

THE PHYSIOLOGIST

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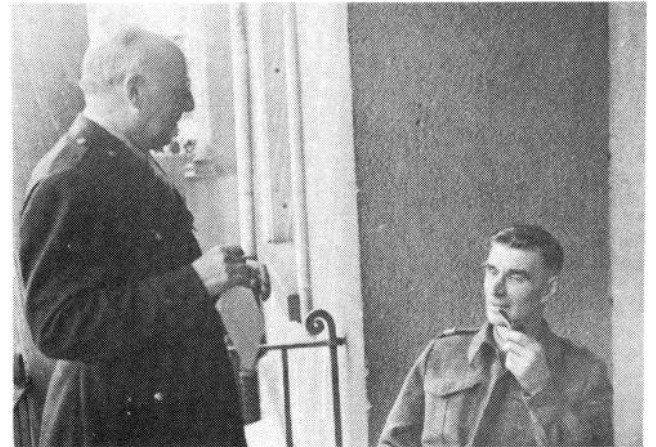
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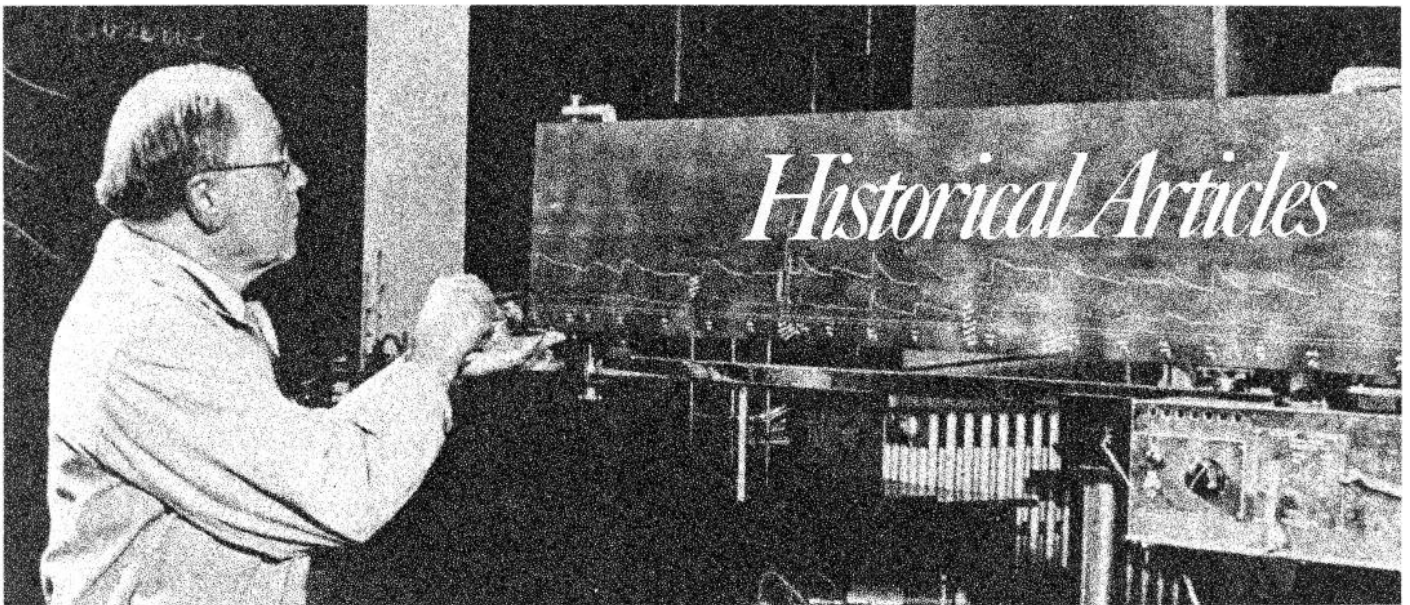
Cover: Sid Robinson, a 1,500-meter runner on the U.S.A. team at the 1928 Olympics in Amsterdam, p. 319.



These pictures of Alan C. Burton were taken by Dr. D. Bruce Dill in his temporary home in Frankfurt, Germany, in September 1945 when Burton came, under the auspices of the Office of the Quartermaster General, U.S. Army, to learn what had happened during World War II to the Kaiser Wilhelm Institute for Work Physiology (now the Max Planck Institut für Arbeitsphysiologie) in Dortmund. Dr. Dill has presented them to the Society's archives with a short account of the circumstances. Can any of our readers help us identify the gentleman standing in the right-hand lower picture?

Correction

Physiologist 25(2): 79, 1984. Association of Chairmen of Departments of Physiology, Analysis of Annual Questionnaire—1983-84. Faculty Salaries (in thousands) are as follows: All Schools Chairmen's Salaries, Mean = \$58.955; Public Medical Chairmen's Salaries, Mean = \$68.437; and Private Medical Chairmen's Salaries, Mean = \$72.642. Other salaries on that page are correct.



Departmental History

Physiology at Indiana University-Bloomington A History

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The Nineteenth Century Background

Established in 1820, Indiana University first offered instruction in a biological science in the spring of 1854. The introduction into the curriculum of a course in "Physiology" marked yet another attempt on the part of the embryonic university to offer "to young gentlemen, who intended ultimately to devote themselves to the medical profession, great advantages" (2). Taught initially by the Reverend Robert Milligan, the course consisted of lectures culled from Wilson's *Anatomy*, Carpenter's *Physiology*, Dunglison's *Laws of Health*, and Agassiz and Gould's *Principles of Zoology*. Since the lectures were not accompanied by laboratory instruction of any kind, natural history and morphology undoubtedly fared better than the little developed study of animal and human functions.

Throughout the latter half of the nineteenth century modified "hygiene" versions of the course in physiology continued to be taught at Indiana University, but each failed for many reasons to advance beyond the introductory lecture level. Significant among these reasons were the small size and parochial character of the university, traits reflected in a limited classical curriculum endorsed by a succession of conservative presidents and

supported by an equally conservative state legislature. It was not, in any case, until 1891 that Indiana University renewed its offer of "advantages" to students seeking premedical education. This was a result of academic and administrative reforms initiated by the zoologist David Starr Jordan, president of Indiana University from 1885 to 1891.

