. Measurements were made at 0, 10, 20, 30, 45, and 60 min during hemorrhage and at 15, 30, 60, 120, 180, and 240 min after hemorrhage. All eight of the normal pigs survived. One of the animals with an elevated temperature died in the course of hemorrhage and two after hemorrhage ($p<0.035$). Before hemorrhage, animals with elevated body temperatures had significantly ($P<0.05$) increased mean arterial pressures (119 ± 5 vs 99 ± 5 mmHg) and heart rates (145 ± 7 vs 112 ± 5 b/m) compared to animals with normal body temperatures. At the end of hemorrhage there was no difference in mean arterial pressures (48 ± 11 mmHg, N=4) for hyperthermia pigs and (48 ± 2 mmHg, N=8) for normals. Heart rates at the end of hemorrhage were greater in the hyperthermic (185 ± 26, b/m) than in the normothermic pigs (146 ± 8, b/m). However, pigs with elevated body temperature, presumably due to some low grade infection at the catheter exit site, had a reduced tolerance to hemorrhage.

50.5

EFFECTS OF PHYSICAL RESTRAINT ON RESPONSES TO HEMORRHAGE IN CONSCIOUS SWINE G.D. Bonner*, C.A. Bossone*, B.F. Williams*, D.S. Trail*, C.E. Wade, and J.P. Hannon Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800.

Responses to hemorrhage (38.5 ml/kg/60 min) were evaluated in chronically catheterized conscious pigs (20-25 kg) restrained in a portable holding cage, n=6, a squeeze cage, n=8, or a Pavlov sling, n=8. Before hemorrhage heart rates of sling (145 ± b/m, x ± SEM) and squeeze cage pigs (129 ± 9 b/m) were elevated ($p<0.05$) compared to holding cage pigs (107 ± 5 b/m). Hemorrhage caused a further increase (to 216 ± 15 B/m) in the sling animals. Initially animals in the squeeze cage had mean arterial pressure (99 ± 4 mmHg), lower than in the holding cage (105 ± 2 mmHg) while sling animals had elevated values (111 ± 7 mmHg). Following hemorrhage mean arterial pressures were similar in holding cage (46 ± 4 mmHg) and squeeze cage animals (43 ± 4 mmHg) while sling animals were elevated (58 ± 8 mmHg) There were no difference in hematocrit between groups either before or after hemorrhage. Plasma glucose and lactate levels were lower in sling before and after hemorrhage. Arterial PO_2 values were elevated and PCO_2 values reduced in squeeze cage and sling throughout the experiment. In studying the cardiovascular and metabolic responses to hemorrhage in conscious swine the method of restraint used alters the qualitative and quantitative nature of the results.

50.6

THE EFFECTS OF A TEN-MINUTE PERIOD OF SEVERE HEMORRHAGE ON CARDIAC CONTRACTILITY. Timothy J. Gawne*, Kristen Gray*, and Robert E. Goldstein. USUHS, Bethesda, Md. 20814

The effects of hemorrhagic shock on cardiac contractility remain unclear. This study investigated the effects of a ten-minute period of severe (25-35 mm.Hg M.A.P.) hemorrhage on the slope of the end-systolic pressure-volume relationship (ESPVR). Young (6-8 wks) domestic pigs were anesthetized with pentobarbital and instrumented with three pairs of sonomicrometer crystals (minor axis, major axis, and equatorial wall thickness) to estimate ventricular volume. Ventricular pressure was measured with a catheter-tip transducer. All studies were conducted in the open chest,open pericardium condition. This technique could reliably identify the depressant effect of 0.8 µg/kg propranolol or 40 mg/kg pentobarbital on ESPVR. 12 animals were hemorrhaged; three died during the hemorrhage; the remainder were reinfused. Seven control animals were used. After reinfusion ESPVR increased significantly (18.14 vs. 10.86 mm.Hg /cc) above control for 50 minutes before returning to baseline, indicating increased contractility. At no time was the ESPVR for the hemorrhaged animals significantly less than for the controls. It is concluded that brief periods of severe hemorrhage that are survived have no short-term effect on the myocardium after restoration of blood volume.

50.7

THE EFFECT OF MYOCARDIAL ISCHEMIA ON THE CORONARY VASCULAR RESPONSE TO BEHAVIORAL STRESS. George E. Billman, Department of Physiology, The Ohio State University, Columbus, OH 43210.

Left circumflex coronary blood flow was measured in 9 mongrel dogs during classical aversive conditioning (a 30 sec tone followed by a 1 sec shock) both before and during acute myocardial ischemia. The anterior wall ischemia was induced by occlusion (hydraulic occluder) of the left anterior descending artery. The conditional response consisted of significant ($P<0.01$ ANOVA $\bar{x} \pm SE$) increases in mean arterial pressure (AP, 13.8 ± 1.9 mmHg), left ventricular (LV) dP/dt (850 ± 117 mmHg/sec) and heart rate (HR, 44 ± 4 beats/min). Mean coronary vascular resistance significantly increased (CVR, 0.52 ± 0.18 mmHg/ml/min) then decreased -0.77 ± 0.12 mmHg/ml/min). In contrast, during myocardial ischemia, the CVR increase was eliminated while both the CVR decrease and the HR increase were reduced (CVR increase 0.08 ± 10 and decrease -0.45 ± 0.15 mmHg/ml/min, HR 20.7 ± 3.8 beats/min). Neither the mean AP nor LV dP/dt was significantly affected by myocardial ischemia (mean AP 12.4 ± 1.8 mmHg, LV dP/dt 650 ± 21.3 mmHg/sec). This attenuated coronary vascular response (mean CVR decrease) to the aversive stress was reversed by alpha adrenergic receptor blockade (phentolamine 1 mg/kg). Thus, during myocardial ischemia both neural (increased alpha adrenergic tone) and non-neural (reduced HR) factors probably contribute to the attenuated mean CVR response. (supported by NIH grant HL 33718).

50.8

THE CARDIOVASCULAR RESPONSE TO HYPERBARIC (6 ATA) OXYGEN (100%) CLOSED CHEST CARDIOPULMONARY RESUSCITATION IN A CARDIAC ARREST (15 MIN) GUINEA PIG. E. R. Mogabgab*, S. J. Whidden, K. W. Van Meter*, Harvey I. Miller.
Baromedical Research Inst., J.E.S.M.C., New Orleans LA 70114

24 male Guinea pigs (400 ± 27 gr) with indwelling arterial venous catheters, Swan-Ganz thermodilution thermistors, intratracheal intubation and EKG electrodes were anesthetized with Ketamine (150 mg/K, IP) and then placed in asystolic cardiac arrest (CA) through the intraventricular use of 2cc .7% NaCl 0.02°C and 2cc 1% KCl 0.02°C. After 15 minutes, these animals were divided into two groups. The first group (A) was treated at 1 atmosphere (ATA) with cardiopulmonary (100 B/M) resuscitation (CPR). The second group (B) were then placed into a multilock multiplace recompression chamber (60 in/12 ft) with two CPR support scientists, and were treated at 6 ATA (160 FSW) O_2 100% with CPR. All showed a decrease in temperature and no cardiac output or EKG at the end of 15 minutes post CA. Overall mortality was 90% in 1 ATA as opposed to 10% in the 6 ATA resuscitated animals at 3 hours post CA. It was found that 100% O_2 at 100 FSW was necessary to induce the return of spontaneous EKG pattern and return of cardiac output. In chamber EKG was monitored by Liteguard Defibrillator monitor and in chamber cardiac output by an American Edwards Cardiac Output Computer 9520.

50.9

DIAPHRAGMATIC ENERGETICS DURING HYPOTENSION INDUCED FATIGUE. F. Rutledge*, S. Hussain*, Ch. Roussos, and S. Magder. McGill University, Montreal, Quebec H3A 1A1.

In low output states, inadequate diaphragmatic blood flow (Qdi) and O_2 delivery (DO_2) are believed to precipitate respiratory muscle failure but they have not been measured during fatigue. We therefore produced respiratory muscle fatigue (F) in 9 dogs by increasing energy demands with oleic acid induced pulmonary edema (OA) and limiting blood flow by cardiac tamponade (T). Qdi was measured with radiolabelled microspheres and the diaphragmatic oxygen consumption ($\dot{V}O_2di$) and DO_2 were determined from the Qdi, and arterial and phrenic venous O_2 contents. Qdi increased from 17.2 ml/100 gm/min ± 1.8 (SEM) at control (C) to 42.2 ± 7.2 at OA. With T (BP 55 mmHg ± 4.2 and CO 0.68 l/min ± 0.08), the Qdi remained elevated (32.8 ± 4), $\dot{V}O_2di$ peaked (3.98 ml/100 gm/min ± 0.48), extraction reached 92%, and phrenic venous lactate (309 μ mole ± 91) exceeded arterial values (97 ± 43). With F, ventilation, frequency, pressure time index and DO_2 fell to control levels without a change in EMG activity of the diaphragm and the Qdi (29.3 ± 6.1) and $\dot{V}O_2di$ (2.3 ± .23) remained above control levels. The O_2 extraction remained maximal (92%) while venous lactates (390 ± 134) remained elevated but equal to arterial values (469 ± 63). We conclude that during respiratory muscle fatigue, $\dot{V}O_2di$ and Qdi remain elevated in spite of reduced work and that $\dot{V}O_2di$ becomes limited by limitations in O_2 delivery. Supported by MRC and the Quebec Heart Foundation.

50.10

VENOUS-ARTERIAL CARBON DIOXIDE GRADIENTS IN PATIENTS WITH CIRCULATORY SHOCK AND CARDIAC ARREST. Martin Griffel,* Eric C. Rackow,* William G. Grundler,* and Max H. Weil. UHS/The Chicago Medical School, North Chicago, Ill. 60064

We have previously reported that mixed venous carbon dioxide increases in patients with shock or cardiac arrest. We subsequently studied the effect of 1) circulatory shock and resuscitation and 2) circulatory shock and cardiac arrest on blood flow, arterial (a) and mixed venous ($\bar{v}$) carbon dioxide (PCO_2). Circulatory shock is defined in all patients as either 1) systolic arterial pressure < 90 mmHg or 2) cardiac index (CI) < 2.2 L/min/M^2 or 3) L > 2.0 mmol/L. In the 19 patients in whom fluid infusion increased CI, $PaCO_2$, $P\bar{v}CO_2$, and $P\bar{v}\text{-}aCO_2$ are reported prior to and after fluid resuscitation. In the 12 patients in whom circulatory shock progressed to cardiac arrest, the data are reported prior to and during cardiac arrest. In 17/19 (89%) in whom blood flow increased, $P\bar{v}\text{-}aCO_2$ decreased and in 12/12 (100%) in whom blood flow decreased, $P\bar{v}\text{-}aCO_2$ increased ($P<.001$).

	Shock & Resuscitation		Shock & Cardiac Arrest	
	Pre	Resuscitated	Pre	Arrested
CI L/min/M^2	2.3 ± 0.2	3.5 ± 0.2*		
$PaCO_2$ mmHg	35.3 ± 2.7	32.9 ± 1.8	30.7 ± 3.7	32.2 ± 3.5
$P\bar{v}CO_2$ mmHg	42.8 ± 2.8	36.3 ± 1.9	41.4 ± 3.7	62.9 ± 8.4*
$P\bar{v}\text{-}aCO_2$ mmHg	7.6 ± 0.9	3.5 ± 0.4*	10.7 ± 3.7	30.8 ± 6.7*

Mean ± SE * $p < .01$

Venous hypercarbia therefore provides another indicator of significant decreases in systemic blood flow.

50.11

DECREASED HUMAN ALPHA-2-MACROGLOBULIN (a2M) FOLLOWING BURN G.D. Niehaus, D. Herndon, R. Swoboda* and M. Stein* -Northeastern Ohio Universities College of Medicine, Rootstown, OH & University of Texas Medical Branch, Galveston, TX

Degradation of tissue can occur if active proteases exceed the inhibitory capacity of available antiproteases. Burn increases proteases by activation of cascade enzyme systems, increased phagocytosis by host defense cells, and altered metabolism. a2M complexes with proteases from all four protease classes, and is rapidly removed from the circulation by the liver. We postulated that an acute depletion of plasma a2M would occur as a result of the burn-induced activation of proteases but that plasma a2M levels would return to normal as protease production decreased and a2M synthesis increased. Immunoreactive a2M was measured in 88 plasma samples from 26 patients (average burn was 68% of surface area). Plasma a2M (mean±SD) for 8 non-burned controls was 3.40+1.11 (mg/ml). The combined values for each 5 day period was:

days	1-5	6-10	11-15	16-20	21-25	26-30
a2M mean	1.64	1.27	1.08	1.06	1.23	1.46
S.D.	.94	.35	.39	.40	.43	.49
(n)	14	15	23	13	12	11

We conclude that thermal injury induces a depletion of plasma a2M. It is not clear whether the sustained inhibitor deletion is the result of a degraded a2M systhesis or maintenance of an elevated protease production.
Supported by: HL 35258 and Shriners Burns Institute

51.1

LUNG MECHANICS DURING CHEST STRAPPING IN THE DOG. S.F. Grinton*, R.E. Hyatt, R.D. Ellefson*, M.S. Rohrbach*. Mayo Clinic, Rochester, MN 55905.

When the human chest is strapped, the lung static pressure-volume (PV) curve is shifted to the right. Mechanical effects of the increased recoil include increased flow and conductance at a given lung volume. However, the cause of the PV curve shift is uncertain. We developed a canine model of chest strapping to further explore shifts in the PV curve. 6 supine anesthetized mongrel dogs were placed in a leather chest restraint; the abdomen was loosely bound with elastic bandages. An esophageal balloon was positioned and the dog placed in a volume body plethysmograph. Control PV curves, ABG (arterial blood gases) and expiratory flow-volume (FV) curves were obtained. Straps were tightened to reduce the vital capacity to 50% of control. Repeat PV and FV curves and ABG's were obtained. All dogs showed a right shift of the PV curve. Slopes of FV curves increased, as did flows at all volumes. Changes in alveolar-arterial gradients were inconsistent. To see if changes in surfactant conformation might account for the PV shifts, we used the methods of Thet et al (J. Clin. Invest. 64:608,1979). We lavaged 2 strapped and 2 unstrapped dogs. There was a relative increase in disaturated phosphatidyl choline in pellets of the centrifuged lavage fluid of strapped dogs. This implies an increase in tubular myelin, a less active surfactant, which may be the cause of the increased lung recoil. (Supported by USPHS grant HL21584).

51.2

CONCEPTS OF A SIMPLE MEASURE OF RESPIRATORY IMPEDANCE. W.E. Fordyce and K.L. Darcy*. SUNY-Upstate Medical Center, Syracuse, NY 13210.

Both average inspiratory flow rate (VT/TI) and mouth pressure developed 0.1 sec after occlusion at end-expiration ($P_{0.1}$) have been suggested as indices of respiratory "drive". The ratio of $P_{0.1}$ to VT/TI is a simple measure of respiratory impedance (IM). We have studied a mathematical model of respiratory mechanics to learn how IM is altered by changed in resistance (R), compliance (C) and inspiratory time (TI). The lungs and thorax are considered a lumped structure with a single C and a single R. Muscle pressure (P_M) was assumed to decrease linearly (1) throughout inspiration and (2) for at least 200 msec during occlusion. Solving,

$$IM = \frac{0.1 * TI}{C * TI - R*C^2*(1-\exp(-TI/(R*C))}$$

With this model, IM was 1.8 cm H_2O/L/sec with normal parameters (R = 4 cm H_2O/L/sec, C = 67 ml/cm H_2O, TI = 1.5 sec). Around normal, the sensitivity of IM to C (i.e., S_C= ∂IM/IM / ∂C/C) = -0.79, S_R = 0.21, and S_{TI} = -0.21. Decreasing TI to 0.5 sec (R and C normal) increased IM to 2.7, a 50% change. Thus, a change in IM of a subject does not necessarily imply changes in R or C. Measurements of IM need to be interpreted in terms of R, C and TI. Supported by N.I.H. Grant HL-30653.

51.3

MECHANICAL VENTILATION WITH LOW-LEVEL POSITIVE END-EXPIRATORY PRESSURE (PEEP) IN RABBITS. Thomas A. Lesh. Center for Medical Education, Ball State Univ., Muncie, IN 47306.

PEEP is a well accepted therapeutic method for preexisting lung disorders. Prophylactic use of PEEP at low pressures has also been advocated, but the benefits of this application are still debated. The present study compared 5 cm H_2O PEEP with intermittent positive-pressure breathing (IPPB) in 2 groups of 6 supine, anesthetized rabbits during 70 minutes of mechanical ventilation at a respiratory minute volume 1.4-1.5 times the spontaneous control rate. Arterial oxygen tension (PaO_2) was sampled, and a small parasternal chest tube monitored intrapleural pressure (Ppl) for calculation of dynamic lung compliance (Cdyn). PaO_2, about 70 mm Hg during natural breathing in both groups, showed a barely significant downward trend during IPPB and a slight upward trend during PEEP. Cdyn declined to approximately 60% of control in both groups; this reduction was gradual during IPPB and prompt during PEEP. Before PEEP was terminated in 3 rabbits, the lungs were briefly inflated to 3 times the tidal volume (by expiratory obstruction); Cdyn then returned to approximately control, and PaO_2 to 10-30 mm Hg above control. The PEEP data suggest hypoinflation of the lung region surrounding the Ppl measurement site and possibly more widespread uneven distribution of ventilation. Airway pressures generated during tidal breathing at 5 cm H_2O PEEP may be insufficient to reopen some alveoli that become closed. (Supported by a grant from Ball State University.)

51.4

DETERMINATION OF PULMONARY MECHANICS ON THE PERSONAL COMPUTER SYSTEM. S.N. Reddy*, M.S. Kannan*, C. Davis*. (SPON: A.K. Grover). Neurosciences Dept., McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

An interactive personal computer (PC) system is described for processing and analysing flow and volume signals acquired from laboratory animals to study lung capabilities and airway parameters. The system consists of an IBM-XT PC equipped with a Tecmar Labmaster analog/digital input and output board, graphics hardware, and various peripherals. Software-wise the PC system has been organized for Basic, higher level programming languages, graphics, signal acquisition, signal processing library, statistics, and word-processing sections to integrate the experimentation, processing, and results together. The acquired flow and pressure signals are digitized through an anti-aliasing filter and processed to remove noise and artefacts. The preprocessed flow signal is then integrated to obtain volume signal. The flow, volume and pressure signals are then processed to obtain airway resistance and compliance from either individual cycles of flow, volume and pressure or from a given number of respiratory cycles. The system has been flexible enough to be run by non-computer oriented personnel. The results have provided reliable volume signal from the flow signal and has enabled the exploration of airway resistance and compliance as a function of time together with flow, volume and pressure signals.

Supported by MRC.

51.5

ISOLATED BRONCHIAL SEGMENT LAVAGE IN SUBJECTS WITH ASTHMA. WL Eschenbacher*, SR Baldwin*, KJ Ciffin* (SPON: L D'Alecy). University of Michigan Medical Center, Ann Arbor MI 48109

A novel technique has been developed for lavaging isolated segments of the central airways of human subjects. A modified pulmonary artery catheter is introduced into a central airway after topical lidocaine and for subjects with asthma after pretreatment with aminophylline. Two balloons at the distal end of the catheter are then inflated creating an isolated airway segment of 3.5 cm in length. Lavage can then be performed in this segment. In a preliminary study, two normal subjects and two subjects with asthma were included. For each subject, three individual lavages were performed: the first lavage was with normal saline, the second with distilled water as a hypo-osmolar challenge, and the third again with normal saline. Cells identified in the samples included ciliated epithelial cells (23-95% of total cells counted) and macrophages (4-58%). Assays for leukotriene C_4 (LTC_4), prostaglandin F_2 ($PGF_{2\alpha}$), and histamine were performed on the lavage samples. The two subjects with asthma who had received aminophylline before the challenge showed no change in either LTC_4 or $PGF_{2\alpha}$, whereas the two normal subjects had increases in LTC_4 (125 to 714 and 427 to 1980 pg/ml), but no increases in $PGF_{2\alpha}$. Histamine assays were not performed on all samples; however, one subject with asthma had an increase in histamine from 1196 to 4970 pg/ml as a result of the distilled water challenge, whereas the control subjects usually had histamine concentrations of less than 700 pg/ml. A new technique has been developed that may provide useful information regarding mechanisms of bronchoconstriction in asthma.

51.6

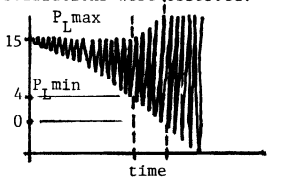
AIRWAY NEUTROPHIL INFLUX INDUCED BY SULFUR DIOXIDE (SO_2) EXPOSURE FAILS TO CAUSE AIRWAY HYPERRESPONSIVENESS IN DOGS. S. Kariya*, S. Shore*, K. Anderson*, J. Pennington*, J. Godleski*, and J. Drazen. Dept. of Environmental Science & Physiology, Harvard School of Public Health and Depts. of Medicine and Pathology, Brigham & Women's and Beth Israel Hospitals, Boston, MA 02115.

We studied the effects of 4 days of exposure to SO_2 gas on bronchoalveolar lavage (BAL) cell counts and airway responsiveness to inhaled methacholine in 6 tracheostomized dogs. Measurements were made before SO_2 exposures were begun, and again 1-2 h after 4th day of exposure (2 h/day at 50 ppm for 3 days and 200 ppm for 2 h on the 4th day). The concentrations of methacholine required to double pulmonary resistance ($ED_{200}R_L$) and to decrease dynamic compliance by 35% ($ED_{65}C_{dyn}$) were unaltered by the SO_2 exposure; however, the BAL fluid demonstrated a dramatic increase in the number of neutrophils and sloughed epithelial cells. Given below are the mean ratios (post-/pre-exposure) and statistical significance of the results: $ED_{200}R_L$ = 1.21 (NS); $ED_{65}C_{dyn}$ = 0.64 (NS); BAL total cell count = 3.40 ($p<.01$); # of polys/BAL = 16.2 ($p<.01$); # of lymphs/BAL = 0.44 ($p<.01$); # of macrophages/BAL = 1.83 (NS); # of epithelial cells/BAL = 19.6 ($p<.01$). We conclude that, in the dog, exposure to inhaled sulfur dioxide induces an epithelial sloughing and neutrophil influx into the airways. These changes, however, were not accompanied by an increased responsiveness to inhaled methacholine. (Supported by HL 19170, HL 01070, HL 27244, and MRC Canada.)

51.7

EFFECT OF END-EXPIRATORY PRESSURE ON THE AMPLITUDE OF VOLUME-PRESSURE OSCILLATIONS IN EXCISED RAT LUNGS. W. Cheng* and D.G. Frazer. NIOSH, Dept. of Physiol. W.V.U. Morgantown, West Virginia 26505.

Excised rat lungs were inflated-deflated in an air-filled plethysmograph for 1 cycle between -5 and +30 cm H_2O. Lung volume at 30 cm H_2O was assumed equal to V_L(max). In cycle 2, lungs were re-inflated to V_L(max), $3V_L$(max)/4, or V_L (max)/2. Following a 2 minute stress-relaxation period a volume perturbation was applied to the lung by withdrawing and replacing a volume of gas in a cosinusoidal fashion {$V_L' \cos[2\pi/3]t$; t=sec}. As V_L' was gradually increased from 0 ml the following transpulmonary pressure (P_L) oscillations were observed. The envelope of the P_L oscillations show that (1) $P_L$max remained constant for $P_L$min > +4 cm H_2O; (2) $P_L$max increased for 0 < $P_L$min < 4 cm H_2O (3) $P_L$max remained constant for P_L(min) < 0 cm H_2O. These results were similar for all initial lung volumes. Since the end-expiratory pressure range between 0 and +4 cm H_2O corresponds to the same range over which minisci form in the airways of rats (Respir. Physiol. 36:121-129, 1979) it seems likely that the formation of menisci in the airways may be responsible for many characteristics of P_L-V_L curves.



51.8

WITHIN BREATH CHANGES OF TRACHEAL SMOOTH MUSCLE TONE IN AWAKE, UNMEDICATED DOGS. E.H. Vidruk and R.L. Sorkness*. John Rankin Laboratory of Pulmonary Medicine, Dept. of Preventive Medicine, Univ. of Wisconsin, Madison 53705.

In anesthetized, paralyzed and ventilated animals, airway tone is reported to vary from breath to breath and within each breath as a function of the central pattern generator for ventilation (VCPG). We asked whether airway tone changes similarly in awake, unmedicated dogs. In 3 dogs, we monitored continuously the pressure changes within an isolated tracheal segment (P_t) as an index of airway tone. Intrabreath changes in P_t ranged from about 1 to 6 cm H_2O during spontaneous breathing. During mechanical ventilation, intrabreath changes in P_t persisted, ranging to 30 cm H_2O at low f and high V_T. P_t increased with inspiration and decreased during expiration at low f. As f increased, intrabreath P_t changes decreased and phase lags between increases in intrabreath P_t and inspiration appeared. Inspiratory effort against an occluded airway produced an increase in P_t without a subsequent decay, while end-inspiratory occlusion caused an exaggerated fall in P_t. Our data are consistent with the idea that airway tone is regulated as a function of the VCPG with tidal feedback from the lungs playing a modulatory role. (Supported by PHS grants HL00780 and HL29043, and an AFPE graduate fellowship.)

51.9

RELATIONSHIP BETWEEN TRACHEAL SMOOTH MUSCLE TONE AND VENTILATION, *TIDAL VOLUME* AND FREQUENCY IN AWAKE MECHANICALLY VENTILATED DOGS. R.L. Sorkness* and E.H. Vidruk. John Rankin Laboratory of Pulmonary Medicine, Dept. of Preventive Medicine, Univ. of Wis., Madison 53705.

Isocapnic changes in ventilation are reported to alter airway smooth muscle tone in anesthetized, paralyzed, and mechanically ventilated animals. We wished to determine how tracheal smooth muscle tone in awake, unmedicated dogs was affected by passive changes in V_T and f under isocapnic, hyperoxic conditions. We monitored continuously the smooth muscle tone in an isolated tracheal segment (P_t). In 3 dogs trained to remain passive during mechanical ventilation, V_T and f were changed by adjusting the controls of the ventilator. When V_T and f were changed reciprocally to maintain a constant alveolar ventilation, P_t did not change significantly (N=18 expts in 3 dogs). However, when either f or V_T was increased with the other held constant (using inspired CO_2 to maintain isocapnia), P_t decreased linearly with increases in log $\dot{V}_E$ (p<.01, N=36 expts in 3 dogs). Changes in V_T had relatively greater effects on P_t than did changes in f (p<.05). We conclude that passive, isocapnic changes in alveolar ventilation via either V_T or f cause predictable changes in tracheal smooth muscle tone. (Supported by PHS grants HL00780 and HL29043, and an AFPE graduate fellowship.)

51.10

RANDOM INPUT FORCING FOR HIGH FREQUENCY VENTILATION. S. Ghazanshahi*, S.M. Yamashiro, and V.Z. Marmarelis*. Biomedical Engineering, USC, Los Angeles, CA 90089-1451.

Virtually all forcings used for high-frequency ventilation (HFV) to date has been cyclic in nature. It is natural to wonder whether or not periodicity is an essential requirement for HFV. According to Taylor dispersion theory, the integral of the velocity autocorrelation function is the main determinant of gas exchange. Thus, even a random non-cyclical input could be used provided the autocorrelation properties were controlled. To test this prediction, gas exchange responses to a band-limited random noise input (6-25 Hz) were studied in 5 anesthetized and paralyzed cats. Gas exchange was assessed by arterial blood gas analysis. Data in all animals verified the importance of the two parameters which determined flow autocorrelation function: airflow variance (σ_F^2) and bandwidth (B). Arterial CO_2 tension was found to vary linearly as a function of B/σ_F^2 (reciprocal of input noise power). A cyclic input does not appear to be a necessary requirement for HFV. (Supported by: NIH Grants HL16390, HL07012).

51.11

THE ROLE OF MEDIATORS IN THE RESPONSE OF THE CANINE PERIPHERAL LUNG TO 1 PPM OZONE. S.R. Kleeberger, J. Kolbe*, S.P. Peters and E. Wm. Spannhake. The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.

As an intitial step in the investigation of ozone-induced hyperresponsiveness in the peripheral canine lung, we have characterized the response to ozone exposure in terms of physiological response, mediators and pharmacology. Resistance to flow through the collateral system (R_{cs}) was measured using the wedged bronchoscope technique and was used as an index of the peripheral lung response. A 5 min exposure of ozone (1 ppm) through the bronchoscope resulted in a mean increase in R_{cs} of 182% immediately after exposure which returned to baseline after 40 min. Analyses of BAL fluid obtained from the isolated segment 1 min after exposure indicated significant increases, compared with control, in mean ($\pm$ S.E.) concentrations of PGD_2 (135.3 $\pm$ 33.3 pg/ml vs. 47.8 $\pm$ 16.0; P<0.02) and histamine (1.43 $\pm$ .19 ng/ml vs. 1.18 $\pm$ .17; P<0.05). Cyclooxygenase inhibition (indomethacin, 5 mg/kg, IV) significantly attenuated the R_{cs} response by 31% (P<0.05) and H_1 receptor blockade (chlorpheniramine, 5 mg/kg, IV) diminished the response by 16%. Four 5 min exposures of ozone over a 3 hr period yielded reproducible R_{cs} responses and analysis of BAL fluid obtained after this period revealed a significant increase in the proportion of polymorphonuclear leucocytes compared to control (13.0% vs 4.1%; P<0.05). These data suggest that cyclooxygenase products of arachidonic acid and histamine are important in the response of the peripheral lung to 1 ppm ozone, and this exposure also induces an inflammatory response.
Supported by ES03505, HL01141, and HL07534.

51.12

GAS EXCHANGE DURING CONSTANT FLOW VENTILATION IN PIGS. Peter M. Webster*, Anil Menon* and Arthur S. Slutsky. Mount Sinai Research Institute, Univ. of Toronto, Toronto, Ont., M5G 1X5.

One of the hypotheses to explain adequate gas exchange during constant flow ventilation (CFV) is that CFV produces pressure differences within adjacent lung regions leading to ventilation via collateral channels. To examine this hypothesis we studied CFV in 6 pigs (mean wt.=32.5 kg), since pigs have high resistances to collateral ventilation. In 2 pigs we examined quasi-steady state gas exchange and in 4 others we studied transient gas exchange at 3 flow rates (20 to 50 l/min 0_2) and 3 catheter positions (0.5 to 2.5 cm distal to tracheal carina). During steady state runs we were unable to attain normocapnia in any of the pigs, with $PaCO_2$'s around 200 mmHg at all flow rates and all positions. These results were markedly different from our results in dogs in which steady state $PaCO_2$ at these flows varied from 20-50 mmHg. During the transient studies we found no systematic effect of either flow rate or catheter position - the rate of rise of $PaCO_2$ during CFV varied between 1.57 - 7.7 mmHg/min. We conclude that flow through collateral channels may be important during CFV, although the rapidly branching airways in the pig may dissipate the CFV jets faster than in the dog, decreasing their penetration and limiting gas transport. (Supported in part by the MRC (Canada) and the PSI Foundation).

51.13

NONINVASIVE DETERMINATION OF PULMONARY BLOOD FLOW ($\dot{Q}c$) AND DIFFUSING CAPACITY (DLCO) IN THE EXERCISING DOG. J.I. Carlin, P. Clifford, S.S. Cassidy, R.L. Johnson, Jr. Univ. of Texas Health Science Center, Dallas, TX. 75235.

We have developed a rebreathing technique for measuring D_{LCO}, $\dot{Q}c$, oxygen consumption ($\dot{V}O_2$) and lung volume in nontracheostomized foxhounds during exercise on a treadmill. A mouthpiece-face mask as described by Montefusco et al. (Angiology 34:340,1983) was used. A dog rebreathed from a 1 liter bag containing a gas mixture of 0.6% acetylene, 0.3% $C^{18}O$ and 9% He. Disappearance of C_2H_2 and $C^{18}O$ were measured with respect to He to estimate $\dot{Q}c$ and D_{LCO}, respectively. $\dot{Q}c$ measurements were made both at rest and exercise, but due to the low respiratory rate in these animals at rest, D_{LCO} was measured only during exercise. $\dot{Q}c$ was 3.9 ± 0.1 $l \cdot min^{-1}$ (Mean ± S.E.) and increased to 8.4 ± 0.1 $l \cdot min^{-1}$ during exercise at an $\dot{V}O_2$ of approx. 900 $ml \cdot min^{-1}$. These values agreed closely with determinations made with the dye dilution technique at rest (4.2 ± 0.6 $l \cdot min^{-1}$) and exercise (8.8 ± 0.9 $l \cdot min^{-1}$). D_{LCO} measured during exercise was 26.7 ± 0.9 $ml \cdot min^{-1} \cdot mmHg^{-1}$ which is increased from that measured in these dogs in the anesthesized condition (16.1 ± 0.9 $ml \cdot min^{-1} \cdot mmHg^{-1}$). This study demonstrates that noninvasive determinations of D_{LCO} and $\dot{Q}c$ can be made in nontracheostomized dogs. (Supported by NIH PO1-HL06296)

51.14

LINEAR PROGRAMMING ANALYSIS OF THE MULTIPLE INERT GAS ELIMINATION TECHNIQUE : THE AVERAGE DISTRIBUTION. K. S. Kapitan and P. D. Wagner Dept. of Med., UCSD, La Jolla, CA. 92093

The defining equations of the multiple inert gas elimination technique are underdetermined, and therefore an infinite number of $\dot{V}A/\dot{Q}$ ratio perfusion distributions exist which identically fit any given set of retentions. Conventional least-squares analysis with enforced smoothing chooses one member of this infinite set whose features are assumed to be representative in some way of the set as a whole. To test this assumption, the geometric center of the perfusion space, which represents the average of all compatible $\dot{V}A/\dot{Q}$ distributions, was calculated using a linear program to identify the boundaries of the space. The average distribution so obtained was then compared to that recovered using enforced smoothing. This approach was applied with Monte-Carlo error simulation to both measured and theoretical retention data from normal and abnormal lungs. In nine typical cases, the distribution recovered with enforced smoothing closely matched the structure of the average distribution. Thus, the distribution of $\dot{V}A/\dot{Q}$ ratios obtained from the multiple inert gas elimination technique using enforced smoothing is representative in an average sense of the family of distributions which are compatible with given retention data. (Supported by HL01310, HL17731)

51.15

THE EFFECT OF PRESSURE ON GASEOUS DIFFUSION IN He-O_2. C. V. Paganelli, P. R. Sotherland*, and J. Africano*. Dept. Physiol. State Univ. New York at Buffalo, Buffalo, NY 14214

Gas-phase diffusion plays a role in both pulmonary gas exchange and insensible water loss from skin. Both these physiological processes are affected by the reduction in gas-phase diffusivity (D) which occurs in hyperbaric environments. Thus it is important to know the dependence of D on absolute pressure (P). According to the Chapman-Enskog equation, D should be inversely proportional to P; thus the product D·P should be constant as P changes. To test this prediction we measured D ($cm^2\ s^{-1}$ at 37°C) in He-O_2 from 6.8 to 68 ata in a closed diffusion tube. Our data are shown in the table below. (For comparison, D for He-O_2 at 1 ata from the literature averages .782 at 37°C.) When the product of D·P is regressed against P, the slope of the line is not significantly different from 0. Thus our preliminary results show that the measured diffusivity of O_2 in He follows that predicted by the Chapman-Enskog equation within the pressure range of our experiments, which encompasses the pressures encountered in human diving activities.

p, ata	6.8	10.2	13.6	34.0	61.2	68.0
D	.120	.0801	.0596	.0241	.0136	.0126
D·P	.819	.817	.810	.820	.833	.857

Supported in part by NIH grants PO1 HL 28542 and F 32 HL 06950.

51.16

CONVECTIVE AND DIFFUSIVE GAS MIXING ALONG AIRWAYS DURING HIGH-FREQUENCY VENTILATION IN DOGS. M. Meyer*, H. Schulz* and G. Hahn* (SPON: J. PIIPER). Dept. Physiology, Max Planck Institute for Experimental Medicine, Göttingen, F.R.G.

Intra-airway gas mixing during high-frequency ventilation (f = 20 Hz, V_T = 20 ml, 10 L/min bias flow) was studied in 5 anesthetized paralyzed dogs (18 kg mean body wt) using a technique of partial clearance of poorly soluble test gases (C_2H_6 and Ar) combined with a single-breath (SB) procedure. After simultaneous washin of 1% C_2H_6 and washout of resident Ar (0.9%) from lung gas (bias flow containing 1% C_2H_6 in Ar free air) the bias flow was switched to atmospheric air for predetermined time intervals followed by instantaneous interruption of HFV and changeover to a servo ventilator for withdrawal of lung gas at constant rate (SB test). Since C_2H_6 and Ar display similar physical properties, test gas partial pressures recorded against expired volume yielded both the standard SB and reversed SB test performed in the same maneuver. These curves were used to construct a partial pressure vs. volume curve that was unbiased by the effects of continuing respiratory gas exchange. By mathematical analysis using Fick's law and Weibel's symmetric bronchial-tree model scaled for dogs' lung volume, a local effective transport coefficient (D_{eff}) was calculated as function of longitudinal distance in the lung. D_{eff} decreased rapidly from about 10^0 cm^2/sec at the airway opening down the endotracheal tube approaching the molecular diffusion coefficient (D_{mol} = 0.23 cm^2/sec) far distant in the airways around the 20th generation. It is concluded that mass transport along the airways during HFV is dominated by convective mixing processes extending into the alveolar region, diffusion playing a minor role in limiting overall mass transport.

51.17

GAS EXCHANGE DURING NORMOBARIC HYPOXIC EXERCISE IN MAN. **M.D. Hammond*, G.E. Gale*, K.S. Kapitan*, A. Ries* and P.D. Wagner. Department of Medicine, UCSD, La Jolla, CA 92093**

Extending earlier work done at simulated altitude in a hypobaric chamber, we studied gas exchange in six subjects breathing 11% oxygen during exercise at sea level (PIO_2=78 torr, PB=760 torr). Ventilation, cardiac output, $\dot{V}_{O2}$, $\dot{V}_{CO2}$, pHa, P_{O2}, P_{CO2}, blood temperature and $\dot{V}_A/\dot{Q}$ distribution (multiple inert gas elimination) were measured under steady state conditions at rest and three levels of exercise (maximum $\dot{V}_{O2}$=20-30 $ml \cdot kg^{-1} \cdot min^{-1}$, minimum PaO_2=37 ± 4 torr, A-a difference = 19 ± 2 torr). $\dot{V}_A/\dot{Q}$ inequality worsened in all subjects with increasing exercise: mean slope of logSDQ (log standard deviation of perfusion) = 0.10/liter $\dot{V}_{O2}$; p<0.03; mean slope of logSDV (log standard deviation of ventilation) = 0.05/liter $\dot{V}_{O2}$, p<0.05. These results were comparable to those of subjects studied earlier while breathing air in a hypobaric chamber (PIO_2=79 torr, PB=429 torr) where the mean slopes of logSDQ and logSDV were 0.07 and 0.08/liter $\dot{V}_{O2}$ respectively. All subjects also showed evidence for diffusion limitation during normobaric hypoxia, again of a similar degree to that seen previously during chamber experiments. Together, these results suggest that during acute exposure to hypoxia gas exchange is independent of gas density, and therefore altitude simulations using either hypobaria or hypoxic gas mixtures are comparable. (Supported by HL-17731 and the British Columbia Health Care Research Foundation)

51.18

GRAVITY INDEPENDENCE OF PHASE IV OF THE SINGLE BREATH WASHOUT TEST IN DOGS. S. Tomioka*, S.Kubo*, H.J.Guy* and G.K. Prisk* (SPON: J.B.West). Depart. of Med. UCSD, San Diego, CA 92093.

To evaluate the effects of posture on phase IV and regional lung volume distribution, Ar-bolus and N_2 single breath washout tests were examined in 10 anesthetized dogs. Quasi-static transpulmonary pressure-volume curves were measured simultaneously to obtain the lung volume at the pressure inflection point (V_{IP}).

While every phase IV for Ar was upward, phase IV for N_2 was variable (prone; up 1/10, up & small 7/10, down 1/10, down & small 1/10: supine; up 9/10, down 1/10). The displacements from extrapolated phase III (ΔIV_{N2}%, ΔIV_{Ar}% in the table), suggest that the tracer gas concentrations were more uniform across the lungs in the prone than in the supine position. Ar-closing capacities indicate that airway closure seemed to be generated at the same lung volumes in both positions. The smaller V_{IP} than CV_{Ar} might be due to collateral ventilation. Body rotation tests in 3 dogs did not cause an inversion of phase IV except one rotation from supine to prone in one dog. We conclude that gravitational gradients, in dogs, have less influence than difference of regional mechanical properties on phase IV. (Supported in part by PHS grant PO1 H2 17731-11).

	PRONE	SUPINE
CV_{Ar}/VC%	42.6	48.1*
V_{IP}/VC%	30.8	27.0*
CC_{Ar}/TLC%	52.5	55.3
V_{IP}+RV/TLC%	43.5	38.6*
ΔIV_{Ar}%	1.7	4.1*
ΔIV_{N2}%	2.0	5.1*

Values are means. CC: closing capacity, CV:closing volume, ΔIV%: see text, *significant differences (paired t-test).

51.19

USE OF THE EXPIRED BREATH TO MONITOR THE AIR QUALITY IN SEALED CAPSULES. D.R. Knight*, J. O'Neill*, J.S. Bowman* and S.M. Gordon*(SPON: K.R. Bondi) Naval Submarine Medical Research Laboratory 06349-5900

Since submarine atmospheres are contaminated by a complex mixture of hydrocarbons, we evaluated the use of COMPUTER-ASSISTED, GAS CHROMATOGRAPHY/MASS SPECTROMETRY for measuring volatile organic compounds (VOC's) absorbed by exposed crew members. STUDY #1: Teflon sampling bags were inflated by 4 crew members in different compartments of a moored submarine. The reconstructed ion chromatograms were remarkably similar between individuals and showed a complex mixture of 468 VOC's per sample. Benzene overloaded the Tenax GC (R) sample collectors. Thirteen of the 17 highest concentrations of VOC's were acyclic, C_7-C_{11} alkanes (15-55 ppb). STUDY #2(in progress): A Teflon breathing manifold was carried aboard a submarine in order to collect samples of expired gas during patrol. A comparison of VOC's in submarine air with those in the lung-body compartment may indicate the biotransformation of some organic contaminants into benzene. Serial collections of expired air immediately after patrol will permit measurement of the body burden and the time-course of body desorption. Preliminary results have indicated that samples of expired breath can be used for the biological monitoring of men confined in sealed capsules. The progress of this work should yield information for understanding human tolerance of air pollutants in spacecraft.

INTESTINAL AND LIVER PHYSIOLOGY

52.1

THE EFFECTS OF Trichinella spiralis (NEMATODA) INFECTION ON THE CRYPT CELL PRODUCTION RATE IN WISTAR RATS. R.G. Bristow, M.V.K. Sukhdeo and D.F. Mettrick. Dept. Zoology, University of Toronto, Toronto, Ontario, M5S 1A1.

The adults of the nematode Trichinella spiralis live intracellularly within the epithelial cells of the small intestine. The worms produce specific lesions and lesion size is a controllable function of the size of infection. Typically, infection presents a wide range of pathophysiological effects of which derangements in solute uptake have been extensively characterized. A stathmokinetic technique that measures the rate of metaphase arrest was used to quantify crypt cell growth. Infection results in a significant increase in crypt cell growth (> 400%) that is dependent on the size and duration of infection. This study is supported by NSERC grant A4667 to DFM.

52.2

DOES AN ACIDIC MICRO-CLIMATE EXIST AT THE SURFACE OF THE INTESTINE? H. M. Said* and J. A. Blair* (Spon.: H. C. Meng). Vanderbilt University, Nashville, TN 37232 and Aston University, Birmingham, U.K.

We investigated the existence and subsequently determined the distribution and characteristics of the intestinal surface acid microclimate (ISAM) in rat and human. Methods: In the rat, in vitro ISAM pH was measured using a flat 1 cm^2 strip of intestinal tissue. In vivo ISAM pH was measured from opened jejunum of anesthetized rat. In human, ISAM pH was measured in vitro from jejunal biopsies. pH measurements were performed using a glass pH-microelectrode *positioned with a* micromanipulator. Krebs-Ringer phosphate buffer served as physiological medium. Results: In the rat in vitro and in vivo ISAM pH of 5.8 and 6.1 were recorded, respectively, compared to buffer pH of 7.4. Lowest ISAM pH was found in the proximal jejunum (5.8). Progressive increase in ISAM pH was noticed distally reaching neutrality in the distal ileum and colon. Glucose (but not galactose), Na+ (but not other monovalent cations) and normal intracellular metabolism are essential for normal existence and maintenance of the ISAM. The ISAM also exists in human jejunum with a pH of 6.1 compared to buffer pH of 7.4. Conclusions: 1) ISAM is a normal physiological phenomenon of the intestine of rat and man, 2) ISAM is the result of normal intracellular metabolism of the mucosa.

52.3

EPITHELIAL AND MUCOSAL PREPARATIONS OF DOG COLON IN USSING CHAMBERS: COMPARISON OF RESPONSES TO AGONISTS. P.K. Rangachari* and D. McWade* (Spon. E.E. Daniel). I.D.R.U., McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

A novel epithelial preparation of the canine colon devoid of its underlying muscularis mucosa was set up in Ussing chambers. Its responses to added agonists were compared with those of a conventional full mucosal preparation where the responses elicited could reflect a direct action on epithelial cells and/or indirect responses mediated by functioning nerves in the attached submucosa. Both preparations responded to a variety of agonists with changes in short-circuit current (I_{sc}), but clear differences were noted. Oscillations in current seen only with the mucosal preparation, were damped by TTX which also reduced basal current. No such effects were noted with the epithelial preparation. On the mucosal preparation, ouabain produced an initial explosive increase in I_{sc} followed by a sharp decline. The initial increase was abolished by TTX. On the epithelial preparation, only a decrease in current was noted, suggesting the absence of functioning nerves. Histamine increased I_{sc} in both preparations, but the responses in the mucosal preparation were abolished by TTX. Differences were also noted in the responses to 5HT, neurotensin, substance P, VIP and $\alpha_2$adrenoceptor agonists reflecting the existence of functioning nerves only in the mucosal preparation. The two preparations appear suitable for evaluating the contributions of neuronal elements to the responses elicited by different agonists.

52.4

GLUCOSE TRANSPORT BY INTESTINAL BRUSH BORDER MEMBRANE VESICLES OF A EURYHALINE TELEOST: SALINITY ADAPTATION. S. J. Reshkin* and G. A. Ahearn, Univ. Hawaii, Honolulu, HI 96822

Glucose transport by upper and lower intestinal brush border membrane vesicles of the African tilapia (Oreochromis mossambicus) was characterized in fish acclimated to either freshwater or full strength seawater. 3H-D-glucose uptake by vesicles from both gut segments was sodium stimulated, phloridzin (0.1 mM) sensitive and electrogenic. Glucose transport was greater in the upper intestine than in the lower intestine and in seawater animals rather than in fish acclimated to freshwater. Salinity adaptation altered vesicle volume for each intestinal segment with significantly smaller vesicles in the seawater fish. Glucose transport by all membrane preparations was standardized to differential vesicle volumes. Glucose influx (10 sec uptake) involved both saturable and non-saturable transport components. Seawater adaptation increased glucose influx K_t, J_{max}, diffusional permeability (P), and the apparent Na affinity of the cotransport system in both intestinal segments, but the stoichiometry of Na-glucose transfer (1:1) was unaffected by saline condition or gut region. It is suggested that increased sugar transport in seawater animals is due to the combination of inhanced Na binding properties and an increase in number or transfer rate of the transport proteins. Freshwater fish compensate for reduced Na affinity of the coupled process by markedly increasing the protein affinity for glucose.
Supported by NFS Grant PCM83-19973.

52.5

STIMULATION OF NA-INDEPENDENT ALANINE TRANSPORT BY H+ AND CL- IN LOBSTER HEPATOPANCREATIC BRUSH BORDER MEMBRANE VESICLES. G. A. Ahearn, M. L. Grover, and R. E. Dunn, Univ. Hawaii, Honolulu, Hawaii 96822.

3H-L-alanine transport by lobster hepatopancreatic BBMV, formed by a Mg precipitation technique, was Na and K insensitive. Initial alanine entry rates (15 sec uptake) were stimulated and transient alanine uptake overshoots were observed when external pH was acidic and a Cl gradient was imposed across the vesicular wall; at pH_o = 7.4 alanine uptake was reduced in rate and hyperbolic in character. Alanine uptake from an acidic extravesicular medium in the absence of Cl responded to a transmembrane electrical potential difference created by an outwardly-directed, valinomycin-induced K diffusional movement. External 5.0 mM L-lysine and L-serine similarly inhibited the influx and overshoot properties of 0.05 mM 3H-L-alanine uptake, whereas 5.0 mM L-leucine was without effect. Trans-stimulation of alanine influx was observed by vesicles preloaded with 1 mM L-lysine, but not with other amino acids. Alanine influx from acidic media in the presence of a Cl gradient occurred by a combination of carrier-mediated transfer and "apparent diffusion." Decreasing pH_o from 6.0 to 4.0 elevated alanine K_t from 0.55 to 2.64 mM, while alanine J_M increased from 55 to 550 pmol/mg prot/15 sec. Alanine transport across these membranes appears to occur by the classical LYS+ system at acidic pH. The extent of this transport is determined by the magnitude of the transmembrane Cl gradient. Supported by NSF grant no. PCM81-18366.

52.6

INFLUENCE OF A LOW PROTEIN DIET ON THE DISTRIBUTION OF TRANSPORT ALONG THE INTESTINAL VILLUS. C.I. Cheeseman. Dept. of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7

The adaptation of the small intestine to a low protein (LP) diet involves changes in morphology and transport kinetics. In this study quantitative autoradiography was used to measure the profile of transport up the villus for L-leucine and glycyl-L-leucine (glyleu) in protein malnourished 6 week old male Sprague-Dawley rats. Animals were maintained on an isocalorific 5% protein diet for 1, 3 or 10 weeks. The mucosal surface of the jejunum was exposed for 45 seconds to 1 mM tritiated leucine or glyleu and then fixed in glutaraldehyde-sucrose buffer for one hour. 2µm sections were coated with Kodak nuclear track emulsion NTB2 and exposed for 3-4 weeks before development. The distribution of silver grains was quantified with a Vickers M85 scanning microdensitometer using a 30µm window. Control tissue showed the same distribution along the villus for leucine and glyleu uptake, i.e. confined to the top 100µm. After one week LP diet the villi were about 30% shorter yet the transport of both substrates was still confined to the tip region. By 3 weeks the villi had almost returned to normal height and the transport profile was the same as the controls. These data indicate that the decreased leucine uptake and elevated glyleu transport which occurs on a LP diet results from changes in the same enterocyte population and not from an altered profile along the villus.

Supported by a grant from MRC Canada.

52.7

AROMATIC AMINO ACID ABSORPTION IS REDUCED IN AGING. Farhad Navab, M.D., University of Arkansas for Medical Sciences, Little Rock, AR 72205

Because of the critical role of aromatic amino acids as precursors in brain neurotransmitter synthesis the present study was performed to see if age related differences in intestinal absorption could be demonstrated. Two jejunal segments each 20cm in length were isolated and cannulated in male Fischer rats 6,12,and 24 months old under ether anesthesia. After recovery in a restraining cage each segment was perfused with Krebs Ringer phosphate buffer 4ml/min, pH 6.5, 37°C, containing 0.5 µmol/ml of ^{14}C-labeled L-tryptophan (L-Trp) and L-Phenylalanine (L-Phe). ^{3}H-inulin was used as nonabsorbable marker. For both L-Trp and L-Phe absorption was linear over 60 min (r=0.97). The amount of L-Phe absorbed (n=8) at 60 min was 23.03± 0.42, 20.61± 0.77, and 15.36± 0.39 µmol/100cm dry length, and for L-Trp 19.98± 0.24, 16.67± 0.77 and 12.04± 0.76 µmol/100cm dry length in 6,12, and 24 months old animals, respectively. The amount of both L-Phe and L-Trp absorbed at 60 min was significantly reduced ($p<0.05$) in 24 month rats compared to 12 months and in 12 months animals compared to 6 months. Rate of absorption for L-Phe was 0.339, 0.337, and 0.251µmol/100cm/min and for L-Trp 0.330, 0.253, and 0.190 µmol/100cm/min for 6,12, and 24 month animals respectively. Results indicate that reduction in absorption of L-Phe and L-Trp occurs as a continuous process with aging.

52.8

THE EFFECT OF BILE ACID (BA) SECRETION RATE ON BILE SECRETORY PRESSURE. M. Cole*, E. Shaffer. GI Research Unit, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Previous studies to assess bile secretion have used exogenour infusions to maintain constant BA secretion rates. Even though the maximum secretory rate (SRm) in rats for taurocholic acid (TCA) has been reported to be ∿2.0 µM/min/100g, we suspected that toxic effects might occur at lower doses. In this study we examined bile secretory pressure (SP) during exogenous BA infusion. Following bile duct cannulation, unanesthetized restrained rats were perfused IV with TCA at rates chosen randomly from .15 to 2.0 µM/min/100g. To obtain low secretion rates the endogenous bile pool was first washed out by intestinal irrigation with NaCL. In each study, bile flow and bile salt secretion were measured over 90 min. SP was then measured by allowing bile to rise to its maximum level in a column manometer. Results: Bile salt secretion and bile flow showed a linear relationship (r=.72). Plots of bile secretion vs SP demonstrated two different responses. At secretion rates <.15 µM/min/g liver, there was little variation in SP, 22.29±1.90 cm $\bar{X}$±SE. Above .15 µM/min/g liver SP dropped significantly, 18.46 ±2.17 cm ($p<.0005$). There was an inverse relation between BA secretion and SP (r=.65). Higher bile salt secretions (and flow) demonstrated lower SP's. Conclusions: In the physiologic range of bile secretion secretory pressure remains constant. Above this range, secretory pressure drops despite increasing bile salt secretion rates. This represents another characteristic of bile salt toxicity.

52.9

THE ELECTROGENIC EFFECT OF SODIUM TAUROCHOLATE (NaTC) ON ISOLATED RAT HEPATOCYTE COUPLETS. C.E. Bear*, J.S. Davison, E.A. Shaffer. Univ. of Calgary, Calgary, Alberta, Canada.

The electrophysiological basis for bile secretion remains obscure. Using a recently developed model for secretion, isolated hepatocyte couplets (Graf, J., PNAS 81: 6516), we studied the effect of NaTC on transmembrane potential. Potential differences were measured using microelectrodes to impale hepatocyte couplets, maintained in medium (M199) pH 7.4 at 37°C. Untreated cells had a resting potential of -40.4±14.6 mV ($\bar{X}$±SD); n=20. In six studies addition of 20 µM NaTC caused depolarization, 25.5±12.6 mV. This significant ($p<0.005$) change occurred within 3-5 min. of adding NaTC. It was not associated with altered cell viability as assessed by trypan blue exclusion. To determine if NaTC-induced depolarization was due to Na^+ influx, the Na^+ transport inhibitor, amiloride 1.0mM, was added to cell preparations. In 4 studies following depolarization with NaTC, amiloride hyperpolarized the cell, 42.2 ±16.4 mV: significantly below the NaTC depolarization ($p<0.05$) and even the resting potential ($p<0.05$). The addition of amiloride in the absence of NaTC (n=5) induced hyperpolarization of 17.5±5.1 mV ($p<0.01$) suggesting that amiloride may block electrogenic sodium transport in resting and NaTC-stimulated cells. In conclusion, NaTC appears to stimulate electrogenic Na^+ influx in isolated rat hepatocyte couplets. The underlying mechanism of this stimulation remains unclear. (This work supported by the Medical Research Council of Canada and the Alberta Heritage Foundation)

58.1

ROLE OF PHRENIC AFFERENTS IN THE RESPONSE TO INCREASES IN LUNG VOLUME. Scott Wilson* and Steve Iscoe. Department of Physiology, Queen's University, Kingston, ON, K7L 3N6.

