THE PHYSIOLOGIST

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Cover: See Public Affairs, p. 112

Congress Has Something For Almost Everyone

The Congress now appears to be in the mood to provide something for nearly everyone concerned with laboratory animal reform legislation.

Committees in both the Senate and the House have amended their versions of the renewal authorization for the National Institutes of Health (NIH) by adding proposals that deal with the care and treatment and the use of animals in research. The bills will now go to the floor of their respective chambers for consideration and then to a joint conference committee to iron out the language differences before final action of a bill by the Congress can be considered.

Most of the activity concerning laboratory animals took place in the House Committee on Energy and Commerce, which spent three days debating research animal amendments during its markup of the NIH Renewal Authorization Bill HR 2350 (formally HR 1555). A total of five amendments were proposed concerning research animals, three of which were finally approved by the Committee. In a somewhat confusing maze of parlimentary procedure, the Committee took the following actions in this order.

- *1*) Approved unanimously an amendment by Rep. Doug Walgren (D-PA) to change the requirement that the Secretary of the US Department of Health and Human Services (DHHS) establish "regulations" for the care and treatment of research animals to a requirement that DHHS establish "guidelines." The requirement for establishing new regulations by DHHS was approved in March by the Committee's Subcommittee on Health and the Environment during its review of the NIH renewal authorization.

The Subcommittee's action at that time was the result of a Walgren amendment that, in addition to the requirement for new regulations, also charged NIH with establishing by June 1984 a plan for developing alternative methods and with initiating a requirement that all NIH-funded research involving animals be monitored by an institutional animal studies committee.

Because of the already existing NIH guidelines, the standards of the Animal Welfare Act, and the criteria for accreditation, the scientific community had been encouraging Walgren not to add still another layer of requirements for the care, treatment, and use of research animals.

- *2*) Defeated an amendment proposed by Rep. Richard Shelby (D-AL) that would have reduced by one-half the $20 million authorization in the Walgren Subcommittee amendment calling for the development of an alternative methods plan by NIH, would have extended by one year the time for which NIH is to complete such a plan, and would have deleted the requirements for institutional animal studies committees and the establishing of guidelines by DHHS.
- *3*) Approved with only two negative votes an amendment by Rep. Edward Madigan (R-IL) to delete the program for NIH's development of a plan for alternative methods, the requirements for institutional animal studies committees, and the creation of guidelines by DHHS and to substitute for the deletions an 18-month study of the issues involving the use of animals in research. This amendment, however, was then defeated by the following action.
- *4*) Approved by a 22-19 vote an amendment by Rep. Matthew Rinaldo (R-NJ) that retains the proposal for NIH to develop a plan for alternative methods, the requirement for institutional animal studies committees, and the establishment of guidelines by DHHS for the care and treatment of laboratory animals and adds the conduct of an 18-month study of research animal issues involving NIH-funded institutions.
- *5*) Approved an amendment protecting commercial firms with NIH research support funds against possible violations of releasing trade-secrets information by the nonaffiliated members of the institutional animal studies committees.

As the House Committee conducted its business on animal reform legislation, the Senate Committee on Labor and Human Resources appended to its version of the NIH renewal authorization (S 773) a bill by Sens. Orrin Hatch (R-UT) and Edward Kennedy (D-MA) that authorizes an 18-month study of the use of animals in research.

Both the Senate and House study amendments are similar in that they call for a five-year review of the types, number, and use of animals in research and an analysis as to whether such use of animals has increased or decreased and whether alternative methods were available; assess the implications and costs to institutions regarding accreditation requirements and standards; review all existing Federal and state laws and regulations governing the use of animals in research and the extent to which animals are protected against inhumane treatment; and evaluate the efforts of DHHS in developing methods to reduce the number of animals used and what assurances are being made by that agency that animals are being treated humanely.

Both bills call for the study to be conducted by the National Academy of Sciences.

Also in the Senate it is now considered a possibility that Sen. Robert Dole (R-KS) may add his bill (S 657) to amend the Animal Welfare Act to the Senate's NIH renewal authorization. The proposal by Dole would add an additional standard of "adequate exercise" for animals and would establish institutional animal care committees to assist the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) in the inspection of research animal facilities.

One of the purposes of the Dole bill is to strengthen APHIS's effectiveness in its responsibilities to inpect research facilities. For the last two years the Reagan Administration has proposed to reduce the APHIS budget by turning over to states, humane societies, industry, and individuals the responsibility for enforcing the inspection provisions of the Animal Welfare Act.

In late April, Rep. Brian Donnelly (D-MA) introduced in the House HR 2633, a bill that would authorize $13 million in each of the next four fiscal years to provide DHHS with grant funds to be awarded for the development of alternative methods. This bill has been

jointly referred to the Committee on Science and Technology and on Energy and Commerce.

In April the American Physiological Society on behalf of seven national organizations presented testimony before the Senate Appropriations Subcommittee on Agriculture, Rural Development, and Related Agencies in support of continuing APHIS funding at its current level of $4.9 million and in opposition of the Administration's proposal to give the enforcement responsibilities to non-Federal entities. Similar presentations last year before Senate and House committees were successful in keeping APHIS funding at current levels so that it could continue its responsibilities as mandated by the Animal Welfare Act.

Primate Center Demonstrations Reported as Being Peaceful

The Mobilization for Animals public protest demonstrations on April 24 were conducted as scheduled but without the fanfare the organizers had desired.

According to reports from the four centers where demonstrations were staged, the largest rally was at the University of California at Davis where several thousand persons turned out to hear Hollywood celebrities discuss animal rights. However, less than 100 persons were reported to have marched in the parade to the regional primate center on the Davis campus.

Rain forced the 3,000 demonstrators from the Boston Commons to a hotel auditorium where the leaders of the Humane Society of the US, People for the Ethical Treatment of Animals, Fund for Animals, and Mobilization for Animals spoke about animal rights and the need for restrictive legislation.

About 1,500 persons were reported to have marched to the regional primate center at Madison, WI, and several hundred attended a rally for animals rights in Atlanta.

It was announced at each demonstration site that vigils will be conducted next year on April 24 at all seven of the regional primate centers.

William A. Samuels, CAE

If Laws Are Needed APS Council Favors Proposal by Sen. Dole

The Council of the American Physiological Society at its meeting in April voted unanimously to favor the legislative proposal of Sen. Robert Dole (R-KS) should the Congress decide to enact laboratory animal reform legislation.

The Council said that it could support the Dole bill (S 657) if certain modifications are made in the proposal because the bill amends the current provisions of the Animal Welfare Act and therefores covers all of the animals involved in research and testing. The Council's primary concerns with the proposals by Rep. Doug Walgren (D-PA) and the 18-month study proposals by Rep. Edward Madigan (R-IL) and Sens. Orrin Hatch (R-UT) and Edward Kennedy (D-MA) are that they are too narrow as well as misdirected in scope by being limited, by and large, to the NIH-supported programs.

Following the Council's action and a report to the Society membership at the FASEB Annual Meeting in Chicago, the Society President Walter Randall, sent the following letter to Sen. Dole.

"If the Congress elects to enact legislation relating to the care and treatment of laboratory animals, the American Physiological Society would encourage the Congress to adopt the proposed 'Improved Standards for Laboratory Animals Act' (S 657) as the most constructive course of action at this time.

"It is the judgment of the Society's Council that S 657 provides a logical approach to ensuring the care and treatment of laboratory animals because it would amend current provisions of the Animal Welfare Act which has established standards for entities involved in biomedical and behavioral research that includes the use of animals.

"A similar legislative proposal, an amendment to the 'National Institutes of Health Renewal Authorization' (HR 1555), is limited in its scope to only those NIH-funded programs and, therefore, fails to address the entities which use the majority of laboratory animals.

"Enactment of S 657 would be complementary to the proposed 'Animal Welfare in Research Study Act 1983' (S 964) which would provide for an 18-month study of all the issues concerning the use of animals in research.

"Therefore, if legislation is to be enacted by the Congress, the American Physiological Society would support the passage of the 'Improved Standards for Laboratory Animals Act' at this time."

APS Statement on NIH Guide Revisions

The American Physiological Society presented the following statement at a public meeting on May 17, 1983 on the preparation of the sixth edition of the *Guide for the Care and Use of Laboratory Animals.* Helene Cecil, a research physiologist for the Agricultural Research Services of the US Department of Agriculture and Chairman of the Society's Committee on Animal Care and Experimentation, presented the statement to the Institute of Laboratory Animal Resources at the National Academy of Sciences.

"The American Physiological Society has 6,000 members. Approximately 4,000 members are teaching and doing research in physiology departments at medical schools, veterinary schools, and other educational institutions, and 2,000 members are scientists in research institutions.

"Physiologists are probably the users of the largest number of animals in academic research. The Society estimates that four out of every five physiologists use live animals. Cardiovascular, neurophysiology, endocrinology, and respiratory research use more than half of the animals, and each of these research areas will continue to require animals for research. Animals are also important in the teaching laboratories at the 126 medical schools and in the 284 academic institutions offering advanced degrees in physiology. A recent survey of 107 schools with physiology departments showed in 1979 that 90% had used classical experiments involving a dog, rat, or frog in their teaching laboratory. In 1982 the percentage using classical experiments had decreased to 66%.

This survey also showed that, of 600 teaching laboratory sessions, 284 sessions used vertebrate animals, 216 sessions were done on the students themselves, and 55 sessions used computers or physical models. When one considers that each year 14,000 medical students and 2,000 physiologists are graduated, the necessity for the use of animals as teaching models cannot be underestimated. Nor can the necessity for guidelines for the care and treatment of research animals be underestimated.

"The American Physiological Society recognizes that medical research must use animals for advancing knowledge, that these research endeavors must carry special considerations and concerns for the health and well-being of all research animals, and that the humane care of research animals is intrinsic to scientific excellence. Seventy years ago, in 1913, Dr. A. J. Carlson of the American Physiological Society proposed the following resolution.

" 'We, the members of the Federation of American Societies for Experimental Biology—comprising the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology—in convention assembled, hereby express our accord with the declaration of the recent International Medical Congress and other authoritative medical organizations, in favor of the scientific method designated properly animal experimentation but sometimes vivisection.

" 'We point to the remarkable and innumberable achievements by means of animal experimentation in the past in advancing the knowledge of biological laws and devising methods of procedure for the cure of disease and for the prevention of suffering in human beings and in lower animals. We emphasize the necessity of animal experimentation in continuing similar beneficent work in the future.

" 'We are firmly opposed to cruelty to animals. We heartily support all humane efforts to prevent the wanton infliction of pain. The vast majority of experiments on animals need not be and, in fact, are not accompanied by any pain whatsoever. Under the regulations already in force, which reduce discomfort to the least possible amount and which require the decision of doubtful cases by the responsible laboratory director, the performance of those rare experiments which involve pain is, we believe, justifiable.

" 'We regret the widespread lack of information regarding the aims, the achievements, and procedures of animal experimentation. We deplore the persistent misrepresentation of these aims, achievements, and procedures by those who are opposed to this scientific method. We protest against the frequent denunciations of self-sacrificing, high-minded men of science who are devoting their lives to the welfare of mankind in efforts to solve the complicated problems of living beings and their diseases.'

"This resolution was adopted by the Federation at its first meeting. To illustrate that concern for research animals is as applicable to animal research today as it was in 1913, in 1953, the Society adopted a set of guidelines for animal care and use which are called 'Guiding Principles in the Care and Use of Animals.' The American Physiological Society was the first Society in the United States to have guidelines for the use of research animals, and it was from this base that 10 years later the Institute of Laboratory Animal Resources developed the *Guide for the Care and Use of Laboratory Animals* for the National Institutes of Health (NIH). It is the intent of the American Physiological Society to keep its guidelines at the level of principles, and, therefore, its Guiding Principles do not include detailed standards for laboratory animals. Rather the Society's Guiding Principles rely on and refer to both the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act for operating standards. In addition to the Society's endorsement of the *Guide* in the Society's Guiding Principles, it also recognizes that the American Association for Accreditation of Laboratory Animal Care (AAALAC) uses the tenets of the *Guide* to accredit animal facilities. Approximately one-half of the medical schools are accredited by AAALAC. The current NIH laboratory animal guidelines to alleviate animal discomfort are well thought out and have been effective. The American Physiological Society endorses the concepts of the *Guide* and will propose specific coments in writing or orally at a future public meeting.

"During the past few years there has been an increased awareness within society in general about ethical issues affecting both humans and animals. The use of animals in research has not been an exception. The American Physiological Society applauds the efforts of the Committee to determine today's requirements for research animals and to assure that the *Guide for the Care and Use of Animals* will continue to define the standards for the humane care and use of research animals."

APS Council Minutes 1913
Trends in Physiology Teaching Laboratories for Medical Students—1982, see p. 148.

GUIDING PRINCIPLES IN THE CARE AND USE OF ANIMALS

Approved by the Council of The American Physiological Society

Animal experiments are to be undertaken only with the purpose of advancing knowledge. Consideration should be given to the appropriateness of experimental procedures, species of animals used, and number of animals required.

Only animals that are lawfully acquired shall be used in the laboratory, and their retention and use shall be in every case in compliance with federal, state and local laws and regulations, and in accordance with the NIH Guide.

Animals in the laboratory must receive every consideration for their comfort; they must be properly housed, fed, and their surroundings kept in a sanitary condition.

Appropriate anesthetics must be used to eliminate sensibility to pain during all surgical procedures. Where recovery from anesthesia is necessary during the study, acceptable technique to minimize pain must be followed. Muscle relaxants or paralytics are not anesthetics and they should not be used alone for surgical restraint. They may be used for surgery in conjunction with drugs known to produce adequate analgesia. Where use of anesthetics would negate the results of the experiment such procedures should be carried out in strict accordance with the NIH Guide. If the study requires the death of the animal, the animal must be killed in a humane manner at the conclusion of the observations.

The postoperative care of animals shall be such as to minimize discomfort and pain, and in any case shall be equivalent to accepted practices in schools of veterinary medicine.

When animals are used by students for their education or the advancement of science, such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research.

Hunting the Wild Vagus

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It is a particularly rewarding experience for me to be selected as the recipient of the Carl J. Wiggers Award this year. I was fortunate enough to have Dr. Wiggers as my physiology professor when I was a medical student at Western Reserve University. Furthermore, after graduation, an internship, and some Army service, I joined Dr. Wiggers' staff during the five years that preceded his retirement in 1953. Dr. Wiggers was very paternal to the members of his department. We were a very closely knit "family," and he was an inspiration to us all.

The outrageous title for my address to the Cardiovascular Group was suggested by a passage in McDowall's book, "The Control of the Circulation of the Blood," which was published in 1938 (14). The author noted that the inhibitory effects of the vagus nerves on the heart were first demonstrated by the Weber brothers in 1845. He then proclaimed that "since that time the vagus has been the *happy hunting ground*[1] of generations of physiologists." I shall now recount some of my adventures during the past two decades in that happy hunting ground.

The story begins with a study that Berne, De Geest, and I did in 1965 on the neural control of the coronary circulation (1). One of the provocative results of that study is illustrated in Figure 1. We used an electromagnetic flowmeter to monitor the left main coronary artery of an anesthetized dog. In confirmation of previous findings, coronary arterial inflow was maximal during diastole, and it decreased during systole, because the cardiac contraction increased the extravascular compression of the intramural coronary vessels. During vagal stimulation (horizonal bars), the principal change in the pattern of coronary arterial inflow in the paced heart was a pronounced attenuation of the systolic reduction in flow. Hilaire De Geest, educated in Belgium, had the audacity to conclude that the extravascular compression must have been diminished by the vagal stimulation, and hence that vagal stimulation exerted a negative inotropic effect on the canine ventricles. Such an interpretation was heretical to Berne and me, both having been educated in the United States and both being disciples of Carl Wiggers! Did not the fifth edition of Wiggers' textbook (16) state that "the evidence seems indisputable that the vagus nerves do not directly affect the ventricles"? There appeared to be crucial differences between the European and American views of the innervation of the heart. Consequently, we decided to pursue this question further, despite the conviction of the American contingent that some other mechanism must account for the vexatious response displayed in Figure 1.

At the time of these coronary circulation studies, De Geest and I had also been using an isovolumetric left ventricle preparation to study certain cardiac reflexes. It struck us that this type of preparation would be ideal for detecting any vagal effect on ventricular contractility. An anesthetized dog was placed on total heart bypass, and a balloon with a fixed volume of saline was inserted into the left ventricle. Heart rate, preload, and the coronary circulation could all be rigorously controlled. We could assess myocardial contractility by recording the pressure generated within the fluid-filled balloon in the left ventricular cavity.

The results of these experiments (2) immediately, consistently, and convincingly confirmed De Geest's bold hypothesis. A representative tracing from these experiments is shown in Figure 2. The right cervical vagus nerve was stimulated supramaximally at various frequencies from 1 to 20 Hz. The resultant changes in heart rate induced in the unpaced heart are shown in the top half of the figure. When the heart was paced at a constant rate, the same vagal stimulations evoked the changes in left ventricular pressure that are displayed in the bottom half of the figure. Vagal stimulation did indeed elicit an appreciable frequency-dependent negative inotropic effect on the left ventricle!

A clue to why certain earlier investigators may not have detected the depressive effects of vagal activity upon the mammalian ventricles was provided by the experiments of Hollenberg et al. in 1965 (4). When these investigators infused acetylcholine (ACh) directly into a major coronary artery in an open-chest dog, they observed only a slight reduction in left ventricular contractile force. However, when the background level of adrenergic ac-

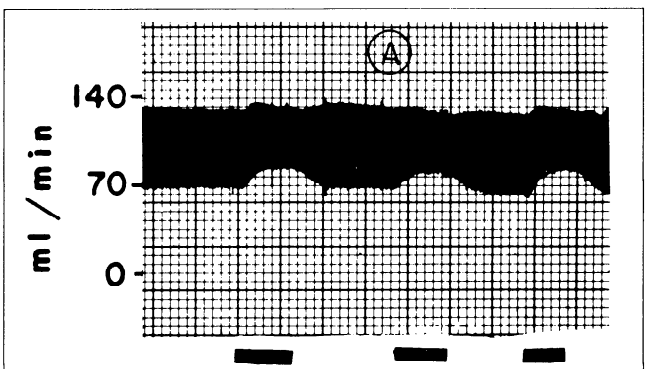

Figure 1
Effect of vagus nerve stimulation (*bars*) on phasic flow (ml/min) in left main coronary artery in an empty dog's heart paced at a constant rate. Left vagus nerve was stimulated supramaximally (15 V) at a frequency of 50 Hz. [From Berne et al. (1).]

The 1983 Wiggers Award Lecture was given at the annual banquet of the APS Cardiovascular Section at the FASEB Spring Meeting in Chicago.

[1]Italics added by the author.

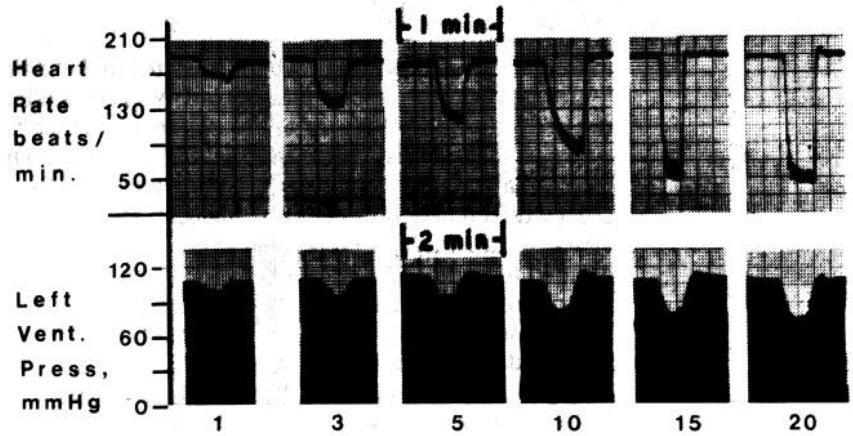

Figure 2

Effects of efferent vagal stimulation on heart rate and left ventricular pressure in a canine isovolumetric left ventricle preparation. In *top tracings*, the heart was beating spontaneously. In *botton tracings*, the heart was paced at a rate of 210 beats/min. *Numbers* at the *bottom* of figure denote frequency (Hz) of vagal stimulation. Order of applying the various frequencies was randomized. [Modified from De Geest et al. (2), by permission from the American Heart Association.]

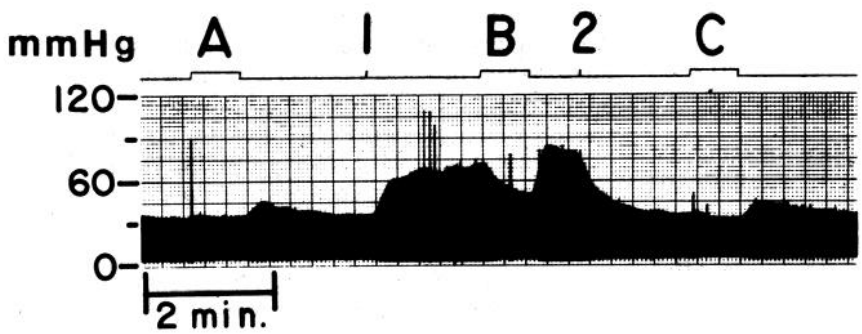

Figure 3

Changes in left ventricular pressure (mmHg) elicited by supramaximal vagal stimulation at a frequency of 20 Hz before (A), during (B), and after (C) stimulation of left stellate ganglion at 2 Hz (between marks 1 and 2) in a canine isovolumetric left ventricle preparation. [From Levy (6).]

tivity was augmented either by sympathetic nerve stimulation or by norepinephrine infusion, the same ACh infusion evoked a much greater depression of ventricular contractile force. Perhaps vagal stimulation as well has only a relatively weak effect on the ventricular myocardium unless an appreciable background level of sympathetic activity also prevails.

These suspicions were confirmed by a series of experiments in which we used vagal stimulation instead of ACh infusion as the cholinergic intervention (6,11). In the absence of concurrent sympathetic stimulation, trains of supramaximal vagal stimulation (A and C) evoked a barely detectable effect during stimulation (Figure 3). However, when the same vagal stimulus (B) was given during concomitant sympathetic stimulation (between marks 1 and 2), the vagal stimuli elicited a pronounced reduction in peak left ventricular pressure. In fact, the vagal stimulation almost completely counteracted the facilitatory effect produced by the sympathetic stimulation.

We also found that similar adrenergic-cholinergic interactions prevail in the neural control of heart rate (12). We applied various combinations of cardiac sympathetic and vagal stimulation in a random sequence in open-chest, anesthetized dogs. The overall chronotropic responses to such combined sympathetic and vagal stimulation in a group of 10 dogs are shown in Figure 4. The upper left edge of the response surface indicated that raising the frequency of sympathetic stimulation from 0 to 4 Hz evokes a substantial positive chronotropic response when there is no background vagal activity ($V = 0$). However, when the vagal activity is substantial ($V = 8$), the same change in sympathetic activity elicits a very weak positive chronotropic response. This accentuated antagonism is reflected by the lower right edge of the response surface.

The accentuated vagal-sympathetic antagonism is mediated by mechanisms that occur at two levels with respect to the neuroeffector junctions in the heart (5,9). At the prejunctional level, the ACh released by vagal endings acts on muscarinic receptor sites on sympathetic nerve endings to diminish the rate of norepinephrine release from those nerve terminals (7). At the postjunctional level, the adrenergic and cholinergic neurotransmitters act on their respective receptors on the cardiac effector cells. The resultant interactions involve changes in cyclic AMP and perhaps also cyclic GMP (15).

In the course of our studies on the autonomic interactions involved in the control of heart rate (Figure 4), a peculiar phenomenon was often observed, particularly

Wiggers Memorial Award

The Wiggers Award was established in 1965 as a memorial to Carl J. Wiggers, twenty-first president of APS. It is awarded annually by the Cardiovascular Section of APS to recognize research contributing to a better understanding of cardiovascular physiology in health and disease. The recipient presents a lecture at the annual meeting of the Section and is awarded a commemorative plaque.

Recipients

F. J. Haddy, 1966
L. N. Katz, 1967
E. H. Wood, 1968
D. E. Gregg, 1969
S. J. Sarnoff, 1970
J. R. Pappenheimer, 1971
A. C. Guyton, 1972
M. B. Visscher, 1973
J. H. Comroe, 1974
R. M. Berne, 1975
J. O. Davis, 1976
D. Bohr, 1977
J. T. Shepherd, 1978
W. C. Randall, 1979
E. Braunwald, 1980
P. C. Johnson, 1981
A. C. Barger, 1982
M. N. Levy, 1983

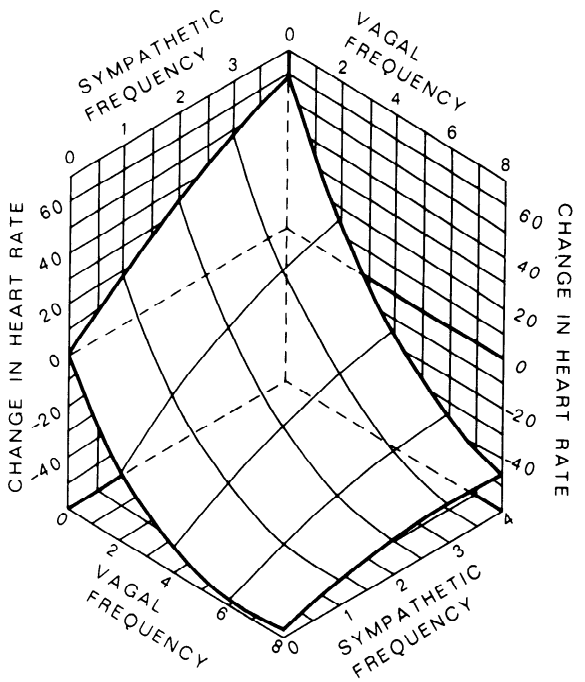

Figure 4

Changes in heart rate (beats/min) evoked by a variety of combinations of vagal and sympathetic stimulation. Response surface represents composite data from a group of 10 anesthetized dogs. [From Levy and Zieske (12).]

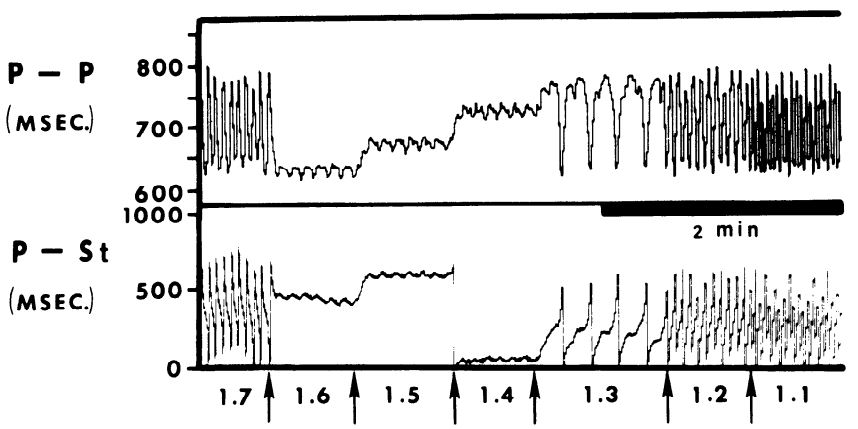

Figure 5

Changes in cardiac cycle length (P-P) and in time from beginning of atrial depolarization to beginning of vagal stimulation (P-St) in an anesthetized dog during vagal stimulation at frequencies (in Hz) denoted by numbers between *arrows*. [Modified from Levy et al. (10), by permission of the American Heart Association.]

when we used relatively low vagal stimulation frequencies. An example of such a puzzling response (10) is shown in Figure 5. At certain vagal stimulation frequencies (1.7, 1.3, 1.2, and 1.1 Hz), the cardiac cycle length (P-P interval) varied rhythmically, whereas at other frequencies (1.6, 1.5, and 1.4 Hz), the oscillations were markedly damped.

What was even more perplexing, the changes in cardiac cycle length were just the opposite of what one would expect from stimulating an inhibitory nerve. As the stimulation frequency was lowered from 1.6 to 1.5 and then to 1.4 Hz, the cardiac cycle length actually increased. Reducing the frequency of stimulation of an inhibitory nerve should result in less inhibition, and hence it should evoke a reduction in cycle length (i.e., an increase in heart rate).

When we converted the cardiac cycle lengths to heart rates, we discovered that heart rate was precisely equal to vagal stimulation frequency. Hence, the vagal neural activity appeared to synchronize the activity of the automatic cells in the sinoatrial (SA) node, such that they fired with each burst of vagal activity. However, the time delay between the vagal stimulus and the pacemaker firing varied, depending on the frequency of stimulation. It is evident in Figure 5 that the time delay (P-St interval) between the onset of artial depolarization (P wave) and the vagal stimulus (St) changed as the frequency of stimulation was varied from 1.6 to 1.5 and then to 1.4 Hz.

This change in the phase relationship of the vagal stimuli to the cardiac cycle (i.e., the P-St interval) is the key to the paradoxical heart rate response. Presumably, more ACh is released each minute when the vagi are stimulated at 1.6 Hz than when they are stimulated at 1.4 Hz. Our studies (10) have shown that when the vagi are stimulated at 1.6 Hz, the timing of the SA nodal discharge is automatically adjusted so that the vagal impulses arrive during a phase of the cardiac cycle at which the pacemaker cells are not very responsive. At a lower stimulation frequency (e.g., 1.4 Hz), on the other hand, the timing is adjusted so that the impulses arrive during a phase of the cycle at which the automatic cells are much more responsive. Thus the negative chronotropic response is greater even though there is less ACh released at this lower stimulation frequency. These shifts in the phase of stimulation are indicated in Figure 5 by the changes in the P-St interval.

Under natural conditions, efferent vagal activity to the heart occurs in pulse synchronous bursts. When we stimulate the vagus nerves with bursts of pulses that mimic the natural activity, this tendency for bursts of vagal impulses to synchronize SA nodal activity is markedly exaggerated (8). Under such conditions, the

paradoxical response noted above can be observed over the entire range of normal heart rates.

The phase-dependent effects of vagal activity on heart rate have been demonstrated to occur reflexly during excitation of the carotid sinus nerves in dogs (13) and during baroreceptor excitation in human subjects (3). Considerably more work needs to be done, however, to establish the physiological role of this perplexing phenomenon. It is highly likely, therefore, that the study of the vagal control of the heart will continue to be the "happy hunting ground" of many more generations of physiologists.

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Departmental Histories

Department of Physiology
Meharry Medical College
Nashville, Tennessee 37208

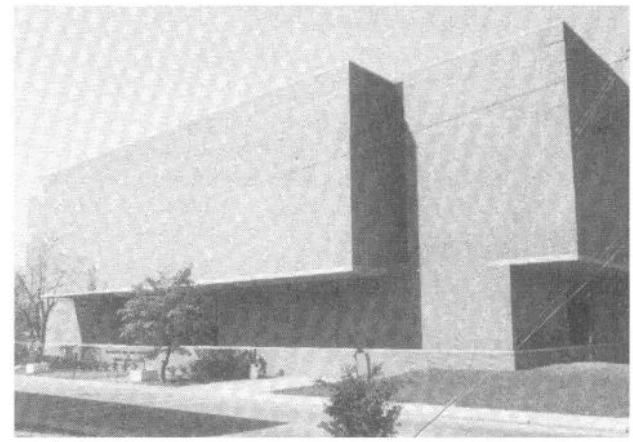


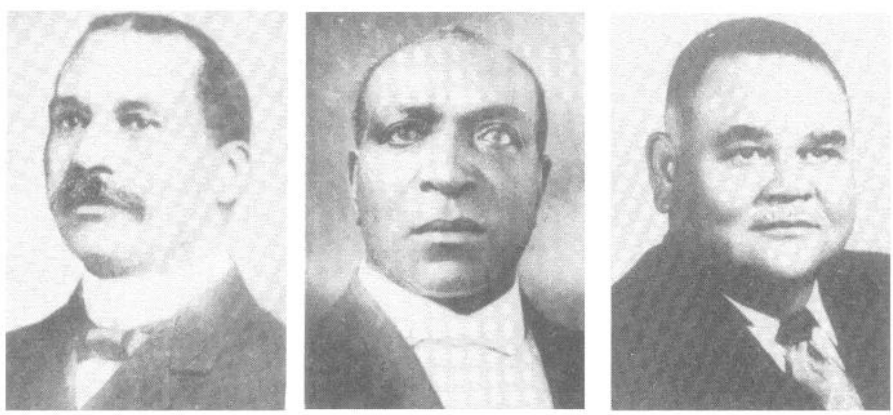
Figure 1

Meharry Medical College was founded in 1876 as the Medical Department of Central Tennessee College of Nashville. Later, a Dental Department, Pharmacy Department, and School of Nursing were added. In 1915 Meharry became an independent professional college with these four components as schools.