In 1891, the year Jordan resigned to take up the presidency of Stanford University, a Medical Preparatory Course was established under the combined direction of the Departments of Chemistry and Zoology, the later founded by Jordan in 1885. Carl Eigenmann, Jordan's former student and fellow ichthyologist, headed the Department of Zoology from which he taught medical preparatory courses in Comparative and Mammalian Anatomy, Histology, Embryology, and Physiology. The last, however, dealt largely with human anatomy, a subject that Eigenmann taught without venturing far afield into the realm of vital functions and their medical importance. As it was, responsibility for the Medical Preparatory Course had forced Eigenmann to venture too far afield from his one-man department already. In 1895 he withdrew from the program, leaving the professor of chemistry, Robert E. Lyons, alone to counsel an ever-growing number of students wanting premedical education.

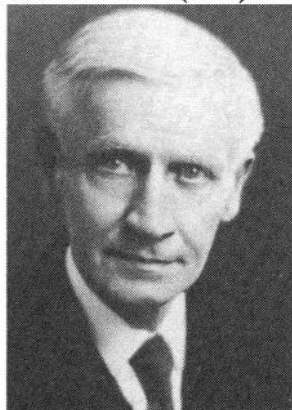
Although unsuccessful, the Medical Preparatory Course drew attention to the fact that the state's largest university had yet to meet the challenge of providing adequate educational opportunities for its professionally minded students. Fortunately, in January 1903, Indiana University gained in William Lowe Bryan, a president committed to broadening the university's educational base by the organization of graduate and professional schools. One of Bryan's first acts concerned directly the neglect of physiology at Indiana. At a meeting of the Board of Trustees in March 1903, Bryan requested that the Board consider seriously the question of establishing a medical department in connection with the university. Not one to demur on crucial matters, Bryan submitted simultaneously a proposal to immediately establish a medical department that would offer the first two years of medical studies. Central to Bryan's proposal was a plan "to increase very considerably the amount of work offered in physiology," beginning with

the assignment of the course in physiology to Dr. William J. Moenkhaus, and to hire an additional man to teach courses in anatomy and assist with the organization of the Department of Medicine (1).

The Board of Trustees approved Bryan's proposal, and in June it invited Dr. Burton D. Myers, a member of the Anatomy staff at Johns Hopkins University, to head Indiana University's new Department of Anatomy. Physiology was, as planned, turned over to the Assistant Professor of Zoology, Dr. William J. Moenkhaus. But whereas Myers was a trained anatomist with an M.D. from the University of Leipzig, Moenkhaus was a zoologist in the Jordan-Eigenmann tradition specializing in the genetics of fish. Of the two men destined to guide the Indiana University School of Medicine through its first and most critical stages, Moenkhaus had certainly the more difficult task: to master a new field and develop a course offering on an equal basis with that of anatomy.

William J. Moenkhaus: A Modest Beginning

A native "Hoosier," **William J. Moenkhaus** earned both the A.B. (1894) and the A.M. (1895) at Indiana



University, with zoology as his major. He next went to Harvard for three years and served for one year as assistant director of the State Museum at São Paulo, Brazil. In 1900 he transferred to the University of Chicago and obtained the Ph.D. in zoology in 1903. His professional association with Indiana University began in 1901, when he joined his former teacher, Eigenmann, in the Department of Zoology as assistant professor. Charged from the start with teaching the traditional course in physiology, Moenkhaus severed his ties with the Zoology Department in 1904 to head the newly established Department of Physiology.

Both the Anatomy and Physiology departments had been established primarily as "teaching departments" that would oversee and offer between them the first two years of medical studies. Nevertheless, their status as independent academic departments entitled each to offer graduate training leading to the Ph.D. Moenkhaus and Myers took seriously the latter purpose of their departments and were determined to train scientists as well as medical students. According to the September 1904 issue of the *Indiana University Bulletin*, the Department of Physiology was prepared to offer three courses for students in medicine, but it was also prepared to offer graduate students the following:

The Department is providing a well-equipped laboratory for experimental work. It is supplied with manikin and other models and charts; kymographs, inductoria, sphygmographs, cardiographs, plethysmographs, stethoscopes, manometers, tambours, haemometers, perimeters, ophthalmoscopes, artificial eyes, and other instruments for the performance of the important experiments in Physiology. About a dozen of the more important English, German and French physiological journals are taken by the Department and it possesses many of the standard works in Physiology.

With these newly acquired instruments and five rooms on

the second floor of the Science Hall, Moenkhaus undertook single-handedly the task of fostering a program of graduate physiological research at Indiana University.

Although the Bryan administration made possible a modest start, it could not guarantee over the years the financial support necessary for the development of a strong research program in Physiology. The funds allotted to the Indiana University School of Medicine, of which the two-year medical program in Bloomington was an intricate part, went of necessity toward the establishment of a four-year clinical school in the neighboring city of Indianapolis. Financially and physically isolated from the main body of the Indiana University School of Medicine, Moenkhaus had neither the time nor the resources to invest in physiological research. As a member of the medical faculty, his first obligation was to teach the prescribed number of medical courses. This he did for nearly twenty years assisted only by a few graduate students and the occasional postdoctoral instructor.

A superb lecturer with a talent for elevating introductory physiology to the level of an intriguing science, Moenkhaus succeeded where his predecessors at Indiana had failed. Encouraged in part by the example and professional support of the anatomist Burton D. Myers, Moenkhaus proved a willing and capable advisor to pre-medical students who chose to major in physiology. His facilities were also improved and expanded when the department moved to Owen Hall in 1911. By 1920 he had supervised work on thirteen masters' theses and three doctoral dissertations, a record which compared favorably with that of other scientific departments at Indiana University. Although the research topics in these works suggested a medical orientation, the range in experimental technique and subject matter indicated that Moenkhaus had mastered over the years the ubiquitous science of "general physiology."