Typical responses to an increase in end-expiratory lung volume in anesthetized animals and conscious man include an increase in phrenic or diaphragmatic activity. Such a response aids in compensating for the decrease in inspiratory muscle length and appears to be reflexogenic in nature, not chemical. The afferents responsible for this response are unknown. To clarify the role of phrenic afferents in eliciting this response, we increased the end-expiratory lung volumes of spontaneously-breathing, anesthetized cats with an expiratory threshold load (ETL) of 10 cm H20 while monitoring changes in phrenic nerve activity. Application of the ETL resulted in an average 63% increase in peak amplitude of "integrated" activity. Increases were unrelated to changes in arterial PCO2. After bilateral dorsal rhizotomy (C4-C7), the increase in peak amplitude to phrenic activity persisted and did not differ significantly from that obtained in intact cats. In neither condition did the average duration of phrenic discharge (inspiratory duration) change during breathing on the ETL. These results indicate that phrenic afferents are not necessary for the increase in phrenic nerve activity that occurs when end-expiratory lung volume is increased by ETL. Supported by the MRC.

58.2

LARYNGEAL MECHANORECEPTOR RESPONSE TO COLD AIR. O.P. Mathew, F.B. Sant'Ambrogio and G. Sant'Ambrogio. Dept. of Physiology and Biophysics and Dept. of Pediatrics, Univ. of Tex. Med. Br. Galveston, Texas 77550

We have previously reported the presence of laryngeal receptors stimulated specifically by cold air. In this study we determined the effect of cold air on laryngeal receptors responding to pressure and contraction of laryngeal muscles. We studied 17 receptors by recording from single fibers of the superior laryngeal nerve in 6 anesthetized dogs spontaneously breathing through a tracheostomy. Air at the chosen temperature was passed through the isolated upper airway. We counted the number of action potentials per respiratory cycle at various laryngeal temperatures and expressed it as a percent of control (lar. temp. 35°-37°C). Nine of the 17 receptors studied were either silenced or markedly inhibited (0%-15% of control) when the laryngeal temperature was lowered by ca. 10°C; 3 receptors were minimally affected (85%-100% of control) and the remaining 5 showed an intermediate sensitivity. The inhibitory effect of laryngeal cooling was most pronounced at temperatures between 32° and 37°C. The most affected receptors showed a considerable hysteresis in their response on rewarming. The reflex bronchoconstriction to upper airway cooling could thus depend not only on the stimulation of laryngeal cold receptors but also on the inhibition of laryngeal mechanoreceptors.
Supported by NIH grants HL20122 and HL01156.

58.3

THE EFFECT OF PYRIDOSTIGMINE ON RESPIRATORY FUNCTIONS, ON BLOOD LACTATE AND PYRUVATE IN DOGS AT REST AND DURING EXERCISE. D. Bassett, A.R. Jayaweera*, W. Ehrlich, T. Guilarte*, H. Abbey. The Johns Hopkins University Medical Institutions Baltimore MD 21205.

One mg/kg pyridostigmine i.m. (pyr) lowered plasma cholinesterase to 40% of control value. The effect of this inhibition on respiration, and on lactate and pyruvate was monitored in 40 experiments with 9 dogs at rest and during exercise (1.5 mph 9° inclination). In resting dogs pyr caused (A) increase in central respiratory drive characterized by an increase in f (22.35/min), in Vmin (3.45 l/min) and a decrease in arterial PCO_2 (1.51 mmHg), (B) an increase in respiratory resistance (1.444 $cmH_2/l/sec$). The elevation of expiratory Ppl (1.99 cmH_2O), the lowering of inspiratory Ppl (2.08 cmH_2O), the increase in Ti/Ttot (0.032) and the increase in Ppl time index (0.707 cmH_2O/min) can be caused by (A) and (B). In the exercising dogs the increase of inspiratory resistance does not reach significance. Pyr enhances lactate in the venous blood of resting and exercising dogs but the lactate/pyruvate ratio remained unchanged. The increase in respiratory drive is probably caused reflexly from the chemoreceptor sites in the blood vessels. The increase in respiratory resistance could be caused by enhanced mucus formation and by parasympathetic bronchoconstriction. In the exercising dogs, enhanced sympathetic activity could mitigate the bronchoconstriction. The lactate and pyruvate values indicated either enhanced glycolytic energy metabolism or decreased utilization of lactate and pyruvate.
Supported by: USAMRDC Contract DAMD 17-83-C-3182

58.4

INHIBITION OF THE DIAPHRAGM BY CUTANEOUS AFFERENTS. F. Bellemare* and N. Garzaniti*. (SPON: M. King). Meakins-Christie Laboratories, Royal Victoria Hospital, McGill University, Montreal, Canada.

Five supine, spontaneously breathing dogs (15-20 kg) were studied under sodium pentobarbital anesthesia (25 mg/kg I.V. corneal reflex abolished). We measured tidal volume (V_T), transdiaphragmatic pressure (Pdi) and the electrical activity of the diaphragm (Edi) and of the parasternal intercostal of the 5th interspace (EIC) with bipolar electrodes implanted intramuscularly. Pinching the skin with forceps in an area of the chest wall about 5 cm above or below the costal margin produced a 52±11% (SE) decrease in V_T and a 82±19% (SE) decrease in Edi and Pdi. In contrast, EIC only decreased by 20±11% (SE). Upon sustained stimulation for 6 consecutive breaths, V_T and Edi showed a partial adaptation to 80±17% (SE) and 36±12% (SE) of their respective control values, while EIC returned to or even above control value at the 2nd or 3rd stimulated breath. This response was independent of intact vagi but was completely abolished after subcutaneous injection of xylocaine at the site of application of the stimulus. Other areas of the chest wall either more caudad or more cephalad elicited a weaker or no response. Repetitive skin stimuli of short duration superimposed on each inspiration (stimulus released in expiration) in vagotomized animals produced a sustained decrease in both Edi and V_T (no adaptation) while EIC remained unchanged. We conclude that the diaphragm can be reflexly and selectively inhibited by the activation of cutaneous afferents in an area of the chest wall where the diaphragm inserts on the ribs. (Supported by MRC Canada and Parker B. Francis Foundation).

58.5

OPPOSITE RESPIRATORY EFFECTS OF PERIPHERAL CHEMORECEPTOR STIMULATION DURING NORMOXIA AND HYPOXIA IN NEWBORN RABBITS. C.M. Schramm*, J.S. Grunstein*, and M.M. Grunstein. Dept. Pediatrics, National Jewish Hospital, Univ. Colorado, Sch. of Medicine, Denver, CO 80262.

Newborn infants have a biphasic ventilatory response to acute hypoxia, wherein transient stimulation is followed by respiratory depression. To determine if the respiratory influence of peripheral chemoreceptor stimulation (PCS) in the newborn may be centrally modulated in the presence of hypoxia, we induced PCS by sodium cyanide (NaCN) infusion in lightly anesthetized newborn rabbits aged 1 to 33 days. The animals were placed in a body plethysmograph to continuously monitor pulmonary ventilation (VE). The VE responses to increasing doses of NaCN (5 to 400 µg/kg) were separately evaluated during normoxia (N) and hypoxia (H) induced by steady-state inhalation of 10% O_2-90% N_2. During N, NaCN infusion established dose-dependent, reproducible increases in VE, with the maximal percent increase ranging from 200 to 480%. During H, NaCN produced dose-dependent decreases in VE, with apnea abruptly occurring in 25% of animals at 200 to 400 µg/kg of NaCN. These effects of hypoxia were readily reversed with return to N. All VE responses to NaCN and to H were abrogated by bilateral carotid body denervation. These observations in newborn rabbits indicate that: (1) during N, PCS with NaCN produces VE stimulation; however, (2) during H, PCS results in VE depression which can lead to apnea. Thus, we speculate that in the acutely hypoxemic newborn, peripheral chemoreceptor input may be centrally modulated or "gated" to inhibitory neurons, resulting in respiratory depression.

58.6

THE EFFECT OF SOMATOSTATIN ON CAROTID BODY NEURAL ACTIVITY. Frank L. Powell and Steven C. Hempleman. Division of Physiology, Dept. of Medicine, University of California, San Diego, La Jolla CA, 92093.

Somatostatin (SRIF) greatly reduces the ventilatory response to isocapnic hypoxia but not hypercapnia in man (Maxwell et al.; Am. Rev. Resp. Dis., 1985). This could result from a depression of either arterial chemoreceptor sensitivity to hypoxia or central integration of afferent information from arterial chemoreceptors. We tested the first hypothesis by recording single unit action potentials from carotid body chemoreceptors in anesthetized Pekin ducks before and after administration of synthetic SRIF (Sigma Chemicals). Duck and human SRIF are chemically identical and avian carotid body cells also have vesicles likely to contain neuropeptides. The birds were unidirectionally ventilated at 3.5 L/min with different concentrations of O_2 and CO_2 in N_2 allowing 3 or 4 point steady state isocapnic O_2 stimulus response curves to be collected in about 20 min. 100 or 200 µg/kg bolus injections of SRIF in 1 ml normal saline or SRIF infusions at 4 or 8 µg/(kg·min) all resulted in a small but consistent decrease in carotid body neural activity; discharge frequency following SRIF was only 79±10% (mean±s.d.) of control at $PaO_2 \simeq 40$ torr and 68±19% of control at $PaO_2 \simeq 90$ torr, $PaCO_2 \simeq 35$ torr in both cases. In two animals there was no effect on the response to isoxic CO_2 changes 30 min after a bolus of SRIF. The effect of SRIF on avian carotid chemoreceptors could be smaller than SRIF effect on man's hypoxic ventilatory response because of species differences, an additional central effect and/or barbituate anesthesia. Further experiments are required to resolve these possibilities. Supported by NIH grants HL-17731 and HL-8L3379.

58.7

PULMONARY AFFERENT ACTIVITY RECORDED FROM REGENERATED NERVE FIBERS FOLLOWING PULMONARY DENERVATION. P.S. Clifford, L.B. Bell, F.A. Hopp* and R.L. Coon. Dept. of Anesthesiology, Med College of Wisconsin and VA Med Ctr, Milwaukee, WI 53193.

The Hering-Breur reflex (HBR) reappears by 12-14 wks after surgical lung denervation in beagle dogs (JAP 54:1451-6,1983). To demonstrate that this is due to reinnervation of pulmonary stretch receptors, we recorded nerve activity from branches of the left vagus in the region previously stripped of all innervation. The HBR was present in 5 beagle dogs studied 19 months following pulmonary denervation. Sectioning the right vagus nerve at the azygos vein did not alter the HBR. The central end of each fiber was sectioned as it joined the vagal trunk and a single or few fiber nerve bundle was placed across a bipolar hook electrode connected to a preamplifier/filter amplifier system. Multifiber pulmonary afferent activity was observed in all 5 dogs with single fiber activity observed in 3. When all pulmonary branches of the left vagus were sectioned, as in the original denervation surgery, the HBR was abolished. In 2 additional dogs, recordings were made from few fiber nerve bundles of the left cervical vagus. Nerve activity was increased during gentle stroking of the surface of the left upper and lower lobes, indicating innervated receptors in both lobes. These data demonstrate that reinnervation of pulmonary stretch receptors does occur and provides evidence that reinnervation of these receptors is responsible for return of the HBR following pulmonary denervation.

(Supported by VA Research)

58.8

RESPIRATORY RESPONSES TO MESENTERIC NERVE STIMULATION. S.B. Gottfried*, A.F. DiMarco*, M.D. Altose. Case Western Reserve University, Cleveland, OH 44109.

Mechanical stimulation of abdominal visceral afferents affects the pattern of respiratory muscle activation. In this study, we evaluated the effects of direct electrical stimulation of the mesenteric nerves on inspiratory muscle activation and thoracoabdominal motion. In 4 thiopental-chloralose anesthetized dogs, diaphragm (DI), parasternal intercostal (IC) and upper airway muscle (alae nasi, genioglossus, posterior cricoarytenoid) (UA) activities were measured during mesenteric nerve stimulation (10 Hz, 10 ms, 6-12 V). Mesenteric nerve stimulation produced a brief apnea followed by marked reduction of DI activity (23 ± SE 12% control). In contrast, IC and UA activities were enhanced (157 ± SE 17%, 209 ± SE 31% control). These changes were associated with a reduction in inspired volume (77 ± SE 7% control), greater outward rib cage expansion and paradoxical inward movement of the abdomen during inspiration. After vagotomy, mesenteric nerve stimulation produced a brief apnea and a similar reduction in DI (25 ± SE 12% control). However, the enhancement of IC and UA was abolished. This was associated with a greater fall in inspired volume (56 ± SE 13% control). We conclude that mesenteric nerve stimulation has differing effects on the activation of the various inspiratory muscles; non-vagal afferents inhibit diaphragm activity while vagal pathways mediate selective enhancement of IC and UA muscle activation.

58.9

REGIONAL DIFFERENCES IN AMINO ACID NEUROTRANSMITTERS IN THE MEDULLA DURING CARBON DIOXIDE NARCOSIS. R.E. Dutton, P.J. Feustel, A. Szema*, G. Renzi* and V. Shih*. Albany Med. Coll., Albany, NY and Mass. General Hospital, Boston, MA.

Respiratory neurones are excited by glutamate and aspartate, and are depressed by GABA and glycine (Toleikis et al., 1979). We measured dorsal and ventral medullary content of these amino acid neurotransmitters in 4 room air control and 7 test dogs that received inspired O_2/CO_2 mixtures which raised Pa_{CO_2} to narcotic levels (117-145 torr) for one hour under pentobarbital anesthesia. Significantly lower amino acid content in ventral medulla for 3 of these neurotransmitters was as follows:

(uM/liter)	Dorsal	S.E.	Ventral	S.E.	P
γ-aminobutyrate	969	61	440	35	<.01
glycine	2,587	82	1,525	166	<.01
aspartate	1,521	136	1,190	118	<.05
glutamate	4,192	129	3,459	258	n.s.

Hypercapnia for one hour led to significant decreases in aspartate in both dorsal (from 2,218 ± 524) and ventral (from 1822 ± 362 M/l) medulla; however, a significant decrease in glutamate only occurred in ventral medulla (from 5173 ± 512 μM/l in control animals). Thus, reduced ventilatory sensitivity to inspired CO_2 or endogenously produced CO_2 may be due to reductions in amino acid excitatory neurotransmitters.

Supported by Grants HL 12564 and GM 15426, USHHS.

58.10

NOCTURNAL PERIODIC BREATHING AT ALTITUDES OF 6300 AND 8050 METERS. John B. West, Richard M. Peters, Jr., Gunnar Aksnes, Karl H. Maret, James S. Milledge and Robert B. Schoene. American Medical Research Expedition to Everest, and Section of Physiology, UCSD, La Jolla, CA 92093.

Nocturnal periodic breathing was studied in eight well-acclimatized subjects living at an altitude of 6300 m (P_B 350-352 torr) for 3 to 5 weeks, and in 4 subjects during one night at 8050 m altitude (P_B 281-285 torr). The measurements at 6300 m included tidal volume by inductance plethysmograph, arterial O_2 saturation by ear oximetry (calibrated by arterial blood samples), ECG and electrooculogram. At 8050 m, periodic breathing was inferred from the cyclical variation in heart rate obtained from a night-long ECG record. All subjects at 6300 m altitude showed well-marked periodic breathing with apneic periods. Cycle length averaged 20.5 seconds with 7.9 seconds apnea. Minimal arterial O_2 saturation averaged 63.4% corresponding to a P_{O_2} of approximately 33 torr. This was probably the most severe hypoxemia of the 24 hour period. At 8050 m altitude, the cycle length averaged 15.4 seconds, much longer than predicted by a theoretical model. Abnormal cardiac rhythms such as ventricular premature contractions seldom occurred. No correlation was seen between the strength of the periodic breathing and the ventilatory response to hypoxia. The severe arterial hypoxemia caused by periodic breathing may be an important determinant of tolerance to these great altitudes. (Supported in part by PHS grant RO1-HL24335.)

CORONARY PHYSIOLOGY I

59.1

MECHANISM(S) OF ADENOSINE (AD) RELAXATION IN THE ABSENCE OF CALCIUM IN BOVINE CORONARY ARTERY. S.J. Mustafa, M. Nakazawa* and M.V. RamaGopal*, Department of Pharmacology, School of Medicine, East Carolina University, Greenville NC 27834

The mechanism for the relaxation of smooth muscle by AD is not fully understood. Several possibilities including the inhibition of calcium (Ca^{++}) influx have been proposed. The purpose of this study was to explore the intracellular mechanism(s) of AD action. LAD branches (around 2 mm o.d.) were dissected out from bovine hearts. 2-3 mm wide rings were mounted in 10 ml (PSS) organ bath (95% O_2+5% CO_2, pH 7.4, 37°C) and equilibrated for 1 hr under 0.5 g tension. After obtaining reproducible contraction with 50 mM KCl, the artery was contracted with $PGF_2\alpha$ (10^{-5} M). Concentration-response curves for AD, NECA and L-PIA were obtained in the presence and absence of 8-phenyltheophylline (8-PT, $3X10^{-6}$M) both in the presence and absence of Ca^{++}. ^{45}Ca efflux was measured after loading the tissues for 2 hrs with ^{45}Ca (2 μCi/ml) in Ca^{++}-free medium. AD and its analogs caused relaxation of the coronary artery both in the presence and absence of Ca^{++} with the same order of potency (NECA>L-PIA> AD). The relaxation was competitively antagonized by 8-PT. $PGF_2\alpha$ had no effect on ^{45}Ca efflux, and AD had no increase in efflux of ^{45}Ca during $PGF_2\alpha$ treatment. These data suggest that AD had a relaxing effect independent of inhibition of Ca^{++} influx, and was not due to an increase in Ca^{++} extrusion. It is likely that AD might have an intracellular effect. (Supported by HL 27339)

59.2

INTERSTITIAL ADENOSINE CONCENTRATION IN ISOLATED PERFUSED RAT HEARTS DURING ADENOSINE INFUSIONS. L.J. Heller, D.E. Mohrman and L.J. Sunnarborg*. Univ. of MN, Duluth, MN 55812

Exogenous adenosine (ADO) has been shown to have potent effects upon several cardiac variables. However, because of various degradation and uptake processes, it is not clear what interstitial concentrations of ADO are achieved when ADO is infused and how these compare to those achieved by endogenous production. In the present study, isolated hearts were perfused at constant flow with salt solutions containing ADO concentrations of 0, 1.0 and 10.0 μM. HPLC techniques were used to determine the concentration of ADO in the venous effluent (v) and in samples of fluid that filtered from the vascular bed through the interstitial space to accumulate on the epicardial surface (s). Results are shown below (Mean ± SE, n = 10):

$[ADO]_a$, μM	$[ADO]_v$, μM	$[ADO]_s$, μM
0	0.03 ± 0.01	0.12 ± 0.02
1.00	0.24 ± 0.02	0.12 ± 0.03
10.00	6.15 ± 0.13	2.70 ± 0.33

Note that 1) under control conditions, the surface solution measurements indicate a higher interstitial [ADO] than do venous measurements and 2) the opposite is true during ADO infusion. Thus, $[ADO]_v$ probably underestimates interstitial [ADO] produced by endogenous processes and overestimates that produced by ADO infusion.

Supported by Am Heart Assn (83-790) and NIH (HL-32686).

59.3

INTRACORONARY ADMINISTRATION OF ADENOSINE DEAMINASE DECREASES RESTING CORONARY BLOOD FLOW AND MYOCARDIAL ADENOSINE. H. Fred Downey, Gary F. Merrill, Carl E. Jones and Arthur G. Williams.* Texas College of Osteopathic Medicine, Fort Worth, Texas 76107 and Rutgers University, New Brunswick, NJ 08903.

The role of adenosine in controlling coronary blood flow is controversal. Thus, experiments were conducted to determine if interstitial degradation of adenosine by intracoronarily administered adenosine deaminase (ADA) would alter coronary blood flow and cardiac nucleosides. In 11 open-chest dogs, anesthetized with sodium pentobarbital, flow in the left anterior descending coronary artery (LAD) averaged 38.5±4.1 ml/min under control conditions. ADA was then infused into the LAD at 5 U/kg/min for 10 min with no change in systemic hemodynamic or blood gas parameters. Five minutes after stopping the ADA infusion, LAD blood flow averaged 27.6±3.1 ml/min (P<.01). To determine if this decrease in resting coronary blood flow was associated with altered cardiac nucleosides, samples of myocardium were analyzed for adenosine and inosine. Seven control hearts had adenosine and inosine concentrations of 1.73±0.12 nmol/g and 0.7±0.1 nmol/g, respectively. Seven hearts administered ADA (5 U/kg/min i.c. for 10 min) had adenosine and inosine concentrations of 0.57±0.18 nmol/g and 2.46±0.56 nmol/g respectively. We conclude that, under resting conditions, coronary flow is directly related to the interstitial concentration of adenosine.

Supported by NIH grants HL35027 and HL29232 and by the Cardiology Fund.

59.4

ADENOSINE DEAMINASE ATTENUATES NOREPINEPHRINE-INDUCED CORONARY FUNCTIONAL HYPEREMIA. Gary F. Merrill, Carl E. Jones and H. Fred Downey. Rutgers University, New Brunswick, NJ 08903 and Texas College of Osteopathic Medicine, Ft. Worth, Texas 76107.

Adenosine deaminase (ADA) was used to investigate the role of adenosine (ADO) in the coronary functional hyperemia produced by norpinephrine (NE, 0.27±0.07 ug/kg/min,i.c.). Experiments were performed in 10 α-chloralose anesthetized dogs instrumented to measure coronary blood flow (CBF). CBF responses to myocardial ischemia (20-sec LAD occl.) and the exogenous ADO (1-20 ug/kg/min,i.c.) were also compared before and during ADA. Results are presented below.

Before ADA				During ADA			
Control	NE	ADO	RH	Control	NE	ADO	RH
53	128	180	168	59	93*	121*	107*
(4)	(11)	(41)	(53)	(5)	(8)	(24)	(24)

CBF (ml/min/100gm); ( )=S.E.M.; *P<0.01 relative to before ADA.

Heart rate and the rate of left ventricular pressure development increased significantly with NE before and during ADA administration. Accompanying the attenuated CBF response to NE was a significant reduction in local myocardial oxygen consumption ($M\dot{V}O_2$ 19.8±1.3 vs 14.0±1.4 ml/min/100gm, P<0.01). The ratio of the change in CBF to the change in $M\dot{V}O_2$ during NE was 7.1±0.8 and 4.7±0.4 (P<0.01) before and during ADA, respectively. These findings support a role for ADO in the coronary functional hyperemia produced by NE.

Supported by USPHS grants HL29232, HL35027.

59.5

MEASUREMENT OF INTERSTITIAL FLUID ADENOSINE CONCENTRATION BY AN EPICARDIAL CHAMBER DURING DIFFERENT LEVELS OF CARDIAC INOTROPY. Jeffrey M. Gidday,* Sander van Cleeff,* Rafael Rubio and Robert M. Berne. Department of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Fundamental to resolving the role of adenosine (ADO) in coronary blood flow (CBF) regulation is the measurement of interstitial fluid (ISF) concentrations of the nucleoside under diverse metabolic conditions. To this end, we utilized a $2cm^2$ plastic chamber that retains 200µl of Krebs-Henseleit solution in a 1mm layer on the surface of the left ventricle. Removal of the sample after various periods of contact time, and analysis by HPLC, yielded the following equilibration data: Under control conditions, ADO reached a steady state concentration of 201pmol/ml in about 4 minutes. Continuous infusion of the β_1 agonist dobutamine increased dP/dt to 210% of control, and increased the steady state concentration of ADO to 377pmol/ml. During a second control period established after the dobutamine infusion, the steady state concentration of ADO returned to 189pmol/ml. The rate of ADO equilibration was independent of the level of cardiac metabolic activity. These findings indicate that ADO is present in the left ventricular ISF at concentrations consistent with previously reported estimates, and suggest that increases in CBF associated with augmented cardiac metabolic activity may be mediated by increases in ISF ADO concentration.

(supported by NIH grant HL 10384-19).

59.6

ASSOCIATION OF CYTOSOLIC GLUTAMIC DEHYDROGENASE WITH THE PURINE NUCLEOTIDE CYCLE IN RAT HEART. Ronald L. Jenkins* and Huey G. McDaniel. VAMC Birmingham, Alabama 35233.

Heart cytosol, 7.5 mg protein/ml, was incubated at 30°C in imidazole-HCl 27mM pH 7 with AMP .35mM, ATP .2mM, GDP .24 mM, MgCl 8.3 mM and either aspartate 3mM or α-ketoglutarate 3 mM and NADPH .23 mM. Every 15 minutes .4ml samples were extracted with 6% perchloric acid. Nucleotides and nucleosides were analyzed by HPLC and ammonia, aspartate, glutamate and malate by enzymatic assays. AMP deamination proceeded at an initial rate of 7 um/min/gm dry wt, which was comparable to the liberation of ammonia. The principal route of AMP degradation was AMP→IMP + NH_3→inosine→hypoxanthine. After 120 min of incubation phosphoenolpyruvate 7.9mM was added to stimulate formation of GTP by endogenous PEP carboxykinase. The regeneration of AMP proceeded linearly at .67 um/min/gm dry wt. Due to the cyclic operation of the purine nucleotide cycle aspartate utilization was twice this rate (1.4 um). In the presence of α-ketoglutarate and NADPH instead of aspartate, the rate of AMP regeneration was the same. Glutamate and aspartate levels paralleled reaching peak values just prior to PEP addition. GTP levels tended to fluctuate opposite to these amino acids.

Conclusion: The most probable route by which α-ketoglutarate could supply aspartate to the purine nucleotide cycle is by amination to glutamate by cytosolic glutamic dehydrogenase (McDaniel AJP 246:H483,1984) followed by transamination to aspartate. This enzymatic route could explain the protection of glutamate perfusion on nucleotide levels in ischemic hearts.

59.7

ELECTROMECHANICAL DISSOCIATION (EMD) AFTER COUNTERSHOCK: HEMODYNAMIC EFFECTS OF EPINEPHRINE (EPI) AND ISOPROTERENOL (ISO). K. Haynes,* J. Niemann,* D. Garner,* G. Jagels,* and M. Laks, Harbor-UCLA Medical Center, Torrance, CA 90509

Asystole or EMD may follow countershock of ventricular fibrillation (VF) in >50% of cases. The adrenergic agonists EPI and ISO are recommended in the management of these rhythm disturbances but their effects have not been studied in this common clinical situation. The purpose of this study was to assess the hemodynamic effects of these adrenergic agents in a clinically relevant animal model. Micro-manometric aortic (AO) and right atrial (RA) pressures, coronary perfusion pressure (AO-RA), and coronary sinus blood flow (CSQ) were measured in 8 dogs after 2 min VF followed by countershock. A nonperfusing rhythm always followed countershock. If restoration of spontaneous circulation (ROSC) did not follow 2 min. of CPR alone, EPI (40 ug/kg) or ISO (2 or 4 ug/kg) was given and CPR continued. The hemodynamic effects of these adrenergic agonists are summarized in the table:

	Ao syst	Ao diast	RA diast	CPP	CSQ	ROSC
CPR	86±34	26±7	8±6	19±5	12±3	0/12
CPR+ISO	84±34	22±8	8±6	15±5**	9±4*	1/12
CPR	78±43	24±9	7±6	17±6	12±4	0/12
CPR+EPI	103±28*	49±21	9±6*	48±25**	37±21**	14/14**

*p<.05 **p<.001

ROSC followed ISO + CPR in only 1/12 (8%) of study episodes. EPI + CPR restored circulation 14/14 (100%) of study episodes. Conclusions: 1) Isoproterenol is of no value in the management of postcountershock rhythm disturbances, 2) epinephrine is the drug of choice in the treatment of postcountershock rhythm disturbances.

59.8

MAINTENANCE OF REGIONAL STROKE WORK DURING CORONARY ARTERY OCCLUSION IN THE CHRONICALLY SYMPATHECTOMIZED VENTRICLE. H.J. Mass,* P.A.Gwirtz, I.Y.S.Liang, and C.E. Jones. Dept. of Physiology, Texas College of Osteo. Med., Fort Worth, Texas.

Previous work indicated that chronic ventricular sympathectomy improves collateral perfusion and preserves myocardial function during acute coronary artery occlusion (CAO). In the present study, myocardial segment length (SL) x left ventricular pressure (LVP) loop areas were analyzed in the circumflex region of conscious dog hearts during 2 min circumflex CAO. 10 nonsympathectomized controls (NS) and 6 hearts which had undergone ventricular sympathectomy 2 (S2), 4(S4), and 8 weeks (S8) earlier were used. HR before and during CAO was similar in all groups (P>0.05). End diastolic segment lengths before CAO were similar in all groups (P>0.05), but SL x LVP loop areas before CAO were less in S2, S4, and S8 than in NS (P<0.05). Loop areas during CAO (expressed as % preocclusion value) were as follows (Mean±SE):

	NS	S2	S4	S8
Preoccl.	100	100	100	100
30 sec CAO	43±5	42±12	71±14*	87±17*
60 sec CAO	16±4	62±17*	91±17*	98±15*
120 sec CAO	8±2	67±18*	85±7*	89±12*

*Indicates P<0.05 when S2, S4, or S8 compared to NS.

The results suggest that preocclusion regional stroke work is less in the chronically sympathectomized ventricle but is maintained during acute CAO (supported by NIH HL-29232 & HL 31144.

59.9

TEMPERATURE EFFECTS ON THE DEPLETION OF MYOCARDIAL CATECHOLAMINES IN THE ISCHEMIC RAT HEART Frank Sellke* and Robert Debski*. Akron City Hospital, Akron, Ohio 44309. Daniel Ely. University of Akron, Akron, Ohio 44325

The hearts of anesthetized (IP Brevital) male, Wistar rats were rapidly excised and immersed in Krebs-Henseleit (K-H) solution at 0°C (n=8), 15° (n=8), and 37° (n=8), or placed in K-H moistened gauze at 37° (n=4). Left ventricular myocardial biopsies were performed at intervals up to 360 min. Specimens were weighed, homogenized in perchloric acid, buffered, and frozen at -20°C. Norepinephrine (NE), Epinephrine (E) and Dopamine (D) levels were determined by radioenzymatic assay. At 37°, NE increased to 1.56+.23 (mean of ratios of concentration/initial concentration) ($p<.05$), then decreased to .52+.07 ($p<.01$) at 225 min. NE depletion was prevented by cooling to 0° or 15°. At 225 min. E fell to .41+.14 ($p<.001$) at 37°, and to .74+.05 ($p<.05$) at 15°. At 0°, E rose to 1.49+.14 ($p<.05$) at 90 min., then decreased to 1.01+.20 at 225 min. At 37°, D fell to .43+.15 ($p<.01$) at 225 min. D decreased to .40+.19 ($p<.05$) at 15°, and to .45+.11 ($p<.01$) at 0° at 225 min. A rapid reduction in tissue levels of NE, E, and D occurs at 37° with a 50% reduction in total catecholamines at 225 min. Depletion of E is temperature dependent, while that of D appears to be independent of temperature from 0° to 37°. NE levels are maintained for 360 min. at both 0° and 15°. Depletion is due primarily to conversion or degradation of catecholamines, not due to leaching into solution.

59.10

EFFECTS OF DOBUTAMINE ON CORONARY RESISTANCE AND CAPILLARY PERMEABILITY-SURFACE AREA DURING REDUCED-FLOW ISCHEMIA IN DOGS. **K.A. Overholser, J.C. Collins, T.R. Harris, N.A. Pou*, and P. Herrero*. Departments of Chemical Engineering, Electrical/Biomedical Engineering, and Medicine, Vanderbilt University, Nashville, TN 37235.**

In 12 anesthetized, open-chest dogs we studied the effect of dobutamine (D) on resistance to myocardial blood flow (R) and sucrose permeability-surface area (PS). We used the multiple-tracer method in which a bolus of mixed isotopes was injected into a carotid-to-left anterior descending coronary artery (LADCA) shunt. Measurements were made at the ends of 2 periods: (a) 40 min of reduced-flow ischemia during which LADCA flow was maintained at 1/3 baseline; (b) 40 additional min of reduced LADCA flow plus IV infusion of D (6 dogs), 15.4 ± 9.8 µg/kg/min, mn ± sd) or of saline only (6 control dogs). Results include:

Group	Period	Resistance (mmHg/ml/min)	Sucrose PS (ml/min)
Control	(a) Ischemia	1.02 ± .60	7.32 ± 1.69
	(b) Ischemia/Infusion	.92 ± .53	7.01 ± 2.47
D	(a) Ischemia	1.30 ± .56	9.34 ± 3.82
	(b) Ischemia/Infusion	.78 ± .30*	9.26 ± 4.01

*different from ischemic value, $p < .05$

Although D reduced coronary resistance during ischemia, it did not change PS. Assuming that recruitment of functioning capillaries would have been reflected in an increase in PS, we conclude that D lowered resistance primarily in larger vessels rather than in the capillary bed. (Supported by a grant from Eli Lilly.)

59.11

ENDOTHELIUM-INDEPENDENT CONTRACTION OF CANINE CORONARY ARTERY TO AGGREGATING HUMAN PLATELETS. Donald S. Houston*, John T. Shepherd and Paul M. Vanhoutte, Department of Physiology, Mayo Clinic, Rochester, MN 55905.

Aggregating human platelets induce endothelium-dependent relaxations in isolated canine coronary artery rings, principally due to release of ADP and ATP. In contrast, in rings without endothelium at basal tension, platelets induce a contraction. Treatment of the rings with methiothepin, a competitive antagonist of serotonin-induced contraction of coronary arteries, abolished the platelet-induced contraction without affecting thromboxane A_2 formation (as assessed by radioimmunoassay of thromboxane B_2 in the organ bath fluid after addition of the platelet suspension) or serotonin release (as determined by HPLC of a sample of bath fluid taken 5 min after addition of platelets). Incubation of the platelets with the thromboxane synthetase inhibitor, dazoxiben (which markedly inhibited thromboxane B_2 release) also inhibited the contractile response to platelets without affecting serotonin-release or the contractile response to separately administered serotonin. These experiments suggest that both serotonin and thromboxane A_2 released from aggregating human platelets may contribute to platelet-induced contraction in the coronary artery. (Supported in part by NIH grant HL 05883.)

59.12

ATRIOPEPTIN II IS A CORONARY VASOCONSTRICTOR. John E. Chimoskey, Roger D. Wangler, Babetta A. Breuhaus*, Hossain H. Saneii* and Harvey V. Sparks. Dept. of Physiology, MI State University, E. Lansing, MI 48823, and Dept. of Cardiovascular Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Atrial natriuretic peptides (ANP) lower arterial blood pressure (BP) and cardiac output (CO). ANP is also a coronary vasoconstrictor in isolated Langendorff-perfused guinea pigs hearts. During constant pressure perfusion, the threshold dose for a single pass through the heart is 5 nmol, the ED_{50} is 22 nmol, and flow nearly ceases at 100 nmol. Neither norepinephrine, .6 nmol, nor adenosine, 10 nmol, can overcome the vasoconstriction. Verapamil, 1.0 umol, can. Table 1. Responses to 35 nmol ANP during constant pressure or constant flow conditions (n=5 and 7 replicates in 5 and 3 hearts, respectively). Values are x̄+SEM. * $p<.05$; ** $p<.01$.

	Constant Pressure			Constant Flow		
	Before ANP	After ANP	% Change	Before ANP	After ANP	% Change
Perfusion P(mmHg)	46	46	--	48+2	71+2**	49
Coronary flow (ml/min/g)	5.5 +.8	1.5** +.4	-74	5.0 +0.3	5.0 +0.3	--
LVP (mmHg)	105+5	57+7**	-53	104+4	81+4**	-22
+LV dP/dt max (mmHg/sec)	1618 +126	1058** +154	-36	1224 +59	1137 +46	-7
-LV dP/dt max (mmHg/sec)	1168 +49	635** +73	-46	1050 +71	871* +75	-17

Vasoconstriction may contribute to the fall in CO. HL-30239.

COMPARATIVE PHYSIOLOGY: LOCOMOTION AND OSMOTIC-IONIC RELATIONSHIPS

60.1

PHYSIOLOGICAL BASIS OF SLOW LOCOMOTION IN CHAMAELEONS. Younis A. Ghalyun*, Thomas E. Hetherington, Lewis Greenwald, and Abbot S. Gaunt.

Neither in the laboratory nor in nature can chamaeleons be stimulated to move faster than speeds on the order of meters per minute. Most other lizards typically can move at speeds on the order of meters per second. To explain this difference in locomotion, we measured force velocity curves for biceps muscle and analyzed muscle fiber type distribution in various limb muscles from Chamaeleo senegalensis and Agama agama (a fast lizard closely related to the genus Chamaeleo). At 32° C, the preferred body temperature for C. senegalensis in the laboratory, the Vmax of the chamaeleon biceps is 1.14 L_o/sec. The Vmax of Agama biceps at 37° C (the preferred T_b for A. agama in the laboratory) is 4.37 L_o/sec. Using a myosin ATPase histochemistry assay for fiber type we found that C. senegalensis limb muscles had about 65% tonic fibers. In Agama limb muscle approximately 20% of the fibers were of the tonic type and 80% were twitch. Thus the difference in speed between Chamaeleo and Agama can in part be explained in terms of differences in Vmax and tonic/twitch ratio. Supported in part by the Ohio State University Graduate School and NIH grant 1-R 23-NS21998 to TEH.

60.2

MITOCHONDRIAL DISTRIBUTION IN RAT SOLEUS AFTER SPRINT AND ENDURANCE EXERCISE TRAINING. S.R. Kayar, K.E. Conley and H. Hoppeler*. Institute of Anatomy, Univ. of Bern, 3000 Bern 9, Switzerland

Rats were subjected to 6 weeks of sprint (S) or endurance (E) running to determine if these programs induced differences in mitochondrial distribution in soleus. S rats ran on a laddermill for 5 min up to a speed that elicited $\dot{V}O_2max$, 1 d/week. E rats ran for 25 min, 5 d/week at 85% $\dot{V}O_2max$. Rats were anesthetised, solei removed, weighed, immersion-fixed and processed for electron microscopy. From cross-sections, fiber areas and volume density of interfibrillar mitochondria (V_V(mi,fim)) were estimated by point-counting. Mitochondria were analyzed in four locations within fibers: next to a capillary, half-way to the center, at the center, and at the border between capillaries. V_V(mi,fim) in E and controls (C) decreased with distance toward the fiber center, whereas V_V(mi,fim) in S was uncorrelated with distance (n=3 each). Absolute mitochondrial volume was 50% higher in E than in C at all locations, but was 80% higher in S than in C only at the fiber center. Thus mitochondrial distribution was significantly different in S compared to E, which may reflect differences in substrate or high-energy phosphate flux in these fibers. Sup. Swiss NSF.

60.3

MUSCLE RESPIRATION LIMITS AEROBIC CAPACITY OF GOATS. J. H. Jones, S. L. Lindstedt, K. E. Longworth*, R. H. Karas*, and C. R. Taylor. Concord Field Station, Museum of Comparative Zoology, Harvard Univ., Old Causeway Rd., Bedford MA 01730.

The morphometrically (MM) determined diffusing capacity for O_2 (D_LO_2) in goat lungs appears to be far in excess of the animal's needs during maximal rates of O_2 uptake ($\dot{V}O_2max$) because a Bohr integral model indicates that the blood is saturated with O_2 after only 1/3 to 1/2 of its transit through the pulmonary capillaries. The MM D_LO_2 therefore predicts that goat blood PO_2 will equilibrate with P_AO_2 down to an F_IO_2 of approximately 0.14. In these experiments goats breathed hypoxic gas mixtures while exercising at $\dot{V}O_2max$ in order to determine the validity of the MM D_LO_2 as well as the rate limiting step for the flux of O_2 from air to mitochondria. $\dot{V}O_2max$ did not decrease until F_IO_2 was < 0.14. $P_{\bar{v}}O_2$ and $P_{\bar{v}}CO_2$ remained constant at all F_IO_2s while P_aO_2 and C_aO_2 decreased linearly. Normoxic $\dot{V}O_2max$ was maintained until P_aO_2 < 50 torr and C_aO_2 < 80% the normoxic value. At these levels of hypoxia O_2 delivery to the tissues was maintained by a > 20% increase in cardiac output, which offset the decreased C_aO_2. These data appear to validate the MM estimate of D_LO_2 and unequivocally demonstrate that $\dot{V}O_2max$ in normoxic goats is not limited by ventilation, pulmonary gas exchange, cardiac output, nor tissue capillary gas exchange, but rather by the inability (or insufficient quantity) of muscle mitochondria to utilize all of the O_2 that is available to them. (Supported by NSF grant PCM-83-17800 and Am. Heart Assoc. grant 84-055006.)

60.4

DETERMINANTS OF STRUCTURE/FUNCTION RELATIONS IN THE RESPIRATORY SYSTEM: SUFFICIENCY vs. LIMITATION. S.L. Lindstedt, J.H. Jones, H. Hoppeler*, and H.A. Thronson, Jr.* Univ. of Wyoming, Laramie WY 82071.

The respiratory system has been described as a series of cascading resistances from the lung to the final oxygen sink in tissue mitochondria. The flow of oxygen across each resistance step is driven by a gradient of oxygen partial pressure. Are each of these resistors "tuned" or does one step represent a substantial limit or gate to maximum oxygen flow ($\dot{V}O_2Max$)? Most often, it is speculated that cardiovascular delivery is limiting because experimental manipulation of delivery capacity of blood usually causes an alteration of $\dot{V}O_2Max$. Tissue aerobic capacity may also limit $\dot{V}O_2Max$; skeletal muscle mitochondrial oxygen consumption is essentially constant among various muscles and even across species ($5ml O_2$/ccmito-min). Likewise, adaptation to altitude or endurance results in increased pulmonary diffusing capacity. Thus, any experimental manipulation must be interpreted with knowledge of the interdependence of each step, plus the apparent "limit" may differ temporally and among different species. Finally, the demonstration of structural sufficiency must be distinguished from the demonstration of limitation. Any drop in oxygen uptake in response to an increase in resistance at any step is a demonstration of sufficiency. Any increase in oxygen consumption in response to a decrease in resistance is a demonstration of limitation. These relations are simply modeled both mathematically and electrically. (Supported by Amer. Heart Assoc. 84-055006.)

60.5

EFFECT OF THERMAL ACCLIMATION ON LOCOMOTOR ENERGETICS AND LOCOMOTOR PERFORMANCE IN A LUNGLESS SALAMANDER. Martin E. Feder, Univ. of Chicago, Chicago, IL 60637

To determine the effects of thermal acclimation upon locomotor performance and the rate of oxygen consumption ($\dot{M}O2$) during activity, small (< 3 gram) lungless salamanders, Desmognathus ochrophaeus, were acclimated to three temperatures (5°, 13°, and 21° C) and exercised at various controlled speeds within an exercise wheel while their $\dot{M}O2$ was measured. The $\dot{M}O2$ increased with speed at low speeds (<14 cm/min). Although animals could sustain greater speeds, the $\dot{M}O2$ did not increase further. These small, exclusively skin-breathing salamanders could increase their $\dot{M}O2$ 9-11X during exercise and could sustain nearly half of the oxygen flux expected across a similar surface area of the mammalian lung. However, their maximum aerobic speed was remarkably slow (14 cm/min) and their minimum cost of transport remarkably large (15-17 ml O2/(g km)). Thermal acclimation affected the $\dot{M}O2$ during activity, the maximum sustainable speed, and locomotor stamina in different ways. During exercise at 13° C, cold-acclimated animals had a significantly greater $\dot{M}O2$ than warm-acclimated animals, but did not differ in stamina or the maximum sustainable speed. During exercise at 21° C, cold acclimation did not affect the $\dot{M}O2$ significantly, but it decreased the stamina and increased the rate of lactate accumulation. Thus, these results suggest that thermal acclimation of the $\dot{M}O2$ is not tightly coupled to thermal acclimation of locomotor performance in salamanders. (Supported by NSF Grants BSR83-07081 and PCM84-16121.)

60.6

EXERCISING WITHOUT LUNGS: ENERGETICS AND ENDURANCE IN A LUNGLESS SALAMANDER, PLETHODON JORDANI. Robert J. Full* (SPON: M.E. Feder) Univ. of Chicago, Chicago, IL 60637

Lungless salamanders (4.1g) were exercised on a treadmill enclosed in a respirometer at a range of speeds (0.05 - 0.24 km h^{-1}). Oxygen consumption ($\dot{V}_{O2}$) was monitored continuously. At the onset of exercise $\dot{V}_{O2}$ increased to a "steady-state" in approximately 5 min. $\dot{V}_{O2}$ (ml O_2 $g^{-1}h^{-1}$) increased linearly with speed (S): $\dot{V}_{O2} = 2.19(S) + 0.12$ ($r = 0.91$). The minimum cost of transport (2.2 ml O_2 $g^{-1}km^{-1}$) was slightly lower than predicted for a vertebrate of the same mass. Maximum oxygen consumption ($\dot{V}_{O2}max$) was attained at 0.16 km h^{-1} (maximum aerobic speed), where this $\dot{V}_{O2}$ was 6 times the pre-exercise rates. The net rate of whole body lactate production (WBL) was insignificant at slow speeds, but did increase at submaximal workloads (85% $\dot{V}_{O2}max$). The highest WBL (0.07 mg $g^{-1}min^{-1}$) was measured at a speed (0.20 km h^{-1}) that exceeded the maximum aerobic speed. Actual endurance measurements were consistent with the metabolic data. Salamanders sustained exercise at slow speeds for more than 2h, while at fast speeds the time to fatigue declined to 5-20 min. For P. jordani, lunglessness does not appear to impose severe limitations on the energetics and performance of terrestrial locomotion. (Supported by NSF Grants BSR83-07081 and PCM84-16121.)

60.7

THERMAL DEPENDENCE AND ACCLIMATION OF ORGANISMAL AND MUSCLE PERFORMANCE IN THE SALAMANDER Ambystoma tigrinum nebulosum. A.F.Bennett and P.L.Else* Univ. of California, Irvine 92717

The thermal dependence of organismal performance capacity and isometric and isotonic muscle function in vitro were studied at 10 and 20°C after 3 weeks of acclimation. Quantitative measurements of organismal performance were made from land and water sprint and endurance capacities. Isolated muscle performance was measured on the musculus extensor iliotibialis par anterior, a true extensor of the leg. Organismal performance measurements showed only slight thermal dependence with Q_{10}s of 0.99 to 1.45 for land and water sprint capacities (i.e. max. speed, leg/tail cycling freq.). Land and water endurance capacities (i.e. distance travelled) had thermal ratios of 1.42-1.87. Force generating capacities in isometric twitch and tetanus were temperature independent (R_{10}=0.86 and 1.11 respectively). Rates of force development for both twitch and tetanus and maximal velocity of shortening are highly thermal dependent (Q_{10}s=1.88 to 2.00). Maximum power output is also highly thermal dependent (Q_{10}=2.32). The maximum power output of the muscle regardless of measurement or acclimation temperature occurred at 38% of maximum (tetanic) force output. No organismal performance capacity measured showed any significant differences between acclimation groups. No differences in isotonic contractile performance were found between acclimation groups. -Supported by NSF PCM81-02331 to AFB and CSIRO Postdoctoral Award to PLE.

60.8

PHYSIOCHEMICAL PROPERTIES OF SECRETAGOGUES ISOLATED FROM MOSQUITO HEADS. K.W. Beyenbach, H.H. Hagedorn, and D.H. Petzel. Cornell University, Ithaca, NY, 14853.

High pressure liquid chromatography (HPLC) of a saline extract of mosquito heads (Aedes aegypti) yields three fractions (from a total of 108) with effects on isolated Malpighian tubules. Fraction I depolarizes the lumen-positive transepithelial voltage (V_T) to zero mV without changing the rate of fluid secretion. Fraction II also depolarizes V_T but it stimulates Na, Cl and fluid secretion. Fraction III, the mosquito natriuretic factor (MNF), hyperpolarizes V_T while also stimulating the secretion of Na, Cl and fluid. The three fractions are remarkably thermostable. Repeated freezing and thawing over a period of 110 days, and boiling the fractions for 5 min does not abolish their biological effects on Malpighian tubules. In contrast, incubation with the proteolytic enzyme Pronase destroys biological activity in all three fractions. Elution from BioGel P-4 sizing columns reveals the following molecular weights: 2400 for Fraction I, 2700 for Fraction II and 1900 for MNF. These results show that the three HPLC fractions are low molecular weight peptides which require intact peptide bonds for expression of biological activity. The effects of MNF on NaCl and fluid secretion and tubule electrophysiology are strikingly similar to those of dibutyryl-cAMP corroborating the protein nature of MNF and suggesting the secondary messenger role of cAMP. Supported by NSF PCM-8403305(KWB+HHH) and a National Kidney Foundation Fellowship (DHP).

60.9

STRUCTURAL BASIS OF THE COUNTERCURRENT MULTIPLIER IN THE BIRD KIDNEY. S. Craig Parks* and Lewis Greenwald. The Ohio State University, Columbus, OH 43210.

Countercurrent multiplication in the mammalian kidney is thought to occur via secondary active Cl reabsorption in the thick ascending limbs. Thin ascending limbs lack transport specialization and play a passive role in Na reabsorption. In the mammalian-type nephrons of bird kidneys (in this case, the budgie, Melopsittacus undulatus) the thin descending limbs surround collecting ducts. Cells of the thin limb posess few mitochondria and no basal infoldings. The descending limb thickens prior to the hairpin turn. Transport specialization (mitochondria-lined basal infoldings) begins to appear in the descending limb, near the hairpin turn, and is present in the entire ascending limb. The ascending limb is thick throughout its length, with no thin segment. Thus the cells of the entire avian ascending limb resemble the cells of the mammalian thick ascending limb in terms of posessing extensive mitochondria-lined basal infoldings. The absence of urea in avian urine and the presence of transport specialization in the entire ascending limb suggests that the two solute (urea and Na) passive countercurrent multiplier hypothesis for the mammalian kidney is inapplicable to the bird kidney. The classic single solute countercurrent multiplier model may be more appropriate. The presence of transport specialization in the thick descending limb (near the hairpin turn) cannot be reconciled by either model.

60.10

SALT GLAND FUNCTION IN THE ATLANTIC GREEN TURTLE (CHELONIA MYDAS MYDAS). David M. Hudson* and Peter L. Lutz. School of Marine Science, Univ. of Miami, Miami, FL 33149.

Cannulation of the sea turtle's lachrymal salt gland enabled the collection of primary secretions. Control turtles secreted small volumes of fluid (400 mOs/kg). Turtles injected intraperitoneally with hyperosmotic saline (sea water) secreted copious volumes (max flow = 0.07 ml/min) of concentrated fluid (1600-1900 mOs/kg) within 30 min. Duration of response depended on volume of saline injected; at cessation response declined rapidly. Hyperosmotic sucrose produced an identical response, but onset was delayed 30 min. Isosmotic saline and sucrose produced similar responses in secretion osmotic pressure and flow rate but of shorter duration. Hyposmotic solutions produced no responses. Secretion appears to be an all or nothing response not dependent on increase either in osmotic pressure or specific ions. Analysis of secretions determined approximately equal concentrations of sodium and chloride (800-900 mM), and potassium at 18-21 mM. Surprisingly, secretion was also rich in bromide (1.6-2.5 mM), calcium (15-25 mM), and magnesium (35-50 mM). Magnesium secretion/blood ratio of 15-30 suggests an active Mg^{++} pump in the gland, and that the gland is involved in multi-ion regulation. Calculations imply that most (75-100%) of salt and osmotic loads are excreted by lachrymal salt glands. Supported by MMS contract # 14-12-0001-30063.

60.11

ELECTROLYTE BALANCE IN THE AIR-BREATHING, FRESHWATER TELEOST, SYNBRANCHUS MARMORATUS: EVIDENCE FOR CUTANEOUS ION TRANSPORT. D.F. Stiffler and J.B. Graham*, Calif. State Polytechnic Univ., Pomona, CA 91768 and Scripps Institution of Oceanography, La Jolla, CA 92093

Teleosts transport ions across their branchial epithelium in order to replace urinary losses. S. marmoratus, however, holds air in its branchial chamber for long periods while engaging in aerial gas exchange across its gill surfaces. Since this behavior would interrupt branchial uptake of electrolytes we have conducted a series of experiments on sodium balance in this fish. Experiments were designed to measure isotopic sodium influx, net flux and efflux from whole animals while either water ventilating or air breathing. Urine collections were made to determine renal Na^+ excretion rates. We found Na^+ influx to be low compared to other teleosts with K_m of 0.8 mM and a J_{max} of 56.2 µEq/Kg-h. Sodium effluxes were also quite low averaging 18 µEq/Kg-h for renal losses and 4 µEq/Kg-h for extrarenal losses. The flux ratio could not be accounted for by electrochemical gradients (PD = -7 mV) suggesting active ion transport. Comparisons of Na^+ fluxes during periods of water ventilation and air breathing showed no significant differences. Partitioning of flux measurements between the head (gills and skin) and body (skin) revealed that 75% of the influx occurs across the body suggesting that the skin is involved in ion transport in this fish.

60.12

WATER CONTENT-PERMEABILITY MODELS OF *Periplaneta* CUTICLE: EFFECTS OF DAMAGE. John Machin. University of Toronto, Toronto, Ontario M5S 1A1.

A new gravimetric technique permitting respiratory and integumental water losses to be separated, shows cockroaches taken directly from the culture have damaged cuticles. Cuticular permeability is an order of magnitude higher and water contents marginally lower than in cockroaches which had been isolated for several days. Other properties of importance in modelling are different. The dependance of damaged cuticle permeability on ambient humidity is reminiscent of inner cuticle layers, showing rapid increase in higher humidities. By contrast repaired cuticle permeability decreases in elevated humidity.

Incorporating a parallel pathway representing the damaged area into the model, somewhat improved the accuracy of its predictions. Remaining discrepancies are attributed to the passive effect of a permeable epidermal layer which nevertheless significantly influences cuticle water content. (Supported by Natural Sciences and Engineering Research Council, Canada, Operating Grant A1717).

TEMPERATURE REGULATION I

61.1

PARTITIONAL CALORIMETRIC STUDIES OF SQUIRREL MONKEYS IN A MICROWAVE ENVIRONMENT. E.R. Adair, B.W. Adams* and G.M. Akel*. John B. Pierce Fndn. Lab., New Haven, CT. 06519

In a microwave environment, autonomic thermoregulation in the steady state obeys a modified heat balance equation

$$M + A_{mw} = (R + C) + E \pm S.$$

Metabolic energy production (M) plus absorbed microwave energy (A_{mw}) must be balanced by that lost by radiation (R), convection (C) and evaporation (E), else storage (S) of energy, occurs. When S = 0, partitional calorimetry allows evaluation of the equation. To determine the fate of energy deposited in the body by microwaves, squirrel monkeys were equilibrated to 1 of 3 ambient temperatures (T_a = 20, 26 and 32°C), then re-equilibrated for 90 min in the presence of 2450-MHz microwaves at power densities (PD) of 10, 15, 20 or 25 mW/cm^2 [specific absorption rate = 0.15 (W/kg)/(mW/cm^2)]. Oxygen consumption, RH of expired air, total body weight loss, foot sweating, colonic (T_{co}) and 4 skin temperatures (T_{sk}) were measured continuously. The monkeys achieved thermal balance at all PD in all T_a by mobilizing appropriate thermoregulatory responses: reduced M at T_a = 20°C, increased thermal conductance at T_a = 26°C, and increased E at T_a = 32°C. Responses (e.g. T_{co} vs $\bar{T}_{sk}$), measured with and without microwaves present, were described by identical functional relationships. (Supported by USAF Contract F-33615-82-K-0600).