T. O. Summers was appointed the first Professor of Physiology in 1881 and served for one year. Between 1882 and 1899, four individuals served in this capacity with short tenures of one to four years. A notable exception was R. F. Boyd (M.D., Meharry Medical School, 1882; D.D.S., Meharry Dental School, 1887), who was Professor of Physiology from 1883 to 1892. Boyd went on to become Professor of Gynecology and Clinical Medicine, to establish the Mercy Hospital of Nashville in which the School of Nursing was born, and to help found the National Medical Association. J. A. Lester (M.D., Meharry, 1895) was Professor of Physiology from 1899 to 1930. To this point the college catalogue describes the physiology curriculum as "Daily recitations are required for two sessions."

In 1930 Daniel Thomas Rolfe (M.D., Meharry, 1927) returned to Meharry as Professor of Physiology after two years of postdoctoral study with Dr. A. J. Carlson at the University of Chicago. In 1936 Physiology attained the status of a division, and in 1938 the Department of Physiology and Pharmacology was established with Rolfe as chairman. Pharmacology became a separate department in 1952, and Rolfe continued as chairman of the department until 1968. During 14 of the latter years he was also dean of the School of Medicine. During Rolfe's tenure, half of the physiology course consisted of basic concepts taught by the small departmental staff and half was applied physiology with a significant portion taught by clinical faculty members and residents. Laboratory experiments evolved from classical physiology with kymographs and smoked paper to the era of modern instrumentation. Departmental research was conducted largely by Professor Landry E. Burgess. Burgess joined the staff in 1948 after receiving his Ph.D. under Dr. Joseph Bodine at the State University of Iowa and was the first staff member to hold that degree. The department awarded several masters degrees during this period. In the 1967-1968 school year, the department had several visiting professors supported by the Porter Development Committee of the American Physiological Society.

After Rolfe's death in 1968 Dr. Burgess served as acting chairman until Clem Russ (Ph.D., University of Pittsburgh, 1952) was appointed chairman in 1969. Russ had been a visiting scientist in the Mayo Clinic Department of Physiology with Dr. Earl Wood. Russ immediately became one of the main forces leading to construction of the spacious Harold D. West Basic Science Building (Figure 1), occupied in 1974. During his twelve-year tenure as chairman, the staff grew to an average size of nine members, and James Townsel (Ph.D., Purdue, 1968) attracted the department's first postdoctoral fellows. Also under Townsel, Eric Thomas became the first person to earn the Ph.D. degree with all of his



Left to right: R. F. Boyd, J. A. Lester, D. T. Rolfe.

research done in the Department of Physiology, although the degree was actually awarded by the Department of Biochemistry. Dr. Thomas joined the departmental staff in 1982 after completing his neurobiology postdoctoral training at Harvard.

In 1981 a college-wide emphasis on academic renewal was initiated, and Joseph M. Stinson (M.D., Meharry, 1964) was appointed chairman of the department. Stinson had taken postdoctoral physiology training under Dr. A. Clifford Barger at Harvard and training in pulmonary diseases at Vanderbilt and had most recently served as Director of Meharry's Division of Pulmonary Diseases. During his short tenure to date, several new faculty members have been recruited, resulting in improved teaching for medical and dental students and significant improvement in research productivity. The department is now planning to reactivate graduate programs in physiology.

Joseph M. Stinson

Department of Physiology Northwestern University Medical School Chicago, Illinois 60611

Northwestern University Medical School (NUMS) traces its origin to its inception as Rush Medical College (1837) through periods as the Medical Department of Lind University (1859) and as a business partner with Northwestern University in Evanston (1870) to the occupancy of a new building on S. Dearborn Street in Chicago in 1893. The history of the Department of Physiology begins with the appointment of the first Nathan Smith Davis Professor and Chairman, W. S. Hall, in 1894.

Dr. Hall, Ph.D., M.D., trained under Ludwig at Leipzig. His first NUMS class numbered 86 students. Physical facilities included a general laboratory measuring 40 by 70 feet, two "special laboratories," an instrument room, and a library. Laboratory equipment, described in the Medical Bulletin and said to have cost "thousands of dollars," included microscopes, kymographs, electrical apparatus, and hemocytometers.

The department was budgeted a full-time instructorship in 1906. W. K. Jaques, Ph.D., M.D., assisted with the teaching until 1910, to be succeeded by C. J. Kurtz, A.M., Ph.D., until 1913. Four medical students per year assisted in the laboratory. One of the students listed in 1917 was Loyal Davis, later a premier neurosurgeon and stepfather-in-law of Ronald Reagan.

Roy G. Hoskins, Ph.D., joined the department as an Associate Professor in 1913. Dr. Hoskins trained with Cannon at Harvard, publishing studies on the thyroid and adrenal glands in the *American Journal of Physiology* (*AJP*) from 1908 to 1916. He published studies on vasomotor effects of various endocrine manipulations from Starling-Ohio Medical School in 1912-13 and from NUMS after 1914. Dr. Hoskins became Professor and Chairman of the Physiology Department in 1917, although both Hall and Hoskins shortly went on leave in the National Service. Neither returned to NUMS.

The Physiology Department was under the guidance of Dr. F. C. Becht, Ph.D., M.D., and Professor of Pharmacology from 1918 until he resigned in 1922.

Dr. L. R. Dragstedt was appointed Chairman of Physiology and Pharmacology at NUMS in 1923. He had worked in Carlson's laboratory at the University of Chicago (1917) and the State University of Iowa (1918) and had returned to the University of Chicago prior to coming to NUMS. His early published reports in *AJP* included studies on intestinal obstruction, extirpation of the duodenum, parathyroid tetany, and parabiosis. Dr. Dragstedt resigned his NUMS position in 1925, to be succeeded by A. C. Ivy as Chairman of Physiology and Pharmacology.

Ivy completed his Ph.D. in Carlson's department at the University of Chicago in 1918. He was an instructor in Physiology at the University of Chicago and an Associate Professor of Physiology at Loyola University School of Medicine while continuing his training at Rush Medical School (M.D. degree in 1922). Ivy then served as an associate professor of physiology at the University of Chicago from 1923 until coming to NUMS in 1925. By this time he had published numerous proceedings, reports, and papers in *AJP*. Ivy first appeared as the author of three reports in *AJP*, volume 49, 1919, on labyrinthine nystagmus, gastric secretion, and experimental ulcers, respectively. Early studies from Loyola and from the University of Chicago identify him as a pioneer in the area of experimental gastrointestinal surgery.

Drs. Ivy and C. A. Dragstedt (Professor of Pharmacology and brother of L. R. Dragstedt) and students moved to the new location of NUMS in the Montgomery Ward Memorial Building, Chicago, in 1926. The Physiology Department occupied the entire fifth floor, which housed a lecture room, 10 student laboratories, research laboratories, offices, animal quarters, and an animal surgery room. Physiology and Pharmacology became separate departments in 1942, with Ivy and Dragstedt as the respective chairman.

During Ivy's chairmanship, 1925-46, the number of students registered for physiology research credit in the Northwestern University Graduate School, Evanston, ranged from 20 to 50 per year. Of a total of 281 registered students, 95 received an M.S. degree, 24 a Ph.D. degree, and 23 earned both degrees, comprising totals of 118 M.S. and 47 Ph.D. degrees. Seventy-three thesis projects dealt with gastrointestinal studies—motor, secretory, or absorptive activities, or liver or gallbladder function. Nineteen endocrine, 15 hematology, 14 cardiovascular, and 7 obstetrics-gynecology thesis studies were completed. One study on altitude exposure and another on explosive decompression constituted an approach to respiratory physiology. Renal and neurophysiological topics were investigated in a total of 7 projects. Dr. Carl Dragstedt and his associates directed 16 pharmacological dissertations.

In addition to the registered graduate students, members of clinical departments were credited with "Summer Research" in the 1930's. For example, 15 names from Medicine, Neurology, Experimental Surgery, or Ophthalmology appear on the 1931 Summer Roster, including one "J. R. Miller," later Dean of NUMS and President of Northwestern University.

A coveted achievement for an individual who was engaged in research in Ivy's department was to be elected to the American College of Dog Surgeons. The motto of this society, invented by Carl Dragstedt, was "No Flea Spitting." Dr. Ivy's policy was to put nearly everyone to work who expressed an interest. If the interest persisted

and was augmented with adequate effort, a degree and/or publications usually resulted.

Dr. Ivy was the first Scientific Director of the Naval Medical Research Institute at Bethesda, MD, in 1942-43. Ivy commuted weekly between Washington and Chicago. Usually with little advance warning, Drs. Gray, Smith, Freeman, or Fredric Jung were called upon to deliver "Ivy's lectures" to the medical students.

Dr. J. S. Gray was appointed Chairman of the Department of Physiology in 1946, shortly after Dr. Ivy accepted the position of Vice President of the University of Illinois and director of the Medical Center in Chicago. Gray had risen from the ranks in the Physiology Department at NUMS as a graduate student (1933-36), instructor, assistant, associate, and full professor. He spent the war years as a civilian physiologist at Randolph Field, TX. His previous experience as a gastrointestinal physiologist little prepared him to teach respiratory physiology to Air Force personnel. In the process of educating himself his mental circuits produced his "multiple factor theory" of the chemical control of ventilation. Gray returned from Randolph Field to NUMS in 1945 and was appointed a full professor. Gray was completing his last required clerkship to earn his M.D. degree when Ivy left Northwestern University and he was appointed Chairman.

In contrast to Ivy, Gray followed a policy of close supervision of the research training of a limited number of graduate students. During Gray's chairmanship, 1946-1970, an average of 8 students were registered for research each year. A total of 80 students completed 67 degrees: 30 M.S., 11 Ph.D., and 13 M.S. plus Ph.D. dissertation projects included 17 on respiratory physiology, 20 on endocrinology and related intermediary metabolism, 13 on neurophysiology, 9 on gastroenterology, and 7 on the cardiovascular system. Research interests of 8 departmental faculty members, headed by John Gray and Fred Grodins, were distributed among these areas.

Dr. Gray resigned from his chairmanship in 1970 and retired from the faculty two years later. Gray was succeeded as chairman by Oscar Hechter. Hechter earned his Ph.D. in Biochemistry at the University of Southern California. He played a major role in the elucidation of steriod hormone synthesis by the adrenal cortex while at the Worcester Foundation. He came to NUMS from the AMA Institute for Biomedical Research in Chicago. Biochemical endocrinology was the major area for thesis research during Hechter's chairmanship, 1970-79. Two M.S. and eight Ph.D. theses were completed. Six additional faculty joined the department during Hechter's chairmanship.

Upon the resignation of Dr. Hechter, James C. Houk was appointed Professor and Chairman of the Department of Physiology. Dr. Houk earned his Ph.D. at Harvard Medical School and came to NUMS from Mountcastle's department at Johns Hopkins. Houk's research addresses the general problem of understanding the neural mechanisms whereby sensory information is used to control movement and posture. Newly recruited faculty are also conducting research on neural aspects of endocrine, respiratory, cardiovascular, and gastrointestinal activities, all making use of extensive computer technology.

The departmental physical plant has increased in size since the completion of the Ward Building. The adjoining Morton Research and Searle Buildings were completed in 1955 and 1966, respectively. At present the department occupies about 12,000 square feet of space, 7,500 of which are research laboratories for individual faculty.

The current departmental roster includes 16 full-time faculty members. Two of these have their major appointment in clinical departments and have research laboratories in divisions of the Departments of Surgery and Medicine. Six faculty with primary appointments in Physiology have joint appointments in clinical departments. One faculty member has a joint appointment in the Division of Biomedical Engineering on the Evanston campus. All 16 faculty members share in the various teaching assignments.

The physiology course content currently taught to medical students compares with that taught in 1925 as the 14th edition of Mountcastle's *Medical Physiology* compares with the 4th edition of Starling's *Human Physiology*. Superimposed on this gradual metamorphosis from a descriptive to an analytical discipline have been changes in placement of physiology in the medical curriculum and in the number of lecture and laboratory hours. Physiology demanded one-third of the students' time for three 10-week quarters, with one or two mammalian laboratory sessions every week, until 1957. For the next 10 years, all of physiology was taught in the third quarter of the freshman year, with laboratory work reduced to 10 sessions. Neurophysiology became part of a first-year interdisciplinary neuroscience course in 1966. The remainder of physiology was taught in the first quarter of the sophomore year. Laboratory exercises were discontinued except as an elective, which was chosen by about six students per year.

Currently, physiology lectures are given in the second and third quarters of the freshman year, overlapping with biochemistry and neuroscience. Small group conferences designed to augment lectures were instituted in 1972, and limited mammalian laboratory work, a cardiovascular and a respiratory exercise, was added in 1978.

Northwestern University Dental School maintained a Department of Physiology and Pharmacology until 1965, when it was incorporated into the then-separate Medical Physiology and Pharmacology Departments. Since 1965, except for a two-year trial of combining medical and dental classes in 1979-80, separate physiology courses have been presented to medical and dental students. The dental class has had a stable size of about 115 students. From 1926 until 1972 the medical class contained about 130 medical students plus up to 30 graduate students from various basic science departments on the Chicago campus. The medical class was expanded to 160 students in 1972.

From 1925 up to the present the faculty of the department of physiology has taught appropriate physiology courses to student nurses, dental hygienists, physical therapists, and biomedical engineering undergraduate students.

Much of the information given in this account was gleaned from Bulletins of the University (1890-1950), *AJP* (volumes 1-60), and registration and grade records kept by the Departments of Physiology since 1925. Lists of faculty (1894-1982) and graduate students (1925-1980) have been deposited in the archives of the APS.

John H. Annegers

Fifty-Sixth President of APS

Alfred P. Fishman of the University of Pennsylvania has been elected President of the American Physiological Society effective July 1, 1983.

Dr. Fishman is internationally known for his research on the pulmonary circulation, the control of breathing, and the interplay between the respiration and circulation. He is the William Maul Measey Professor of Medicine at the University of Pennsylvania, having served as Associate Dean for Research from 1969 to 1976. The extent of his research is extremely broad, ranging from comparative physiology to the integrating response of intact animals and humans. He has published over 260 papers on the various research problems and is also the author or editor of eight books and monographs. After completing his medical residency and a fellowship in pathology, Dr. Fishman embarked upon an extended program of training and research in circulatory and respiratory physiology, first as a fellow and subsequently as an Established Investigator of the American Heart Association. The experience was not only broadly based but extended to a variety of mentors and institutions. His mentors included L. N. Katz at Michael Reese Hospital, Homer W. Smith at New York University, Cournand and Richards at Bellevue Hospital, Landis and Pappenheimer at Harvard, and Dawes at Oxford.

Following these initial experiences in physiology, Dr. Fishman assumed the directorship of the Cardiopulmonary Laboratory at the Columbia Presbyterian Medical Center, where he contintued until 1966 when he succeeded Dr. Katz as Director of the Cardiovascular Research Institute at the Michael Reese Hospital and became Professor of Medicine at the University of Chicago. He moved to the University of Pennsylvania as Head of the Cardiovascular-Pulmonary Division in 1969 and was designated as the William Maul Measey Professor of Medicine in 1972.

Dr. Fishman's connections with the American Physiological Society are long-standing and his contributions have been most notable with respect to its publications. He served as a member and then Chairman of the Handbook Editorial Committee (1967-1972). He was the Editor of "Physiology in Medicine," which appeared as a monthly component of the *New England Journal of Medicine* (1970-1978). He was Chairman of the Publications Committee from 1974 to 1981 and is currently the Editor of the *Journal of Applied Physiology*.

Dr. Fishman has served the National Institutes of Health in various capacities ranging from member of study sessions and special task forces to membership on the National Advisory Heart-Lung Council and then on the Pulmonary Advisory Council.

His contributions to physiology have been recognized not only by membership on editorial boards of scientific publications but also by lectureships in this country and abroad. He served the American Heart Association as co-chairman of its Research Committee and is currently a member of the Health Policy Board of the Institute of Medicine, National Academy of Sciences. He is currently a member of the Board of Directors of the Philadelphia Zoological Gardens and of the American Associates of the Ben Gurion University.

Dr. Fishman is deeply concerned about future directions in physiology and was instrumental not only in reorganizing the journals of the American Physiological Society and in initiating the Clinical Physiology Series but in extending the traditional areas into cell physiology and integrative physiology. These interests are reflected in the current journals of the Society. He has also played a large part in attempting to promote interplay and communications within the American Physiological Society. Finally, he is committed to greater participation of the American Physiological Society in international exchanges and endeavors.

APS Election Results

Dr. **John B. West**, Chief, Physiology Section, University of California Medical School, San Diego, was elected President-Elect. The two new Councillors are Dr. **Howard E. Morgan**, Chairman, Department of Physiology, Pennsylvania State University, Hershey, for a four-year term and Dr. **Norman C. Staub**, Cardiovascular Research Institute, University of California, San Francisco, to complete Dr. West's term expiring in 1985.

APS President-Elect's Tour

From Whence Cometh Future Physiologists?

I have long-standing interests in why and how people choose a career in physiology. APS membership is almost equally divided between M.D. and Ph.D. degree holders. What attracts quality physicians into physiology? Why did many of the brightest undergraduate science majors enter the profession in the 1950s and 1960s and why has such recruitment declined during the 1970s and 1980s? Were federal training grants that important in attracting young people into biomedical science? Does the severe cutback in such programs account for the present deficit, not so much in quantity, but in quality of applicants? How important was the immediately postwar research into high-altitude physiology, oxygen lack, antigravity and low temperature, space medicine, and stress of all kinds? Similarly, how significant were developments of the National Institutes of Health, National Science Foundation, National Aeronautics and Space Administration, and other federally supported research agencies? Are today's undergraduate science majors convinced all research problems have been solved? If so, who gave them that impression?

The cynical think immediately of differences in financial rewards in medical practice vs. teaching-research careers as primarily responsible for the greater popularity of medicine. But such discrepancies have always existed, and I personally doubt this is as important as many believe. Certainly, the highly publicized deficits in jobs and research funding contribute importantly, but here again, many of us remember our excitement for such careers during the depths of the depression of the 1930s when jobs were also scarce and external research funding was hardly known.

It became clear quite early in my tour that simple answers to my questions were not forthcoming, and I decided to conduct my own survey among undergraduate science majors and basic science graduate students, together with their teachers. I wrote to a number of college science departments within convenient travel distance from Chicago and offered to visit their campuses for a research seminar and to discuss careers in physiology and the basic medical sciences with their science majors. I also propositioned each medical school physiology professor who invited me to present a seminar that I would come *only* if he would arrange for me to spend some informal discussion time with the department's faculty and graduate students. When I talked with the college science majors, I asked what they knew about physiology and how much their major professors had told them about it as a potential career. Then, in separate conversations, I asked the science faculty how they advised their students and how they acquired their information, and I attempted to assess how up-to-date their information was.

Following is a table of institutions visited. In several instances I was able to visit both undergraduate and graduate divisions within the same institution.

Undergraduate	Graduate
North Illinois University (NIU)	
Oral Roberts	Oral Roberts
(NIU students visited lab)	Northwestern University
St. Louis University	St. Louis University (canceled)
George Williams	Chicago Medical School
(Wheaton students in lab)	
Dalhousie University	Dalhousie University
Taylor University	
(Taylor students in lab)	University of Georgia
Wheaton College (canceled)	
Loyola University	
	Uniformed Services (scheduled)
	University of Kansas (scheduled)

An invitation was generally issued during my visit for interested faculty and students to visit our own research laboratories at which time we would put them into scrub suits and let them actually *participate* in a day of research. Several college teachers responded positively to the suggestion, and such visits are indicated in the table. (Incidentally, we have recruited several of our best students via this device.) The students invariably react strongly and favorably to the experience.

I am convinced the best college training for a career in physiology is based in substantial course backgrounds in chemistry, physics, and mathematics, so I generally requested opportunity to chat with faculty and students from these departments as well as biology. To achieve rapport, we sat around a coffee table or in as informal arrangement as possible. My objective was primarily to obtain information; I listened carefully to many different opinions and points of view. Inviting questions and encouraging exchange of ideas and information, I worked as many of the following kinds of questions into the conversations as possible.

Interactions with Students

How much do you know about physiology? Can you define what physiologists do? Have you had a course(s) in physiology? What was its primary emphasis and orientation? What text did you use? Can you differentiate between organ-system, comparative, general, or cellular physiology? Are you *aware* of career opportunities in physiology? Do you separate, in your mind, a career in medicine from one in physiology? Do you have an impression of how research in medicine is accomplished? Who does this research? Have you considered the possibility of a career in such research (in physiology or medicine)? To whom do you turn in seeking advice concerning career opportunities? Do you know a physiologist among your personal friends or acquaintances? How many physicians do you know well? Have you seen any literature describing careers in physiology? Are there brochures or announcements of graduate school openings on your departmental bulletin board? Have they seemed attractive? Have you heard physiologists speak about their research and/or teaching in your departmental seminars or science club meetings? Do you consider any of your teachers to be professional physiologists? Do careers in biochemistry, pharmacology, neuroscience, or other basic science disciplines seem more exciting to you? Do you feel molecular biochemistry is where the action is? Do you see any connections with engineering? What image has your faculty advisor painted of graduate training? Have you talked with him about opportunities of going to

medical school? Does he point out differences between graduate and medical school? How does he compare qualifications for admission? How attractive does he paint opportunities in research and teaching in comparison with practice of medicine? Do you know anyone who has applied for admission to graduate school? Do you know any who have been accepted? Rejected? Have you taken or are you considering taking GRE and/or MCAT examinations?

Conversations with Faculty

What has changed to make graduate school (which was so attractive to bright students 20 years ago) so relatively unattractive today? How important is the apparent deficit in jobs for Ph.D.'s? Is lack of research funding a major deterrent? How well informed do you feel you are about the breadth and depth of job markets for newly graduating Ph.D. scientists? What advice do you give your science majors concerning careers in basic medical sciences? Do students ever inquire specifically about training and careers in physiology? Do you feel students know about opportunities in physiology? Do they have substantial knowledge of research in distinction from practice of medicine? Do they know who actually does research? How important are the relative financial returns in teaching-research compared with practice of medicine in the student's career selection? If incomes in medicine and basic science were equivalent, would careers in teaching and research be more attractive? Other than his science teachers in college, what other sources are available to the student to acquire information? Where do you get your information regarding graduate school recommendations? Do you belong to the professional society of your discipline? Are you or are any of your institution's faculty members of APS? Would you like to belong to APS? Would you be willing to pay current dues for Regular membership in APS? Do you feel qualified for Regular membership in APS? For Associate membership? Is there need for Affiliate membership? Do you have access to *The Physiologist*? In your opinion are there too many Ph.D. training programs in physiology? Are too many physiologists being trained? Are some Ph.D. training programs doing a poor job? Is there need for more fully qualified physiologists to teach at the college level? Should the training for college physiology teachers be significantly different from that for medical school level faculty?

My Overall Reactions

I found striking discrepancies in real up-to-date understanding among college faculties concerning requirements for graduate school admission, career choices, career opportunities, and research and teaching challenges in physiology and the other basic medical sciences. Many faculty members still advise their students to "try to gain admission to medical school, and if you don't make it, you can always go to graduate school." I was strongly impressed by the fact that those faculties that contained APS members provided better advice (concerning physiology) to their science majors. Also, those college teachers having close interactions with active research departments were better informed. The college (graduate) students were often less inhibited and more vocal than were their teachers. Once I gained their confidence, they were generally ready, willing, and able to talk. They provided the following reasons for the current preference among college students for medicine compared with physiology.

The M.D. doesn't have to worry about job opportunities. He is prepared to move in any (combination) of three (practice, teaching, research) directions. They were generally unaware of the lack of experience and training for research by the typical practicing physician. The M.D. doesn't have to explain or justify working with animals (e.g., cats, dogs, rabbits). There was litte appreciation for problems of research on human patients or in applying research data to patients. Everyone *knows* what the M.D. does; few know or care what a Ph.D. scientist does, other than teaching for a living. Few lay people know or care who does research, or even what creative research is. The Ph.D. scientist has less *status* in his living community that does the M.D. Sometimes he is considered a kind of "egg-head," a dreamer and not a "doer." Students often focus on *security* more than any other factor in scientific career selection. They reiterate their confidence in their ability to pass necessary coursework of an academic program but express concern for the uncertainty of completing a successful dissertation. They have relatively little real knowledge of what is involved in the dissertation (thesis) aspects of a graduate training program. They have only rarely had mammalian laboratory experience and frequently no laboratory experience at all outside organic and elementary chemistry. Thus a prescribed 4-year course can be accomplished successfully—they have been passing examinations and courses all their lives, but they are unsure of a 4- to 6-year program including vague and indefinite research requirements. Scholarship support during graduate school removes some of the reservations. Students see the physician living at a significantly higher level of sophistication and comfort. He and his family can have symphony or opera tickets or almost any pleasurable diversion (ski trips to Colorado or even the Alps, winter vacations in Florida or Jamaica, and so forth), whereas most Ph.D.'s cannot. Basic scientists, in general, work very had with long hours of intense concentration and commitment. They receive relatively little in return in terms of status or finanical income. Their intangible rewards in terms of personal satisfaction are generally not apparent.

Faculty generally reported the differences in financial return as being the most important difference in career choices for college students. It is interesting that the students did *not* list this very high, but they did place *security*, *flexibility* of options, and *stability* of career outlook very high in their perception of the future. College students are "people oriented." They are idealistic and want to "help people." Students are much better informed about medicine than about research. They have very little "feel" for research and generally do not recognize it as a career choice. Most do not emphasize teaching as an attractive profession. Physiology is not sufficiently advanced in most colleges to allow for much research (except in the larger departments of major universities), and while chemistry and computer sciences are highly visible and therefore quite well recognized, the basic medical sciences are not.

Conclusions

Physiology is not well recognized as a potential career pathway by the general public or by undergraduate science majors. The role of physiologists in creative research is also poorly understood, and few lay people associate teaching and research. They often attribute advances in medicine to research done by physicians, without insights

into the special training and experience required for such career choices. Better interactions between undergraduate college teachers and the basic medical sciences would markedly help the science major in seeking a career pathway. Membership in APS would greatly stengthen this bond betwee the college teacher and physiology as a profession.

Unless some attention is given to the problem of attracting the *brightest* young people into physiology, there is danger of deterioration in quality of teaching and research in the relatively near future. This tends to parallel deficiencies in recruitment of teachers and investigators in clinical departments. Governmental authorities appear undisturbed until a crisis is on them, and as is currently happening in science and mathematics training at elementary levels, one can predict "crash" programs to fill the needs for excellence in teaching and investigation in the medical sciences.

Walter C. Randall

Ray G. Daggs Award, 1983

The Ray G. Daggs Award was presented to Clifford Ladd Prosser by Dr. Walter C. Randall, who said, "This year the Ray G. Daggs Award is bestowed upon **Clifford Ladd Prosser**, 42nd President of the American Physiological Society.

"Since receiving his doctorate at Johns Hopkins University 51 years ago, Ladd Prosser had devoted his entire professional life to the promotion of physiological science in its various facets, as teacher to his 40 doctoral students, as author of the first modern comprehensive book on Comparative Physiology, as US Editor of the *Journal of Comparative Physiology*, Managing Editor of *Physiological Zoology*, and member of the Editorial Board of various other journals.

"He was elected to our Society 48 years ago and has served on our Eduction Committee, the Editorial Board of the *American Journal of Physiology* and the *Journal of Applied Physiology*, on Council, as our representative to the Federation Board, and as our President.

"Ladd Prosser is not only an eminent physiologist, he is one of the great biologists of our time whose background, training, and research activities have given him an unprecedented grasp and outlook on biological sciences in general. His great contribution to our Society was to expand the parochial perspective of our medical school-oriented membership by bringing in physiologists from other areas. This was not a simple task quickly accomplished. However, no one was better prepared to accomplish this gradual evolution and to bring together the scattered interests of physiological activities than Ladd Prosser. Not only had he been recognized by the Society of General Physiologists to become their President in 1958 but also by the American Society of Zoologists to lead their Society as President in 1961 before being elected as our President in 1969.

"Today Ladd Prosser, Professor Emeritus at the University of Illinois, is still serving our Society as a member of the Committee on Senior Physiologists.

"During his long and distinguished career through the last half-century he has been rewarded with many honors, here and abroad. It is only fitting that on this occasion we acknowledge our great debt to Ladd Prosser for expanding our horizons and for his long and devoted service to our Society and to physiological sciences in general by bestowing upon him the Ray G. Daggs Award for 1983."

Dr. Prosser responded, "This is a very unexpected honor, and I appreciate it tremendously. I am delighted; in fact, the Society has accepted some of the policies I tried to promote at various stages in my service in the Society. I might take a moment to say a bit about my association with Ray G. Daggs and one other idea with respect to the Society.

"As the President said, I joined the Society in 1934. My first principal assignment was membership on the Education Committee in 1952. The Committee was composed of Edward Adolph, who is in the audience, as Chairman, William Anderson, and myself. We had a variety of very interesting projects most of which did not seem to strike a favorable response from the National Institutes of Health and were supported for only a few years.

"I moved on to a variety of other committees, and many of these led to meetings at Beaumont House through a period of 15 or more years. I must say that Ray Daggs was very supportive. As you may know, it is not very easy to have a quick lunch in the Beaumont House neighborhood, and Ray and Mary Daggs, who had a house on the Federation property, served many nice lunches to our small committees at their home, and these were appreciated.

"Ray and I had our disagreements. He was very much a purist and lived by the letter of the law. I thought he should have been a little more flexible. On the other hand, I certainly came to respect him and recognize the tremendous contributions he made. I was happy to be able to recommend Orr E. Reynolds as Executive Secretary to Council at the time I was finishing my term on Council, and I think Orr has carried on beautifully the traditions established by Ray.

"Just one other item that might be of interest to our Long Range Planning Committee. Many of you have spoken at times about the fragmentation of our Society. I would like to make one or two comments. This Society is the parent of a great many societies in American Biomedical Research. In 1948, when the biophysics group wanted to have a division of biophysics, a separate very

Recipients of the Ray G. Daggs Award

1974	J. H. Brookhart
1975	M. B. Visscher
1976	J. D. Hardy
1977	J. H. Comroe
1978	H. Rahn
1979	J. R. Pappenheimer
1980	J. R. Brobeck
1981	A. C. Guyton
1982	R. W. Berliner
1983	C. L. Prosser

successful society was established. Shortly thereafter, the general physiologists organized a society. The comparative physiologists set up a division in the American Society of Zoologists, and the biomedical engineers split off. The most recent, and perhaps more serious, has been the establishment of the Society of Neuroscience which has become a very large and prosperous organization.

"I had some experience with organizing the divisions of the American Society of Zoologists, which worked very well, and I had hoped we could do the same in APS.

"One of the real disappointments of my professional life came at a meeting when I was President of the Society. I had convinced the Council that we should, in fact, go to divisional organization. However, when it came to the vote of the entire membership, it was defeated. The upshot of it was the idea had to have time to percolate. Therefore, I was delighted when the journals were divided, which, I think, is all to the good. On the other hand, perhaps we should consider ourselves as parents, and most of us, being good parents, are proud of our children. I am sure the establishment of the new societies has fragmented our Society to some extent, but our offspring are successful. They have led to improved science, and I do not think we should be too disturbed. Perhaps our role now, as it has been in the past, is to parent new societies. Many of the divisions now functioning may split up and become societies. However, I do not think we should be too worried about this. It changes the character of the meetings from time to time, but I have the feeling we have swung too far toward wanting to maintain the same format all the time. I just give this as a bit of a suggestion that perhaps we are serving our purpose by sponsoring new societies.

"Again, I wish to thank the Council and membership for honoring me with this award."

Election to the National Academy of Sciences

Richard J. Havel, director of the Cardiovascular Research Institute at the University of California, San Francisco, and APS member, has been elected to the National Academy of Sciences.