Two of the first men to have earned the Ph.D. under Moenkhaus were Dennis E. Jackson (1908) and Paul Montgomery Harmon (1920). Jackson subsequently became one of the leading American experts in pharmacological physiology. Harmon, who had written a rather exceptional dissertation on "The Influences of Temperature and Other Factors Upon the Two-Summitted Contractility Curve of the Gastrocnemius Muscle of the Frog," chose to remain with Moenkhaus and was appointed Assistant Professor of Physiology in 1922. His appointment effectively ended the era of Moenkhaus' one-man department.

In 1926-27 **Paul Montgomery Harmon** served as Visiting Instructor in Physiology under the eminent



Walter B. Cannon at the Harvard Medical School. From Cannon, Harmon absorbed a lifelong interest in the area of homeostasis and the concept of physiology as an active investigative science, two things that were to play an important role in his future as department chairman. Until Moenkhaus' retirement in 1941, he and Harmon worked together to see the department through the financial gloom of the 1920's and to augment its size and status in the 1930's.

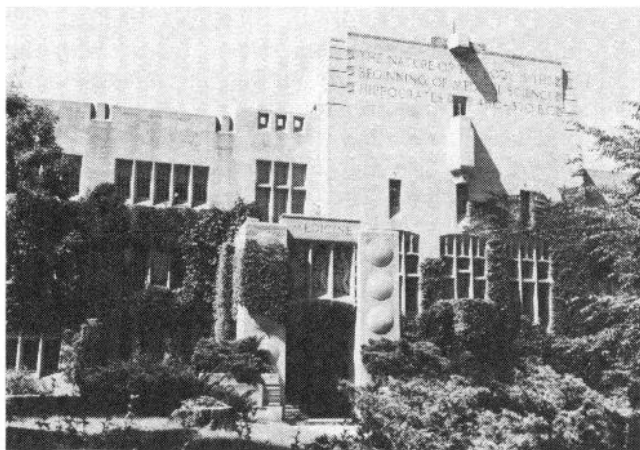
With the 1930's came not only President Roosevelt's "New Deal," but also President Bryan's "Brass Tack" approach to financing a university known for its lean budget and professional salaries. Although the Physiology Department was not targeted for immediate increased support, Moenkhaus' position as Chairman of the Indiana University Athletic Committee occasioned a rewarding relationship with the new assistant track coach, Sid Robinson.

Sid Robinson was brought to Indiana in 1930 by his former track coach, Billy Hayes. Although hired initially as Assistant Professor of Physical Education, Robinson's growing interest in the physiological determinants of athletic performance led him to take graduate courses from Moenkhaus and in chemistry. Eventually a mutually beneficial arrangement ensued between the very successful cross-country coach and the Physiology Department. While still a salaried member of the Athletic Department, Robinson was made Assistant Professor of Physiology, and with his help a course in exercise physiology was added to the curriculum. In turn, Robinson requested and was granted a leave of absence for the years 1936-1938 to earn the Ph.D. in exercise physiology under the direction of Dr. David Bruce Dill at the Harvard Fatigue Laboratory. With Moenkhaus and Harmon's full backing, it was agreed that Robinson would return to Indiana as a salaried member of the Physiology Department.

In 1937, the Departments of Anatomy and Physiology moved from Owen Hall to the new School of Medicine Building (renamed Burton D. Myers Hall in 1958), one of the spacious WPA/PWA buildings erected in the final years of the Bryan administration. The move signaled the beginning of a transition period punctuated by the addition of new young faculty, Moenkhaus and Myers' retirement and the outbreak of World War II. Harmon succeeded Moenkhaus as chairman of the department in the summer of 1941. But of the three young physiologists who had joined the department since the opening of Myers Hall, only Dr. Paul A. Nicoll remained. Dr. V. Scott Brown, who had served as assistant professor in the department since 1938, had left to pursue his medical research elsewhere. Sid Robinson returned to the Harvard Fatigue Laboratory in 1942 to serve his country through his science.



Sid Robinson: track star, coach, scientist, teacher; chairman of the newly formed Department of Anatomy and Physiology from 1959 until 1964.



Myers Hall: home of the Indiana University School of Medicine's Medical Science Program and physiology on the Bloomington Campus. Built from locally quarried limestone and occupied in 1937 as the Medical School Building. It was renamed Burton D. Myers Hall in 1958.

Faced with the sudden unanticipated loss of two very promising faculty members, Harmon shrewdly arranged for Dr. Theodore J. B. Stier, Associate Professor of Physiology at Harvard, to fill in for the absentee Robinson. With Robinson's return in 1943, Stier was offered and accepted an appointment at Indiana. Thus Harmon succeeded in preserving through the war years the manpower necessary to transform the department from a teaching appendage of the Indiana University School of Medicine into a small but cohesive group of research-oriented physiologists. His influence was great, and it resulted in many medical students becoming interested in research in physiology. Consequently many of his students entered academic medicine in clinical departments after completing their formal medical education.

From Lectern to Laboratory

When Sid Robinson first returned to Indiana in 1939, only his salary, faculty position, and space in Myers Hall were guaranteed. However, Robinson returned to Indiana with a grant from the Research Committee of the Edible Gelatin Manufacturers of America. With this money he purchased a human treadmill and designed and equipped a research laboratory specifically for studies in the physiology of exercise and temperature regulation. In accordance with the terms of the grant, Robinson began his research career at Indiana University by investigating the effects of gelatin consumption on the athletic performance of human treadmill subjects.

Funding, however, was not the only critical factor in Robinson's early career. From the outset he received absolute support and assistance from Harmon. Above all, the latter was determined to make research an important function of the department he was due to inherit. The combination of Robinson's ability as a research scientist and Harmon's backing as a fellow physiologist, superior, and friend proved fortunate indeed. Within a few years, the former track coach authored a half-dozen papers on the physiological effects of training, exercise, and temperature. These early studies drew attention to Indiana's budding research program in exercise physiology.

In 1942 the exigencies of global war interrupted Robinson's work at Indiana. At the request of David Bruce Dill and Arlie V. Bock, Robinson returned to the

Harvard Fatigue Laboratory to direct war time research on heat and cold environmental stress. He took with him his research assistant Eugene Turrell, who turned in later years to a profession in psychiatry.