61.2

OSCILLATING HEAT FLOW FROM THE EAR OF THE RABBIT (ORYCTOLAGUS CUNICULUS). Forrest S. Mohler* and James E. Heath. University of Illinois, Urbana, IL 61801

Infrared thermography of the ear permits direct visualization of vascular adjustments for thermoregulation. Rabbits exposed to 16, 20 and 24°C show surface temperature oscillations at periods (in seconds) of 51.5 ± 18.7, 54.2 ± 19.8, and 56.2 ± 23.1, respectively. By image analysis the image of the ear surface can be divided into 10 isotherms and the area of each calculated. Radiative and convective (convection coefficient = 0.014 cal/cm^2·°C·min) heat loss were summed for all isotherms to calculate an instantaneous total heat loss across the ear. The heat loss was averaged over time at each ambient temperature to give an average heat loss in cal/min · 100 cm^2 (6.94 ± .30 at 16°C; 30.60 ± 1.38 at 20°C; 26.96 ± .72 at 24°C). The rabbit ear may prove a useful model of the oscillatory thermal control system proposed by Gordon and Heath (Comp Biochem Physiol 74A:479, 1983). Supported by training grant PHS 5T32 GM07143.

61.3

THERMOREGULATORY RESPONSES OF THE IMMATURE RAT TO THERMAL STRESS: NOCTURNAL DIFFERENCES. D.E. Spiers. John B. Pierce Foundation Laboratory, New Haven, CT. 06519.

Immature rats were tested to determine if steady-state metabolic and thermal responses to ambient temperature (T_a) were different during light and dark phases of the daily cycle. Pups were maintained with dams in litters of 9 animals and received illumination from 0700 to 1900 h. Tests were conducted at 2, 7, 11, and 15 days of age within a temperature-controlled test chamber. Rats were tested individually in cylinders during exposure to T_a = 25.0, 30.0, 32.5, and 35.0°C. Total test duration was approximately 7 h, with day-tests beginning at 0800 - 0900 h and night-tests of littermates beginning at 2000 - 2100 h. During the test, effluent air from each cylinder was analyzed periodically for oxygen content to provide an estimate of metabolic rate (M). Both colonic (T_{co}) and tail-skin temperatures were continuously monitored during the test. Immature rats at 2 days of age displayed significant differences in M and T_{co} at each test T_a, with higher values noted during the dark phase. Both M and T_{co} differences decreased in magnitude from 7 to 15 days of age, at which time there were no significant differences in the responses. These results indicate that the rat neonate does display light:dark thermoregulatory responses characteristic of the adult and provide evidence to conclude that such differences in response change with age.

61.4

THERMOREGULATION IN SHRs AND WKYs: FEMALE VS. MALE. R. Hess* and K. Taubert. School of Pharmacy, University of Pacific, Stockton, CA 95207.

We have shown that male hypertensive rats(SHR) do not thermoregulate as well as normotensive controls(WKY) and that thermoregulatory ability of both groups decreases with age (Fed Proc 44: 1194, 1985). The present study explores this phenomenon in female rats. SHR and WKY females were separated into age-matched groups of: (A)3-6mos; (B)9-12mos; and (C)>18 mos. Core body temperature(Tc) was measured rectally. Control Tcs were taken and rats were placed in a rat warmer set at 40°C for 2 hr. When control Tcs were compared between strains and age groups, no differences were found. Control Tcs were also compared with our previously studied males and again no differences were noted. When thermoregulatory ability was measured at 2 hrs, Tcs in WKYs showed an increase of 0.9±0.08°C in A, 0.9±0.07°C in B, and 1.7±0.19°C in C. The increase in C was greater than A or B(both $p< 0.02$). For SHRs, Tc increased 1.6±0.14°C in A, 2.5±0.08°C in B, and 4.2±0.36°C in C. The increase in C was greater than A or B and B was greater than A (all $p<0.001$). When these data were compared to data collected in males of the same strain and age, no differences were observed at any time during the 2 hr period. SHRs of both sexes achieved higher Tcs than WKYs in each age group and males and females within either strain show similar decreases in thermoregulatory ability as age increased. Therefore the altered thermoregulatory ability is independent of sex.
(Supp. in part by a grant from the Calif. Heart Assn.)

61.5

THE EFFECTS OF BROMOCRIPTINE ON SWEAT GLAND FUNCTION DURING HEAT ACCLIMATIZATION. F.L.Kaufman,* D.E.Mills,* R.L.Hughson, and G.T.Peake* and L.Hoffman-Goetz. University of Waterloo, Waterloo, Ont. and University of New Mexico, Albuquerque, N.M.

Endocrine involvement in the acclimation of the sweat gland during chronic heat exposure is controversial. Neither manipulation of Aldosterone nor Vasopressin fully explain sweat gland changes which occur. This study examined the possible involvement of Prolactin (PRL) in sweat gland function during heat acclimatization. Two groups of male subjects (n=8) were tested: placebo and bromocriptine treated. Both groups performed cycle ergometer exercise at 50% of maximal oxygen uptake on 10 consecutive days in an environmental chamber at 39°C and 39% relative humidity. Duration of exercise was 90 minutes on days 2-4 and 6-9 and 45 minutes on test days 1, 5 and 10. On test days PRL levels were measured at time 0 (pre), 20 and 45 minutes of exercise. Sodium concentration ([Na+]) in sweat was determined by total body washdown. PRL increased ($p<.01$) during exercise on day 1 in the control group but not on days 5 and 10. In contrast, PRL was suppressed by bromocriptine and did not rise during exercise. The [Na+] in sweat decreased ($p<.05$) in the control group from day 1 to 10 but was unchanged in the treatment group. Sweat volume tended to increase in the control group ($p<.07$) with no change in the treatment group. These data suggest that acclimatization-related changes in sweat gland function may be attenuated by increases in central dopaminergic activity and implicate PRL in sweat gland function.
(Supported by The Heart and Stroke Fdn. of Ontario and NSERC.)

61.6

THE ROLE OF GABA IN THERMOREGULATION. David H. Smullin and John Bligh. Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

Gamma-aminobutyric acid (GABA) is evidently the primary central inhibitory neurotransmitter in thermoregulation, and may mediate reciprocal crossing inhibition between the pathways of heat loss and heat production. The role of GABA was studied using intracerebroventricular (ICV) injections of GABA, its agonist muscimol (Mu) and its antagonist biccuculine-methiodide (Bi) into unshorn sheep at high (41°C) and shorn sheep at low (3°C) ambient temperature (Ta). Both GABA (39 µmol) and Mu (35 nmol) depressed respiratory frequency (RF) at high Ta and oxygen consumption (VO_2) at low Ta. Bi (39 nmol) caused large increases in VO_2 at both high and low Ta. At high Ta Bi caused a biphasic RF response. RF initially fell below the pre-injection rate for 5-15 min followed by a rise above the pre-injection rate that remained high for over 2 hrs. At low Ta, Bi caused a slight rise in RF. Prior ICV injection of Bi at high Ta attenuated the depression of RF by the acetylcholine agonist carbamylcholine (CCh)(1), while maintaining a high VO_2. Prior injection of Bi in the cold attenuated the inhibitory action of 5-hydroxytryptamine (5HT) on VO_2(1) and allowed 5HT to increase RF for a short period. These data suggest that GABA acts as the neurotransmitter of crossing inhibition, and perhaps also of recurrent or convergent inhibition, on the thermoregulatory pathways. (Supported in part by the University of Alaska).
1. Bligh,J.,Cottle,W.H.&Maskrey,M.1971.J.Physiol.212:377-392

61.7

DO SALICYLATES ACT WITHIN THE VENTRAL SEPTUM OF THE RAT TO SUPPRESS PGE HYPERTHERMIA? S.J. Maitland*, A.M. Naylor*, W.D. Ruwe and W.L. Veale. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Perfusion of the antipyretic peptide, arginine vasopressin (AVP), within the ventral septal area (VSA) of the rat has been shown to suppress a hyperthermia evoked by bacterial pyrogen or PGE. In addition, PGE directly injected into this area causes a marked hyperthermia. These experiments were carried out to determine whether an inhibitor of prostaglandin synthesis, acetylsalicylic acid (ASA), might act in this AVP-sensitive area to suppress such a hyperthermia. Stainless steel guide cannulae were implanted stereotaxically above the VSA and a lateral cerebral ventricle (LCV) of male Sprague-Dawley rats (280-325 g). Colonic temperature was monitored continuously. Artificial CSF (aCSF) or ASA (30 µg/µl) was microinfused at 1.0 µl/hr for 1 h prior to and for 1 h following an infusion of PGE_1 (200 ng-2.0 µg/10 µl) into the LCV. With infusion of aCSF, the PGE_1 evoked a hyperthermia of 1.1°C. However, during infusion with ASA, the hyperthermic response was significantly reduced (< 0.6°C). These data suggest that the classical antipyretic, ASA, may act in brain sites in which the endogenous peptide, AVP, exerts a similar action. Moreover, these results indicate that an additional action of ASA may be involved in the fever reducing properties of this drug. Supported by the MRC of Canada.

61.8

VERAPAMIL PERFUSED IN THE HYPOTHALAMUS OF THE CAT ALTERS NORMAL BODY TEMPERATURE. Amir H. Rezvani,* D.B. Beleslin* and R.D. Myers. Univ. of North Carolina School of Medicine, Chapel Hill, N.C. 27514

When the ionic milieu of the posterior hypothalamus (PH) is perturbed in terms of a Ca^{++} imbalance, the body temperature of the animal is markedly affected. In this study, a Ca^{++}-channel antagonist perfused within the hypothalamus was found to act directly on the two distinct regions proposed to underlie the mechanism for thermoregulation, the anterior hypothalamic, preoptic area (AH/POA) and PH. In the unrestrained cat, artificial CSF and either 0.4 or 2.0 mg/ml verapamil were perfused at thermoactive sites by push-pull cannulae at 25 µl/min for 30 min with colonic temperature monitored continuously. Verapamil perfused in AH/POA induced a significant decline in core temperature, but within PH verapamil produced a significant rise in body temperature. Whereas an intense thermogenesis or marked hypothermia was site-dependent, the magnitude of either response was concentration-dependent. It is concluded that the Ca^{++}-channel blocker exerts differential effects on amine-containing neurons within AH/POA by altering the release of endogenous monoamines. In contrast, verapamil's functional effect on the PH is identical to that of EGTA, thus suggesting that any interference with Ca^{++} in PH results in a "set-point" shift with concomitant thermogenesis.
Supported in part by Grant BNS-84-10663 from the National Science Foundation.

62.1

MICROVASCULAR FLOW ADJUSTMENTS WITH POSTURAL CHANGES IN HUMANS. Peter N. Sfakianos*, Alan R. Hargens, and Wayne H. Akeson*. Div. of Ortho. (V-151), Univ. of Calif., La Jolla, CA 92093

Relative changes in lip endothelial capillary flow were monitored continuously by laser doppler velocimetry in 7 normotensive volunteers. They were placed in the following spacial orientations: Upright (0°), head-up (30°), horizontal (90°), head-down (150°), total inversion (180°), return to upright (0°). The subjects were held in each position on a tilt table for 2 minutes. During that time, their pulse and BP were recorded. Relative flow is expressed as a percentage of normal capillary flow achieved in the upright position (100%). It decreased with each change in orientation: 87±4.5% mean ± S.E.M. @ (30°), 76±6.2% @ (90°), 66±6.4% @ (150°), 52±6.6% @ (180°). Flow essentially returned to normal (99±4.5% mean ± S.E.M.) when the subject was returned to an upright position. The subjects pulse generally decreased and their pulse pressure increased (increased systolic, steady diastolic) as the subject assumed a more inverted spacial orientation. Return to an upright position was followed by an immediate and transient increase in pulse beyond resting and return of the BP to baseline (upright position). The microvascular flow adjustments may be secondary to constriction of the precapillary sphincters in responses to an increase perceived pressure head. Loss and reversal of the hydrostatic pressure gradient from head to heart and (to a lesser degree) the centrally mediated increased systolic pressure could account for the increased precapillary transluminal pressure.

62.2

CARDIOVASCULAR DYNAMICS DURING THE FIRST HOUR OF 6° HEAD-DOWN TILT. M.A.B. Frey, C.M. Tomaselli*, R.A. Kenney, and G.W. Hoffler*. NASA Biomedical Office and The Bionetics Corp., Kennedy Space Center, FL 32899; and Dept. of Physiology, George Washington University, Washington, DC 20037.

A 6° head-down tilt induces fluid shifts which simulate weightlessness. Electrocardiogram, impedance cardiogram, systolic time intervals, arterial pressure, and calf circumference were monitored in 12 male subjects, ages 30-39 years, during one hour of 6° head-down tilt to elucidate the early effects of microgravity. Observations indicate a biphasic pattern of response (ANOVA, alpha = 0.05). The initial period was characterized by an elevated stroke volume, cardiac output, and mean stroke ejection rate, and a decrease in heart rate and intrathoracic fluid volume (increased thoracic impedance). The only change in systolic time intervals was an early increase in left ventricular ejection time. Within 15 minutes, this pattern had reversed. The cardiovascular response was thereafter characterized by a reduced stroke volume, cardiac output, and mean stroke ejection rate, and an increase in mean arterial pressure, total peripheral resistance and intrathoracic fluid volume. Calf circumference progressively decreased. The initial response suggests that blood is moving out of the legs and great veins and into extrathoracic spaces. The later trend suggests intrathoracic sequestration of fluid volume, redistributed to the pulmonary vasculature rather than being retained in the great veins.

62.3

DIURESIS, NATRIURESIS AND CARDIOVASCULAR RESPONSES TO WATER IMMERSION IN TRAINED RUNNERS AND SWIMMERS. V.A. Convertino, R.B. Rogan*, D. Hale*, and L. Smith*. The Biomedical Office, NASA, and The Bionetics Corporation, Kennedy Space Center (KSC), FL 32899; and University of Arizona, Tucson, AZ 85721.

The purpose of this study was to determine if the diuresis, natriuresis and cardiovascular adaptations which accompany multi-hour water immersion differ in individuals who undergo hypogravic aerobic training (swimming). Three groups were examined: (1) 6 long-distance runners (R); (2) 5 competitive swimmers (S); and (3) 6 sedentary control (C) subjects. Each subject underwent 5 hr of immersion in water to the neck and a submaximal cycle ergometer exercise test was preformed before and after immersion. Water immersion induced significant increases in urine volume, Na clearance, and a 3-5% decrease in plasma volume. Urine volume during immersion was greater ($P<.05$) in R (2.4 ± .2 ml/min) compared to C (1.3 ± .2 ml/min). However, changes in PV, Na clearance and free water clearance during immersion were not different between R, S, and C. After 5 hr of immersion, there was an increase ($P<.05$) in submaximal exercise HR of 9 ± 3 bpm and 10 ± 3 bpm in both R and C, respectively, but no significant change was observed in S. These data suggest that hypogravic aerobic training may provide a protective mechanism against acute cardiovascular deconditioning which was not associated with the degree of dehydration and associated diuresis and natriuresis.

62.4

MECHANISMS FOR NEGATIVE WATER BALANCE DURING WEIGHTLESSNESS: IMMERSION OR BED REST. John E. Greenleaf. NASA Ames Research Center, Moffett Field, CA 94035

The mechanism for the apparent decrease in body fluid volume in astronauts during spaceflight remains obscure. The widespread postulate that the hypohydration is the result of the Gauer-Henry reflex has not been established with measurements on astronauts. An hypothesis is proposed which accounts for fluid - electrolyte shifts during weightlessness. Upon entering orbit, a moderate but transient increase in central venous pressure occurs that is insufficient to activate the Gauer-Henry reflex, but sufficient to stimulate the release of atrial natriuretic peptides. Increased sodium excretion would facilitate some increased urinary water loss. The resulting relatively dilute plasma and interstitial fluids would cause fluid to shift into the cellular space resulting in edema in the head and trunk and inhibition of thirst and drinking. Thus, the negative water balance in astronauts would be caused by a gradual natriuresis and diuresis, coupled with reduced fluid intake, until the proper equilibrium level of total body water is reached. Responses during immersion and bed rest will be discussed as they relate to this hypothesis.

62.5

VASCULAR SMOOTH MUSCLE ALPHA RECEPTOR RESPONSIVENESS DURING ACUTE ORTHOSTASIS FOLLOWING SIMULATED WEIGHTLESSNESS. C.A. Blamick*, D.J. Goldwater*, and V.A. Convertino. University of Arizona, Tucson, AZ 85721; and NASA-Ames Research Center, Moffett Field, CA 94035.

To determine the effect of simulated weightlessness on alpha-adrenoceptor responsiveness of leg vasculature during lower body negative pressure (LBNP), 10 men (35-49 yr) underwent two randomized graded presyncopal-limited LBNP tests before and after 10 days of continuous 6° headdown bedrest (BR): one test with intravenous saline placebo and one with phenylephrine (PE). Impedance plethysmographic leg pulse volume (LPV), heart rate (HR), and mean arterial pressure (MAP) were measured during resting control (preLBNP) and at 1 min of -30 torr LBNP. BR-induced deconditioning was manifested by decreases ($P<.05$) in plasma volume (17%), maximal oxygen uptake (16%), and LBNP tolerance (17%). BR decreased ($P<.05$) placebo LPV during preLBNP and at 1 min of -30 torr LBNP (-14.8% and -13.3%, respectively). However, BR had no effect on LPV in the PE treatment. LPV during placebo and PE treatments was reduced ($P<.05$) from preLBNP to 1 min -30 torr LBNP by the same degree before and after BR. PreLBNP HR was unchanged after BR, but HR was increased ($P<.05$) at 1 min of -30 torr LBNP postBR in placebo and PE. These results suggest no apparent alteration in vascular smooth muscle alpha-adrenoceptor responsiveness during orthostatic stress after BR.

62.6

DIRECT EVIDENCE FOR A 'NEW' ORTHOSTATIC REFLEX VENOPRESSOR MECHANISM: VENOUS AFFERENT ELICITED POTENTIALS IN LEG MUSCLE MOTONEURONS. F.J. Thompson and B.J. Yates . Dept. of Neuroscience, College of Medicine and Veterinary Medicine, University of Florida, Gainesville, Florida 32610.

Recent findings suggest that leg vein distentions, typical of orthostatic blood shifts, activate stretch sensitive afferents which arise from the walls of leg veins (Thompson et al, 1984). Previous studies suggested that leg venous afferents make reflex connections on leg skeletal muscle motoneurons (Thompson et al, 1982.) The studies reported here examined excitability changes in motoneurons of leg skeletal muscles elicited by activation of low threshold femoral venous afferents in decerebrate spinal cats. Motoneuron intracellular potentials were recorded from identified calf muscle motoneurons. These experiments revealed reflex connections of low threshold venous afferents to leg skeletal muscle motoneurons which produced excitatory postsynaptic potentials of up to several mv amplitude. Since it is known that leg muscle tone is a major factor (venopressor) in the prevention of blood pooling in the legs, these findings are compatible with the hypothesis that 0-G lability of this reflex may account for a portion of decreased muscle tone reported during orbital flight missions and increased blood pooling (orthostatic intolerance) upon return to 1-G (Thornton, NASA Publication,1982).(cont.#F33615-82-0627, Sch. Aerospace Med., Brooks AFB)

62.7

FLUID AND ELECTROLYTE RESPONSE OF A PRIMATE MODEL TO LOWER BODY POSITIVE PRESSURE INDUCED CENTRAL VOLUME EXPANSION. S.E. Churchill, M.E. Natale* & M.C. Moore-Ede, Dept. Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

To simulate the fluid and electrolyte response to weightlessness, we have developed a primate model which uses continuous lower body positive pressure (LBPP,20 torr) to induce a cephalad fluid shift. Seven adult, male, chair-trained squirrel monkeys with indwelling vascular catheters were studied in isolation under both LBPP and control (no LBPP) conditions for the length of a Space Shuttle mission (7 days). LBPP induced a rise in CVP of 1.76±1.16 torr that returned to the pre-LBPP value by 4 hrs. In the following 24 hrs sodium excretion increased from 1.56±.42 to 3.55±.56mEq ($p<.04$), urine volume increased from 29.6±2.8 to 50.2±8.7ml ($p<.02$) and urine osmolality decreased from 1575±129 to 972±104mOsm/L($p<.01$). By day 2 only U_{Osm} was different from control. Potassium excretion was unaffected. Plasma osmolality, sodium, potassium, renin activity, aldosterone and hematocrit were not different from control values on any day. Withdrawal of LBPP caused an immediate fall in CVP of 1.96±1.26 and in $\bar{P}_A$ of 16±3 torr ($p<.01,n=4$). When the day after LBPP was compared to the last day of LBPP, a modest ($p<.05$) antinatriuresis, antikaliuresis and antidiuresis were apparent. We conclude that LBPP-induced central volume expansion causes a reflex diuresis and aldosterone-independent natriuresis to reduce plasma volume. This response is rapid and confined to the first 1-2 days of stimulus, after which a new steady state volume level is reached. On removal of LBPP the redistribution of volume induces a modest renal conservation of fluid and electrolytes.

62.8

CENTRAL VENOUS PRESSURE AND PLASMA ARGININE VASOPRESSIN DURING LOWER BODY POSITIVE- AND NEGATIVE PRESSURE IN MAN. Norsk, P., F. Bonde-Petersen, and J. Warberg. University of Copenhagen, August Krogh Institute Universitetsparken 13, DK 2100, Copenhagen Denmark, and Panum Inst. Med Fysiol. C.

After overnight food and fluid restriction, 9 healthy males were examined in the supine position before, during and after lower body positive (LBPP) and negative (LBNP) pressures of +11±1, -10±1, -20±2 and -30±2 mm Hg respectively (mean=SE). Central venous pressure (CVP) during supine rest was 7.5±0.5 mm Hg, and varied between 13.4±0.8 mm Hg during LBPP and 2.0±0.5 mm Hg during LBNP. Plasma arginine vasopressin (pAVP) did not change significantly in face of this large variation in CVP of 11.4 mm Hg. Mean arterial pressure increased significantly during LBPP from 100±2 to 117±3 mm Hg ($p<0.001$) and from 102±1 to 115±5 mm Hg ($p<0.05$) at 15 min of LBNP at -30±2 mm Hg. Heart rate did not change during LBPP but increased slightly from 51±3 to to 55±3 bpm ($p<0.05$) at 7 min of LBNP at -30±2 mm Hg. Plasma osmolality, sodium and potassium did not change during the experiment. Plasma volume decreased by 3.0±1.1 % ($p<0.05$) during LBPP and by 5.6±1.1 % ($p<0.05$) during LBNP of -30±2 mm Hg. Esophageal pressure, measured during short periods of LBPP and LBNP decreased slightly during LBPP but was unchanged during LBNP. We conclude that cardiopulmonary mechanoreceptors in man do not play a major role in regulation of AVP secretion during short term changes in CVP.

62.9

CARDIOVASCULAR HYSTERESIS DURING LOWER-BODY NEGATIVE PRESSURE C.M. Tomaselli*, M.A.B. Frey, R.A. Kenney, and G.W. Hoffler*. NASA Biomedical Office and The Bionetics Corp., Kennedy Space Center, FL 32899; and Dept. of Physiology, George Washington University, Washington, DC 20037.

This study examined whether cardiovascular variables during lower-body negative pressure (LBNP) are influenced by the previous cardiovascular status and/or LBNP level as well as the concurrent pressure level. Twelve male subjects, ages 30-39 yrs, underwent an LBNP which involved stepwise descent to -50 mmHg followed by stepwise ascent back to 0 mmHg. Fluid shifts and hemodynamic parameters were evaluated using systolic time intervals, impedance cardiography, and measurement of calf circumference. Stroke volume, cardiac output, total peripheral resistance, electromechanical systole, left ventricular ejection time (LVET), pre-ejection period (PEP), PEP/LVET, calf circumference, and thoracic impedance displayed a difference in the overall means between descending and ascending LBNP. Variables that decreased during stepwise descent of LBNP had lower values during the stepwise return; those that increased during stepwise descent of LBNP had higher values during the stepwise return. LVET (msec) serves as an example:

LBNP	0mmHg	-8mmHg	-16mmHg	-30mmHg	-40mmHg	-50mmHg
Descending	315	309	307	284	264	241
Ascending	313	304	293	271	253	

Cardiovascular ability to cope with orthostatic stress may depend on the previous status of the system.

62.10

GRAVITY DEPENDENT INTRAPLEURAL ALBUMIN TRANSFER IN DOGS. G.Miserocchi*,D.Negrini*,M.C.Gilardi*,F.Fazio*,M.Pistolesi*,F.Rossitto*. Ist.Fisiol.Umana,Univ.Milano; Osp.S.Raffaele,Milano; CESNEF, Politecnico Milano, Italy. (SPON: J.Milic-Emili).

We studied in 5 spontaneously breathing anesthetized dogs subject to change in posture, the gravity dependent distribution of 99mTc labelled albumin (2mg/ml in 1ml saline; 250 μCi) injected intrapleurally. After 30 min from injection the animal posture was suddendly changed following three protocols: supine to prone, prone to supine, supine to head-up. Intrapleural vertical label transfer was detected via a suitably placed γ-camera, whose field was divided in 4 superimposed equal regions scanned up to 50min by 64sec acquisition frames. After moving from supine to prone and viceversa, the average activity in the top regions decreased by 28.7%, while it increased by 12.2% in the bottom ones, total activity decreasing by 14.4% over the experimental period. Tilting from supine to head-up resulted in about 13% decrease in top regions with a corresponding equal increase in the bottom ones, total activity remaining essentially constant. Data point to the existence of an intrapleural top to bottom label transfer in all postures studied. A net albumin egress, as mirrored by the decrease in total activity, only occurred in the horizontal postures.

62.11

EFFECTS OF INCREASED FOOT-TO-HEAD ACCELERATION (+Gz) ON CARDIOPULMONARY RESPONSE TO EXERCISE. D.R. Pendergast, A. Olszowka, M.A. Rokitka, B.E. Shykoff* and L.E. Farhi. Dept. of Physiology, SUNY at Buffalo, Buffalo, NY 14214.

We studied the combined effects of increased foot-to-head acceleration (+Gz) and upright leg exercise on O_2 uptake ($\dot{V}O_2$, l/min), cardiac output ($\dot{Q}$, l/min), heart rate (HR, b/min), stroke volume (SV, ml) and arterio-venous O_2 concentration difference [$(a-v)O_2$, ml/100 ml]. Five male subjects sat at rest for 8 min, and then exercised for 8 min at 25, 75 and 175W; on another occasion the exercise levels were 50, 100 and 150W. ECG was monitored continuously, providing HR. In each condition, triplicate measurements of $\dot{V}O_2$ and $\dot{Q}$ (rebreathing technique) were obtained. SV and $(a-v)O_2$ were calculated from these data. At rest, Gz did not affect $\dot{V}O_2$ but decreased $\dot{Q}$ (5.0 ± 1.8 at 1Gz → 4.2 ± 1.2 at 2Gz → 3.8 ± 1.0 at 3Gz), raised heart rate (81 ± 15 → 93 ± 17 → 116 ± 9) leading to a drop in SV (58 ± 13 → 49 ± 8 → 41 ± 8) and an increase in $(a-v)O_2$ (6.6 ± 1.4 → 7.8 ± 2.7 → 8.9 ± 2.3). At exercise, $\Delta\dot{V}O_2/\Delta\dot{W}$ was unaltered, $\Delta\dot{Q}/\Delta\dot{V}O_2$ dropped (7.0 ± 3.0 → 5.9 ± 1.5 → 3.5 ± 1.0), as did $\Delta SV/\Delta\dot{V}O_2$ (34 ± 21 → 25 ± 14 → 15 ± 10), while $\Delta(a-v)O_2/\Delta\dot{V}O_2$ rose (2.1 ± 1.6 → 3.5 ± 1.5 → 6.0 ± 2.5). Of particular interest is the fact that the HR of subjects exposed to 2 or 3 Gz did not increase at 25 or 50W workloads. This indicates that in moderate exercise the beneficial effects of mechanically enhanced venous return overrode those of the increase in metabolic demand.
(Supported by NASA Contract No. NAS9-16042.)

62.12

AEROBIC FITNESS AFFECT ON HEART RATE RESPONSE DURING AIR COMBAT MANEUVERS. Guy R. Banta*, L. C. Meyer*, and C.A. DeJohn* (SPON: W. G. Lotz, Ph.D.) Naval Aerospace Medical Research Laboratory, Pensacola, FL 32508-5700.

Concerns about the effect of aerobic fitness on G-tolerance during high performance flight has initiated monitoring of G-load and heart rate response during actual Air Combat Maneuver (ACM) training. Eleven Naval aviators flying 23 ACM training flights on a Tactical Air Combat Training System (TACTS) range were used as subjects. Heart rate response was collected every 2.5 seconds during flight by Vitalog and continuous G-loading by TACTS. Aerobic fitness as determined by treadmill stress testing, grip strength, and body composition as determined by % body fat were assessed prior to flight. Aerobic fitness, body composition, and muscular strength assessment revealed a mean $\dot{V}o_2$ max of 49.9 ± 5.2 ml·kg^{-1}·min^{-1}, % fat of 13.0 ± 4.5, and grip strength of 54.5 ± 7.8 kg. During ACM mean max G was 5.3 ± 1.2 and HR increase was 58.6 bpm as compared to starting HR. The most significant HR increase was seen the first 60 seconds following ACM ($P<0.01$). Significance was not found between peak G and aerobic fitness. However, HR response for total G-loading and during the 60-second lag period was inversely related to aerobic fitness ($r = -0.565$, $P<0.05$). Aerobic fitness demonstrates a significant influence on cardiovascular response to G-loading during ACM flights.

63.1

INDOMETHACIN ATTENUATES ACUTE ANTIGEN-INDUCED INCREASES IN BRONCHIAL ARTERY BLOOD FLOW IN ALLERGIC SHEEP. W.M. Long*, L.D. Yerger*, C.L. Sprung*, W.M. Abraham, and A. Wanner. Univ. of Miami, Vet. Admin. Med. Ctr., Miami, FL 33125 and Div. Pulm. Dis., Mt. Sinai Med. Ctr., Miami Beach, FL 33140

We previously showed that aerosol challenge of allergic sheep with Ascaris suum antigen caused a mediator dependent increase in bronchial artery flow (Qbr) and that the principal mediator was not histamine (Am Rev Resp Dis 131:A 335, 1985). To assess the role of cyclo-oxygenase products in antigen induced (AI) increases in Qbr, we pretreated sheep with indomethacin (2mg/kg) before antigen challenge and compared responses to allergic sheep challenged with antigen alone. In control sheep, Qbr increased 124±39% (N=6; x̄±SE; P<0.05) from baseline immediately post challenge and remained significantly elevated 30 min post challenge. In 4 of 6 indomethacin treated sheep, the AI increase in Qbr was abolished; no protection was observed in the other animals. Indomethacin did not affect AI bronchoconstriction. These results suggest a possible role for cyclooxygenase products of arachidonic acid in AI increases Qbr, but not in AI bronchoconstriction. (Sup. by Amer. Lung Assoc. FL)

63.2

SUBSTANCE P IS A POTENT PULMONARY VENOCONSTRICTOR. W.M. Selig*, K.E. Burhop, and A.B. Malik. Dept. of Physiology Albany Medical College, Albany, NY 12208.

The effect of substance P (SP) on pulmonary hemodynamics and fluid balance, monitored as lung weight gain (LWG), was examined in Ringer's-albumin isolated perfused guinea pig lungs (constant flow). Pulmonary arterial pressure ($P_{\overline{pa}}$) and capillary pressure ($P_{\overline{cap}}$, double occlusion method) were measured (cmH_2O) and arterial (Ra) and venous resistance (Rv) calculated (cmH_2O/ml/min). Measurements ($\bar{x}$ ± SEM) were compared to baseline for 30 min post-SP (10^{-7}M) in the presence or absence of the cyclooxygenase inhibitor, meclofenamate (50 µg/ml) or the vasodilator, papaverine (0.1 mg/ml).

$P_{\overline{pa}}$	7.6±0.6	13.6±0.6	6.0±1.4	8.5±0.1
$P_{\overline{cap}}$	5.2±0.8	11.0±1.1	3.9±0.2	7.2±0.3
Ra	.09±.01	.10±.01	.08±.02	.05±.01
Rv	.11±.03	.32±.04	.08±.01	.20±.02

SP caused a sustained increase in Rv (peak 5 min post-SP) which was attenuated by meclofenamate and abolished by papaverine. SP-induced increases in LWG (measured 30 min post-SP) of 2.3±0.4 g were reduced to 0.6±0.4 g and 0.2±0.1 g by meclofenamate and papaverine, respectively. SP-induced pulmonary microvascular responses are predominantly hydrostatic and partially cyclooxygenase dependent. (Supported by HL-17355; HL-26551; HL-07529).

63.3

THE RELATION BETWEEN PLASMA ETHANOL (ETOH) CONCENTRATION AND THE ACUTE PULMONARY HYPOXIC PRESSOR RESPONSE. R.J. Porcelli, P.C. DEVINE* AND E. BRONSTEIN*. VAMC @ Northport, N.Y. 11768

The present study investigated the relationship between plasma ETOH levels and the pulmonary pressor response to acute hypoxia (FIO_2=0.08) in the isolated, blood perfused lung. Acute hypoxia raised pulmonary vascular resistance (Rpv) by 24.8±2.5%* during control conditions (n=8). As plasma ETOH concentrations rose to 85.8±4.2 mg/100cc, the hypoxic pressor was potentiated (41.5±5.9%Δ Rpv*). When plasma ETOH concentrations rose to higher levels, 125.4±5.4 mg/100cc and then to 169.2±5.7 mg/100cc, the hypoxic pressor responses were enhanced to 43.1±6.0%Δ Rpv* and to 53.7±11.9%Δ Rpv*, respectively. Finally, when ETOH levels reached 233.5±7.5 mg/100cc the hypoxic pressor response peaked at 56.0±9.7%Δ Rpv*. These observations were specific for hypoxia, since norepinephrine did not show similar results. Since the effect of ETOH on Rpv was variable and averaged only -3.4±2.4%, the ETOH-induced changes in the hypoxic pressor response could not be correlated to changes in the base line tone resulting from the plasma ETOH concentrations. Although systemic blood flows were not measured, hypoxia raised systemic arterial pressure (PSA) by 29±3 torr during control conditions and that hypertensive ability, though reduced, persisted throughout the ETOH administration, (16±6 torr*, 28±7 torr, 15± torr*, 9±5 torr*, respectively). These data demonstrate that ETOH potentiates the pulmonary hypoxic pressor response in a dose-dependent fashion. This effect was specific for hypoxia and independent of baseline tone.* p<0.05.

63.4

LOSS OF HYPOXIC PULMONARY VASOCONSTRICTION FOLLOWING EXPOSURE TO HYPEROXIA. S. Matalon and J.A. Krasney. SUNY at Buffalo, Buffalo NY 14214

We sought to determine whether prolonged exposure of sheep to 100% O2 at one atmosphere would diminish the hypoxic pulmonary vasoconstriction. Six chronically instrumented conscious sheep were exposed to 100% O2 till death. They all developed uncompensated respiratory acidosis after 40-60 hr in 100% O2. At different intervals the animals breathed a mixture of oxygen and nitrogen adjusted to lower their arterial oxygen tension to 40 torr. Pulmonary artery pressure (PA), cardiac output (Q) and arterial blood gases were measured fifteen minutes later and compared to their immediately preceeding hyperoxic values. During this hypoxic challenge, PA, Q and input pulmonary vascular resistance (PIVR) increased by 44, 17 and 31% respectively, at the early stages of exposure (0-20 hr), and by 40, 38 and 1% after 65 hrs in 100% O2. Infusion of meclofenamate failed to restore the increase of the PIVR. We concluded that prolonged exposure to hyperoxia decreased the ability of the pulmonary vasculature to respond to hypoxia and this effect was not mediated by the production of dilator prostaglandins.

(Supported by NIH grant HL31197)

63.5

The effects of zymosan activated plasma (ZAP) on the canine lung. M.I. Townsley, R.J. Korthuis and A.E. Taylor. Dept. of Physiology, Univ. of South Alabama, Mobile, AL 36688.

The effects of complement fragments on pulmonary hemodynamics and permeability were evaluated in isolated, blood perfused canine lung lobes (n=4, wt=47.2±11.3g, mean ±SE) after administration of ZAP. We measured vascular pressures, isogravimetric capillary pressure ($P_{c,i}$), blood flow ($\dot{Q}$) and the capillary filtration coefficient ($K_{f,c}$), and calculated total vascular resistance (R_T). These parameters were measured during a control period and 30, 60 and 120 min after ZAP. ZAP was prepared from autologous plasma and added as 5 ml boluses (28.5±4.7 ml total ZAP) over 65±15 min. ZAP resulted in a small increase in $K_{f,c}$ (0.20±0.04 ml/min/cmH_2O/100g at 30 min) compared to control (0.14±0.01, p<.05) which was sustained for the 2 hr observation period, although $P_{c,i}$ remained at the control value (9.7±0.9 cmH_2O). Pulmonary hemodynamics were unaltered during ZAP administration, and further, R_T and $\dot{Q}$ after ZAP were no different from control values of 13.5±1.1 cmH_2O/L/min/100g and 827±99 ml/min/100g, respectively. The lack of any hemodynamic effect of ZAP in the isolated canine lung was confirmed in two intact anesthetized dogs. In these animals, iv boluses of ZAP did not elicit any change in pulmonary or systemic arterial pressures or in cardiac output. We conclude that, in the canine lung, complement activation may induce only a small increase in microvascular permeability or filtration surface without altering pulmonary hemodynamics.

63.6

EFFECT OF LEUKOTRIENE C_4 (LTC_4) AND HISTAMINE ON REGIONAL BLOOD FLOW (RBF) IN PIG LUNG. H Ohtaka, A Foster, R Cory, JC Hogg and RR Schellenberg. UBC Pul Res Lab, St. Paul's Hosp., Van. BC, Canada

LTC_4 and histamine are potent constrictors of porcine pulmonary artery both in vitro and in vivo. This study aimed to determine their effects on pulmonary blood flow. Nine female pigs (15±1 kg) were anesthetized and positioned supine with a catheter in the pulmonary artery to measure cardiac output ($\dot{Q}$) (l/min) by thermodilution. Microspheres were injected before (^{153}Gd) and 40 sec after (^{113}Sn) the injection of either 10^{-8} M/kg of LTC_4 or $6x10^{-7}$ M/kg of histamine or saline. RBF (ml/min/g blood-free lung) was calculated from the frozen lungs which were sliced at 2 cm intervals from the dorsal (slice 1) to ventral (slice 5) portion.

	Saline n=3		LTC_4 n=3		Histamine n=3	
Slice	Pre	Post	Pre	Post	Pre	Post
1	189±47	185±39(-1%)	197±85	151±51(-21%)	164±54	105±38(-33%)
2	176±36	177±26(+3%)	202±87	146±43(-25%)	193±32	156±31(-18%)
3	145±50	145±50(+2%)	162±79	132±55(-16%)	184±41	153±38(-16%)
4	133±25	126±29(-5%)	112±64	104±52(- 1%)	152±41	133±26(- 8%)
5	64±22	63±22(-1%)	68±60	92±54(+61%)	86±32	66±23(-16%)
$\dot{Q}$	4.5±.7	4.4±.6(-2%)	4.3±1	3.1±.9(-28%)	4.2±1	3.3±.7(-21%)

These results show that both LTC_4 and histamine caused decreases in RBF which were accounted for by the fall in Q, and that LTC_4 also caused a disproportionate redistribution of RBF to the upper lung region.

63.7

EFFECTS OF HYPOXIA ON PULMONARY ENDOTHELIAL CELL (EC) AND VASCULAR SMOOTH MUSCLE CELL (VSMC) ARACHIDONIC ACID (AA) METABOLISM IN-VITRO.

Mitchell Friedman, Michael C. Madden*, Harriette P. Nichols* and Robert L. Vender*. Univ. of N. Carolina, Chapel Hill, N.C. 27514.

AA metabolites may play a role in pulmonary hypoxic vascoconstriction. We studied the effects of hypoxia on AA metabolism of confluent bovine pulmonary artery EC and VSMC grown in 5% CO_2, air. The cells were washed, serum-free media with 50mM HEPES added, and exposed to room air (4H) and the media removed. New media was added and the cells exposed to 100% N_2 (4H). The media was assayed for 6-keto $PGF_{1\alpha}$ and Thromboxane B_2 (TxB_2) by radioimmunoassay. The data are (mean ± SEM):

	Room Air (n=6)	Hypoxia (n=6)
6-keto-$PGF_{1\alpha}$ (ng/ml)	7.2±1.7	0.1±0.0*
TxB_2 (ng/ml)	0.4±0.1	0.1±0.0*

(*Significant difference ($p<.01$) from 21%O_2 data.) Synthesis of 6-keto-$PGF_{1\alpha}$ after AA (20 μM for 5 min) was decreased by 75±20% after hypoxia ($p<.01$). No changes in VSMC AA metabolism occurred after hypoxia. Thus hypoxia results in the decreased ability of EC to produce prostacyclin from endogenous or exogenous sources and may be important in hypoxic pulmonary vasoconstriction.

63.8

LUNG SURFACE BLOOD FLOW DURING MICROPUNCTURE EXPERIMENTS.

G Nicolaysen, MA Gropper, NC Staub and J Shepard. Cardiovasc Res Inst and Dept Physiol, Univ of Calif, San Francisco, CA 94143.

Our direct micropuncture measurements of microvascular pressure in subpleural pulmonary vessels (FED PROC 44:1759, 1985) requires statically inflated lung lobes. To determine whether this condition affects regional distribution of blood flow, we prepared 6 dogs for lung micropuncture and held the left lower lobe at 5 cmH_2O for at least 1.5h. We injected 1.5 mCi of Tc-labeled macroaggregates i.v. and after 5 min froze the inflated lungs in liquid nitrogen. We cut 4-7 blocks from the frozen left lower lobe, each 2x2cm and penetrating the lobe. We measured the radioactivity in and weight of 1-2mm thick layers cut parallel to the surface. The table summarizes the data for the outermost surface layer relative to all other layers, to the second subpleural layer and the surface inside the ring versus outside the ring, as percent difference (mean±SD), compared by a two-tailed sign test (n=6).

Surface/all layers	Surface/second layer	Ring/non ring
86 ± 6.5	90 ± 7.5	79 ± 26
$P<.05$	$P<.05$	$P>.05$

Flow in the surface layer, which included the pleura, averaged 14% less than the mean lobar flow and 10% less than the adjacent subpleural layer. The flow may be slightly affected by the suction ring used to stabilize the lobe. We conclude that subpleural flow in statically inflated lung lobes during micropuncture is only slightly less than in the interior of the lung. (Supported in part by HL25548)

63.9

Micropuncture measurement of microvascular pressures in the intact dog lung under Zone III conditions.

JM Shepard, MA Gropper, C Fike, NC Staub and J Bhattacharya. Cardiovasc Res Inst and Dept Physiol, Univ of Calif, San Francisco, CA 94143.

We recently micropunctured the intact dog lung (FED PROC 44:1759, 1985) to measure microvascular pressures under Zone II conditions (alveolar > left atrial pressure). Using the same preparation, we now report micropuncture data with Zone III conditions (alveolar < left atrial pressure). In 5 anesthetized dogs, we measured pressures in a pulmonary artery and the left atrium. We held the left lower lobe constantly inflated to a pressure of 5 cmH_2O while we separately ventilated the right lung with 100% O_2. We measured pressure by micropuncture (JAP 52:643, 1982) in subpleural arterioles and venules (10-50 μm diameter). PaO_2, $PaCO_2$, and pH averaged > 400 mmHg, 38 ± 8, 7.3 ± .1, respectively. Extravascular lung water in the left and right lower lobes averaged 3.9±.3 and 3.8±.5 g/g dry, respectively. The table shows the measured pressures (cmH_2O mean ± SD referred to the hilum).

	Pul Artery	Arteriole	Venule	Left Atrium
Zone III	16.4±2.5	13.5±1.6	9.3±1.5	7.7±1.6
Zone II	15.6±2.3	12.8±1.6	6.6±2.4	0.2±1.2

In Zone III, pressure drops in the arterial, microvascular, and venous segments averaged 33, 48, and 18%, respectively. Venous pressure drop was less in Zone III than in Zone II ($p<0.01$). We conclude that in the intact lung in Zone III, the largest pressure drop occurs in the microvascular segment. (Supported by HL25548)

63.10

REGIONAL PULMONARY BLOOD FLOW DURING 96 HOURS OF HYPOXIA IN CONSCIOUS SHEEP.

D. Curran-Everett* and J.A. Krasney. Dept. of Physiology. SUNYAB, Buffalo, NY 14214

The effects of acute hypoxia on regional pulmonary perfusion have been studied previously in anesthetized, artificially ventilated sheep (Neumann et al. J. Appl. Physiol. 56:338-342, 1984). These studies have generally indicated that a rise in pulmonary artery pressure is associated with a shift of pulmonary blood flow toward the dorsal (nondependent) areas of the lung. The present study examined the relationship between the pulmonary artery pressor response and regional pulmonary blood flow in seven conscious, standing ewes during 4 days of normobaric hypoxia. The sheep were made hypoxic by nitrogen dilution in an environmental chamber (PaO_2 = 40 torr). Regional pulmonary blood flow was determined by injections of 15 μm radiolabelled microspheres into the superior vena cava during normoxia and at 24 hour intervals of hypoxia. The reference sample was withdrawn from the pulmonary artery. Pulmonary artery pressure increased significantly by 43% for the duration of hypoxia. Despite the large, sustained increase in pulmonary artery pressure, there was no significant change in the perfusion (expressed as either % of cardiac output or $\dot{Q}$/g lung tissue) of any lung region during hypoxia. This lack of change of regional lung perfusion may be because the conscious, standing sheep lacks zone 1 in its lung. (Supported by HL-27683)

63.11

CYCLOOXYGENASE INHIBITION ALTERS THE VASCULAR RESPONSIVENESS TO REPEAT DOSES OF HISTAMINE.

J.E.Hall*, W.F. Hofman, I.C. Ehrhart. Medical College of Georgia, Augusta, GA 30912.

The effect of cyclooxygenase inhibition (COI) on the amount of histamine (H) needed to bring about repeated pulmonary vasoconstriction was studied in the isolated, ventilated canine right lower lung lobe, pump-perfused with blood at constant flow. H diphosphate was given via the venous reservoir until pulmonary artery pressure (Pa) doubled. The first dose response to H (DR1) was repeated after 2 hours (DR2). Group H (n=4) received only H, while Group H+I (n=4) received 40 μM indomethacin (I) prior to DR1. Given below are the cumulative H doses in μg/ml perfusate volume needed to increase Pa 10%.

	DR1	DR2
H	3.67 ± 1.80	501.98 ± 119.73*†
H+I	1.22 ± 0.19	1.53 ± 0.26

*$p<0.05$ for H vs. H+I at DR2; †$p<0.05$ for DR2 vs. DR1

COI with I appeared to restore the responsiveness to a repeat dose of H (DR2). Some of the diminished vascular responsiveness to repeat H administration in the lung may be due to cyclooxygenase products. (Supported by American Heart Association, Georgia Affiliate, and American Lung Association, Georgia.)

63.12

EFFECT OF CYCLOOXYGENASE INHIBITION ON LUNG PERMEABILITY AND VASCULAR RESPONSE TO SEROTONIN INFUSION.

I.C. Ehrhart and W.F. Hofman. Medical College of Georgia, Augusta, Ga., 30912

Effect of continuous serotonin (5-HT) infusion on pulmonary hemodynamics and the microvessel filtration coefficient (Kf) was studied in the isolated canine lower left lung lobe (LLL) blood perfused at constant flow. The pre- to post-capillary resistance ratio (RR) was determined by venous occlusion. Lobes were infused with 500 μg/ml 5-HT at 0.11 ml/min and then 0.21 ml/min over 80 min (n=6). In a second group cyclooxygenase inhibition (COI) was induced by 45 μM meclofenamate (n=3) or 40 μM indomethacin (n=3) prior to 5-HT infusion. Controls received only saline (n=6). Pulmonary vascular resistance (PVR, Torr/L/min), RR and the Kf (ml/min/Torr/100 g LLL) are presented below.

	0.11 ml/min infusion		0.21 ml/min infusion		
Group	PVR	RR	PVR	RR	Kf
Saline	22±3	1.7±0.4	22±3	1.6±0.4	0.083±0.016
5-HT	38±6	2.8±0.6	46±6	2.6±0.6	0.054±0.011
COI + 5-HT	78±20*	4.0±0.4*	96±24*	3.0±0.3	0.043±0.010

* $p<0.01$ compared to saline control

5-HT did not change the Kf and increased PVR and RR only after COI. Thus, cyclooxygenase products may modulate the vasoactivity of 5-HT in the lung. However 5-HT does not appear to increase vascular permeability either in the presence or absence of cyclooxygenase products. (Supported by American Lung Association, Georgia.)

63.13

CYCLOOXYGENASE INHIBITION ENHANCES VASOPRESSOR RESPONSE TO SEROTONIN IN THE DOG LUNG. W. F. Hofman and I. C. Ehrhart, Department of Physiology, Medical College of Georgia, Augusta, GA 30912.

We examined whether cyclooxygenase inhibition (COI) would alter the vasopressor response to serotonin (5-HT) in the isolated blood perfused lower left lung lobe (LLL). LLs were challenged with graded doses at 5-HT (50 to 250 µg) before and after either 40 µM indomethacin (n=5) or 45 µM meclofenmate (n=3). 5-HT produced a dose-dependent increase in pulmonary artery pressure (PAP) of 39 to 108% which was nearly doubled (70-210%) after COI. Pulmonary vascular resistance ($cmH_2O/ml/min \times 10^{-2}$) at the peak vasopressor response for each dose of 5HT before and after COI is presented below.

	Baseline	50 µg	100 µg	250 µg	500 µg
Pre-COI	3.7±0.6	5.6±1.1	6.6±1.2	8.0±1.2	8.8±1.5
Post-COI	4.4±0.6	8.7±1.4*	11.1±2.0*	14.9±3.1	19.1±4.4*

Means ± SE; n=7; *p < 0.01 from Pre-COI

5-HT vasoconstriction was enhanced after COI. Thus, the vasopressor response to 5-HT in the lung may be modulated by vasodilator prostaglandins. Supported by American Lung Association of Georgia.

63.14

ANOXIA DECREASES PHAGOCYTOSIS BY PULMONARY ALVEOLAR MACROPHAGES (PAM). Sandra L. Woodford* and John W. Mills* (Spon: D. Bartlett, Jr). Dartmouth Medical School, Hanover, NH 03756

Alveolar hypoxia occurs in a variety of clinical conditions. PAM have a high rate of O_2 consumption, especially during phagocytosis, and thus may be sensitive to low O_2 levels. To investigate the effects of lack of O_2 on PAM phagocytic function, rabbit PAM were exposed in vitro to glutaraldehyde-fixed red blood cells (GRBC) and incubated in bicarbonate Ringer's solution equilibrated with air/5% CO_2 or N_2/5% CO_2. The percent of PAM containing GRBC was determined at intervals by fluorescent microscopy after exposure to trypan blue, which shifts the normal green fluorescence of GRBC to red, permitting differentiation between phagocytosed and surface-adherent GRBC. Phagocytosis was significantly less in PAM exposed to anoxia for 60 minutes or longer. More than 90% of the PAM were viable at all time points in both air and anoxia. Return to aerobic conditions after 150 minutes of anoxia resulted in recovery to control levels of phagocytosis by 90 minutes. These results show that anoxia decreases phagocytosis by PAM and that returning anoxic PAM to aerobic conditions allows recovery of phagocytic ability. Supported by NIH Grants HL 07449 and HL 06664.

Table I

Phagocytosis of GRBC by PAM[1]

Time(min)	30	60	90	150
Air	63±2	80±2	82±2	80±2
Anoxia	62±3	54±3	39±3	31±2

[1]Percent ± S.E. of total PAM with phagocytosed GRBC; n=4-8 experiments at each time point.

63.15

HYDRAULIC CONDUCTIVITY OF SUBCUTANEOUS AND PULMONARY INTERSTITIUM DURING EDEMA. James C. Parker, Julia A. Cartledge*, A.E. Taylor, and M.I. Townsley, Dept Physiology, University of South Alabama, Mobile, AL 36688

The hydraulic conductivity (KA/η) was measured in the interstitial spaces of excised pieces of dog skin and the hilar region of lung. The flow of saline (NS) was measured between two implanted porous intracatheters (Δx=.4cm) after imposing a $25cmH_2O$ pressure gradient between a fluid reservoir and drainage catheter while maintaining the inflow pressure $1cmH_2O$ subatmospheric. Edema was induced by periodically raising the reservoir. The relationship of conductivity to weight gain (WG) was determined using NS in the reservoir. The following relationship of KA/η in $cm^6 dyn^{-1} s^{-1}$ to WG in g were determined for linear regressions of log-log plots. For skin with NS(n=94):

$$KA/\eta = 1.897 \times 10^{-12} + 3.51 \times 10^{-7} WG, \; r=0.76 \quad (1)$$

For hilar interstitium with NS(n=58):

$$KA/\eta = 2.61 \times 10^{-6} + 1.61 \times 10^{-7} WG, \; r=0.69 \quad (2)$$

The addition of xanthine oxidase and purine to the reservoir to generate toxic free radicals increased the hydraulic conductivity. Thus, hydraulic conductivity was significantly increased with increased tissue hydration and also with free radical damage. Supported by HL-24571.

63.16

ANALYSIS OF WEIGHT GAIN TRANSIENTS IN ISOLATED DOG LOBES BY A MULTICOMPARTMENT INTERSTITIAL MODEL. T. Izumi* and J. Hildebrandt. Virginia Mason Research Center, Seattle, WA 98101.

We sought to analyze the early phases (e.g. 0-3 min) of the weight gain curve of isolated canine lobes in response to sudden elevations of vascular pressure. Assuming that vascular space, perimicrovascular space and central interstitial space each comprise resistance-capacitance systems (i.e., each weight change increases in an exponential manner) weight change is given by: $\dot{W}(t) = dw(t)/dt = Wf_1/\tau_1 \cdot e^{-t/\tau_1} + Wf_2/\tau_2 \cdot e^{-t/\tau_2} + Wf_3/\tau_3 \cdot e^{-t/\tau_3}$, where Wf_i = the final weight increase of compartment i, τ_i = its time constant, and t = time. From semilog plots of $\dot{W}$ versus t we obtained successive slopes ($1/\tau i$) and intercepts ($Wi/\tau i$) giving the filtration coefficient (Kf=1/R) and the compliance (C) of each compartment. Average values ± SD were obtained from 9 measurements made on 4 lobes using Tyrode's solution containing 5g BSA per L. The time constants of vascular space, perimicrovascular space, and central interstitial space averaged 4, 14 and 164 sec, respectively. Endothelial membrane conductance (Kfmv = 5.18 ± 2.00) was about 7 times interstitial tissue conductance (Kfis = 0.68 ± 0.09); the total series conductance was 0.60 ± 0.09g/min/cm H_2O/100g lung. Central interstitial compliance (Cis = 1.60 ± 0.18) averaged about 1.7 times perimicrovascular space compliance (Cpmv = 0.98 ± 0.26g/cm H_2O/100g lung). It is probable that another compartment could be resolved from data extended to 15 min or more, having an even smaller conductance and larger compliance. We conclude that Kf and C depend significantly on the time intervals analyzed. Endothelial permeability is probably best obtained from $\dot{W}$ occurring within the first min, after the initial vascular transient of about 15 sec is complete.