At 58, Havel has developed an international reputation for his work on lipoproteins and cholesterol, which are associated with development of atherosclerotic plaques. In 1955 Havel developed a method for separating lipoproteins from human plasma, a technique that is the basis for all separation methods in use today.

He went on to find the cause of a rare inherited disorder, called familial type 1 hyperlipidemia, in which triglycerides build to high levels in the blood. Havel discovered that the condition is caused by a genetic deficiency of an enzyme, lipoprotein lipase, which normally breaks down triglycerides. In a classic series of studies, Havel then worked out the pathway by which droplets of dietary fat, called chylomicrons, enter the bloodstream from the intestine and are delivered to various tissues to be metabolized. In 1973 Havel helped to unravel the cause of another genetic disorder, type 3 hyperlipidemia, which produces elevated blood lipid levels and can lead to premature atherosclerosis. He found that patients with the disease accumulate a mutant form of apoprotein E – one of the protein components of high-density lipoprotein – in their blood. High-density lipoprotein normally helps to scour cholesterol from the blood by carrying it to the liver, where it can be broken down and excreted from the body. But first, the lipoprotein must bind to receptors on the liver which only recognize apoprotein E. The mutant form of apoprotein E is not recognized by the liver receptors, Havel found. Because it cannot bind to the receptors, it cannot deliver its load of cholesterol.

Havel was born in Seattle and received his M.D. degree from the University of Oregon Medical School in 1949. He was a clinical and research associate at the National Heart Institute from 1953 to 1956, when he joined UCSF as an assistant professor. He was named a professor in 1964, became associate director of the Cardiovascular Research Institute in 1961, and was appointed director in 1973.

Also elected to the NAS was endocrinologist Dr. **Samuel M. McCann**, Chairman, Dept. of Physiology, the University of Texas Health Science Center at Dallas, former APS Council member, and current Chairman of the APS Membership Committee. He is internationally recognized for his work in "executive" brain hormones called neuropeptides.

McCann has received numerous awards for his research in reproductive endocrinology and neuroendorcrinology including the Ernst Oppenheimer Award and the Fred Conrad Koch Medal, both from the Endocrine Society. He came to Southwestern Medical School, a component of UTHSCD, as chairman of Physiology in 1965. Previously he was on the faculty of University of Pennsylvania School of Medicine with a brief stint as assistant in Physiology at the Royal Veterinary College of Sweden in Stockholm.

After receiving his M.D. at the University of Pennsylvania School of Medicine in 1948, he did an intership and residency in medicine at Massachusetts General Hospital. He has served on numerous NIH committees, chairing the NIH Reproductive Biology Study Section from 1980 until 1982. An organizing member of the International Society of Neuroendocrinology, he is now serving as vice president of that organization. The author of more than 500 scientific papers, he currently serves on the editorial boards of *Proceedings of the Society for Experimental Biology and Medicine*, *IRCS Review of Medical Science*, *Life Sciences*, *Neuroendocrinology Letters*, *American Journal of Physiology*, *Peptides*, and *Italian Journal of Physiological Sciences.*

129th APS Business Meeting

Time: 9:45 A.M., Wednesday, April 13
Place: Conrad Hilton Hotel, Chicago, IL

I. Call to Order

The meeting was called to order by the President, W. C. Randall, who welcomed the members to the 129th Business Meeting of the Society. The agenda, a legislative fact sheet, a letter to Sen. Dole about Bill S 657, and an announcement of a public meeting on laboratory animal care and use were distributed to the membership.

D. C. Randall, J. P. Filkins, T. K. Akers, and R. D. Foreman were appointed as tellers. The tellers distributed ballots for items III and IV (below) to Regular members in attendance.

II. Report on Membership

A. P. Fishman, President-Elect, reported on the status of the Society membership and deaths since the last meeting.

A. Summary on Membership Status. The current membership of the Society is 6,124, of which 4,476 are Regular members, 560 Emeritus, 10 Honorary, 98 Corresponding, 718 Associate, and 262 Student members. Each year, the Society continues to admit approximately 180-200 new members.

B. Deaths Reported Since Last Meeting. Dr. Fishman read the names of those members whose deaths have been reported since the previous meeting and asked the membership to stand for a moment of silence in tribute to them (p. 141).

III. Election of Members

It was announced by O. E. Reynolds that all candidates were elected to membership (p. 122).

IV. Proposed Resolution on Nuclear War and Nuclear Weapons

In discussion of a proposed resolution on nuclear war and nuclear weapons, signed by some active members of the Society, Dr. Randall stated that everyone agrees the threat of nuclear war is extremely important. However, this particular resolution raises societal as well as purely scientific issues. As a consequence, there are members of the Society who question the appropriateness of the Society taking a position on societal issues. Therefore, at Council's request, the ballot appended to the resolution raises the question of whether the proposed resolution is appropriate for Society consideration. A majority of the members voting in the affirmative will result in a mail ballot being sent to all APS Regular members.

Dr. Reynolds announced the voting results as 94 affirmative and 61 negative votes. Therefore, a ballot will be mailed to all Regular members.

V. Election of Officers

Reporting the results of the Election of Officers by mail ballot, Dr. Reynolds announced that the new President-Elect is John B. West and the new Councillors are Howard E. Morgan for a four-year term ending June 1987 and Norman B. Staub to complete Dr. West's term, which expires in 1985. The total ballots cast for President-Elect were 1,604 and for Councillor 1,434.

VI. Actions of Council

A. The Council received the recommendation of the Committee on Committees for appointments of Society committee members and representatives to other organizations listed in the August issue of *The Physiologist* reported Dr. Randall. Following the practice established several years ago, other standing committee reports submitted to Council have been posted outside the APS headquarters office for perusal of the membership in addition to their publication in the August issue of *The Physiologist*.

B. The Finance Committee report presented no surprises to Council, and Dr. Randall was pleased to inform the membership that the finances of the Society seem to be substantial at this time and do not require a dues increase. There was an applause from the audience.

C. Of extreme importance to the Society are its activities in public affairs and animal care legislation. A very significant action of Council deals with the recommendations of the Public Affairs Committee. To make a contribution to the legislative process in relation to animal care and experimentation, a mail-o-gram (p. 113) was sent Sunday night to Sen. Dole stating support of Bill S 657. This bill calls for amending the Animal Welfare Act by adding a provision relating to proper care and treatment of animals used in all research facilities. A copy of the APS letter of endorsement and a fact sheet outlining the rationale for support of the letter were included with the agenda. The APS Public Affairs Consultant, William Samuels, who was introduced by the President, has been extremely active in keeping on top of the many facets of the legislative issues.

Mr. Samuels stated that the current situation is fluid and rapidly changing. At the present time, in addition to S 657, the Society is also concerned with HR 1555, which is the House version of the NIH renewal authorization, and Senate Bill S 964, which would require an 18-month study of all issues involving the use of research animals. Mr. Samuels said he will continue to keep the membership apprised of current legislation pertaining to animal research in his regular column in *The Physiologist*.

At the local level, "humane" groups in nine states (South Carolina, North Carolina, New Hamphshire, Oregon, Vermont, Connecticut, Minnesota, Massachusetts, and California) are working to repeal the dog pound release laws. In California, Bill S 883, which was defeated last year, is being reintroduced. In addition to the repeal of pound release, three additional provisions have been included. It will be a misdemeanor carrying a fine of $500 each for *1)* anyone in California to import dogs or cats for research unless the animals are bred for research purposes, *2)* a person to conduct research and fail to provide pain-relieving drugs, or *3)* a pound to release an animal to an institution for research.

With the continued increase in legislative activity at both the state and local levels, Dr. Barger expressed concern about the few letters written by the scietific community to legislators supporting the use and need of animals in research. Science would be in a much better position if more such letters were written. It also would be desirable to have physicians and patients who have benefited by such research write letters, and Mr. Samuels was urged to look into ways this might be accomplished.

Mr. Samuels agreed that letters are extremely important. There is need for the bench scientists to write about

the areas in which they work, being specific about how their work will be affected if the animals are taken away. He advised, "Write the letter in your own terms. If you have the same concerns as the Society, express them. If you have other concerns, express them." If there are questions related to a particular piece of legislation, Mr. Samuels said he would be happy to assist wherever possible.

Dr. Bade said that Congress is proposing legislation for alternative methods and, on the other hand, alternative systems that already exist cannot be used. Her work is on cold-blooded animals, and the Child Health and Human Development Institute at NIH will only consider supporting research on warm-blooded animals. She expressed the hope that there could be some type of coordination of effort. Dr. Fishman responsed, "That it is a problem of educating NIH which sometimes can get confused with legislative problems. However, we must not lose sight of someone dictating what alternative system can be used in our research. The Society is trying to protect the right to use any system that is appropriate under the proper conditions, and Congress is attempting to dictate whether we work on vertebrates or invertebrates, what you can do, and how it will be done, with external reviewers, who are not scientists, looking over your shoulder and thinking what you do is inapprorpriate."

A member said he has the misfortune of living in David LaVerde's district and is chairman of the animal care committee at his institution as well. As a consequence, he was interviewed on a local television program developed for animal welfare. His host, sitting on a couch stroking his cat, not once gave him the opportunity to have the last word. As a result, he received many phone calls with some suggestions, such as using prisoners or his own children instead of dogs. However, there were some calls from reasonable people who had no idea of some of the developments that have come from animal research. One lady said she would not have an operation or any treatment that involved the use of laboratory animals. It turned out she was using insulin, and when told to throw it away because it came from animal experimentation she was amazed. The scientist tends to take for granted that people are informed. It is extremely worthwhile to have this meaningful knowledge brought to the attention of the public. It is within the Society's interest and the responsibility of FASEB to inform the public of the advances in medicine that have been made in the basic animal laboratory.

Dr. Randall expressed his deep appreciation to those members who have been responsive and active related to the use of animals in research.

D. Council was informed of the Program Executive Committee's planning for the 1984 Spring and Fall Meetings. There will be 29 symposia sessions presented at the 1984 Spring Meeting in addition to the theme on Physiological Regulation, and nine symposia are planned for the 1984 Fall Meeting in Lexington, Kentucky, supplemented by tutorial lectures and refresher course.

E. There was a report from the Long Range Planning Task Force chaired by Robert Berne, and the recommendations of the Task Force will be the principal item of business at a special meeting of Council to be held in the Fall, and it is hoped that representatives of Society Sections will participate.

F. Dr. Robert Krauss, Executive Director of FASEB, reported on the Federation's plans to build an additional wing to the Lee Building in Bethesda, MD, which is to be available by early 1985. The requirements of the Society, mainly because of the great increase in its publications program, have been a major contributor to the need for FASEB to provide additional space.

G. A report of the Centennial Celebration Committee (p. 130) was accepted by Council, and the information on the status of a number of Centennial projects including plans for lectures to be presented at the 1986 Congress in Vancouver. The theme of the 1987 FASEB Spring Meeting will be "A Century of Progress in Physiology" and will be held in Washington, DC. A number of publications are scheduled in celebration of the Centennial, some of which are the *Centennial History of the Society* by John Brobeck and Orr Reynolds, the *History of Physiology in America* by Gerald Gieson of Princeton University, and a series of books following the pattern of *Circulation of the Blood: Men and Ideas* by Alfred P. Fishman.

Plans are also underway to encourage participation of societies outside the FASEB family which have their origins in close relation to the American Physiological Society and some foreign societies which contributed to the early history of the APS to encourage participation in Centennial activities of the Society.

H. Dr. Randall announced with regret that this will be the last Society meeting attended by Mr. Herbert Brownstein, Head of the Membership Services Department, who is retiring in December after eight full and productive years with the Society. One of his principal contributions has been the organization of the meetings.

VI. Awards

A. Ray G. Daggs Award (see p. 125).

B. Caroline tum Suden Professional Opportunity Awards. The Caroline tum Suden Professional Opportunity Awards were approved by Council last year, and Dr. Randall expressed delight in joining the Chair of the APS Women in Physiology Committee, Dr. Marie Cassidy, in presenting the first six awards to James Blank (University of Texas), Reed Hoyt (University of Pennsylvania), Valerie Kalter (University of Iowa), Robert Knabb (University of Virginia), Jeri Taylor (Howard University), and Virginia Zinsmeister (University of Cincinnati).

Dr. Cassidy announced that Caroline tum Suden was one of the very first women members of this Society. Her life has been researched, and an account has been published in *The Physiologist* 24(6): 1-11, 1981. She had a long and productive career as an investigator, a teacher, and a mentor. When Caroline tum Suden died, a sizable portion of her very substantial estate was left as an undesignated bequest to the American Physiological Society. For several years, it has been a source of support to the Society for various functions and activities. The Women in Physiology Committee thought it would be appropriate to devise a mechanism to honor her name and this substantial legacy. With some considerable thought and approval of Council, the Committee established a series of awards to be presented each year at the FASEB meeting.

The Caroline tum Suden Professional Opportunity Awards are open to graduate students or postdoctoral fellows who are submitting an abstract and presenting a paper at the FASEB Spring Meeting. A letter of recommendation from their sponsors or the chair of their

departments is necessary. The Award entitles the recipients to a $500 check to attend the meeting, free registration, and access to the FASEB Placement Service. This year, there were 40 applicants of which 29 were male and 11 female. The Committee, in general, was very impressed with the overall excellence of all applications. With some difficulty, six recipients were selected. Dr. Cassidy said, "It is our hope and wish that this is one early step in a long and productive career for all of you and that you will become very active and functional Society members."

VII. New Business

In response to a question from the floor concerning the possibility of the Society ever having a Hispanic American Congress, Dr. Reynolds asked that a letter be written to him which he, in turn, would take to the IUPS General Assembly meeting in Australia, August 28.

With no other business, the 129th Business Meeting was adjourned at 10:45 A.M.

Alfred P. Fishman, President-Elect

Committee Reports

Animal Care and Experimentation

Revision of the NIH Guide

The Institute of Laboratory Animal Resources (ILAR) is revising the National Institutes of Health (NIH) Guide ("Guide for the Care and Use of Laboratory Animals," revised 1978, reprinted April 1980) and is considering written comments from interested parties. The Committee will review the "Guide" to determine what comments the Society should forward to ILAR. Written comments can be sent to Dr. Errol W. Grogan, Executive Secretary, ILAR, National Academy of Science, 2101 Constitution Ave., Washington, DC 20418.

Action of Animal Rights Groups

The "Mobilization for Animals" scheduled mass demonstrations at four Primate Centers (Boston, Atlanta, Madison, and Davis) for April 24, 1983, and has a goal to develop a grass-roots network to campaign against all animal research. In addition, a political action committee will be lobbying the US Congress to decrease the Primate Centers budget by approximately 25%.

The Animal Liberation Front has been active in the Washington, DC area and has stolen research cats from Howard University and dogs from the Bethesda Naval Hospital. Last year another group stole rabbits from the University of Maryland. Are there any special precautions which could prevent or discourage unlawful entry and animal stealing from research facilities?

AAALAC

Orville Smith represented the APS at the December 7, 1982 meeting of the American Association for Accreditation of Laboratory Animal Care (AAALAC) Board of Trustees. Because of an apparent widespread belief in Congress that AAALAC is strongly in favor of current bills legislating accreditation of research animal facilities, the Board of Trustees voted to send letters to both Representative Waxman and Senator Dole indicating that AAALAC supports the use of animals in research and supports the idea that the individual scientists and local animal care committees should continue to be responsible for regulating the use of research animals. Dr. William Raub, Associate Director of NIH for Extramural Programs, discussed the authority delegated to the NIH to carry out and oversee animal research.

National Society for Medical Research

Helene Cecil represented the APS at the December 14, 1982 annual meeting of the National Society for Medical Research (NSMR). The appointment of three members to the Board of Directors was approved: John Jennings, Virginia Weldon, and Sheldon Wolff. The NSMR adopted a policy position that the routine use of the quantitative LD_{50} test is no longer scientifically justified. The Treasurer reported an annual budget of $200,000 with deficits during four of the last five years. After the business meeting the NSMR sponsored a forum on "Animal Care Committees" with representatives from industry, academia, and government discussing the responsibilities of their Committees. Dr. William Raub addressed "Developing NIH Policy and Procedures on the Care and Use of Animal Research." NIH will be conducting selected site visits focusing on the assurance statement as it applies to facilities, procedures, etc., related to the care and use of laboratory animals. Dr. Bruce Ewald described the membership of the American Association for Accreditation of Laboratory Animal Care and the procedures AAALAC uses in granting accreditation to research animal facilities.

The NSMR is coordinating the development of a national coalition of associations, academic health centers, industrial research firms, and agricultural groups in an effort to counteract the campaign of the Mobilization for Animals to close two of the seven regional primate centers.

Mobilization for Animals is a recently organized militant international coalition of approximately 100 animal welfare groups of which 70 are located in the United States. Its headquarters is in Jonesboro, TN, and its efforts are being coordinated by a long-time activist, Dr. Richard Morgan, who is a professor of English at East Tennessee University.

Mobilization for Animals is attempting to pressure both the Senate and House appropriations committees to reduce or eliminate funding for the two regional centers in Fiscal Year 1984. The two centers targeted specifically are at Beaverton, OR, and Covington, LA. The reasons being cited for closing those centers are "relative inaccessibility, high disease and mortality rates, geographical redundancy, and duplication of work."

Mobilization for Animals (MFA) wants the funds that would be appropriated for the two centers to be used to "repatriate resident primates to natural habitats or wildlife refuges, or to place them in MFA-approved research facilities (and) under the direction of MFA member groups." Mobilization for Animals is also seeking within each of the primate centers and related facilities and at NIH to have MFA-designated members be given 25% of the voting membership on all policy-making committees and review and advisory committees concerned with the treatment and care of animals, the conduct of experiments, pain classification, and funding requests.

To call public attention to this campaign, the Mobilization for Animals scheduled mass demonstrations for April 24, designated by animal welfare groups as "World Laboratory Animal Day," with demonstrations set for

the regional primate centers at Southborough, MA, Madison, WI, Davis, CA, and Atlanta, GA. Mobilization for Animals also plans a series of full-page ads in 50 metropolitan daily newspapers calling attention to the seven primate centers and the efforts to close two of them.

The coalition to be coordinated by NSMR will be identified in newspapers ads that will appear in newspapers within a day or two after the Mobilization for Animals' ads. The coalition also will prepare and present testimony before the Congressional Appropriations Committees in support of the primate centers.

The coalition is expected to be supported by several hundred member organizations and institutions including nonmembers of NSMR. There is to be no cost to any organization or institution supporting the coalition.

Helene Cecil, Chairman

Centennial Celebration

1987 Centennial Meeting

The Centennial Celebration of The American Physiological Society will be held April 5-10, 1987, in Washington, DC in conjunction with the FASEB Meetings. The overall theme for this meeting is "A Century of Progress in Physiology."

Historical Lecture Series

The History of Physiology lecture series was initiated at the 1982 Fall APS Meetings (in San Diego) where Dr. Arthur Otis spoke on the "History of Respiratory Mechanics." Dr. Otis's talk has been published in the May issue of the *Journal of Applied Physiology* (54: 1183-1187, 1983). At the 1983 FASEB Meetings, Dr. Al Fishman presented the next lecture in the series entitled "The Growth of Ideas about Pulmonary Circulation and Pulmonary Edema." Two historical lectures are scheduled for presentation at the 1984 FASEB Meetings by Drs. John West and Robert Frank. Other speakers are currently being recruited. All speakers will be strongly encouraged to publish their lectures in the appropriate sections of the *AJP* or *JAP*. The Centennial Celebration Committee (CCC) would appreciate your suggestions and recommendations.

Historical Vignettes

The Historical Section of *The Physiologist* is the medium for publishing vignettes. A number of very interesting vignettes have appeared in recent issues, and the CCC would like to have more published as the APS looks toward its Centennial. Dr. Ralph Kellogg is spearheading this effort and would welcome suggestions of physiologists to prepare historical vignettes. Properly written, these vignettes provide valuable insight and serendipity that will be lost forever, if not recorded and published.

Department Histories

Nine histories of departments of physiology have been submitted this year in response to a letter sent by Dr. Arthur Otis to all Department Chairmen inviting them to prepare histories of their departments. Nearly 100 departments have indicated an interest in pursuing this goal. Several of these departmental histories have appeared in recent issues of *The Physiologist*. The CCC again urges all those interested in documenting the "genesis and evolution" of their department to send completed manuscripts to Dr. Otis.

APS Centennial Celebration Fund

The Centennial Celebration Fund was established to provide financial assistance for the various publications and activities planned for the Centennial Celebration. Recent issues of *The Physiologist* have presented opportunities for members to make a contribution and to receive special jewelry of their choice bearing the APS logo in recognition of their contribution. Also, at the 1983 Spring Meetings, attractive T-shirts, with the anatomy of the thorax on the front and the abdomen on the back and bearing the phrase "Physiologists know the inside story," were offered for sale ($8.00) for the first time. Further information can be obtained from the APS office in Bethesda.

APS Historian/Archivist

Dr. Toby Appel, a trained historian and archivist, will start her appointment with the Society in September 1983. Her initial activities will focus on the development of several written historical works in preparation for the Centennial Celebration.

"Circulation of the Blood: Men and Ideas"

This book, originally edited by Drs. Fishman and Richards, has been reprinted and made available by the APS. Dr. Fishman has added a preface to this book highlighting the contributions of the individual scientists to the upcoming APS Centennial Celebration. Copies of this book may be purchased through the APS office. Other "People and Ideas" books are currently being planned and should be available by the time of the 1987 Centennial Meeting.

History of the APS, 1887-1987

The History of the Society is being prepared under the coeditorship of Drs. Brobeck and Reynolds. Work on this volume is progressing nicely.

APS Centennial Roster, 1887-1987

A roster of all APS members, past and present, is also being prepared. This Centennial Roster will be bound with the History of the APS and given to all 1987 FASEB Meeting registrants as a memento of the APS Centennial.

Program Activities for 1987 Meeting

Fall. "Kick-off" of APS Centennial at IUPS Meeting in Vancouver. The CCC is considering names of people, representing Canada, US, and Mexico, to present historical lectures at the IUPS Meeting. Names of recommended speakers will be presented to the IUPS at their meeting in Sydney in August 1983.

Spring 1987. Opening plenary session, 10:00 A.M. No other papers scheduled. Talk wil be a "Janus," looking backward and to the future. Major (45-60 min) historical lectures one by each FASEB member society tracing its roots to the APS. Minor (30 min) historical lectures preceding various symposia within APS and other FASEB societies that focus on related historical developments. APS Banquet with speaker, for members and spouses.

Peter A. Chevalier, Chairman

Education

Continuing Medical Education (CME) Projects

Review Tapes. A contract for the production and marketing of review tapes was signed by Dr. Orr Reynolds (on behalf of the APS) and Audio Visual Medical Marketing, Inc. (AV/MD), the company which markets our audiovisual materials. The underlying concept for these audio cassettes is to produce up-to-date material in the form of reviews on current advances in physiology relevant to clinical medicine.

The selection and review of materials to be included in this program is the responsibility of an editorial board, which was assembled by Dr. Joel Michael and consists of the following APS members: Drs. Joel A. Michael, James Houk, Weil A. Kurtzman, and Allen Rovick.

Each selection is projected to consist of a 45- to 60-min taped presentation accompanied by a printed study guide and a 10-question multiple-choice examination. The cassettes will be copyrighted in the name of APS.

Dr. Michael has already contacted some potential authors, and the Board continues to expand the list of potential contributors. Physicians completing and submitting the exam to the APS would be awarded category I CME credits.

Slide-Tape Programs. A total number of 43 audiovisual presentations, previously produced by the APS are currently being developed into appropriate CME tools by the generation of 6–10 questions per tape. A batch of questions initially prepared by AV/MD was not suitable for our purposes. Therefore the writing of questions is currently undertaken by several members of the Committee. Drs. Perry Hogan and Allan Mines are doing the electrophysiology programs, and Dr. Michael is taking care of the renal sets. Dr. Michael is also searching for volunteers to do other components of the series.

Program on Aging. Dr. Spitzer has initiated inquiry into the possibility of producing instructional material on the physiology of aging for the CME market. Dr. Paola Timiras, a former member of this committee and a recognized authority on several aspects of aging was approached. After negotiations involving Dr. Reynolds, AV/MD, and Drs. Spitzer, Michael, and E. J. Masoro, an agreement was reached. Dr. Timiras and co-workers will produce the script for a series (possibly 20 or so) of cassettes on various topics in the physiology of aging. An advisory board including Drs. Masoro (recruited by Dr. Spitzer for his expertise on gerontology), Michael, and Spitzer will assist with thematic selection, appropriate format for presentation, and "packaging" in general. AV/MD will bear the cost of producing and marketing the cassettes. Members of the "advisory board" offered to serve in this capacity at a "reduced rate" in order to permit Dr. Timiras to proceed with the project.

Refresher Course for 1983

The refresher course for the 1983 Fall Meeting (in Honolulu, on August 24) will be on "Receptor Physiology," organized by Dr. Spitzer. Topics to be covered include diverse aspects of the insulin receptor mechanism, catecholamine, lipoprotein, and putative calcium receptors.

Application of Computers to Teaching Physiology

The Education Committee strongly supports the concept of the use of computers in the teaching and research aspects of physiology. Many APS members already own or have access to some form of computing device. Numerous programs have been and will be developed by such persons that could be useful for educational and research purposes. However, no effort has been made so far to coordinate and oversee these individual efforts. The Committee perceives the need for *1)* a possible central software resource bank set up by the APS, utilizing FASEB computer facilities, and *2)* disseminating information to the scientific community regarding the availability and usability (e.g., matching of different hardware and software systems) of these resources. We are fortunate in having several people on the Committee with expertise in this area. Dr. James Randall is a pioneer in the development of microcomputer-based teaching tools in phyiology; Drs. Michael and Perry Hogan have relevant experience and interest.

A meeting on "Computers in Physiology Teaching: How Can APS Help?" was held during the 1983 FASEB Meeting. The meeting was organized by Drs. Michael, Hogan, and Randall. The purpose of this session was to apprise physiologists and other interested members of FASEB of the current status of computer-based teaching methods, the "ways and means" whereby such methods can be used, to poll these colleagues as to their present and future needs in this area, and how they think the APS can help to meet these needs. About 85–90 people attended the session. Several participants urged the APS to catalog and possibly review available teaching programs. As a result of the high level of interest in computers both for simulation and for providing "lessons" in teaching physiology, a workshop on the application of computers is planned for the 1984 Spring meeting in St. Louis.

Judy A. Spitzer, Chairman

Financial Development

The Financial Development Committee has continued to seek ways to increase the income of the APS and to reduce its dependence on income from mandatory dues. To achieve these goals, it has fostered voluntary contributions from regular and emeritus members of the Society; called the attention of members to the possibility of bequests to the Society and Living Trust arrangements to benefit APS and take advantage of current tax laws; encouraged expansion of the Sustaining Associate Membership of the Society; and approached a number of foundations about supporting Society activities. Recent developments follow.

Endowed Lectureships

A pharmaceutical firm has generously agreed to endow a prestigious annual lecture at the Spring Meeting of APS, and the first lecture in this series in now being scheduled.

New Sustaining Associate Members

In addition to increasing the number of industial concerns which are Sustaining Associate Members of the Society and setting up mechanisms to increase communication between the Society and the Sustaining Associates, we have begun to explore the possibility of professional societies becoming Sustaining Associate Members. In this regard, we are very pleased that, in view of the close relation between physiology and clinical medicine, the American Medical Association has become

a Sustaining Associate Member. We are in the process of exploring the possibility of other professional societies becoming Sustaining Associate Members.

Support by the American Heart Association

The Cardiopulmonary Council of the American Heart Association has begun to make regular contributions to support an annual symposium at an APS meeting. The possibility of similar support by the Council on High Blood Pressure Research of the American Heart Association for a basic symposium on blood pressure regulation and hypertension is being pursued. In addition, it has been suggested to the leadership of the American Heart Association that, in view of their ongoing support through the Cardiopulmonary Council, the American Heart Association might become a Sustaining Associate Member of our Society.

William F. Ganong, Chairman

Porter Development

The Porter Development Committee is now supporting three postdoctoral fellows and two predoctoral fellows in physiology: Dr. Jose E. Garcia-Arraras, who is continuing his fellowship in the laboratory of Dr. Nicole Le Dourain at the Institut d'Embryologie in the Centre National de la Recherche Scientific, Nogent-sur-Marne, France; Dr. Nelson Escobales, who is a graduate of the Dept. of Physiology and Biophysics, University of Puerto Rico, and is now a postdoctoral fellow in the laboratory of Dr. Mitzi Canessa in the Dept. of Physiology and Biophysics, Harvard Medical School; Dr. Jorge R. Mancillas, who is a graduate of the Dept. of Neurosciences, University of California, San Diego, and is starting a postdoctoral fellowhsip in the laboratory of Dr. Floyd E. Bloom at Salk Institute; Jean A. King, who is a candidate for the Ph.D. degree in the Dept. of Biological Sciences, Hunter College, in the laboratory of Dr. James H. Wyche; and Darlene K. Racher, who is a candidate for the Ph.D. degree in the Dept. of Physiology and Biophysics, Chicago Medical School, in the laboratory of Dr. Warren W. Tse.

The Committee has also continued funding for the Atlanta and New Orleans consortia. Two former Porter Development Committee Fellows, Drs. Pamela Gunter-Smith and John C. S. Fray, have been Visiting Porter Lecturers in the Atlanta Complex.

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

A. Clifford Barger and
Edward W. Hawthorne, Co-Chairmen

Program Executive

The Program Advisory and Executive Committees met in Chicago to consider programs for both 1984 Spring and Fall Meetings.

The 1984 Spring Meeting will take place in St. Louis, April 1-6. Other FASEB societies to be present at the meeting include ASPET, AAP, and AIN. In addition, the Society of General Physiologists, the Biomedical Engineering Society, the Society for Mathematical Biology, and the Society for Experimental Biology and Medicine will be guests of APS at the St. Louis meeting. Representatives of all four guest societies attended the meeting of the Program Advisory Committee and made valuable suggestions for the 1984 Spring Meeting program.

1984 FASEB Themes

APS has agreed to organize a theme on Regulatory Mechanisms, and Dr. Gene Yates has assumed responsibility for the coordination of this theme. The Biomedical Engineering Society has indicated an interest in participating in this theme, and BMES representatives are working with Dr. Yates in structuring the program. Other FASEB themes at the 1984 Spring Meeting will include Receptors, organized by Dr. Loh of ASPET, and Response to injury, organized by Dr. L. M. Buja of AAP.

APS-Sponsored Symposia at 1984 Spring Meeting

The following list gives the symposia approved for presentation at the 1984 Spring Meeting, the organizers, and the APS section or guest society sponsoring the symposium: Capillary endothelium: cellophane wrapper or metabolic barrier? H. V. Sparks, Cardiovascular Section, APS; Membrane ATPase function in vascular smooth muscle during hypertension. R. K. Hermsmeyer, Cardiovascular Section, APS; Mediator mechanisms in shock. R. F. Bond, Cardiovascular Section, APS; A history of neurophysiology and the latest developments. J. Trubatch, Nervous System Section, APS; Sexual differences in neural development/sexual dimorphism of CNS. C. D. Toran-Allerand, Nervous System Section, APS; Neurobiology of aging. K. E. Finch, Nervous System Section, APS; Functional interaction/developmental neurobiology. G. Pilar, Nervous System Section, APS; Role of guanine nucleotide binding proteins in biology. M. Vaughn or A. G. Gilman, Endocrinology & Metabolism Section, APS; Role of tyrosine phosphorylation in the action of hormones and growth factors. R. Kahn or I. Pastan, Endocrinology & Metabolism Section, APS; Membrane biogenesis. G. Blobel, Cell & General Section, APS; Membrane turnover. G. Ashwell, Cell & General Section, APS; Membrane modification by fusion events. Q. Al-Awqati, Epithelial Transport Section, APS; Intestine transport: structural and functional adaptive responses. H. Binder, Gastrointestinal Section, APS; Renal transport: structural and functional adaptive responses. J. Wade, Renal Section, APS; Regulation of renal phosphate transport. V. Dennis, Renal Section, APS; Central mechanisms in the control of salt and water intake. D. J. Ramsay. Water & Electrolyte Homeostasis Section, APS; Regulation in physiological systems during exercise in untrained and trained—five year update. F. W. Booth, Env. Ex. Therm. Section, APS; Thermogenesis: its role in the development and maintenance of obesity (two sessions). J. Stern and B. A. Horowitz, Env. Ex. Therm. Section, APS; Skeletal muscle fiber regeneration following injury. J. A. Faulkner and B. M. Carlson, Env. Ex. Therm. Section, APS; Regulation of phosphoprotein photophatase activity. J. DiSalvo, Society for Experimental Biology and Medicine; The control of cell volume. A. L. Finn, Society of General Physiology; Theoretical trends in neuroscience. H. Lecar & J. Rinzel, Society of Mathematical Biology; Systems analysis of biocontrol (to be included in Regulatory Mechanisms theme). (No organizer listed), Biomedical Engineering Society; Respiration: histamine and the lung's

circulation. A Hyman. Respiratory Section, APS; cosponsored by AHA-Cardiopulmonary Council.