Despite the interruption, the impact of World War II on Robinson and his program at Indiana translated into growth and stability. The most immediate result of Robinson's wartime research on the combined effects of climate, clothing, and work on the performance levels of soldiers was the acquisition of expertise and profound interest in the general area of environmental stress. Needless to say, the US Government and military agencies also developed a conspicuous interest in this area. Consequently, Robinson became like hundreds of other American scientists, a direct beneficiary of the war that shocked the country into recognition and treatment of science as a national resource rather than an academic pastime.

Robinson's scientific interests and prospects for a research career were not, however, the only things which had changed with the war. The physiology department to which he returned at the end of 1943 was enriched by the presence and research activities of Dr. Paul A. Nicoll and Dr. Theodore J. B. Stier. Recruited by Harmon for their teaching and investigative abilities, these two physiologists had weathered the war years with unusual resourcefulness and drive. Nicoll, a graduate of Washington University and former Instructor at the University of Chicago, turned a chance domestic encounter with a bat in 1942 into a major, now classic study of the micro-circulation. Together with Dr. Richard L. Webb of the Anatomy Department, Nicoll published in 1946 a fundamental paper on "Blood Circulation in the Subcutaneous Tissue of the Living Bat's Wing." Dr. Theodore J. B. Stier, who had trained and worked at Harvard in comparative and cellular physiology, survived his temporary appointment with equal enterprise. The recipient of a considerable grant from the Seagram's Company, Stier spent his first years at Indiana investigating the growth of yeast cells under anaerobic conditions. In 1946 Harmon, Robinson, Nicoll, and Stier were joined by Dr. Howard H. Rostorfer from the University of Arkansas. A graduate of Ohio State University, Rostorfer's work centered around the biochemical aspects of physiological processes; he studied for some years the metabolic changes in hemoglobin induced by malaria.

From 1946 until the mid-1950's life at Myers Hall was characterized by stability and improved rapport with the School of Medicine at Indianapolis. Only the death of Moenkhaus on June 7, 1947, cast a shadow over the department. That same year Robinson was appointed full professor and the Harvard Fatigue Laboratory closed its doors. In 1949 a woman joined the faculty for the first time. Dr. Grace E. Wertenberger, a graduate of the University of Chicago, came to Indiana as Associate Professor and devoted herself to teaching. Due largely to Harmon's qualities as chairman, an acceptable balance was struck between serving the School of Medicine, training graduate students, and laboratory research. But the latter continued to depend very much on each individual's ability to secure outside financial support or to make the most of the laboratory facilities available.

By 1955 a number of factors combined to warrant the hiring of additional faculty in physiology at Bloomington. The postwar increase in medical students led to the continued physical growth of the School of Medicine in

Indianapolis. Its transformation into a large-scale academic medical center had accelerated enrollment and teaching demands, particularly for the first year of medical school in Bloomington. Thus in 1955 two new assistant professors joined the department, Dr. Leon K. Knoebel from the University of Rochester and Dr. Ward W. Moore, a graduate of the University of Illinois. They were followed in 1956 by Dr. Robert W. Bullard, a graduate of the University of Rochester.

After decades of administrative unease over the physical separation of the first year of medical studies from the main body of the Medical School at Indianapolis, a decision was reached to move the first year, along with some of the faculty at Myers Hall, to the new Medical Sciences Building in Indianapolis. However, beneficial to the Indiana University School of Medicine, the proposed move threatened to terminate the autonomy and scientific life of Harmon's beloved department in Bloomington. Knoebel, Moore, and Bullard had been recruited by Harmon on the basis of their promise as researchers and teachers with the provision when they were hired that they would move to Indianapolis.

According to plan, the first year of Medical School was transferred to Indianapolis in 1958. The three new faculty members, Knoebel, Moore, and Bullard, moved to Indianapolis and joined the Department of Physiology headed by Dr. Ewald E. Selkurt. Nicoll, who had headed an Indiana University/AID Project in Pakistan from 1955 to 1961, was assigned to Indianapolis where he remained until 1971. Harmon, Robinson, Stier, Rostorfer, and Wertenberger remained in Bloomington and formally merged with Anatomy into a new Department of Anatomy and Physiology on the Bloomington campus. The merger automatically terminated Harmon's chairmanship and led to Sid Robinson's appointment as chairman of the Department of Anatomy and Physiology. The anatomists joining the new department included Drs. Richard Webb, Raymond Murray, Stanley Rafalko, and Martha Strong.

The Department of Anatomy and Physiology came under the administrative auspices of the College of Arts and Sciences, but the official dissociation from the School of Medicine was replaced by that body's promotion of the new department's involvement in a pioneering program in medical education. In obvious recognition of the importance and reputation of the physiology and anatomy programs at Myers Hall, Dr. Herman B. Wells, President of Indiana University, and Dr. John D. Van Nuys, Dean of the Medical School, singled out the Department of Anatomy and Physiology for its unique liberal arts-medicine-research curriculum. The Program was designed for exceptional medical students interested in teaching and scholarly research; specifically, it aimed to prepare students for careers in academic medicine. Since the program's inception in 1959, its medical graduates (1964-1983) earned 51 Ph.D. and 75 Master's degrees in one of the basic medical sciences.

Accent on Stress:

The Human Physiology Laboratory

External affirmation of the importance of Bloomington's physiology program came in 1959. That year Sid Robinson applied for, and received, the first of the major government research contracts with which he financed one of the world's best human stress physiology laboratories. In the fall of 1961, national attention

focused on both the Bloomington- and Indianapolis-based physiology programs when the two departments jointly hosted the annual meeting of the American Physiological Society. Again in 1970 the two departments hosted the American Physiological Society Meeting as part of the 150th Anniversary Celebration of Indiana University.