63.17

ISOGRAVIMETRIC PRESSURE (Pisog) AND FILTRATION COEFFICIENT (Kf) MEASURED IN ISOLATED RABBIT LUNGS. S.J. Lai-Fook, and M.R. Kaplowitz*. Univ. of Calif., San Francisco, CA. 94143

We studied lungs whose artery and vein were connected to a weighed reservoir filled with autologous blood. With alveolar pressure (Palv) constant, changes in lung weight were measured during 20 minute periods following step changes in vascular pressure. We lowered vascular pressure by 2 cmH_2O every 20 minutes until lung weight decreased rather than increased. Pisog (relative to pleural (ambient) pressure at the lung base) was the mean of the two consecutive vascular pressures between which a reversal of the gain in lung weight occurred. Kf was calculated by dividing the flow averaged over the last 10 minutes of the 20 minute periods by the difference between the two vascular pressures spanning Pisog. The table summarizes the data (mean ± SD, n=5) at deflation Palv values (cmH_2O):

Palv	3	5	15	25
Pisog	4.0±0.6	4.8±1.2	6.1±2.1	7.8±2.7
Kf	.083±.06	.074±.071	.079±.055	.070±.01

Kf, (g). $(min)^{-1}(cmH_2O)^{-1}(100g \text{ wet lung})^{-1}$, was constant even though conditions changed from zone 1 to zone 2 with lung deflation. This suggests that leakage occurred through small extra-alveolar vessels. Pisog was near Palv at low lung volumes, but fell increasingly below Palv at higher Palv values, consistent with the behavior of alveolar liquid pressure (Resp Physiol 57:61, 1984). (Supported by HL-1071 and HL-25816 from NHLBI).

63.18

ALVEOLAR LIQUID PRESSURE IN EXCISED AIR-INFLATED LUNGS OF MATURE AND IMMATURE FETAL RABBITS. J. Usha Raj* (SPON: R.M. Effros), Harbor-UCLA Medical Center, Torrance, CA 90509.

Lung surfactant, by lowering alveolar surface tension (T), is thought to reduce the pressure drop across the curved alveolar air-liquid interface, and thus reduce the driving force for pulmonary edema. To obtain direct evidence for this hypothesis, we measured alveolar liquid pressure (P_{liq}) using the micropipette-servonulling method in excised air-inflated lungs of fetal rabbits at 27 and 31d gestation (term 31d), at transpulmonary pressures (P_{tp}) between 5 and 25 cm H_2O. We also determined the total amount and composition of phospholipids recovered by alveolar wash and the static pressure volume behavior of the lungs. Results: Mean ± S.D.

Rabbit Lungs	P_{liq} (cmH_2O)		Vol of Air (ml)		Total Lipids/Lung (mg)
Deflation P_{tp}	25	5	25	5	
Mature (31d) n=9	8.4±1.7	2.3±0.6	1.55±0.38	0.86±0.29	0.70±0.23
Immature (27d) n=8	2.6±1.0	1.5±0.2	0.17±0.11	0.07±0.03	0.05±0.02

P_{liq} was significantly more negative at each P_{tp} in immature lungs compared to mature lungs due to increased alveolar T. If pericapillary interstitial liquid pressure equals P_{liq}, then surfactant deficiency may lead to a hydrostatic pressure gradient favoring fluid accumulation in immature lungs.

63.19

SEQUENCE OF FLUID ACCUMULATION IN NITROGEN DIOXIDE (NO_2)-INDUCED LUNG EDEMA IN DOGS: A MORPHOMETRIC STUDY.
M. Vassilyadi*, R.P. Michel. Lyman Duff Labs, Dept. Pathology, McGill Univ., Montréal, QC, H3A 2B4, Canada

In hydrostatic and drug-induced permeability (eg. alpha-naphthylthiourea) edema, fluid first accumulates in the interstitium, then floods alveoli. To test the hypothesis that this sequence may be altered when a toxin enters the lung via the airways, 7 anesthetized supine spontaneously breathing dogs were exposed to a mean dose of 212±27 ppm.hour NO_2 in a chamber. After a mean of 90 min. post-exposure, the lower lobes were inflated to 5cm H_2O and frozen in liquid nitrogen. Wet/dry weight ratios, corrected for blood water, averaged (±SE) 7.42±0.62 (control 4.53±0.12, n=3). We measured with morphometry (Lab Invest 51:97, 1984) interstitial edema ratios (ER) around arteries and airways within 290 bronchovascular bundles with respiratory bronchioles and bronchioles, each separated and connected. We also graded alveolar edema semiquantitatively in 10% increments from 0 to 100%. We found that the mean % alveolar edema was 25.4±7.7; the ER for airways ranged between 0.20 and 0.37, while for vessels they ranged between 0.25 and 0.54; these values are no different from previously reported control ER. We conclude that NO_2 causes edema with preferential alveolar flooding and appears to bypass the usual sequence of fluid accumulation. This may be related to preferential epithelial permeability changes.
Supported by MRC of Canada Grant No. MT-7727.

AGING, ENDOCRINOLOGY, AND METABOLISM

64.1

CAROTID-CARDIAC BAROREFLEX RESPONSES DECREASE WITH EARLY AGING IN WOMEN. C.J.M. Porth, L. Groban* and J.J. Smith. Univ. of Wis.-Milwaukee, 53201, VA Med. Ctr. and the Med. College of Wis., Milwaukee, WI 53226.

The purpose of this study was to examine carotid baroreflexes (Eckberg method) and respiratory sinus arrhythmia in eight normal 20 to 29 yr old young females (YF) and ten normal 40 to 49 yr old middle-aged females (MAF). Random five second alterations of neck pressure (-40, -20 and +15 mm Hg) were induced repeatedly at a fixed time in the cardiac cycle. Previous data indicate that carotid-cardiac baroreflex responses are vagally mediated.

There were lesser R-R interval responses and longer times to maximum slowing and speeding of heart rate with carotid stimulation in MAF than YF. Baroreflex sensitivity in MAF (1.35±0.13 msec/mm Hg) was less than in YF (3.76±0.58 msec/mm Hg) (p=0.001).

The standard deviation of 100 consecutive R-R intervals (SD) during supine, quiet breathing is a measure of respiratory sinus arrhythmia and a reflection of resting vagal cardiac activity. The SD of the R-R intervals was less in MAF (43.39) than in YF (59.38).

The results indicate a reduction in baseline cardiac vagal outflow at rest and a lesser vagal responsiveness to carotid baroreceptor stimulation during early aging in normal male subjects.
(Supported by a NRSA postdoctoral nurse fellowship, NIA grant AGO 3064 and the Veterans Administration.)

64.2

ROLES OF RIBOFLAVIN DEFICIENCY AND AGE ON ADENOSINE RECEPTOR BINDING IN ADIPOCYTES. P. Dutta* and J. Pinto*. (SPON: G.B. Raiczyk) Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, N.Y. 10021

Our previous studies show that an age-related decline in beta-adrenergic receptor (BAR) binding is absent during riboflavin (Rf) deficiency. In young but not old animals, Rf deficiency decreases BAR binding. This study examines whether adenosine receptor (AR) binding is influenced by either age or Rf deficiency. Female Holtzman rats were fed a Rf deficient diet for 3 to 40 weeks. Age-matched animals were pair-fed an identical diet but supplemented with Rf. Isolated fat cell membranes from pooled parametrial adipose tissue were incubated at 37° for 15 min with 14 nM (-)N^6-(^{3}H)phenylisopropyladenosine in Tris buffer. AR binding is decreased in old (>17 weeks) compared to that in young animals (<17 weeks) in both control (242 ± 26 vs 327 ± 17 fm/mg protein) and deficient (267 ± 26 vs 377 ± 4 fm/mg protein) rats. No difference in AR binding is observed between Rf deficient and supplemented groups at any age. The weight of parametrial fat in rats in early stages of Rf deficiency is less than 25% that of controls. As rats continue to feed on the Rf deficient diet, the weight of fat increases progressively and, after 30 weeks, no difference can be observed between Rf deficient and supplemented groups. The erythrocyte glutathione reductase activity coefficient, a marker for Rf deficiency, is higher in animals at an early stage (<17 weeks) than at a later stage (>17 weeks) of Rf deficiency (1.90 ± 0.01 vs 1.60 ± 0.03). These results show that rats on Rf deficient diets for prolonged periods slowly adapt to the deficiency state and that the age-related decline in binding of AR, unlike that of BAR, is unaffected by Rf deficiency.

64.3

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

The calciuric effect of some drugs, such as glucocorticoids and thyroid extract is well known. In order to determine the effect of other medications on the excretion and intestinal absorption of calcium, a controlled study was carried out to investigate these aspects. Urinary calcium was determined for several months during the long term use of the antibiotic Tetracycline and during the use of the antituberculous drug Isoniazid (INH). The effect of the diuretics Furosemide and Hydrochlorthiazide, of aluminum-containing antacids, and of corticosteroids on calcium and phosphorus metabolism was also studied. Metabolic balances of calcium, phosphorus were determined, as well as the intestinal absorption of calcium, using oral doses of ^{47}Ca. Plasma levels, urinary and fecal excretions of ^{47}Ca were determined. All drugs tested increased urinary calcium with the exception of the diuretic Hydrochlorthiazide. The increase depended on the duration of the use of some of the drugs. Of interest is the effect of corticosteroids: the absorption of calcium was unchanged after its short term use and was very high after long term use. The studies have shown that several widely used drugs induce an increase in urinary calcium and may thereby contribute to calcium loss. (This study was supported by a grant from USDA, CSRS, S&E.)

64.4

POTENTIATION OF GROWTH HORMONE SECRETION IN DOGS BY D-PROPRANOLOL. N. Altszuler and B. Winkler*, New York University School of Medicine, New York, NY 10016.

The secretion of growth hormone (GH) can be influenced by specific adrenergic agents. Since the various adrenergic agonists and antagonists lack absolute specificity, characterization of adrenergic receptors modulating GH secretion is incomplete. The present studies, utilizing physiologic and pharmacologic agents, suggest that different sites of action of the agonists may be involved. Plasma GH levels were measured in trained, conscious normal dogs. Intravenous infusion of epinephrine (EPI), norepinephrine (NE) or isoproterenol (ISO) at 0.2 μg/kg/min had no effect on plasma GH, while clonidine, 0.1 μg/kg/min, produced a marked rise. Addition of EPI, NE or ISO to clonidine prevented the usual rise seen with clonidine. Infusion of EPI and NE, along with d,l-propranolol, resulted in marked elevation of plasma GH, suggesting that removal of the β-adrenergic activity unmasked the stimulatory effect of the α-activation. However, a similar rise in plasma GH was observed when EPI or NE were infused with d-propranolol, which is devoid of β-blocking action. The rise in plasma GH induced by clonidine was not enhanced by propranolol, but was blocked by the α-blocker phentolamine. Addition of the α-blocker to the combined NE or EP plus propranolol infusion, did not prevent the enhanced GH response. These findings suggest that adrenergic receptor sites within the brain and inaccessible to circulating EPI or NE, nor to α-blocking agents, may affect GH secretion.

64.5

LACK OF EVIDENCE FOR RETROGRADE BLOOD FLOW IN THE PITUITARY STALK. Alex J. Baertschi* (SPON: B. Duling). University of Virginia, Charlottesville, VA 22908

Fluorescent microspheres were injected into anaesthetized rats, and their direction of motion observed by fluorescence-microscopy up to 300μm below the surface of the pituitary stalk. Images were monitored with an image-intensifying TV camera, displayed on a TV monitor together with elapsed time, and recorded on Video tape (10-20msec resolution). Frame-by-frame analysis showed that transit times were 200-300 msec (3.5-5mm/sec velocity) through small arterial branches, 300-500msec through capillary loops of the median eminence, 200-300msec (2-2.5mm/sec) through the long portal vessels from the capillary loops of the median eminence to the anterior pituitary gland, 500-600msec (0.8-1mm/sec) through the pituitary portal vessels, and 10-40sec through the pituitary sinuses. The pituitary stalk was scanned in depth at large magnification. Without exception, direction of blood flow in 5 normal preparations was from median eminence to pituitary gland. Retrograde blood flow through one single vessel was observed in 2 dying animals, and in preparations where a portal vessel had been lesioned at the pituitary level. Hemorrhage of 20% of blood volume and vasopressin infusions (1-5mU) did not cause flow reversal. The results do not provide evidence for a vascular transport of pituitary hormones to the brain. (Supported by N.I.H. grant RO1 HL31133.)

64.6

VASOPRESSIN REPLACEMENT LOWERS THE RELEASE OF OXYTOCIN-ASSOCIATED NEUROPHYSIN IN THE HOMOZYGOUS BRATTLEBORO (DI) RAT. Savio W.T. Cheng* and William G. North* (SPON: H. Valtin) Dartmouth Medical School, Hanover, NH 03756

We have recently shown that the release of oxytocin-associated neurophysin (OT-RNP) during acute salt-loading is greater in the DI rat which lacks vasopressin (VP) than in the LE rat. This study was performed to determine if acute (1μg) and chronic (3000ng/day, 12 days) VP replacement in the DI rat would correct this difference in the release of OT-RNP. Infusion of 18% NaCl (10μl/100g bw/min, 60 min) was carried out at 1 hour after acute replacement and on day 5 and 12 of chronic replacement. Plasma OT-RNP levels and plasma osmolality (Posm) were measured at varoius times of infusion.

		Increases in plasma OT-RNP conc. (fmole/ml)			
Time	(min)	10	20	40	60
DI	(n=5)	477±112	842±278	1105±159	1927±288
DI+1μg AVP	(n=5)	119±16	236±19	433±45	603±61
DI+5d AVP	(n=5)	104±16	248±38	419±94	467±124
DI+12d AVP	(n=7)	31.3	99±27	206±36	393±120
LE	(n=7)	221±60	486±101	734±100	682±40

Despite similar increases in Posm when compared with control DI animals, the rises in OT-RNP levels in VP-treated DI rats were significantly reduced ($p<0.001$) and were even less (for all treatments) than those for LE rats. These results suggest that VP may negatively modulate the secretion of oxytocin. (Supported by CA 19613, the Albert J. Ryan Foundation and the New Hampshire Heart Association.)

64.7

INDUCTION OF NONSHIVERING THERMOGENESIS AND REPRODUCTIVE REGRESSION BY TWO DAILY MELATONIN INJECTIONS IN FEMALE WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS). Eric S. Hall and G. Robert Lynch. Wesleyan University, Middletown, CT 06457

Twice daily injections of saline, 5, 10, 50 or 100 ug melatonin (MEL) were administered to 102 adult female white-footed mice at 2 and 12 hours after lights on (AM and PM, respectively) for 7 weeks under a long day photoperiod (LD 16:8). MEL (50 or 100 ug) administered in the PM induced gonadal regression and increased nonshivering thermogenesis (NST) regardless of the AM injection. Injections of 5 or 10 ug in the PM resulted in intermediate reproductive tract weights. Injections of 10, 50 or 100 ug MEL increased NST regardless of the timing of the injections. In contrast to Syrian hamsters (Chen, Brainard, and Reiter, Neuroendocrinology, 31:129-132, 1980) AM injections had no effect on the ability of PM injections to induce gonadal regression in white-footed mice. Also, the mechanisms of melatonin-induced increase in NST and reproductive regression are probably different. Increased NST can be induced by a cumulative daily dose of MEL of 10 ug or more given at any time of the day, whereas melatonin-induced gonadal regression occurs only after PM injections.

Supported by NSF grant PCM8216768, NIH grant NS15503 and the Whitehall Foundation.

64.8

CYCLIC AMP AND THE CONTROL OF JUVENILE HORMONE III BIOSYNTHESIS IN THE ADULT FEMALE COCKROACH DIPLOPTERA PUNCTATA. R.R. Aucoin* and S.S. Tobe. Dept. of Zoology, Univ. of Toronto, Toronto, Ont.

In the cockroach, the corpora allata (CA) are paired endocrine organs. They are the site of biosynthesis and release of juvenile hormone (JH) which is essential for metamorphosis and reproduction in most insect species. Mated adult females of D. punctata undergo a precisely timed cycle of JH biosynthesis associated with oocyte maturation which appears to be inversely correlated with levels of cyclic AMP (cAMP) in the CA. Virgin CA by comparison undergo no significant changes in JH biosynthesis or in levels of cAMP. Use of the adenylate cyclase activator forskolin, and the phosphodiesterase inhibitor IBMX, has allowed us to demonstrate a CA-adenylate cyclase capable of eliciting cAMP accumulation up to 20-fold greater than controls. Sensitivity of the CA to stimulation appears to be age-dependent, with days 4-5 (highest rates of JH biosynthesis) being least sensitive. Incubation of CA in the presence of 50 uM forskolin and .1 mM IBMX, resulted in a reversible inhibition of JH biosynthesis by as much as 85% over controls. High K^+ medium also inhibited JH biosynthesis and elevated cAMP levels although not when used in combination with high Mg^{2+}. These findings suggest inhibition of JH biosynthesis early in the gonotrophic cycle may involve cAMP-mediated mechanisms.

64.9

ADJUVANT-INDUCED PROGESTERONE SECRETION IN THE RAT. Brent Bruot*, Kent State University, Kent, OH 44242, Vimal Kishore*, Xavier University, New Orleans, LA 70125 and Neal Latman†, Southwestern Oklahoma State University, Weatherford, OK 73096. (Spon: W.C. Adams).

Progesterone and/or estrogens have been implicated in the reduction of the symptoms of rheumatoid arthritis (RA) during the luteal phase of the menstrual cycle and during pregnancy. In addition, the incidence of RA is reduced in women taking oral contraceptives. These observations suggest a relationship between sex steroids and RA. This study was undertaken to determine if progesterone is secreted in response to adjuvant-induced arthritis in the rat. Twenty male rats were divided into two groups. One group served as control while the other group was injected with 0.5 mg Mycobacterium butyricum in Freund's adjuvant. Paw edema and progesterone were measured 21 days later. Paw volume increased 0.53 ± 0.14 ml in the adjuvant injected group. This represents a 33% increase in paw volume. Progesterone secretion also significantly ($P<.001$) increased in the adjuvant injected group. Control rats secreted 0.50 ± 0.07 ng/ml compared to 1.20 ± 0.29 ng/ml in the adjuvant injected group. When grouped by degree of edema, the correlation coefficient between edema and progesterone was 0.98. These results suggest an anti-inflammatory role for progesterone in the adjuvant-induced arthritic rat.

64.10

PROSTAGLANDINS IN LUMINAL EPITHELIUM AND STROMA FROM ENDOMETRIUM OF RATS SENSITIZED FOR THE DECIDUAL CELL REACTION. E.J. Psenicka* and T.G. Kennedy. Dept. of Obstetrics & Gynaecology, University of Western Ontario, London, Canada N6A 5A5.

Prostaglandins (PGs) have been implicated in the early stages of implantation and decidualization. The source of PGs is unknown. To determine if the endometrium is a source of PGs, epithelial sheets were removed as described by Bitton-Casimiri et al(J.Endocr. 1977,73,537-38) from uteri of ovariectomized rats treated to mimic day 5 of pseudopregnancy. Stromal fractions(S) were separated enzymatically. Both fractions were centrifuged, resuspended in serum-free medium and incubated in the absence or presence of 10^{-5}M Indomethacin(I) (PG synthesis inhibitor) at 37°C for 0-4h. Medium was removed after centrifugation and PG content measured. Because of between experiment differences, experiments were considered separately. PGE2 content in E increased significantly with time in 2 of 3 experiments. The presence of I reduced this increase. $PGF2_{\alpha}$ content of E in one experiment increased with time. Presence of I reduced $PGF2_{\alpha}$ content in a second experiment. In a third experiment, I treatment increased $PGF2_{\alpha}$ content over that of untreated cells by 1h. This was no longer significant by 4h. The presence of I in S reduced the increase in PGE2 and $PGF2_{\alpha}$ content which was seen with time in untreated cells. These results suggest that both epithelial cells and stroma have the capacity to produce and secrete PGs in vitro and thus may be a potential source of PGs in implantation and decidualization. [Supported by the MRC of Canada.]

64.11

THE POSSIBLE ROLE OF CALMODULIN ANTAGONIST W-7 ON THE ACROSOME REACTION OF GUINEA PIG SPERMATOZOA AT HIGH pH. T. NAGAE* AND P.N. SRIVASTAVA. Department of Biochemistry, University of Georgia, Athens, Georgia 30602.

Although a large amount of calmodulin (Calm) is located in the acrosome of mammalian sperm (Jones et al., 1978), its function is still unclear. W-7, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, inhibits the acrosome reaction (AR) of guinea pig sperm (Lenz and Cormier, 1982) but not human sperm (Nagae, 1984). The present report is the first demonstration that W-7 reacts with sperm at high pH independent of Calm. Guinea pig sperm were incubated in isotonic acidic saline (IAS) with W-7 at 37°C in air. At least one hundred motile sperm were examined to assess the AR. The AR % ± SD at different time, pH and W-7 concentration were as follows: At 15 min, pH 8.19, 5μM, 17.5 ± 2.5; 25μM, 5.5 ± 2.5; 50μM, 1.5 ± 0.9; control, 0.8 ± 0.4. At 30 min; pH 8.32, 5μM, 71.3 ± 11.4; 25μM, 30.0 ± 10.0; 50μM, 4.3 ± 1.3; control, 1.5 ± 2.1. At 60 min; pH 8.39, 5μM, 86.3 ± 6.5; 25μM, 85.0 ± 5.0; 50μM, 67.5 ± 8.3; control, 1.8 ± 1.7. The sperm incubated in IAS containing W-7 at 37°C, 5% CO_2 in air (pH 7.7 ± 0.1) did not undergo the AR. After one hr of incubation in a Ca-free IAS with 5μM W-7, the addition of Ca caused 70.0 ± 5.6 of sperm to undergo the AR compared to 7.0 ± 1.0 in the control. Kd of W-7 for Calm is 11μM in the presence of Ca. Thus this effect of W-7 on the AR appears to be independent of Ca-Calm reaction. Supported by NIH grant # HD14947-06 and Yanase fund from Toho University.

64.12

THE EFFECT OF SCHISTOSOMIASIS ON TESTOSTERONE LEVELS AND ANDROGEN REGULATED GENE EXPRESSION IN THE MOUSE. Hadar Isseroff, Paul W. Sylvester*, and William A. Held*. Biology Department, SUNY College at Buffalo, Buffalo, NY 14222, Grace Cancer Drug Center, and Department of Cell and Tumor Biology, Roswell Park Memorial Institute, Buffalo, NY 14263.

Schistosomiasis mansoni is an important human tropical disease. The major pathology of the disease is due to worm ova that become trapped in the liver. There they are encapsulated in fibrous granulomas which may result in chronic illness and death. The present investigation was undertaken to determine whether schistosomiasis selectively alters gene expression in the liver. Two-dimensional gel electrophoresis of liver mRNA translation products revealed that the production of mouse urinary proteins (MUP) was inhibited in infected mice. MUP production is androgen regulated and a dot-blot hybridization analysis of RNAs from various mouse tissues with a variety of cDNA probes indicated that all androgen regulated mRNAs were depressed in infected mice. Administration of testosterone to infected animals restored MUP production. Direct measurement of serum testosterone levels and seminal vesicle weights confirmed that chronic schistosome infection reduces testosterone to castration levels in male mice.

64.13

EFFECT OF HYPERTENSION ON UTEROPLACENTAL BLOOD FLOW IN TERM-PREGNANT, SPONTANEOUSLY HYPERTENSIVE RATS (SHR). S.L. Reynolds*, R.A. Ahokas and G.D. Anderson*. Univ. of Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

It is generally believed that hypertension during pregnancy reduces maternal placental blood flow. Studies on the effect of hypertension on the uteroplacental circulation are scarce, however, due to the lack of availability of animal models of hypertension during pregnancy. The fall in blood pressure which normally occurs during the last week of gestation in the SHR can be prevented by overfeeding carbohydrate throughout gestation. Thus, to determine the effect of hypertension on hemodynamics at term of pregnancy, we measured cardiac output and organ blood flows on day 21 of gestation, using 15 μm radioactive labeled microspheres, in ad libitum fed SHR given water only (C-fed), or 10% sucrose (S-fed), to drink throughout gestation. Mean arterial blood pressure of the S-fed SHR was significantly higher than that of C-fed SHR (169±13 vs 112±5 mmHg, $P \leq 0.01$); cardiac output was similar in both groups, thus, the high blood pressure was due to an increased total peripheral vascular resistance. Although there were no significant differences in the organ blood flows measured between groups, blood flows to the placentas, and to the skeletal muscle, were inversely related to blood pressure in the S-fed, but not in the C-fed SHR. These results indicate that the hypertension induced by carbohydrate overfeeding is due to a generalized increase in total peripheral vascualr resistance, and suggest that high blood pressure during pregnancy results in a reduction of maternal perfusion of the uteroplacental circulation.

64.14

EFFECT OF CARBOHYDRATE OVERFEEDING ON BLOOD PRESSURE IN THE PREGNANT, SPONTANEOUSLY HYPERTENSIVE RAT (SHR). R.A. Ahokas, S.L. Reynolds*, and G.D. Anderson*. Univ. of Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

Although the SHR is considered a good model of human essential hypertension, it is not a good model of hypertension during pregnancy. Blood pressure falls progressively during the last week of gestation reaching normotensive levels by term. Carbohydrate overfeeding stimulates sympathetic nervous activity and increases blood pressure in male SHR. To determine whether carbohydrate overfeeding might prevent the fall in blood pressure during pregnancy, systolic blood pressure (SBP) was measured semidaily by tail-cuff plethysmography in ad libitum fed, pregnant (P), and nonpregnant (NP), SHR given water only (C-fed), or 10% sucrose (S-fed), to drink throughout gestation. The S-fed, P and NP SHR consumed 21 and 6% more kcal/day, respectively, than their C-fed counterparts. SBP of the P, C-fed SHR decreased 60 mmHg during the last week of pregnancy and was significantly lower than that of the NP, C-fed SHR by term (127±6 vs 177±8 mmHG, $P < 0.01$). SBP of the P, S-fed SHR also decreased slightly, but it was not significantly lower than that of the NP, S-fed SHR at term (164±11 vs 180±7 mmHg). There were no significant differences in litter size, number of stillbirths or mean birth weight between treatment groups. The results indicate that carbohydrate overfeeding will maintain hypertension throughout pregnancy in the SHR without affecting reproductive performance. This may be a useful experimental model of essential hypertension during pregnancy.

64.15

EFFECTS OF ANEMIA ON BLOOD FLOW AND O_2 DELIVERY TO MATERNAL ORGANS IN PREGNANT SHEEP. D. I. Edelstone, M. Paulone,* M. Hagberg,* J. Salerno,* Dept. of Obstetrics and Gynecology, Univ. of Pittsburgh, Sch of Medicine, Pittsburgh, PA 15213.

Anemia in nonpregnant animals results in large increases in perfusion to the brain and heart and only modest increases or no changes in perfusion to all other organs. Because of these blood flow responses, oxygen delivery (DO_2 = blood flow X arterial blood O_2 concentration) is constant for the brain and heart, but decreases to all other tissues. It is unknown how organ perfusions and DO_2's change during anemia in pregnant animals. In six chronically catheterized unanesthetized pregnant sheep, we measured organ blood flows (Q) and DO_2 at basal hematocrits (mean = 26%), and during moderate anemia (mean = 19%) and severe anemia (mean = 14%). Anemia was induced by slow isovolemic exchange transfusions with sheep plasma. Blood flows were measured with the radioactive microsphere technique. Blood flows to the maternal brain and heart increased with moderate and severe anemia. These increases were of sufficient magnitude so that DO_2's to these organs were constant. In contrast, perfusions to the kidneys, stomach, small intestine, colon, spleen, liver, and uterus were unaffected by moderate anemia, but decreased with severe anemia. Thus, DO_2 to these latter organs decreased linearly as a function of decreasing maternal hematocrit. Our results indicate that anemia during pregnancy induces the same responses of organ perfusion and O_2 delivery as occur in the non-pregnant state.

64.16

MATERNAL AND FETAL CARDIOVASCULAR RESPONSE TO MARIJUANA SMOKING IN LATE OVINE PREGNANCY. Richard F. Pekala*, Margaret K. McLaughlin*, and James F. Clapp* (SPON: J. Evans) UVM College of Medicine, Burlington, VT 05405

We studied the effect of chronic marijuana exposure designed to mimic human exposure on maternal and fetal cardiovascular parameters. Singleton gestation ewes (105-125 days, term = 145±5) were chronically instrumented with uterine flow probes, fetal and maternal vascular catheters, and fetal electrocortical leads. Following a one hour control period marijuana was administered (1.82-2.46% tetrahydrocannabinol) by tracheal inhalation. Maternal respiratory rate was depressed by 15-25 respirations per minute from a control level of 30-50 RPMs in all animals studied by 30 minutes post inhalation. Respiratory rate gradually increased to control levels during a 2-4 hour post-smoking monitoring period. Maternal heart rate consistently slowed by 5-35 beats per minutes when compared to control heart rates. Heart rates returned to control levels in 1-3 hours. Maternal arterial pressure decreased 10-20 mmHg in 4 of 5 animals with a variable decrease in uterine blood flow. In 3 of 5 ewes this was accompanied by an increase in uterine vascular resistance and an increase in fetal arterial pressure of 10-20 mmHg. Fetal heart rate was suppressed 15-60 BPM in 4 of 5 animals. Despite these minimal cardiovascular changes, daily control fetal pO2 values dropped with repetitive exposure, and fetuses either delivered prematurely or expired in utero. Supported by NIH DA03722.

64.17

EFFECT OF SUCKLING ON PITUITARY GTPase ACTIVITY IN THE LACTATING RAT. R. Ravindra* and C.E. Grosvenor. Dept. of Physiology, Univ. of Tenn., Memphis, TN 38163

It is well established that tubulin possesses an intrinsic GTPase activity, and in the present study, we employed this as a parameter to study pituitary tubulin function and to understand better the nature of the microtubule involvement in PRL secretion. Primiparous rats of the Holtzman strain (6 pups/litter) were not suckled for 4-5 h on day 12-14 postpartum. Groups of mothers were then suckled for 0, 15, 30, 60, and 90 min, then killed. The anterior pituitaries were collected and processed immediately to obtain the tubulin fraction which represents the monomeric form of the protein and the cold-stable and cold-labile fractions of polymerized microtubules. The GTPase activities in three fractions were estimated using [γ-^{32}P] GTP. A significant ($p<0.05$) increase was observed in the GTPase activity in the tubulin and in the cold-labile and cold-stable microtubular fractions prepared from the anterior pituitaries of rats suckled for 15 min compared to that in the non-suckled controls. The GTPase activity of the cold-labile fraction decreased significantly ($p<0.05$) between 15 and 30 min, while a substantial increase in the tubulin fraction occurred between 30 to 60 min ($p<0.05$), indicating a shift in the equilibrium between the polymerized and monomeric tubulin. These results suggest that tubulin is rapidly mobilized in the pituitary of the suckled rat and that the pituitary phosphatases are involved in PRL secretion. (HD-04358)

64.18

DHEA inhibition of [^{3}H] uridine uptake and incorporation in normal and transformed liver cells. Mohammed Kalimi and William Regelson. Med. Col. of Virginia, VCU, Richmond, VA 23298

Dehydroepiandrosterone (DHEA), a major hormone secreted by the adrenal cortex in humans, has been shown to have antitumorogenic and anti-obesity effects. In order to understand the biochemical mechanisms by which DHEA exerts its various effects on the cellular functions, we have studied the uptake and incorporation of ^{3}[H] uridine in rat hepatoma (HTC) and hepatocyte (H) cells. 2 x 10^6 HTC or H cells were treated with 10^{-5} to 10^{-10} M DHEA for 24h in BME supplemented with 5% fetal calf serum, pulse labelled with 5μci of ^{3}[H] uridine and both TCA soluble (uptake) and insoluble (incorporation) radioactivity were determined. Interestingly, DHEA treatment resulted in significant inhibition of both [^{3}H] uridine uptake and incorporation in HTC and H cells. The optimum inhibition was observed at 10^{-5} M DHEA. The inhibition was more pronounced in HTC cells (60%) than in H cells (40-45%) over control untreated cells. The DHEA effect was observed to be specific in that 10^{-5} M testosterone, 17β estradiol or hydrocortisone were without any significant effects. In conclusion, our findings suggest that DHEA (a) significantly inhibits both [^{3}H] uridine uptake and incorporation in HTC and H cells and (b) the inhibition is more pronounced in transformed HTC cells.

64.19

MILRINONE, A BIPYRIDINE CARDIAC INOTROPIC AGENT, COMPETES WITH THYROID HORMONE FOR BINDING SITES ON HUMAN SERUM PREALBUMIN (TBPA). Paul J. Davis*, Vivian Cody, Faith B. Davis* and Marion Schoenl*. VA Medical Center and Medical Foundation of Buffalo, Buffalo, NY 14203.

Milrinone (2-methyl-5-cyano-[3,4'-bipyridin]-6[1H]-one), a cardiac inotropic agent, is thyromimetic in a rabbit myocardial membrane Ca^{2+}-ATPase model, whereas amrinone, its 2-H-5-NH_2 analog, is not. In the present studies, milrinone (M) and thyroid hormone were compared for activity as competitors for binding sites on human serum thyroxine(T_4) transport proteins. Polyacrylamide gel electrophoresis of sera equilibrated with [^{125}I]T_4 showed that M (10-100 μM) competed with T_4 for sites on TBPA (10 and 100 μM, 61% and 73% reduction, respectively, in T_4-binding to TBPA, $P<0.01$), displacing T_4 to TBG. [^{14}C]-Milrinone was shown electrophoretically to bind to purified TBPA. Amrinone had <5% of the capacity of M to compete with T_4 for TBPA sites. Added in vitro, M had no effect on dialyzable fraction T_4 or T_4 RIA standard curve. Computer graphic modeling of M-binding to the T_4 site in the crystal structure of TBPA showed that M best occupies this site when the substituted bipyridine ring overlaps the phenolic ring of T_4. In this orientation, the cyano group occupies a similar volume and electronegativity as the 5' iodine of T_4. The 5-amino group of amrinone lacks these characteristics. Thus, M and thyroid hormone share structure and bioactivity homologies and compete for the same binding site on TBPA.
(Supported by NIH AM15051 and VA Research Funds)

64.20

THE LONG-TERM EFFECT OF CHRONIC EXOGENOUS HYPERINSULINEMIA ON THE DEVELOPMENT OF GLOMERULOPATHY IN NON-DIABETIC UNI-NEPHRECTOMIZED RATS. Susan M.R. Thompson*, Charles R. Smith* and Walter Zingg. Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

Thirty eight male Wistar rats underwent a unilateral nephrectomy immediately prior to assignment to one of three treatment groups. Group A (n=22) received a daily subcutaneous (sc) injection of six units of monocomponent porcine protamine zinc insulin (PZI) and a dextrose-supplemented diet. Group B (n=11) received a daily sc injection of PZI diluent along with a dextrose-supplemented diet. Group C (n=5) received PZI diluent along with a normal diet. After six months all rats were euthanized and renal tissue examined by light and electron microscopy; morphometric analysis was performed. Relative normoglycemia with pronounced hyperinsulinemia was evident in rats in Group A. Focal segmental glomerulosclerosis developed in all three groups with changes in Group A being the most severe (poorly perfused hypertrophied glomeruli with thickened capillary loops and an increase in mesangial cellularity); changes in Group B and C were mild in comparison. Glomerular basement membrane width was significantly increased in Group A. These findings demonstrate that chronic exogenous hyperinsulinemia accelerates the development of glomerulopathy in uni-nephrectomized non-diabetic rats.

SMRT is a Medical Research Council of Canada Fellow.

64.21

EFFECTS OF CHRONIC GLUCAGON ADMINISTRATION ON GLUCOSE HOMEOSTASIS IN PARTIALLY PANCREATECTOMIZED RATS. John A. Duncan, III* and Nancy R. Stevenson. Rutgers Medical School, Piscataway, New Jersey 08854.

The effects of sustained, 14 week glucagon administration were studied in adult, partially pancreatectomized rats. Sprague Dawley rats, 300-350 grams, underwent surgery reducing pancreatic mass 50-65%. Group A received continuous, subcutaneous glucagon (lot# 6ED44A, Eli Lilly) via implantable, osmotic minipumps (Alza, 2002). In the same manner, Group B received succinylated glucagon (biologically inactive, immunologically reactive) and Group C received hormone carrier. Group D was intact, untreated rats. All animals were fed ad libitum. Weight gains were normal throughout the experiment. During treatment, in Group A, fasting (18 hrs), plasma glucagon levels were 180% (140-220%) of corresponding controls over the 14 week period. From the 4th through 14th week, fasting plasma insulin levels in this group were decreased 73% (64-85%), compared with other groups ($p<0.001$). Irrespective, glucose homeostasis under fasting conditions and during glucose tolerance was maintained within normal limits. In response to oral and intraperitoneal glucose challenge (2.0 g/kg), plasma insulin and glucagon levels responded appropriately before; 3 and 10 days after cessation of glucagon treatment. In addition, there was no difference in pancreatic insulin and glucagon content (per mg protein) after treatment. Immunohistochemical staining and light microscopy revealed normal islet and acinar tissue. Thus, a chronic, elevated, physiologic, steady state level of glucagon, does lower plasma insulin levels without altering glucose homeostasis in partially pancreatectomized rats. (Supported by FUMDNJ #36-84.)

64.22

EFFECT OF DIBUTYRYL-cAMP AND INSULIN ON ERYTHROCYTE METABOLISM. Janice L. Podolski and Akira Omachi. Univ. of Illinois at Chicago, IL 60680.

Although human erythrocytes have little or no adenylate cyclase activity, cAMP can enter these cells. The effect of cAMP on red cell metabolism, however, is not well understood. This nucleotide reportedly increases lactate production (LP) by red cells previously kept under storage conditions (Biochim Biophys Acta 279:587,1972), but apparently has no effect on LP by freshly drawn cells (Clin Physiol Biochem 2:227,1984). To further investigate this phenomenon, stored cells were washed and incubated in Tris-Ringer's buffer with or without 5 mM dibutyryl-cAMP (db-cAMP), 5.8 nM insulin or both compounds. LP and glucose utilization (GU) were determined from samples of suspensions taken before and after 2.5 hr of incubation at 37°. LP was increased by 24.3% in the presence of db-cAMP ($p<0.05$). LP in suspensions with both db-cAMP and insulin was 45.7% greater than controls and 36.4% greater than those treated with insulin alone ($p<0.05$). Although an increase in GU was suggested in suspensions containing either db-cAMP or insulin alone, these changes were not significant. However, cells treated with both db-cAMP and insulin utilized 20.4% less glucose than cells treated with db-cAMP alone ($p<0.02$). These results support an earlier suggestion that db-cAMP directly increases LP by previously stored erythrocytes. Further, they suggest that insulin may modify the metabolism of db-cAMP-treated red cells.

64.23

MAMMALIAN METABOLITE FLUX RATES IN A TELEOST: LACTATE AND GLUCOSE TURNOVER IN TUNA. Jean-Michel Weber, Richard W. Brill, and Peter W. Hochachka. Southwest Fisheries Center, National Marine Fisheries Service, NOAA, Honolulu, Hawaii 96812.

Turnover rates were measured by bolus injection of U-^{14}C-lactate and 6-^{3}H-glucose in cannulated, lightly anaesthetized skipjack tuna, Katsuwonus pelamis, to: 1) assess the importance of these metabolites as fuels for tuna muscle, and 2) find out whether the high rates of lactate clearance observed during recovery from burst swimming could be accounted for by high lactate fluxes. Measured lactate turnover rates ranged from 112 to 431 umol $min^{-1}kg^{-1}$ and were correlated with blood lactate concentrations. Glucose turnover rates averaged 15.3 umol $min^{-1}kg^{-1}$. After correction for body mass and temperature, skipjack tuna shows higher lactate turnover rates than those reported for mammals. Their glucose turnover rates are similar to that of mammals but much higher than levels found in other teleosts. Even the highest lactate turnover rate measured in tuna cannot fully account for the rate of lactate clearance from the blood observed during recovery, suggesting that part of the lactate produced in skeletal muscle during exercise must be metabolized in situ during recovery from exercise. After injection of U-^{14}C-lactate, less than 5% of total blood activity was recovered in glucose, indicating that the Cori cycle is probably not an important pathway of lactate metabolism in tuna.

64.24

ADRENERGIC CONTROL OF PHOSPHORYLASE a ACTIVITY IN HEPATOCYTES FROM FEMALE RATS. **Rebecca K. Studer, Univ. of Pittsburgh, Pittsburgh, PA 15261.**

Although in adult male rats activation of glycogen phosphorylase is mediated by α-adrenergic pathways, in females sampled randomly throughout the estrous cycle a β component is present. To determine the relative roles of the α and β mediated pathways, and whether they are affected by changes in hormone levels during the normal estrous cycle, hepatocytes were isolated from females on the morning of metestrus, diestrus, proestrus and estrus. Phosphorylase a + b and basal and stimulated (epinephrine, 5 X 10^{-8}M alone, or with 10^{-5}M phenoxybenzamine, or 10^{-5}M propranolol) phosphorylase a activities were measured. Phosphorylase a + b was similar in cells from all phases of the cycle, however basal phosphorylase a did show some cyclic changes. The average activation by the α component ranged from 65% to 77% of the epinephrine alone stimulation, while the β component varied from 53% to 61%. It is concluded that both α and β mediated pathways are significant at all phases of the estrous cycle, and although small fluctuations in the α and β contributions to phosphorylase a activation are seen during the cycle, randomly sampled females can be utilized for future comparisons between male and female rats of the adrenergic regulation of glycogenolysis.

64.25

RAT MUSCLE METABOLITES FOLLOWING EXERCISE: COMPARISON BETWEEN INTENSE SWIMMING AND TETANIC ELECTRICAL STIMULATION.

Michael I. Lindinger*, George J.F. Heigenhauser and Lawrence L. Spriet*. Dept. of Medicine, McMaster University, Hamilton, Ont., Canada. L8N 3Z5.

The isolated perfused rat hindlimb has gained acceptance as a model for muscular exercise. We compared changes in intramuscular metabolites (glycogen, lactate, creatine phosphate (CP) and adenosine triphosphate (ATP)) after 5 min electrical stimulation in the isolated perfused rat hindlimb with those obtained following intense swimming (swim time = 4'23" ± 33"; mean ± SEM). Pre-exercise metabolites were within the normal range in both groups. The soleus showed little change in glycogen and ATP, whereas the largest changes were associated with the white gastrocnemius. Compared to the swim group, lactate accumulation in the stimulated group soleus and red gastrocnemius was small. These differences can be accounted for by high arterial lactates (20.0 ± 0.8 $mmol.l^{-1}$) after the swim, whereas one-pass perfusion kept arterial lactate below 2 mM in the stimulated group. In all other respects, the changes in muscle metabolites and wet:dry weight ratios between the two treatments were similar. In conclusion, the isolated stimulated perfused rat hindlimb preparation appears to be an appropriate model of in vivo energy metabolism in mammalian skeletal muscle during high intensity exercise.

Supported by the Medical Research Council of Canada.

64.26

THE EFFECTS OF METABOLIC STRESSES ON HEPATIC ATP LEVELS IN NORMAL AND MALARIAL MICE *IN VIVO* AND *IN VITRO*: A ^{31}P NMR STUDY. Yves Geoffrion, K. Butler*, I.C.P. Smith* and R. Deslauriers*. Div. Biol. Sci., NRC, Ottawa, Canada K1A OR6.

It has been reported that isolated liver mitochondria from mice infected with *Plasmodium berghei* malaria are functionally impaired (Biochem. J. 76: 41-44). We have used ^{31}P NMR to compare the steady state level of ATP in the liver *in vivo* and in the isolated perfused liver from normal and malarial mice. We observed a lowered steady state ATP level in perfused livers from malarial animals, but a similar P_i signal. The decrease in ATP and increase in the P_i and sugar phosphate (SP) signals caused by hypoxia was more drastic in perfused livers from normal rather than malarial animals. A fructose load (10 mM) in the perfusate also affected less the ATP level of the malarial than that of the normal preparation. *In vivo* steady state spectra from conscious (restrained) normal and malarial mice were identical. Two fructose loads (10 mM/kg ea.; 45 min apart) were given *i.p.* while the animals were secured within the probe inside the magnet. While this had little effect on the normal animals, the hepatic ATP level in malarial mice was drastically reduced and these animals did not survive the matabolic challenge. These results suggest that one of the causes of the proposed mitochondrial defect in the liver of malarial animals could be secondary to an impaired liver circulation which is known to occur in malarial animals.

64.27

ENERGY UTILIZATION IN FOOD-RESTRICTED FEMALE RATS. J.O. Hill*, C. Talano*, M. Nickel*, and M. DiGirolamo. Division of Endocrinology, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30303.

To assess if female rats differ in adaptation to calorie deprivation from males, sexually mature (250 g) female rats were exposed to varying degrees of food restriction and then kept at constant reduced body weights for up to 72 days. Energy intake was partitioned into 1) carcass storage; 2) cost of carcass storage; 3) energy required to maintain carcass energy content (maintenance). During underfeeding, all food-restricted rats showed considerable energy conservation according to our newly developed index of energy conservation (IEC). This was due to a reduction in the energy required for daily maintenance. Fasting for 3 or 6 days produced relatively lower (1/2 to 1/3) loss of fat and of caloric storage than underfeeding (60% of ad lib) for 15 or 32 days to reach comparable body weights. By contrasting results of the present study with those of food-restricted male rats of comparable age, we found that female rats lost relatively more (1/3 to 1/2) calories from fat than male rats subjected to comparable food restriction, but preserved lean mass to a far greater extent. Since fat is calorically denser than lean mass, females lost less body weight for a given reduction in carcass energy than males. We speculate that this relative sparing of lean mass and greater use of fat with food restriction in females may be linked to preservation of reproductive potential and may also influence capacity for weight loss.

64.28

METABOLIC RESPONSES OF THE PYGMY NUTHATCH (Sitta pygmaea) TO CHANGING ATMOSPHERIC PRESSURE. Douglas B. Hay‡ (Spon: D. Byman). Northern Arizona Univ., Flagstaff, AZ 86011.

Captive flocks of pygmy nuthatches were maintained in outdoor aviaries for use in studies of oxygen metabolism. Experimental analyses at 0°C revealed a significant positive correlation between atmospheric pressure(BP) and weight specific oxygen metabolism($\dot{V}_{O_2}$)(p .01, n=22). These data included the $\dot{V}_{O_2}$ of communal groups of four birds (r^2=.85, p .001, n=8), which displayed an average 20% difference in metabolism over a range of 10 mmHg. An additional experiment tested the association of paired individuals' metabolism to BP at 10°C. Analyses of $\dot{V}_{O_2}$ were made every other night over a period of 20 days and a range of 6 mmHg in actual atmospheric pressure. The paired birds' positive metabolic response to BP was significant (p=.007, n=40). A low r^2 of .23 in the second experiment appears the result of the birds' response to changing BP,rather than absolute, through time, such that the magnitude and direction of the change in $\dot{V}_{O_2}$ tracks that of BP. Frequent calibration of the O_2 analyzer, a consistent flow rate, and correction of the metabolic data to STP assured these data were both real and accurate. The birds' ability to sense BP and to respond to this exogenous cue by reducing their resting metabolism allows appreciable energetic savings prior to periods of bad weather which often accompany low pressure systems. Thus the observed reduction in metabolism represents an alternative to torpor, generally employed after periods of energetic stress.

64.29

SUBSTRATE UTILIZATION OF MITOCHONDRIA FROM HEART AND MOSAIC MUSCLE OF RAINBOW TROUT AND RESPONSE TO CHANGES IN EXTRAMITOCHONDRIAL MILIEU. J.M Donaldson* and P.W Hochachka. Univ. of British Columbia, Vancouver, B.C., Canada, V6K 1L6

Mitochondria were isolated from heart and mosaic muscle of rainbow trout (Salmo gairdneri,R.). Respiratory rates were determined at 5 and 15°C using pyruvate, malate, lactate, glutamate or acetyl-carnitine as substrate. pH profiles were generated for the final 3 substrates. Pyruvate was the substrate of preference for mitochondria in all cases, although at 15°C malate was equally good for heart and glutamate or acetyl-carnitine were equally good for muscle. Maximal oxidation rates of heart mitochondria were greater than or equal to those of muscle. Q_{10} for most substrates was about 2. Heart had a Q_{10} of 3 for malate while muscle had a Q_{10} of 4 for acetyl-carnitine and 7 for glutamate. Q_{10} was generally higher for muscle mitochondria. At pH above 7.6 respiratory rates decreased with increasing pH. Heart mitochondria showed an increase of respiratory rate as pH decreased over a wide range, while in muscle no such pH dependence was observed. RCRs were above 4 in all experiments except at high pH. Heart mitochondria have a higher oxidative capacity than muscle and are less affected by an acute temperature drop. Temperature and pH may alter intramitochondrial enzymes or transport mechanisms and in turn affect overall mitochondrial function in these tissues.

64.30

CITRATE PRODUCTION BY ISOLATED EPITHELIAL CELLS OF RAT VENTRAL PROSTATE. L.C. Costello, R.B. Franklin and V. Akuffo*. University of Maryland, Baltimore, Maryland 21201.

Prostate secretory epithelial cells have the unique function of accumulating and secreting extraordinarily high levels of citrate. The carbon sources for net citrate production have not been elucidated. Our recent studies support the proposal that aspartate provides the 4-carbon source of intramitochondrial oxalacetate. In contrast, the 2-carbon source for acetyl CoA remains unresolved. Suggested sources have been glucose (via pyruvate oxidation), fatty acid, and acetate. A prostate cell preparation containing low levels of citrate (and other metabolites) is essential for these studies. Recently, we prepared epithelial cell suspensions by collagenase treatment of ventral prostate. These cells are viable, propagate in culture, and respond to testosterone; thereby indicating their functional integrity. Harvested cells were suspended in basal medium (pH 7.2, 37°C) to which 5 mM pyruvate (to generate AcCoA) and glut + asp (to generate OAA) were added. Citrate levels at zero time were negligible. After 60 min. incubation the citrate level was 23 nmols. Reaction flasks containing pyruvate alone, glutamate + aspartate alone, and pyruvate with glut + asp were compared for citrate production. Only the reaction system containing the combined substrates generated citrate production. We now have a model system to study the exogenous sources for citrate production by prostate epithelial cells.

(Supported by NIH Grants HD16193 and AM28015)

64.31

A CASE OF COMPUTER MODELING IN VLDL METABOLISM. J. Yarmush, A. Robin, Loren Zech, K. Gil, J. Askanazi*, D. Elwyn. Columbia University College of Physicians & Surgeons. New York NY 10032.

Plasma triglyceride (TG), mainly VLDL, synthesis rates were calculated by single (traditional) compartment method or using a multicompartmental computer model (JCI 63:1262, 1979) in trauma (n=10) and depleted (n=8) patients. A primed constant infusion of C^{14} labelled FFA was administered and intermittent blood samples withdrawn for approximately 4 hrs. Plasma concentration and activities of FFA and TG were measured and TG synthesis rates were calculated. This study was approved by the Institutional Review Board, Health Sciences Center, Columbia University.

TG Synthesis Rates (umol/kg/min) Mean ± SEM

	TRADITIONAL	MULTI	MULTI WITH SLOW COMPONENT
Trauma	.092 ± .014	.099 ± .016	.214 ± .035
Depleted	.123 ± .035	.133 ± .040	.283 ± .088

A four hour study, using a continuous infusion, reveals TG synthesis rates at .1 umol/kg/min with no significant differences between trauma and depleted patients. However, recent studies with a pulse infusion reveal a second (slow) component of TG synthesis not seen until 8 - 12 hours. The computer model can simulate an experiment of longer duration incorporating the slow component and predicts an approximately two-fold increase in synthesis rates which are closer to experimental results of others.

64.32

METABOLIC AND HORMONAL RESPONSES TO PHYSICAL RESTRAINT IN CONSCIOUS PIGS. Carol A. Bossone*, Marjorie M. Hunt*, Charles E. Wade and John P. Hannon. Letterman Army Institute of Research, San Francisco, Ca. 94129

The effects of restraint on certain metabolite, hormonal and blood gas levels were studied in conscious swine. Seven to 10 days prior to study carotid artery catheters were implanted in Duroc pigs (20-25 kg, N=18). On the day of study, the pigs were allowed to assume a recumbent position for 30 min in a portable holding cage after which two control samples were drawn. Animals were then randomly divided into two groups. The first group remained in the cage while the second group was placed rapidly in a Pavlov sling. Blood samples were then taken at 2.5, 5, 10, 30, 60, 120 and 240 min. Sling placement led, within 10 min, to significant ($p \leq 0.05$) increased values (mean ± S.D.) for plasma epinephrine (69 ± 25.4 to 337 ± 139.8 pg/ml), norepinephrine (178 ± 89.7 to 363 ± 198.2 pg/ml), glucose (77 ± 6.2 to 101 ± 17.9 mg/dl), lactate (7.5 ± 2.50 to 46.5 ± 30.78 mg/dl), and arterial PO_2 (84 ± 6.4 to 94 ± 14.2 torr), and decreased values for plasma free fatty acids (0.78 ± 0.208 to 0.55 ± 0.237 μM/ml), and arterial PCO_2 (39 ± 2.2 to 35 ± 1.9 torr). A nonsignificant increase in insulin (3.72 ± 2.3 to 5.62 ± 2.72 μU/ml) and decrease in glucagon (225 ± 64.6 to 212 ± 30.6 pg/ml) led to a significant increase in the insulin to glucagon ratio (0.016 to 0.026). Plasma cortisol levels rose progressively over the 4 hour period of sling restraint (4.5 ± 1.69 to 8.3 ± 2.12 μg/dl). While cortisol, glucose and lactate values showed sustained elevations, other variables returned to basal values within 30 min. Pigs remaining in the cages showed no significant changes. The results of this study show that placement of the pigs into a Pavlov sling results in long and short term metabolic and hormonal changes.

64.33

NOCTURNAL SURGES AND REFLEXIVE RELEASE OF PROLACTIN IN PARENTALLY BEHAVING VIRGIN FEMALE AND MALE RATS. J. Terkel and Jakubowski, M. Department of Zoology, Tel Aviv University, Israel

Maternally behaving virgin rats enter a state of pseudopregnancy (PSP) during which prolactin (PRL) is secreted autonomus in a pattern of nocturnal surges. As long as maternal virgins continue cycling they are also capable of releasing PRL reflexively in response to pup stimulation. In the present study we examined (a) the initiation of the nocturnal PRL surges, (b) the role of estrogen in the reflexive release of PRL (c) the influence of gender on these two modes of PRL secretion. It was found that nocturnal PRL surges are already present on diestrus 1 and 2 of pup-induced PSP. At this stage, however, the surges are not yet autonomous. The capacity of reflexive PRL release was abolished by ovariectomy, and restored by estrogen replacement. The nocturnal PRL surges could not be generated in paternally behaving male rats. Paternal male rats were also incapable of releasing PRL reflexively in response to pup stimulation. These results demonstrate that the two modes of PRL secretion are sex dependent, and that the maternal virgin rat, unlike the postpartum rat, requires estrogen for releasing PRL in response to stimulation by the young.