The 1984 Fall Meeting will take place on the campus of the University of Kentucky at Lexington, July 29-August 3. Dr. Don Frazier, Chairman of Physiology of Lexington, attended the meeting of the Program Advisory Committee and described the local committee's remarkably well-developed plans for the meeting. Members of the Program Advisory Committee made additional suggestions for symposia to be held at the meeting. The following list gives the approved symposia and other highlights, the organizers, and the responsible group: Special Lecture: Experiences of a blue grass physiologist in space. S. Musgrave, Local Committee; Workshop: Integrative approaches to physiology education. J. E. Engelberg, Local Committee; Workshop: Physiologists' approach to age-dependent changes in function. D. Wekstein and B. Peretz, Local Committee; Loaded breathing. Load compensation and respiratory sensation. F. W. Zechman, Jr., Local Committee; Current topics in neuroendocrine control of reproduction. S. J. Legan, Local Committee; Quantitative approaches to the study of cardiovascular regulation. D. Randall, Local Committee; Intrarenal hemodynamics. J. C. Passmore, Cardiovascular Section, APS; Alternation in microcirculatory function during hypertension. P. D. Harris, Cardiovascular Section, APS; Vasoactive agents in control of the mesenteric circulation. E. D. Jacobson, Cardiovascular Section, APS; Neural control of renal function. G. F. DiBona, Comparative Section, APS; Respiratory muscle fatigue as a limiting factor in exercise. H. Welch, Env. Ex. Therm. Section, APS; Thermoregulation in the elderly. L. Blatties, Env. Ex. Therm. Section, APS.

Michael Jackson, Chairman

Public Affairs

The Public Affairs Committee (PAC) has concentrated its efforts since October largely on the Congressional activities relating to proposed research animal legislation.

Committee members and Society staff provided both House and Senate members with recommendations, information, and data concerning the proposed "Humane Care and Development of Substitutes for Animals in Research Act" (HR 6928 and S 2948). The Society also participated in the December 9 hearing on HR 6928 conducted by the House Subcommittee on Health and the Environment.

The hearing focused on two issues: alternative methods and the cost to institutions for accreditation and for administration of the bill's requirements. The Society, the Association of American Medical Colleges, and the National Society for Medical Research coordinated their efforts to secure Dr. Michael DeBakey to present the viewpoints of the scientific community concerning alternative methods.

Although the bills died with the December 23 adjournment of the 97th Congress, the Committee and staff continued working with Congressional members on proposed amendments that could be included when the bills are reintroduced in the 98th Congress.

While many of the Society's recommendations were technical amendments, one amendment proposed by the Society was that the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" be used as interim standards and that the Secretary of the US Department of Health and Human Services (HHS) be instructed to consult with the Director of the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) in developing standards for animal care in research.

A revision of the proposed House bill in the final days of the last Congress charges the Secretary of HHS to develop standards, thus opening the possibility of conflicting standards between HHS, the accrediting entity, and APHIS. Furthermore, the Senate version calls for a one-year study of the costs of accreditation, based only on the current standards.

Committee Workshops

The Committee conducted a workshop in October on problems and methods in dealing with state and local legislative issues. The workshop was held during the Society Fall Meeting in San Diego and featured the University of California's chief health lobbyist in Sacramento and two representatives from the University of Southern California who are responsible for the legislative affairs of that institution.

A workshop was held during the FASEB Spring Meeting on how to meet the press and media when an animal rights group has charged your institution with animal abuse.

State Activities

Committee members in both California and Massachusetts have reported successful efforts in defeating legislative proposals that would have prohibited the use of pound animals for research. Similar legislation still is pending in other states and is being considered in several major cities.

John Shepherd, Chairman

Publications

Innovations continued in the Society journals in 1982. The experiment with a special category of manuscripts, Rapid Communications, was expanded to include all of the journals of *American Journal of Physiology.* Book reviews and a conference report appeared in the *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. A 15-year cumulative index to *Physiological Reviews* was distributed to all subscribers to the journal.

New members of the Society were invited to subscribe to one of the journals at a special price during the first year that they are in the Society.

The Publications Committee hosted a meeting in New Orleans with all involved as editors, associate editors, or members of the editorial boards for the journals or books. The status of the publications program was reviewed, and special attention was paid to the responsibilities of editors and reviewers toward the use of animals in research and the handling of manuscripts in which there is a suspicion of fraudulent data.

The Publications Committee initiated a "search committee" approach to selecting new editors for journals. By this process, which included consultation with sections of the Society, meetings, and interviews, the Committee selected editors for three journals for which the present editors' terms are scheduled to end in 1983.

Manuscripts

The number of new manuscripts received for the journals increased by 7% (213 manuscripts) in 1982, surpassing the increases of recent years (175 manuscripts in 1981 and 155 manuscripts in 1980). Changes ranged from +39% for *AJP: Cell Physiology* to −10% for the *Journal of Neurophysiology*. The *American Journals of Physiology* received 203 more manuscripts (+12%) than last year. *AJP: Endocrinology and Metabolism*, which experienced a drop of 37 (12%) in 1981, was up by 78 (29%).

Rapid Communications

Rapid Communications were received for *AJP: Cell Physiology* and *AJP: Heart and Circulatory Physiology* during the entire year. They began to be received for the other journals of *AJP* in September. It is clear that Rapid Communications are handled more quickly by the editors and the editorial office. The time from receipt to acceptance is usually shortened by close to 3 months and the time from acceptance to publication by more than a month.

Articles and Pages Published

The amount published since the journals were reorganized continues to increase; the number of pages printed has about doubled.

	Amount Published	
	Articles	Text Pages
1976	1031	8121
1977	1131	9825
1978	1177	10665
1979	1287	11705
1980	1435	14138
1981	1560	14352
1982	1734	16110

The number of articles and pages published in the journals increased in all journals except *AJP: Endocrinology and Metabolism*; 174 manuscripts (+11%) and 1758 pages (+12%) more were published than last year. The greatest increases in the number of pages published occurred in *AJP: Regulatory, Integrative and Comparative Physiology* (+41%), *AJP: Cell Physiology* (+36%), and *Physiological Reviews* (+35%).

Printing

Printing costs increased in 1982 as more pages were printed and inflation continued.

	Increases in Amount and Cost of Printing		
	Pages, No.	Pages, %	Cost, %
AJP	+ 946	+ 13	+ 19
JAP	+ 318	+ 10	+ 17
JN	+ 138	+ 5	+ 14
PRV	+ 356	+ 35	+ 38
Overall	+ 1758	+ 12 (Avg)	+ 20 (Avg)

Subsciptions

From 1981 to 1982 the number of paid subscriptions decreased for most of the journals; only three of the journals showed slight gains. The total number of paid subscriptions declined by 389 (-2%). The number of member subscriptions increased for the consolidated *AJP*, *JAP*, and four of the six individual *AJPs*. The number of member subscriptions for *JN* and *PRV* declined. Between 1970 and 1982 the total number of paid subscriptions has remained fairly constant. This small variation has existed despite the changes in the publishing program that have resulted in a different mix of journals being offered for sale.

Since the journals were reorganized in 1976-77 the anticipated drop in the number of subscriptions to the consolidated *American Journal of Physiology* has continued as the number of subscriptions to the individual journals of *AJP* increased. The *Journal of Applied Physiology* reached its peak number of subscribers in 1975, dropped in 1976 and 1977 but since then has plateaued at about 4250 subscribers. There has been an increase of 8% from 1970 to the present. The *Journal of Neurophysiology* subscription list remained at about 2700 through the early 1970s but has been declining since then. The number of paid subscriptions for *Physiological Reviews* has dropped from 5615 in 1970 to 4579 in 1982 (−27%). Subscription prices for 1982 were increased by 17–38%, with a median of 22%. It is anticipated that the number of subscriptions will continue to decline as long as purchasing practices by libraries and individuals remain as selective as they are under the existing economic conditions.

Page Charges

Page charges remain an important source of income for the journals. The percentage of authors arranging for page charges to be paid was 86% overall, the same as in 1981.

Reprints

The number of reprints of each article ordered continues to decline, although the number of authors ordering at least 100 reprints was about the same as last year (96% in 1982, 95% in 1981).

Physiology in Medicine

By the end of the year the decision had been reached that "Physiology in Medicine" would be published in *Hospital Practice*, with T. E. Andreoli serving as Editor of the series. Earlier in the year contact had been reestablished by A. J. Vander with David W. Fisher, the Editorial Director of *Hospital Practice* and the President and Editorial Director of HP Publishing Co., Inc. The new series will be directed more toward physicians than research oriented clinicians. The editor will relate to the Publications Committee as do editors appointed for the Society journals.

Financial Summary

In 1982 total income increased by 26% and total expenses by 22% over 1981. All major items of income and expenses were higher than last year. The increases in expenses are consistent with the additional amount published and inflation. The journals continued to be operated in the black. Total income including interest, dividend, royalty, and miscellaneous income was $3,029,224. Total expenses were $2,929,312.

Books

In 1982 the Publications Committee diligently pursued the selection of topics and editors for new books to be published by the Society, while the editorial staff in

Bethesda emphasized work on the several new editions of the *Handbook of Physiology*. Two books were completed, *Circulation of the Blood: Men and Ideas* and *Excitation and Neural Control of the Heart.*

Handbook of Physiology

Work in Progress. Toward the end of the year chapters began to be set in pages for the much delayed *Sensory Processes*. This volume will be a continuation of the encyclopedic section of *The Nervous System* in which *Cellular Biology of Neurons* and *Motor Control* have already been completed. Authors have been invited to prepare manuscripts for three additional volumes in the series.

Galley proofs of the first three chapters of *Peripheral Circulation and Organ Blood Flow* were sent to the printer to be set in pages in December. Completion of this volume is expected early in 1983. The volume will be the third in the section, *The Cardiovascular System*, and builds on the two earlier volumes, *The Heart* and *Vascular Smooth Muscle*. The section will be concluded with a volume entitled *Microcirculation*. Most manuscripts have been received for this volume, which should be completed in 1983.

Four volumes of the section *The Respiratory System* are in preparation. By the end of the year manuscripts had been received and accepted for 20 of an anticipated 135 chapters.

The last two manuscripts for *Skeletal Muscle* arrived late in the year. Work on the volume should now progress smoothly toward a 1983 completion.

New Commitments. The Pulications Committee approved the preparation of one new section of the Handbook and new editions of two existing sections. J. F. Hoffman and J. S. Cook are to edit a new section on cellular physiology. S. G. Schultz is to organize a new edition on gastrointestinal physiology and E. E. Windhager and G. H. Giebisch a new edition on renal physiology.

The Publications Committee favors producing future volumes limited to several hundred pages, so that parts purchased by individuals are more affordable. It is anticipated that Handbooks will become updates of special areas, not complete packages reviewing and analyzing entire fields.

Financial Summary. In 1982, 3071 copies of the Handbooks were sold, providing an income of $242,484 (1981 income was $136,796). The cost of the series from 1959 through December 1982 totaled $3,504,484; the income was $3,305,575. The total deficit is $198,909. The cost in inventory is $764,266.

Clinical Physiology Series and Special Publications

A symposium "Man at High Altitude" sponsored by the Subcommittee on Clinical Sciences was held in San Diego in October. Articles derived from the papers presented at this symposium are being developed into the next book in the Clinical Physiology Series. The book is being edited by J. B. West and S. Lahiri. No new books in this series were published in 1982.

Between 1977 and 1981 review articles on specific themes appeared in the *American Journal of Physiology: Heart and Circulatory Physiology.* These carefully refereed articles were brought up-to-date by the authors and edited by M. N. Levy and M. Vassalle for a book entitled *Excitation and Neural Control of the Heart*. The book was published in April.

Circulation of the Blood: Men and Ideas, edited by A. P. Fishman and D. W. Richards, was originally published in 1964 by Oxford University Press. With the endorsement of the Centennial Celebration Committee, the Publications Committee oversaw the reprinting of this elegant book. A new preface, which emphasizes the pivotal role of Harvey in the beginning of modern physiology and the celebration of the Society's centennial, was written by A. P. Fishman. The book was completed in September.

The momentum resulting from the preparation and appearance of *Circulation of the Blood: Men and Ideas* and the historical interest of the Society engendered by the approaching centennial led to plans for other books of this type, i.e., a People and Ideas Series. S. M. McCann agreed to edit a book on endocrinology, D. C. Tosteson one on membrane transport, and A. P. Fishman to do an update version of the just reprinted book on circulation of the blood. Additional topics and editors probably will be selected in 1983.

At the spring meeting in New Orleans a symposium on pain perception in animals was sponsored jointly by the American Physiological Society, the American Veterinary Medical Association, and the American Society for Pharmacology and Experimental Therapeutics. The manuscripts from the presentations at this symposium are being developed into a book by H. H. Erickson and R. L. Kitchell.

The Publications Committee was asked by T. G. Smith, Jr., to publish a book on voltage clamping with microelectrodes as an outgrowth of a workshop to be held in September 1983. The Committee approved the project.

Financial Summary. In 1982, 1521 copies of the first six books in the Clinical Physiology Series and 1160 copies of the two Special Publications were sold. The cost of these books through December 1982 was $229,056; the income was $247,161. The cost in inventory is $73,948.

H. E. Morgan, Chairman

Women in Physiology

Selection Mechanisms for Caroline tum Suden Professional Opportunity Awards

These are designed to provide $500 to meritorious graduate students (both male and female) who are presenting a paper at FASEB meetings and who also receive complimentary registration for the meeting and free access to the Placement Service. The solicitation of applicants was achieved by having them check the appropriate box on the abstract form and by having letters of nomination from their professor. A letter announcing the availability of this opportunity was also sent to Chairmen of Physiology. We were gratified at the response but suspect that the number of applicants will be significantly greater in ensuing years.

Summary. Total number of applications = 40 (male 29, female 11). Present academic status of applicants: currently enrolled in Ph.D. programs graduating in 1983-84 = 17; currently enrolled in M.D. programs = 2; and currently postdoctoral fellows = 19. Approximate distribution by disciplinary field representation (a necessarily subjective judgment): Cardiovascular 14; Renal 6; En-

docrinology 8; Gastrointestinal 3; Respiratory 4; Cellular 4; Neurophysiology 1.

With little difficulty and based on appropriate status for award and meritorious presentation, six awardees and four alternates were ranked in order. The awardees were James Blank, University of Texas; Reed Hoyt, University of Pennsylvania; Valerie Kalter, University of Iowa; Robert Knabb, University of Virginia; Jeri Taylor, Howard University; and Virginia Zinsmeister, University of Cincinatti.

It was suggested that, in future years, the preliminary appraisal by field be developed by the Committee and that the Editorial Board of the Journals might be approached for their input to the ranking decision. We would like to approach the editors with this idea, realizing, however, the extent of their present duties. Dr. Reynolds has created attractive Certificates of Merit to be awarded by the President of APS at the Business Meeting at which time the students will receive their checks. We feel that this public recognition will contribute to the prestige of the awards and encourage the awardees to become and remain active members of the Society.

Women in Society Leadership

A matter of some concern to the Committee is the continued lack of representation of women physiologists in the Society leadership. It seems that some short-term and long-term strategies are required to address this problem. Printouts of committees and their memberships are being sent to members of this committee for anaylsis and suggestions. It has not escaped our attention that advising our constituency to write textbooks in new areas could be very effective. We are also developing a list of women physiologists from 1905–1970 from computerized membership files. For several reasons we would like to identify productive, even eminent, women physiologists of an earlier era. This exercise would provide us with *1*) historical vignettes for *The Physiologist*, a subject of great interest to our younger membership; *2*) the possibility of creating materials for use in the Centennial exhibition; and *3*) participation in an elegant poster series (for sale to schools, libraries and academic institutions). In essence, we need to recover the past as we also try to project the future.

Recognition for Women

It seems important to interest women physiologists and help them achieve recognition to develop some programs and special events. There are some fields in which women cluster (perhaps a role-modeling effect). There are also some unique difficulties encountered by women scientists which reflect sociocultural roadblocks. Hence we have evolved a two-pronged thrust: *1*) to promote and initiate symposia on scientific topics in which there are active and productive women scientists; and *2*) to sponsor talks on subjects of broad career interest, e.g., career development, grant-getting techniques, academic vs. industrial careers. To this end, Dr. Sandra Tangri discussed "Dual Professional Career Strategies" at the 1983 FASEB Meeting.

Requests for APS Brochure

The Committee also discussed the new APS brochure. An increasing number of the college students requesting information from APS are young women, with a startling number professing an interest in exercise physiology. We would like an opportunity to survey the publications, in draft, to be certain that it is adequately sensitive for this purpose.

Women in *The Physiologist*

Upcoming columns for *The Physiologist* include a report on the physiological training of new women astronauts; the current funding status of the Women in Science Bill at NSF; an interview with Dr. Margaret Rossiter on her new book, "Women Scientists in America"; and a report on our FASEB activities, the coffee lounge, dinner arrangements, room-sharing, and new ideas from the Women's Caucus. We welcome any advice and suggestions.

Marie M. Cassidy, Chair

APS Representative's Report on AAALAC Board of Trustees

This is a preliminary report on those items of immediate concern to APS that were raised at the December 7, 1982 meeting of AAALAC Board of Trustees.

Update on Congressional Legislation

Waxman will hold a hearing on Thursday, December 9. Testimony will be limited to one antivivsectionist and one scientist. There is apparently great dissatisfaction on the part of some of the antivivisection groups who feel both bills have "sold out" to the scientists. No information was available on status of Senate bill. The best guess is that there will be no law passed in this "lame-duck" session, but that there will be legislation early next year.

Clarification of Position of AAALAC Regarding Animal Research

Because of an apparent widespread belief in Congress that AAALAC is strongly in favor of the current bills, the Board of Trustees voted to send letters to both Waxman and Dole indicating that AAALAC supports the use of animals in research and supports the idea that the individual scientists and local animal care committees should continue to be responsible for regulating the *use* of animals. I attempted to have added to the letter that AAALAC also recommends that if legislation is passed it should include provisions for newly appropriated funds to cover the costs of upgrading facilities. This latter point was, however, considered to be a separate issue and was not included. The letter stopped short of recommending either approval or disapproval of the bills.

Presentation of Dr. William Raub, Associate Director of NIH for Extramural Programs

Dr. Raub pointed out that both bills have been unequivocally opposed by NIH on the basis that there is already adequate legislation and that the cost benefit is not acceptable. NIH is legally mandated to carry out animal research and has a moral obligation to do so as well.

He indicated that AAALAC and NIH have similar goals, although NIH is not a regulatory agency except in the case of human subjects. He stated that the idea of AAALAC doing the accrediting is appealing; however, the NIH is still ultimately responsible, e.g., Taub case. NIH apparently has the authority to regulate.

The negative issues regarding accreditation are the uncertainty of costs (he was responsible for the 500 million dollar estimate that is being mentioned), concern about who will meet the costs, and the effect of this responsibility upon the organization doing the accrediting. Obviously, if AAALAC is asked to be the accrediting agency, it will find itself with the power to decide who gets grants and who does not.

Dr. Raub closed by pointing out that NIH credibility has been challenged by Congress and the public. Therefore it has started several activities. *1*) Several laboratories close to NIH have been identified as "test" sites. These organizations all have AAALAC accreditation. A group from NIH will conduct an intensive review of these sites and will determine the adequacy of the assurance statements that all NIH grant recipients must file with the government. *2*) They will take a random sample of nonaccredited facilities for intensive review. *3*) They are specifically questioning the adequacy of the local Animal Care Committees.

The request of the Scientist Center for Animal Welfare to become a member of AAALAC was deferred until further information could be obtained about the organization.

At the end of the meeting I moved that the Bylaws be amended to read that applicant organizations must provide a copy of their bylaws, funding sources, a roster of members, and copies of recent professional publications to the Board so that eligibility for membership could be adequately assessed. The motion was referred to the Executive Committee for action.

Orville A. Smith

Letter to APS from AAALAC Board of Trustees

As Chairman of the Board of Trustees of the American Association for Accreditation of Laboratory Animal Care, I have been instructed to inform you that the following motion was passed by the Board of Trustees of the American Association for Accreditation of Laboratory Animal Care relative to HR 6928 and S 2948:

> Whereas there is important legislation before Congress which could affect biomedical research and laboratory animal care, and the American Association for Accreditation of Laboratory Animal Care (AAALAC) has a purpose, stated in its Bylaws, "of promoting a program for accreditation of laboratory animal care facilities which will encourage, promote, and facilitate scientific research which includes the use of experimental animals."
>
> It is resolved that *1*) AAALAC strongly endorses the use of animals for research, testing, and teaching where the use for these purposes is appropriate; *2*) AAALAC strongly supports the freedom and responsibilities of investigators, teachers and testers, institutional animal care committees, and institutional administrations to determine when use is appropriate; *3*) AAALAC reaffirms its role as a voluntary accrediting body for laboratory animal facilities and programs, which includes assurance of the humane and skillful care and use of laboratory animals.

In addition to passing the above resolution, the Board of Trustees of AAALAC questioned the suitability of the implied relationship between the federal government and accrediting agencies. Finally, the Board expressed concern that funding the proposed legislation might have an adverse effect on the research productivity of institutions involved.

George C. Christensen, Chairman

APS Sections

Cell and General Physiology

The Cell and General Physiology Section of the American Physiological Society held their Second Annual Banquet-Lecture during the FASEB meeting in Chicago on April 12. This event was held at a local restaurant, and there were 76 people present. Dr. Charles F. Stevens of Yale University presented a lecture on "Contributions of Single Channel Recording to Cell Physiology."

APS-Cell has initiated a research award competition for young investigators whose research as represented by an abstract submitted to the FASEB meetings in the field of cell physiology is judged to be an outstanding contribution. The winners of the 1983 Research Award were announced at the banquet-lecture and are Dr. **E. Krasny, Jr.,** Dept. of Physiology and Biophysics, University of Alabama in Birmingham, Dr. **Edward J. Olender,** Dept. of Surgery, Upstate Medical Center, and Dr. **Carole M. Liedtke,** Dept. of Pediatrics, Case Western Reserve University. Each of the recipients received an award of $100.

Dr. Jean Marshall (Brown University) was recently elected Councillor to replace Dr. Mortimer Civan (University of Pennsylvania). Dr. Caroline Pace (University of Alabama in Birmingham) was reelected as Secretary/Treasurer. The other officers are Dr. Margaret Neville (University of Colorado), Chairperson, Dr. Joe Handler (NIH), Councillor, and Dr. Robert Gunn (Emory University), Program Representative.

The officers of APS-Cell encourage those members of APS who are working in the general field of cell physiology to request membership status in the Cell and General Physiology Section by writing to Mr. Herbert Brownstein, 9650 Rockville Pike, Bethesda, MD 20814.

Caroline S. Pace
Secretary/Treasurer

Annual Meeting of American Society for Cell Biology

The 23rd Annual Meeting of the American Society for Cell Biology will be held in San Antonio, TX, Nov. 29–Dec. 3, 1983. *Deadline for receipt of Abstracts:* July 8, 1983. Registration forms may be obtained from the ASCB National Office, 9650 Rockville Pike, Bethesda, MD 20814. Phone: (301) 530-7153.

APS Fall Meeting

August 20-24, 1983, Honolulu, HI

1983 Bowditch Lecture
Sunday, August 21, 4:30 P.M.
Functional mapping in cardiovascular reflexes and the heart using C^{14}-2-deoxyglucose
David Kostreva
Veterans Administration Hospital, Milwaukee

Past President's Address
Monday, August 22, 4:30 P.M.
Crises in physiological research
Walter C. Randall
Loyola University, Maywood, IL
Followed by APS Business Meeting

Saturday, Aug. 20

6-8 P.M. Opening Reception

Sunday, Aug. 21, A.M.

Symposium: Autonomic control of coronary tone: facts, interpretations and consequences.
Chaired by P. M. Vanhoutte
*Symposium: Processes of passive and active H^+ transport in isolated gastric and renal membrane vesicles

Sunday, Aug. 21, P.M.

Symposium: Neurohumoral regulation of circulation.
Chaired by V. S. Bishop
Tutorials
Calcium regulation in osteoporosis.
B. L. Riggs
Calcium exchange in the heart.
G. A. Langer
Physiology of in bile.
A. Hoffman
*Poster Discussion: Transport in isolated gastric and renal membrane vesicles

Monday, Aug. 22, A.M.

Symposium: Physiology of water immersion.
Chaired by Y. C. Lin and S. K. Hong
*Symposium: Epithelial H^+ transport processes and electrophysiology

Monday, Aug. 22, P.M.

Symposium: Factors influencing vasopressin in body fluids.
Chaired by J. R. Claybaugh
Tutorials
Structural basis of visual cortical function.
C. Gilbert
Neuronal basis of plastic adaptation in gaze control.
B. W. Peterson
Nutrition as a modulator of the aging process.
E. J. Masoro
7:00 *Poster Discussion: Regulation of H^+/HCO_3 transport in gastrointestinal epithelia

Tuesday, Aug. 23, A.M.

Symposium: Prostaglandins, leukotrienes and lung fluid balance.
Chaired by A. B. Malik
Tutorials
Comparative physiology of the renin-angiotensin system.
R. L. Malvin
Hypothalamic control of body temperature.
J. A. Boulant
Long-term reflex regulation of the cardiovascular system.
A. M. Scher
*Poster Discussion (time is tentative): Regulation of HCO_3 transport in kidney epithelium

(Note: Sessions marked with an asterisk are part of the Conference on Hydrogen Ion Transport organized by J. G. Forte and F. C. Rector, Jr.)

Tuesday, Aug. 23, P.M.

Symposium: Sea bird energetics. Session I.
Organized by G. C. Whittow and H. Rahn
Tutorials
Regulation of blood flow and oxygen transport in skeletal muscle.
B. Duling
The aging lung. M. G. Levitzky
Contractile properties of vascular smooth muscle.
R. A. Murphy

Wednesday, Aug. 24, A.M.

Symposium: Sea bird energetics. Session II

Wednesday, Aug, 24, A.M. & P.M.

Refresher Course: Physiology and biochemistry of receptors.
Organized by J. A. Spitzer.

Announcement

1984 Research Opportunities Offered by Leukemia Society

The Leukemia Society of America, a national voluntary health agency, is now accepting applications for 1984 grants to support research in the fields of leukemia and related disorders. The grants are intended to encourage studies at both the basic science and clinical levels. The Society offers three awards for individuals whose work is concentrated on uncovering cures for leukemia, the lymphomas, Hodgkin's disease, and multiple myeloma. Five-year scholarships for a total of $125,000 are available for researchers who have demonstrated their ability to conduct original investigations in the specified fields. Two-year special fellowships and fellowships, for $37,000 and $30,000, respectively, are offered for those in the intermediate and entry stages of career development. In all categories, candidates must hold a doctoral degree but may not have attained the tenured status of associate professor.

Deadline for filing applications: September 1, 1983. *For application forms and further information*: Research Grant Program, Leukemia Society of America, 800 Second Ave., New York, NY 10017.

Future Meetings

1983	
APS "Fall" Meeting	Aug 20-24, Honolulu
IUPS Congress	Aug 28-Sep 3, Sydney
1984	
FASEB Annual Meeting	Apr 1-6, St Louis
*APS "Fall" Meeting	Jul 29-Aug 3, Lexington
1985	
FASEB Annual Meeting	Apr 21-26, Anaheim
*APS "Fall" Meeting	Aug 4-9, Buffalo
1986	
FASEB Annual Meeting	Apr 13-18, St. Louis
IUPS Congress	July 12-20, Vancouver, Canada

*Campus meeting

Membership Status

Regular	4,476
Emeritus	560
Honorary	10
Corresponding	98
Associate	718
Student	262
Total	6,124

NEWLY ELECTED MEMBERS
The following, nominated by Council, were elected to membership in the Society at the Fall Meeting, 1983.

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Integrative Physiology: On Mapping the Organism

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A physiologist's outlook tends to span all levels of organismal organization (molecule, cell organelle, cell, tissue, organ) and to focus on the question, What is the organization of the organism at the level of its wholeness? How, one might ask, however, can the study of the organism at the level of its wholeness be approached? I would like to describe a simple, humble, and partial answer to this question, which in spite of its simplicity can be of great value to the teacher and student of physiology. It involves "mapping": the diagrammatic representation of the "structure" of an organism.

What is a map? A map is a drawing whose points and lines bear a one-to-one representation to the features of some geographic locale. The power of the map, however, lies not in its completeness and detail. It is but a poor shadow of what it tries to represent: a whole city may be represented by but a single point. Nevertheless, though it does not provide all the information one would ever wish to know, it is still a powerful guide to thought and enables us to move purposefully through a locale from some point *A* to some point *B*.

Physiologists are fond of organismal maps, and they abound in the physiological literature. A variety of conventions of representation are used. I would like to consider one very useful convention of this kind and examine its consequences. It was proposed by Riggs (1) in connection with his mathematical studies of physiological systems and involves the use of solid and dotted arrows, the former representing stimulation, the latter inhibition.

Let there be two variables, *A* and *B*, between which there lies a causal connection such that an increase (decrease) in *A* tends to induce an increase (decrease) in *B*. Riggs represents this relationship by a solid arrow

$$A \rightarrow B$$

When an increase (decrease) in *A* tends to lead to a decrease (increase) in *B*, the relationship is represented by a dotted arrow

$$A \dashrightarrow B$$

The relationship between "plasma glucose level" and "insulin secretion rate," for example, is represented by a solid arrow, the relationship between "carotid sinus blood pressure" and "sympathetic activity" by a dotted arrow.

In Figure 1 a cause-and-effect diagram using this convention is shown. It is well to meditate on this figure, for it contains a number of truths helpful to the understanding of integrative aspects of organismal structure.

First, cause-and-effect relationships tend to take the form of cycles: one departs along an arrow from a given element and returns to thd same element around a continuous pathway. In Figure 1 there are four such pathways of return, four *cycles of cause-and-effect* (4).

Second, each of the four cycles tends to hold at constant levels all the variables contained within it. Imagine, for example, that plasma calcium is increased, say, by injecting a quantity of calcium lactate. This raises "plasma Ca^{2+}" in Figure 1. Let us trace the consequences of this rise in calcium around cycle 1 (Figure 2). (The rise in calcium tends to decrease parathyroid hormone secretion, the resulting decrease in hormone secretion tends to decrease the plasma parathyroid hormone level, and so forth.) We find the effect of this cycle of cause-and-effect to be the reduction of the initial rise in plasma calcium. Thus the cycle in question acts to stabilize the plasma calcium level (as well as all other levels in the cycle): it tends to hold these levels constant in the face of perturbations which might tend to raise or lower them.

Third, cycle 1 contains one dotted arrow. The reader can readily demonstrate that had the cycle consisted of only solid arrows, the effect of the cycle would have been not to reduce alterations in calcium level but to amplify such alterations. It can be shown more generally (5) that if a cycle contains an odd (1,3,5,...) number of dotted arrows, it is a *stabilizing cycle* (negative feedback). Such a cycle tends to hold all variables within it at constant levels. On the other hand, if a cycle contains an even (0,2,4,...) number of dotted arrows, it is a *growth cycle* (positive feedback). Such a cycle tends to amplify disturbances rather than reduce them, leading to explosive behavior rather than stability.