At this time (1961) the promise of generous government grants enabled the department to begin building a new Human Stress Physiology Laboratory. Over the next few years a major addition was built onto Myers Hall to house the laboratory. New sophisticated equipment was installed, which allowed one to simulate environmental conditions affected simultaneously by a variety of factors, such as high altitude and hot and cold temperatures. The laboratory was designed to foster and serve a comprehensive research program that would focus on the combined effect of various external and internal stresses and permit study of their implication of renal and cardiovascular diseases, endocrine disorders, and human aging. The latter studies would be carried out by faculty members of the department, while the former were aimed specifically at bringing to Bloomington Dr. Bullard (then at Indianapolis) and Robinson's lifelong mentor and friend, Dr. David Bruce Dill. In addition, Dr. E. M. Bogdanove, a graduate of Iowa, joined the department in 1961. His expertise in neuroendocrinology added breadth to the department. He left the department in 1971 to join the faculty of the Medical College of Virginia.

The Human Physiology Laboratory opened in June 1963, with Robinson as its director, and Dill, in a somewhat ironic reversal of roles, as a visiting Research Scholar. The following year Dr. Bullard returned, as anticipated, from Indianapolis to devote himself to the study of the effects of high altitude, cold, heat, and low O₂ levels on humans and animals. Two new people joined the department on Bullard's return in 1964: Dr. Charles Barnes, a neurophysiologist and graduate of Iowa, and Dr. Mukul Banerjee, an environmental physiologist from LSU. Barnes left the department in 1971 and Banerjee left in 1973. That same year (1964) the Air Force awarded Indiana's Human Physiology Laboratory a large grant to develop a research and training program that would apply human stress physiology to problems in space biology. At this juncture, Robinson's triple role as laboratory director, principal investigator, and department chairman produced its own peculiar kind of "executive" stress. Furthermore, Harmon's death on July 16, 1964, meant the loss of an invaluable counselor and assistant where departmental matters



were concerned. That summer Robinson resigned as chairman of the Department of Anatomy and Physiology, leaving Bullard at the helm. **Robert Winslow Bullard** served as acting chairman until 1967 and subsequently as chairman until his death in 1971.

The combination of Robinson, Dill, and Bullard made Indiana's Human Physiology Laboratory and its research program internationally famous. With Bullard

also attracting research contracts on a large scale, stress physiology inevitably dominated scientific life within the department throughout the 1960's. But the program thrived to some extent on input and expertise from other areas of physiology brought to the department by new faculty. Demonstrating exceptional sensitivity and professional acumen, Bullard especially encouraged the participation and collaboration of all the members of his department, anatomists, physiologists, and graduate students alike. Bullard's tenure as chairman was also characterized by the recruitment of additional faculty and a large expansion of the Department's graduate program which has continued to flourish. Between 1964 and 1983 the department awarded 83 Ph.D. and 48 Master's degrees.

The first of Bullard's new men, Dr. Roderick A. Suthers, arrived in 1966. A Harvard graduate, Suthers specialized in the sensory physiology and behavior of echolocating bats and birds, a field known today as bioacoustics. The powerful trend in the life sciences toward quantitative methods and biophysics led to the appointments of Dr. Alfred Strickholm as associate professor and Dr. James R. Hippensteele as an assistant professor in 1967. A graduate of the University of Chicago, Strickholm trained in neurophysiology and applied his background in physics to the study of communication (transport and excitation) between neural and muscle membranes. Hippensteele, a graduate of the University of California (Berkeley), applied his background in physics to the study of biophysics and rheology. That same year, Dr. Reynaldo S. Elizondo joined the department as a postdoctoral research fellow. Brought to Indiana from Tulane University by Bullard, Elizondo turned from research on the renal and cardiovascular systems to research in thermoregulation. But as a convert to stress physiology, his quantitative approach set him apart from Robinson, and to a lesser extent Bullard, as did his treatment of the thermoregulatory system as a subject of study in its own right. The last two new men to represent the era's dramatic shift from descriptive to quantitative physiology under Bullard were Dr. James E. Randall and Dr. Nelson Leatherman. Randall, an investigator who specialized in quantitative mechanistic studies of the dynamic characteristics of physiological systems, joined the department as full professor in 1968. An undergraduate in electrical engineering, Randall held a Ph.D. in Biophysics and Physiology from Ohio State University. Leatherman, a Michigan graduate in Biomedical Engineering who specialized in the Biophysics of the circulation, joined the department in 1969 but left in 1974 to join the faculty of the University of Vermont. Anatomists appointed to the department from 1964 to 1976 were Sherwin Mizell (1965), Robert Murphy (1967), David Potter (1969), Ilsa Schwartz (1970), Stanislav Gajisin (1971), Alton Floyd (1972), Dolores Schroeder (1974), and Dwight Hector (1975).

The products of a vastly different scientific era, the new physiologists and their research activities altered the image and function of the Department of Anatomy and Physiology. By the end of the decade, the physiology program at Myers Hall was no longer exclusively synonymous with Robinson's Human Physiology Laboratory. On this progressive note Bullard ended his days as chairman to serve as a Visiting Fellow of the John B. Pierce Foundation at Yale University. There he continued work on cardiac metabolism under hypoxic

stress. His colleague and co-worker in hibernation studies, the anatomist Dr. Sherwin Mizell, took over as acting chairman for one year.

On June 24, 1971, Bullard fell to his death down a glacial crevasse on Mt. McKinley. He was engaged at the time in a study of the effects of work and stresses at high altitude. His untimely death at the age of 42 left a painful vacuum in the Department of Anatomy and Physiology, and it deprived his science of a prodigious discerning disciple. Happily, the policy of expansion and diversification instituted by Bullard continued to bear fruit under two successive chairmen, Dr. Rostorfer (1971-74) and the anatomist Dr. Raymond Murray (1974-76).

The Medical Sciences Program at Indiana University

Sid Robinson's retirement in 1973 signified the end of a classical era for Indiana's physiology program at Bloomington. The department, which owed to him much of its scientific foundation, had become at this time the principal candidate for conversion once again into a supportive research-teaching center of the Indiana University School of Medicine. Then committed to the policy of massive reorganization and modernization known as the "Indiana Plan," the School of Medicine proposed to foster and finance a research and teaching program in medical sciences that would include members of the faculty of the Department of Anatomy and Physiology. The proposal was accepted and implemented. Subsequently, Bloomington's physiology program (along with anatomy) reunited in 1976 with the School of Medicine in Indianapolis as a vital section of the Medical Sciences Program at Myers Hall.