64.34

CYCLOOXYGENASE PRODUCT SYNTHESIS BY ISOLATED PERFUSED RAT KIDNEYS FOLLOWING WHOLE BODY γ-IRRADIATION. M.J. Schneidkraut, P.A. Kot, P.W. Ramwell and J.C. Rose. Georgetown University Medical Center, Washington, D.C. 20007

The present study determined the involvement of the kidneys in radiation-induced changes in cyclooxygenase product excretion. Male Sprague-Dawley rats were anesthetized (30 mg/kg sodium pentobarbital i.p.) and exposed to 20 Gray (Gy) γ-irradiation with the abdomen or thorax shielded. A third group was exposed to 15 Gy without shielding. Urine thromboxane (iTXB2) and 6-keto prostaglandin Fla (i6KPGFla) values in these three groups were compared to sham irradiated controls. Irradiation (15 Gy) significantly increased urine iTXB2 and i6KPGFla 4 hrs after exposure. Both thoracic and abdominal shielding prevented the radiation-induced increase in these cyclooxygenase products. A second series of studies evaluated cyclooxygenase product release from isolated perfused kidneys. The animals were anesthetized, anticoagulated (1000 USP units heparin/kg i.v.) and the kidneys perfused in situ for 10 min, 4 hrs after exposure to 20 Gy or sham irradiation. The radiation-induced increase in urine iTXB2 was absent in the isolated perfused kidney urine but increased i6KPGFla excretion was observed. These data suggest that radiation-induced increases in iTXB2 excretion are due to alterations in non-renal synthesis but increased urinary i6KPGFla values following whole body irradiation are partially due to altered renal synthesis and/or metabolism.

(Supported by U.S. Army Contract DAMD 17-84-C-4006)

68.0

GRAVIPERCEPTION BY PLANT ORGANS. D.G. Heathcote. Univ. College Cardiff.

No abstract submitted

68.0

ROLE OF PLANT HORMONES IN GRAVITROPISM. K. Dörffling. Inst. of Botany, Hamburg.

No abstract submitted

68.0

INVESTIGATIONS OF HIGHER PLANTS IN WEIGHTLESSNESS. A.I. Merkis. Inst. of Botany, Hamburg.

No abstract submitted

68.1

CRITICAL ASSESSMENT OF HYPOGRAVITY SIMULATIONS. Allan H. Brown and David K. Chapman. Univ. of Pennsylvania, Philadelphia, PA 19104-4288.

The only unambiguous method of validating hypogravity simulations by clinostatting is to compare results of tests on clinostats with tests in satellites. Very few such comparisons have been accomplished. Less convincing test comparisons include pairs of putatively equivalent clinostat configurations that should yield the same biological result. If not, that would call into question assumptions on which the effects of the clinostat environment were predicted. We believed that, in principle, only three such testable pairs of configurations are possible. Data suitable for such tests were collected and these tended to support the validity of clinostat simulations of hypogravity. Nevertheless more definitive data from a Spacelab 1 experiment, HEFLEX, demonstrated that, in the case of hypocotyl circumnutation, the results obtained in true microgravity were not quantitatively the same as those from tests on clinostats. We conclude that, for some applications, the results of tests on clinostats may support useful if not definitive conclusions about biological effects of a hypogravity environment; however, we now can be sure that the clinostat environment cannot in all cases affect biological systems the same as does true microgravity.

68.2

GRAVITY SENSING IN ANIMAL CELLS.
Augusto Cogoli. ETH-Zentrum, CH-8092 Zürich, Switzerland

In this paper I am reviewing the results of experiments performed in micro-G in space, in low-G simulated in the clinostat, and in hyper-G in the centrifuge, clearly showing that in most cases animal cells are sensitive to the G-environment. While G-sensitive organelles, the statolyths, were identified in root cells of higher plants, no equivalent G-receptors were discovered in animal cells so far. However, since any change of the environmental parameters, like temperature, concentration or pressure, is followed by an adaptation reaction of the cell, it is justified to expect that a change of the G-environment may result in a change of cellular functions. We have seen that the activation of human lymphocytes by mitogens in-vitro (a typical example of cell differentiation) is reduced by 50% in the rapidly rotating clinostat (0.2 x g) and by more than 90% in micro-G (Spacelab-1). Conversely, lymphocytes and other cell lines grow faster (by 20 to 30%) at 10 x g than at 1 x g. Talas et al. reported that human lymphocytes produce 5-times more interferon when cultured in space (Salyut-6) than on the ground. WI-38 human embryonic lung cells cultured in Skylab (Montgomery et al.) reduced their glucose consumption by approx. 20%, without changing their proliferation rate or motility. Hyper-G speeds up the differentiation process of mouse palate cells in-vitro (Duke et al.), while the number of mouse oocytes reaching metaphase II is significantly reduced in the clinostat rotating at 100 rpm (Wolgemuth and Grills). Surprisingly, experiments with amphibian eggs in the clinostat (Neff et al.) led to the prediction that Xenopus eggs fertilized and allowed to develop in space will undergo normal morphogenesis. In conclusion, hyper-G tends to increase cell proliferation rate, whereas hypo-G has a depressing effect on mitosis and differentiation of mammalian cells. The G-effects seem to be more pronounced in cells undergoing differentiation than in non-differentiating cells.

68.0

INVESTIGATIONS OF VESTIBULAR-OPTIC-MOTOR INTERACTION IN WEIGHTLESS CONDITIONS. I.B. Kozlovskaya (with co-authors).

No abstract submitted

68.0
ORGANIZATION OF NYSTAGMUS: INVOLVEMENT OF OTOLITH ORGANS AND SEMICIRCULAR CANALS. I. Orlov.
I.P. Pavlov Inst. of Physiology, Leningrad.

No abstract submitted

CONTROL OF BREATHING: CENTRAL MECHANISMS AND INTEGRATED RESPONSES

69.1

HYPOTHALAMIC STIMULATION CHANGES RESPIRATORY NEURON MEMBRANE POTENTIALS. **H.R. Holmes and J.P. Farber. Dept. Physiology, Univ. Oklahoma HSC, Oklahoma City, OK 73190.**

This study examined the modulation of brainstem respiratory neurons by hypothalamic pathways. Constant current bipolar stimulation of anterior hypothalamic sites in chloralose anesthetized, paralyzed, and vagotomized cats was used to change breathing patterns. To examine excitatory and inhibitory synaptic inputs, membrane potentials of inspiratory (I) and expiratory (E) neurons in dorsal and ventral respiratory groups were recorded intracellulary, and compared to whole phrenic nerve activity during control, electrical activation of the hypothalamus and negative current injection. A common response pattern to hypothalamic stimulation was reduced phrenic output, with prolonged inspiration and little effect on expiration. During stimulation, excursion of membrane potential of both projecting and non-projecting I (11 of 15) and E (18 of 23) was reduced; neither depolarization nor hyperpolarization reached control levels. Typically there was no evidence of decreased synaptic input. During excitatory breathing patterns such as panting, 14 I and E cells followed phrenic output with increased depolarization. In non-cycling phrenic output patterns such as apneusis and apnea, some I (5 of 12) and E (8 of 9) membrane potentials continued to cycle, albeit with reduced excursion. Hypothalamic stimulation alters synaptic input to brainstem respiratory neurons in both I and E phases. (NIH HL-00619).

69.2

EFFECTIVENESS OF THE VENTILATORY PUMP AT INCREASED LUNG VOLUMES. Steve Iscoe. Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Increases in lung volume may impair inspiratory function at increased lung volumes because of both shortening of the inspiratory muscles and the increased radius of curvature of the diaphragm. To quantitatively evaluate this impairment, I had anesthetized cats rebreathe CO_2 both at control functional residual capacity (FRC) and at an elevated lung volume produced by an expiratory threshold load. The rate of rise of "integrated" phrenic activity served as an index of respiratory drive and the rates of generation of tracheal pressure during occlusions and volume change during unobstructed breaths as indices of inspiratory muscle output. FRC increased an average 44 ml, approximately half that predicted on the basis of the passive compliance. At levels of inspiratory drive equivalent to those obtained during eupnea at control FRC, there was no deficit in pressure or flow generation at increased FRC. At twice control drive, however, average pressure and flow generation decreased significantly by 25 and 20% respectively at increased FRC. No further decreases occurred at three times control values. These results suggest that at normal levels of respiratory drive, nondiaphragmatic inspiratory muscles can compensate for the mechanical impairment produced by an increased FRC but are less successful under conditions of increased respiratory drive. Supported by the MRC and Ontario Thoracic Society.

69.3

LARYNGEAL MUSCLE ACTIVITIES DURING HYPERCAPNIC HYPERPNEA IN UNANESTHETIZED DOGS. S.J. England, R. Harding*, J. Stradling* L. Kozar*, S. Andrey*, and E. Phillipson. Respiratory Physiology, Hospital for Sick Children and Dept. of Medicine, Univ. of Toronto, Toronto, Canada.

The response of respiratory muscles, especially the intrinsic laryngeal muscles, during ventilatory stimulation induced by hypercapnia have not been well documented in unanesthetized animals. These responses were studied in four adult dogs with chronically implanted electromyographic electrodes in the diaphragm, internal and external intercostal, thyroarytenoid (TA), posterior cricoarytenoid (PCA), and cricothyroid (CT) muscles. Hypercapnia was induced by hyperoxic rebreathing during wakefulness, slow wave sleep, and rapid eye movement sleep (REM). As ventilation increased, inspiratory activities of the diaphragm, external intercostal, PCA, and CT muscles were augmented. When expiratory flow rates had increased to 2-3 times control, PCA, CT and internal intercostal muscles were activated during expiration. No qualitative state-dependent differences in respiratory muscle activities were apparent. TA activity was observed in only one dog during what appeared to be forceful expirations as hypercapnia progressed in wakefulness and slow wave sleep but not REM. In conclusion, in all behavorial states, the intrinsic laryngeal muscles are activated in concert with the diaphragm and intercostal muscles to decrease both inspiratory and expiratory laryngeal resistance during hypercapnic hyperpnea.
Supported by MRC Grants MA 7844 and MT 4606.

69.4

RELATIONSHIP BETWEEN RESPIRATORY MUSCLE ELECTRICAL ACTIVITY AND INSPIRATORY SHORTENING DURING HYPERCAPNIA. E. van Lunteren* and N.S. Cherniack. Case Western Reserve Univ., Cleveland, OH

Conventional indices of respiratory muscle mechanical output (tidal volume, occlusion pressure) assess the integrated output of multiple muscles whose responses to respiratory stimuli may differ; very little data exists comparing the electrical activity of individual thoracic respiratory muscles with their in situ mechanical behavior. In 9 anesthetized dogs we compared EMG's of costal diaphragm (D) and 3rd parasternal intercostal (IC) muscles with their respective respiratory changes in length (measured by sonomicrometry) during CO_2 rebreathing. Hypercapnia (CO_2 up to 90 torr) increased inspiratory shortening of D from 6.4% of resting length (L_R) to 12.9% L_R ($P < 0.001$) and of IC from 7.7% L_R to 11.8% L_R ($P < 0.005$). There were significant linear correlations between CO_2-induced increases in EMG's and increases in inspiratory shortening for each muscle. However, the EMG CO_2 response was greater for IC than D, whereas the mechanical (muscle shortening) CO_2 response was greater for D than IC. During occluded breaths, D shortened 2.2% L_R and IC 5.7% L_R; these amounts increased with hypercapnia to 8.1% L_R for D and 8.6% L_R for IC. The results suggest that (1) a close linear relationship exists between the electrical and mechanical behavior of individual thoracic muscles during CO_2 rebreathing, (2) at higher levels of ventilation IC may act increasingly to stabilize the thoracic cage, and (3) during occluded breaths substantial shortening of both D and IC occur. Support: NIH HL-25830.

69.5

OPTIMIZATION MODEL OF VENTILATORY CONTROL: EFFECT OF MECHANICAL LIMITATION. **C.S. Poon. North Dakota State Univ., Fargo, ND 58105.**

In a previous paper (Poon, C.S. In: Modelling and Control of Breathing, NY: Elsevier, 1983, pp. 189-196) I postulated that ventilation may be controlled to minimize the conflicting challenges due to chemical drive and respiratory mechanical work. The proposed model was shown to yield the normal ventilatory responses to exercise and various chemical stimuli. In addition, the model suggested that hypercapnia and exercise had purely multiplicative effect on $\dot{V}_E$. Recent experimental findings from our laboratory, however, reveal both additive and multiplicative components in the interaction. In the present study, I considered a modified optimization index of the form $J = (\alpha\ PaCO_2 - \beta)^2 + 2 \ln [\dot{V}_E/(1 - \dot{V}_E/\dot{V}_{max})]$, where α, β are the chemoreceptor sensitivity and threshold, respectively, as previously defined, and $\dot{V}_{max}$ is the maximal sustainable ventilation representing the mechanical limit of the respiratory pump. This formulation led to a predicted CO_2-exercise interaction with additive-plus-multiplicative effect on $\dot{V}_E$. The extended model yielded good fits to ventilatory response data obtained in eight healthy subjects during hypercapnic exercise, with estimated $\dot{V}_{max}$ values similar to the subjects' maximal voluntary ventilations. These results lend further support for the optimization hypothesis and suggest a significant role for respiratory mechanical limitation in ventilatory control. **(Supported by NIH grant HL-30794 and AHA grant DA-84-G-12).**

69.6

VENTILATORY RESPONSE TO CO_2 AND O_2 NEAR EUPNEA IN AWAKE DOGS. S.M. Yamashiro, W. Hwang*, D. Sedlock*, and F.S. Grodins. Biomedical Engineering, USC, Los Angeles, CA. 90089-1451.

To investigate the ventilatory response to arterial CO_2 tension near the resting point more thoroughly, a method of high-frequency ventilation was employed to unload metabolically produced CO_2 from 5 awake resting dogs. Steady state iso-oxic ventilatory responses to CO_2 above and below the eucapnic level were measured under hyperoxic, normoxic, and hypoxic conditions. A quadratic regression equation was fit to pooled minute ventilation - arterial CO_2 tension data, and a statistically significant nonlinear response was evident in all 5 animals for conditions of hyperoxia, and hypoxia. The response was linear down to apnea during normoxic conditions for 3 of 5 animals. The mean ventilatory sensitivity to CO_2 was 40% less at the apneic CO_2 level than 5 torr above it during hyperoxic conditions, while the mean ventilatory sensitivity was 55% greater at the apneic CO_2 level than at 5 torr above it during hypoxic conditions.

These results indicate that the ventilatory sensitivity to CO_2 near the operating point is not markedly different than predicted by conventional CO_2 inhalation techniques, and the effect of arterial O_2 tension on the ventilatory response to CO_2 is important even during a change from hyperoxic to normoxic conditions. (Supported by: NIH Grants HL16390 and HL07012).

69.7

RESPIRATORY INTERACTIONS BETWEEN CHANGES IN METABOLIC RATE AND TEMPERATURE. **J. S. Humphery* and E. M. Adams. Div. Cardiothoracic Surgery, University Hosp., Cleveland, OH, 44106.**

Increased metabolic rate (VCO2) results in proportional increases in the ventilatory drive (Ve). However, one consequence of increased VCO2 is often a rise in core body temperature (Tb). This study was designed to examine the thermal contribution to respiration at elevated VCO2. Respiratory and CO2 responses to increased VCO2 produced by 2,4-dinitrophenol (DNP) were studied over a range of Tb (35-42 C). Cats were anesthetized with Nembutal and respiration, diaphragmatic EMG (Edi), end-tidal and mean expired PCO2's were measured. VCO2, Vt/Ti, Edi/Ti, and Ti/Ttot were calculated. VCO2 increases (+100 - 400%) were produced by DNP administration. Baseline Ve and VCO2 were measured after each VCO2 or Tb change and the CO2 response was assessed by CO2 rebreathing. The Ve/VCO2 and Ve/Tb relationships were linear but parallel shifted when temperature was increased at any VCO2 level. Increasing either Tb or VCO2 decreased B and increased CO2 response slope. The combined stimuli appear to act additively. Tb stimulated increases in Ve resulted in higher respiratory rates and lower Vt than did the VCO2 stimulus. We conclude that: 1) temperature inputs to the respiratory controller are independent of those associated with VCO2, and 2) the interaction between these inputs is additive in nature.

69.8

FASTIGIAL STIMULATION AND OUTPUT OF RESPIRATORY MUSCLES IN THE DEVELOPING OPOSSUM. **J. P. Farber and S. R. Gates*. Dept. Physiol. Univ. Oklahoma HSC, Oklahoma City, OK 73190.**

Pathways from the cerebellum are capable of modulating breathing patterns in adult mammals, but developmental effects have not been considered. In the present study, constant current cerebellar stimulaton using bipolar electrodes was performed in Inactin-anesthetized opossums between 20 and 70 days of age. For animals > 30 days, positive pressure breathing was used to induce expiratory EMG activity in intercostal (IC) and abdominal (ABD) muscles; these were measured in addition to the diaphragm (inspiratory) EMG. Stimulation of the fastigial region showed strong expiration-phased effects. At low current density, the expiratory ABD and IC EMGs were reduced; often, the ABD EMG was suppressed more than the IC EMG. With higher stimulation intensity, ABD EMG was typically excited (presumably as the result of current spread); this effect was also observed in animals too young to show spontaneous activation of ABD muscles. In animals of all ages, fastigial stimulation prolonged the expiratory phase; optimal stimulation frequency for prolongation of expiration was usually lower for younger vs. older opossums. Since pathways from the fastigial region may differentially influence motor groups with comparable respiratory function, it could contribute to developmental inhomogeniety previously observed in ABD and IC responses during positive pressure breathing (Federation Proc. 44:461, 1985). (NIH HL-24865; HL-00619).

69.9

VIBRATION OF HUMAN EXPIRATORY MUSCLES ALTERS THE OUTPUT OF RESPIRATORY NEURONS. T. Kondo* and B. Bishop. Dept. of Physiology, State University of N.Y. at Buffalo, Buffalo, N.Y. 14214.

To determine whether input from expiratory proprioceptors modifies respiration we asked 1) How does sustained vibration (SV) of intercostal muscles (IC) modify breathing? 2) Does SV of the external oblique (EO) muscle alter abdominal motoneuron excitability? In an IC series, subjects rebreathed for 6 min from a 5L spirometer initially filled with 100% O_2. Vibration (4mm, 100Hz) was applied uni- or bilaterally to the T_7 space, where IC function is expiratory. Vibration was sustained for 6 breaths after every 10 non-vibrated breaths. Bi-, but not unilateral SV of IC reduced tidal volume and inspiratory duration. As hypercapnia progressed, these reductions decreased. In an EO non-rebreathing series, the abdominal monosynaptic reflex (AMR) was elicited repeatedly by taps to a bar (3x20 cm) held against the linea alba. AMR amplitudes were determined from surface EMGs made before, during and after unilateral SV of EO. AMR was suppressed by SV but recovered in 2 min. In conclusion, bilateral IC vibration exerts inhibitory effects on the central "off-switch" mechanism, whereas EO vibration exerts inhibitory effects at the spinal level.

69.10

UPPER AIRWAY MUSCLE (UAM) ACTIVITIES IN THE FETAL SHEEP. Alastair A Hutchison*, Robert M Abrams, Sidney Cassin, September L Evans*. Depts of Peds, Ob-Gyn, and Physiol, Univ of Florida, Med Ctr, Gainesville, Fl 32610

In utero, UAM activities are related to sleep state, movemements, arousals, swallowing, and lung liquid flow. The aim of this study was to re-examine these activities and, by enlarging the number of muscles studied in each behavioral state, to demonstrate clearly the nature of the above observations. Four fetal sheep, gestational ages 130-132 days, were chronically instrumented with electrocorticogram electro-oculogram (EOG), nuchal electromyogram (NEMG), and genioglossus (GG), posterior cricoarytenoid (PCA), thyroarytenoid (TA), inferior constrictor (Inf Con), intercostal (IC) and costal diaphragmatic (DEMG) EMGs. A fifth fetus had an exteriorized tracheal loop in situ. Studies were performed 3-5 days post-operatively. Movements in NREM were associated with increased NEMG, GG, TA, IC, and DEMG activities, with minimal increase in PCA activity. They were often related to, and at times preceded by, an arousal. These may be the "gaspings" seen in NREM. Arousal in REM, characterised by NEMG activity and a change from phasic to tonic GG activity, produced the same pattern. Only movements in REM which caused momentary IC activity were accompanied by increased TA activity, the phasic PCA and DEMG activities ceasing. Swallowing, noted with GG, TA, and Inf Con and no PCA or DEMG activities, was seen primarily in REM, where it accounted for almost all of the TA activity. With a tracheal loop, UAM activities were decreased, especially in REM. During absence of upper airway lung liquid flow, these decreases were more marked, although phasic GG and PCA activities were seen with changes from NREM to REM.

69.11

Concepts of the ventilatory exercise gain. F.M. Bennett and W.E. Fordyce. Worcester Polytechnic Institute, Worcester, MA 01609 and SUNY Upstate Medical Center, Syracuse, NY 13210.

Confusion exists as to the strict definition of ventilatory exercise gain. The estimate commonly used is G=$\Delta \dot{V}E/\Delta \dot{V}CO2$. This is the overall system gain. This gain reflects exercise-specific stimuli as well as changes in arterial blood gases. Only if the response to exercise is strictly isoxic, isocapnic, and without metabolic acidosis will G be equal to the gain of the exercise stimuli (GEX). We examined the difference between G and GEX with a steady-state respiratory model (Resp. Physiol. 59:55, 1985) where the response was not isocapnic. The resting operating point was held constant while GEX was varied. When the response was hypercapnic ($\Delta PaCO2$=3 torr), G/GEX = 2.0 and when the response was hypocapnic ($\Delta PaCO2$= -3 torr), G/GEX = 0.7. When the resting operating point was changed and GEX was held constant, G systematically decreased with increasing resting PaCO2. This could lead to the incorrect conclusion that the ventilatory exercise gain had changed. In general, G is an inappropriate and potentially misleading estimate of the gain for the exercise-specific stimuli. Supported by N.I.H. Grant HL-30653.

69.12

NEURO-MECHANICAL INTERACTIONS BETWEEN LARYNGEAL AND CENTRAL INSPIRATORY MECHANISMS. L.L.FAN,* C.M.SCHRAMM,*J.S.CRUNSTEIN,* AND M.M.GRUNSTEIN. Univ. of Colorado, Denver, CO 80262.

To evaluate the interrelationship between laryngeal function and respiratory pattern, we tracheostomized spontaneously breathing anesthetized rabbits and isolated the larynx from the lower airway. Translaryngeal resistance (R_{TL}) was measured by relating changes in translaryngeal pressure to a continuously applied airflow; lower airway (LA) airflow and tracheal pressure (P_T) were concomitantly measured. During control breathing, R_{TL} progressively decreased during the course of inspiration. During subsequent sustained, multiple-breath occlusion of the LA at end-expiration: 1) the time course of inspiratory decrease in R_{TL} was the same in the first occluded breath as in the preceding control breath, indicating an absence of volume feedback modulation; 2) within the following occluded inspiration, an inverse linear relationship was obtained between instantaneous decreases in R_{TL} and P_T; and accordingly 3) the maximal progressive decreases in R_{TL} at end-inspiration were inversely linearly related to the maximal changes in occluded P_T. The relationship between changes in R_{TL} and P_T was abrogated by administering increasing doses of sodium pentobarbital. These findings indicate a close interaction between the mechanisms regulating central inspiratory activity and laryngeal function, wherein this interaction is unaltered by phasic volume feedback from the lungs or changes in the magnitude of central inspiratory output, but disrupted with increasing levels of anesthesia.

CEREBRAL CIRCULATION

70.1

CEREBRAL BLOOD FLOW RESPONSE TO HYPOXIA: EFFECT OF DECREASED OXYHEMOGLOBIN AFFINITY. Raymond C. Koehler, Richard J. Traystman and M. Douglas Jones, Jr. The Johns Hopkins Medical Institutions, Baltimore, MD 21205

The increase in cerebral blood flow (CBF) during hypoxic hypoxia (HH) maintains bulk O_2 delivery (OD=CBF X CaO_2). With CO hypoxia, the CBF response is greater and OD rises in association with the concurrent decrease in P_{50} (PO_2 at 50% O_2-Hb saturation). Because CO may have other effects, we investigated whether P_{50} interacts with the CBF response to isocapnic HH for similar reductions in CaO_2. CBF (microspheres) was measured in 9 unanesthetized neonatal lambs during normoxia (N: CaO_2 = 13.8 vol%) and 2 levels of HH (HH1: CaO_2 = 8.7) (HH2: CaO_2 = 6.7) at baseline P_{50} (26.3 ± 1.7 Torr) and then repeated at high P_{50} (36.6 ± 2.0 Torr). P_{50} was increased by isovolemic exchange transfusion with low affinity, adult sheep donor blood. Elevated P_{50} reduced CBF by 22% during N (90 ± 12 to 71 ± 4 $ml \cdot min^{-1} \cdot 100g^{-1}$). The slope of the CBF response to HH was similarly reduced by 26%; i.e., the HH response was reduced in proportion to the new normoxic level. With elevated P_{50}, OD ($ml\ O_2 \cdot min^{-1} \cdot 100g^{-1}$) was reduced at N (12.4 ± 1.8 to 9.8 ± 0.6), HH1 (11.9 ± 1.2 to 9.5 ± 0.8) and HH2 (13.5 ± 1.8 to 10.0 ± 0.9) by similar amounts. OD during HH was unchanged from N at both low and high P_{50}. Transfusing with matched P_{50} blood in control lambs did not lower OD. Therefore, O_2-Hb affinity influences the CBF response to HH independent of the level of CaO_2 and it may be important in the postnatal regulation of CBF. (Supported by NS-20020)

70.2

THE EFFECTIVENESS OF DESFERRIOXAMINE ON IMPAIRED REPERFUSION AFTER TOTAL CEREBRAL ISCHEMIA IN DOGS, R.C. Crumrine*, V.H. Heyman*, T.J. Ricci*, J.C. LaManna, Univ. Hospitals of Cleveland and Case Western Reserve Univ. Med. Sch., Cleveland, Ohio 44106

Iron catalyzed, free-radical induced membrane damage may limit survival from cerebral ischemia. Dogs were subjected to 11 min of total cerebral ischemia produced by inflation of intravascular occlusion balloons in the inferior vena cava and the aortic arch. A loading dose of an iron chelator, desferrioxamine (15 mg/kg), was administered over 1 hour starting immediately post occulsion with a maintenance dose (3 mg/kg/hr) for the next 5 hours. Regional cerebral blood flow (RCBF) measurements by radiolabeled microspheres were taken before and 3 hours after ischemia. Control, untreated animals could be split into two groupings; those animals that survived 7 days and those that did not. The nonsurvivors had a lower cortical RCBF than the survivors at 3 hours (34 ± 6% vs. 59 + 10% expressed as percent of pre-ischemic flow). The cortical RCBF obtained from the desferrioxamine animals at 3 hours follows the pattern of the nonsurvivor control group (35 + 7%). All dogs given desferrioxamine exhibited signs of drug toxicity (pulmonary edema, elevated HCT and elevated temperature). While desferrioxamine does not alter post ischemic hypoperfusion, its effectiveness on long-term, post ischemic survival remains to be determined. Supported by NIH grant, R01, HL23583.

70.3

THE EFFECTS OF CBS-645 and PROSTACYCLIN (PGI_2) ON THE GERBIL MODEL OF CEREBRAL ISCHEMIA. R.L. Saldanha,* M.D. Cruze,* O.S. Bunnell,* and T.M. Louis. ECU School of Medicine, Greenville, NC 27834.

The effects of CBS-645 and PGI_2 on gerbils undergoing a left common carotid artery occlusion were evaluated using the MacGraw Stroke Index. The gerbils were observed for stroke signs for a period of 1 hr., followed by removal of the occlusion clip. Twenty-five percent of the gerbils showed stroke signs and were immediately given 1 of 4 treatments.

Group	Treatment	Number	% Recovered From Stroke
I	Saline control	21	29
II	PGI_2	21	57
III	CBS-645	9	22
IV	PGI_2+CBS-645	10	10

After a recovery period of 30 min., each gerbil was scored for stroke signs at 10 min. intervals for 1 hr., then at 24 and 48 hrs. Twenty-nine percent of the gerbils in the control group showed no stroke signs at 24 and 48 hrs. Groups III and IV showed no improvement as compared with controls. However, gerbils in Group II showed significant improvement at 24 and 48 hrs. (p=0.045 by the Fisher's Exact Test). Comparison of the 4 treatment groups suggests that PGI_2 given alone reduced the neuropathologic symptoms induced by ischemia. However, CBS-645 alone or combined with PGI_2 given after the ischemic insult did not appear to help in the recovery from stroke in gerbils.
Supported in part by the NC United Way to RLS.

70.4

A MODEL OF TOTAL CEREBRAL ISCHEMIA (TCI) IN THE NEWBORN PIGLET. A. Kopelman,* M.D. Cruze,* S.C. Young,* T.M. Louis, J.P. Harris* and R.H. Ray. ECU School of Medicine, Greenville, NC 27834

Recent evidence suggests that neurologic damage in the newborn often results from brain ischemia. Animal studies of brain blood flow during and following brain ischemia have been hampered by difficulty in re-establishing cardiovascular stability, or by the requirement for major surgery. We have adapted a model of TCI (Jackson, D. L., Stroke 12, 66, '81) produced by occluding the ascending aorta by inflating a balloon catheter inserted via the femoral artery in the anesthetized, ventilated newborn piglet. A second balloon cathether is first inflated in the IVC to reduce right heart filling and prevent pulmonary edema. Monitoring axillary B.P., carotid blood flow and EEG confirms TCI during the 12 min. occlusion. Coronary perfusion is unobstructed and H.R. and B.P. rapidly return to baseline after the balloons are deflated.

	HEART RATE (beats/min.)	MEAN BLOOD PRESSURE (mmHg)	CAROTID BLOOD FLOW (ml/min.)
Pre-occlusion	257	79	42
During occlusion	302	12	0
Post-occlusion	240	74	47

The advantages of this technique for producing TCI are that it avoids thorocotomy and protects cardiac function.

70.5

CEREBRAL BLOOD FLOW AUTOREGULATION WITH LOCAL HYPOTENSION IN THE NEWBORN LAMB. J. Timothy O'Neill, Gregory A. Franklin*, Errol R. Alden*. Pediatrics, USUHS, Bethesda, Md. 20814

We have been studying the cerebral blood flow (CBF) response of newborn lambs to decreases in mean arterial pressure (MAP). In previous studies, hypotension (HT) was induced by hemorrhage. While CBF was maintained down to a MAP of 30 mmHg, one group of lambs became anemic with HT. In a group of lambs in which hematocrit was maintained, cerebral O_2 consumption (MVO_2) increased with HT. In an attempt to study cerebral autoregulation in the absence of these factors, we used carotid constriction to produce local HT. Eight newborn lambs (<7 days old) were anesthetized with α-chloralose and urethane, paralized and ventilated. Catheters were placed in the left ventricle, axillary arteries and the dorsal sagittal sinus for radioactive microsphere blood flow measurements and blood sampling. Snares were placed around each common carotid artery and a 22 gauge angiocath was placed in each carotid distal to the snares to measure cephalic blood pressure (CBP). CBP was reduced, by progressive occlusion of the snares, from control 86 ± 3 to 50, 40 and 30 mmHg. Total and 19 regional CBF's were not changed with decreases in CBP. MVO_2 did not significantly change in these experiments although brain O_2 extraction did increase 30% ($p<0.01$). These data indicate that the newborn lamb can autoregulate CBF to 30 mmHg MAP (representing a perfusion pressure of 23 mmHg) and it appears that HT is a stimulus for the brain to increase fractional O_2 extraction. Supported by USUHS Grant C08631.

70.6

EFFECT OF THEOPHYLLINE ON HYPOXIC AND FUNCTIONAL HYPEREMIC RESPONSES OF CEREBROCORTICAL MICROCIRCULATION. Arisztid G. B. Kovách and Eörs Dóra. Exp. Res. Dept. and 2nd Inst. of Physiol., Semmelweis Univ. Med. Sch., H-1082 Budapest

In the present study, we investigated the effects of topical /superfusion/ of the brain cortex with a CSF solution containing 10^{-4} M theophylline/ and systemic /intraperitoneal injection of 2×10^{-4} mol/kg theophylline/ theophylline /THEO/ treatment on the arterial hypoxia- and epilepsy-induced increases of CBF in the chloralose anaesthetized cat. Changes in cerebrocortical microcirculation were measured with reflectometry /1/. Under control conditions, transient arterial hypoxia /6-7% FiO_2/ and epileptic seizures increased CBF by approximately 150% and 300%, respectively. The CBF increasing potency of arterial hypoxia was not diminished by THEO treatments, but topical and systemic THEO treatments resulted in an approximately identical attenuation in the CBF increasing potency of epileptic seizures. The epilepsy-induced CBF increase was decreased by approximately 25% by THEO. In accordance with our previously published data /2/, the present study indicates that extracellular adenosine is not a critical factor in the regulation of CBF in the cat during arterial hypoxia, but it may have some role in the functional hyperemic response of cortical microcirculation.

A. Eke et al., Am. J. Physiol. H759, 236, 1979.
E. Dóra et al., J. Cereb. Blood Flow Metabol. 447, 4, 1984.

70.7

MECHANISMS AND SITE OF RELEASE OF ADENOSINE IN NERVOUS TISSUE. M. Bencherif*, R. Rubio and R. M. Berne. Dept. of Physiology, University of Virginia, Charlottesville, Va.

Adenosine plays a role in the control of neurotransmission and blood flow. To assess the function of adenosine, the mechanism and site of its release must be known. We have used the isolated rabbit and frog sympathetic ganglia in which the nucleotide pool was pre-label with ^{3}H-adenosine. In both preparations the ^{3}H-purine release was determined by washout. In these preparations the afferent and efferent inputs can be stimulated individually. The ^{3}H-purine release was determined as the decay of the label in the ganglion which in a semilogarithmic plot yields a straight line which defines the rate of adenosine production. Preganglionic electrical stimulation enhanced the ^{3}H-purine release in a frequency-dependent manner (1 to 20 Hz) and the frequency vs the rate of decay yielded a sigmoidal curve. This effect was blocked by curare plus atropine. A similar graded enhancement of ^{3}H-purine release was induced by carbachol (10^{-6} to 10^{-2}M). In other experiments in which we incubated ganglia with ^{3}H-adenosine and ^{14}C-choline, we have found that there was a concurrent release of ^{3}H-purines and ^{14}C-acetylcholine during electric field stimulation. Interestingly, only the stimulation-induced ^{14}C-ACh was blocked when a Ca++ free solution was used. Antidromic stimulation also caused a frequency-dependent release of ^{3}H-purines. These results indicate that the release of adenosine is not the sole result of transmitter release but is due to postsynaptic activation.

70.8

ROLE OF ADENOSINE IN CEREBRAL BLOOD FLOW (CBF) REGULATION DURING HYPERCAPNIA. John W. Phillis, Robert E. DeLong,* Julie K. Towner* and Dominic J. Sanfilippo.* Wayne State University, Detroit, MI 48201

Hypercapnia causes arteriolar dilatation and increased CBF. The coupling mechanism which links $PaCO_2$ to vascular tone has not been established. The possibility that endogenously released adenosine, a potent vasodilator, is involved in the response to hypercapnia has been investigated in an anesthetized (Penthane), paralyzed (Pavulon) rat model. The left retroglenoid vein is cannulated and cerebral venous blood flow measured with a drop counter. Venous blood is returned to the animal via a femoral vein. Femoral arterial pressure and intracisternal pressure are recorded. Animals are ventilated mechanically with a 40% oxygen, 60% nitrogen gas mixture. At 20 min intervals, at a constant rate of flow, the inspired gas mixture is altered to 10% carbon dioxide, 40% oxygen, 50% nitrogen for periods of between 30-90 sec. This brief hypercapnic challenge induces a rapid increase in CBF in the absence of any change in MABP. The involvement of adenosine in this response has been evaluated by using an adenosine antagonist, caffeine, and an adenosine uptake inhibitor, dipyridamole. Caffeine (10 and 20 mg/kg i.p.) 20 min prior to a hypercapnic challenge significantly ($p< 0.001$) decreased the peak increase in CBF from 132.1 ± 13.5% to 57.8 ± 6.9 (10 mg/kg). Dipyridamole (0.2 mg/kg) enhanced the peak increase in flow from 31.7 ± 3.4% to 78.2 ± 12.3% ($p< 0.01$). These results indicate that adenosine plays an important role in coupling $PaCO_2$ to CBF.

70.9

REGIONAL DISTRIBUTION OF THE CEREBRAL VASODILATION INDUCED BY PHYSOSTIGMINE. O.U. Scremin, K. Allen* and A.M.E. Scremin* Rehabilitation Medicine and Research Services. Veterans Administration Medical Center, Albuquerque, NM 87108.

Physostigmine, a cholinesterase inhibitor that permeates the blood-brain barrier, induces a cerebral hyperemia without a concommitant metabolic activation (J Cereb Blood Flow Metab 2:24, 1982). Regional variations of this phenomenon have not been systematically studied thus far. Regional cerebral blood flow (CBFr) was studied in unanesthetized rats with the Iodo-^{14}C-antipyrine autoradiographic technique. After cannulation of femoral arteries and veins under Halothane, the animals were left to recover from anesthesia 2 hrs in a restraining cage. One group was injected with Physostigmine Sulfate (PHY) (0.05 mg/kg i.v.) and the other with saline, followed by the flow measure procedure within 5 min. Vasodilation induced by PHY was limited to the neocortex and the rostral portion of the Caudate-Putamen. A sharp demarcation was observed at the rhinal fissure between the hyperemic neocortex and olfactory cortex that remained unaffected. The CBFr increase, due to PHY, ranged in the neocortex from 170% of control (Motor Cortex) to 52% (Striated Cortex). The rostral Caudate-Putamen showed a 159% increase. The rest of the cerebral hemispheres, brain stem, Cerebellum and Spinal Cord showed no changes. Observed differences do not correlate with known brain parenchymal concentrations of Acetylcholine or Acetyl-cholinesterase and suggest that cholinergic cerebrovascular control is not mediated by neuronal activation. (Supported by funds from the Research Advisory Group, Veterans Administration.)

70.10

PERFUSED CEREBRAL CAPILLARY AND ARTERIOLAR MORPHOMETRY DURING MIDDLE CEREBRAL ARTERY OCCLUSION. Ellen Buchweitz and Harvey R. Weiss. UMDNJ-Rutgers Med. Sch., Heart and Brain Circ. Lab., Piscataway, NJ 08854.

We examined various morphometric indices of the total, perfused and percent perfused rat cerebral arteriolar and capillary bed 1 hr after middle cerebral artery (MCA) occlusion. Regional cerebral blood flow (CBF) was monitored with ^{14}C-iodoantipyrine. FITC-dextran was injected i.v. 20 sec, 3 min or 6 min after 1 hr of MCA occlusion. The animals heads were frozen in liquid N_2 and 5 brain regions isolated: ischemic cortex, contralateral cortex, pons, medulla and thalamus. CBF decreased 48% in the ischemic cortex. There were no significant regional differences in any anatomic parameter in the total arteriolar or capillary bed. Total capillary volume fraction (Vv) averaged 0.075±0.001 mm^3/mm^3 (mean±SEM) in the contralateral cortex. Percent perfused Vv in ischemic cortex was significantly lower (19.4%) than in contralateral cortex (60.3%). A similar change was noted in the arteriolar bed. 6 min post FITC injection > 90% of the arteriolar and capillary beds were perfused in all brain regions. Thus, the decline in the perfused portion of the microvasculature is not due to vessel blockage but may be related to vasoconstriction or partial obstruction.

70.11

CEREBRAL CIRCULATION AND CORTICAL O_2 UTILIZATION DURING 2.66% AND 3.99% END-TIDAL SEVOFLURANE ANESTHESIA IN HEALTHY ISOCAPNIC SWINE. Murli Manohar. University of Illinois College of Veterinary Medicine, Urbana, IL 61801.

In 7 previously instrumented swine regional brain blood flow (Q; 15 μm radionuclide labelled microspheres) was studied while awake and during 2.66% (1 MAC) and 3.99% (1.5 MAC) sevoflurane (SF) anesthesia. In 6 additional pigs superior sagittal sinus (SS) was also catheterized to permit cortical $\dot{V}O_2$ determination. Awake Q in the cerebral cortex, white matter and deep gray matter was 117 ± 9, 38 ± 2 and 105 ± 8 ml/min/100 g. At 1 MAC SF, Q in these regions decreased to 66%, 76% and 75% of awake value and these values were not different from 1.5 MAC SF. Mean aortic pressure was 78% and 61% of the awake value at 1 and 1.5 MAC SF. Vascular resistance did not decrease in any region of the brain at 1 MAC SF; however, vasodilation occurred in cerebral white matter, cerebellum, pons and medulla at 1.5 MAC SF.

Cerebral cortical $\dot{V}O_2$ decreased by 50% and 52% at 1 and 1.5 MAC SF from control (7.66 ± 0.45 ml/min 100 g), but the hemoglobin-O_2 saturation in SS blood (57 ± 3% and 69 ± 3% at 1 and 1.5 MAC SF) consistently exceeded control value (42 ± 1%). This suggested that cortical O_2 supply during SF anesthesia remained adequate. It is concluded that unlike isoflurane, and halothane which increased cerebral blood flow, sevoflurane anesthesia decreases cerebral perfusion and cortical O_2 consumption.

70.12

THE EFFECT OF LIDOCAINE ON REGIONAL SPINAL CORD BLOOD FLOW IN THE DOG. Gerald A. Burger, Frank Collins & J. Timothy O'Neill. Department of Anesthesiology and Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

The spinal cord blood flow (SCBF) effect of lidocaine administered in the lumbar subarachnoid space of anesthetized dogs (n=7) was measured using the radioactive microphere technique. Extent of sensory anesthesia was assessed using somatosensory evoked potential response in the posterior tibial (PT) and median nerves (MN). Measurements of SCBF were made after induction of anesthesia, after spinal needle placement, and after injection of 5% lidocaine (50 mg). Temperature, pH, and pCO_2 were maintained constant. SCBF was not altered by spinal needle placement. Perfusion pressure fell significantly after lidocaine from 133.3 ± 4.9 torr to 119.7 ± 3.6 torr. Evoked potentials in the PT uniformly disappeared after lidocaine administration, while those in the NM were not changed. Blood flow to the cervical cord (CC), thoracic cord (TC), lumbar cord (LC) and both kidneys were measured at necropsy. CC values did not change from control (17.7 ± 6.4ml/100gm/min). TC values rose significantly from 12.4 ± 1.5ml to 23.8 ± 4.4ml/100 gm/min after lidocaine. LC values also rose significantly from 14.9 ± 2.0 to 40.9 ± 6.9ml/100gm/min after lidocaine. Renal blood flow was not significantly altered. This study shows the regional effect of lidocaine on SCBF is distributed in a pattern consistent with sensory evoked potential changes. Supported by USUHS Grant RO8014 and CO8613.

CORONARY PHYSIOLOGY II

71.1

VASCULAR WATERFALL HYDRAULICS MAY NOT BE APPARENT IN THE CORONARY BED EVEN IF INDIVIDUAL VESSELS BEHAVE AS COLLAPSIBLE TUBES. R.F. Bellamy, V.L. Gildengorin*, and D.W. Shosa*. Letterman Army Institute of Research, San Francisco, CA 94129.

By way of analogy with collapsible tubes, the coronary zero flow pressure (PZF) has been viewed as a surrounding pressure (SP). Although the coronary bed is usually modeled as equivalent to a single tube and SP, a more realistic model would consist of multiple parallel tubes with a distribution of SPs. A collapsible tube can exist in three configurations: closed, partially collapsed, or distended. For thin-walled collapsible tubes, the transition zone pressure over which the tube opens or closes is a few mm Hg. If the range of Sps is large compared to the transition pressure, most collapsible tubes will be either open or closed rather than in the transitional state in which waterfall hydraulics are possible. To explore this, a Data General MV 8000 was programmed to sample from a distribution of SPs, to obtain the corresponding pressure-flow relations and to add the data from multiple tubes. Given a log-normal distribution of SPs with a range of 25 mm Hg, a transition zone of 5 mm Hg and a PZF and flow similar to that of the basal coronary bed, the computer predicts that 97% of the total flow will pass through tubes that are distended. The value for the computer simulated vasodilated bed is 82%. Only near PZF will flow be controlled by waterfall hydraulics. The model suggests that right atrial pressure is the coronary back pressure. PZF, which is the lowest SP, is not the back pressure when flow is in the physiologically relevant range.

71.2

Characterization of Coronary Venous Outflow in Conscious Dogs Using a Volumetric Ultrasonic Flowmeter. J.M. Canty, Jr. and A. Brooks*. SUNY at Buffalo, NY 14215

Previous studies of volumetric coronary venous outflow have been performed in open-chest dogs by cannulating a coronary vein and diverting outflow through an extracorporeal flow probe. The present study was performed to characterize phasic coronary venous flow in conscious animals. An implantable ultrasonic transit time flow probe capable of measuring volume flow rates was placed on the great cardiac vein or coronary sinus of five mongrel dogs. Several days later, volumetric venous flow was determined in the awake state before and during vasodilation with adenosine (2.5 mg/min IV). Coronary venous (CV) flow in both situations showed a nadir approaching zero at the onset of systole and a peak at end-systole or early diastole. The ratio of systolic to diastolic flow was ~2:1 under control conditions and ~3:1 during adenosine:

	CV Flow ± SEM (ml/min)		Percent	
	Mean	Peak	Systolic	Diastolic
Control	27 ± 2.6	63 ± 6.5	69 ± 2	31 ± 2
Vasodilation	73 ± 6.4	166 ± 9.8	77 ± 2	23 ± 2

These flow patterns are qualitatively similar to those observed in cannulated open-chest preparations although phasic components are more pronounced. (HLB-01168, HLB-15194, AHA 83-717).

71.3

LONG-TERM PROTECTION OF ISCHEMIC MYOCARDIUM. Race L. Kao, Joy Chen*, Rosa G. Williams*, and George J. Magovern*. Allegheny-Singer Res. Inst., Pittsburgh, PA 15212

Isolated perfused working hearts subjected to elective cardiac arrest were used to test the hypothesis that ischemic myocardial damage could be prevented if an optimal cardioplegic solution and condition had been achieved. When oxygenated asanguineous arresting solution at 8°C containing 0.5% glucose was utilized to initiate the cardioplegia and reinfused for one minute at 30 minute intervals, hearts could be cross-clamped for 60 minutes with no measurable changes in hemodynamic and metabolic performances during reperfusion, as compared to normal hearts. Calcium and adenosine have been identified as key components regulating reperfusional damage in the arresting solution. When hearts were arrested at 16°C for closer simulation of open-heart surgical conditions, 200 μM calcium and adenosine resulted in the best hemodynamic and metabolic recovery of ischemic heart. When hearts were arrested at 16°C for 3 hours with modified Tyers' cardioplegic solution equilibrated with 95% O_2:5% CO_2 and containing 25 mM glucose, 0.2 mM calcium, 0.2 mM adenosine, 2.5 mU/ml insulin, and reinfused every 30 minutes, the hearts recovered their normal physiologic functions during reperfusion. We concluded that ischemic myocardium can be protected for at least 3 hours under the elective cardiac arrest conditions and suffer no measurable damage during reperfusion.
Supported by NIH Grant HL-32231.

71.4

CORONARY ARTERY BALLOON OCCLUSION (CABO): FOR THE PRODUCTION OF EXPERIMENTAL MYOCARDIAL INFARCTION IN DOGS. D. Garner,* L. Ginzton,* and M. Laks, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509

Previous techniques for the production of experimentally induced myocardial infarctions (MI) in dogs have used either a thoracotomy, coronary artery injection of occlusive agents, or externalized intracoronary balloon catheters. These investigators report a survival rate of 50-70%. The purpose of this presentation is to describe a new technique with a higher survival rate. A catheter with an inflatable, detachable balloon for intravascular use is introduced into the aorta via the carotid artery, and can be readily engaged in the orifice of the right, left anterior, or circumflex coronary arteries. The balloon is inflated and disconnected by pulling back on the catheter. The proximal left anterior descending and circumflex arteries were occluded in 12 dogs (18-30 kg). In the initial studies 2 out of 3 dogs went into VF and died. These dogs were anesthesized with 30 mg/kg sodium pentobarbital. We changed anesthesia to 3-5 mg/kg subcutaneous morphine followed in 30 minutes by 5-8 mg/kg intravenous sodium thiopental (ST). The ST (250-400 mg total) was given as needed throughout the procedure which lasted 30-60 minutes. When the procedure was completed 0.8-1.2 mg of Narcan(R) was given, reversing the effects of morphine resulting in a walking animal within 15 minutes. Nine out of 9 dogs have survived for 3 months. Infarction has been documented acutely and chronically (3 mo) at autopsy.
Conclusion: 1) The new technique of CABO can be used to produce a chronic degree of infarction with a high survival rate due to our use of short acting anesthesia. 2) Because of rapid rate of recovery, this technique permits earlier studies of hemodynamics and arrthymias of MI in the conscious dog.

71.5

DETERMINATION OF ADENOSINE AT LOW FEMTOMOLE CONCENTRATIONS BY AN ELECTROCHEMICAL METHOD. Robert M. Berne, Richard R. Curnish*, Jeffrey M. Gidday*, and Rafael Rubio. Univ. of Virginia, Charlottesville, VA. 22908.

Because of the physiological importance of adenosine (ADO) a method was developed to measure its concentrations in very small samples of body fluid and tissue. After protein precipitation and centrifugation, the supernatant is subjected to standard HPLC. The Ado fraction (as determined by retention time) is evaporated to dryness and the residue dissolved in 0.5 ml KH_2PO4/Na_2HPO_4 buffer (0.05M, pH 7.4). Aliquots (0.1 or 0.2 ml) are enzymatically degraded to uric acid (UA) by incubation at 37° for 2 hrs. with a mixture of adenosine deaminase, nucleoside phosphorylase and xanthine oxidase (total enzyme volume 0.4 or 0.8 µl). The reaction is stopped with perchloric acid, (final conc. 0.2M), the precipitate removed by centrifugation and the UA determined by electrochemical detection (ECD). The ECD system has a mobile phase of monochloroacetic acid (0.05M) at pH 3.0. Flow rate is 1ml/min. through a C-18 reverse phase column (5µ, 4mm I.D. x 25 cm) and past a glassy carbon electrode (oxid. pot. 0.8V). Accurate measurements of 25 x 10^{-15}M (4.2pg) UA are made with an amperometric detector of 0.5 nA full scale and the concentrations are linear over the range of 25 - 2000 femtomoles. This method can be used with low volumes (1-100µl) as well as low levels of Ado (25 fmoles) or other compounds readily converted to UA. With this technique the basal level of cardiac interstitial fluid Ado was found to be 0.15µM (range 0.08-0.24µM).

71.6

CORONARY VASCULAR ACTIONS OF STROMA-FREE HEMOGLOBIN SOLUTIONS (SFHS). G.P. Biro, G.C. Taichman, B. Lada*, W.J. Keon*, L.S. Sehgal*, A. Rosen. University of Ottawa, Ottawa, Ont., K1H 8M5, Canada and Michael Reese Hospital and Medical Center, Chicago, Ill. 60616.

Two preparations of SFHS were compared in their coronary vascular actions with autologous blood in the separately perfused L.A.D. vascular bed of the in-situ dog heart. Unmodified SFHS (p50=16 mmHg, HGb-conc.=7.2 g/dl) and pyridoxylated, polymerized SFHS (p50=20 mmHg, HGb-conc.=13.7 g/dl) were compared. In the baseline state, switching from autologous blood-perfusion, to SFHS-or poly-SFHS-perfusion was followed by resistance-changes of -6±8% and -29±9%, ($p<.05$) respectively. The corresponding changes in coronary vascular hindrance were +87±19 and -5±11% ($p<.05$). The slope of the coronary pressure-flow relationships, determined during long diastoles, changed from 1.34±.63 to 2.19±1.25 during SFHS-perfusion, and from 1.44±.68 to 0.77±.24 during poly-SFHS-perfusion ($p<.05$). The responses to adenosine-infusion (resistance-change of -57±18 and -62±16%), were abolished during both perfusions. Electron microscopic examination of the perfused myocardium showed endothelial damage, intracellular edema and the accumulation of Fe-containing vacuoles, more pronounced in the SFHS-perfused hearts. The findings suggest that SFHS possesses significant coronary vasoconstrictor potency and that both preparations interfere with adenosine-mediated dilatation.
(Supported by the Ontario Heart Foundation)

71.7

EFFECT OF CARBON MONOXIDE ON THE ISOLATED PERFUSED RAT HEART. Steve J. McFaul* and James J. McGrath. Texas Tech University Health Sciences Center, Lubbock, TX 79430.

We investigated the direct effects of carbon monoxide (CO) on the isolated potassium-arrested perfused rat heart. Hearts from male Sprague-Dawley rats (350-390g) were perfused via the aorta with oxygenated Krebs-Henseleit solution. The recirculating system contained 70 ml of perfusate maintained at 36° C. Coronary flow was determined by timed collection in a 10-ml graduated cylinder. After a 6-min washout, the potassium concentration of the perfusate was raised by 20 mM, and the heart ceased beating within 1 min. When the perfusate was equilibrated with either 95% O_2:5% CO_2 or 5% N_2:90% O_2:5% CO_2, coronary flow rates were 24 ± 0.96 and 24 ± 1.8 ml/min/g dry wt., respectively. When the perfusate was equilibrated with 5% CO:90% O_2:5% CO_2, however, coronary flow was 28 ± 1.2 ml/min/g dry wt., a 17% increase. A similar increase was observed also with 2% CO. The 5% CO exerted a vasodilating effect even in the presence of 4.8 µM propranolol. Our results indicate that CO exerts on the coronary vasculature a vasodilating effect that is not caused by hypoxia nor mediated by beta receptors. Supported by NIH training grant #HL07289.

71.8

EFFECTS OF LEUKOTRIENE D_4 ON FLOW RESISTANCE OF EPICARDIAL ARTERIES IN SITU . FRM Laurindo*,D Ezra*,J Czaja*,G Feuerstein* and RE Goldstein. USUHS, Bethesda, MD 20814

The capacity of vasoconstrictor agents to modify epicardial coronary artery(CA) flow resistance(RES) of in situ hearts is unclear. Leukotriene D_4(LTD_4), an arachidonate metabolite, constricts isolated CA and decreases coronary flow(CBF) and epicardial CA diameter in vivo. We infused LTD_4(2 mcg/min) or saline into CA of 8 open-chest pigs instrumented with CA flowmeter and catheters in aorta(Ao), left ventricle(LV) and proximal and distal CA. LTD_4 caused initial CBF drop of 72±4 % (from 46±3 ml/min), followed by partial CBF escape despite continued infusion. For steady-state saline or LTD_4 infusions, diastolic CBF was plotted vs. PD (pressure difference Ao - distal CA) during reactive hyperemia(RH) after 20 sec total CA occlusions. Epicardial CA RES was taken as slope(SL) of the regression line CBF(ml/min) vs. PD. Small vessel diastolic RES (svRES)=(distal CA pressure - LV pressure)/CBF.Results [mean±SE] :

		AoP(mmHg)	MEAN CBF	svRES#	SL #
SALINE	before RH:	81±5	43±3	.72±.06	
	peak RH:	78±5	150±16	.18±.01	.09±.02
LTD_4	before RH:	89±3	37±4	.90±.08**	
	peak RH:	87±2	77±11*	.42±.07*	.12±.04

*p<.01 or **p<.05 vs saline # mmHg.min/ml

Thus, at doses that produce significant small vessel constriction, LTD_4 has no discernible action on epicardial CA flow RES, even during hyperemic CBF.