Fourth, it may be observed that the enormous stability of any organism resides in the innumerable stabilizing

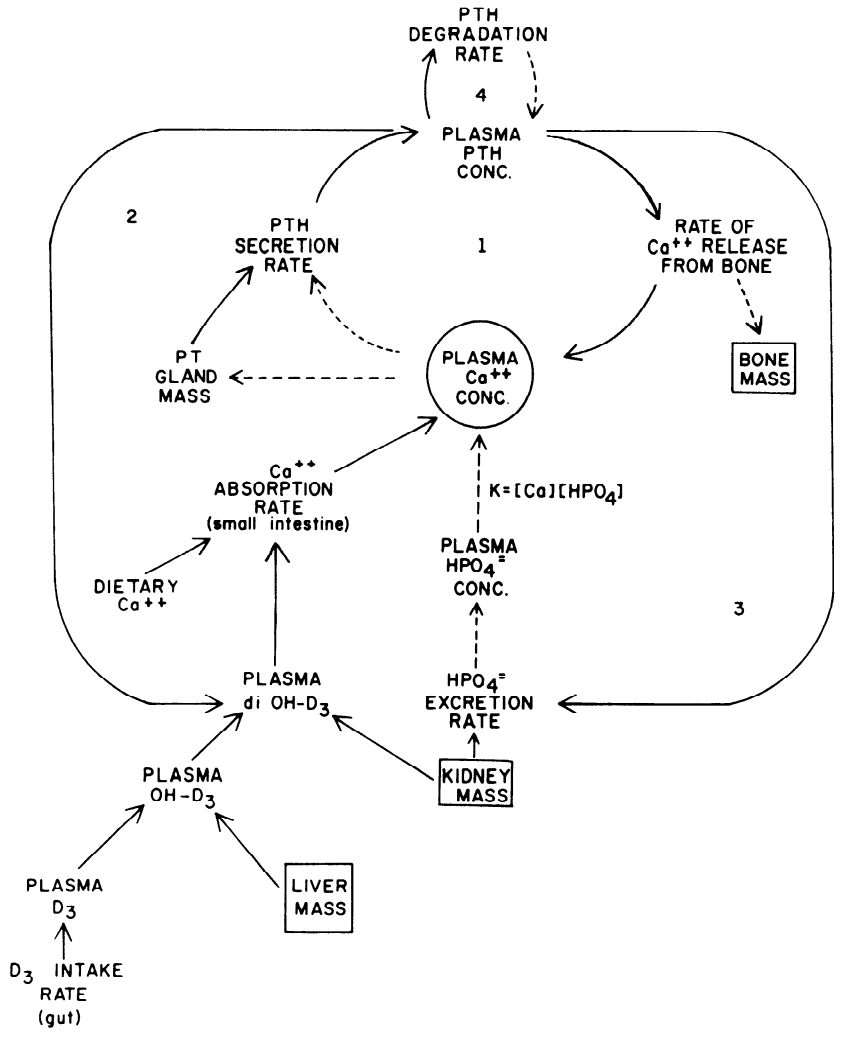

Figure 1
Regulation of plasma Ca^{2+} (see Notes 2 and 3). This is a partial map of cause-and-effect relationships that tend to hold the plasma calcium level constant. Effects of calcitonin, calcium excretion by the kidneys, and so forth, not shown. Abbreviations: PT, parathyroid; PTH, parathyroid hormone; D_3, cholecalciferol; OH-D_3, 25-hydroxycholecalciferol; diOH-D_3, 1,25-dihydroxycholecalciferol.

cycles of cause-and-effect which constitute its structure. These resist alterations at every level of organization.

Fifth, one needs to use the words "tend to" in describing cause-and-effect relationships. This is because one can, in general, say only that some variable *A tends to* increase some other variable *B* (rather than that *A* increases *B*); for, generally, *B* will be under the influence of many other variables. Hence, though an increase in *A tends* to increase *B*, *B* may actually decline due to other influences.

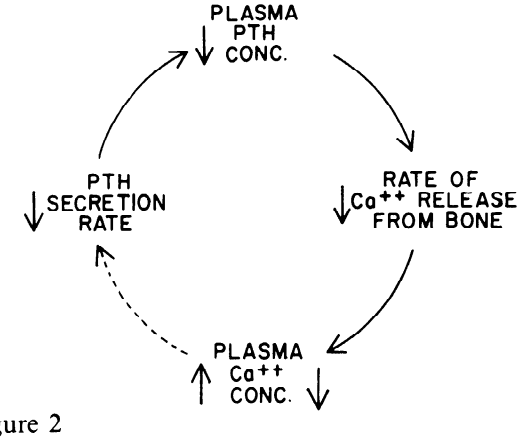

Figure 2
Response of cycle 1 (Figure 1) to a sudden increase in plasma calcium level. Parathyroid hormone secretion rate is reduced, reducing plasma level of the hormone. This, in turn, reduces calcium release from bone. Reduction in calcium release tends to reduce plasma calcium level.

In summary, physiological chains of cause-and-effect tend to form cycles. These cycles are fundamental units of organismal organization. The oddness or eveness of the number of inhibitory (dotted) arrows in a cycle provides significant information as to the nature of the cycle's contribution to the economy of the organism.

The physiological literature exhibits a variety of diagrammatic conventions to represent cause-and-effect relationships. The lack of a universal convention can breed confusion, particularly in the classroom. It would be useful for physiologists to seek some common, convenient scheme. The simple and powerful scheme of Riggs (1) described above deserves consideration.

I am indebted to Dr. Henry Hirsch for introducing me to the Riggs convention, to Dr. Robert Siegel for introducing me to calcium metabolism, and to Sarah Johnson for drafting the figures.

Notes

1. Riggs, D. S. *The Mathematical Approach to Physiological Problems.* Baltimore, MD: Williams & Wilkins, 1963, p. 88.

2. Knox, F. G. (Editor). *Physiology of Calcium and Phosphate Metabolism.* Bethesda, MD: Am. Physiol. Soc., 1980 (Refresher Course Syllabus).

Coburn, J. W., and D. L. Hartenbower. Physiology of calcium, phosphorous and magnesium, and disorders affecting their metabolism. *Curr. Nephrol.* 1: 99-171, 1977.

3. Figure 1 contains 4 cycles of cause-and-effect. Cycle 1 involves calcium release from bone. Cycle 2 involves calcium absorption from the gut (plasma calcium --►hormone secretion →hormone level →diOH-D_3 synthesis →calcium absorption →plasma calcium). Cycle 3 involves phosphate excretion by the kidneys (plasma Ca^{2+} --► hor-

mone secretion → hormone level → phosphate excretion --► plasma phosphate level --► plasma calcium level). Cycle 4 involves hormone degradation. All of the cycles contain an odd number of dotted arrows: cycle 1, 1; cycle 2, 1; cycle 3, 3; cycle 4, 1. Hence, they are all stabilizing cycles (negative feedback).

The sequence, plasma calcium →gland mass →hormone secretion rate, is not a *cycle* of cause-and-effect. The arrows in this sequence do not follow. . .-head-tail-head-. . . as they should if it were a true cycle.

4. To simplify the numbering of the cycles we have considered the two sequences, plasma calcium --► hormone secretion rate, and plasma calcium --► gland mass →hormone secretion rate, to be parallel pathways of the same cycle, namely, cycle 1.

5. A causal sequence containing one dotted arrow and any number of solid arrows is equivalent to and can be replaced by a single dotted arrow, e.g., $A \rightarrow B \dashrightarrow C \rightarrow D \equiv A \dashrightarrow D$. A sequence containing two dotted arrows and any number of solid arrows is equivalent to and can be replaced by a single solid arrow, e.g., $A \dashrightarrow B \rightarrow C \dashrightarrow D \rightarrow E \equiv A \rightarrow E$.

Consider a cycle which contains n dotted arrows. If n is even, the dotted arrows can be paired, with each pair equivalent to a single solid arrow; hence, such a cycle (e.g., $A \rightarrow B \dashrightarrow C \rightarrow D \dashrightarrow E \rightarrow A \equiv A \rightarrow A$) can be replaced by a single solid arrow. This makes it a growth cycle (positive feedback): a perturbation which increases A produces a reaction around the cycle which further increases A.

If n is odd, then $n-1$ is even. The $n-1$ dotted arrows cancel and reduce to solid arrows. Hence, the whole cycle is equivalent to one dotted arrow plus solid arrows. It can therefore be replaced by a single dotted arrow (e.g., $A \dashrightarrow B \rightarrow C \dashrightarrow D \dashrightarrow E \rightarrow A \equiv A \dashrightarrow A$). This makes it a stabilizing cycle (negative feedback): perturbation which increases A produces a reaction around the cycle which tends to decrease A and thus reduces the perturbation.

Analysis of Physiological Systems via Mathematical Models

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The process of scientific inference is dependent on conceptual models whether they are formulated consciously or subconsciously. Physiological investigation is an attempt to use controlled observations or experiments to rationally build, test, and modify conceptual models. Some steps involved in the modeling process are listed in Table 1. The ideas are discussed in more detail by Ackerman and Gatewood (1), Aris (2), Bender (4), Gold (12), and McIntosh and McIntosh (16).

Goals

The purpose of the model should be clearly defined. Some common goals are listed in Table 2. The first is that of data description. An empirical model is required. It reflects observed relationships between variables. Its primary function is to summarize those relationships in terms of a small number of parameters for purposes such as discussion, prediction, or control. A model may be proposed for the purpose of discriminating between certain physiological states. The goal could be diagnostic or prognostic classification. Such models are usually empirical but may have a theoretical basis as well.

Theoretical models embody the modeler's concepts of what causes the observed behavior. The model still reflects observed relationships, but its structure constitutes an explanation of the mechanisms that underlie the relations. These models have goals such as *1*) estimating parameters and variables inaccessible to direct measurement, *2*) testing hypotheses, *3*) designing experiments, and *4*) increasing insight and understanding.

It is rare in physiological systems to have adequate knowledge for the derivation of a fully theoretical model. More often the theoretical concepts are supplemented by empirical hypotheses and previously observed relationships. The remainder of this paper is addressed to physiological models that have a significant theoretical component.

Model Formulation

Once the goal is defined, the next step is the formulation of the model. First, the system needs to be identified and distinguished from its surroundings. In physiology such things as function (the cardiovascular system, the respiratory system), structure (red blood cells, mitochondria), or a chemical (widely distributed anatomically) may delineate the system.

Next, the system components must be named and their interactions described. These interactions may be based on physical, chemical, or biological laws, or they may be based empirically on previous observations or on

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Table 1
Steps in Modeling Process

Definition of Goals
Model Formulation
Qualitative Examination
Parameter Estimation
Model Fitting
Model Evaluation
Design of New Experiments

Table 2
Goals of Modeling

Data Description
Discrimination Between States
Theoretical Models
  Estimating unobservable parameters
  Testing hypotheses
  Designing experiments
  Increasing insight and understanding

hypotheses. Any real physiological system contains a large number of internal components and interactions. When described in fine detail, the complexity is unmanageable. One of the chief advantages of using a model is the capability it provides for studying the minimum number of components and interactions that influence the behavior of interest. Since the relevance of all factors is not known ahead of time, the physiologist must draw heavily on his experience when simplifying the prelimary model.

How can models be simplified? It may be feasible to combine certain components. If they behave in a similar manner or only their average behavior is of interest and influences the remaining components, such lumping is valid. It may be possible, or even necessary in the absence of sufficient information, to isolate a subsystem and consider only the input-output relationship of its variables. This is reasonable when the intermediate steps in the subsystem are of no interest to the modeler and are not influenced by other changes in the system. Another simplification can be achieved by restricting the range of conditions over which the model must be valid so that the effects of some components (or interactions between them) become negligible. Definition of the time scale of interest can result in simplifications. Variables changing significantly only over time intervals much longer than those considered can be treated as constants. Alternatively, variables reaching equilibrium very quickly relative to the time scale of interest can be replaced by their equilibrium values.

Mathematical Description

Once the system has been defined in appropriate detail, it should be described mathematically (15,18). What are the advantages of a mathematical description? First, the very process of defining precisely the biological meaning of mathematical symbols, of determining which components vary independently, which vary dependently, and which are constant, and of defining the rules under which the components interact clarifies one's thinking and reveals deficiencies in our knowledge of the system. Second, if a model conforms to certain known mathematical equations, then the possible consequences of the hypotheses and the relationships of its variables have already been logically deduced. Third, applying the logic of mathematics to the equations can reveal inconsistencies and redundancies in the mathematical descriptions and, hence, in our understanding of the physiological system.

The type of mathematical equation appropriate depends on the nature of the variables and their interactions. Differential equations are the single most important type in physiology. Ordinary differential equations have one continuous independent variable, the most common example being time. The dependent variables must be continuously differentiable over the time range of interest. Often inherently discrete quantities such as the number of molecules or the number of radioactive atoms present can be treated as continuous variables when a single event produces a very small effect on the large number present. When other independent variables such as those defining spatial location must also be considered, partial differential equations are employed. When past events influence system behavior, i.e., when the system has memory, integrals and delays may appear in the mathematical description. The assumption of steady-state or equilibrium conditions can reduce differential equations to algebraic equations. When the independent variable is discrete, the mathematical description is in terms of difference equations.

Qualitative Evaluation

Now that the model has been formulated and mathematically described, it must be tested by comparing its predictions with preliminary observations and experimental data. One of the most compelling reasons for describing the model mathematically is that powerful tools exist for solving the mathematical equations. Before launching into extensive computations though, it is well to examine the model further. Can the number of variables be reduced? Algebraic equations are useful here. Consider special cases such as steady-state or equilibrium conditions that yield simpler solutions. Consider limiting cases, or cases with fixed parameter values for which solutions in closed form are known. Unless these studies negate the present model, proceed to examine it further.

If an analytic solution to the equation can be found in closed form, the characteristics of the model are known. It is possible to assess whether the model can qualitatively reproduce experimental results. More often such solutions cannot be obtained; so one must resort to computer simulations (17). The response of the model is calculated by substituting values for its parameters and for a range of values of the independent variables. The behavior of the model under a variety of conditions can be observed.

Parameter Estimation

Suppose there are some ranges of parameter values for which the model is in qualitative agreement with preliminary observations. The next question is whether all of the parameters are identifiable given the data to be obtained from experiments performed on the physiological system represented by the model (6). The answer is particularly important when the parameters are equivalent to physical quantities whose indirect estimation is a prime objective of the study.

A number of tests for analyzing the identifiability of parameters in linear systems have been developed (8). Sometimes it is possible to show that a given parameter

could not be identified uniquely with the proposed experimental design even in the absence of noise. Such tests are generally not available for nonlinear systems, however; so heuristic methods are employed (14). Parameter estimates are sought that will bring the model predictions into the best possible agreement with the observed responses. As the words imply, the agreement will not be perfect. There is uncertainty in physiological investigations introduced by measurement error, by modeling error, and by biological variability.

The parameter estimates sought are those that bring the model predictions into the "best possible agreement" with the observations. How can the phrase "best possible agreement" be defined in quantifiable terms? One such definition is provided by the maximum likelihood method, which is widely employed in scientific research (19). An experiment is performed in which responses are observed under a given set of experimental conditions. The observations are assumed to be a random sample from a population characterizable by a probability distribution. The form of the distribution may be based on theoretical principles, it may be determined from previous observations, or it may be an additional hypothesis to be tested. The probability distribution is a function of unknown parameters (denoted by the vector $\underline{\eta}$) that can be related to the parameters of the physiological model.

Once experiments have been performed, the responses and experimental conditions are known. Now the distribution, which is a function of the unknown parameters only, becomes the likelihood function, denoted $L(\underline{\eta})$. The values of $\underline{\eta}$ for which $L(\underline{\eta})$ assumes its maximum value are the parameter values for which the observed responses are most likely to have occurred (within the framework of the given model and experimental conditions). Hence, these values are termed maximum likelihood estimates, denoted $\underline{\hat{\eta}}$.

The heuristic criterion for parameter identifiability says essentially that if $L(\underline{\eta})$ has a unique maximum in some region of the parameter space, then the parameters are identifiable in that region (3). $L(\eta)$ can be evaluated over a grid of values of $\underline{\eta}$ covering the physiologically meaningful range in order to test for uniqueness.

If the parameter are not identifiable, the effects of measurement errors and/or modeling errors must be reduced. Once new proposals are made, the identifiability analysis should be repeated.

Model Fitting

Suppose the parameters are identifiable in the presence of random noise. The next step is to locate those values of $\underline{\eta}$ that maximize the likelihood function within the bounds of any constraints imposed by the physiological considerations.

This is the problem to which computer optimization or model-fitting algorithms are addressed (10,11). Such algorithms are subject to inadequacies due to the nature of the problem (9). First, although $L(\underline{\eta})$ must have a greatest value, that value is not necessarily finite, as can be appreciated by considering the case of one parameter where $L(\underline{\eta})$ is a linear function of η, i.e., a straight line. Alternatively, $L(\underline{\eta})$ may have several maxima. Computer algorithms cannot distinguish, in general, between local maxima and a global maximum. Nevertheless, there are optimization algorithms available in software packages that produce satisfactory results in most cases.

Note that if the responses are independently, identically, and normally distributed with a constant variance, the maximum likelihood estimates are equivalent to least-squares estimates.

Evaluation

Now we have the best fit of the model to data. How good is it? Is there evidence for rejecting the model? The answer is inevitably subjective, but there are guidelines. If there are no modeling errors, the residuals (the differences between an observed value and a predicted value) should measure random experimental error only. Distribution-free tests requiring ranking of residuals or examination of the number of sign changes ("runs") may be useful in evaluating randomness (7). Too few or too many sign changes are evidence of an inadequate model. When replicate experiments are performed, the sample variance can be calculated under each set of experimental conditions. If there is no modeling error, the sample variances should be comparable in magnitude to those calculated from the residuals. Examine the parameter estimates. Are the values reasonable in the light of independent knowledge about the physiological system? A negative answer suggests an inadequate model.

Suppose the model is judged to be inadequate. What next? Numerical errors may have occurred in the digital computer solution of the model equations (7a). If the model predictions are correct, the model structure must be reexamined. Simplifications were introduced when passing from the preliminary model to the mathematically described model. One or more of these may be invalid. Theoretical considerations may dictate a likely candidate. If the residuals are not random, the pattern of their departure from randomness may suggest which simplifying assumptions are invalid. The model fitting and evaluation procedures must be repeated, with questionable simplifying assumptions eliminated. If these efforts fail, the structure of the preliminary model must be reexamined.

If the model is judged to be adequate, confidence intervals can be placed on the parameter estimates and on the responses using the maximum likelihood estimates to evaluate the likelihood function.

Design of New Experiments

Consider the case where the model is tentatively accepted. More information is desired. Generally, more experiments will be performed. The model can be used to design them. For example, a proposed experiment can be simulated using the best available estimates for the model parameters. The simulations are often performed to explore the effects that would be produced by altering those experimental conditions under the investigator's control. In this case an entire family of synthetic curves should be produced with each member simulated with a different selection of experimental conditions. A set of curves should be generated from each family member by the addition of random noise to the simulated responses. If the problem is to distinquish between two competing hypotheses (models), a family of curves should be generated (with random noise added) for both models. The simulated data can be analyzed statistically (5,13).

Suppose that the model simulated is believed to be correct but that better parameter estimates are sought. One

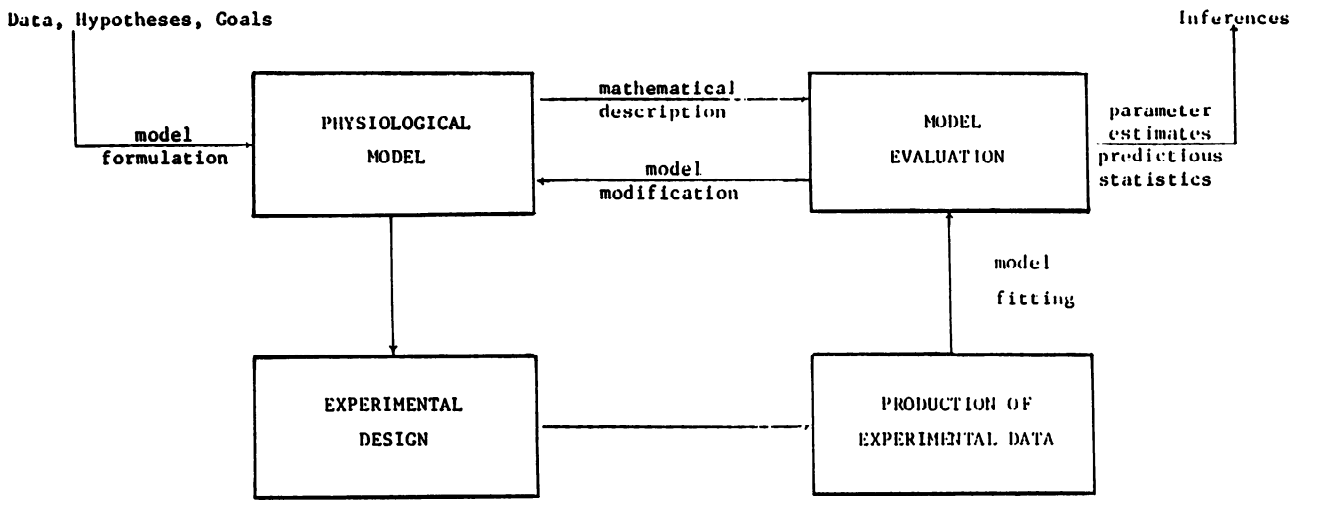

Figure 1
A scheme for making inferences about physiological systems.

might select the family member exhibiting the least correlation between parameters. If a single parameter is of special interest, select the family member producing the smallest confidence interval for the desired estimate. Suppose, instead, the problem is to choose between two competing models. Select the family member for which the experimentally observable response (simulated with the first and second models, respectively) differ most. Once the design is completed new experiments will be performed; so the process has come full circle.

Summary

Table 1 showed a list of steps to be executed when studying physiological systems with theoretical mathematical models. However, the interactive nature of the process is better revealed by the diagram in Figure 1. This is actually a scheme for making inferences about physiological systems. It includes the model formulation, together with its evaluation (by comparing model predictions with experimental data and observations) and its use in designing new experiments. The presence of a loop implies that, as a result of new data being produced and used to evaluate the model, new models will be formulated. Models should, in fact, become obsolete. Hence, it is not the model itself but rather the entire intellectual process implied by the term modeling that serves the physiologist in the search for new insights and understanding.

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Correction

Physiologist 25(3): 109, 1983. The price of *Ecology of Bats* is $49.50 instead of $9.50.

Trends in Physiology Teaching Laboratories for Medical Students – 1982

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What teaching tools other than lecturing are we now using with our medical students? This survey of approaches to teaching physiology was designed to describe changes since 1969 and 1973 when similar surveys were made (1). The members of the Association of Chairmen of Departments of Physiology were polled during the summer of 1982, and 107 of 139 (77%) of those who teach medical students in North America responded. The department heads were not unanimous in their opinions or approaches, but it is clear that the use of classical, animal-lab experiments is waning.

During the last ten years, 64 departments (60%) decreased the emphasis on laboratory experiences for their medical students, and only 11 (10%) increased the emphasis. Classical, student laboratory experiences were used by only 64% of the 107 departments responding -- a marked decrease from 90% reported in the earlier surveys. Although, two-thirds of all responding departments considered the time allocated to the laboratory program (Table 1) adequate, of the remaining one-third, 31% felt this to be less than adequate, and only 2% felt it was more than adequate. Only 5 respondents, of 64 departments with decreased emphasis on laboratories, considered this decrease in time and emphasis to be beneficial in terms of quality of education, while 60% considered the decreased emphasis to be detrimental. The decrease in emphasis seems to match the reduced time scheduled for "laboratory". Non-lecture scheduled time averaged about 70 hours compared to an average of 113 hours in 1969. (With only 38% of the schools responding to the 1973 survey, the validity of comparisons to 1973 is doubtful. Of the 17 departments in which the respondents to this survey stated that they changed laboratory objectives during the last 10 years, most indicated a reduction in experimental laboratory programs, less emphasis on manipulative skills and less use of live animals. Budgetary and curricular time restraints, lack of enthusiasm on the part of faculty, motivation of students for simply passing the National Board Examinations, and antivivisectionist attitudes of the students were listed as reasons for reduced emphasis on the laboratories.

The average time available for lecturing (102 hrs) seems to have increased during the last decade (Table 1). Lecturing only was used in 7% of the departments responding, compared to 22% in 1973 that had no laboratories. Classical, pre-assigned experiments were used by only 64% of the departments (Table 2). Of these, only about half (54%) of the students' scheduled non-lecture time was used for experiments, because other teaching approaches were also used. Attendance in laboratories was required in only 65% of the departments (71% in 1969 and 64% in 1973), but actual average laboratory attendance was 88%. In the 65 departments using laboratory conferences, the attendance averaged 83%. Class discussions, seminars and presentations continued to be popular. Demonstrations were used, but less than in the previous decade. Nearly half of the departments assigned problem sets. The students averaged about 18 hours on these problems (26% of 68 hours). Video, films, and slide-tape packages were used by only about one third of the departments, with the students spending about 7 hours with these teaching tools. Computer-assisted instruction was used in 11 (10%) of the departments. The trend was to replace predesigned laboratories with problem sets and video or slide/tape packages. Computers are not yet widely used.

Table 1. Time available for Physiology other than lecture.
(Percentage of departments responding)

	1969	1973	1982
0 (Lecture only)	2	22	7
1 - 25 hrs	14	25	13
26 - 75 hrs	26	32	49
76 - 125 hrs	35	13	23
More than 125 hrs	22	6	6
Lecture (Mean ± SD in hrs)	85	89	105 ± 40
Non-lecture (if used) (hrs)	113	73	68 ± 52
Average total scheduled time:	-	-	164 ± 49
Number of Schools in North America:	102	102	139
Response to Survey (%)	62	38	77

Table 2. Non-lecture Time Spent by Students.

	1969		1982	
	Depts[1]	Time[2]	Depts	Time
Pre-assigned experiments	90	55	64	54
Class discussion, seminar	70	12	64	29
Demonstrations	75	14	53	18
Solve problem sets	--	--	43	26
TV, film, slide/tape	--	--	35	10
Computer Assisted Instr.	--	--	10	17
Laboratory research proj.	--	10	6	35
Other (Clin. Corr. Conf.)	--	--	7	28
Other (Exams, free, etc.)	--	--	8	46

Average scheduled time was 68 ± 54 (SD) hours for the 99 (93%) departments using non-lecture programs.
1 Percent of departments providing this option.
2 Percent of total non-lecture time scheduled used in this activity.

Self-teaching packages are not highly popular (Table 3). Slide-tapes and video tapes were used about equally. In 23 departments, computer simulation and/or computer-assisted instruction (CAI) was used, with 11 using CAI. Independent study was used by 18 departments. Except for 3 or 4 departments in which more than 90% of the students used self-teaching

Table 3. Self-teaching Methods Used

	Number Depts Offering	(%)	M[1]	Percent Using[2]
Slide-tape Programs	37	35	3	22 ± 28
Video Tapes	32	30	2	21 ± 26
Computer Simulation & CAI	23	21	4	43 ± 35
Independent Study	18	17	4	40 ± 38

M[1] Number of departments in which more than 90% of the students use this method.

2 Percent of students using this option where available (Mean ± SD).

methods, this approach was used either as supplemental material or for remedial help in that less than half of the students chose these options. Many respondents (67) commented about the trend toward using self-teaching approaches. Most, but not all, considered the trend to be increasing, especially toward more computer assisted instruction or simulation. The limited time available for the students and the quantity and quality of available materials were listed as current hindrances to wider use of self-teaching packages.

The students themselves were the most popular experimental subject (Table 4). Dogs continued to be used extensively, with 4 departments having 6 or more sessions and 23 having 2 dog labs. Amphibians were utilized by numerous departments that scheduled one or two laboratory sessions. Computer and physical models were used, but not extensively. About a third of the respondents would like to see a change in the mix of laboratory subjects used. About half of these would like more animal laboratories, but the cost and student and faculty time are serious constraints. More labs with the students themselves and more computer models and/or physical models were also suggested as desired changes.

Table 4. Experimental Subjects used in Physiology Labs

(Numbers of departments using each.
For example, 23 departments have 2 labs with dogs.)

Laboratory Sessions with:	1	2	3	4	≥5	Sum*
Students themselves	10	21	15	16	11	216
Dogs	16	23	9	11	7	168
Amphibians	27	12	2	2	-	65
Rats	18	6	-	1	1	39
Computer Models	7	7	3	-	-	30
Physical Models	14	4	1	-	-	25
Cats	8	2	-	-	-	12
Invertebrates	-	1	-	-	-	2
Other	14	5	1	1	2	41

*Sum = Sum(Number of lab sessions)x(Number depts. using)

Many respondents considered physiology laboratories to be essential for the education of physicians. Experiences in acquiring and handling data, development of problem solving skills, and interaction with the faculty were considered adequate justification for the effort, time and money required. The most important laboratory objectives are given in Table 5.

Table 5. Relative Importance of Laboratory Objectives.

(Percent of Departments considering objective "very important" or "important".)

	1969	1973	1982
Supplement and reinforce lectures	75	90	95
Appreciate experimental methods	71	71	64
Student-faculty relations	36	43	64
Experience with live animals	53	53	58
Acquire manipulative skills	21	23	33
Interpret clinical lab findings	24	23	25

Based on rating of each as: 1 = very important to 4 = minor objective.

Number of respondents to this section was 88 (82%).

Interpretation of clinical laboratory findings and acquisition of manipulative skills were considered to be very important by less than 10% of respondents. Nonetheless, these were "important" objectives for about one-third of the respondents. Pathophysiology clinical case studies were considered effective for teaching physiological principles to medical students. No clear trend was apparent.

One respondent who reported no evidence of a decline in National Board scores felt that if formal lectures were designed to present the subject matter with an emphasis on the experimental basis of our current knowledge, then the need for the type of laboratory experience of "by-gone days" will vanish. This appears to be the problem: How to teach the experimental basis of physiology, rather than spoon-feed facts to pass a multiple choice examination.

Only 45 (42%) of the departments considered laboratory work in the determination of a final grade and, on the average, laboratory work counted only about 10% toward the final grade. If we want the students to acquire problem solving skills, be able to handle data and understand the experimental basis of physiology, should we not develop ways to grade on the basis of these skills, in addition to skill in answering multiple choice examinations?

A total of 32,950 students was taught by a full-time faculty of 1385 and 473 part-time instructors. This load averaged 17.7 students per faculty member.

In the 1969 and 1973 surveys, there was a trend toward an integrated organ system approach to teaching medical students (Table 6). This trend has waned. Only 8% of the responding departments use an integrated approach, although another 8% use some combination. Over two-thirds of the schools have departmental courses. In 11% of the schools, a single department has the undivided attention of the students for a segment of the academic year. The curricular design used was satisfactory for 83% of the respondents. Of the 14 respondents (13%) that desired a change, 5 departments were currently using an integrated (organ system) teaching program. Of the 14 desiring a change, most would prefer departmental courses.

Table 6. Type of Program for Medical Students

	(Percent of departments using) 1969	1973	1982
Integrated (organ system) program	15	25	8
Departmental (discipline)	81	73	71
Departmental (only one at a time)	--	--	11
Other	4	2	10

In conclusion: Laboratory emphasis has decreased, but labs continue to be considered important and used extensively in teaching physiology to medical students in over two-thirds of the responding schools. There seems to be a modest trend toward the use of problem solving sets, video, slide/tapes, computer simulation, and computer-assisted instruction. A hands-on approach to problems with the help of an enthusiastic professor effectively helps students understand physiological mechanisms. The concluding sentence of Poland et al (1) continues to be relevant: ". . . Any of the possible approaches used in laboratory teaching must reflect the convictions of the local staff to be successful."

ACKNOWLEDGMENTS

The attention of the Chairmen of the Departments of Physiology and the support of the American Physiological Society and the Department of Physiology, Indiana University School of Medicine, are greatly appreciated, as is the help of Marsha Hunt and Helen Glancy. Results of the survey were reported at the symposium, "Teaching of Cardiovascular Physiology Outside the Lecture Hall", October 12, 1982, in San Diego.

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Book Reviews

Vision D. Marr

San Francisco, CA: Freeman, 1982, 389 pp., illus., index, $9.95

Vision as conceived by David Marr is a different kind of book. It is an attempt to find a new approach in examining how human vision functions. Marr, in association with his colleagues at Massachusetts Institute of Technology, has used computational theory in trying to understand the abstract nature of the visual process. The question Marr asked is whether it is implemented in neurons and which algorithm is used in the process.