Moenkhaus, Harmon, and Robinson had not struggled in vain. Physiology within the School of Medicine at Bloomington was no longer restricted in its service to the classroom, or its representatives kept to a bare minimum. Given its role and importance as a basic medical science, additional faculty had been recruited. Paul Nicoll returned to Bloomington in 1971, and Dr. Maurizio Mirolli, a Harvard-trained neurophysiologist, joined the program in 1972. He was followed in 1974 by Dr. David Robertshaw, a graduate of Glasgow University of Scotland. Dr. Barbara Randall, a graduate of Iowa State University, became a part-time assistant professor in 1975; she was followed in 1976 by Dr. Henry D. Prange, a graduate of Duke University specializing in thermoregulation and comparative respiratory physiology.

In 1976 Dr. Ward W. Moore, who had been hired by Harmon in 1955 and was currently Associate Dean for Basic Medical Sciences of the School of Medicine, accepted the additional assignment of Director of the Medical Sciences Program. His research interest in the physiology of the neuroendocrine system added yet another dimension to the physiology program at Myers Hall. Rostorfer retired in 1975, and in 1978 Nicoll retired, leaving Moore to carry on as the last of Harmon's recruits on the Bloomington Campus.

Presently the Physiology Section of the Medical Sciences Program at Indiana University-Bloomington consists of the same faculty members joined by Moore in 1976, except for Robertshaw, who left in 1979 to become chairman at Colorado State University. Another exception is Dr. Bruce J. Martin, Sid Robinson's last graduate

student; Martin was appointed assistant professor of physiology in 1979. Indiana's physiologists represent among them a wide range of research areas and technical expertise: Reynaldo Elizondo (Head of the Physiology Section), Comparative Thermoregulation and Environmental Physiology; Bruce Martin, Exercise Physiology and Control of Respiration; Maurizio Mirolli, Cellular Neurophysiology and Mechanoreceptors; Ward W. Moore, Neuroendocrine Physiology; Henry Prange, Comparative Physiology of Respiration, Locomotion, and Thermoregulation; James E. Randall, Biomedical Engineering and Computer Applications; Alfred Strickholm, Biophysics and Membrane-Cellular Physiology; and Roderick Suthers, Sensory Physiology, Animal Sonar Systems, and Bioacoustics.

In addition to housing one of the best research laboratories for environmental and exercise physiology, Myers Hall on the Indiana University-Bloomington Campus of the School of Medicine provides today excellent research facilities for all of its investigators and graduate students. Although the emphasis is on outstanding research and scientific contribution, the program as a whole is dedicated to promoting through teaching and example the field of Physiology as a vital and rewarding profession.

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Knowledge of the scientific basis of the mechanism of pain in animals has advanced substantially in the last two decades. Therefore a symposium on this topic was held at the 1982 Annual Meeting of the Federation of the Societies for Experimental Biology. It was sponsored by the American Veterinary Medical Association Council on Research, the American Physiological Society, and the American Society for Pharmacology and Experimental Therapeutics. Increasing public concern about animal welfare has added urgency to the need to learn more about animal pain.

Now you can bring the results of this landmark symposium to your own work and concerns. **Animal Pain: Perception and Alleviation** provides the factual background on animal pain from which more detailed and specific information can be developed. Plus, you'll find a timely review of the status of pain research today... including areas in which future research is needed.

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Historical Section

Physiology at Indiana University School of Medicine-Indianapolis

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Until about 25 years ago, the disciplines of both medical physiology and anatomy of Indiana University were taught at Myers Hall on the Bloomington campus. With the completion in 1958 of the Medical Science Building on the Michigan Street campus in Indianapolis, the time had arrived to bring together all disciplines as a unified four-year program in Indianapolis. This essentially involved only the organization of new departments of physiology and anatomy, since biochemistry, pharmacology, and pathology were already well established in Indianapolis.

Dr. Ewald E. Selkurt of Western Reserve School of Medicine in Cleveland, Ohio, accepted the physiology chair in 1958 and brought together a department prepared to offer courses to 160 medical students, about 125 dental students, and 40 students in allied health (occupational therapy and physical therapy). Four full-time faculty members came up from Bloomington: Drs. Robert Bullard (Rochester, 1956), Leon K. Knoebel (Rochester, 1955), Paul Nicoll (Washington University, 1936), and Ward Moore (Illinois, 1952). Selkurt also brought in Drs. J. J. Friedman (Tulane, 1953), Paul E. Johnson (Michigan, 1955), Sidney Ochs (Chicago, 1952), and Carl F. Rothe (Ohio State, 1955). A technical staff

was organized, and several candidates for graduate training were enlisted.

The research and teaching specialties of the new department were quite varied: kidney, splanchnic circulation, circulatory shock (Selkurt); temperature regulation, exercise physiology (Bullard); gastrointestinal physiology (Knoebel); microcirculation (bat wing) (Nicoll); endocrinology (Moore); circulatory shock, peripheral circulation (Friedman); peripheral circulation, microcirculation (Johnson); neurophysiology, spreading depression, axoplasmic flow (Ochs); circulatory shock, cardiodynamics (Rothe).

Early Departmental Organization

The later months of 1958 were occupied with the hiring of technical personnel (machinist, diener, electronics man, secretary) and the selection and purchase of equipment, including that needed for teaching and for equipping the machine shop and electronics facility.

Laboratory manuals had to be compiled and a textbook for medical physiology selected. Fulton and Houssay were considered, the latter being favored. In 1960, a switch was made to Bard. The Youmans text was selected for the dental students and Langley and Cheraskin for the allied health students.

Because smoked paper recording was still in vogue in 1958, both monodrums and long-paper kymographs were purchased and smoking and shellacking equipment was installed. A battery-driven "Flexlab" system was installed to supply current to each laboratory bench for signal magnets and so forth. AEL stimulators were purchased. The principle of using "handout trays" was adopted to aid in the distribution of special items necessitated by the particular experiments to each pair or quartet of students. By early 1959, electronic recorders began to make their appearance in teaching and research laboratories. The department examined the Offner (Beckman) machine and began using some of these machines by 1962.