CARDIAC DYNAMICS

72.1

COMPARISON OF STANDARD AND IMPEDANCE-DERIVED INDICES OF CARDIAC CONTRACTILITY. J.F.M. van Brederode*, J.L. Seagard, J.J. Smith and J.P. Kampine. The VA Medical Center and The Medical College of Wisconsin, Milwaukee, WI 53226.

In chronically instrumented, mongrel dogs (n=6), under halothane anesthesia (1 MAC), left ventricular pressure, aortic flow (EM flowmeter), and transthoracic electrical impedance were recorded before and after infusions of epinephrine, norepinephrine, isoproterenol and propranolol. Ten second periods of control (C) and peak response during drug infusions (D) were digitized, stored and averaged using a computer. The percent change of maximum rates of change from C to D of ventricular pressure (dP/dt_{max}), aortic flow (dF/dt_{max}), and impedance (dZ/dt_{max}) during systole were calculated and compared using linear regression. The correlation coefficient (r) between dZ/dt_{max} and dP/dt_{max} was +0.69 ($p<.01$); between dZ/dt_{max} and dF/dt_{max}, +0.78 ($p<.01$); between the time from beginning of systole (BS) to peak dZ/dt, and time from BS to peak dP/dt was +0.75 ($p<.01$); and between time from BS to peak dZ/dt and time from BS to peak dF/dt, +0.80 ($p<.01$).

Results suggest that dZ/dt_{max} and time from BS to peak dZ/dt compare favorably with conventional indices of cardiac contractility over a wide range of cardiac output measurements in the dog. This investigation was supported by VA 7793-02P and NHLB grant 0-27185.

72.2

THE EFFECTS OF INSULIN LEVEL ON THE RESPONSES TO BILATERAL CAROTID OCCLUSION AND DOBUTAMINE IN CONSCIOUS, CHRONICALLY INSTRUMENTED DOGS. D. Fitzovich and D.C. Randall, Dept. Physiology & Biophysics, Univ. of KY, Lexington, KY 40536.

We have reported (Fed. Proc. 43: 526, 1984) the development of a preparation in conscious, chronically instrumented dogs in which insulin is reduced below physiologically significant levels by Alloxan treatment, and then replaced by continuous IV infusion via a tether system. This allows us to acutely alter insulin and glucose levels while maintaining the dog in a normal metabolic state. We now report the effects of altering insulin and glucose levels for one hour prior to performance of bilateral carotid occlusions (BCO, 30 sec. duration) and bolus injection of 8, 16 and 32 ug/Kg of dobutamine (D), as assessed by the maximum rate of rise of left ventricular pressure (d(LVP)/dt max). Data (n=2) are reported for control (N, prior to Alloxan), 1/5 basal insulin with high glucose (L), and 5X basal insulin with high glucose (H). Resting values were (N) 3622±86, (H) 3248±200 and (L) 3567±123 mm Hg/sec ± SEM. Responses are given as % change from resting ± SEM. BCO responses were (N) 8±3, (H) 1±4, and (L) 0.7±3. 32 ug/Kg D caused (N) 136±3, (H) 179±9 and (L) 140±2. The trend for lower doses of D was similar, an apparent increase with H but not L. The BCO response seems lower in both H and L. In this preparation, altering insulin and glucose levels affects both resting d(LVP)/dt max and responses to BCO and D. These conclusions, based upon preliminary results, are to be considered as tentative. (Supported by NIH grant HL 19343)

72.3

HALOTHANE ANESTHESIA DURING CARDIOPULMONARY BYPASS: EFFECT ON THE LEFT VENTRICULAR DIASTOLIC PRESSURE VOLUME RELATIONSHIP. William Y. Moores*,Diane Sansonetti*,Richard B. Weiskopf, Walter P. Dembitsky*,Robert Mack*,David C. Willford. (SPON: E. P. Hill) UCSD, SDVAMC, San Diego, CA 92161.

To determine whether the depression associated with Halothane is due to systolic performance or to the diastolic pressure volume relationship, we used cardiopulmonary bypass in 10 swine and examined myocardial function(stroke volume (SV,ml), dp/dt Max(mmHg)). Function curves were constructed before and after administration of 0.5% Halothane during controlled LVEDP(14 Torr), constant heart rate(140) and constant mean blood pressure(65 Torr). Diastolic ventricular volumes (LVEDV) and segment lengths(LVEDL), were evaluated by thermodilution and sonomicrometry. An additional 5 animals, serving as non-Halothane controls, were subjected to the same protocol without Halothane and had no significant changes in the values.

RESULTS: (mean ±SEM, *p<0.05, 0.0% vs. 0.5% Halothane) At equal LV loading pressures we noted significant decreases after Halothane administration in SV(20+2 vs. 11+2*) and dp/dt Max(2336±422 vs. 1101±288*). We also found a significant decrease in LVEDL(919±0.1 vs. 913±0.1*) signifying a stiffer ventricle(smaller LVEDL at equal LVEDP). However we failed to document a significant decrease in SV at equal LVEDV(13±2 vs. 14±1), or equal LVEDL(18±1 vs 16±2), but did find a significant increase in LVEDP(equal LVEDV:9±2 vs. 16±1, equal LVEDL: 11±1 vs. 18±3).

72.4

ROLE OF CIRCULATING CATECHOLAMINES IN THE CARDIAC RESPONSE TO INTRAVENOUS NICOTINE. Arthur G. Williams* and H. Fred Downey. Department of Physiology, Texas College of Osteopathic Medicine Fort Worth, Texas 76107.

Nicotine increases myocardial contractile function (MCF) by directly causing release of norepinephrine from cardiac stores and indirectly by activation of the sympathetic nervous system. Since MCF did not increase when the coronary circulation was perfused from a reservoir, sympathetic stimulation of myocytes cannot account for the effect of intravenous nicotine on MCF (Fed. Proc. 44:1901, 1985). To differentiate further between direct and indirect effects of nicotine on MCF, additional experiments were conducted in open-chest dogs, anesthetized with pentobarbital. The dose-response relationship between intracoronary nicotine and MCF revealed that an intracoronary concentration of 8 ug/ml plasma was required to increase MCF to the same extent observed at the peak response to nicotine, 36 ug/kg/min, i.v. (n=7). However, this intravenous infusion of nicotine resulted in a concentration of only 0.7 ± 0.1 ug/ml plasma (n=9). Thus, direct intracardiac action of nicotine cannot account for the increase in MCF observed during intravenous nicotine. The role of adrenal-released catecholamines was examined in 4 dogs subjected to bilateral adrenalectomy. Adrenalectomy abolished the MCF response to nicotine, 36 ug/kg/min, i.v. We conclude that adrenal release of catecholamines is primarily responsible for the increase in MCF during intravenous nicotine. Supported by Smokeless Tobacco Research Council, Inc.

72.5

SKELETAL MUSCLE PUMPING VIA VOLUNTARY AND ELECTRICAL INDUCED CONTRACTIONS. S.N. Rattan,* R.M. Glaser, F.J. Servedio* and S.R. Collins.* Wright State Univ. Sch. of Med., Miami Valley Hospital, and VA Medical Center, Dayton, OH, 45435

Electrical stimulation (ES) induced contractions of thigh and calf muscles have been shown to decrease venous pooling as indicated by increases in stroke volume (SV) and cardiac output ($\dot{Q}$). The present study determined metabolic and cardiopulmonary response differences for contractions of these muscle groups induced voluntarily (VOL) or via ES. For this, 10 healthy adult volunteers underwent ES of the thigh and calf muscles for 3 min duration in the sitting position. ES (Biphasic pulses of 250 μsec duration at 35 Hz) induced 12, 2.5 sec tetanic contractions per min. Intensity was adjusted to be tolerable to the subjects while producing desired contractions. VOL contractions of these muscle groups were performed during another session. Heart rate (HR), SV and $\dot{Q}$ were determined by impedance cardiography. Blood pressure (BP), oxygen uptake ($\dot{V}O2$) and minute ventilation ($\dot{V}E$) were also monitored. ES induced contractions elicited 15-20% increases in SV and $\dot{Q}$ without significantly affecting HR, BP, $\dot{V}O2$ and $\dot{V}E$. In contrast, VOL contractions produced significant increases in HR, systolic BP, $\dot{V}O2$ and $\dot{V}E$, along with similar increases in SV and $\dot{Q}$. It thus appears that mechanical milking of blood from leg veins can be accomplished to the same degree with ES as for VOL contractions, but with lower metabolic and cardiopulmonary stresses. (Supported in part by the VA Rehab. R&D Service).

CARDIAC ELECTROPHYSIOLOGY

73.1

LARGE K^+ CONDUCTANCE CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS. G. Bkaily, R. Sauvé*, M.D. Payet* and N. Sperelakis, Dept. Biophysics, Fac. of Medicine, Univ. of Sherbrooke, Sherbrooke, Quebec, Canada. Dept. of Physiology, Fac. of Medicine, Univ. of Montreal, Montreal, Quebec, Canada. Dept. of Physiology, Univ. of Cincinnati, Ohio 45267, USA.

Single potassium channels were recorded from cultured aortic single cells of rabbit using cell attached patch clamp technique. A very large amplitude of opening of K+ channel was found in vascular smooth muscle (VSM) cells of rabbit, with a slope conductance of 80 pS. The single channel I/V curve was linear over the entire applied voltage range studies, i.e. from -200 mV to +200 mV. The channel amplitude, open time duration and open probability were voltage dependent. At positive patch potentials, the amplitude and the opening probability increased and at +150 mV patch potentials, the channel was most of the time in the open state. The present results demonstrate the presence of a very large potassium conductance in aortic VSM cells and this would explain why this muscle does not produce action potentials upon electrical stimulation in normal conditions.
This study was supported by grants from MRCC, FRSQ and FQMC to G. Bkaily and MRCC to R. Sauvé, and QHF, MRCC to M.D. Payet and NIH to N. Sperelakis.

73.2

SODIUM CURRENT INACTIVATION: WHOLE CELL AND SINGLE CHANNEL RECORDINGS. N. Morier* and M.D. Payet* (SPON: G. Bkaily). Dept. Biophysics, Fac. Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.

Sodium current (I_{Na}) inactivation was studied on isolated cardiac cells from newborn rats. The patch clamp technique was used in whole-cell and on-cell configurations. Macroscopic currents inactivate with two time constants for voltage depolarizations between -30 mV and +30 mV. At more negative or positive voltages only one time constant could be measured. The faster time constant τ_1 decreases from 8 ms to 1.5 ms. The voltage relationship for the slower time constant τ_2, is U-shaped, the minimum value is reached at -20 mV. Single channel recordings are averaged after capacitive transient compensation and filtered at 1.3 KHz. The inactivation time constants are measured from the averaged currents. The values obtained for τ_1 are similar to what was obtained on whole cell current but shifted by 30-40 mV toward more negative membrane potentials. The second inactivation phase could not be recorded in all cases. If τ_2 is present, its value is identical as in whole cell current however it is shifted toward negative potentials.
This study was supported by "Fondation du Québec des Maladies du Coeur". M.D. Payet is scholar from the Canadian Heart Foundation.

73.3

FORSKOLIN-INDUCED REDUCTION OF INTRACELLULAR Na^+ ACTIVITY IN CARDIAC PURKINJE FIBERS FROM SHEEP. Rudolfs K. Zalups and Shey-Shing Sheu*. Dept. of Pharmacology, University of Rochester Medical Center, Rochester, NY 14642.

The effects of the diterpene forskolin (a unique promoter of cytosolic cyclic AMP production) on intracellular sodium activity (a^i_{Na}) and membrane potential (Vm) were studied in quiescent cardiac Purkinje fibers from sheep using Na^+-sensitive and conventional microelectrodes. In 11 fibers, forskolin (12 μM) caused a^i_{Na} to decrease by an average of 2.7±0.4 mM (mean±SEM), from a resting value of 8.2±0.5 mM to a value of 5.6±0.6 mM. In addition to decreasing a^i_{Na}, forskolin caused an 11.2±0.7 mV cellular depolarization, changing Vm from -77±1.3 mV to -65±1.7 mV. To determine if the forskolin-induced decrease of a^i_{Na} was related to the activity of the Na^+ pump, the effects of forskolin on a^i_{Na} were examined while the Na^+ pump was inhibited by strophanthidin (1 μM) or 0 mM extracellular K^+. During the inhibition of the Na^+ pump by either strophanthidin (7 fibers) or 0 mM extracellular K^+ (2 fibers), forskolin did not have an effect on a^i_{Na}. Forskolin did cause a marked depolarization (7.9±1.4 mV) in the presence of strophanthidin, but not in the presence of 0 mM extracellular K^+. These results indicate that forskolin causes a^i_{Na} to decrease and Vm to become less negative in the quiescent cardiac Purkinje fiber from sheep. Furthermore, they indicate that the forskolin-induced decrease in a^i_{Na} may be directly related to the activity of the Na^+ pump. (AHA Grant-In-Aid & Fellowship)

73.4

A ROLE OF CALCIUM IN STROPHANTHIDIN INOTROPY. Pasquale Abete* and Mario Vassalle, S.U.N.Y., Downstate Med. Ctr., Brooklyn, N.Y. 11203.

Cardiac steroids are generally believed to increase contractile force by increasing cellular calcium through an enhanced cellular sodium. We tested strophanthidin under conditions that decrease intracellular sodium activity (a^i_{Na}) but increase directly (norepinephrine, high $[Ca]_o$) or decrease indirectly (tetrodotoxin, TTX) calcium influx. Purkinje fibers from dog and sheep hearts were perfused in vitro and membrane potential, contractile force (F) and a^i_{Na} were recorded simultaneously and continuously. Increasing $[Ca]_o$ from 2.7 to 3.6 mM or administering norepinephrine (NE, 10^{-7} M) increased F and decreased a^i_{Na}. A low strophanthidin concentration (5 x 10^{-8}M) increased F and a^i_{Na}. Occasionally, there was a small transient decrease in a^i_{Na} at the beginning of strophanthidin perfusion. High $[Ca]_o$ or NE decreased a^i_{Na} less and increased F more in the presence than in the absence of strophanthidin. Increasing $[Na]_o$ to 176.9 mM in the presence of strophanthidin further increased a^i_{Na} and F. Adding TTX (2.06 x 10^{-6} M) to the high Na-strophanthidin solution decreased a^i_{Na} toward the level in strophanthidin alone but decreased F well below that in Tyrode solution. When high $[Na]_o$ was perfused first, strophanthidin increased F more than a^i_{Na}. TTX decreased a^i_{Na} toward and F below control values. It is concluded that the mechanism for potentiation of strophanthidin inotropy by high $[Ca]_o$ and NE and the depression of inotropy by TTX may include an enhancement and a decrease in calcium influx, respectively. (Supported by NIH grants HL17451 and HL 27938)

73.5

POTENTIATION BY NIFEDIPINE OF ACETYLCHOLINE-INDUCED BRADYCARDIA William Wood, Janice Fong*, David Meyer* and Steve Charles*. Univ. TN Center for Health Sciences and Crippled Childrens' Vitreoretinal Res. Fndn., Memphis, TN 38163.

Transitory ventricular asystole was induced in sodium pentobarbitalized dogs by i.v. bolus injection of 50 μg/kg acetylcholine chloride (ACh) before and after 100 μg/kg i.v. nifedipine (N). Following N, the duration of ventricular aystole to ACh injection was significantly increased over control ($p<0.005$). In the same animals, comparable ventricular asystole evoked by electrical stimulation of the intact right vagus nerve was not significantly changed after N. Vasodilation and hypotension approximating that of N was induced in 4 additional dogs by i.v. nitroglycerin. Ventricular asystole to injected ACh was potentiated after nitroglycerin whereas ventricular asystole to vagal stimulation was not. These data suggest that N at a dose level of 100 μg/kg: (1) does not significantly interfere with normal exocytotic vagal release of ACh, (2) does not perceptibly modify reactivity of cardiac muscarinic receptors to ACh, (3) probably potentiates ventricular asystole to exogenous ACh by dilating coronary vessels and reducing perfusion pressure, both of which could increase exposure of cardiac receptors to injected ACh. Presumably the same principle holds true for other cardiac agonists or antagonists administered concurrently with N.

(Supported by Crippled Children's Vitreoretinal Research Fndn.)

73.6

ON THE MECHANISMS BY WHICH HYPOXIA ABOLISHES THE ARRHYTHMIAS INDUCED BY CARDIAC STEROIDS. Marco Di Gennaro*, Mario Vassalle, Giovanni Iacono*, Marco Pahor*, Roberto Bernabei* and Pierugo Carbonin*. Dept. of Medicine, Catholic University, Rome, Italy, and S.U.N.Y., Downstate Med. Ctr., Brooklyn, N.Y.

The mechanisms by which hypoxia abolishes the tachyarrhythmias induced by cardiac steroids (beta-methyldigoxin and strophanthidin) were studied in dog and guinea pig cardiac Purkinje fibers, ventricular muscle fibers and in guinea pig perfused A-V blocked hearts. In ventricular muscle fibers, hypoxia reduces markedly excitability, reduces the size and increases the time to peak of the oscillatory potentials and of aftercontractions induced by cardiac steroids, thereby quickly abolishing the fast discharge. In Purkinje fibers, hypoxia decreases the oscillatory potential less than in muscle and abolishes the tachyarrhythmias only if repeated or prolonged. In perfused A-V blocked hearts, hypoxia quickly abolishes or prevents digitalis-induced arrhythmias and fibrillation. In Purkinje-muscle preparations, hypoxia blocks the conduction of impulses from the fast discharging Purkinje fibers to the myocardial fibers when the latter are still little intoxicated. It is concluded that hypoxia abolishes the tachyarrhythmias induced by cardiac steroids by decreasing the size of oscillatory potentials (probably through an impaired calcium uptake into the sarcoplasmic reticulum), by causing a conduction block at the Purkinje-muscle junction and by reducing excitability. (Supported by grants from C.N.R., Italy, and NIH HL17451)

73.7

ROLE OF CALCIUM AND PROCAINE DURING HYPOXIA IN CANINE VENTRICULAR MUSCLE. M.L. Bhattacharyya, Meharry Medical College, Nashville, TN. 37208.

The effects of procaine and calcium were studied during hypoxia, hyperkalemia, acidosis (an altered solution produced by changing KCl, $NaHCO_3$, NaCl to (mM) 6.0, 5.54, 150 respectively in Tyrode solution and gassing the solution with 95% N_2 and 5% CO_2) and reoxygenation (in Tyrode) by simultaneous recording of electrical (microelectrode technique) and mechanical activity (force) in ventricular muscles. We reported earlier that contracture (increased resting tension) developed in muscle but not in Purkinje fibers. Contracture in muscle developes sooner in altered solution with high Ca^{++} (10.8 mM) or with zero substrate (0 mM Glucose). Procaine (reported earlier) and verapamil (0.05ml/100ml) both stabilized the rise in resting tension during altered solution superfusion of the muscle tissue. During reoxygenation after 40 min of exposure to altered solution, the ventricular muscle action potentials exhibited oscillation (oscillatory after potentials) in diastole which persisted even when the electrical stimulation was withdrawn. The oscillations disappeared in the presence of procaine (0.1 mM). We conclude that increased $[Ca^{2+}]_o$, aggravates the situation in hypoxia; reoxygenation may cause $[Ca^{2+}]_i$ to increase further causing oscillatory after potential and procaine abolishes these oscillation by reducing $[Ca^{2+}]_i$ through Na-Ca exchange.

73.8

CA-BLOCKING AGENTS PROMOTE HEALING-OVER IN CARDIAC MUSCLE. Walmor C. De Mello. Department of Pharmacology, Medical Sciences Campus, UPR, GPO Box 5067, San Juan, P.R. 00936.

When cardiac muscle is damaged the depolarization elicited by lesion is quickly reversed (healing-over). It is assumed that the sealing process is related to the binding of Ca to gap junctions and their transformation into high resistance barriers (De Mello, 1972). In the present work I investigated the influence of Ca-blocking agents (Verapamil, Mn and Co) on the sealing process. For this, canine Purkinje fibers were exposed to free-Ca low Na solution for 25 min and then verapamil (10^{-5} M), Mn (2 mM) or Co (2 mM) were added to the bath. Sixty minutes later the resting potential and V_o/I_o were measured and the fiber was damaged. The results indicate that: 1) the depolarization produced by lesion is quickly reversed by Ca-blocking agents; 2) V_o/I_o is appreciably increased near the cut-end and the values are compatible with the establishment of a high resistance barrier near lesion. The results might indicate that these agents bind to gap junctions and promote healing. (Supported by Grant HL 30614-02 from NIH.)

73.9

A NEW METHOD FOR THE KINETIC ANALYSIS OF EXCITATORY SODIUM CURRENT IN CARDIAC CELLS. S.R. Besch* and P.M. Hogan, Ph.D., Department of Physiology, State University of New York at Buffalo, Buffalo, NY 14214

If the rapid depolarization phase of the cardiac action potential is a nearly pure sodium event, then a family of non-propagating action potential upstrokes, initiated from a range of resting potentials, contains all the information needed to describe the kinetics of excitatory sodium current. Such a set of upstrokes, transformed to the phase plane, may be analysed for constants associated with the kinetics of sodium activation and inactivation. The method depends on separating each trajectory into two distinct but overlapping regions, a lower region dominated by sodium activation and an upper region by sodium inactivation. These regions may be analysed by permuting the constants of functions which represent the appropriate processes (activation or inactivation) until a best fit is obtained based on a least squares criterion. Constants extracted for one process are used to condition the original trajectories prior to the extraction of constants for the other process. This alternating analysis of inactivation then activation continues until a family of theoretical upstrokes calculated using the constants a_v derived becomes congruent with the actual data. The method was applied to a family of six upstrokes generated using the McAllister, Noble and Tsien model of the Purkinje fiber action potential. The twelve constants describing the sodium current in this model (V_{Na}, G_{Na}/C_m and five each for the kinetics of activation and inactivation) were retrieved by the analysis to within 0.1-2.5% of their known values. Calculated trajectories using these extracted constants compared favorably with those generated by the model. This method promises to be a productive alternative to voltage clamp analysis in the study of sodium current in cardiac Purkinje preparations.

(Supported by USPHS Grant PO1-HL28542.)

73.10

A METHOD FOR ELIMINATING ACTION POTENTIAL PROPAGATION IN CARDIAC PURKINJE FIBER BUNDLES USING EXTRACELLULAR CURRENT APPLICATION P.M. Hogan and S.R. Besch*, Department of Physiology, State University of New York at Buffalo, Buffalo, NY 14214.

Impulse propagation, with its attendant electrotonic current, has been a source of significant error in the analysis of the action potential upstroke in cardiac Purkinje fiber preparations. Elimination of propagation would increase the precision of upstroke measurements sufficiently to permit the application of new methods of analysis which avoid the pitfalls of voltage clamp. Theoretically, application of extracellular current along hyperbolic flux lines produces a longitudinal potential profile which matches that inside the fiber, resulting in a uniform membrane potential displacement along several space constants of fiber length. Using a method based on this theory, we were able to control membrane potential in a spatially uniform way in both the depolarizing and hyperpolarizing directions. The same method was used to 'field' stimulate fibers preconditioned to membrane potentials in the range of -100 to -60 mv, resulting in action potentials with upstroke velocities from 800 to 50 v/s. Propagating action potentials show a significant decrease in conduction velocity over this range of conditioning potentials, producing an increase in delay to the recording microelectrode, while 'field' stimulated action potentials demonstrate a nearly constant delay regardless of recording site in the preconditioned region. Equivalent results were obtained when potassium depolarization was used in place of 'field' preconditioning. We conclude that the 'field' stimulated action potential is nonpropagating, and as such provides an excellent experimental model for careful study of the upstroke of cardiac Purkinje fibers.

(Supported by USPHS Grant PO1-HL28542.)

COMPARATIVE PHYSIOLOGY: CARDIOVASCULAR, RESPIRATORY, OSMOTIC-IONIC, AND THERMAL EFFECTS

74.1

FACTORS INFLUENCING THE ONSET AND MAINTENANCE OF DIVING BRADYCARDIA IN THE MINK. Nigel H. West* and Bruce N. Van Vliet* (SPON: J. Thornhill). University of Saskatchewan, Saskatoon, Sask. Canada S7N OWO

In the mink, a small semi-aquatic carnivore with a high metabolic rate, three types of bradycardia were produced by separating or combining stimuli presumed to be present in voluntary asphyxic submersion. In paralysed mink water flow through the upper airways with lungs deflated produced a rapid onset (<2s) bradycardia, qualitatively similar to that observed on voluntary head submersion. Neither of the stimuli presented alone was as effective in reducing cardiac frequency; water flow alone produced a transient bradycardia, while lung deflation caused a slowly developing bradycardia. The role of the peripheral chemoreceptors in engendering or maintaining bradycardia in mink was ambiguous; hyperoxic anaesthetized mink showed no alleviation of the bradycardia resulting from tracheal water flow in expiration, but in paralysed mink hyperoxia partially alleviated and hypoxia accentuated the bradycardia associated with lung deflation, suggesting an influence from peripheral chemoreceptors under these conditions. The adaptive significance of the rapid bradycardia in conscious mink may be that prompt cardiovascular adjustments serve to maintain PaO_2 and therefore aerobic tissue metabolism. Certainly in paralysed animals PaO_2 was significantly higher at 30s in asphyxic trials in which rapid bradycardia was induced (41.4 ± 8.4 mmHg vs 23.0 ± 2.5 mmHg, N, n=6).

Supported by MRC (Canada) and NSERC (Canada) grants to N.H.W.

74.2

EFFECTS OF VARIOUS RESTRAINT CHAIRS ON HEART RATE RESPONSE IN RHESUS MONKEYS. Bruce M. Halpryn*, Eileen Waterman*, Harold Sandler. NASA/Ames Research Center, Moffett Field, Ca. 94035.

Heart rate (HR) was monitored in 4 male rhesus monkeys while unrestrained in a primate cage (C) and sitting in 3 types of primate restraint chair. Two chairs were commercially available (Plas-Labs (PL), Primate Products (PP)), the third was similar to that used aboard Soviet Kosmos spaceflights (K). Monkeys were pre-trained to each chair in order to minimize stress. HR was monitored on the third day in each chair using surface EKG electrodes and continous telemetry from units worn in a protective jacket. Average 24 hour HR (mean +/- SE) was 114+/-5.7 (C), 98+/-1.2 (K), 108+/-2.0 (PP), and 173+/-2.4 (PL). HR differences (compared to C) were significantly lower ($p<.05$) with K and higher with PL and insignificant with PP. Minimal HR's, during lights off, ranged from 66-105 BPM (C), 54-78 BPM (K), 80-92 BPM (PP), and 109-123 BPM (PL). The lower HR in K are inconsistent with a high degree of stress and are best explained by the high degree of hypokinesia associated with this chair. The higher HR with PL was not accompanied by elevated serum 17-OH Corticosteroid levels (16-28 ug/100ml), but elevated blood pressure data suggest an increased sympathetic nervous tone. This comparison of rhesus heart rates while caged and chair restrained suggests that chair restraint may not be a major stress in properly conditioned animals.

74.3

INFLUENCE OF AGE ON ADRENERGIC CONTROL OF HEART RATE AND BLOOD PRESSURE IN TURKEYS. John C. Lee and Alvah T. Leighton, Jr.* Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia 24060.

The present investigation describes developmental changes in adrenergic control of heart rate (HR) and blood pressure (BP) of Broad Breasted White turkeys at 4, 8, 12, 16, 20 and 24 weeks (WK) of age. A total of 138 unanesthetized male turkeys were used in this study. BP and HR were measured directly from the left brachial artery. The average MABP increased with age from 110 mmHg in 4 WK to 183 mmHg in 24 WK, while the average HR decreased from 336 to 165 beats/min. Administration of the β-adrenoceptor blocker, propranolol (PP, 1 mg/kg) significantly reduced the HR at all ages tested, but a more pronounced bradycardia occurred in the 4 and 8 WK groups. PP also significantly lowered the BP in these two groups. In contrast, administration of α-adrenoceptor blocker, phentolamine (2 mg/kg) reduced BP significantly in older birds but had no effect on 4 and 8 WK birds. I.V. infusion of norepinephrine (NE) elicited an age dependent pressor dose-response. However, NE-elicited bradycardia was less in older than in younger turkeys. Pretreatment of PP (1 mg/kg) largely prevented the NE-elicited bradycardia but it had no effect on the pressor response. These data suggest that 1) the α-adrenergic tone increases with age; and 2) the β-adrenergic system plays an important role in control of HR.

74.4

STIMULATION AND RECORDING OF VAGAL DEPRESSOR AFFERENTS IN THE TOAD (BUFO MARINUS). Bruce N. Van Vliet* and Nigel H. West* (SPON: J.X. Wilson). University of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N OWO.

An arterial baroreflex has previously been demonstrated to originate within the pulmocutaneous artery (PCA) of Bufo marinus. In chronically prepared toads we have recently found that stimulation of the recurrent laryngeal branch of the vagus (rLN), which contains the baroreceptor afferents, elicits reductions in heart rate and systemic and PCA blood pressures. Since two depressor responses could be distinguished based on the magnitude and time course of the fall in heart rate, and could be selected for via the stimulus intensity, two populations of depressor afferents were postulated to be carried in this nerve. Single unit recordings confirmed the presence of baroreceptors in the rLN. The baroreceptors originated near the separation of the PCA from the truncus arteriosus and were characterized by applying pressure steps and ramps to the isolated PCA. Recordings from the rLN also demonstrated the presence of afferents originating from the glottis, which may represent the second population of depressor afferents in the rLN. Receptors originating near the glottis and arterial baroreceptors were also recorded in fine pharyngeal branches of the vagus, demonstrating that these vagal afferents are not exclusively confined to the rLN.

Supported by NSERC (Canada) and MRC (Canada) grants to N.H.W.

74.5

ENDOTHELIUM-DEPENDENT RESPONSES IN AORTAE OF REPTILES. V.M. Miller, V.J. Vanhoutte* and P.M. Vanhoutte. Dept. Physiol. and Biophys., Mayo Clinic, Rochester, MN 55905.

Endothelium-dependent responses to a variety of substances occur in both mammalian arteries and veins. Except for endothelium-dependent relaxations to serotonin in the jugular vein from the chick (Imaizumi, et al., Eur. J. Pharmacol. 97: 335, 1984), such responses are not defined in other classes of vertebrates. To investigate the phylogenetic distribution of endothelium-dependent responses, rings with and without endothelium cut from the descending aorta of the red eared turtle (Pseudemys scripta elegans) and the cayman (Caiman crocodilus) were suspended for isometric force measurements in buffered salt solution (95% O_2-5% CO_2; 30°C). Rings from the turtle showed spontaneous activity and concentration-dependent increases in tension to norepinephrine and acetylcholine. In the presence of norepinephrine, the Ca^{++} ionophore, A 23187, caused contraction only in rings with endothelium. Rings of aorta from the cayman were not spontaneously active but showed concentration-dependent increases in tension to norepinephrine. Acetylcholine and the Ca^{++} ionophore caused concentration-dependent decreases in tension; the effect of acetylcholine was inhibited by methylene blue. These results indicate that endothelium-dependent responses are present in vessels of reptiles and that the quality of such responses varies with the species. (Supported in part by grants HL 07111 and NIH 31183.)

74.6

ANEMIA AND CUTANEOUS GAS EXCHANGE IN ADULT AQUATIC RED SPOTTED NEWTS AT 5°, 14°, and 21°C. Ruthanne B. Pitkin, Allegheny College, Meadville, PA 16335

To investigate the role of hemoglobin in oxygen transport, the oxygen consumption rates at different temperatures of normal and anemic newts submerged in small jars were determined by a Micro-Winkler technique. Anemia was induced with intraperitoneal injections of 1-acetyl-2 phenylhydrazine dissolved in 10% ethanol. The normal newts were injected with comparable volumes of 10% ethanol. The mean hemoglobin concentration of the 30 anemic newts was 0.73 ± 1.0 g/100 ml of blood whereas that of 28 controls was 5.46 ± 1.99 g/100 ml. A Two-Way ANOVA (normal - anemic vs. 5°, 14°, and 21°C) on the oxygen consumption values was significant ($p<0.05$). A Newman-Kuels Multiple Comparison Test revealed that at 5° and 14° there was no significant difference in the oxygen consumption rates of the anemic and normal newts, but that there was a significant increase in the rate with temperature. However, at 21°C the anemic newts' oxygen consumption rate (54.7 mg/g·hr) was significantly lower than the rate of normal newts at 21°C (84.2 mg/g·hr). Thus the presence of hemoglobin seems not to be necessary to transport oxygen at low temperatures in submerged Notophthalmus viridescens viridescens. Perhaps at lower temperatures where the metabolic call for oxygen is decreased, sufficient oxygen may be physically dissolved in the blood plasma.

74.7

VENTILATORY RESPONSES OF CHRONICALLY HYPOXIC HAMSTERS TO HYPOXIA AND HYPERCAPNIA. **E.M. Adams, B.R. Walker, and N.F. Voelkel. Case Western Reserve Univ./University Hosp., Cleveland, OH, 44106, Tulane Univ., New Orleans, LA, 70112, and Univ. Colorado HSC, Denver, CO, 80262.**

The Syrian hamster (Mesocricetus auratus) is fossorial in its native habitat and demonstrates cardiovascular adaptations to chronic hypoxia. The present experiments were performed to compare the hamster ventilatory responses to hypoxia (10% O2 + 5% CO2) to those of the non-fossorial rat after both species were exposed to 6 weeks of hypobaric hypoxia (simulated alt.=4250m). Ventilatory measurements were obtained from conscious animals using the barometric method in a sealed plethysmograph. Basal Ve/100g and 5% CO2 response in hamsters are normally lower than those of the rat; however, after six weeks of chronic hypoxia these differences were eliminated. The response to either hypoxia or hypercapnic hypoxia was not different between the two species before or after chronic hypoxic exposure. We conclude that the hamster's cardiovascular adaptations are not associated with comparable changes in hypoxic ventilatory response.

74.8

EFFECTIVE AND MORPHOMETRIC O_2 DIFFUSING CAPACITY OF THE GILLS OF THE ELASMOBRANCH SCYLIORHINUS STELLARIS. **J. Piiper, P. Scheid and G.M. Hughes*. Dept. Physiology, Max Planck Institute for Experimental Medicine, Göttingen/F.R.G.**

Determinations of the effective diffusing capacity (conductance or transfer factor) of fish gills for O_2 (D_{eff}), obtained from experimental data of gill O_2 exchange, were compared with the predicted O_2 exchange properties of gill models based on morphometric measurements in the elasmobranch Scyliorhinus stellaris. D_{eff} was calculated from O_2 uptake and Po_2 in gill water and blood, using a modified Bohr integration technique. In the morphometric gill model, O_2 conductance was considered for both the water/blood tissue barrier (D_m) and the interlamellar water (D_w). D_m was calculated from the total secondary lamellar surface area, the harmonic mean water-blood barrier thickness, and an assumed Krogh O_2 diffusion constant for gill tissue. D_w was estimated from the dimensions of the interlamellar spaces, the mean respiratory water flow velocity and the diffusion coefficient of O_2 in water. The combined membrane-to-blood diffusing capacity, D_{m+w} ($1/D_{m+w} = 1/D_m + 1/D_w$), was similar to D_{eff}, the ratio D_{m+w}/D_{eff} being 1.64 for quiescently resting, 1.02 for resting alert, and 0.92 for swimming fish. The reasonable agreement between D_{m+w} and D_{eff} estimates validates the approach, and leaves, at least for the alert and swimming fish, little space for functional inhomogeneities which are expected to reduce D_{eff} as compared to D_{m+w}.

74.9

Differences in weight-standardized oxygen consumption, growth and weight loss between low- and high-growth Mytilus edulis (Mollusca). **Walter J. Diehl, Patrick M. Gaffney* and Richard K. Koehn*. SUNY, Stony Brook, N.Y. 11794**

Small (S individuals: 4 mm initial shell length) and large (L individuals: 10 mm initial SL) Mytilus edulis spat were grown singly in plastic racks in a tidal salt marsh for 72 days, then starved in the laboratory for up to 2 months. Oxygen consumption was measured during the period of starvation and after a pulse of food (Isochrysis galbana) was given to individuals starved 2 months. Heterozygosity at five polymorphic enzyme loci was measured for individuals starved less than 20 days. L individuals were significantly more heterozygous and always had significantly lower weight-standardized oxygen consumption ($\dot{V}O_2$) than S individuals. There was generally a negative relationship between $\dot{V}O_2$ and dry weight gain in the racks for all individuals. During starvation in both groups, a positive relationship existed between $\dot{V}O_2$ and dry weight loss; after prolonged starvation, the postprandial $\dot{V}O_2$ was uniformly greater than the starved $\dot{V}O_2$. The slopes of these relationships were significantly heterogeneous between the S and L groups, with the slopes of the S group being greater than those of the L group. Reduced respiratory costs, as low $\dot{V}O_2$, are associated more with resistance to weight loss than with the capacity for weight gain. The metabolic response to food, however, is not a function of either resistance to weight loss or capacity for weight gain. This research was supported by NSF grant DEB 7908862.

74.10

EFFECTS OF IONOPHORE A23187 ON CYTOSOLIC SODIUM AND CALCIUM IN PAROTID CELLS RELATIVE TO ACETYLCHOLINE. Robert J. Stark. DePauw Univ. Greencastle, IN, 46135

A23187 is frequently used to stimulate changes in cell function, presumably by increasing cytosolic calcium ([Ca]i). However, in this study, A23187 also increased cytosolic sodium ([Na]i) and the basolateral membrane potential (Em). Induced changes in Em, [Na]i, & [Ca]i were measured with ion-selective and open-tip microelectrodes in mouse (ICR) parotid cells during A23187 (10-7 to 10-5 M) and acetylcholine (ACh)(10-9 to 10-5 M) stimulation. Both stimulii produced similar dose-dependent Em hyperpolarizations. A23187 also induced a linear increase in [Na]i (2.25 mM/ 10-fold A23187 change) from a rest value of 9.2± 0.4 mM and a consistant increase in [Ca]i to approx. 1.0 uM from 0.44± 0.04 uM when the ionophore was above 10-7 M. ACh, while inducing a similar sharp increase in [Ca]i (to approx. 1.0 uM) with 10-8 & 10-7 M, at higher conc. reduced the [Ca]i increase. A similar pattern was observed for [Na]i. At least two mechanisms appear to be regulating ionic activities during stimulation of parotid acinar cells: one limiting the [Ca]i increase following a calium challenge to approx. 1.0 uM; and a second acting on the ACh receptor or on membrane channels to reduce ion permeability when ACh is above 10-7 M. (Grant support NIH grant PHS AM26246 & Indiana Acad. of Science)

74.11

ALLOMETRY OF THE SAURIAN KIDNEY: IMPLICATIONS FOR THE ONTOGENY OF OSMOREGULATION IN AMNIOTE VERTEBRATES. Carol A. Beuchat and Eldon J. Braun. Colgate University, Hamilton, NY 13346 and University of Arizona, Tucson, AZ 85724

In reptiles there are two functional kidneys at birth, the mesonephros and the metanephros. As in all amniote vertebrates, the metanephros is retained as the kidney in adults, but the function of the mesonephros is unknown. In the lizard Sceloporus jarrovi, the metanephric kidney is smaller at birth and has fewer glomeruli than would be predicted from the allometric relationship between kidney mass and body mass in adult lizards. However, the sum of mesonephric and metanephric kidney mass conforms to this allometric prediction. Other amniote vertebrates appear to follow this pattern as well. In marsupials, which also retain the mesonephros after birth, the sum of mesonephric and metanephric mass conforms to the predicted allometry. The mesonephros of eutherian mammals, however, degenerates before birth, and the metanephric kidney alone is of the predicted size. Because the scaling of kidney mass in amniotes retaining the mesonephros after birth conforms to that of adults if metanephric and mesonephric kidney mass are summed, this suggests that the mesonephric kidney in these vertebrates may play a significant role in the regulation of water and ion balance for a short time after birth. (Supported by NIH NRSA AM07063-03.)

74.12

OSMOTIC AND IONIC REGULATION IN THE AQUATIC CAECILIAN TYPHLONECTES COMPRESSICAUDA AND THE FOSSORIAL CAECILIAN ICTHYOPHIS BIANGULARIS. Marie L. DeRuyter* and Daniel F. Stiffler, Calif. State Polytechnic Univ., Pomona, CA 91768.

Renal and extrarenal hydromineral balance was assessed in caecilians (order apoda). The aquatic Typhlonectes has a higher affinity Na^+ transport system (K_m = 0.1 mM) than Icthyophis (K_m = 0.5 mM) however both species have similar J_{max} of about 2 µEq/10g-h. Similarly, the aquatic caecilian has a higher affinity for Cl^- (K_m = 0.1 mM) than does the fossorial species (K_m = 0.7 mM) however Icthyophis does have a higher Cl^- capacity than does Typhlonectes (J_{max} = 2.3 vs. 0.8 µEq/10g-h). There were also differences in transepithelial potential difference between the species. Icthyophis had a TEP of 58 mV (inside positive) while Typhlonectes' TEP averaged 14 mV (inside positive). The osmotic uptake of water in Typhlonectes was 0.08 ml/10g-h while Icthyophis absorbed water at a rate of 0.28 ml/10 g-h. Renal clearance measurements on Typhlonectes yielded a urine flow of 0.13 ml/10g-h and a GFR of 0.139 ml/10g-h. Fractional tubular reabsorption of Na^+ (95.8%), K^+ (88.7%), and water (9.5%) were similar to other amphibians.

74.13

RENAL RESPONSE TO FEEDING AND SALT-LOADING IN THE YELLOW-BELLIED SEA SNAKE, PELAMIS PLATURUS. S. Benyajati, S.D. Yokota*, W.H. Dantzler, I. Rubinoff*, and J.B. Graham*. Dept. Physiology, Univ. Arizona, Tucson, AZ 85724; Smithsonian Trop. Res. Inst., Balboa, Panama and Scripps Inst. Oceanography, La Jolla, CA 92093.

Renal function was assessed in conscious, free-swimming P. platurus (BW 27.5±4.2g, n=12) at 25C. Extracellular fluid volume (ECFV), estimated with ^{3}H-PEG, was 35.5±2.2%BW. Mean glomerular filtration rate (GFR), estimated by the plasma clearance of a single injection of PEG over a 7d period, was 3.53±0.40 ml/kg•h (n=9) and was not significantly different from the renal clearance determined simultaneously. The total rate of ion excretion (salt gland + renal) in unfed animals (n=9) was 2.2±0.5 µmol/kg•h for Na and 0.085±0.057 µmol/kg•h for K. The renal excretion rate of ions determined in 3 animals was 0.94±0.28 µmol Na/kg•h (=42% total) and 0.11±0.02 µmol K/kg•h (=127% total). After a fish meal (10%BW), ECFV increased by 9% and returned to control values by Day 6 (n=8). GFR increased from 4.06±0.50 to 6.55±0.53 ml/kg•h ($p<0.05$). Total Na and K excretion increased 3-9 fold while plasma Na and K remained unchanged. NaCl loading (2%BW of 1M NaCl, subcutaneously) increased total Na excretion and GFR within 24h (n=4). Na excretion increased to 17.9±5.5 µmol/kg•h, primarily by the secretion of the salt gland. The Na load was completely eliminated by 24h when the GFR also returned to normal. It appears that the kidney is important in Na elimination during normal function in these sea snakes. [Supported by NSF-PCM82-02360(WHD), NIH-AM33246(SDY), NIH-HL07249, Smithsonian Scholarly Studies Program and the Tupper Foundation.]

74.14

FLUID SECRETION BY PROXIMAL TUBULES OF A EURYHALINE TELEOST IN SEAWATER. W.H. Cliff and K.W. Beyenbach. Cornell University, Ithaca, NY, 14853.

Tubular fluid secretion has been demonstrated in glomerular kidneys of an evolutionarily diverse group of marine species including the dogfish, flounder and sea snake. We now report the presence of spontaneous fluid secretion in isolated proximal tubules of the common killifish, Fundulus heteroclitus, adapted to 100% seawater. In vitro fluid secretory rates were 49 ± 9 pl/min mm tubule length (mean ± SE, n=16 tubules). Wavelength dispersive spectroscopy electron probe analysis revealed that secreted fluid consisted primarily of Na (128 ± 5 mM), Mg (29 ± 3 mM), Cl (159 ± 3 mM) and S (11 ± 2 mM). Concentrations of K, Ca and P were similar in secreted fluid as in the bathing saline. Fluid secretion continued in the absence of bath $MgSO_4$ but at a reduced rate (51% of control) indicating that $MgSO_4$ secretion can not account entirely for tubular fluid secretion. Our preliminary findings indicate that the proximal tubule of the euryhaline killifish may be a unique model for investigating the functional transition of a renal proximal tubule from a net reabsorbing to a net secreting segment for water and electrolytes. Supported by NIH AI 14771.

74.15

EFFECT OF SALINITY ON THE ACTIVITY-RELATED O_2 CONSUMTION RATE AND GROWTH OF AN OREOCHROMIS MOSSAMBICUS ♀ X O. HORNORUM ♂ HYBRID (PISCES). RICARDO FEBRY AND PETER L. LUTZ. Univ. of Miami, RSMAS-BLR, Fl. 33149

Oxygen consumption rates ($\dot{V}O_2$) were measured in three groups of fish (@ 20-170g) acclimated to fresh (0 °/₀₀), brackish (12 °/oo), and seawater (35 °/oo), respectively, while swimming at controlled speeds of 10, 20, 30, and 40 cm/s. Salinity (S °/oo) had no effect on the standard $\dot{V}O_2$, estimated for each fish by extrapolation to zero activity, when $\dot{V}O_2$ variation due to weight differences was removed ($P=0.47$). Under active conditions however, S °/oo was significant after variations in $\dot{V}O_2$ due to weight and swimming speed were removed as well ($P=0.09$). In an ideal, 63.0g fish, $\dot{V}O_2$ at 0 and 35 °/oo, relative to $\dot{V}O_2$ at 12 °/oo salinity, increases @ 14 and 21%, respectively as swimming speed increases from 0 to 40 cm/s. Other experiments on growth in fish held at 0, 12, and 35 °/oo salinities showed that fish at 12 and 35 °/oo salinities grew @ 93 and 67% more, respectively, than fish held at 0 °/oo salinity after a period of 4 weeks of controlled feedings ($P<0.05$). These data show that Oreochromis hybrids in our study were more efficient organisms in 12 °/oo salinity, suggesting that a lower osmoregulatory demand is imposed by the environment at such level. Separate measurements of plasma osmotic concentration in fish acclimated to 0, 6, 12, 24, and 35 °/oo salinities ranged from 317.3 + 7.2SD mOsm/kg at 0 °/oo to 371.7 + 28.0SD mOsm/kg at 35 °/oo acclimated fish, around the osmotic concentration of 12 °/oo salinity.

74.16

CA^{2+} INFLUX FOLLOWS A HYPOOSMOTIC STRESS AND CALMODULIN ANTAGOGONISTS ALTER CELL VOLUME RECOVERY IN INVERTEBRATE BLOOD CELLS. Alexander D. Politis, Laurens H. Smith Jr., and Sidney K. Pierce, Univ. of Maryland, College Park, MD 20742.

Cell volume recovery during hypoosmotic stress is sensitive to external $[Ca^{2+}]$. While several investigators have suggested that intracellular $[Ca^{2+}]$ changes regulate the volume recovery mechanism, studies on mammalian cells have failed to demonstrate a change in intracellular free Ca^{2+} following an osmotic stress. We have tested for changes in Ca^{2+} flux (using ^{45}Ca) following a hypoosmotic stress. A net ^{45}Ca influx occurs within seconds of a hypoosmotic stress in both the red blood cells of Noetia ponderosa and red coelomocytes of Glycera dibranchiata. The net ^{45}Ca influx lasts less than 1 minute and the level of intracellular ^{45}Ca remains elevated for at least 10 minutes. ^{45}Ca efflux during the hypoosmotic stress is negligible. Thus, an increase in intracellular $[Ca^{2+}]$ occurs in response to the osmotic change in both cell types. In addition, A23187 potentiates volume regulation in hypoosmotically stressed Glycera cells. Furthermore, the calmodulin inhibitors, trifluoperazine and chlorpromazine modify cell volume regulation in both cell types. The inhibitors did not affect the amino acid efflux from hypoosmotically stressed Noetia cells. These results suggest that the mechanism of hypoosmotic cell volume regulation may be initiated by a Ca^{2+} influx and mediated by calmodulin. (Supported by NIH GM23731)

74.17

SULFIDE OXIDATION AND SULFATE ADENYLYLTRANSFERASE ACTIVITY IN GILL OF THE CLAM Solemya velum. C.S. Hammen, Chong Chen*, and Bruno Rabourdin*. Univ. Rhode Island, Kingston, R.I. 02881.

Solemya velum is one of several bivalves recently discovered to contain symbiotic sulfide-oxidizing bacteria in the gill tissues. Rates of carbon dioxide fixation by these chemoautotrophs have been reported by Cavanaugh (1983), but the sulfide metabolism has not been closely examined. Whole clams, placed in a Gilson respirometer, consumed oxygen at an average rate of 2.91 micromoles/h per g at 15C. When sulfide was added at a final concentration of 0.50 mM, oxygen uptake increased by more than 100 percent. The supernatant fraction of centrifuged homogenate of Solemya gill contained adenylate kinase (EC 2.7.4.3) activity, 2.52 micromoles/min per g, which was inhibited 94% by di-adenosine pentaphosphate (DA5P) 0.25 mM. The reaction catalyzed by sulfate adenylyltransferase (EC 2.7.7.4) was started with the addition of pyrophosphate, and proceeded rapidly in the presence of DA5P. An extract of gills from 12 clams gave a maximum velocity of 3.54 micromoles/min per g at 21C, and an apparent Michaelis constant of 0.18 mM. Calorimetry with 42-50 animals in sea water showed heat output equivalent to 3.6 - 5.0 times oxygen consumption during the aerobic phase, which suggests that total metabolism in this symbiosis is ordinarily 70-80% anaerobic.

74.18

THERMAL STEP RESPONSE OF CHICKEN EGGS IN ALTERED ENVIRONMENTS. H. Tazawa, H. Takenaka* and Y. Suzuki*. Muroran Institute of Technology, Muroran 050, Japan.

Avian embryos are poikilothermic in terms of poor capacity of thermoregulation. Conspicuous resistance to ambient temperature changes appears just after the time of hatching, although emergence from the shell is not necessary. Therefore, once prenatal embryos are exposed to lowered temperature, the egg temperature follows it in an exponential fashion. Heat is lost from the embryo to environment through thermal resistances arranged in series. Among them, the boundary layer surrounding eggs has been suggested to provide a major resistance to heat loss (Sotherland et al., The Physiologist 27: 260, 1984). To assure it, thermal step responses of eggs (embryonated, non-embryonated and dead) and boundary layer under atmospheres of which thermal conductivity was altered (i.e., in N_2, He and SF_6) were determined. Besides, the step response of egg temperature was measured in water. Temperature gradient from the egg to environment was reduced and egg temperature response was accelerated in He atmosphere compared with those in N_2, and they were facilitated remarkably in water. Reverse changes occurred in SF_6. Results were direct evidence showing the major resistance in the air boundary layer. The heat transfer coefficient of boundary layer calculated from response curve was about 10 kcal/(cm^2·h·K) and the heat transferred by convection at an onset of exposure was ca. 1 Kcal/h which surpassed eminently the heat production of embryos.

74.19

CONTROL OF PERIODIC BREATHING IN HIBERNATING GROUND SQUIRRELS. W.K. Milsom, M.D. McArthur* and C.L. Webb*. Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 2A9.

A breath by breath analysis has been made of the breathing patterns of both Columbian (S. columbianus) and golden-mantled (S. lateralis) ground squirrels, awake and in deep hibernation. While awake, both species breathe continuously (50-60/min), respond briskly to hypoxic exposure but show blunted hypercapnic sensitivity relative to non-hibernating rodents. Entrance into hibernation is accompanied by a transition to periodic breathing which may consist of single breaths separated by short periods of apnea (1-2 min) (S.c. always, S.l. only at body temp. (Tb) <4°C) or clusters of breaths separated by longer apneic pauses (5-10 min) (S.l at Tb >5°C). In hibernation, hypoxic responses were slight or absent; in many instances only hypoxic depression of ventilation was observed at levels of inspired O_2 <3%. Hypercapnic sensitivity was reduced in absolute terms but increased when normalized to metabolic rate. Hypercapnia led to increases in tidal volume but no changes in inspiratory or expiratory duration. Regardless of breathing pattern, changes in frequency were due mainly to shortening of the apneic pause. Carotid denervation had no effect on either the breathing patterns or ventilatory responses of the hibernating animals. The data suggest that periodic breathing in hibernating mammals is quite different from that seen in mammals with central sleep apnea (such as at altitude) but strikingly similar to that seen in reptiles. (Supported by NSERC of Canada)

TEMPERATURE REGULATION II

75.1

THERMOREGULATION DURING ACTIVE AND PASSIVE HEATING DURING THE MENSTRUAL CYCLE. Margaret A. Kolka and Lou A. Stephenson. US Army Research Institute of Environmental Medicine, Natick, MA 01760-5007.

Thermoregulatory responses were studied in five women in both the follicular (F) and luteal (L) phases of the menstrual cycle. Continuous measurements of esophageal temperature (T_{es}), mean skin temperature ($\bar{T}_{sk}$, 8 site), metabolism, chest and forearm sweating ($\dot{m}_s$) were made during both passive (PAS) heat exposure and seated cycle exercise (ACT, 80%$\dot{V}O_2$ peak) in ambient conditions T_a=50.0°C and T_{dp}=18.5 °C. Blood samples taken during T_{es} transients (0.2 °C increments) were analyzed for norepinephrine (NE), and epinephrine (E). We matched the T_{es} in PAS and ACT when blood samples were taken. The normal L increase in T_{es} (0.3 °C) occurred in all five subjects indicating elevated levels of circulating progesterone. Resting NE was significantly higher (74%) during L. Circulating NE and E were higher during ACT than PAS due to the exercise. Chest and arm local sweating rates were higher during ACT at the same T_{es} drive. $\bar{T}_{sk}$ was lower during ACT than PAS, and decreased with time of exposure during ACT. $\bar{T}_{sk}$ was higher during L in both PAS and ACT. During ACT the T_{es} threshold for the onset of sweating was higher (0.3°C) in L. There was no change in the slope of either arm or chest $\dot{m}_s$: T_{es} between F and L. The relationships between circulating NE or E and local sweating although positive, were not consistent. The higher local sweating rate during ACT, but lower $\bar{T}_{sk}$, at the same T_{es} as PAS implies other inputs in the control of sweating. Higher circulating NE and E may be one input.