The text is divided into three parts. Part I is a general introduction that includes complex information-processing systems and the importance of computational theory as related to vision. Part II is concerned with vision itself, i.e., all aspects including intensity and color. Also such subjects as spatial arrangements, ways in which surface information is encoded in images, and how image formation takes place are discussed. This is followed by the relationship of images to surfaces and vice versa, including that to motion. Part III, the epilogue, is based on lunchtime conversations at the Salk Institute in La Jolla, CA, among Francis Crick, Tomaso Poggio, and the author. Here questions are asked about vision, perception, nerve cells, the retina, the brain, and the computer and are answered in the form of a kind of round-table discussion. Next to the introduction (part I), this part (III) of the book was the most interesting to this reviewer. However, the meat of the text is in part II.

The author describes his work as an adventure to be enjoyed, and it can be that, but for the generalist it will take some effort. To visualize many of the figures in part II, *Vision*, stereoscopic viewers will be helpful for obtaining three-dimensional images of the figures illustrated. I can recommend the book to all those interested in human vision and pattern recognition, as well as those working in artificial intelligence and robotics. The book can be read in sections, and the topics in part II should be explored as an experiment in the process by the reader. This part contains much that should be considered in trying to understand human vision. It if unfortunate that David Marr did not live to see his book in final form, but he has left his mark here. The book has a helpful glossary, a good bibliography, and an index.

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Metamorphosis: A Problem in Developmental Biology (2nd ed.). L. I. Gilbert and E. Frieden (Editors)

New York: Plenum, 1981, 578 pp., illus., index, $9.50

This second edition of *Metamorphosis* is not actually an update of the first edition but represents a second volume of articles about various problems in developmental biology. There is only a slight overlap with the contents of the first edition, and a new group of contributors has been used. The editors have brought together a variety of informational and research material on metamorphosis reviewing much of the recent work and written by recognized and authoritative specialists in developmental biology. The material contained in the book is intended for the graduate and advanced undergraduate student.

The book is divided into two sections: invertebrate metamorphosis with emphasis on the insects, and vertebrate metamorphosis with emphasis on the amphibians. Many of the chapters in the book end with a discussion, concluding statements, and/or summaries, which are most useful for a text or reference book. The invertebrate section consists of eight chapters, beginning with an overview of metamorphosis by G. Wald and covering both the invertebrates and vertebrates. Chapter 2 by K. C. Highnam is a general survey of the range and pattern of metamorphosis throughout the invertebrate phyla and includes a comparison of insect metamorphosis with that of other invertebrates. Chapter 3 by M. Locke is a review of cellular structural changes during insect metamorphosis. Chapter 4 by N. A. Granger and W. E. Bollenbacher covers hormonal control and includes a discussion of the methods of studying the endocrinology of insect metamorphosis, the complexity of the endocrine system, and the chemistry and control of the endocrine glands. Chapter 5 by L. I. Gilbert and W. Goodman is on the chemistry, metabolism, and transport of hormones controlling insect metamorphosis and is a review of the prothoracicotropic, molting, and juvenile hormones. Chapter 6 by S. Sridhara covers macromolecular changes, essentially those in protein and RNA in insect metamorphosis. Chapter 7 by J. W. Fristrom contains a discussion of *Drosophila* imaginal discs as a model system for studying metamorphosis, and Chapter 8 by J. D. O'Connor and E. S. Chang is on the use of established cell lines to study the effects of the ecdysteroids and the juvenile hormones. The vertebrate section also consists of eight chapters, beginning with a survey of chordate metamorphosis including the urochordates, cephalochordates, fish, and amphibians (Chapter 9 by J. J. Just, J. Kraus-Just, and D. A. Check). Chapter 10 by H. Fox is on cytological and morphological changes in various organs and systems during metamorphosis. Chapter 11 by B. A. White and C. S. Nicoll consists of a review of hormonal control with studies of the thyroid, prolactin, adrenocortical hormones, and osmoregulation and metamorphosis. Chapter 12 by B. G. Atkinson is a discussion of the basis of tissue regression and synthesis. Chapter 13 by J. J. Kollros is a review of transitions and changes in the nervous system during amphibian metamorphosis. Chapter 14 by R. H. Broyles is on the changes in the blood during amphibian metamorphosis. Chapter 15 by S. J. Smith-Gill and V. Carver covers biochemical transitions and changes of several tissues (liver, kidney, intestine, integument, and the eye) during metamorphosis. Chapter 16 by E. Frieden is a review and discussion of the role and mechanism of action of thyroid hormones in vertebrate development and calorigenesis.

This book presents an excellent review of the recent work and thinking on insect and amphibian metamorphosis. I recommend it for persons interested in developmental biology and related fields of study.

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Oxygen Radicals and The Microcirculation

A Symposium Presented
At The Microcirculatory Society

New Orleans, Louisiana
April 24, 1982

Organized and edited by: A. E. Taylor
President-Microcirculatory Society

Faculty: J. D. Crapo, D. N. Granger, H. A. Kontos,
J. M. McCord, J. E. Repine and A. E. Taylor

Typing and matting done by Mrs. Sandy Worley

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INTRODUCTION

Last year, we had the opportunity to review the extensive literature on the formation of alveolar edema, especially in regard to changes in capillary permeability. In reviewing this vast amount of new information, it became increasingly clear that many seemingly unrelated types of pulmonary pathologies had common components - the involvement of leukocytes and/or the oxygen radical system. Because of the broad scope of this area, I designed this symposium around several areas of research currently in progress. So, basic biochemists, physiologists and clinical researchers have provided their expertise for the content of this symposium.

Oxygen Radical Generation and Tissue Damage

Figure 1 is a very schematic diagram indicating how tissue damage causes the release of superoxides (O_2^-) which are converted to hydrogen peroxide (H_2O_2) and hydroxal radicals (OH•). All three compounds are extremely toxic to tissues and it has also become quite clear that the endothelium of the microcirculation is extremely sensitive to these compounds. These tissue damaging compounds are referred to as oxygen radicals and the enzymes and compounds which either increase their conversion to another, less toxic compound or scavenge these radicals are usually referred to as "free radical scavengers".

In this symposium, Dr. Joe McCord will present the biochemistry of the superoxides and how the variuos compounds and enzymes shown in Figure 1, i.e., superoxide dismutase, catalase, dimethylsulfoxide (DMSO) and mannitol, decrease the amount of superoxides, hydrogen peroxide and hydroxal radicals in tissues (15).

Figure 2 shows a computer model which describes the superoxide system as related to tissue damage. Tissue damage can cause the release of leukotrines through the prostaglandin system, which stimulates neutrophil infiltration into the tissues and increases O_2^- within the tissues (blocks 8 and 9) (10). Also, when prostaglandin G_2 is converted to D_2, O_2^- increases (12) which can also feed back to

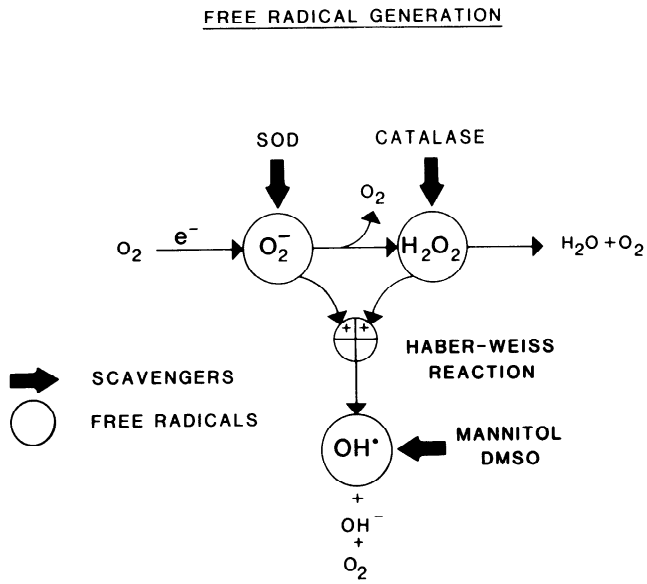

Figure 1. Schematic representation of the formation of oxygen radicals: superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxal radical (OH•). The dark arrows refer to either enzymes or other substances which will convert these oxygen radicals into other substances, i.e., superoxide dismutase, catalase, dimethylsulfoxide and mannitol inactivate O_2^-, H_2O_2 and OH•, respectively.

increase the neutrophil sequestration into the tissues (block 8). In addition, hyperoxia is known to increase O_2^- production (block 7) and this O_2^- can feed back to cause neutrophil movement into the damaged tissues. The depletion of neutrophils has been shown by several investigators to decrease the damage associated with microemboli (5, 11), endotoxin (10), hyperoxia (Crapo, this symposium), thiourea compounds (Repine, this symposium; 20).

Tissue hypoxia results in an increased hypoxanthine which produces superoxides when xanthine is formed (blocks 14 and 15). Again, the superoxides can feed back to cause neutrophil attraction into the damaged tissues (9).

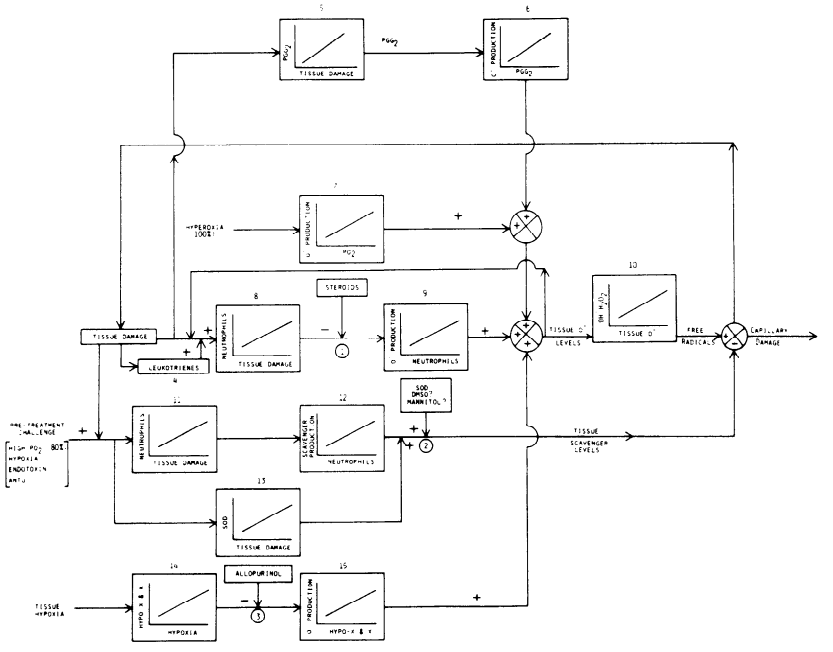

Figure 2. A schematic representation of the oxygen radical system and tissue damage as related to increased capillary permeability to macromolecules. The numbered boxes are the assumed relationships between the independent variables (input) and dependent variables (output). The circled numbers refer to interventions which will either block the formation of O_2^- or inactive H_2O_2 and $OH^\cdot$ or convert O_2^- to H_2O_2.

Blocks 11, 12 and 13 demonstrate a very interesting finding - some challenges cause an increased production of superoxide dismutase, which converts O_2^- to H_2O_2. The mechanism associated with this increased SOD production is presently unclear, but it is most likely related to the tissue production; however, the neutrophils may also provide more tissue superoxide dismutase. Only small challenges are used to produce the effect, e.g., endotoxin levels 1/50 of the LD50 dosage, produce a marked increase in SOD production by the lung tissue (2, 6, 7, 8, 19).

Although all these phenomena do not occur in the same pathological states, they are shown to be additive in Figure 2 to produce H_2O_2 and $OH^\cdot$ at block 10. The free radicals, if greater than the tissue scavenger levels (shown as a summation on the far right of Figure 2), will produce tissue damage and this results in an increased capillary permeability to macromolecules. In fact, it appears that changes in vascular permeability are very sensitive indicators of free radical levels within any damaged tissue (2, 3, 5, 9, 10, 13, 17, 18, 20). Dr. Karl Arfors and coworkers have presented microscopic studies from a cheek pouch preparation in which xanthine + xanthine oxidase and hypoxia produce oxygen radicals and increased microvascular permeability, and in addition, these effects are blocked by SOD, mannitol, and DMSO (3).

Superoxide Studies Presented in this Symposium

Dr. Neil Granger will present his work on the generation of superoxide radicals in ischemic smallbowel. This study is unique since it shows very quantitatively that ischemia increases vascular permeability which is associated with the production of oxygen radicals. The osmotic reflection coefficient for plasma proteins was shown to decrease from 0.9 to 0.6[1] in ischemia and the effect is totally reversed with SOD, but not by indomethacin, methylprednisolone, and H_1 (benadryl), and H_2 (cimetidine) histamine blockers (9).

Dr. Hermes Kontos will discuss how free radicals generated by the prostaglandin system (specifically during conversion of G_2 to D_2) are related to the cerebral vascular dilitation associated with brain damage. This study indicates that vascular reactivity as well as permeability may be greatly effected by the generation of superoxide radicals. Dr. James Crapo will discuss how intracellular generation of O_2^- radicals are related to the damage associated with O_2 toxicity using very elegant biochemical, structural and cultured cell techniques. In addition, leukocytes trapped by platelets are a later event which may amplify the initial intracellular damage. Dr. John Repine will present his work relative to how superoxides are involved in the formation of pulmonary edema. In Repine's studies, free radicals are shown to

increase vascular permeability which produces pulmonary edema, and the effect can be blocked by catalase (an H_2O_2 scavengers) and dimethylthiourea (an $\dot{O}H$ scavengers) (18).

Lung Endothelial Damage Associated with ANTU

Finally, I would like to briefly discuss data from Dr. Dennis Martin's laboratory in regard to the alteration of the pulmonary vascular membrane to plasma proteins associated with α-napthlthiourea (ANTU) challenge - a compound known to cause extensive lung damage. In these studies, lymph flow, the concentration ratio of plasma protein in lymph and plasma (C_L/C_P) and lung water were used in a dog lung model to assess the ability of the different scavengers to reverse the damage associated with ANTU challenge. Table 1 shows the average results of these studies. Shown in row one are control values for lymph flow, C_L/C_P and lung water. When left atrial pressure is increased in control lungs, C_L/C_P decreases to 0.4, lymph flow increased 4-fold and lung water increased to 4.7, a level which is not associated with intra-alveolar edema. The next row shows the effects of ANTU on these parameters. Lymph flow increased 9 times, but C_L/C_P was not greatly altered and intra-alveolar edema always developed. A value for C_L/C_P of 0.6 at this high lymph flow rate indicates that the capillaries are extremely permeable to plasma proteins since:

$$\sigma = 1 - C_L/C_P$$

or, $\sigma = 1 - 0.6 = 0.4$. This indicates that the capillary permeability has increased to such a level that only 40% of the colloid osmotic gradient across the capillary wall will be effective in opposing increased capillary filtration. When DMSO was present, partial protection was observed but catalase failed to alter the endothelial damage. However, when the circulating white blood cell count was decreased with hydroxy urea pretreatment or when superoxide dismutase was present, the protection against the ANTU damage was very evident since both lung water and C_L/C_P were similar to controls. These studies indicate that the superoxides are likely involved in lung pathology associated with ANTU challenge. However, it is only fair to point out that the protection with SOD was variable since 50% of the animals were fully protected from the damage while 50% were only partially protected or not protected at all! But, it is important to note that these interventions prevented the formation of intra-alveolar edema seen with ANTU and this is an impressive result! Perhaps other systems, such as prostaglandins are involved in the generation of toxic substances and future research may unmask several other systems which are important modulators of tissue damage.

SUMMARY

All studies presented in this symposium are shown diagrammatically in Figure 2, and even a casual reading of the following symposium papers should stimulate the reader, since for the first time, a definite system is involved in several forms of tissue pathology which can be blocked by using various interventions as shown diagrammatically in Figure 2: 1) steroids (acting at 1) may inhibit the inflammatory response and the tissue infiltration of leukocytes (3). 2) The addition of SOD, DMSO, or catalase (shown at 2) to pathological tissues will scavenge O_2^-, $OH^\cdot$ and H_2O_2, respectively (1, 3, 4. 13. 14. 18. 18, 20). 3) The addition of allopurinol to an ischemic tissue (shown as 3) will inhibit the xanthine oxidase and thus decrease O_2^- production (9). 4) There is no doubt that SOD tends to reverse capillary permeability changes associated with microemboli (4), ANTU (13, 17, 20), ischemia (9), and paraquat poisoning (1). This indicates that either damaged tissues release O_2^-, H_2O_2 and $OH^\cdot$ or leukocytes attracted to damaged tissue by these products are responsible for higher tissue levels of these tissue destroying compounds. The reason that low levels of tissue damage cause an increased production of SOD is presently not known, but probably indicate the tissues ability to respond to minor tissue damage by producing more O_2^- scavengers. Future work in this area should be most promising not only as it applies to the etiology of tissue pathology but also as it relates to treating the critically ill patient.

ACKNOWLEDGEMENTS

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[1] The osmotic reflection coefficient (σ_d) is a measure of membrane selectivity. If the molecule cannot cross the membrane, then σ_d=1. If the molecule is not hindered by the membrane, σ_d=0. A change in σ_d from 0.9 to 0.6 is a large change in membrane selectivity.

TABLE 1

REVERSIBILITY OF ANTU LUNG ENDOTHELIAL DAMAGE WITH SUPEROXIDE DISMUTASE (SOD), DIMETHYLSULFOXIDE (DMSO), CATALASE AND WHITE BLOOD CELL DEPLETION (WBCD)

Condition	Lymph Flow (µl/min)	C_L/C_P	Lung Water (g/g BFDW)
Control	14	0.7	3.9
Left Atrial Pressure Elevation (LAP)	56	0.4	4.7*
ANTU + LAP	120	0.6	7.0
ANTU + LAP + DMSO	225	0.5	6.0
ANTU + LAP + SOD	180	0.5	4.6*
ANTU + LAP + Catalase	225	0.65	8.0
ANTU + LAP + WBCD	94	0.5	4.8*

* These lungs only had interstitial edema, others challenged with ANTU demonstrated intra-alveolar edema.

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THE BIOCHEMISTRY AND PATHOPHYSIOLOGY OF SUPEROXIDE

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INTRODUCTION

Most of the lifeforms on this planet derive their energy from electron transport systems which are based on the controlled reduction of molecular oxygen to water. In man, about 98% of all oxygen reduction is brought about by the cytochrome a/a_3 complex (cytochrome oxidase) in the mitochondria. This enzyme effects a concerted four electron reduction of the oxygen molecule to produce two molecules of H_2O, a completely innocuous product, with no detectable release of intermediate forms of partially reduced oxygen. How cytochrome oxidase performs this chemical feat is still a subject of speculation and investigation, but we are fortunate that the enzyme has this ability to avoid massive production of the toxic intermediates represented in Table 1.

TABLE 1
REDUCTION STATES OF MOLECULAR OXYGEN

No. of e^-	Unprotonated	Protonated
0	O_2	O_2
1	O_2^-	$HO_2^{\cdot}$
2	$O_2^{=}$	H_2O_2
3	$O^- + O^{=}$	$OH^{\cdot} + H_2O$
4	$O^{=} + O^{=}$	$H_2O + H_2O$

The one, two or three electron reduction of molecular oxygen gives rise to superoxide (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radical ($OH^{\cdot}$) plus water, respectively. Hydrogen peroxide is cytotoxic because of its moderate oxidative abilities, and is commonly used as an antiseptic or disinfectant. It is reasonably stable and concentrated solutions may be stored for long periods. The hydroxyl radical is an extremely reactive, unstable, and powerful oxidizing free radical (5) which can be produced by a metal-catalyzed reaction between O_2^- and H_2O_2 (10). Several metalloproteins such as transferrin can catalyze the reaction, as well (Lee and McCord, unpublished data). Biologically, the hydroxyl radical is capable of producing widespread oxidative damage, as well as initiating free radical chain reactions. The superoxide radical results from the univalent reduction of oxygen. Its biochemistry and pathophysiology are the subjects of this discussion.

SUPEROXIDE AS A CYTOTOXIC SPECIES

Chemically, superoxide is not extremely reactive, as free radicals go. It is nonetheless a good reducing agent, a fair oxidizing agent, and can initiate free radical chain reactions. A moderate amount of organic chemistry of superoxide has been described (8) and new reactions continue to appear in the literature.

Biologically, an overwhelming amount of evidence attests to the cytotoxicity of superoxide under a wide variety of circumstances. Since 1969 several thousand reports dealing with this subject have appeared in the literature.[1] Substantial biological toxicity from a substance with modest to low chemical reactivity may appear surprising at first glance, but is not at all unusual. Numerous examples may be cited where substances of low chemical reactivity (e.g., potassium chloride, sodium cyanide, or cobra venom toxin) can produce lethal reactions when injected into an animal. Clearly, cytotoxicity is not synonymous with organic reactivity. And while the mechanism of cyanide toxicity, e.g., is well understood, the mechanisms of superoxide toxicity largely remain to be discovered.

When a biological system is exposed to superoxide two kinds of results may be observed: "damage" and "evoked tissue response." Damage appears to be the result of direct or indirect attack at the molecular level by superoxide or

1. No attempt will be made here to cover comprehensively all the manifestations of superoxide toxicity. The interested reader is referred to Michelson *et al.* (16), Bannister and Hill (3), and Bannister and Bannister (2) for reviews of this area.

secondarily generated radicals. The metal-catalyzed Haber-Weiss reaction mentioned above:

$$O_2^- + H_2O_2 \xrightarrow{Me^{+n}} O_2 + OH^\cdot + OH^-;$$

provides a mechanism for converting the relatively unreactive superoxide radical into the strongly oxidizing hydroxyl radical (10). This appears to be the mechanism which accounts for the superoxide-dependent degradation of hyaluronic acid, for example (9), as well as for the cytotoxicity of superoxide shown toward neutrophils in vitro (18). On the other hand, a free radical chain oxidation of lactate dehydrogenase-bound NADH is initiated and propagated by superoxide per se (4, 7). Although this phenomenon has not been shown to occur in vivo, it seems plausible enough. Thus, any cell exposed to a flux of superoxide exceeding its ability to scavenge the radical might be subjected to a substantial drain of reducing power and energy production. It is likewise important to note that a single superoxide radical, by initiating a chain reaction, can bring about the oxidation of many NADH molecules. In this particular case, "damage" results from energy deprivation rather than from direct molecular destruction.

The second and altogether different category of superoxide-induced toxicity may be described as "evoked tissue response." The nature of this response is not yet well documented and is somewhat speculative. The rationale for why tissues may have evolved the ability to respond to superoxide lies in the fact that the radical plays key metabolic roles in the phagocyte-mediated inflammatory response. If, for example, increased capillary permeability is advantageous by facilitating the infiltration by phagocytes of an infected tissue, then the tissue's ability to recognize superoxide (which signals the presence of activated phagocytes) and to respond by lowering its permeability barriers is entirely appropriate. A key feature which distinguishes response from damage is rapid resolution or reversibility when the stimulus (O_2^-) disappears.

At present, superoxide appears to be centrally involved in three major pathophysiological states: oxygen toxicity, phagocyte-mediated inflammation, and post-ischemic tissue injury. Two of these areas, oxygen toxicity and ischemic injury, will be reviewed by other participants in this publication.

SUPEROXIDE AND INFLAMMATION

Because normal oxidative metabolism results in the production of a small but significant flux of superoxide, all aerobic cells are equipped with a protective enzyme, superoxide dismutase, which eliminates the radical by catalyzing its disproportionation (11, 12):

$$O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 .$$

Traces of superoxide dismutase may be found in extracellular fluids, but the enzyme is, for all practical purposes, an intracellular enzyme. It was empirically discovered nearly twenty years ago that superoxide dismutase displayed antiinflammatory activity in certain animal models. At that time the enzymic activity of the protein was unknown, and it was given the name "orgotein" (15). The biochemical basis for the antiinflammatory activity was not understood until Babior et al. (1) reported that phagocytosing neutrophils produce superoxide, probably for bactericidal purposes. Johnston et al. (6) established the role of superoxide in the destruction by phagocytes of ingested microbes. A rational hypothesis for the antiinflammatory activity of superoxide dismutase came with the recognition that inflammatory cells produce the cytotoxic radical in the course of their bactericidal activity: if an inflamed tissue were infiltrated by active phagocytes it would be vulnerable to radical-induced damage; if superoxide dismutase were present in the extracellular fluid, however, it could scavenge the radical and protect the tissue (9).

We have examined the antiinflammatory activity of intravenously injected derivatives of superoxide dismutase in three laboratory models of induced inflammation (13, 14, 17): the reverse passive Arthus reaction in the rat; carrageenan-induced foot edema in the rat; and passive immune complex induced glomerulonephritis in the mouse. In all three models, treatment with superoxide dismutase produced dramatic suppression of the inflammatory response. Several observations, however, rendered the working hypothesis inadequate to explain all the data. The first was that the enzyme caused nearly complete suppression of symptoms. Phagocytes possess some cytotoxic mechanisms that do not involve superoxide; the protection afforded by superoxide dismutase should have been partial, at best. Secondly, catalase and mannitol had no effect in vivo, whereas they had been found to prevent cytotoxicity in vitro, presumably by preventing the secondary formation of the hydroxyl radical. Third, histological examination showed that a dramatic decrease in the extent of inflammatory cell infiltration occurred in superoxide dismutase treated animals. Finally, the intradermal injection of an enzymatic superoxide-generating system (xanthine oxidase and purine) did not produce the grossly observable signs of inflammation, but did result in a histologically observable heavy infiltration of neutrophils to the injection site. All of these observations were reconciled with the formulation of a new and testable hypothesis: that the normal accumulation of neutrophils specifically requires the generation of extracellular superoxide. We proposed that extracellular fluids contain a latent but superoxide-activatable chemoattractant. Thus, when the first cell to encounter an inflammatory stimulus begins to liberate superoxide into the microenvironment, this superoxide, by activating the latent factor, serves as a chemical beacon to

guide additional cells to the site. The newly arriving cells will in turn be stimulated to produce more chemoattractant and thereby amplify and/or maintain the gradient until all the stimulus is eliminated by phagocytosis. The existence of the hypothetical latent factor in plasma was readily verified (17). The factor consists of a specific lipid of presently unknown structure bound non-covalently to serum albumin. While the lipid may be made soluble by the presence of other proteins such as ovalbumin, serum albumin is required for expression of biological activity. We have subsequently found that neutrophils themselves release the lipid precursor to the medium when metabolically stimulated.

Unlike most chemoattractants for neutrophils (such as complement fragment $C5_a$ or formyl methionyl peptide), the superoxide dependent factor does not induce degranulation or superoxide production. If it did, it would catalyze its own generation, and the process would be self-sustaining. Rather, the factor is purely a chemoattractant, depending on the presence of a stimulus of the respiratory burst to effect its production.

The superoxide-dependent chemotactic factor appears to play a major role in the development of neutrophil-mediated inflammatory responses. The primary mechanism of the antiinflammatory activity of superoxide dismutase appears to be via the prevention of the activation of this factor in the extracellular fluids.

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ROLE OF OXYGEN RADICALS IN THE PATHOGENESIS OF INTESTINAL ISCHEMIA

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INTRODUCTION

Ischemia-induced tissue injury plays a major role in heart disease, stroke, bowel disease and several other pathologic states. In spite of the extensive effort given to elucidating the mechanisms involved in the pathogenesis of these disorders, the biochemical basis of ischemic injury remains ill-defined. Recently, however, a mechanism has been proposed which may have far-reaching implications regarding the biochemical causes and physiological consequences of post-ischemic tissue injury (20, 39). This mechanism implicates the superoxide radical (O_2^-), an unstable and cytotoxic form of molecular oxygen, with the appearance of tissue damage in the post-ischemic state. Although superoxide radicals are well known to play a major role in oxygen toxicity and the neutrophil-mediated acute inflammatory response (36), the source and actions of the radical during ischemia appear to be quite different from that reported for these two pathological states (37, 39). In this review, we summarize the available data which implicates O_2^- in the vascular endothelial and parenchymal cell damage associated with intestinal ischemia. The mechanism(s) of superoxide production during intestinal ischemia is also discussed.

Vascular Permeability Studies

A major consequence in the pathogenesis of intestinal ischemia is enhanced transcapillary fluid filtration and interstitial fluid accumulation (25). Studies on the small intestine indicate that ischemia, induced by prolonged local arterial hypotension (30 mmHg arterial pressure), increases vascular permeability. This conclusion is based upon estimates of capillary filtration coefficients (24) and capillary osmotic reflection coefficients (21) following reperfusion of the ischemic small bowel. In these studies the osmotic reflection coefficient (σ_d) was estimated from the steady-state relationship between intestinal lymph flow (capillary filtration rate) and lymph-to-plasma protein concentration ratio (C_L/C_P) assuming $\sigma_d = 1 - C_L/C_P$ when C_L/C_P is filtration rate independent (22). Reperfusion after one hour of ischemia significantly reduces the osmotic reflection coefficient for total proteins from a normal value of 0.92 to 0.59 (Figure 1), indicating a dramatic rise in vascular permeability.

Although capillary permeability is increased there remains a high degree of selectivity to macromolecules on the basis of molecular size by intestinal capillaries in the post-ischemic state. Application of the σ_d values to pore theory reveals a preferential effect of ischemia on the "large" pore system of intestinal capillaries (19), i.e., the size of the large pores increases from 200 A° to 330 A°, while the small pores remain relatively constant 47-50 A°.

Numerous vasoactive substances released by the ischemic bowel are known to increase vascular permeability in the intestine or other organs. These include histamine, prostaglandins, lysosomal enzymes, and bacterial endotoxins. The increased intestinal vascular permeability produced by ischemia is not prevented when animals are pretreated with either anti-histamines, indomethacin, or methylprednisolone (20), suggesting that histamine, prostaglandins and lysosomal enzymes do not play a role in the ischemia-induced increase in vascular permeability. Since very high (lethal) doses of *E. coli* endotoxin were required to decrease σ_d and the magnitude of the reduction in σ_d observed with endotoxin was small relative to ischemia, endotoxins most likely play a minor role in the ischemia-induced increase in intestinal vascular permeability.

In contrast to the results acquired with the other agents, pretreatment with superoxide dismutase (SOD), an O_2^- scavenging enzyme, significantly attenuates the increased capillary permeability induced by ischemia (Figure 1); σ_d was reduced to only 0.86 in the ischemic bowel pretreated with SOD (20). The maximal protective effect of SOD was observed when both kidneys were ligated, presumably due to the fact that SOD is rapidly cleared by the kidney. These results indicate that superoxide radicals are primarily responsible for the increased vascular permeability in the post-ischemic small bowel.

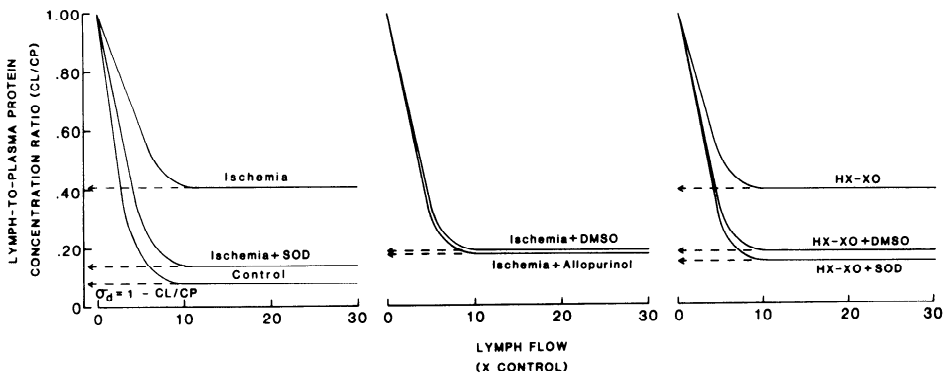

Figure 1. Effects of various experimental conditions on the osmotic reflection coefficient (σ_d) of intestinal capillaries. σ_d is assumed to equal $1-C_L/C_P$ when C_L/C_P no longer changes as lymph flow is increased. Note that ischemia dramatically reduces σ_d from control, indicating an increased vascular permeability to proteins. The ischemia-induced rise in vascular permeability is attenuated by pretreatment with either superoxide dismutase (SOD) or allopurinol. Intra-arterial infusion of hypoxanthine-xanthine oxidase (HX-XO), an O_2^- generating system, reduces σ_d. The HX-XO induced reduction in σ_d is largely prevented by dimethylsulfoxide (DMSO), a hydroxyl radical scavenger and SOD.