In January 1959 Dean Ashton of the graduate school informed the department that the graduate program which had been planned was satisfactory. This initiated planning for recruitment of students and new courses designed to cover broadly all aspects of physiology. A highlight of the spring of 1959 was the official dedication of the Medical Science Building (Figure 1).

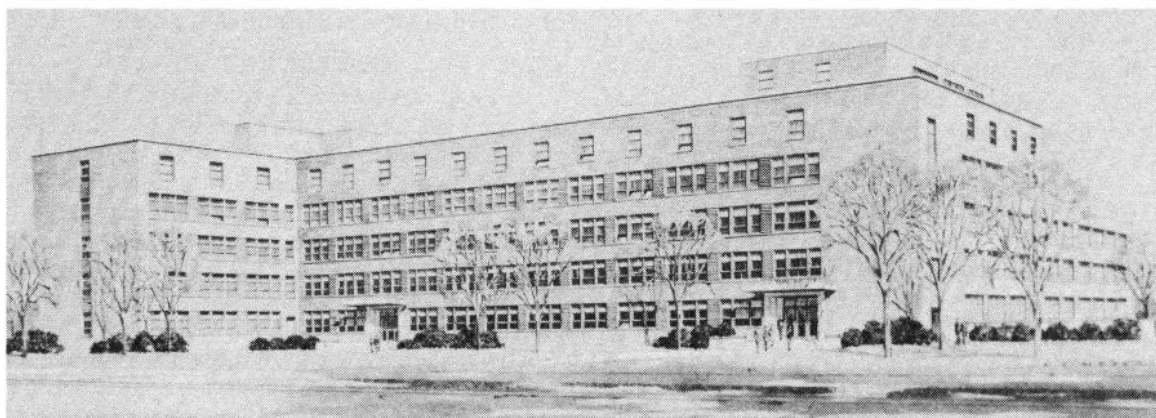


Figure 1

Medical Science Building occupied by the basic science departments of the first two years of the medical curriculum.

By September 1959, the medical course enrollment had grown from 160 to 175. Since the laboratory capacity was 160, the class was divided into a two-way rotation (with three subsections in each), utilizing two large laboratories with 32 places in each and four laboratories with 24 places each.

Development of Graduate Program

The graduate program continued to grow. In addition to biophysics, courses in circulation and cell biology were introduced in 1960. A continual screening process sifted through applicants to the graduate program, but exceptional talent was not the order of the day and some students were admitted on probation. Candidates for postdoctoral fellowship were also considered. Funds for graduate assistance during the first year were derived from grant funds in accordance with availability. After the initial year, students were expected to select an area of interest and the staff in that area would bear expense of support. Alternative consideration for a training grant to support students was strongly recommended by staff. Although the question of student availability had delayed applying for a grant, in 1960 a US Public Health Service Training Grant application was planned.

Departmental Training Grant Application. An application was submitted for a training grant to include funds for a biophysicist, secretary, and three graduate and one postgraduate student per year for four years. The amount requested for first year was \$50,000 to increase to about \$75,000 by third year. A three-man group (Drs. Fenn, Brown, and Cotton) visited the department on January 20, 1961. The outcome was favorable. The departmental training grant was approved for \$170,000 for 5 years. The grant was under the directorship of Dr. Selkurt with Dr. L. K. Knoebel as assistant director. Application for a five-year renewal beginning in 1966-67 was approved. Regrettably, 1971 saw the end of training grant support for the department.

Departmental Texts. In November 1960, a contract was sealed with Little, Brown and Company of Boston for the writing of a text entitled *Physiology*. This was a collaborative effort by the departmental members with E. E. Selkurt as editor. Arrangements were made with Mr. Fred Belliveau of Little, Brown. This text is now in its fifth edition. Another more elementary departmental text (for undergraduates), *Basic Physiology for the Health Sciences*, was published in 1975 and is now in its second edition (1982).

American Physiological Society Meeting

In February 1961, plans were formulated for a Fall Meeting of the American Physiological Society in Bloomington and Indianapolis. Dr. Sid Robinson of Bloomington suggested the possibility of arranging demonstrations and symposia at the Indianapolis Center after the close of the Fall Meeting in Bloomington. The staff was agreeable with this suggestion, so arrangements went ahead. The Indianapolis component fell on Friday, September 8, 1961, following the program in Bloomington during the earlier part of the week. The local committees were: P. C. Johnson, J. J. Friedman, C. F. Rothe, and R. W. Bullard (Demonstrations); E. E. Selkurt, S. Ochs, and W. W. Moore (Symposia); and W. W. Moore and L. K. Knoebel (Facilities).

Plans were made for publication of the symposia (Kidney, Endocrinology, and Neurophysiology). Overall attendance at the Indianapolis symposia was good, with

an excellent showing of members and guests. A tour of the city aided by the Eli Lilly firm was arranged for the spouses and families.

Pakistan Program

Dr. Paul Nicoll was director of the program in Karachi from 1955 to 1961. The program there emphasized mammalian physiology and led to an M.S. degree. Nicoll was instrumental in setting up a two-year program to be taken after the M.S. training and leading to a Ph.D. degree. In addition, Nicoll announced that a position was open in Karachi for teaching and research at a salary of \$12,500 plus 20% and the rank of associate professor if taken for four years. Dr. W. W. Moore (who was director of physiology at Rockefeller Medical School, Bangkok, Thailand, from 1968 to 1971) was a participant in the program in 1963-64 and Dr. F. R. Abel in 1964-65. The negotiations led to several Pakistanis spending two-year periods with the department and obtaining Ph.D. degrees. The first of these was Dr. Gulzar Ahmed, who already held an M.D. degree. He joined Dr. Nicoll, who in 1961 returned to the U.S. and joined the Indianapolis department. Other Pakistanis followed the same course. Dr. Nicoll urged flexibility with these students, since they had only two years to complete the Ph.D. requirements (superimposed on the M.S. degree obtained in Karachi). The recommended Ph.D. language requirements were German or French in addition to English.