75.2

PLASMA VOLUME DYNAMICS DURING HEAT STRESS AND EXERCISE IN WOMEN. Lou A. Stephenson and Margaret A. Kolka. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760-5007

Resting plasma volume (PV) is lower during the mid-luteal phase than the mid-follicular phase of the menstrual cycle. To determine whether PV dynamics were affected by the menstrual cycle, we studied five women during exercise (HE) and passive heat stress (HP). An exercise bout (80% $\dot{V}O_2$ max) on a modified cycle ergometer and a passive heating bout were done in a hot environment (T_a = 50°C, T_{dp} = 18.5°C) during the follicular (F) and luteal (L) phase. Esophageal temperature (T_{es}) and $\dot{V}O_2$ were measured continously. Venous blood samples were drawn after each 0.2°C increase in T_{es}. Hemoglobin concentration (Hb) and hematocrit (Hct) were measured in each sample. Initial PV was estimated at rest during F. PV changes from rest were calculated at each T_{es} from Hb and Hct. The results are shown below.

		Δ PV (l)		FINAL PV (l)	
		F	L	F	L
HP	$\bar{X}$	0.156	0.300	2.83	2.47
	S.D.	(0.08)	(0.10)	(0.09)	(0.18)
HE	$\bar{X}$	0.463	0.381	2.50	2.50
	S.D.	(0.09)	(0.07)	(0.11)	(0.23)

During HP, initial PV was lower in L than F and decreased more rapidly to a lower absolute volume. These data indicate that there is a menstrual cycle effect on PV dynamics during HP. Also, PV is defended at a higher absolute volume during HP-F than in HP-L. Furthermore, final PV during HE-F and HE-L is the same as HP-L.

75.3

SKIN WETTEDNESS AND FABRIC ACCEPTABILITY. A.R. Gwosdow*, J.C. Stevens* and L.G. Berglund* (Spon: A.P. Gagge). J.B. Pierce Fndn. Lab and Yale Univ., New Haven, CT 06519.

The influence of skin wettedness on perception of fabric texture and pleasantness (acceptability) was studied by exposing eight men to the following sequence of air and dew-point temperatures (T_a:T_{dp}): 23:15°C, 35:15°C, 35:29°C and 23:15°C. Air speed was 0.05 m/s. For each condition, which lasted 20 min, six different fabrics (wool, brushed cotton, cotton, silk, linen and burlap) were slowly pulled across the subject's forearm. Each material was presented twice. Force of fabric pull across the forearm and the subject's ratings of texture and pleasantness were recorded. Arm skin temperature and skin wettedness were measured continuously. The results indicate that skin wettedness was positively correlated ($P < 0.05$) with the force required to pull each fabric over the skin. As skin wettedness and force increased ($P < 0.05$), subjective responses indicated all fabrics felt more textured and less pleasant ($P < 0.05$). Increased perception of fabric texture significantly decreased fabric acceptability. On return to the initial condition all parameters returned to their original values. Conclusion: Moisture on the skin surface enhances perception of fabric texture which decreases the acceptability of clothing worn in hot environments.

75.4

HYPERTHERMIA AUGMENTS HUMAN GAMMA INTERFERON INDUCTION POSSIBLY BY TWO DISTINCT MECHANISMS. **R. S. Elizondo, J. F. Downing*, K. M. Wei*, L. A. Mangels* and M. W. Taylor*. Indiana Univ. Sch. of Med., Physiology Section and Biology Dept., Bloomington, IN 47405.**

Studies on the effects of febrile temperature on human gamma interferon (IFN-γ) production have revealed two possibly distinct mechanisms which elevate the levels of this important lymphokine. Firstly, incubation (60 hrs.) of human mononuclear cells in the presence of T cell mitogen at 37°C or 39°C showed that the elevated temperature resulted in a significant 69% increase in IFN-γ production. Using staphylococcal enterotoxin (0.5 ug/ml) as a mitogen and a sensitive radioimmunoassay, control (37°C) cultures produced IFN-γ at a concentration of 445 units/ml while parallel hyperthermic cultures (39°C) produced IFN-γ equal to 650 units/ml. Secondly, IFN-γ production at 37°C was higher in cultures obtained from hyperthermic humans compared to controls. Subjects were immersed in a warm water bath (40-45°C) to induce a steady rise in core (T_{re}) temperature. Blood samples were obtained via venous cannulation at T_{re} equal to 37°, 38°, and 39°C. A significant relationship was observed between IFN-γ production and increasing T_{re}. Interferon production increased from 380 ± 75 units/ml at T_{re}=37°C to 4,000 ± 425 at 39°C. These data are consistant with the hypothesis that hyperthermia augments human IFN-γ induction by two possibly distinct mechanisms. **(Supported in part by PHS AI 21898)**

75.5

HYPERTHERMIA-INDUCED PULMONARY EDEMA. K.Y. Mustafa*, W.M. Selig*, K. Burhop*, and A.B. Malik. Departments of Physiology, Albany Medical College, Albany, New York 12208.

We investigated the effects of increases in temperature (41-47°C) on pulmonary microvascular permeability using the isolated-perfused guinea pig lung and bovine pulmonary arterial endothelial cell monolayer grown on a gelatinized membrane. A step increase in the temperature of Ringer's-albumin perfusate produced an increase in the lung weight with no change in pulmonary artery pressure and capillary pressure (double occlusion method). The lung weight increased by 25% above baseline (37°C) at 45°C and by 300% at 47°C. In the monolayer system an increase in temperature (37-47°C) had no effect on the permeability of the gelatinized membrane alone. ^{125}I-albumin clearance (µl/min) across the endothelial cell monolayer at 45°C showed a 3 fold increase (0.295±0.035 to 1.020±0.258), an effect comparable to that of positive controls of trypsin (0.272±0.046 to 1.595±0.138) or oleic acid (0.278±0.043 to 0.672±0.26). We conclude that hyperthermia in the clinically encountered range 41°-47°C (e.g. heat stroke) produces pulmonary edema by an increase in pulmonary endothelial cell permeability. (K.Y.M. is on a sabbatical leave from the Dept. of Physiology, Faculty of Medicine, Kuwait University

75.6

HIBERNATION IN THE NORTHERN WATER SNAKE, NERODIA SIPEDON: SEASONAL VARIATIONS IN PLASMA AND TISSUE CHEMISTRY. **Jeremy S. Wasser.* (SPON: H. D. Prange). Indiana Univ., Medical Sciences Program, Bloomington, IN 47405**

Although it has become apparent that reptiles that become dormant in the winter show seasonal adaptations similar to those of hibernating mammals, few unifying characteristics of hibernation in ectotherms have been established. I therefore examined the seasonal changes in plasma and tissue chemicals in the northern water snake in order to characterize chemical compositional changes typical of hibernation in this reptile. My conclusions were: (1) there were no seasonal variations in total body water or in plasma and tissue water; (2) plasma magnesium concentrations were higher in the winter; (3) plasma osmolality was lower in the winter; (4) there were no seasonal variations in fat body weight; (5) variations in some chemical element concentrations and a lack of change in others indicated that snakes avoided excessive diffusion of intracellular and extracellular constituents in the winter; (6) there was no apparent storage of potassium in the kidneys in the winter; (7) chemical profiles of snakes kept outdoors were different than those of snakes experimentally acclimated to winter or summer conditions suggesting that nonseasonal acclimation studies may be subject to error. Therefore, water snakes do not stop regulating their plasma and tissue chemical compositions in the winter but instead possess adaptations, probably at the membrane level, that allow them to survive long periods of seasonal cold.

75.7

CARDIORESPIRATORY RESPONSES TO COLD AIR FOLLOWING REPEATED COLD WATER IMMERSION. S.R. Muza, A.J. Young, M.N. Sawka and K.B. Pandolf. US Army Research Institute of Environmental Medicine, Natick, MA 01760-5007

The effects of cold acclimation (CA) on the cardiorespiratory responses to a cold air stress test (CAST) were studied in seven males before and after a CA program (daily 90 min cold (18°C) water immersion, repeated five times a week for five consecutive weeks). The CAST consisted of a 90 min resting exposure to cold (5°C, 30% rh) air during which rectal temperature (T_{re}), oxygen consumption ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), heart rate (HR) and cardiac output ($\dot{Q}$) were periodically measured. After CA, resting T_{re} were lower ($p < 0.01$) prior to CAST and the decrease in T_{re} during CAST was greater ($p < 0.01$) after CA than before. The $\dot{V}O_2$ increased ($p < 0.01$) during the first 10 min of CAST then continued to gradually rise. However, following CA, the $\dot{V}O_2$ at 10 minutes was lower ($p < 0.02$) compared to before CA. The $\dot{V}_E$ increased ($p < 0.01$) during CAST as a function of both increased tidal volume and frequency. The CA had no effect on $\dot{V}_E$ responses to CAST. The CA had no effect on $\dot{Q}$ which increased ($p < 0.01$) during the first 45 min of CAST then remained stable. The increased $\dot{Q}$ during CAST was a function of small increases in HR and stroke volume. The arteriovenous blood O_2 difference increased ($p < 0.01$) during CAST but was not altered by CA. These results suggest that CA attenuated the onset of metabolic heat production but did not alter its ultimate magnitude. The relationships between the cardiorespiratory variables and metabolic requirements were not changed by CA.

75.8

ALTERED HUMAN THERMOREGULATORY RESPONSES TO COLD AIR FOLLOWING REPEATED COLD WATER IMMERSION. A.J. Young, S.R. Muza, M.N. Sawka, R.R. Gonzalez and K.B. Pandolf. US Army Research Institute of Environmental Medicine, Natick, MA 01760-5007

The effects of repeated cold water immersion on thermoregulatory responses to cold air were studied in seven males. A cold air stress test (CAST) was performed before and after an acclimation program of a daily 90 min cold (18°C) water immersion, repeated five times a week for five consecutive weeks. The CAST consisted of resting for 90 min in cold (5°C, 30% rh) air while metabolism (M), rectal (T_{re}) and mean weighted skin (T_{sk}, forearm, calf, chest) temperatures were measured. Pre- and post-acclimation, M increased ($p < 0.01$) by ~ 85% during the first 10 min of CAST, and thereafter rose slowly. After acclimation, M was lower ($p < 0.02$) at 10 min of CAST compared to before, but by 30 min M was the same as before. Therefore, onset of shivering may have been delayed following acclimation. After acclimation, T_{re} were lower ($p < 0.01$) both before and during CAST, and the drop in T_{re} during CAST was greater ($p < 0.01$) than before. The T_{sk} was lower ($p < 0.01$) following acclimation compared to before, at least at the three sites measured. Acclimation resulted in a larger ($p < 0.01$) T_{re} to T_{sk} gradient which would facilitate heat flow from core to shell. However, localized redistribution of heat to the shell was not detected probably because T_{sk} was measured at only three sites. These results suggest that following repeated cold water immersion, man exposed to cold air responds with lower core temperatures which may be related to altered metabolism and cutaneous blood flow.

76.1

SPACE STATION LIFE SCIENCE RESEARCH FACILITY. Roger Arno* and John Hilchey*, (SPON: C.M. Winget). NASA-Ames Research Center, Moffett Field, CA 94035, NASA-Marshall Space Flight Center, AL 35812

Space Station will become a reality by the early 1990's, opening a new era in the U.S. Space program--an era in which basic research on biological systems can be conducted in microgravity for relatively long periods of time. Significant contributions can be made to many life sciences disciplines through the use of laboratory facilities and extended mission duration that a Space Station will provide. Life scientists will utilize one of four habitable modules that constitute the initial Space Station configuration. Typical non-human life sciences equipment includes animal holding facilities or "habitats", plant growth chambers, work stations specially designed to operate in the absence of gravity, video cameras, and recording systems, data analysis systems, refrigerators, freezers, storage systems, and a variety of scientific instruments, tools and supplies. The current Space Station Program includes a significant life sciences research capability. The initial operational configuration (IOC) provides for four habitable modules, two of which will be laboratories. One laboratory will be for materials, experiments and commercial research. Most of the other laboratory module will be split equally between human and non-human life sciences research. Later, perhaps as early as 1994, another module, dedicated entirely to nonhuman life sciences, is scheduled to be launched.

76.2

CONCEPTS, STRATEGIES AND POTENTIALS USING HYPO-G AND OTHER FEATURES OF THE SPACE ENVIRONMENT FOR COMMERCIALIZATION OF HIGHER PLANTS. A.D. Krikorian. State University of New York at Stony Brook,Stony Brook, NY 11794

In a micro-g or hypo-g environment where there is no gravity vector, no polarity, no buoyancy, no convective current, no stratification of layers and where surface tension dominates, we can anticipate major impacts on metabolism that may well be reflected in the morphogenesis of cells, tissues and organs. Since morphology is tightly linked to physiology, physiological perturbations of various sorts will have significance for distribution and partitioning of solutes and assimilates in cells, tissues and ultimately in the whole plant body, and even in the further expression of diverse biosynthetic potentials. Opportunities for releasing, capturing, constructing and/or fixing the differential expressions or response potentials of the higher plant genome, both nuclear and cytoplasmic, exist using the "new biotechnologies". One cannot guarantee that these will lead immediately to commercialization but the studies needed to probe all this in depth will necessarily involve utilization of aseptically cultured morphogenetically competent cells, tissues, organs and intact plants. The disclosure of new facts from such studies should go far to helping us understand what development is all about as well as have practical benefits for agricultural and other biology based technologies. Supported by NASA NSG-7270 "Cells, Embryos and Development in Space."

76.3

THE USE OF SPACE STATION IN PLANT GROWTH STUDIES. Tom K. Scott. Univ. North Carolina at Chapel Hill, Chapel Hill, N. C. 27514 and Thora W. Halstead. Life Science Division, NASA Headquarters, Washington, D.C. 20546

The impact of gravity on plant cell growth and the mechanisms of its control are still not understood. The Space Station, by providing a prolonged near-weightless environment, will provide an opportunity to explore and possibly answer these questions. The ready availability of general laboratory facilities and sophisticated equipment over protracted periods of time together provide the essential elements to allow unambiguous determinations of various characteristics of plant growth and how they may be altered in microgravity and how they compare or contrast to those in a 1g environment. Examples of significant parameters which may change according to gravitational status are: 1) the biophysical and biochemical elements which govern cell elongation; 2) the perception, transduction, transmission and translation steps which lead to gravitropic responses of roots and shoots; 3) the nature of light-induced tropisms (and other tropic and nastic responses) which occur in the absence of the interactive influence of gravity-induced responses and; 4) the potential stress of a 1g force which may have had major evolutionary impact. Significantly, it will be possible to perform experimentation concerning these and other questions during any or all stages of the plant's life cycle and repeat them over successive generations in the Space Station.

76.4

MORPHOLOGY OF HUMAN EMBRYONIC KIDNEY CELLS IN CULTURE AFTER SPACE FLIGHT. Paul Todd*, M. Elaine Kunze*, Kimberly Williams*, Dennis R. Morrison*, and Marian L. Lewis* (SPON: A. H. Brown). The Pennsylvania State University, University Park, PA 16802

As a result of an electrophoresis experiment on Space Shuttle flight STS-8 in 1983, cultured human embryonic kidney cells at the first passage were subjected to nearly a full week of space flight conditions, including microgravity, in suspension, in a quiescent state. Microscopic examination of cells cultured from returned separated fractions was used to evaluate the extent to which space flight conditions affected the ability of cells to differentiate into four morphological types: small epithelioid, large epithelioid, "domed" (single cells with an upward bulge), and "fenestrated" (one or two large holes through the cytoplasm). The average percentages of each of these cell types after two passages in culture after space flight were 70, 16, 9, and 5, respectively. This distribution did not differ significantly from those found in control cultures. Cells cultured from low electrophoretic mobility fractions were rich in domed cells, while those from high mobility fractions were rich in small epithelioid cells, irrespective of exposure to space flight conditions. Apparently quiescent storage under microgravity conditions does not significantly affect the morphological differentiation of human embryonic kidney cells in low-passage culture. Work supported by NASA.

76.5

MULTIPLE GENERATIONS IN SPACE - AMPHIBIA AS A MODEL SYSTEM FOR ANIMAL DEVELOPMENT. G.M. Malacinski and A.W. Neff. Department of Biology and Medical Sciences Program, Indiana University, Bloomington, Indiana 47405.

Results from extensive ground based (e.g. clinostat) as well as space flight research have indicated that most major morphogenetic events during animal development are not catastrophically affected by hypogravity. Subtle defects due to microgravity probably, however, would go undetected during relatively short term experimentation. In the case of the amphibian embryo, ground based novel gravity orientation (e.g. clinostat, egg inversion) experiments have revealed that alterations in symmetrization, pigmentation pattern, and primordial germ cell number occur. In order to assess the long term effect of microgravity on the development of the amphibian embryo an experiment for rearing multiple generations in a space station is being designed. The following specific questions will be answered: Do oocytes develop normally in hypogravity? Is the germ cell line preserved in microgravity? Is metamorphosis affected?

76.6

MAMMALIAN DEVELOPMENTAL PHYSIOLOGY ON THE SPACE STATION J. Richard Keefe Case Western Reserve University, School of Medicine, Cleveland, OH 44106

Research potential in developmental physiology will be markedly enhanced with the advent of the long-duration and variable gravity exposure capabilities to be provided on the U.S. Space Station in 1992. The ability to establish and maintain a permanent population of selected, null-gravity adapted, experimental avian and mammalian species should provide: (1) fully-adapted specimens for comparative study of gravity re-adaptation upon return to earth-based laboratories; (2) studies of developmental responses to a complete range of variable gravities, both in terms of intensity and duration;and, (3) unprecendented opportunities in comparative physiological studies utilizing cell/tissue/organ cultures. Suggested applications in the study of fluid electrolyte regulation, cardiovascular dynamics, immune responsiveness, neurophysiological mechanisms of proprioception, structures and mechanisms of gravity perception/response, role of the calcium ion and its regulation, and the role of gravity in animal development and evolution will be presented.

76.7

PHYSIOLOGICAL AND NEUROSCIENCE RESEARCH IN THE MICROGRAVITY OF SPACE STATION. Charles A. Fuller. Univ. of California, Davis, CA 95616.

The next decade will bring the advent of a new era for space research in physiology and the neurosciences. Current space station planning provides for life science research on a portion of one pressurized module. These capabilities will include animal holding facilities, a variable gravity research centrifuge, integrated and automated physiological instrumentation (human and animal), a laminar flow work station, and animal development and plant growth chambers. Such facilities will allow research to be conducted in various disciplines and species. Gravitationally sensitive systems, particularly those related to structural loading (i.e., bone and muscle) and energetics (i.e., temperature regulation, metabolism, sleep, etc.), and their regulation/control mechanisms (neural and endocrine) can be studied at zero, fractional, one, and hypergravity in the space environment. In addition, for the first time the adaptability of various systems to chronic microgravity exposure can be examined. Crew participation in research will allow not only for their involvement as experimental subjects, but also as experimenters capable of performing complex procedures and care of animal subjects not possible in an unmanned environment. Thus, we can begin to prepare for the truly permanent presence of humans in space.

76.8

SPACE STATION OPPORTUNITIES FOR CARDIOVASCULAR RESEARCH. C. Gunnar Blomqvist. University of Texas Health Science Center, Dallas, TX 75235

The operation of a permanent space station will present new challenges and opportunities for basic and applied cardiovascular research. The space station crew will face many physically and mentally demanding tasks. Crew members are also likely to represent a wide range with respect to experience and personal characteristics. A prerequisite for the maintenance of crew health and optimal performance is a detailed knowledge of the physiological mechanisms involved in the adaptation to space flight of long duration and in readaptation to normal gravity.

Principal cardiovascular problem areas with important clinical correlates include the effects of prolonged weightlessness on (a) myocardial metabolism, function, and mass, and (b) neurohumoral control mechanisms. The instrumentation should include systems for cardiac imaging and for non-invasive and direct systemic and regional hemodynamic measurements at rest and during various forms of stress. Metabolism and neurohumoral control mechanisms may be studied using a wide spectrum of biochemical and immunological probes. Studies in different species at multiple levels of integration will be required, including clinical trials of various means of facilitating adaptation to weightlessness and readaptation to 1G.

76.9

OPPORTUNITIES FOR BIOLOGICAL RESEARCH ON SPACE STATION: THE MUSCULOSKELETAL SYSTEMS. Victor S. Schneider* and Adrian LeBlanc*. University of Texas Medical School and Baylor College of Medicine, Houston, Texas 77025. (SPONL: T. Halstead). NASA Hdq., Washington, D.C.

Short term space flight has proven that people can live and work in zero G without significant clinical medical problems. Skylab extended our data base to 84 days of continuous flight. Significant loss of bone as measured by a negative calcium balance in all three Skylab IV astronauts and a decline in bone densitometry in the two who lost the most calcium was observed. Muscle loss was also found as measured by a decrease in lean body mass, increases in urinary muscle breakdown products and a significant decrease in muscular peak strength. Although similar findings have been demonstrated during the inactivity of bed rest, the mechanism causing the apparent disuse osteoporosis is still unknown.

Some skeletal muscle atrophy was probably prevented by vigorous exercise during the Skylab IV mission; however, no exercise "prescription" is currently used by the astronauts for space flight.

NASA will encourage the research community to participate in scientific studies on the Space Station to determine the clinical and biological relevance of the musculoskeletal loss.

76.10

EXOBIOLOGY EXPERIMENT CONCEPTS FOR SPACE STATION. D.L. DeVincenzi* and L.D. Griffiths. NASA Headquarters, Washington, D.C. 20546 and MATSCO, Washington, D.C. 20546

The Science Lab Module (SLM) of the Space Station orbiting complex may provide an ideal setting in which to perform certain classes of experiments which form the cornerstone of exobiology research. These experiments could demonstrate the pathways and processes by which biomolecules are synthesized under conditions that simulate the primitive Earth, planetary atmospheres, cometary ices, and interstellar dust grains. For some of these experiments, gravity is a critical factor. Others may require exposure to the ambient space environment for long periods of time. Still others may require on-orbit preparation, servicing, maintenance, fixing, and analysis of samples. The pressurized SLM provides sufficient duration in the space environment and the crew interactions needed to assure implementation of these investigations. Exobiology experiments proposed for Space Station generally fall into four classes: interactions among gases and grains (nucleation, accretion, gas-grain reactions), novel high-energy chemistry for the production of biomolecules, physical and chemical processes occurring on an artificial comet, and tests of the theory of panspermia.

81.1

RECONSTRUCTION OF ALVEOLAR CAPILLARY NETWORKS IN LUNGS PERFUSED WITH OSMIUM IN ZONE II. Joan Gil and Yuyen Ho Tsai.* Pathology Dept., Mt. Sinai Medical School New York, NY. 10029

A rabbit lung inflated at 10 mm Hg with air was perfused with OsO_4 at 13-15 mm Hg. 2x2 cm blocks were embedded in Quetol and serially sectioned at 3 μm with Ralph knives. Three holes were drilled to serve as fiducial marks. Alignment was achieved in a computerized video system by superimposing a real time image of the marks with the previous stored image; after this, a motorized scanning stage traveled to the preselected area of interest. Images were recorded in a laser disc device. Primary object of the study was the distribution of wide open capillaries in corner pleats and of patches of derecruited capillaries in relation to recognizable landmarks of lung anatomy. It was found that pleats are located at the bottom (primary wall) of alveolar spaces and that they often extend symmetrically into alveoli at both sides. Areas of wide open capillaries arising from the pleats at the alveolar bottom first climb around the air space; as the alveolar space widens, the open capillaries suddenly fail to penetrate into certain whole lateral (secondary) walls. Some individual open corner pleats are in communication with both an artery and a vein; others form a network which always begins in an artery and ends in a vein. It is concluded that bundles of wide open capillaries inside pleats form a short cut between arteries and veins which is protected against air pressure and that secondary side walls of alveoli are variably perfused collaterals (HL 34196).

81.2

PULMONARY VASCULAR RESISTANCE IN BEAGLES: EFFECT OF CIGARETTE SMOKING. T.S. Hakim, M. King, R.P. Michel, C.G. Wang*, M. Cosio*. McGill University, Montreal, Quebec, Canada.

Left lower lobes from 12 control beagles and from 6 beagles who smoked cigarettes (50 cig/wk for 40 wks) were perfused in situ to study their vasculature using the arterial and venous occlusion technique and pressure-flow relationships. Both measurements suggested that the pulmonary vascular resistance (PVR) in smokers was about 40% less than non-smokers. Vasoconstriction with infused prostaglandin F_2, norepinephrine and histamine occurred predominantly in the veins. The changes in total, arterial and venous pressure drops in response to hypoxia (H, 5% O_2) and infusion of serotonin (5HT) and methacholine (M) are shown (X ± SE; C = control; S = smoker). We conclude that in beagles 1) Smoking

	n	ΔTotal	ΔArterial	ΔVenous
H-C	8	0.1 ± 0.4	0.1 ± 0.2	0.2 ± 0.3
H-S	6	4.5 ± 2.4	-0.3 ± 0.2	-0.3 ± 0.3
5HT-C	8	11.4 ± 1.4	3.0 ± 0.6	7.5 ± 1.8
5HT-S	6	12.7 ± 2.4	4.0 ± 1.3	5.5 ± 2.4
M-C	9	3.7 ± 0.8	-0.9 ± 0.4	3.9 ± 0.7
M-S	6	2.3 ± 1.0	-0.8 ± 0.2	1.0 ± 0.6

causes a decrease in PVR and in venomotor reactivity 2) Smoking enhances hypoxic vasoconstriction 3) The distribution of PVR among the 3 segments and the site of vasoconstriction were different than in mongrel dogs. Supported by MRC Canada and CTMC.

81.3

BRONCHOCONSTRICTOR RESPONSE OF SMALL AIRWAYS OF THE FERRET TO METHACHOLINE, HISTAMINE AND SEROTONIN. SA Vitkun, WM Foster, AF Rapisarda, EH Bergofsky, PJ Poppers. Depts. of Anesthesiology and Medicine, SUNY, Stony Brook, New York 11794.

In a specially perfused ferret bronchial tree we investigated the reactivity of the small airways to agonists associated with the asthmatic state: methacholine(M), histamine(H) and serotonin(S). The bronchus of excised lung lobes was cannulated at the center of several lobules. Needle scarifications were made on the pleural surface to allow perfusate to exit, with the main pathway for perfusate flow being through several small bronchi. Lungs were perfused with gassed(95%O_2/5%CO_2)and warmed Krebs-Ringers solution at constant flow; perfusion pressure was measured as a gauge of airway resistance. Concentration dependent increases in resistance to perfusate flow(bronchoconstriction)were observed when M, H or S were added to the perfusate. Responses to all agonists were less when compared to responses in a similar lung preparation of guinea pig(Vitkun, et.al., Fed. Proc., 44:491, 1985). In the ferret, the max H response(n=15)averaged only 22% of the max M response and the max S response(n=13)averaged 50% of the max M response. The amplitude of the max M response of the ferret airways was only 20% of the max response found for guinea pig airways. We concludethat in this in vitro preparation M induces greater bronchoconstriction than H or S, and these agents are less active in the ferret as compared to our previous studies in the guinea pig. (Supported by the VA and NHLBI HL-31429-02, Washington, D.C.)

81.4

CHOLINERGIC STIMULATION OF MUCUS GLYCOPROTEIN SECRETION BY ISOLATED CAT TRACHEAL GLAND CELLS IN PRIMARY CULTURE. David K.P. Lee*, David J. Culp*, David P. Penney*, and Matthew G. Marin. University of Rochester, Rochester, New York 14642.

We studied cholinergic control of mucus glycoprotein secretion by isolated cat tracheal gland cells cultured on gels of polymerized rat-tail collagen. On reaching confluency cultured cells were polygonal in shape and contained granules that stained with periodic acid-Schiff. By electron microscopy the cells contained microvilli at their apical surfaces, tight junctions at their apical lateral borders, lateral interdigitations, granular basement membrane material in the basal extracellular space, rough endoplasmic reticulum, Golgi apparatus, and granules of mixed electron density. To label mucus glycoproteins, we added 35-sulfate to the culture medium for 48 hours. Secretion of glycoprotein was quantitated by measuring radiolabel in the void volumes of Sepharose 6B-CL columns during consecutive 1-hour periods (basal and test). The secretory index (CPM in test/CPM in basal periods) was used to compare secretion rates under control and stimulated conditions. Secretory index under control conditions (n=5) was 1.06 ± 0.06 (mean ± SE). In the presence of 10^{-4}M carbachol (n=5), the secretory index rose to 1.36 ± 0.05. Thus, isolated cat tracheal gland cells in primary culture secrete mucus glycoproteins and retain their responsiveness to cholinergic stimuli. Supported in part by NIH grants HL21314-08, HL07216-09, and HL32949-01.

81.5

EFFECTS OF HIGH OR LOW TAR CIGARETTE SMOKE ON AUGMENTATION OF ELASTASE-INDUCED EMPHYSEMA. **Y.-L. Lai and L. Diamond. University of Kentucky, Lexington, KY 40536**

Whether the low tar cigarette is less apt to induce emphysema than its high tar forerunner was examined in a rat model of cigarette smoke-augmented, porcine pancreatic elastase (PPE)-induced emphysema. Sixty-eight female Long-Evans rats were divided into seven groups: control, PPE, PPE + sham smoke, high tar cigarette (1R1), low tar cigarette (1R4F), PPE + 1R1, and PPE + 1R4F. Three days after intratracheal administration of PPE (400 IU/kg), animals in the smoke treated groups were exposed to 10 puffs of cigarette smoke each day, 7 days/wk. Sham treated animals received air. All exposures were continued for three months, at which time pulmonary function tests were performed under general anesthesia. Smoke exposure alone (1R1 or 1R4F) did not produce any significant (P>0.05) changes in pulmonary function. Both PPE groups demonstrated significant (P<0.05) changes in total lung capacity (↑22%), functional residual capacity (↑67%), lung compliance (↓32%), carbon monoxide (CO) diffusing capacity (↓15%) and CO diffusion coefficient (↓25%). Both 1R1 and 1R4F cigarette smoke significantly (P<0.05) enhanced most of the emphysematous changes produced by PPE, with no significant (P>0.05) differences between their effects. These data indicate that smoking low tar cigarettes does not lessen the degree of cigarette smoke augmentation of PPE-induced emphysema in the rat. (Supported by University of Kentucky Tobacco and Health Res. Inst.).

81.6

The Ultrastructure of the Oxygenated Premature Rabbit Lung. D.L. Forman*, S.R. Hilfer*, T.H.Shaffer, Department of Physiology, Temple University Medical School, Department of Biology, Temple University,Philadelphia PA 19140.

The structural effects of oxygenation were investigated in premature rabbits delivered at 27 days gestational age. Anesthesia was induced with 2mg/kg Ketalar® given IV to the restrained doe. The fetuses were delivered by cesarian section and a tracheostomy was performed under local anesthesia. GR I fetuses were sacrificed immediately as non-breathing controls. GR II animals were mechanically ventilated with oxygenated fluorocarbon (RIMAR 101) for 3hrs while monitoring physiological parameters. After sacrifice, each lung was prepared for transmission electron microscopy (TEM) using standard procedures. Analysis of cell structure from EM micrographs showed no disruption of alveolar membranes. Changes in mitochondrial shape included elongation and an increase in the number of cristae after liquid ventilation (LV) in GR II. Epithelial cells in GR I appeared semi-cuboidal, however, those in GR II were more rectangular and had regular borders. These data suggest that the structural integrity of the prematurely delivered rabbit lung is maintained during LV. Moreover, the morphologic changes due to ventilation may reduce diffusion distances and improve lung expansion. Finally, alterations in mitochondrial shage suggest an increase in cellular aerobic metabolism during LV. Thus, lung maturation after premature delivery may be facilitated by this procedure. Supported by BRSG #RR05417, HL/HD 30525, HL/HD 32131.

81.7

DOES MIXED EXPIRED P_{CO_2} ($P_{E\bar{C}O_2}$) EXCEED ARTERIAL P_{CO_2} (Pa_{CO_2}) DURING CO_2 INHALATION? H.V. Forster, L.G. Pan*, C. Flynn*, and G.E. Bisgard, Dept. of Physiol., Med.Col.Wis., Milwaukee, VA Med.Ctr., Wood, WI, and Sch.Vet.Med., U.Wis., Madison.

There are reports of $P_{E\bar{C}O_2}$ exceeding Pa_{CO_2} when $P_{I\bar{C}O_2}$ is above normal (J.Appl.Physiol. 38:382-388,1975). However, it is controversial whether this difference is physiologic or due to measurement errors (J.Appl.Physiol. 52:1177-1180, 1982). We presently studied 8 awake ponies during 30 min. at $P_{I\bar{C}O_2}$'s of <0.7, 14, 28 and 42 mmHg. Each pony was studied 3 times during nares breathing (N.Br.) and 3 times breathing through a chronic tracheostomy (T.Br.). Physiologic dead space at a $P_{I\bar{C}O_2}$ of <0.7 mmHg was 438 ml and 255 ml during N.Br. and T.Br. respectively. Neither arterial nor rectal temperature changed significantly at any level of $P_{I\bar{C}O_2}$. At a $P_{I\bar{C}O_2}$ of 0.7 mmHg, Pa_{CO_2} exceeded $P_{E\bar{C}O_2}$ by 21.7 and 13.0 during N.Br. and T.Br. respectively. The Pa_{CO_2}-$P_{E\bar{C}O_2}$ differences decreased as $P_{I\bar{C}O_2}$ was increased. During T.Br. at a $P_{I\bar{C}O_2}$of 28 mmHg and during N.Br. at a $P_{I\bar{C}O_2}$ of 42 mmHg, there was no significant difference between Pa_{CO_2} and $P_{E\bar{C}O_2}$ (P>.10). During T.Br. at a $P_{I\bar{C}O_2}$ of 42 mmHg, $P_{E\bar{C}O_2}$ exceeded Pa_{CO_2} by 2 mmHg (P<.01). With the observed tidal volume during the latter condition, Pa_{CO_2} would need to exceed $P_{E\bar{C}O_2}$ by 1 mmHg if V_D was unchanged from control. In other words, if $\dot{V}_D$ is unchanged and the negative P_{CO_2} gradient is artifactual, then there must be a 3 mmHg P_{CO_2} measurement error. Since an error of this magnitude is unlikely, we believe the negative Pa_{CO_2}-$P_{E\bar{C}O_2}$ gradient is physiological. Supported by USPHS 25739 and Med.Res.Serv. of Vet. Adm.

81.8

DIFFUSING CAPACITY OF THE PULMONARY MEMBRANE. R.A. Klocke, Dept of Medicine and Physiology, SUNYAB, Buffalo, NY 14214.

The diffusing capacity of the pulmonary membrane for oxygen (D_m) was measured in 8 isolated perfused lungs from rabbits (body weight 1.13 kg ± .16 SD). Lungs were inflated with air to a pressure of 10 cm H_2O and perfused at 130 cc/min with buffered saline containing 3 gm% albumin. The rate of oxygen uptake following injection of a 1.0 cc bolus containing 50 mM sodium dithionite was obtained from the resulting plethysmographic pressure change. This process is determined by a) the rate of oxygen transfer from the alveoli into the capillaries which contain dithionite and b) the distribution of transit times to the gas-exchanging vessels. The latter was estimated from the rate of plethysmographic pressure change occurring after injection of a 1.0 cc saline bolus saturated with acetylene. D_m was calculated from the volume of oxygen uptake, alveolar oxygen tension and mean capillary transit time (computed from the transit times of the dithionite and acetylene injections). D_m averaged 0.347 (± 0.130) ml/min/mm Hg. D_LCO measured in the same animals prior to removal of the lungs for perfusion was 0.456 (± 0.102) ml/min/mm Hg. Using the data of Powers et al. (J. Appl. Physiol. 31:438, 1971) we corrected D_LCO to 25°C. The relative magnitudes of D_m (0.347) and D_LCO corrected to 25°C (0.200) suggest that physiological measurements of D_m are much less than morphological estimates and the pulmonary membrane offers significant resistance to oxygen transfer (Supported by NHLBI 15194).

81.9

CHARACTERIZATION OF PULMONARY ARTERIAL INPUT IMPEDANCE WITH LUMPED PARAMETER MODELS. B.J.B. Grant and L.J. Paradowski
Dept. of Medicine, SUNYAB, Buffalo, NY 14215.

A variety of electrical networks have been devised to characterize pulmonary arterial input impedance. The purpose of this report is to evaluate the use of these lumped parameter models systematically. Pulmonary arterial input impedance was estimated from the Fourier series of pulmonary arterial pressure and flow in cats that were anesthetized with chloralose. The Marquardt parameter estimation procedure was used to obtain the best fit by least squares criteria to the data points weighted with experimental error for each of the eight lumped parameter models that we tested. From a simple Windkessel model with two elements: a capacitor (C) and a resistor (R1) in parallel, one or more elements were added to this basic design in various combinations. Analysis of variance was used to assess the most appropriate lumped parameter model which was a three element model with a resistor (R2) in series with the Windkessel. To determine the significance of these parameters, this model was fitted to impedance spectra calculated from a distributed parameter model of the feline pulmonary vasculature. The lumped parameter values approximated to the distributed parameter model values for total pulmonary arterial compliance (Ca) and characteristic impedance (Zc). From the experimental data ($n=12$) we estimated that Ca to be 0.24 ± 0.14 SD (kdynes^{-1}.cm^{5}), and Zc to be 0.25 ± 0.17 SD (kdynes.sec.cm^{-5}). (Supported by USPHS RCDA HL01418 and AHA Grant-in-Aid).

81.10

EFFECTS OF ENDOTOXEMIA ON SHEEP PULMONARY HEMODYNAMICS: A COMPARISON OF PULMONARY ARTERIAL (PPA), PULMONARY WEDGE (PW) AND LEFT ATRIAL (PLA) PRESSURES. R. E. Parker. Pul. Circ. Cntr, Vanderbilt Univ., Nashville, TN 37232

E. coli endotoxin (0.5-1.0ug/kg) was infused over 15 minutes (time 0-15 min) into 5 unanesthetized sheep. PW was measured via a deflated 7 French Swan-Ganz catheter. PPA and PLA were measured directly from the main pulmonary artery and left atrium. PPA and PW began to increase at 15 and 20 minutes, respectively; and reach maximal values at 25 and 35 minutes, respectively. PLA began to decrease at 30 minutes and reached a nadir at 45 minutes. The data suggest that the venular percentage of total vascular resistance significantly increases during endotoxemia as evidenced by the (PW-PLA)/(PPA-PLA) values of 0.28 and 0.63 at baseline and 35 minutes, respectively. The table shows the pressures for baseline (0) through 120 minutes (mean ± SE).

	Time (min)					
	0	15	30	45	60	120
PPA (cmH_2O)	21.6 ±1.7	35.8* ± 2.8	65.2* ± 1.9	46.6* ± 1.9	36.6* ± 2.4	36.8* ± 5.1
PW (cmH_2O)	8.0 ±1.5	9.6 ± 1.9	35.2* ± 3.5	24.0* ± 2.6	13.6* ± 1.7	12.0* ± 2.8
PLA (cmH_2O)	2.8 ±1.4	2.4 ± 1.8	-3.2* ± 2.2	-5.4* ± 1.3	-2.4* ± 0.9	-2.8* ± 2.3

Supported by HL 27169 and HL 19153 (SCOR in Pulmonary Vascular Diseases)

81.11

REMOVAL OF AIRWAY EPITHELIUM (EPI) REDUCES THE POTENCY OF VERAPAMIL AGAINST AGONIST-INDUCED CONTRACTIONS OF RABBIT AIRWAY SMOOTH MUSCLE. D. Raeburn*, D.W.P. Hay*, V.A. Robinson*, W.W. Fleming* and J.S. Fedan* (SPON: D.G. Frazer). Physiol. Sect., LIB, NIOSH, and Dept. of Pharmacol. and Toxicol., WVa Univ., Morgantown, WV 26505

Removal of the EPI cell layer from rabbit airways variably affected the reactivity of the the smooth muscle to histamine, methacholine, and K^+. In the secondary bronchus, EPI removal potentiated responses to methacholine and histamine, but not K^+. In the primary bronchus, only responses to methacholine were consistently augmented. In the trachea this potentiation was not observed following EPI removal. Verapamil (1 μM) attenutated responses to these agents, but was less potent in primary bronchus, and substantially less potent in secondary bronchus, following EPI removal. In the case of methacholine (the only agent which contracted each generation of airways studied), contractions of the lower airways were more sensitive to verapamil. These results indicate that 1) the EPI layer modulates airway smooth muscle reactivity; this modulation is widespread in mammals, having now been observed in dogs, guinea pigs and rabbits, and 2) that the modulatory effect increases in the smaller airways. The results also suggest that the relative ineffectiveness of Ca^{2+} entry blockers in asthma may be related to the EPI damage/denudation which had been reported to accompany this disease.

81.12

HIGH FREQUENCY CHEST WALL OSCILLATION (HFCWO) PHYSIOTHERAPY IN ASYMPTOMATIC SMOKERS. M. King, M. Dolovich*, C. Chambers*, J.F. Chrome*, D. Gross, and M. Newhouse. Meakins-Christie Laboratories, McGill Univ., Mtl., Que. and St. Joseph's Hospital, Hamilton, Ont.

HFCWO, which involves oscillating the pressure in a thoracic cuff at high frequency, is a proposed method of physiotherapy that enhances particle clearance in dogs (J. Appl. Physiol. 58: 1157, 1985). We tested the effect of HFCWO in six smokers (age 38 yrs, pack yrs 26, FEV_1 109% pred., 1/5 prod. a.m. cough) and one non-smoker. The subjects inhaled a 99m-Tc sulfur colloid aerosol (Devilbiss 646 nebulizer) for 2 min., while seated and breathing quietly. One-min. gamma camera images of the right lung were then taken every 15 min. for 1 hour. HFCWO at 13 Hz and peak cuff pressure 50 cmH_2O was then applied for four 5-min. intervals over 45 min. Images were taken between HFCWO periods and for a further one hour. On a separate day, each subject repeated the protocol, including wearing the inflated cuff, but without oscillation. Total and regional (upper/lower, inner/outer) lung retention ratios were determined; the clearance index CI was defined as the change in retention between 30 and 135 min (pre- and post HFCWO). Five subjects (4 smokers, 1 non-smoker) showed a higher total lung CI on the HFCWO day while CI was lower for the other two (CI 5.58% vs. 4.33%, N.S.). There was no consistent pattern in regional lung clearance. Most of the variation in CI was due to differences in inner/outer deposition ratio, with a small positive component due to HFCWO. Physiotherapy by HFCWO in asymptomatic subjects may enhance mucus clearance, but the effect is small in comparison with natural mechanisms. (Supported by Can. C.F. Foundation).

MECHANICS OF BREATHING: VENTILATORY MUSCLE FUNCTION

82.1

NATURAL FREQUENCY OF THE PULMONARY SYSTEM WHEN GAS DENSITY IS HIGH. Hugh D. Van Liew, Physiology Dept., Univ. at Buffalo, SUNY, Buffalo, NY. 14214

Could high gas density cause bizarre breathing during hyperbaric exposures because the respiratory system reaches its natural frequency (resonant frequency)? Data in the literature for normal density show that the respiratory system is analogous to a surprisingly simple electrical circuit -- a capacitance, a resistance, and an inductance in series; pulmonary analogs are compliance, flow resistance, and inertance. At the natural frequency, pressures for inertance and compliance cancel each other so that pressure to cause flow is the only energy-requiring entity. Calculations show that the natural frequency of the human respiratory system moves from the normal 6 to 1.7 and 1.2 cycles/sec when density is 10 and 20 times normal. Thus large increases in density do not decrease the natural frequency enough to be in the range of frequencies expected even in a strenuously-exercising diver; furthermore, the inertance effects are insignificant compared to flow-resistance increases. Inertia may have second-order effects, but it appears that the main troublesome aspect of dense-gas environments is flow resistance.

82.2

EXPONENTIAL ANALYSIS OF QUASI STATIC PV CURVES IN BEAGLES EXPOSED TO CIGARETTE SMOKE. G. DeSanctis*, S.M. Kelly*, M. Saetta*, J.L. Stril*, R.J. Shiner*, M. Cosio*, and M. King. Meakins-Christie Labs., McGill University, Montreal, Canada.

Ten tracheostomized beagles were exposed to cigarette smoke for up to ten months (10 cigarettes a day over 2.5 hr., 5 days a week). A 35cc bolus of smoke was generated from unfiltered 70 mm cigarettes (20 mg tar, 1.2. mg nicotine), delivered to the inspiratory line each 20 sec. Quasi-static PV curves were obtained from the smokers and from sham-smoking controls at 2, 6 and 10 months of exposure. The dogs were anesthetized and placed in a volume displacement, body plethysmograph in the prone position. Transpulmonary pressure was measured by the standard esophageal balloon technique. Each dog was inflated from FRC to 30 cmH_2O and deflated down to RV at constant flow rates. Of 10 smoking dogs PV data were obtained for 8 after two months, 6 after six months, and 6 after ten months of smoking. Similar data were obtained on 4 sham-exposed controls. The PV data between TLC and FRC were fitted to a exponential function of the form $V = A - Bexp(-kP)$, using an r^2 optimization procedure. In smoking dogs, the B/A (%) ratios (an index of hyperinflation) were 98, 92, and 103 at 2, 6 and 10 months respectively and did not differ significantly from the non-smoking dogs whose respective values were 94, 95, and 95. The respective k values (indicating distensibility) for smokers were 154, 148, and 150 cm^{-1} and did not differ significantly from those of the non-smokers (138, 150, and 145 cm^{-1}). We therefore conclude that there was no significant change in elastic recoil pressure after ten months of cigarette smoke exposure. (Supported by CTMC)

82.3

PLEURAL PRESSURE AND OTHER RESPIRATORY PARAMETERS IN AWAKE DOGS DURING EXERCISE. A.R. Jayaweera* and W. Ehrlich. The Johns Hopkins University Med. Inst. Baltimore, MD 21205.

Pleural pressure (P_{pl}) and respiratory flow values during exercise were compared with resting values in 87 experiments with 9 awake dogs. P_{pl} was monitored with a fluid-filled catheter and also with a "Konigsberg" electronic transducer in a shielded locus of the pleural space. Respiratory flow was monitored with a pneumotachograph. Light treadmill exercise (1.5 mph at 9° inclination) increased cardiac output by 38%, minute volume by 94% and oxygen consumption by 77% over resting values. During this exercise, mean P_{pl} fell by 2.5 cmH_2O (from 12.1 to 14.6 cmH_2O) even though P_{pl} at FRC and P_{pl} at end inspiration remained unchanged. Peak expiratory P_{pl} was elevated by 4 cmH_2O. However, lowest inspiratory P_{pl} was decreased by 4.6 cmH_2O and the relation of inspiratory time to total respiratory cycle time was elevated by 0.109. The pleural pressure time index, an indicator of inspiratory muscle work was increased by 85% over resting value. The derived values for inspiratory pulmonary resistance, dynamic compliance and breathing effectiveness during exercise were not different from the resting values. The finding that mean P_{pl} during exercise is lower than the resting value is related to the increase in the respiratory flow and is important for the distending pressures in the circulatory compartments in the chest.

Supported by USAMRDC contract DAMD 17-83-C-3182.

82.4

EFFECT OF ALTERATIONS IN RIBCAGE/ABDOMINAL CONTRIBUTIONS TO TIDAL VOLUME ON ACCURACY OF RESPIRATORY INDUCTIVE PLETHYSMOGRAPHY. Glenn Bowes, Catherine Owen* and Margaret M. Smith* Monash Medical School, Alfred Hospital, Prahran, Victoria, 3181 Australia.

The influence of alterations in ribcage and abdominal motion on respiratory inductive plethysmograph (RIP) accuracy has not been extensively studied. We investigated the effect of changing ribcage and abdominal contributions on RIP accuracy in awake normal subjects. RIP was calibrated against volume measured at the mouth by a rolling seal spirometer (SPIR). Twenty breaths were recorded in each of two body positions, standing and supine, and calibration factors determined by the least squares method. Seven subjects with normal lung function were studied. Following calibration, accuracy was assessed in the supine posture under 3 conditions: (1) normal breathing (NB), (2) ribcage breathing (RCB), (3) abdominal breathing (AB). RCB and AB were produced using an elastic binder placed around the abdomen and chest respectively. Twenty breaths of tidal volume (Vt) were recorded for each mode of breathing. Group means for percent ribcage contribution to Vt were 54%, NB; 84%, RCB; and 21%, AB. Accuracy of RIP Vt with respect to SPIR Vt was judged by; (1) linear regression coefficient, (2) mean of absolute breath-by-breath differences. In all subjects, RCB and AB altered the linear regression coefficient, however the direction of change varied. Hence, we chose to look at the magnitude of deviation of each linear regression coefficient from unity; group means (± SEM) were 0.05 ± 0.02 for NB, 0.12 ± 0.029* for RCB and 0.16 ± 0.028* for AB (*,P 0.05). Mean absolute breath-by breath differences between RIP Vt and SPIR Vt were 29 ± 6 ml for NB, 114 ± 26ml* for RCB and 83 ± 14 ml* for AB. We conclude that variations in ribcage and abdominal contributions to Vt can produce marked changes in RIP accuracy.

82.5

FUNCTIONAL COUPLING BETWEEN PARASTERNAL INTERCOSTALS (PS) AND TRIANGULARIS STERNI (TS) DURING BREATHING IN DOG. Vincent Ninane*, Marc Decramer* and André De Troyer. Respiratory Research Unit and Chest Service, Erasme Univ. Hospital, 1070 Brussels, Belgium.

Using sonomicrometry, we have measured the length changes of the PS and TS muscles in ten supine anesthetized dogs breathing quietly. The length changes were defined relative to the muscle length during mechanically induced apnea (Lr). Inspiration was always associated with an active shortening (below Lr) of the PS and passive lengthening (above Lr) of the TS. These changes progressed as inspiration proceeded, and at end-inspiration the PS shortening and TS lengthening in the 10 animals averaged 4.03 and 7.51 % Lr, respectively. At the onset of expiration, both muscles first rapidly came back to Lr, and then the TS always showed an active shortening that caused most PS to passively lengthen. For the 10 animals, the TS active shortening and PS passive lengthening during the expiratory pause averaged 7.17 and 0.71 % Lr, respectively. This pattern was essentially unchanged after bilateral phrenicotomy. We conclude that: 1) Expiration in the dog is an active process; 2) The persistent contraction of the TS during expiration often causes passive lengthening of the PS, while contraction of the PS during inspiration causes passive lengthening of the TS; and 3) These two muscle groups thus can be considered as acting synergically on the rib cage during breathing. (Supported by FRSM Belgium and Erasme Foundation).

82.6

DIAPHRAGMATIC RELAXATION IN THE DOG: A COMPARISON OF IN SITU AND IN VITRO RATES. G. Griggs,* G.A. Farkas,* and D.F. Rochester. Pulmonary Division, Dept. of Internal Medicine, University of Virginia, Charlottesville, Virginia 22908.

Prolongation of relaxation rate is characteristic of diaphragmatic fatigue. We wished to determine if the relaxation rate of transdiaphragmatic pressure (Pdi) in situ is the same as relaxation of muscle force in vitro, and also to compare the exponential relaxation time constant (Tr) with half relaxation time (1/2 RT). We studied 7 anesthetized mongrel dogs, measuring Pdi at supine FRC, with 1, 20 and 70 Hz stimulation of both phrenic nerves. Subsequently, costal diaphragm bundles were removed, mounted to a force transducer in a 37° C bath, and stimulated directly at the same frequencies. Results (msec, mean ± SD) are tabulated;† denotes difference ($p<.01$) between tetanic vs twitch stimulation,* denotes difference ($p<.05$) between in vitro and in situ data.

	1/2 RT,1 Hz	Tr,1 Hz	20 Hz	70 Hz
In vitro	68±8	66±10	53±10†	51±8†
In situ	67±4	54± 9	36± 9†*	35±8†*

We conclude that relaxation rate after a twitch is similar by both methods. The decrement of Tr after tetanic stimulation in vitro may result from greater recruitment of fast fibers. The larger decrement in Tr after tetanic stimulation in situ suggests that relaxation of Pdi is affected by muscle shortening and/or outward recoil of the rib cage. (Supported by NIH HL 21500, P.B. Francis Foundation and ALA of Virginia).

82.7

IN SITU OPERATING LENGTHS OF CANINE NECK INSPIRATORY MUSCLES. D.F. Rochester and G.A. Farkas*. Pulmonary Division, University of Virginia, Charlottesville, Virginia 22908.

The scalenes (SCA) and sternomastoids (SM) are recruited to support breathing under numerous conditions. Evidence suggests that the SCA has a greater effect on the rib cage than the SM. We hypothesize that the SCA's relative advantage derives from a more advantageous force-length relationship. Therefore, we measured the in situ operating lengths of SCA and SM sonomicrometrically in 14 bundles of each from 7 anesthetized dogs. Resting lengths were measured at supine FRC and TLC (+30 cm H_2O airway pressure). The muscles were then removed with crystals in place, mounted to a force transducer in a 37° C. bath, and adjusted to optimal length (Lo). In vitro length-tension characteristics were virtually identical for both muscles. In contrast, in situ lengths (%Lo, mean ± SEM) of the SM were shorter ($P<.01$) at both lung volumes. (table).

	FRC	TLC
SCA	86.0 ± 1.5	80.5 ± 1.9
SM	72.9 ± 1.9	70.8 ± 2.2

At TLC the SCA was shorter than at FRC ($P<.05$) but SM was not. We conclude that although in vitro contractile properties are similar in situ, the SM operates at greater mechanical disadvantage than the SCA. However, force generating capacity of both neck inspiratory muscles is relatively unaffected by increasing volume from FRC to TLC. (Supported by NIH HL 21500, Parker B. Francis Foundation, and ALA of Virginia).

82.8

EFFECTS OF LUNG VOLUME AND STIMULATION RATE ON IN SITU CANINE DIAPHRAGM LENGTH. G.A. Farkas*, and D.F. Rochester, Pulmonary Division, Department of Internal Medicine, University of Virginia, Charlottesville, Virginia 22908.

The in situ performance of the diaphragm (DPM) is determined by its force-length and force-frequency charcteristics. The purpose of this study is to indicate how these two relationships interact in the dog DPM at various lung volumes. We studied 6 anesthetized mongrel dogs, measuring DPM length (DL) sonomicrometrically at 3 lung volumes ranging from RV (-20 cm H_2O) to TLC (+30 cm H_2O). At each lung volume, DL was measured at rest and during stimulation of both phrenic nerves at 5, 20 and 100 Hz with the airway occluded. Subsequently, costal DPM bundles containing the sonomicrometric crystals were removed, mounted to force transducer in a 37° C bath, and adjusted to optimal length (DLo). DLo in vitro was compared to the DL obtained in situ. Results (mean ± SEM) are tabulated.

	Length (% in vitro DLo)			
	Rest	5 Hz	20 Hz	100 Hz
RV	100±4	97±4	84±3	63±7
FRC	93±3	86±3	69±2	53±3
TLC	61±4	54±4	51±3	41±4

We conclude that in situ force-frequency response must differ from in vitro response because of a marked shortening consequent to tetanic stimulation at all lung volumes. That is, the effect of high frequency stimulation on Pdi would be counteracted by marked DPM shortening. (Supported by NIH HL 21500, P.B. Francis Foundation and ALA of Virginia.)