Support for the hypothesis that oxygen radicals (superoxide or a secondary radical) are involved in the intestinal vascular permeability changes induced by ischemia is provided by the observation that experimentally generated oxygen radicals increase vascular permeability in the non-ischemic small intestine (40). Local intra-arterial infusions of hypoxanthine-xanthine oxidase in non-ischemic preparations reduced σ_d from a control value of 0.92 to 0.66, suggesting a rise in vascular permeability comparable to that produced by ischemia (Figure 1). Pretreatment with superoxide dismutase (SOD) or dimethylsulfoxide (DMSO), a hydroxyl radical scavenger, significantly attenuates the reduction in σ_d (Figure 1) produced by the hypoxanthine-xanthine oxidase infusion, suggesting that hydroxyl radicals are primarily responsible for the hypoxanthine-xanthine oxidase induced change in vascular permeability. The finding that enzymatically-generated oxygen radicals increase vascular permeability in the intestine supports the recent studies of Del Maestro et al. (14) where oxygen radicals were shown to increase microvascular permeability in the hamster cheek pouch.

Morphologic Studies

Perhaps the most characteristic feature of intestinal ischemia is the damage to the mucosal membrane. Mucosal lesions have been reported in man and experimental animals following both hemorrhagic shock and regional intestinal ischemia (25). The mucosal lesions produced by 3 hours of regional intestinal ischemia are characterized by massive epithelial lifting from the villi (particularly in the tip region), epithelial necrosis, disintegration of the lamina propria, hemorrhage, and ulceration. Such damage is associated with an increased permeability of the mucosal barrier to molecules with molecular weights ranging between 680 and 68,000 (7, 23, 29) and widespread functional impairment (8, 44).

Many hypotheses have been proposed to explain the epithelial damage produced by intestinal ischemia. However, the most widely accepted is that hypoxia per se is the key factor causing the ischemic lesions. According to this hypothesis the villus tips become virtually anoxic in spite of an unaltered mucosal blood flow due to arteriovenous shunting of oxygen in the villus countercurrent exchanger (32). Support for this theory is provided by the frequent observation that ischemic injury to the small bowel is most severe at the villus tip and diminished by intraluminal perfusion with oxygenated saline (1). The observation that most of the injury associated with ischemia (produced by either hemorrhage or regional hypotension) occurs at the time of reperfusion (46) is inconsistent with the countercurrent hypothesis since there is a temporal dissociation between the hypoxic insult and the development of mucosal lesions. Recent evidence, however, indicates that oxygen radical formation rather than hypoxia per se accounts for the mucosal lesions produced by reperfusion after 3 hours of regional intestinal ischemia in the cat (39, 46) and one minute of superior mesenteric artery occlusion in weaning rats (12). Pretreatment with SOD significantly attenuated villus and crypt epithelial necrosis and effectively prevented the reduction in villus height produced by ischemia in the cat and largely prevents the transmural bowel necrosis observed 48 hrs following reperfusion in the rat.

Indirect evidence which supports the possibility of a central role for O_2^- in producing ischemic injury to the intestinal mucosa include: 1) local intra-arterial infusion of hypoxanthine-xanthine oxidase, a O_2^- generating system, increases mucosal membrane permeability to albumin (23) to a level comparable to that produced by 2 hours of regional hypotension (42). The increased mucosal permeability produced by intra-arterial hypoxanthine-xanthine oxidase infusion is prevented by SOD, and 2) the morphologic changes produced by intestinal ischemia are very similar to that observed after irradiation (17), a condition well known to involve oxygen radicals (16).

Although oxygen radicals appear to be the primary mediators of mucosal injury produced by 3 hours of partial intestinal ischemia, there is evidence which indicates that oxygen radicals play a more minor role in the tissue damage produced by long durations (1-4 hours) of complete ischemia, i.e., arterial occlusion. Superoxide dismutase pretreatment does not modify the structural changes produced by 4 hours of arterial occlusion (41) nor does it prevent the mucosal permeability increase produced by one hour of complete ischemia (23). Presumably other factors, such as anoxia, play a more important role under these conditions.

Mechanism of Oxygen Radical Formation during Ischemia

There are several potential biological sources of O_2^-. These include cellular enzymes involved in catalyzing oxidation reactions (e.g., xanthine oxidase), the mitochondrial electron transport chain, and NADPH oxidase found on the surface of phagocytic cells (polymorphonuclear leukocytes) (13). The major source of O_2^- in the ischemic small intestine appears to be the enzyme xanthine oxidase. This conclusion is based largely upon the observation that allopurinol, a competitive inhibitor xanthine oxidase, is as effective as SOD in preventing the increase in vascular permeability produced by one hour of ischemia (Figure 1) and the mucosal lesions resulting from 3 hours of ischemia (41).

Xanthine oxidase was the first documented biologic source of O_2^- (36) and has been studied extensively. The intestinal mucosa and liver are the richest sources of the enzyme. In the intestinal mucosa the enzyme appears to be more concentrated in the villus tip region (2) where ischemic damage is most severe. There is considerable evidence indicating that in a normal healthy cell the enzyme exists as a NAD+-reducing dehydrogenase (D). Conversion to the superoxide-producing oxidase (O) can be induced by a number of conditions including proteolysis or incubation under low oxygen tension in the presence of substrate (3). In the rat intestine nearly complete conversion of D-to-O takes place within less than one minute of ischemia (45). This rapid conversion is completely prevented by pretreatment with soybean trypsin inhibitor. The finding that soybean trypsin inhibitor greatly attenuates the increased vascular permeability induced by 1h intestinal ischemia is consistent with a role for proteases in D-to-O conversion (38).

The appearance of xanthine oxidase activity in the ischemic intestine is not sufficient to cause radical-mediated damage; there must be substrates. One substrate, hypoxanthine, has been shown to accumulate during ischemia as a result of adenosine 5'-triphosphate (ATP) catabolism (10). During hypoxia, ATP is discharged to increase the intracellular concentration of AMP. The AMP is catabolized to adenosine, inosine and finally to hypoxanthine. The remaining substrate, molecular oxygen, is supplied during reperfusion of the ischemic tissue; with it comes a burst of superoxide radical production (Figure 2). The superoxide anion may then undergo further reduction to form hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$).

Mechanisms of Oxygen Radical-Mediated Injury during Ischemia

Although a role for oxygen radicals in ischemic damage to the small bowel has been established, the mechanism(s) by which oxygen derived free radicals increase vascular permeability and damage the mucosal membrane remain unclear. Oxygen free radicals are very reactive and therefore very unstable oxidizing species which are cytotoxic. The cytotoxic effects of oxygen derived free radicals result from peroxidation of lipid components of cellular

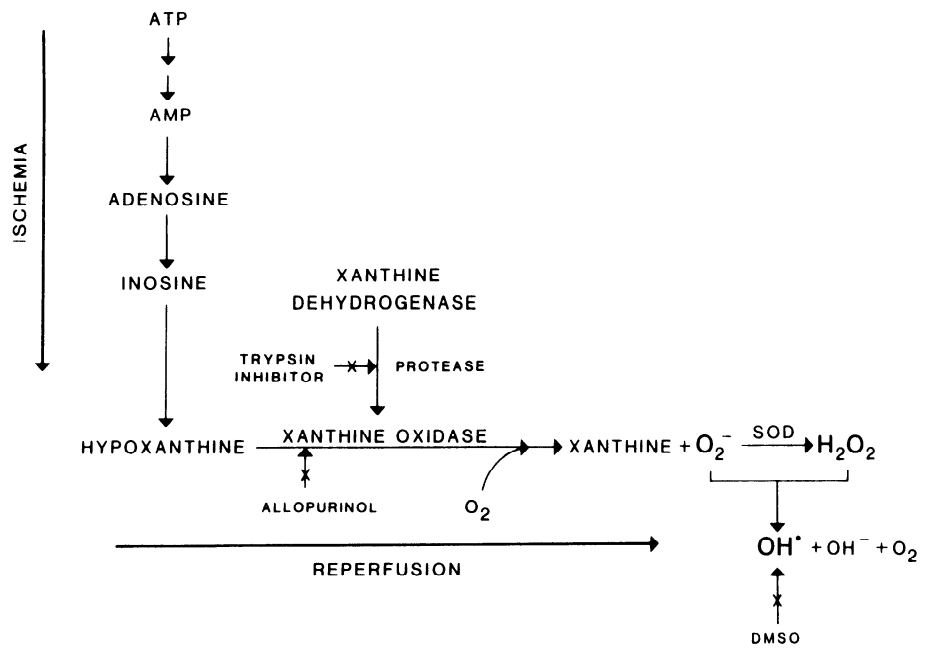

Figure 2. Proposed mechanism for oxygen-derived free radical production in the ischemic bowel. During ischemia, ATP is broken down to hypoxanthine and xanthine dehydrogenase is converted to xanthine oxidase. At reperfusion, hypoxanthine, xanthine oxidase, and molecular oxygen react to form superoxide anion (O_2^-), which may undergo further reduction to form hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$).

and mitochondrial membranes (18). Therefore, following ischemia, damage to the mucosal membrane and increased vascular permeability may result from lipid peroxidation of epithelial and endothelial cells, respectively. The effects of oxygen radicals on the integrity of the capillary and mucosal membranes may also be related to the fact that oxygen radicals degrade hyaluronic acid and collagen, constituents of basement membranes and the extracellular matrix (4, 34). Disruption of capillary and epithelial basement membranes could account for the increased vascular permeability and epithelial lifting produced by ischemia in the intestine.

The effects of oxygen radicals on capillary endothelium and mucosal epithelium during ischemia may also be related to the chemotactic properties of these agents (36). The superoxide radical produced by the reaction of hypoxanthine and xanthine oxidase, following reperfusion, may lead to leukocyte accumulation and activation. Substances liberated by the leukocytes may, in turn, produce the endothelial and epithelial damage associated with ischemia.

Role of Oxygen Radicals and Xanthine Oxidase in Ischemic Injury in Other Tissues

Oxygen derived free radicals have also been implicated in the damage induced by ischemia in heart, brain, kidney, and skin. In the heart, superoxide dismutase activity decreases significantly following coronary artery ligation (43). Pretreatment with SOD reduces the infarct size produced by coronary artery occlusion to one-third the size observed in untreated animals

(5). Further support for a role of O_2^- in ischemic damage to the myocardium is provided by reports that 1) O_2^-, generated experimentally by the hypoxanthine-xanthine oxidase reaction, reproduce the changes seen in sarcoplasmic reticulum from ischemic myocardium (27), 2) free radical scavengers also prevent the leakage of creatine kinase and interstitial edema produced by ischemia in the myocardium (31), and 3) addition of SOD to cardioplegic solutions significantly reduces the ischemic injury associated with reperfusion of the ischemic myocardium (47). Studies employing free radical scavengers (SOD and mannitol) in the brain indicate that ischemia or trauma leads to free radical damage to capillary endothelium and the parenchyma (30). Manson et al. (33) have shown that administration of SOD prior to the reperfusion of island skin flaps exposed to 8h of total arterial occlusion substantially decreased the incidence of necrosis compared to untreated animals.

There is considerable evidence indicating that xanthine oxidase is involved in ischemic damage to a variety of tissues. Crowell et al. (9) demonstrated a 6-fold increase in the survival time of dogs in hemorrhagic shock when pretreated with allopurinol. Allopurinol has also been shown to prevent the diminished myocardial contractility and dysrhythmias produced by coronary artery ligation (15). Furthermore, xanthine oxidase inhibition reduces the infarct size produced by coronary artery occlusion to one-third the size observed in untreated animals (5). Allopurinol also dramatically improves the survival rate and function of kidneys subjected to 40-120 minutes of ischemia (6, 26). In most of these studies allopurinol was considered to exert its beneficial effect against ischemia by preventing the loss of purine bases from the hypoxic cell. Once degradation of a nucleotide has proceeded beyond the xanthine-hypoxanthine level to uric acid, it becomes irreversibly lost from the nucleotide pool. Prevention of the purine loss by inhibition of xanthine oxidase should preserve the nucleotide pool during the hypoxic stress. While a role for this mechanism in allopurinol's prevention of ischemic injury to tissues cannot be dismissed, an equally likely explanation for allopurinol's effects is the prevention of O_2^- formation via the hypoxanthine-xanthine reaction. This possibility is supported by the fact that SOD exerts equal protection to allopurinol in myocardium (5) and skin (28, 33).

CONCLUSIONS

The involvement of superoxide in ischemic injury represents a new and therapeutically important facet of the pathobiology of oxygen radicals. The source and actions of superoxide in intestinal ischemia appear to differ from that proposed for other pathologic states, i.e., oxygen toxicity and neutrophil-mediated inflammation. Future studies should be directed towards determining the role and source of oxygen radicals in ischemic disorders of other organs.

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FREE OXYGEN RADICALS IN CEREBRAL VASCULAR RESPONSES

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INTRODUCTION

The sequential univalent reduction of oxygen leads to the generation of intermediate compounds, namely superoxide anion radical, hydrogen peroxide, and the free hydroxyl radical, which are very reactive (9). They are ordinarily scavenged effectively in tissues so that their concentration under normal conditions is severely limited. If their production increases in excess of the capacity of the tissue to eliminate them, they may produce serious damage (24). Free oxygen radicals have, for example, been shown to inactivate viruses (18), kill bacteria (2), lyse cells (14), destroy cell membranes (21), inactive enzymes (1), and alter important tissue components (13, 26). Because of their capacity to cause cellular damage, which may frequently be irreversible, free oxygen radicals have been implicated as mediators for such damage under a number of pathological conditions which include ischemia (4), inflammation (22), acute hypertension (15), and traumatic brain injury (28).

The purpose of this paper is to consider the effects of free oxygen radicals on cerebral vessels. We will examine first the effects of generation of free oxygen radicals by exogenous sytems on the caliber, functional characteristics and morphology of cerebral vessels *in vivo*, and on their metabolism *in vitro*. Secondly, we will consider the involvement of free oxygen radicals in the pathogenesis of the vascular changes from experimental brain injury and acute hypertension.

EFFECTS OF FREE OXYGEN RADICALS ON CEREBRAL VESSELS *IN VIVO*

We examined the effects of free oxygen radicals on cerebral vessels in anesthetized cats equipped with cranial windows for observation of the pial microcirculation of the parietal cortex. Free radicals were generated by introducing in the space under the cranial window dissolved in artificial cerebrospinal fluid (CSF) the agents listed below: 1) Xanthine (0.1 mM) plus xanthine oxidase (0.02 mg/ml). This system is known to generate superoxide anion radical (23). 2) Hydrogen peroxide (1 µg/ml). 3) Hydrogen peroxide (1 µg/ml) plus ferrous sulfate (1 µg/ml; Fenton reagent). This combination is known to generate free hydroxyl radical (9). 4) Sodium arachidonate (200 µg/ml). Arachidonate is metabolized via the cyclooxygenase or lipoxygenase pathways. Its metabolism by either pathway generates a powerful free oxygen radical which resembles very closely in its reactivity to the free hydroxyl radical (17). 5) 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) (200 µg/ml). This fatty acid is hydroperoxidized to 15-hydroxy-eicosatetraenoic acid (15-HETE). In the process of this conversion a powerful oxidant siilar to that generated from arachidonate metabolism is produced (17). 6) Cyclic endoperoxide G_2 (PGG_2) (10 µg/ml). This intermediate of the cycloxygenase metabolism of arachidonate is converted to PGH_2 by peroxidation and in the process generates a free oxygen radical very similar, if not identical, to the free hydroxyl radical (17).

The typical experiment involved control measurements followed by the introduction of these agents under the cranial window, measurements after a 15-minute contact of these agents with the brain surface followed by a washout with fresh artificial CSF and repeat observations one hour later. All agents which generate free radicals produced similar effects on cerebral arterioles. These consisted of pronounced vasodilation during the application of the agents, persistent residual dilation after the agents were washed from the brain surface, and reduced responsiveness to the vasoconstrictor effects of hypocapnia. After the termination of the functional studies, the vessels were fixed by perfusion, harvested and examined by scanning and transmission electron microscopy (6). Scanning electron microscopy disclosed discrete destructive lesions in the endothelium. These were generally localized at the junctions between cells. They consisted of localized destruction of the tissue in the form of a vacuole. When they burst into the lumen of the vessel, they gave rise to a crater. The density of these lesions varied depending on the agents used. Morphological abnormalities of the vascular smooth muscle, identified by transmission electron microscopy, consisted of necrosis of individual smooth muscle cells, sometimes more confluent necrosis, increased density of cells, inclusion bodies and myelin figures. In general, the number of smooth muscle cells which had

TABLE 1

EFFECTS OF 15-HPETE ON CEREBRAL ARTERIOLES WITH AND WITHOUT SUPEROXIDE DISMUTASE (SOD) AND CATALASE

A. 15-HPETE (200 μg/ml)

	Control	During	After
Small Arterioles (n=28) (μm)	71 ± 2.4	100 ± 4.0	83 ± 3.7
Large Arterioles (n=18) (μm)	176 ± 11.0	226 ± 16.0	196 ± 14.0

B. 15-HPETE (200 μg/ml) plus SOD (20 μg/ml)

	Control	During	After
Small Arterioles (n=13) (μm)	70 ± 3.0	71 ± 3.8	75 ± 5.2
Large Arterioles (n=9) (μm)	150 ± 4.7	156 ± 6.5	162 ± 9.2

C. 15-HPETE (200 μg/ml) plus Catalase (20 μg/ml)

Small Arterioles (n=10) (μm)	66 ± 4.8	66 ± 11.2	67 ± 3.8
Large Arterioles (n=11) (μm)	166 ± 14.6	163 ± 13.9	169 ± 14.9

Values are mean ± SE.

abnormalities in any one vessel was relatively small, 5-10%.

These results show clearly that free oxygen radicals are capable of causing cerebral vascular damage manifested by morphological abnormalities of the vascular smooth muscle and endothelium, relaxation of vascular smooth muscle and abnormal responsiveness to vasomotor influences. Since all free radicals examined, including superoxide anion radical, hydrogen peroxide and the free hydroxyl radical, have the same effect, we suggest that either each one of these radicals is itself damaging to vascular tissue or more likely each radical leads to the generation of new radicals and a single radical is the immediate cause of damage. A possible mechanism for this is the production of free hydroxyl radical from superoxide anion radical and hydrogen peroxide by means of the Haber-Weiss reaction (11). The free hydroxyl radical may then be the destructive radical.

We studied the effects of various scavengers on the functional and morphological damage induced by the free oxygen radicals outlined above in the cerebral vessels. We found that the vasodilation, the reduced responsiveness and the morphological abnormalities induced by arachidonate were inhibited by mannitol, a scavenger of the free hydroxyl radical (16). They were also inhibited by pretreatment with indomethacin suggesting that the free radical from arachidonate metabolism was generated via the cyclooxygenase pathway. The effect of PGG_2 was partially inhibited by superoxide dismutase (SOD), suggesting that the superoxide anion radical is involved (16). The effects of 15-HPETE were inhibited completely by either SOD or by catalase (Table 1). These results show that the free radical from 15-HPETE is probably the free hydroxyl radical generated by the interaction of superoxide anion radical and hydrogen peroxide. The inhibition of the effects of arachidonate by mannitol also suggests that the free hydroxyal radical is involved in the damage caused by arachidonate.

EFFECTS OF OXYGEN FREE RADICALS ON CEREBRAL VESSEL METABOLISM *IN VITRO*

We studied the effects of free oxygen radicals on cerebral vessel metabolism *in vitro* (19). Vessels were removed from the brain surface of anesthetized cats, divided into three or four pieces and incubated with the appropriate agents in artificial CSF. After 15 minutes of incubation, the vessels were washed and placed in the Cartesian diver microrespirometer where their

oxygen consumption rates were determined over the succeeding 5-6 hours (Table 2). We found that superoxide anion radical generated via the xanthine oxidase reaction acting on acetaldehyde as substrate, reduced the oxygen consumption of

TABLE 2

EFFECT OF ARACHIDONATE ON CEREBRAL ARTERIOLAR OXYGEN CONSUMPTION

Group		Pial Arteriolar Oxygen Consumption (µl/hr per mg dry wt)
1	Control	1.101 ± 0.113
	AA	0.075 ± 0.054
	AA+C	0.610 ± 0.201
2	Control	1.670 ± 0.080
	AA	0.089 ± 0.027
	AA+SOD	0.151 ± 0.050
	AA+SOD+M	0.124 ± 0.028
3	Control	1.248 ± 0.232
	AA	0.048 ± 0.039
	AA+C+SOD	1.525 ± 0.248
4	Control	1.390 ± 0.168
	AA	0.158 ± 0.085
	AA+C+SOD+M	1.547 ± 0.296

the vessel wall and this reduction was inhibited by superoxide dismutase (SOD, 20 µg/ml) and catalase (designated C, 20 µg/ml). Similarly, arachidonate (AA, 200 µg/ml) depressed the oxygen consumption of the vessel wall severely. As shown in Table 2, this reduction in oxygen consumption was reversed by the combination of SOD and catalase, but not by each one separately. The addition of mannitol (M, 20 mM) had no effect. These results show that free oxygen radicals are capable of depressing the resting oxygen consumption of the vessel wall. Because this effect is inhibited by SOD and catalase, we suggest that the agent immediately responsible for this effect is the free hydroxyl radical which originates from the interaction between superoxide anion radical and hydrogen peroxide.

ORIGIN OF FREE OXYGEN RADICALS IN TISSUES

Free oxygen radicals can be generated in tissues from a variety of sources. It is known that mitochondria produce small amounts of superoxide anion radical and hydrogen peroxide (3). A number of oxidative enzymatic reactions lead to the univalent reduction of oxygen and the generation of superoxide anion radical (24). Phagocytes produce superoxide anion radical which they use to kill bacteria (2). Of particular interest is the production of free oxygen radicals in the metabolism of arachidonate. As noted above, these can be generated in the metabolism of arachidonate by either cyclooxygenase or lipoxygenase (17).

INVOLVEMENT OF FREE RADICALS IN THE VASCULAR ABNORMALITIES FROM EXPERIMENTAL BRAIN INJURY AND HYPERTENSION

Experimental brain injury brought about by fluid percussion in cats produces vascular abnormalities very similar to those we found from free radical generation on the brain surface (27). After such injury, the cerebral arterioles show sustained dilation for several hours, reduced or absent responsiveness to the vasoconstrictor effects of arterial hypocapnia, and reduced ability to respond to changes in arterial blood pressure. Electron microscopic examination of the vessels which show these functional abnormalities disclosed discrete endothelial destructive lesions as well as abnormalities of the vascular smooth muscle similar to those seen from action of free radicals. Such vessels had reduced oxygen consumption rates when compared to vessels of comparable size from normal animals.

Cerebral blood flow changes after injury were consistent with the changes in vascular caliber (5). Immediately following injury there was hyperemia which subsided rapidly, although vascular resistance remained low because of an associated decrease in perfusion pressure. Responsiveness to CO_2 and autoregulation were disturbed (20).

Immediately following this type of brain injury, there is a marked increase in sympathetic activity and a sudden marked increase in arterial blood pressure which is short lasting. This acute hypertensive episode was essential for the production of the abnormalities outlined above (27). If the blood pressure was raised suddenly by the intravenous administration of vasoconstrictor agents, such as angiotensin or norepinephrine, similar functional, metabolic and morphological abnormalities were found in cerebral arterioles (15).

Although no platelet aggregates were seen after brain injury, platelet aggregation in response to other stimuli, e.g., UV light following sensitization with fluorescein, was enhanced, showing that brain injury of this type had a proaggregatory effect though, by itself, it was not sufficient to lead to spontaneous platelet aggregation (25).

The functional and morphological abnormalities after brain injury and acute hypertension were inhibited by agents which inhibit cyclooxygenase, such as indomethacin or sodium amfenac (AHR-5850), showing that the abnormalities were dependent on activation of arachidonate metabolism via cyclooxygenase (15, 28). These abnormalities were also inhibited partially or completely by SOD, mannitol or by nitroblue tetrazolium (NBT), a dye which is a free radical scavenger (15, 28).

Our present knowledge of the sequence of events which account for the vascular changes in experimental brain injury and acute hypertension is as follows: The initial essential step seems to be a marked rise in arterial blood pressure. In brain injury, this is related to massive sympathetic discharge from the Cushing response. The rise in arterial blood pressure causes

phospholipase activation and release of free arachidonate. We demonstrated an increase in the activity of phospholipase C in brain tissue following brain injury (29). No comparable studies are available in acute hypertension induced by vasoconstrictor agents. The precise mechanism by which the rise in blood pressure activates phospholipase is not known. It may involve the increase in vascular permeability and entry of blood constituents into the vessel wall and into the brain parenchyma or release of normal constituents of the brain tissue which activate phospholipase, such as polypeptides. The increase in concentration of free arachidonate stimulates prostaglandin synthesis. A rise in prostaglandin concentration in brain tissue was demonstrated shortly after injury in cats (8). This increase in concentration involved prostaglandins which are mainly produced by brain parenchyma and to a lesser extent by blood vessels. It is clear from this evidence that increased prostaglandin synthesis takes place in the parenchyma; whether the same is true in blood vessels is not known with conviction. If the increase in prostaglandin synthesis occurs in a burst of very high activity, it is conceivable that the associated production of free radicals might overcome the normal defenses of the tissues and that these may then escape into the extracellular fluid. We have preliminary evidence demonstrating the occurrence of SOD-inhibitable reduction of NBT after brain injury, suggesting strongly the appearance in the extracellular fluid of superoxide anion radical. Once this occurs, it is conceivable that a chain reaction might begin with sequential production of new radicals which eventually destroy cell membranes and cause the vascular abnormalities we observed. The endothelial destructive lesions can be explained as a result of the action of free radicals generated in this manner upon the membrane of endothelial cells. The localized nature of the lesions is of interest, but the reasons for this localization are not clear. The loci of damage may represent particularly vulnerable portions of the cell or they may represent the location of enzymes involved in the production of radicals. The enchancement of platelet aggregation may be explained by the fact that free radicals have been shown to selectively destroy prostacyclin synthetase (12).

The vascular smooth muscle abnormalities including relaxation and unresponsiveness can probably be explained by the damage done by free radicals to the vascular smooth muscle. Whether the morphological lesions are sufficiently extensive to explain the marked vasodilation and unresponsiveness is not known with certainty (15). If, however, one assumes that those smooth muscle cells that have been damaged are less able to develop tension, then the proper conditions would exist for heterogeneous internal contraction of contractile elements within the vessel wall. Since the vascular smooth muscle cells are in series, shortening of one smooth muscle cell may produce lengthening of another (15). It is known that vascular smooth muscle activated at long length in vitro may display reduced ability to generate tension (7). At times, agents which cause contraction of vascular smooth muscle, such as norepinephrine may actually cause dilation of the vessels under these circumstances (7). This phenomenon has been explained as due to an unequal shortening of contractile elements in series and is known under the name of attenuation. It seems to be a plausible explanation for the abnormalities we observed.

We ascribe the reduced oxygen consumption of the vessel wall to damage of mitochondrial components.

From the practical standpoint, it should be noted that although several agents can prevent the vascular damage from acute hypertension and experimental brain injury, preliminary experiments attempting to find out whether these same agents have any beneficial effect when administered after the acute hypertensive epidose have yielded discouragingly negative results.

A number of other abnormal conditions have been described which are characterized by marked vasodilation and reduced responsiveness of the cerebral circulation. Consequently, the participation of free oxygen radicals in the production of the vascular abnormalities in these conditions seems worth exploring. These conditions include ischemia, acute severe intracranial hypertension and repeated seizures. In ischemia, it was shown that there is an increase in brain tissue concentration of free arachidonate (10). After reperfusion is established, arachidonate concentration decreases, and there is a corresponding increase in cyclooxygenase products (10). Production of free oxygen radicals associated with increase in prostaglandin synthesis, therefore, seems very likely.

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MECHANISMS OF HYPEROXIC INJURY TO THE PULMONARY MICROCIRCULATION

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INTRODUCTION

The lung is the primary target organ for injury during exposures of animals to hyperoxia at normal barometric pressures (4). Damage to several lung tissue compartments has been shown to occur, but the pulmonary capillary endothelium is a critical site of cellular injury (5). Destruction of the pulmonary capillary bed is the event most closely associated with death of animals exposed to hyperoxia. Pathologic changes in injured endothelial cells include condensation of nuclear chromatin, vacuolization of cell cytoplasm and mitochondrial swelling (5). Enhanced production of hydrogen peroxide and superoxide has been postulated to occur in lung tissue exposed to hyperoxia and to be an important component of the cytotoxic response. In addition, it has been suggested that leukocytes are necessary for the full manifestation of oxygen induced lung injury. Production of severe neutropenia by the use of agents such as nitrogen mustard protects against many of the lethal manifestations of oxygen induced lung injury (17). That the polymorphonuclear leukocyte plays a substantial role in oxygen induced lung injury is clear; however, controversy has existed as to whether it initiates the injury or acts in the later stages to amplify the injury. The studies which are summarized here evaluate the rates of production of toxic oxygen species within endothelial cells and compare the time course of injury to the pulmonary capillary endothelium with the morphologic pattern of accumulation of blood-borne cellular elements with the lung.

EXPERIMENTAL METHODS

Animals and Exposure Conditions

Specific pathogen-free Charles River CD strain male rats weighing 300-350 g were exposed to various oxygen concentrations in polystyrene chambers using procedures previously described (8). The O_2 concentrations varied less than 2% and CO_2 concentration was maintained at less than 0.5% by adjusting gas flow rates per hour to a volume equal to seven to eight times the chamber volume. During the exposures food and water were provided *ad libitum*. The animals were kept in 12 hour on, 12 hour off light at all times.

Histologic Studies

The lungs of rats were fixed using intratracheal infusion of 2% glutaraldehyde and tissue samples were prepared for electron microscopic morphometric analysis (7). Sixty random electron micrographs were taken from a total of four different sites in the left lung of each animal and these photographs were enlarged to 8500X on 11x14" photographic paper. The volume of platelets and polymorphonuclear leukocytes in the pulmonary capillary bed was determined by placing each 8500X micrograph under a plastic overlay containing 224 points. The number of points falling on capillary lumen and the proportion of those points falling on platelets or polymorphonuclear leukocytes was counted. The ratio of these point counts is directly proportional to the volume densities of each of these blood elements in the lung. The absolute volume of platelets and polymorphonuclear leukocytes was calculated by multiplying their volume densities by the total volume of the vascular compartment in the alveolar region. The vascular volumes of these animals have been reported previously (5).

Cell Culture

Porcine thoracic aorta segments (20 to 25 cm) were obtained from the slaughterhouse and transported in iced Hanks' salt solution (without added substrates) containing 20X concentrations of penicillin-streptomycin-fungizone (GIBCO, Grand Island, NY). Cells were harvested after incubation in medium 199 (GIBCO) with 0.1% collagenase (Sigma, St. Louis, MO) at 37°C for 20 minutes, using the general methods described by Jaffe *et al.* (13). Cell isolation was complete within 3 hours of the animals' death. The harvested cells were washed by centrifugation in fresh medium 199 supplemented with L-glutamine (0.7 mM), Hanks' salts and 10% fetal calf serum (GIBCO). Cells were seeded into 25 cm^2 flasks (Corning Glass Co., Corning, NY) at densities of about 10^4 cells.cm^{-2} and grown in the same medium at 37°C. Cells were grown in room air-equilibrated flasks or in 24-well culture plates (2.4 cm^2/well) which were equilibrated with the specified oxygen concentrations. Media was changed every 3 days or sooner. The concentration of fetal calf serum was reduced stepwise to 1% as cells achieved confluency. Within 24 hours of confluency, cells were rinsed

with Ca^{+2} and Mg^{+2}-free Hanks' salt solution (GIBCO), harvested from the flasks with 0.1% trypsin-EDTA solution (GIBCO), centrifuged, and resuspended in fresh medium for routine plating into new flasks at split ratios of 1:4. Identification of cultured cells as endothelial cells was done by a variety of methods including noting their phase contrast microscopic appearance as regular polygonal (cobblestone) cells which exhibited contact inhibition. Subcultures were fixed in 2% buffered glutaraldehyde and specific immunofluorescent staining was performed for angiotensin converting enzyme (goat-antiporcine antibody, courtesy or Dr. S. Alex Stalcup), factor VIII antigen (antihuman factor VIII antibody, Cappel Laboratory, Cochranville, PA) and α_2-macroglobulin (Cappel), demonstrating that all cultures were greater than 95% endothelial cells by these criteria. A few cultures were also fixed in 2% glutaraldehyde, osmicated and embedded in Epon. Their morphology in the electron microscope was typical of cultured endothelial cells, as described by Ryan et al. (16).

Biochemical Measurements

Oxygen consumption in lung tissue slices was measured polarographically in the presence or absence of 1 mM KCN according to Freeman and Crapo (11). Endothelial cell oxygen consumption was measured similarly in Roswell Park Memorial Institute (RPMI) 1640 medium containing 5 mM glucose.

Superoxide formation was measured by the SOD-inhibitable reduction of acetylated cytochrome c (1) or oxidation of epinephrine to adrenochrome (15). Adrenochrome formation and cytochrome c reduction was monitored in a split-beam Cary 118 spectrophotometer at 480 nm ($E = 4\ mM^{-1} \cdot cm^{-1}$) or 550 nm ($E = 21\ mM^{-1} \cdot cm^{-1}$), respectively.

Hydrogen peroxide production was monitored in an Aminco Chance dual wavelength spectrophotometer at 417-402 nm, by measuring the rate of formation of Compound I between hydrogen peroxide and horseradish peroxidase, 0.5 μM, Sigma Type VI, Sigma Chemical Co., St. Louis, MO. ($E = 50\ mM^{-1} \cdot cm^{-1}$) (3).

Phospholipid Vesicle Preparation and Cultured Endothelial Cell Treatment

Phospholipid vesicles were prepared using sterile technique. Multilamellar vesicles were first made by vortexing lipid (dried in vacuo) at 47-50°C in the presence of superoxide dismutase-containing 10 mM NaCl, 1 mM phosphate buffer, pH 7.4. Unilamellar vesicles were made from multilamellar vesicles by sonication for four 30 second intervals at 47-50°C. Vesicles contained 450 U bovine CuZn superoxide dismutase/μmol phospholipid and consisted of dipalmitoylphosphatidylcholine:cholesterol:stearylamine (7:1:2, mol/mol). Enzyme-loaded vesicles were removed from enzyme-containing aqueous medium by centrifugation at 100,000 x g x 60 min. Confluent cell cultures in 24-well plates were pretreated for 2 hours with 50 nmol/ml vesicle phospholipid in culture medium, washed 3 times with Hanks' buffered salt solution and further cultured in serum-containing culture medium for 24 hours. Endothelial cell lactate dehydrogenase (LDH) release into culture medium was measured using an LDH assay described by Bergmeyer (2).

Statistics: All comparisons of statistical significance were done using analysis of variance procedures and Duncan's multiple comparison test (10).

RESULTS

Previously reported morphometric studies of the time course of injury to the pulmonary capillary bed during exposure to 100% oxygen have shown little injury during the first 40 hours of exposure (5). After this point rapid destruction of the capillary bed occurs and by the time the animals die (at a mean of 66 hours) approximately 50% of the pulmonary capillary endothelial cells are destroyed (Figure 1). Destruction of these endothelial cells is associated with loss of a corresponding proportion of capillary bed surface area and of the total mass of capillary endothelium found within the lung (Table 1). A slight increase in total endothelial cell mass appears to occur between 60 hours of exposure and the death of the animals as shown in Figure 1.

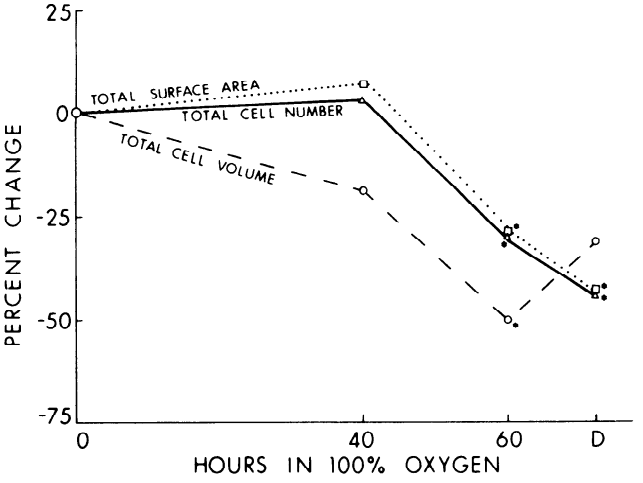

Figure 1. Alterations in pulmonary capillary endothelial cells in response to exposure of rats to 100% oxygen (5). Percent changes in total endothelial cell number (solid line), total volume of endothelial cells (dashed line) and in total pulmonary capillary surface area (dotted line) are shown. An asterisk indicates points where the difference from control was statistically significant ($p < 0.05$). D is the time of death of animals and occurred at a mean of 66 hours of exposure. N = 8 for controls and 4 for all other time points.

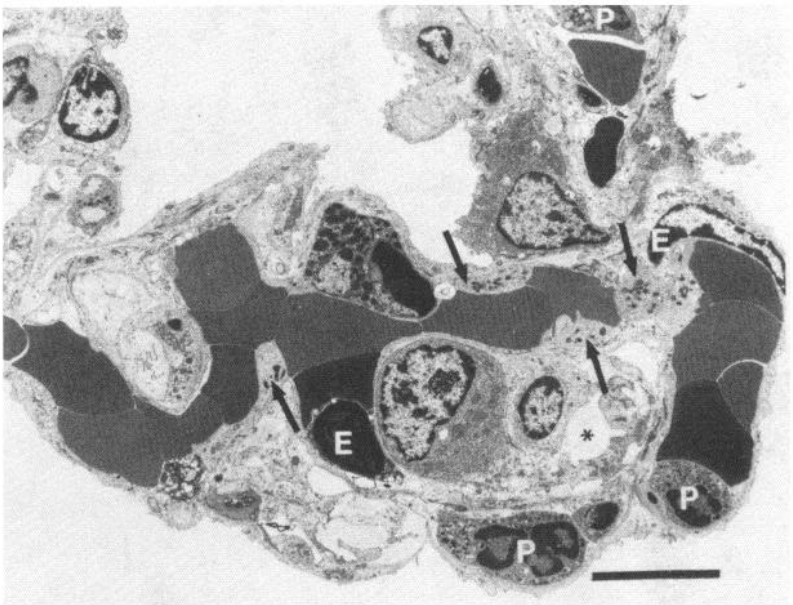

Figure 2. Transmission electron micrograph of the alveolar region of a rat lung after exposure of the animal to 100% O_2 until death. The alveolar septum is dramatically widened due to cell infiltrations and edema (*) in the interstitium. The existing capillary lumen are plugged with red cells and contain numerous platelets (arrows) and polymorphonuclear leukocytes (P). Endothelial cells (E) show substantial evidence of injury including pyknotic nuclei and areas of cytoplasmic swelling. Bar = 5 microns.

This is probably caused by cell swelling in the terminal phase of oxygen induced injury. Substantial injury also occurs to the majority of the endothelial cells remaining after 60 hours exposure to 100% oxygen as illustrated in Figure 2. At this point, the pulmonary capillary bed is congested with red cells, suggesting little or no flow in major regions of the remaining capillary bed. Endothelial cells which remain, frequently have pyknotic nuclei, swollen perinuclear cisternae and substantial cytoplasmic injury (Figure 2). There are dramatic increases in numbers of platelets and polymorphonuclear leukocytes in the pulmonary microvasculature (Figure 2) and numerous inflammatory cells are present in the interstitial spaces.

Cyanide resistant oxygen consumption of whole lung and subcellular organelle preparations has been shown to correlate with the rate of production of partially reduced oxygen species (11, 12, 18, 19). Direct measurement of superoxide or hydrogen peroxide production cannot be made in a whole lung preparation or in intact cells because of the presence of scavenging enzymes such as superoxide dismutase, catalase and peroxidases. Cyanide resistant respiration is a useful index of rates of production of partially reduced oxygen species in intact cells (11, 12, 18). Cyanide resistant oxygen consumption represents less than 4% of total oxygen consumption in lung slices (Table 1, see Appendix), but it increases more than 2-fold when the oxygen tension is changed from 21% to 85% oxygen. Endothelial cells in culture have a cyanide resistant oxygen consumption which is proportionately higher, representing almost 20% of the total oxygen consumption, and show a similar doubling of cyanide resistant oxygen consumption in the presence of 85% O_2 (Table 1, see Appendix). This implies that cultured endothelial cells have greater rates of oxygen radical production than the lung cells represented by whole lung preparations. Cyanide-resistant respiration is, however, an indirect measurement, highlighting the importance of measuring the actual oxygen species being produced by lung cells.

To evaluate the possible subcellular sites

where partially reduced oxygen species may be produced, submitochondrial particles and microsomes were prepared from rat and porcine lungs. Both submitochondrial particles and microsomes were found to have significant rates of superoxide and H_2O_2 production and the rate of production of these partially unsaturated oxygen species increased dramatically with increasing oxygen tension (Table 2, see Appendix) (11, 18, 19).

The time course of accumulation of platelets and polymorphonuclear leukocytes in the lung of oxygen exposed rats is illustrated in Figure 3. After 40 hours of exposure the volume of platelets in the lung microvasculature had increased significantly and there was ultrastructural evidence of some injury to the pulmonary capillary endothelium (5). However, these changes were not associated with an increase in the volume of polymorphonuclear leukocytes in the pulmonary microvasculature. Rapid accumulation of polymorphonuclear leukocytes occurred after the increase in intravascular platelets and this was associated with overt damage to the pulmonary capillary bed and death of the animal (Figure 3).

To determine whether or not endothelial cells are injured by exposure to hyperoxia in the absence of polymorphonuclear leukocytes and platelets, cultured porcine aortic endothelial cells were challenged with various oxygen concentrations. The loss of LDH from these cultured endothelial cells increased almost three-fold with an increase of oxygen tension from 5% to 95% (Table 3, see Appendix). Superoxide dismutase added to the cell culture medium did not significantly alter the cell injury, as indicated by LDH release, suggesting that superoxide radicals produced in the extracellular space were not a significant component of the cell injury. These same cultured cells were treated with phospholipid vesicles containing superoxide dismutase. Cell-associated concentrations of Mn superoxide dismutase increased 14-fold in this experiment, to 9.8 U Mn SOD/mg endothelial cell protein. A statistically significant decrease in LDH release during a 24 hour incubation occurred at all oxygen concentrations tested. Phospholipid vesicles not containing superoxide dismutase failed to produce the same degree of protection against the oxygen-induced injury (Table 3, see Appendix).

DISCUSSION

A substantial body of data suggests that the pulmonary microvasculature is the primary target for oxygen induced lung injury and that destruction of this specific compartment is closely associated with death of the animal (5, 14). Our finding that cyanide resistant oxygen consumption in lung tissue slices is proportional to oxygen concentration suggests that hyperoxia increases cellular production of partially reduced oxygen species. Endothelial cells in culture were found to have a proportionally higher rate of cyanide-resistant oxygen consumption. This suggests that this cell type may be more sensitive to oxygen injury because it may have comparatively high rates of superoxide and H_2O_2 production (Table 1, see Appendix).

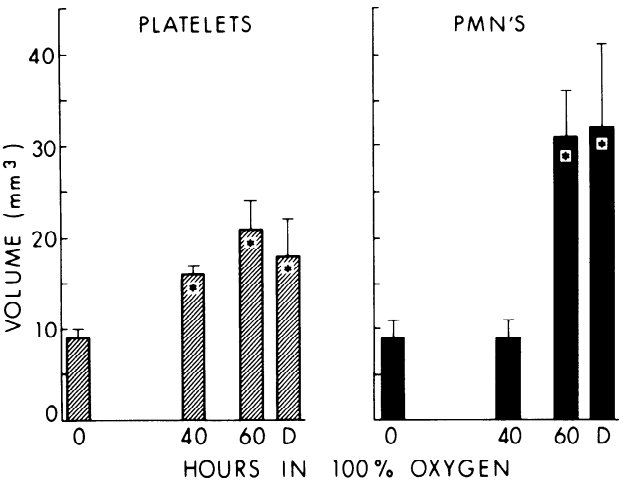

Figure 3. Changes in volume of platelets and polymorphonuclear leukocytes (PMN) in the pulmonary capillary bed of rats during exposure to 100% oxygen. All data are mean ± SEM. N = 4 for all groups. Changes which were significantly different from controls ($p < 0.05$) are indicated by an asterisk. Note that the increase in intravascular platelets precedes the increase in polymorphonuclear leukocytes.

Since submitochondrial particles and microsomes can be prepared in forms that are free of superoxide dismutases, catalase and peroxidases, it is possible to directly measure superoxide and H_2O_2 production by these isolated organelle fractions. These two organelle fractions account for a substantial fraction of the total cyanide resistant respiration of lung tissue (11, 18, 19). Furthermore, cyanide resistant respiration is almost entirely accounted for by superoxide production in these organelles. Hydrogen peroxide may be produced by the organelles both directly and as a byproduct of superoxide dismutation (18, 19).

SUMMARY

Increased production of partially reduced oxygen species has been shown to occur immediately upon exposure to lung tissue to high

oxygen tensions (11, 18, 19). The production of these potentially toxic oxygen species can be associated with direct endothelial cell injury, demonstrated by increased LDH release in oxygen challenged cultured endothelial cells. This index of endothelial cell injury can be decreased by augmenting intracellular superoxide dismutase with phospholipid vesicles containing SOD (Table 3, see Appendix). Extracellular superoxide dismutase added to the cell culture medium was ineffective in reducing cell injury, suggesting that the critical site of toxic oxygen species production is intracellular.

The time course of accumulation of platelets and of polymorphonuclear leukocytes in the pulmonary microvasculature suggests that these blood components are not primary agents in the initiation of oxygen induced lung injury. The initiation of injury is more likely to be mediated by the increased intracellular production of partially reduced oxygen species. This injury (perhaps by inhibition of normal prostaglandin metabolism) could lead to platelet adherence and aggregation, which may then attract polymorphonuclear leukocytes and enhance their activation and adherence to the pulmonary capillary endothelium (9). Secretion of superoxide radicals by leukocytes adherent to the pulmonary capillary endothelium may be an essential amplification process in the development of the later, severe stages of oxygen induced lung injury. These data thus suggest that the initial events in oxygen induced lung injury occur in the pulmonary capillary bed via the intracellular production of partially reduced oxygen species and that blood elements enter later and play a critical role in the amplification of this injury.

ACKNOWLEDGEMENTS

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APPENDIX

TABLE 1

Oxygen Consumption and Cyanide-Resistant Oxygen Consumption in Whole Lung Slices and in Cultured Endothelial Cells

	Whole Lung ($\mu mol\ O_2 \cdot min^{-1} \cdot lung^{-1}$)	Endothelial Cells ($nmol\ O_2 \cdot min^{-1} \cdot 10^6\ cells^{-1}$)
Total O_2 Consumption	1.68 ± 0.10	1.93 ± 0.20
CN resistant O_2 consumption at 21% O_2	0.07 ± 0.02	0.36 ± 0.10
Percent of Total	8	19
CN resistant O_2 consumption at 85% O_2	0.33 ± 0.02	0.83 ± 0.20
Percent of Total	18	43

Values are mean ± SEM.

TABLE 2

Hydrogen Peroxide and Superoxide Production by Porcine Lung Organelle Fractions

Percent Oxygen	O_2^- Production ($nmol \cdot min^{-1} \cdot mg\ protein^{-1}$)		H_2O_2 Production ($nmol \cdot min^{-1} \cdot mg\ protein^{-1}$)	
	Submitochondrial[b]	Microsomes[c]	Mitochrondria[d]	Microsomes[e]
5	0	0.7 ± 0.4	0	0.5 ± 0.1
21	0.70 ± 0.10[a]	4.2 ± 0.3[a]	0.06 ± 0.01[a]	2.3 ± 0.3[a]
95	1.70 ± 0.09[a]	8.2 ± 0.6[a]	0.60 ± 0.04[a]	4.5 ± 0.6[a]

All values are mean ± SEM.

[a] $p < .05$ for comparison to values at next lower O_2 concentration.

[b] Submitchondrial particles (0.4 mg protein/ml) were incubated with 6 mM succinate and 2 μM antimycin in the presence of 10 μM cytochrome c.

[c] Microsomes (0.2 mg protein/ml) were supplemented with 0.1 mM NADPH in the presence of 1 mM epinephrine.

[d] Lung mitochondria (0.3 mg protein/ml) were incubated with 6 mM succinate in the presence of 5 μM horseradish peroxidase.

[e] Microsomes (0.1 mg protein/ml) were supplemented with 0.1 mM NADPH and 0.5 μM horseradish peroxidase.

TABLE 3

Percent of Total Cell LDH Release from Cultured Porcine Aortic Endothelial Cells Challenged with Various Oxygen Concentrations[a]

Condition	Percent Oxygen 5	21	95
Control Cells	3.6 ± 0.4	6.4 ± 0.8	9.0 ± 0.5
SOD in medium[c] (100 U/ml)	3.6 ± 0.3	6.2 ± 0.7	8.8 ± 0.5
SOD Vesicle-treated cells	1.5 ± 0.4[b]	4.5 ± 0.4[b]	5.9 ± 0.6[b]
Enzyme-free vesicle treated cells	4.5 ± 1.0	5.5 ± 0.3	8.0 ± 0.3[b]

[a] Confluent third passage cells grown in multiwell plates were challenged with 5, 21 and 95% oxygen in gas phase for 24 hours. Data represents the percent of total cell monolayer LDH released into culture medium (Mean ± SEM, n = 12).

[b] Represents significant difference from control cells, $p < 0.05$, challenged with the same oxygen concentrations.

[c] Human Mn SOD purified according to Crapo et al. (6) was used.

OXYGEN RADICALS AND LUNG EDEMA

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INTRODUCTION

Acute noncardiogenic pulmonary edema (Edematous lung injury) is an important clinical problem which contributes to the death of nearly 75,000 people annually. Most cases of edematous lung injury are included under the heading of Adult Respiratory Distress Syndrome (ARDS) (18). ARDS is associated with many potential inciting events, including sepsis, microembolism, hyperoxia, shock, trauma, aspiration, burns and gastrointestinal disorders. As a result, it has been very difficult to clearly determine a common denominator which accounts for the loss of alveolar-capillary membrane integrity and permeability pulmonary edema seen in ARDS. Indeed, the association of ARDS with multiple inciting events has suggested that under certain circumstances many factors might be involved in the pathogenesis of ARDS including alveolar macrophages, platelets, arachidonic acid metabolites, kinins, complement by-products, fibrinogen degradation products, endotoxin, neutrophils, granular products and oxygen radicals. This brief review will focus on the emerging role of neutrophils and O_2 radicals in the development of acute edematous lung injury.

NEUTROPHIL'S PARTICIPATION IN LUNG INJURY

Numerous observations suggest that neutrophils participate in the development of edematous lung injury. First, increased numbers of neutrophils are often found in lung lavages of patients with ARDS and along damaged endothelial cells in the lungs of animals with edematous lung injury (1, 6, 24). In addition, increased levels of C5a are present in the blood of patients with ARDS suggesting that factors which could cause neutrophil accumulation and O_2 radical release may be circulating in the lungs of patients with ARDS (14). Moreover, the ability of alveolar macrophages to release factors which recruit, adhere and stimulate release of O_2 radicals and other toxins from neutrophils suggests another mechanism by which neutrophils could become involved in acute lung injury, especially in those cases initiated by air borne insults (15). The above findings demonstrate that neutrophils accumulate in the lungs in clinical and experimental ARDS, and the mechanisms underlying this accumulation are being unravelled. But, are neutrophils directly participating in the development of lung injury in these settings? Several studies have now suggested that neutrophils are critical for the development of some experimental edematous lung injuries. For example, permeability edema from pulmonary microembolism with air or glass beads is decreased in neutrophil-depleted animals (10, 19, 42). Similarly, injection of large amounts of activated complement produces an increased flow of highly proteinaceous lung lymph and increases deposition of labelled albumin in the lungs of normal but not neutropenic animals (5). In addition, neutrophils are a necessary component in a complement-mediated model of alveolar-capillary injury (17). Finally, neutrophil depletion decreased edematous lung injury in rabbits exposed to hyperoxia - an experimental model of acute edematous lung injury (32, 33). A third line of evidence which supports the potential role of neutrophils in the development of edematous lung injury is that neutrophils can release O_2 radicals, granular enzymes and arachidonic acid metabolites which can cause tissue injury, alter vascular permeability and perturb hemodynamics (22, 41). Thus, neutrophils appear to be crucially involved in some lung injuries, and they certainly have potent mechanisms for such participation.

HYPEROXIA AND NEUTROPHIL ACTIVATION

To further investigate the contribution of neutrophils and O_2 radicals to the development of acute edematous lung injury, our laboratory conducted a series of investigations of pulmonary edema caused by hyperoxia or the injection of phorbol myristate acetate (PMA) - a chemical which stimulates neutrophil adherence and O_2 radical production (30). This research can be summarized briefly! Our overall hypothesis is shown in Figure 1. First, after exposure to hyperoxia for approximately 66 hours, a rapid increase in mortality occurred in either rats or rabbits. Importantly, just before death, hyperoxia exposed animals, either rats or rabbits, developed a protein-rich pulmonary edema characterized by marked increases in lung weights and lung lavage albumin concentrations (11, 32). In addition, sharp increases occurred in the number of neutrophils recoverable from lavages and were paralleled by similar increases in the ability

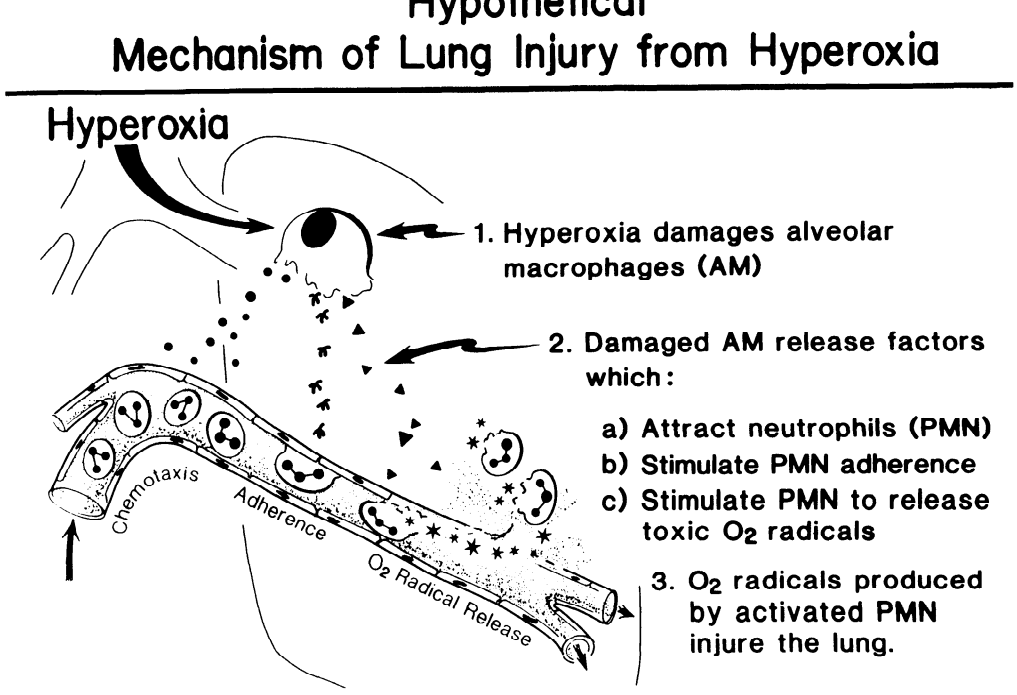

Figure 1. Schematic of the hypothetical mechanism of acute edematous lung injury from hypoxia.

of lavages to stimulate neutrophil chemotaxis. Furthermore, lavages from the lungs of hyperoxia exposed rats augment O_2 radical production by neutrophils. The close temporal association between influx of neutrophils, neutrophil chemotaxins and neutrophil O_2 radical stimulants and the death of these animals from hyperoxia led us to ask if neutrophils contributed to lung edema in pulmonary oxygen toxicity. This possibility was supported when hyperoxia-exposed rabbits rendered neutropenic with nitrogen mustard had less lung weight and lung lavage albumin concentration increases than non-neutropenic rabbits (32, 33). Furthermore, significant correlations existed between the numbers of neutrophils circulating initially or the numbers of neutrophils in lung lavages and the amount of lung injury as reflected by increases in lung lavage albumin concentrations. In addition, pretreatment of rats with dimethylthiourea (DMTU) - an O_2 radical scavenger - reduced lung edema associated with hyperoxia (12). The aforementioned indicated that neutrophils might participate in the development of lung edema and prompted us to seek the source of lavage factors that had the capacity to stimulate neutrophil recruitment and activation. Our hypothesis that alveolar macrophages which had been damaged by hyperoxia had released these factors was supported when cultured alveolar macrophages exposed to hyperoxia for 48 hours in vitro also released chemotaxins for neutrophils which were similar in molecular weight to neutrophil chemotaxins isolated from the lung lavages of rabbits exposed to hyperoxia. Supernatants from alveolar macrophages exposed to hyperoxia in vitro also contained increased amounts of factors which augmented adherence and O_2 radical production by neutrophils (2, 16).

Because of similarities in the neutrophil activating effects of factors derived from alveolar macrophages exposed to hyperoxia and the chemical agent, PMA, we injected PMA into rabbits to see if it would produce lung edema (34). Injection of PMA caused a rapid accumulation of neutrophils in the lung and the development of profound increases in lung weights and lung lavage albumin concentrations. In contrast, in nitrogen mustard pretreated neutropenic rabbits, injection of PMA did not increase lung weights or lung lavage albumin concentrations. Moreover, statistically significant correlations again existed between the initial numbers of circulating neutrophils or the numbers of neutrophils in lung lavages and the degree of lung injury as reflected by amounts of albumin in lung lavages (34). Furthermore, pretreatment with mepacrine, a scavenger of O_2 radicals and a phospholipase A_2 inhibitor, prevented the

development of edematous lung injury following injection of PMA (3).

DAMAGE BY O_2 RADICALS, ESPECIALLY H_2O_2

To focus more directly on the possible role of neutrophils and O_2 radicals in the development of lung edema (Figure 2), experiments were next conducted using the

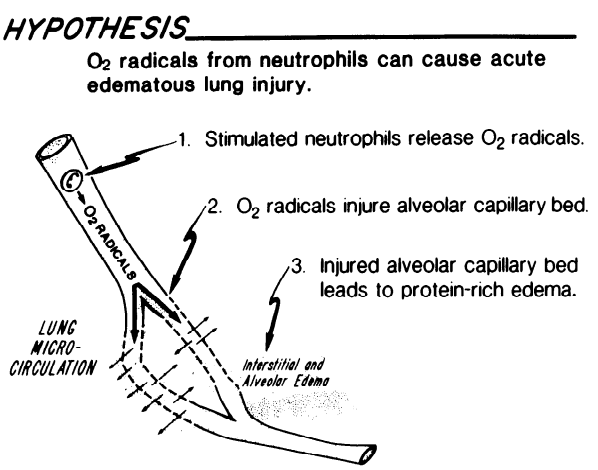

Figure 2. Schematic of hypothesis by which O_2 radicals from neutrophils cause permeability edema.

isolated perfused rabbit lung model. This approach has the advantage that the lung circulation can be washed essentially free of blood components thus allowing more direct assessment of specific mechanisms of injury. Addition of neutrophils and PMA to the perfusates of isolated lungs caused sharp increases in lung weights and lung lavage albumin concentrations which were not seen in controls in which neutrophils or PMA were added alone (34). The role of O_2 radicals in these processes was suggested by three additional lines of investigation. First, dimethylthiourea (DMTU) blocked the development of lung edema following addition of neutrophils and PMA. Second, addition of O_2 radical-deficient neutrophils from a patient with chronic granulomatous disease (CGD) and PMA did not cause lung edema (34). Third, instillation of purine and xanthine oxidase, a chemical system which generates O_2 radicals, also caused lung edema which was preventible by O_2 radical scavengers, DMTU and catalase, but not SOD (38). Fourth, addition of β-D-glucose and glucose oxidase, a chemical system which generates H_2O_2, but not O_2, caused catalase inhibitable lung edema (37). The latter two observations suggested that H_2O_2, or perhaps (·OH) or some other product of H_2O_2, was toxic to the alveolar-capillary barrier (29). Furthermore, these studies also suggested that chemically generated O_2 radicals caused pulmonary vasoconstriction (37, 38). Again, these pressure changes were preventable by addition of O_2 radical scavengers or papaverine, a smooth muscle relaxant. In more detailed investigations, it was possible to demonstrate that O_2 radicals increased permeability of the alveolar capillary membrane in addition to the pulmonary vasoconstriction (39).

Additional evidence from other laboratories which indicates that O_2 radicals might contribute to the development of edematous lung injury includes the following observations. First, stimulated neutrophils can damage endothelial cells in culture (31). Close approximation of neutrophils and C5a caused release of small amounts of ^{51}Cr from prelabelled endothelial cells in culture. Chromium release did not occur when neutrophils came from a patient with chronic granulomatous disease (CGD) or when SOD and catalase were added to the complete system, suggesting that O_2 radicals were involved in endothelial damage. Furthermore, xanthine plus xanthine oxidase also caused release of ^{51}Cr prelabelled endothelial cells in culture. Subsequently, similar studies suggested that H_2O_2 or one of its byproducts may be the key reduction product of oxygen involved in damage to cultured endothelial cells (40), an observation which parallels our findings implicating H_2O_2 or its byproducts in the development of edema in isolated perfused lungs.

Other studies which support the possible pathogenetic role of O_2 radicals in acute lung edema are that addition of superoxide dismutase (SOD) decreases lung edema in sheep following microembolism (9) and that addition of SOD and catalase decrease lung injury following intratracheal administration of xanthine and xanthine oxidase or glucose oxidase and myeloperoxidase (20). Catalase also protected lungs following intratracheal administration of PMA which was believed to stimulate release of toxic O_2 radicals from alveolar macrophages (21). Finally, it has recently been observed in dogs injected with endotoxin and DMSO that increased amounts of methane are generated suggesting that hydroxyl radical (·OH) production was occurring in this model of endotoxin-induced pulmonary edema (35) since interaction of ·OH with DMSO produces methane (28).

MECHANISMS OF O_2 RADICAL TOXICITY

The mechanisms and cellular targets which account for the toxicity of O_2 radicals are unknown (13). However, it is generally believed that key cell components, such as proteins, membrane lipids, and/or nucleic acids, are highly susceptible to O_2 radicals. While most cells have certain intracellular protective enzymes, such as SOD which degrades O_2 to H_2O_2, or catalase, which degrades H_2O_2, these

enzymes may not be in the right locations to protect cells from extracellular O_2^- radical attack. Moreover, if O_2^- and H_2O_2 are not degraded, they can react to form $^{\cdot}OH$, a very powerful oxidant capable of mediating significant tissue injury (25). Since there are no known enzymatic cellular scavengers for $^{\cdot}OH$, its potential toxicity may be great. Another mechanism of tissue injury is reaction of O_2^- radicals with unsaturated fatty acids forming lipid peroxide radicals. These reactions initiate chain reactions between lipid peroxides and membrane fatty acids which damage cell structures (8, 36). This chain reaction can be interrupted by antioxidants, such as Vitamin E. Lipid peroxide radicals can also decompose to form malondialdehydes which can crosslink and damage free amino groups of proteins, nucleic acids, and phospholipids. Lipid peroxides can be converted by selenium-sensitive glutathione peroxidases to less toxic hydroxy fatty acids. O_2^- radicals could also contribute to cell injury by reacting with arachidonic acid intermediates (7, 23, 26, 27) or by inactivating antiprotease defense mechanisms (4).

SUMMARY

In summary, neutrophils and O_2^- radicals clearly can contribute to the pathogenesis of experimental lung injury. The exact mechanisms underlying their capacity to injure and the relevance of these findings to clinical ARDS remain to be established. However, it seems appropriate, on the basis of current evidence, to seriously consider a role for neutrophils and their toxic O_2^- radicals in the pathogenesis of ARDS.

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