82.9

IMPEDANCE AND RELATIVE MOTIONS OF THE CHEST WALL OF HUMANS DURING SINUSOIDAL FORCING AT 0.2 TO 4 Hz. G.M. Barnas*, K. Yoshino*, K.B. Kelly*, S.H. Loring, and J. Mead. Dept. Environ. Sci. & Physiol., Harvard School of Public Health, Boston, MA 02115

We measured the effective chest wall resistance (R) and elastance (E) of relaxed human subjects at FRC during sinusoidal volume changes (250 to 300 ml at 0.2 to 4 Hz) delivered at the mouth. Subjects sat in a "head-out" body plethysmograph, and transthoracic pressure was measured with an esophageal balloon. In all 4 subjects, R ($cmH_2O \cdot l^{-1} \cdot s$) was highest (2.5-6.0) at the lowest frequency and fell sharply to a minimum (0.5-0.8) at 1 to 4 Hz. E ($cmH_2O \cdot l^{-1}$) increased from 10 at the lowest frequency to about 15 at 1 Hz, reached a plateau between 1 to 3 Hz and decreased above 3 Hz. In the same subjects, we measured the relative magnitude and phase between the motions of different parts of the chest wall with magnetometers (including antero-posterior and transverse diameters of the rib cage and abdomen) during similar sinusoidal forcing. These measurements indicate that the chest wall expands and deflates uniformly at frequencies up to 1 Hz. Thereafter the relative magnitude and phase of motion between different points of the chest wall show complex changes. We conclude that R and E measured in relaxed human subjects during sinusoidal forcing is extremely frequency dependent at 0.2 to 4 Hz. However, at 0.2 to 1 Hz this frequency dependence is not due to non-uniformities in displacement of different parts of the chest wall. (Supported by NIH grants HL19170, HL07118, HL33009, and HL00943.

82.10

REGIONAL ABDOMINAL PRESSURE IN DOGS. K. Yoshino*, G.M. Barnas*, W.R. Kimball*, and S.H. Loring, Dept. of Environ. Sci. & Physiol., Harvard School of Public Health, Boston, MA 02115

We measured abdominal pressure (Pab) at 1 cm intervals along the inner abdominal wall of dogs in different postures (supine, lateral, prone and upright) during quiet spontaneous breathing, mechanical ventilation and apnea (with or without muscle paralysis with d-tubocurarine). Pab was measured with a saline-filled catheter drawn in 1 cm steps through the peritoneal cavity, roughly parallel to the mid-sagittal plane. Gastric pressure (Pga) also was measured with an air-filled balloon catheter. Both Pab at a given position in the abdomen and Pga varied with posture. Fluctuations in Pab with mechanical ventilation were generally constant or decreased only slightly from upper to lower abdomen. During spontaneous breathing, differences in fluctuations from upper to lower adbomen were variable and depended on expiratory muscle activity. Pab in the upright posture, especially during apnea, was closely related to height within the abdomen. The effective specific gravity of the abdominal contents, calculated from changes in Pab with height, was between 0.8 and 1.0. In one dog, specific gravity of abdominal contents (excluding the liver) measured in vitro was 0.97. We conclude that Pab under static conditions has a nearly hydrostatic gradient. However, Pab under dynamic conditions depends on the location in the abdominal cavity and on respiratory muscle activity. (Supported by NIH grants HL07118, HL19170, HL00943 and HL33009).

82.11

EFFECTS OF SWIM TRAINING ON LUNG VOLUMES AND INSPIRATORY MUSCLE FUNCTION IN VARSITY FEMALE SWIMMERS. T.L. Clanton, G. Dixon,* J. Drake.* The Ohio State Univ. Pulmonary Division, Columbus, OH 43210.

Lung volumes and inspiratory muscle (IM) function tests were performed on 16 competitive female swimmers (age = 19 ± 1 yrs) before and after 12 weeks of intense swim training. Eight swimmers underwent additional IM training; the remaining eight served as controls. Significant increases in vital capacity (VC) occurred in both groups which could be attributed entirely to a rise in expiratory reserve volume (ERV = + 0.30L ± 0.36, $p<0.01$). Maximum inspiratory mouth pressure (PImax) at functional residual capacity (FRC) changed -43 + 18 cm H_2O ($p<0.005$) in swimmers undergoing IM training and -29 ± 25 ($p<0.05$) in controls. The time that 65% of prestudy PImax ($PImax_i$) could be endured, with controlled breathing patterns, increased significantly in IM trainers ($p<.001$) and controls ($p<0.05$). All results were compared to a similar IM training study on normal females (age = 21.1 ± 0.8 yrs) which demonstrated significant increases in PImax and endurance in IM trainers only, but no changes in VC or ERV (Clanton, et al., Chest, 87:62-66, 1985). We conclude that a) intense swim training in mature females results in increased VC by increasing ERV, b) swim training increases IM strength and endurance measured near FRC and c) the increases in VC do not appear to be a direct result of increases in IM strength near FRC. Supported in part by the Bremer Foundation and the Ohio State University Small Research Grants Program.

82.12

QUANTITATIVE ERRORS IN STUDIES OF MECHANICS OF FETAL LUNG AERATION AT ROOM TEMPERATURE. M.T. Antonio-Santiago*, R. Mathew*, B.C. Clutario* and E.M. Scarpelli. Pulmonary Div., Res. Ctr. Schneider Children's Hospital-LIJMC, New Hyde Park, N.Y. 11042.

Mechanics of the first aeration are studied conventionally in excised lungs at room temperature. We examined *mature* rabbit fetal lungs, with pulmonary fluid intact and chest opened, by stereomicroscopy during the first inflation and deflation with air. Pairs of fetuses from the same litter, one at 22 °C and one at 37 °C, were studied simultaneously (n=5 pairs). Pressure was changed in 5 cm H_2O steps, 2 min at each step. Analysis of stereomicroscopic anatomy and volume-pressure diagrams revealed the following significant differences ($p<.05$): Compared to lungs at 22 °C, saccular recruitment began ("opening pressure") at lower pressure (15 vs 20 cm H_2O), proceeded at a faster rate (63.8±3 vs 17.5±2 $ml \cdot kg^{-1} \cdot min^{-1}$) and achieved larger volumes (86.3±8 vs 56.4±6 $ml \cdot kg^{-1}$) at lower maximal pressure (25 vs 30 cm H_2O) at 37 °C. At end-deflation, zero distending pressure, warm lungs retained more air (60.5±7 vs 25.7±8 $ml \cdot kg^{-1}$) than did lungs at room temperature. Air retention, an index of lung stability, was the result of intrapulmonary bubble formation in both groups, a phenomenon that characterizes the mature lung both in vitro and in vivo. We conclude that (1) studies at room temperature misrepresent, quantitatively, lung mechanics at 37 °C; (2) distensibility, bubble production and stability are significantly greater at 37 °C; and (3) the first aeration is not as difficult a mechanical process as was once believed.

SPACELAB 3 BIOSCIENCE MISSION RESULTS

83.1

RODENT BODY, ORGAN, AND MUSCLE WEIGHT RESPONSES TO SEVEN DAYS OF MICROGRAVITY. R. Grindeland, T. Fast, M. Ruder, M. Vasques, P. Lundgren, S. Scibetta, J. Tremor, P. Buckendahl, L. Keil, O. Chee, T. Reilly, B. Dalton, and P. Callahan. NASA/Ames Research Center, Moffett Field, CA 94035.

Nineteen male rats flown on the 7 day Space Laboratory 3 mission were sacrificed 11-17 hrs after landing. At recovery the body weights of 7 large rats were 396 ± 6 g (large flight; LF), and 407 ± 7 g (large control; LC) ($p>0.05$); for 12 small rats the weights were 248 ± 3 g (small flight; SF) and 252 ± 5 g (small control; SC) ($p>0.05$). Spleens (wet weight) of LF rats were 26% smaller than those of LC rats ($p<0.001$). The brains (-3%), spleens (-20%), thymus glands (-16%), and testes (-8%) were smaller for SF than for SC animals ($p<0.05$). Anterior pituitary, heart, liver, kidney, and ventral prostate weights of LF or SF rats did not differ from their controls ($p>0.05$). Significantly, adrenal gland weights of LF and SF rats did not differ from their 1G controls ($p>0.05$). LF and SF rats had smaller muscles; compared to LC the soleus (Sol; -24%), gastrocnemius (Gastroc; -14%), plantaris (Plant; -11%), tibialis anterior (TA; -9%) and extensor digitorum longus (EDL; -10%) of LF were smaller ($p<0.05$), whereas the adductor longus (AL) was not. For SF rats the Sol (-36%), Gastroc (-21%), Plant (-22%), TA (-11%), EDL (-16%), and AL (-26%) were smaller than those of SC rats ($p<0.05$). Moreover, for LF rats all 6 muscles, and for SF rats the Sol and AL, were smaller than for rats sacrificed 20 hrs before launch. Carcass composition was similar for SF and SC rats ($p>0.05$).

83.2

RAT MAINTENANCE IN THE RESEARCH ANIMAL HOLDING FACILITY DURING THE FLIGHT OF SPACELAB 3. T. Fast, R. Grindeland, M. Ruder, M. Vasques, P. Lundgren, S. Scibetta, J. Tremor, P. Buckendahl, L. Keil, O. Chee, T. Reilly, B. Dalton, and P. Callahan. NASA/Ames Research Center, Moffett Field, CA 94035.

To test the husbandry capabilities of the Research Animal Holding Facility (RAHF) during space flight, 24 male rats were flown on Space Lab 3 for 7 days. Twelve large rats(400 g, LF), 5 of which had telemetry devices implanted (IF), and 12 small rats (200 g, SF) were housed in the RAHF. Examination 3 hr after landing (R+3) revealed the rats to be free of injury, well-nourished, and stained with urine. When examined at R+10 the rats were lethargic and atonic with hyperemia of the extremities; they were well groomed except for a middorsal area stained with urine and food. Both LF and SF rats showed weight gains comparable to their 1G controls ($p>0.05$). However, IF rats grew less than controls (8 ± 1 vs. 24 ± 5 g; $p<0.05$). Daily food and water consumption was essentially constant throughout flight and similar for flight and control groups (30 g/day, 35 ml/day). Plasma concentrations of total protein, sodium, albumin and creatinine did not differ between flight and control groups($p>0.05$). LF and SF rats had elevated plasma glucose, and SF rats had increased blood urea nitrogen, glutamic pyruvic transaminase and potassium ($p<0.05$). These observations indicate that rats maintained in the RAHF were healthy, well-nourished, and experienced minimal stress; physiological changes in the rats can thus be attributed to the effects of space flight.

83.3

CENSUS OF OSTEOBLAST PRECURSOR CELLS IN PERIODONTAL LIGAMENT (PDL) OF SPACELAB-3 RATS. W.E. Roberts, P.J. Fielder, L.M.L. Rosenoer, A.C. Maese and M.R. Gonsalves. Bone Research Laboratory, Univ. of Pacific, School of Dentistry, San Francisco, CA 94115 and NASA Ames Research Center, Moffett Field, CA 94035.

Right mandible, ulna/radius, both sides of the maxilla, last two thoracic and first two lumbar vertebrae were recovered postmortem from six small (about 200 gm at launch) and five large (about 350 gm at launch) rats flown aboard Spacelab-3. There were two control groups of the same size. The large rats were perfused in situ while bones and teeth of the small animals were fixed by immersion in neutral buffered formalin. Maxillary halves were demineralized in 10% EDTA containing 2% formalin for two weeks. Specimens were divided with a razor blade along the midsagittal plane of the mesial root of the first molar and the medial surface was embedded in plastic flat against the bottom of a glass vial. First molars and surrounding periodontium were serially sectioned at 3 μm with a heavy sledge-type microtome, stained with hematoxylin and eosin/phloxine. Nuclear length and width of 100 fibroblastlike cells in the midroot area of the PDL are measured with an ocular micrometer at 1000X under oil immersion. Nuclear volume (V) of each cell is calculated according to $V=4/3\pi ab^2$, where **a** is the major and **b** the minor radii. Cell types are classified according to nuclear size as: **A/A'** (40-79), **B** (80-119), **C** (120-169) and **D** ($\geq$170 μm^3). Respectively, these cells are less differentiated precursors/committed osteoprogenitors, nonosteogenic cells, θ_1 stage preosteoblasts and θ_2 stage preosteoblasts. Analysis of SL-3 specimens is in progress to determine the relative influence of weightlessness on cell census of an osteogenic tissue. Supported by NASA Ames Cooperative Agreement NCC 2-224.

83.4

ELECTRON MICROPROBE ANALYSES OF Ca, S, Mg, and P DISTRIBUTION IN INCISORS OF SPACELAB-3 RATS. Gary D. Rosenberg* and David J. Simmons+. * Dept. of Geology, Indiana/Purdue Univ., Indianapolis, IN 46202; + Orthopedic Surgery, Medical School, Washington Univ., St. Louis, MO 63110

The distribution of Ca, S, Mg, and P was mapped within the incisors of Spacelab-3 rats using an electron microprobe. Preliminary evaluation of the data suggests that flight rats maintained in orbit for 6 days have significantly higher Ca/Mg ratios in dentin due to both higher Ca and lower Mg content than in dentin of ground-based controls. There is also evidence that mean concentrations of P and S and/or their distributions across the enamel and dentin are more variable in flight animals. These results are consistent with those obtained on a previous NASA/COSMOS flight of 18.5 days duration, although they are not as pronounced. The results further suggest that continuously growing rat incisors provide useful records of the effects of weightlessness on Ca metabolism.

83.5

RESPONSES OF AMINO ACIDS IN HINDLIMB MUSCLES TO RECOVERY FROM HYPOGRAVITY AND UNLOADING BY TAIL-CAST SUSPENSION. Marc E. Tischler, Erik J. Henriksen*, Stephan Jacob* and Paul H. Cook*. University of Arizona, Tucson, AZ 85724

Amino acids were assayed in muscles from rats exposed to 7 days of hypogravity and 12 hr of gravity (F) or 6 days of suspension with (R) or without (H) 12 hr of loading. In these groups, lower asp + asn concentrations were common only to the soleus (SOL) relative to control muscles, the smallest difference being in the R group. This difference in asp + asn for F and H, but not for R, correlated with lower malate suggesting diminution of citric acid cycle intermediates. The R SOL value was increased over the H SOL. Thus, despite 12 hr of loading, the F SOL was more comparable to the H SOL. Other data concerning muscle protein balance (Henriksen etal, this meeting) support this contention. The role of stress in preventing recovery of the F SOL was apparent from the ratios of gln/glu. Synthesis of gln can be enhanced by glucocorticoids and is reflected by an increase in this ratio. In 4 of the 5 F muscles studied, this ratio was 29-79% greater than in controls. In contrast, the ratio in all R muscles was similar to controls and showed recovery from the values in H muscles. Hence, the post-flight treatment of F rats may have produced additional stress. Despite this stress, in some respects the SOL responses to hypogravity were similar to its responses to unloading by suspension. (Support: NASA grant NAGW-227 and an Estbl. Invest. from the Amer. Heart Assoc. to MET.)

83.6

MUSCLE PROTEIN AND GLYCOGEN RESPONSES TO RECOVERY FROM HYPOGRAVITY AND UNLOADING BY TAIL-CAST SUSPENSION. Erik J. Henriksen*, Marc E. Tischler, Stephan Jacob* and Paul H. Cook*. Univ. of Arizona, Tucson, AZ 85724

Muscle mass, protein, glycogen and tyrosine were studied in hindlimb muscles from rats exposed to 7 days of hypogravity and 12 hr of gravity (F) or 6 days of suspension with (R) or without (H) 12 hr of loading. Ground control and F rats grew similarly, while H rats grew slower than R or control rats. In F, H and R animals the soleus (SOL) atrophied, while the gastrocnemius, plantaris and extensor digitorum longus showed only reduced growth. The tibialis anterior showed little response. Changes in mass and protein content correlated in these muscles. Muscles from the F animals showed dramatic increases in glycogen, the SOL being most responsive. H rats showed a greater glycogen concentration in the SOL only, with an even greater concentration in R SOL. Changes in glycogen in F SOL appears due specifically to recovery from unloading and additionally to hypogravity or the post-flight treatment. Only in F SOL was tyrosine greater than the control, suggesting a more negative muscle protein balance, as substantiated in previous studies using H rats. In this study, recovery from suspension decreased SOL tyrosine. These results suggest that the additional stress placed on the F rats post-flight may have prevented the SOL from showing evidence of recovery from hypogravity. (Supported by NASA grant NAGW-227 and an Established Investigatorship from the Amer. Heart Assoc. to MET).

83.7

HEMATOLOGIC PARAMETERS OF ASTRORATS FLOWN ON SL-3. Robert D. Lange, Richard B. Andrews, Linda Gibson, Paul Wright, C.D.R. Dunn, and J.B. Jones. (Spon: C. Schatte). University of Tennessee Memorial Research Center, Knoxville, TN 37920.

Previous studies have shown that a decrease in red cell mass occurs in astronauts as well as, in some studies, a leukocytosis. Similar changes may occur in laboratory animals exposed to microgravity. A life science module with small and large rats as well as monkeys was flown on SL-3. After landing at Edwards Air Force Base the animals were flown to Kennedy Space Center for sacrifice. A group of control animals were sacrificed two days later. Results on the flight (F) and control (C) 200 gm. rats are presented. Complete blood counts, bone marrow and spleen cell differential counts as well as erythroid colony unit-forming ability of the bone marrow and spleen cells were evaluated by standard methods. Statistically significant differences ($p < 0.001$) were found as follows:

	F	C
1. Hematocrit (%)	43.60±1.30	40.70±1.50
2. RBC ($x10^{12}$ l)	6.46±0.40	5.85±0.28
3. Hemoglobin (g/dl)	14.76±0.59	13.47±0.46
4. WBC differential (%)		
a) lymphocytes	77.8±8.1	89.8±4.8
b) neutrophils	11.5±4.6	8.2±4.1

Flight animals demonstrated significantly more erythroid colonies in 11 of 14 erythropoietin treatment groups. The results indicate the need for further studies utilizing isotopes to determine the red cell mass and plasma volume.

83.8

SPACE LAB 3: HISTOMORPHOMETRIC ANALYSIS OF THE RAT SKELETON. T.J. Wronski*, E.R. Morey-Holton*, A.C. Maese* and C.C. Walsh* (SPON: D.D. Buss). University of Florida, Gainesville, FL 32610 and NASA-Ames Research Center, Moffett Field, CA 94035

To study the physiologic effects of space flight, male Sprague Dawley rats weighing an average of 385g were placed in orbit for 7 days aboard the space shuttle. Rats housed under similar environmental conditions in a ground-based vivarium served as controls. Calcein labeling allowed histomorphometric analysis of bone formation during preflight and flight periods. Trabecular bone mass in the proximal humerus and lumbar vertebra was not altered by weightlessness. Flight rats exhibited a nonsignificant decline (15-20%) in the rate of longitudinal bone growth at these skeletal sites. The rate of periosteal bone formation (PBFR) in the tibial diaphysis appeared to be diminished during the flight period in flight rats relative to ground-based control rats (13.2 ± 2.4 (SD) $\times 10^{-3}$ mm^2/day, N=4 vs. $19.9 \pm 6.6 \times 10^{-3}$ mm^2/day, N=5), but this difference was not statistically significant. However, if data for PBFR during the flight period are expressed as a percentage of PBFR during the preflight period, the mean value for flight rats ($44.0 \pm 8.0\%$) is significantly different ($P<0.05$) from that of control rats ($66.1 \pm 16.0\%$). These preliminary findings suggest that 7 days of weightlessness may be of sufficient duration to inhibit bone formation. Additional studies with a greater sample are needed before more definitive conclusions can be reached.

83.9

MICROGRAVITY ASSOCIATED CHANGES IN PITUITARY GROWTH HORMONE (GH) CELLS PREPARED FROM RATS FLOWN ON SPACE LAB 3: PRELIMINARY RESULTS. W. C. Hymer, R. Grindeland+, M. Farrington, T. Fast+, C. Hayes K. Motter and L. Patil. Penn State University, University Park, PA 16802 and +Ames Res. Ctr., Moffett Field, CA.

To determine if changes in GH cells occurred in glands of flight animals, structural, secretory and implant studies were done. Preliminary results. First. Distribution analysis by flow cytometric immunofluorescence showed changes in GH cell numbers. An average of 202,000 cells were "counted" in each of 4 treatment groups; small rat flight (SF), small control (SC), large rat flight (LF) and large control (LC). Percentages of somatotrophs in glands of SF and SC were 44.7 and 42.5 respectively; in LF and LC these were 43.7 and 36.7. Increased frequency of GH cells in LF was accompanied by a 5% decrease in PRL cells. Second, the concentration of bioactive GH (ng GH/1000) somatotrophs was: (SF), 1.91;(SC) 0.53;(LF) 0.48;(LC) 0.95. Third, total ng bioactive GH released from 250,000 cells into culture media over 6 days was 221 (SF), 240 (SC), 89 (LF) and 290 (LC). Encapsulation of cells into XM-50 hollow fibers followed by intracranial implantation into hypox rats resulted in recipient growth of the same order from SF and LF relative to their corresponding controls. Fourth, six GH variants (Western blot) appear only marginally affected by flight. Tentative conclusions: flight affects somatotroph number, GH content/ cell, and GH release in vitro. Since results from the implant study showed no differences in growth parameters, these "defects" maybe reversible. Supported by NASA A21991C (VAB).

83.10

HOMEOSTASIS AND BIOLOGICAL RHYTHMS OF THE RAT DURING SPACEFLIGHT. Charles A. Fuller and Dale M. Edgar. Univ. of California, Davis, CA 95616.

During Spacelab-3, body temperature (T_B) and heart rate (HR) were monitored from 4 Sprague-Dawley rats implanted with telemetry transmitters. These data were compared with preflight control information from the same animals. Ambient temperature was maintained at 24°C and the light-dark cycle consisted of 12 hrs of light and 12 hrs of darkness. Preliminary analysis indicated considerable variability in individual responses. Diurnal rhythms persisted in both T_B and HR with no apparent change in phase. However, T_B showed prominent ultradian components, which became more prominent as the flight progressed, obscuring the 24-hr rhythm in one animal. Further, the HR rhythm was less prominent in microgravity in all animals. T_B rhythm amplitude decreased in three of the animals, while the 24-hr mean decreased in only one. The HR mean decreased in three of the animals. Thus, there appears to be some readjustment of the internal regulation of both these variables as a result of spaceflight. Further analysis is being performed on the activity, feeding and drinking patterns of these and other rats from Spacelab-3. Future life science flights will provide further data necessary to an understanding of these changes. [Supported by NASA Contract NAS 2-10536.]

83.11

EARLY ADAPTATION TO ALTERED GRAVITATIONAL ENVIRONMENTS IN THE SQUIRREL MONKEY. Charles A. Fuller, University of California, Davis, CA 95616.

Astronauts exposed to spaceflight may exhibit a series of debilitating symptoms collectively called Space Adaptation Syndrome (SAS). At present, however, no means of predicting sensitivity to SAS is available. During the recent flight of Spacelab-3 one of the two squirrel monkeys on-board exhibited behavior similar to that reported for crewmen suffering SAS. This animal required 96 hr to recover normal behavior, while the second animal adapted immediately. After this, feeding and activity were, with some exceptions, comparable to pre-flight levels. Six unrestrained monkeys have also been exposed to 1.5 G via centrifugation for 7 days. Their feeding behavior showed an average recovery period (2.5 days; range 0-4 days) similar to that seen during spaceflight. The pre-centrifugation control feeding rhythms were similar to the average rhythms of days 4-7 at 1.5 G. Thus, the role of steady-state changes in the ambient gravity environment may be similar for both hyper- and hypogravitational fields. Since prolonged +G fields can be produced artificially on earth, whereas zero G cannot, it may eventually be feasible to develop an earth-based model of SAS which is predictive of etiology in the microgravity environment. However, this will first require an understanding of the mechanisms behind the zero G and +G responses to elucidate their similarities, whether functional or superficial. [Supported by NASA contract NAS 2-10536;Grant NAG 2-349.]

83.12

EFFECTS OF WEIGHTLESSNESS ON NEUROTRANSMITTER RECEPTORS IN SELECTED BRAIN AREAS. J. D. Miller,* B. A. McMillen,* D. M. Murakami,* Mona M. McConnaughey,* H. L. Williams* and C. A. Fuller. Univ. of Calif, Davis, CA; E. Carolina Univ., Greenville, NC.

Six male Sprague-Dawley rats were exposed to microgravity for 7 days aboard Spacelab-3. These rats and 6 age matched controls were sacrificed (12-14 hrs postflight), and standard receptor binding assays for receptor number (B_{max}) and affinity (K_d) were performed on frozen brain tissue. The brains were dissected and used for these receptor assays: prefrontal cortex (D2 and 5HT2), lateral frontal cortex (5HT1), olfactory tubercle (GABA), rest of cortex ($\alpha1,\alpha2,\beta$, and muscarinic), hippocampus (5HT1), hypothalamus ($\alpha1,\alpha2,\beta$, and muscarinic) suprachiasmatic nucleus (5HT2), amygdaloid area (D2 and 5HT2), striatum (D2 and adenosine-1), midbrain ($\alpha1,\alpha2,\beta$, and opiate), pons-medulla ($\alpha1,\alpha2,\beta$, muscarinic and opiate) and cerebellum ($\alpha2,\beta$, muscarinic, GABA and adenosine -1). In addition, activity of the Na^+/K^+ ATPase in nonspecfic cortex was determined. Two significant differences were observed: 1) an elevation in S-1 B_{max} in the hippocampi of the experimental group ($p<0.05$) with a corresponding trend towards elevation of S-1 K_d; 2) a decrement in the activity of the Na^+/K^+ ATPase in the cortices of the flight animals ($p<0.05$). These results suggest a joint serotonergic supersensitivity coupled with a decrement in synaptic activity in neocortical/allocortical regions. [Supported by NASA Grant NAG 2-349 and Contract NAS 2-10536.]

83.13

CHANGES IN FUNCTIONAL METABOLISM IN THE RAT CENTRAL NERVOUS SYSTEM FOLLOWING SPACEFLIGHT. D. M. Murakami,* J. D. Miller* and C. A. Fuller. Univ. of California, Davis, CA 95616.

Many physiological systems are affected by the microgravity condition of space flight (e.g., muscle, bone, cardiovascular). Since these physiological systems are directly or indirectly regulated by the nervous system, it is important to know how central control mechanisms are affected by weightlessness. This study examines the pattern of metabolic activity in the brains of 5 Sprague-Dawley rats following flight on Spacelab-3 and 5 controls. The rats were sacrificed and perfused with fixative 12-18 hours postflight. Alternate coronal 40 μm sections were stained with thionin or for cytochrome oxidase. Three flight animals exhibited a decrease in metabolic activity in the paraventricular and supraoptic nuclei. This may reflect the prominent fluid shifts to a new steady-state commonly observed under microgravity. Two of the flight animals were dehydrated and exhibited a significant increase in metabolic activity in the paraventricular and supraoptic nuclei compared to controls. Soma diameters of neurons in these nuclei were significantly larger in the dehydrated flight animals. These preliminary results suggest that the neural mechanisms involved in fluid homeostasis functioned normally in response to dehydration. Preliminary examination of other hypothalamic and motor system nuclei has not revealed obvious changes in metabolic activity. [Supported by NASA Grant NAG 2-349, Contract NAS 2-10536, and DMM recipient of NASA Space Biology Fellowship.]

83.14

MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN SOLEUS AND EXTENSOR DIGITORUM LONGUS MUSCLES OF RATS ORBITED IN SPACELAB 3. D.A. Riley*, S. Ellis*, G.R. Slocum*, T. Satyanarayana*, J.L.W. Bain* and F.R. Sedlak*. (SPON: H. Leon) Department of Anatomy, Medical College of Wisconsin, Milwaukee, WI. and NASA Ames Research Center, Moffett Field, CA.

Twelve rats (384 ± 4 g) were exposed to hypogravity for 7 days, and 12 control animals (392 ± 5 g) remained on earth in simulated flight cages. Muscles of 7 flight rats were harvested 12-16 hours postflight and quick frozen for biochemical and histochemical analyses; 5 flight animals were perfused for electron microscopy. Controls were processed similarly 48 hours later. Soleus fiber area decreased by 40%, and extensor digitorum longus (EDL) slow fibers were 17.5% smaller than controls. Lysosomal dipeptidylaminopeptidase II activity was unchanged; tripeptidylaminopeptidase and Ca^{++} activated protease activities were elevated at least 75%. Myofibrils were eroded by multiple focal losses of myofilaments. Overall, soleus and EDL fibers showed reduced histochemical mitochondrial NADH dehydrogenase, elevated alpha glycerophosphate dehydrogenase and unchanged myofibrillar ATPase activities; fibers generally possessed increased glycogen. These findings demonstrate that soleus atrophies more than the EDL following spaceflight. In general, muscle metabolism shifts from oxidative to glycolytic. Primary degradation of myofibrils appears to involve focal breakdown of myofilaments rather than lysosomal autophagy.

83.15

MICROGRAVITY CHANGES IN HEART STRUCTURE AND c-AMP METABOLISM. D.E. Philpott*, A. Fine*, M. Mednieks*, K. Kato*. NASA Ames Research Center, Moffett Field, CA 94035. New York V. A. Medical Center, N.Y., N.Y., 10010. N.I.H.-N.I.D.R., Bethesda, MD. 20205.

Male rats were flown on Space Lab-3 for 7 days and sacrificed 12 hours after recovery. Flight animal (FA) ultrastructural heart changes include: increase in glycogen and small lipid globules, decrease in cytoskeletal tubules. Cyclic AMP (cA) mediated hormonal regulatory responses were evaluated by measurements of the specific activity of cA-dependent protein kinase (SA, cA-PK), activation ratios (AR), inhibition by a specific inhibitor (INH) and photoaffinity labeling of cA-PK regulatory (R) subunits with azido cA ($8\text{-}N_3cA$). These parameters are decreased in FA cell fractions, compared to controls (SA cA-PK with a $p < 0.1$, AR and INH by 20 to 30%, and $8\text{-}N_3cA$ labeling showed variation in R subunit distribution). Mednieks found similar trends in secretory cells from FA salivary glands. Phosphorylation studies show that a high salt extract of the particulate fraction in the FAs has a different phosphoacceptor banding pattern than in the controls. Adenylate cyclase (basal, F^--stimulated) and low Km cA phosphodiesterase (PDE) specific activities were unaltered in the FAs. A loss in the high Km cA-PDE activity occurred in the FA, $p < 0.1$.

83.16

REDUCTION OF THE SPERMATOGONIAL POPULATION IN RAT TESTES FLOWN ON SPACE LAB-3. D.E. Philpott*, W. Sapp*, C. Williams*, J. Stevenson*, S. Black*, R. Corbett*. NASA, Ames Research Center, Moffett Field, CA 94035. Tuskegee Institute, Tuskegee, AL 36088.

It is well documented that spermatogonial populations exhibit a sensitive response to radiation and stress. Six rats were flown for 7 days on SL-3. Twelve hrs. after recovery, the testes were excised, weighed, slit open under "Triple Fix", refrigerated and flown to Ames Res. Center. Two micron sections of the tubules were stained with Toluidine Blue and alternate sections containing maturation stage 6 were used to count the surviving spermatogonia. The average weight loss during flight was 7%. The decrease in spermatogonial cell counts was 6.9% (significant in one tail "t" test). Unfortunately, dosimetry aboard the shuttle has been reduced and no dosimetry was carried out in the experimental area. Only TLD dosimetry occurred in the forward areas. An estimated (total) dose is 0.05r. It would take about one rad of cosmic rays to reduce the population by the observed amount. Stress from adapting to weightlessness and the final jet flight to the Cape, or other factors may be the major cause. These important findings need to be repeated with dosimetry to ascertain the cause/causes for the cell loss.

83.17

EFFECT OF FLIGHT IN MISSION SL-3 ON INTERFERON-GAMMA PRODUCTION BY RATS, Cheryl L. Gould*, Jo Ann Williams*, Adrian D. Mandel*, and Gerald Sonnenfeld*. University of Louisville, Louisville, KY 40292 and NASA Ames Research Center, Moffett Field, CA 94035.

Several rats were flown in Space Shuttle mission SL-3 and data were analyzed to determine the effects of the flight on interferon-gamma production. Interferon-gamma is an important antiviral and immunoregulatory agent. The rats were weightless for approximately seven days, and a twelve hour delay after landing ensued prior to placement of spleens from the rats in tissue culture. Spleen cells from the rats were stimulated with concanavalin-A for 48 hrs to induce interferon-gamma. Supernatant fluids were harvested from the cultures and were assayed for interferon-gamma activity by determining the greatest dilution of sample (titer) that decreased vesicular stomatitis plaquing by 50%. 63% of control rats produced interferon-gamma titers of a mean value of 25 units. This is within the normal range of what would be expected for this procedure. None of the flown rats produced any detectable levels of interferon. These data indicate that the production of interferon-gamma is severely inhibited in tissue from rats subjected to space flight and the attendant period of weightlessness. The data are in agreement with previous studies utilizing antiorthostatic, hypodynamic, hypokinetic suspension modeling that also showed that simulated weightlessness resulted in inhibition of interferon induction. C.L. Gould is a NASA Postdoctoral Research Associate.

83.18

BIOCHEMICAL AND MORPHOLOGICAL EVALUATION OF THE CONSEQUENCES OF SPACE FLIGHT CONDITIONS ON THE STRUCTURE AND FUNCTION OF SALIVARY GLANDS. M.I. Mednieks, L.F. Cheng and A.R. Hand. NIH, National Institute of Dental Research, Bethesda, MD 20205

Cellular reactions mediated by cyclic AMP and the ultrastructure of salivary glands have been assessed in rats from SL-3 (FA) and corresponding ground controls (GC). Environmental stimuli such as the action of catecholamines are known to result in altered cell morphology and in changes of cyclic AMP-dependent protein kinase (cA-PK) activity and cellular localization (Mednieks and Hand, Eur. J. Cell Biol. 28, 1982). Assay of cA-PK showed decreased activity in the soluble fraction but an increase was observed in a 0.4 M NaCl extract (P_2) of the particulate cell fraction of FA. Similarly, photoaffinity labeling of cA-PK regulatory (R) subunits in P_2 showed a marked increase when compared to GC. LM and EM examination of parotid glands from FA revealed significant differences from CC animals in two cases. These included reduced acinar cell size, fewer secretory granules, increased numbers of lysosomes, autophagic vacuoles containing degenerating secretory product, irregular RER configuration, and basal lipid droplets. Since these rats had a significant weight loss, the changes may be due to a lack of reflex masticatory stimulation, as occurs during acute starvation or feeding a liquid diet (Hand and Ho, Arch. Oral Biol. 26, 1981). This dual assessment of the effects of space flight on salivary glands showed changes which are similar to those induced experimentally by physiological or pharmacological manipulation.

83.19

MICROPROBE ANALYSES OF EPIPHYSEAL PLATES FROM SPACELAB 3 RATS. J. Duke, L. Janer* and M. Campbell*. Univ. of Texas Dental Branch, Houston, TX 77225.

Ultrastructural studies of epiphyseal plates from Cosmos 1129 rats showed that production and mineralization of matrix vesicles was delayed by the 18.5 day flight. To determine if differences in differentiation could be detected in seven days, proximal tibial epiphyseal plates were obtained from rats flown aboard Spacelab 3, and prepared for microanalysis using a freeze-substitution method. 200 nm sections were placed on beryllium grids, and examined in a Cameca electron microprobe. Concentration of elements was measured in the matrix of longitudinal septa of the growth plate in the proliferative, prehypertrophic, hypertrophic, and calcifying zones. In control plates, all four zones had high levels of Na, and, in unmineralized regions, low levels of Mg, P, and Ca. K and S levels were high and increased from the proliferative to the calcifying zones. The level of P rose in the mineralized regions of the matrix and Ca/P ratios ranged from 1.2-1.4. In contrast, flight animals had very low Na and K values, although Mg levels were unaffected. S levels were less than half of control values, and Ca values were less in both unmineralized and mineralized regions, although the Ca/P ratio was similar to that of controls. These data indicate that even a short space flight can alter bone mineralization, and that the primary defect is at the level of initial matrix production. This hypothesis is being explored using TEM and image analysis. (NASA contract A21999C-VAB)

83.20

OTOCONIAL MORPHOLOGY IN SPACE-FLOWN RATS. *Muriel D. Ross, *Kathleen Donovan, and *Oliver Chee. *The University of Michigan, Ann Arbor, MI 48109 and **Ames Research Center, Moffett Field, CA 94035

Inner ears from 6 rats flown on Spacelab-3 were fixed by intralabyrinthine perfusion with 2.5% glutaraldehyde + 0.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for up to 12 hrs. Six controls from rats of matched age were prepared similarly. Tissues were washed in buffer, post-fixed in 1% osmium tetroxide and brought through graded alcohols to 70% for microdissection. Otoconial complexes were removed to aluminum stubs and air-dried for scanning electron microscopy. Maculas were prepared for transmission electron microscopy by usual methods. Transmission work is incomplete at this time. Otoconial complexes were sputter-coated with gold-palladium and studied in an ISI-130 DS scanning electron microscope. Results showed that otoconial complexes from space-flown rats were similar in size and morphology to controls. No demineralization was evident. Whether a slight increase in mineral occurred as result of short-term exposure to microgravity cannot be determined by the method employed. The conclusion is that short-term exposure to microgravity is not deleterious to otoconial complexes of gravity receptors.

Supported by NASA Contract #NAS2-10535.

83.21

EFFECT OF SEVEN DAYS OF SPACEFLIGHT ON HINDLIMB MUSCLE PROTEIN, RNA AND DNA IN ADULT RATS. J. M. Steffen and X. J. Musacchia, Dept. Physiol. Biophys., School of Med., Univ. of Louisville, Louisville, Ky. 40292

In whole body suspended rats muscle disuse is accompanied by decrements in protein and RNA contents and no changes in DNA. Effects of space flight on skeletal muscle protein, RNA and DNA contents were determined. Male Sprague-Dawley rats (360-410g) were weightless for 7 days (12 hrs. elapsed between shuttle landing and sacrifice). Controls (C) were maintained in 1g, for a similar period. Soleus (S) and extensor digitorum longus (EDL) muscles were excised, frozen in liquid nitrogen; protein, RNA and DNA were differentially extracted and determined. Soleus and EDL of flight animals atrophied 18% and 11%, respectively. Muscle protein contents (mg) were reduced in parallel with muscle weight. There were no significance changes in absolute (mg) DNA content in S or EDL, but DNA concentration (mg/g) increased ($P<.05$) in both S ($.336 \pm .022$ vs $.257 \pm .017$) and EDL ($.281 \pm .010$ vs $.234 \pm .026$). Absolute (mg) RNA content was decreased ($P<.05$) in both S and EDL from flight animals, but RNA concentration was significantly reduced only in the S ($.483 \pm .056$ vs $.368 \pm .022$). Loss of muscle mass during weightlessness can be due to loss of protein, perhaps a reduction in synthetic capacity (decreased RNA content), with no alteration in muscle cell number (no change in DNA content). The results support using whole body suspension models for studies simulating effects of weightlessness on skeletal muscle. (Supported by NASA).

83.22

1,25-DIHYDROXYVITAMIN D_3 RECEPTORS IN SPACE-FLOWN VS GROUNDED CONTROL RAT KIDNEYS. David J. Mangelsdorf*, Samuel L. Marion*, J. Wesley Pike*, and Mark R. Haussler* (SPON: M. E. Tischler). Univ. of Arizona, Tucson, AZ 85724

Under conditions of hypogravity, the skeleton undergoes significant bone demineralization, resulting in a sharp rise in urinary calcium excretion. In the kidney, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) facilitates the readsorption of renal calcium, presumably via its receptor in a classic steroid hormonelike manner. To study the possibility that changes in $1,25(OH)_2D_3$ receptors may be responsible for inhibiting $1,25(OH)_2D_3$'s normal ability to retain calcium in space-flown kidneys, we utilized rat kidneys from NASA's Spacelab-3 Mission to quantitatively and qualitatively assess $1,25(OH)_2D_3$ receptors via Scatchard analysis. Extracts prepared from frozen rat kidneys were labeled with increasing concentrations of tritiated $1,25(OH)_2D_3$ ± 100 fold excess radioinert hormone to determine specific saturable hormone binding. Scatchard analysis revealed no statistical difference in $1,25(OH)_2D_3$ receptors between space-flown ($Kd = 0.23 \pm 0.13$ nM; $N_{max} = 27.6 \pm 10.1$ fmol/mg protein; n=5) and grounded control ($Kd = 0.60 \pm 0.27$ nM; $N_{max} = 27.9 \pm 8.0$ fmol/mg protein; n=5) animals. These results support a view in which kidney $1,25(OH)_2D_3$ receptors play neither a causal or effectual role in regulating renal calcium excretion due to hypogravity-induced bone loss. An important caveat to interpreting these data is NASA's 12h postflight lag time before animal sacrifice. Supported by NIH Grant AM 15781-14.

83.23

MORPHOLOGIC AND HISTOCHEMICAL STUDIES OF BONE CELLS FROM SL-3 RATS. Stephen B. Doty, Columbia University, NY, NY, 10032.

Morphological and histochemical studies of the skeletal tissue from rats obtained from SL-3 are now underway. Preliminary findings include the following: (1) Alkaline phosphatase activity (an indicator of new bone formation) is abundant in the control and flight animals. However, the morphology of the cells from the flight group indicates a depression in cell size and perhaps cell number, at the bone surface. (2) Intracellular dipeptidase activity (a potential indicator of cytoplasmic collagen or procollagen degradation) is equally abundant in control and flight animals. However, if flight animals are degrading procollagen at an equivalent rate as the controls, but are making bone matrix at a slower rate than controls (as suggested by results from (1) above) the overall effect could be a significant bone loss in the flight animals. (3) The calvaria, a non-weight bearing bone, from control and flight animals is being used to compare results from the weight bearing tibias discussed in (1) and (2). Supported in part by NASA NCC 2-325.

83.24

THE INFLUENCE OF SPACE FLIGHT ON THE RAT SOLEUS. T.P. Martin, V.R. Edgerton (Dept. Kinesiology, UCLA) and R.E. Grindeland (NASA-Ames)

The influence of 7 days of space flight (F) (NASA space shuttle challenger) was investigated in the rat soleus (Sol). Animals housed in identical cages during the flight acted as ground controls (C). Muscle fibers were analyzed by quantitative histochemical techniques for succinate dehydrogenase (SDH) and α-glycerolphosphate dehydrogenase (GPD) activity, relative myosin ATPase density, and cross sectional area (CSA). The body weights of F and C were not different whereas the sol and sol/body weight were significantly less in the F group. The CSA of both ST and FT fibers were decreased by 40% in the F group. The SDH activities of C and F fibers were not different. THe GPD activity was increased in both fiber types in the F sol. The increase in GPD is consistent with the 28% increase in FT fibers in the F group and the apparent shift from SO to FOG fibers following space flight.

	CSA (um^2)		SDH (OD/min X 10^4)		GPD (OD/min x 10^4)	
	ST	FT	ST	FT	ST	FT
C(N=4)	2801	2078	218	320	6	10
F(N=6)	2640	1237	257	342	16	33

83.25

RESULTS OF EXAMINATION OF THE RESPIRATORY SYSTEM IN SPACELAB-3 FLIGHT RATS. Lisbeth M. Kraft. NASA/Ames Research Center, Moffett Field, CA 94035. Histopathological examination of the nasal epithelium and lungs of rats revealed that members of all groups (pre-flight controls, flight, simulation controls, and vivarium controls) manifested varying degrees of a patchy pneumonitis. Microscopically the lesions were consistent with those known to be caused by a virus infection; therefore attempts were made to determine a most probably etiological agent by means of retrospective electron microscopy and histoimmunofluorescence or histoimmunochemistry. The significance of these findings for interpretation of the results of other investigators studying these animals will be discussed. Further, these results will be compared with those of similar studies conducted on the respiratory system of pocket mice that flew on Apollo XVII and of rats that flew on Cosmos 1129, in both of which diverse respiratory virus infections were shown to have been present during flight. The use of "defined flora" rather than "SPF" animals with the aim of reducing to a minimum the possibility of similar occurrences on future spaceflights involving rodents will also be discussed.

83.26

OSTEOCALCIN (OC) AS AN INDICATOR OF BONE METABOLISM DURING SPACEFLIGHT. P.E. Buckendahl, C.E. Cann, R.E. Grindeland, R.B. Martin, G. Mechanic, and S.B. Arnaud. NASA/Ames Research Center, Moffett Field, California 94035.

Reduced bone mass and remodeling activity, and increased fragility in the skeleton of rats following 18.5 days of spaceflight have been reported. Biochemical changes may precede these skeletal changes. We compared calcium (Ca), phosphorus (Pi), alkaline phosphatase, OC (a bone specific noncollagenous mineral-binding protein) and 1,25 dihydroxyvitamin D in the serum, and Ca, Pi, OC, and collagen in a vertebra (L3) of 250 g. rats after a 7 day flight. Bending strength of the humerus was also tested in these animals and was reduced 28%. Serum parameters were the same in flight (F) and ground controls (S), except for OC which was 28% lower in F, 249 ± 36 ng/ml (mean ± SD), vs 347 ± 42 (S), $p<.001$. The dry weight of L3 was significantly lower in F than in S rats ($.318 \pm .018$ mg/g body weight vs $.354 \pm .009$, $p<.01$). Ca content in F was 180 ± 13 ug/mg bone vs 179 ± 3.7, whereas OC content was $2.19 \pm .22$ ug/mg bone in F vs $2.43 \pm .11$ in S. These data suggest that major changes occur in the rate of growth and accumulation of OC in trabecular bone during the first days of spaceflight and that the level of OC in serum is a sensitive indicator of these changes. Serial measurements in the first 1-5 days of future flights will be necessary to determine if these changes are due to an abrupt or a gradual decrease in bone formation.

83.27

HEPATIC ENZYMES OF SPHINGOLIPID AND GLYCEROLIPID BIOSYNTHESIS IN RATS FROM SPACELAB 3. Alfred H. Merrill, Jr.*, Elaine Wang* and James L. Hargrove* (SPON: John Manning). Depts. of Biochemistry & Anatomy & Cell Biology, Emory Univ. School of Medicine, Atlanta, GA 30322.

Lipid metabolism is a major function of liver, which synthesizes these molecules for membranes and lipoproteins. The activity ratio of serine palmitoyltransferase (SPT) and glycerol 3-phosphate acyltransferase (GPAT), the initial enzymes of sphingolipid and glycerolipid synthesis, is a probable determinant of tissue lipid composition (Merrill et al., J. Lipid Res., in press). SPT activities of rats on Space Lab 3 were significantly lower than controls handled similarly on the ground and were, respectively (Mean ± SE, n=6): 17.4 ±2.1 & 29.0±2.7 pmol/min/mg microsomal protein (P< 0.01) (microsomal protein for the flight and control rats was 4.2±0.3 & 5.6±0.3 mg/g liver, P<0.02); 783±69 & 1600 ±190 pmol/min/liver (P<0.01); and 330±36 & 622±77 pmol/min/100g body weight (P<0.01). Microsomal GPAT was not significantly different (P>0.1) for the flight and ground groups: 1.0±0.24 & 1.06±0.14 nmol/min/mg. There were no significant differences in body weight, weight change during the experiment, or liver weight. These findings indicate that hepatic sphingolipid metabolism is specifically affected by space flight, which may reflect hormonal changes or cellular adjustments to a zero gravity environment. Supported by NIH grant EM 33369 with samples provided by NASA.

83.28

HEPATIC ENZYME ADAPTATION IN RATS AFTER SPACE FLIGHT. James L. Hargrove* and Dean P. Jones* (SPON: John Manning). Dept. of Anatomy and Cell Biology, and Dept. of Biochemistry, Emory University, Atlanta, GA 30322.

Adaptive changes in many hepatic enyzmes result from environmental stressors, therapeutic agents, and changes in content of plasma hormones or nutrients. Since exposure to microgravity during space flight may *alter secretion* of adrenal hormones, we have begun a survey of liver enzymes to ascertain whether changes in hepatic function may have occurred during the flight of Spacelab 3. Samples were obtained from animals 12 hours after return to earth, and from ground controls taken at the same time of night two days later. For two cytosolic enzymes, no significant difference was found between means as analyzed by a two-tailed T test. Values for ground and flight animals, respectively were: tyrosine aminotransferase, 0.021±0.007 versus 0.026±0.005 units /mg protein (P<0.2); aspartate aminotransferase, 1.50±0.24 versus 1.49±0.19 units /mg (P>0.5). For two microsomal enzymes, values were: cytochrome b5, 0.71±0.30 units/mg versus 0.62±0.32 (P>0.5), and cytochrome P450, 3.38±1.23 versus 1.71 ±0.35 units/mg (P<0.01). Conclusion: A significantly lower value was found in hepatic cytochrome P450 content in animals exposed to hypogravitation. Enzyme adaptation during space flight should be confirmed and its possible consequences studied on future Spacelab missions. (Supported by USPHS Grants AM32154 and HL30286 with samples provided by NASA).

83.29

ATRIOPEPTIN (AP-3) IN ATRIA AND PLASMA OF RATS ORBITED ABOARD NASA SPACELAB (SL3) FOR SEVEN DAYS. Walter H. Inge* and Diane K. Hartle* (SPON: John W. Manning). Emory University School of Medicine, Atlanta, GA 30322

The diuretic, natriuretic, and vasodilatory actions of AP-3 may be important in adaptation to microgravity. Cephalad shifts in body fluids theoretically should increase AP-3 secretion. Six rats were orbited aboard SL3 for 7 days, landed in CA and flown to FL where they were anesthetized (halothane), decapitated, blood collected, and plasma separated. Ventricular samples and atria were resected. All samples were frozen at -70 degrees C. Samples from 6 ground control animals were similarly prepared. AP-3 immunoreactivity (APir) was determined for plasma and both right and left atria. All plasma levels were an order of magnitude higher than unstessed basal levels, presumably due to anesthesia. No differences between control and flight rat plasma APir were found (p<0.64). Both right and left atrial APir content in flight rats were slightly elevated over controls but not significantly (Rt:p<0.5,L:p<0.33). In the table, plasma levels are in ng/ml; tissue levels in ug/mg wet weight, ±S.E.

	PLASMA	RT.ATRIUM	L ATRIUM
Gnd	2.8±0.48	0.15±0.01	0.14±0.04
Flt	2.0±0.67	0.19±0.04	0.18±0.04

We conclude that an adequate test of the effects of microgravity on AP-3 secretion requires serial inflight plasma sampling in rats to avoid the confounding effects of reentry and postflight delays. NASA PO A21993C(VAB).

83.30

PLASMA RENIN CONCENTRATIONS (PRC) OF RATS ORBITED FOR 7 DAYS ABOARD NASA SPACELAB 3. Diane K. Hartle* and Walter H. Inge* (SPON: John W. Manning). Emory Univ. School of Medicine, Atlanta, GA 30322

Cardiovascular and neural adaptations to space flight involve many factors that would influence renin secretion and thereby affect hemodynamic and electrolyte homeostasis. PRC were determined in 6 rats (SL3R) orbited aboard Spacelab 3 for 7 days and in 6 ground control rats (GC). SL3R were flown from CA to FL after landing; SL3R and GC were transported similarly from Kennedy Spaceport to Kennedy Res. Labs, anesthetized with halothane and decapitated. Plasma samples were obtained from heparinized trunk blood and frozen at -70 deg C. PRC were estimated by measuring the conversion of rat renin substrate (RRS) to angiotensin I (AI) per ml plasma per hr at 37 deg C. (excess RRS insured a zero order rate constant). AI was measured by radioimmunoassay. PRC of SL3R were 35.6+6.6 and of GC 44.2+7.3, p=0.4. No conclusions can be drawn concerning the effects of prolonged microgravity on the regulation of renin secretion because of the relatively short half-life of renin compared to the 12-14 hr time delay between reentry and sampling. Although there were no significant differences in PRC between SL3R and GC, PRC were elevated in both groups above levels usually obtained in unstressed rats (15-20 ng AI/ml/hr range), indicating that handling and halothane anesthesia increased PRC before sampling. These results underscore the need in future experiment to obtain cardiovascular data and plasma samples in flight. NASA PO# A21993C.

83.31

BONE MATURATION IN RATS FLOWN ON THE SPACELAB-3 MISSION. Jean E. Russell, Jeri Webber, and David J. Simmons, Washington University, School of Medicine, St. Louis, Mo. 63110

The effects of short-term spaceflight on the maturational profile of bone calcium was measured in rats flown aboard the Shuttle Spacelab-3 Mission. Femurs obtained at autopsy from those animals and from a group of 6 age & weight matched rats maintained in a land-based vivarium were fixed in 100% ethyl alcohol. The bones were divided into (marrow & soft tissue-free) trabecular (TB) and cortical (CB) regions. These were frozen, lyophilized, and ground to 40um particles which were then separated by a bromoform-toluene gradient into 4 specific gravity fractions (1.3-1.7, 1.8-1.9, 2.0-2.1, & 2.2-2.9 sp.gr.). The lowest sp.gr. fraction represents mineral deposited in newly calcifying bone, while the highest sp.gr. fraction represents mineral in the most mature moiety of the tissue. In the SL-3 rats, total femoral Ca-concentrations in CB and TB were normal (TB= 201-207 mgCa/g bone; CB=213-225 mgCa/g bone). However, the two groups could be distinguished by the distributional patterns of Ca within the sp.gr. fractions. In CB, the SL-3 femurs showed significantly less Ca than normal in the 1.8-1.9 fraction (P<.025), and a corresponding elevation of Ca in the 2.0-2.1 fraction (=38% difference). This reciprocal pattern was also observed in the TB from the SL-3 femurs (P<.01 for the 2.0-2.1 sp.gr. fraction; 20% difference). These Ca-profiles indicate that SL-3 femurs are relatively more "mature" than bones from control rats, perhaps due to a spaceflight associated decrease in bone turnover.

84.1

A COMPUTER ASSISTED TUTORIAL ON BODY FLUID COMPARTMENTS. John Allen Bettice. Office of Medical Education and the Department of Physiology, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

This tutorial consists of a written syllabus and a computer based problem set. The syllabus contains an introduction to the topic of Body Fluid Compartments, and the problem set is designed to aid students both in developing their understanding of fundamental principles and in learning to apply these principles to practical problems. The computer program contains both short and detailed, step-by-step solutions to the problems.

Topics contained in this tutorial include:

1. A compartment definition and size,
2. Measurement of compartment size,
3. Distribution of fluid between compartments,
4. Experimental changes in compartment size,
5. Clinically oriented case problems.

This tutorial can be used for either the routine or the remedial study of body fluid compartments.

The computer program is written in BASIC for use with an MS-DOS operating system (IBM compatible) and is contained on one 5.25 inch floppy disk.

84.2

FULLY AUTOMATED DETERMINATION AND COMPUTATION OF KINETIC AND THERMODYNAMIC PARAMETERS OF ENZYMES. David Hoak*, S. Banerjee* and G. Kaldor, VAMC, Allen Park, MI 48101, Depart. of Pathology, W.S.U. Detroit, MI 48201.

A fully automated system has been designed suitable to perform enzymatic assays and also to compute some kinetic constants such as Km, Vm and Vo (initial velocity) from the progress curves. We used an IL Multistat III centrifugal analyzer equipped with 32K RAM programmable microcomputer to implement our program. The programming language is Focal 8. One advantage of this method is that it is amenable to direct on line computer analysis. A further advantage is that the kinetic constants may be estimated from one single experiment. Obviously the accuracy of the method is enhanced if several progress curves are analyzed and statistically evaluated. We have used the equations suggested by Cornish-Bowden (Biochem. J., 140, 305-312, 1975), to compute Km, Vm and Vo. The computation of Km is at least two, preferentially more than two temperatures permits the calculation of the standard free energy F°, enthalpy H° and enthropy S° of the reaction. The determination of Vmax at two or more temperatures facilitates the computation of the enthalpy ($H_+^{\ddagger}$) free energy ($G_+^{\ddagger}$) and enthropy ($S_+^{\ddagger}$) of the activated complex formation. This system can be employed in a clinical laboratory for clinical research as well as in a teaching laboratory to actually determine or simulate the effect of inhibitors natural or iatrogenic on various enzymes.

AUTHOR INDEX