Further Growth and Development of the Department

Committees were organized for M.S. and Ph.D. candidates. The Masters committee consisted of three members and the Ph.D. committee of five or six. Two members of the Ph.D. committee were invited from the split-minor department. The staff was urged to organize committees for their respective graduate students. New students who had not expressed a specific research interest were routed through the various laboratories in the department to become acquainted with the different areas of research in the department.

By 1962 there were 189 students in medical physiology. This increased enrollment necessitated the addition of several new faculty members: Dr. William McD. Armstrong (University College, Ireland, 1953), a cell physiologist; Dr. Francis Abel (M.D., Harvard, 1957; Ph.D., Wisconsin, 1960), biophysics of circulation; Dr. Frank Nash (Indiana, 1958), renal physiology; Dr. Regina Frayser (Duke, 1960), respiratory physiology (joint with Medicine); and Dr. Kalman Greenspan (Down State, Brooklyn, 1960), electrophysiology of the heart.

State-Wide Medical Education

In 1964, there were 216 students in the medical physiology course. A committee was appointed in 1964 by the president of the university to evaluate the need for increased medical education in Indiana. Their proposals were to expand the Bloomington program, to expand the Medical Center program, or to form a new medical school. A compromise was reached in the "regional centers" plan (now 9 centers at various initial locations in the state).

The first phase of the "Indiana Program" started in 1967. A special state act and appropriation provided for "the establishment of a regional hospital-affiliated internship residency program and development of an expanded continuing medical education program. . . ."

In the fall of 1968 the Indiana University School of Medicine in cooperation with Purdue University and the University of Notre Dame undertook a pilot program of enrolling a small number of students as first-year medical students and "temporary" graduate students. Initially there were three students at Purdue and two at Notre Dame. This pilot program was continued in 1969, and in 1970 it was expanded to include six students at Purdue, ten students at Notre Dame, and four students at Ball State University in Muncie. On April 1, 1971, Governor Whitcomb signed into law an act to create the "Indiana Statewide Medical Education System."

In January 1969, the Boards of Trustees of Indiana University and Purdue University approved a plan to unify the operations of two institutions in Indianapolis. The combined system is known as Indiana University-Purdue University at Indianapolis (IUPUI). The Indiana University Medical Center (School of Medicine, School of Dentistry, School of Nursing, and the University Hospitals) and affiliated hospitals are in the midst of a continually developing major campus in Indianapolis, the IUPUI.

Early in the new program, first-year medical students entered in Indianapolis, Bloomington, Purdue University at Lafayette, the University of Notre Dame in South Bend, and Ball State University at Muncie. Later the system added additional centers: Evansville, Gary Northwest, Terre Haute (Indiana State), and Fort Wayne. Twenty students enter through each satellite center, and a second-year program has been developed in each. The regional center courses are led by a "division head," e.g., of physiology. All junior students spend the third year at the Indianapolis center. Senior students are placed in approved electives on the Medical Center Campus and in community hospitals throughout the state.

Further Curricular Developments

During 1964-65 Dr. Paul Johnson took over as acting chairman while Dr. Selkurt was in Göttingen, West Germany, sabbatical leave. Not long after this, Dr. Johnson left to take the chair of physiology at Tucson, Arizona. Dr. W. W. Moore directed the medical physiology course; Dr. P. A. Nicoll continued in charge of dental physiology and K. Greenspan, allied health; and Dr. Armstrong continued as liaison man for cell biology.

Ron Wathen (Ph.D., 1965) from the Bloomington program became available for teaching and research activities in the capacity of instructor. He later left to pursue his M.D. degree at Dallas and was replaced by Dr. William Judy (West Virginia, 1971). Dr. Alan Freeman (Hahneman, 1962) also joined the department in 1971.

In 1965 the Curriculum Committee of the medical school prepared a revised schedule for the freshman year. The total number of class hours was reduced to a maximum of 30 per week, including examinations, and courses were to run for an entire semester, with anatomy and cell biology the first and physiology and biochemistry the second. The revised schedule provided the students with time following lectures that could be used to study the material just covered. It was hoped that this arrangement would be of benefit to the students.

Correlation Between Courses. In 1971, the basic science chairmen discussed more correlation between courses, possibly leading to changes in curriculum and shifting of the basic science courses to the undergraduate years. If this was to occur, it was important that the

physiology department establish close contact with the biological sciences department of IUPUI (and in particular with Dr. Pflanzner).

With the cooperation of Dr. Gibson (chairman of Biochemistry) an attempt was made for several years to more closely correlate the physiology and biochemistry courses. For example, Dr. Bryan (Biochemistry) gave lectures in acid-base and electrolyte balance to the physiology students, and Dr. Beck (Physiology) gave the lectures in endocrinology.

Liaison with Biology Department. Dr. Richard Pflanzner of the Biology Department, IUPUI, was given a joint appointment in physiology. His Ph.D. degree was earned in the Physiology Department on the Bloomington campus. He had been teaching introductory physiology to as many as 300 undergraduate students per year and was also interested in teaching a comparative physiology course on the graduate level. So that they could gain teaching experience and also learn how to use recording equipment, some of our graduate students were sent to assist Dr. Pflanzner. This arrangement eliminated the need for the Department of Physiology to teach such a course and remains to the present time.

Educational Retreat for Physiology

A retreat was held in July 1969 at the Brown County home of Dr. Paul Nicoll. New faculty members, Drs. Tom Lloyd (Pennsylvania, 1955), George Tanner (Harvard, 1964), Ron Beck (Ohio State, 1968), and Arthur Nunn (Iowa, 1960) joined the group. (Lloyd's interest was in pulmonary reflexes, Tanner's nephron micropuncture, Beck's endocrinology, and Nunn's gastrointestinal physiology.) The topics included objectives of medical physiology, methods, laboratories, audiovisuals, conferences, and evaluation (examinations and grading). The retreat proved stimulating and productive of new ideas and led to the organization of working committees on improvement of teaching (Rothe, chairman) and audiovisual aids (Nicoll, chairman). "Fallout" from the retreat continued through the autumn of 1969.

Recommendations on teaching experience for graduate students proposed by the recently organized Committee to Study Graduate Training in Physiology were approved. Students were to be informed of their teaching assignments as early as possible. Lecturing by graduate students would be part of their training and would be properly supervised.

The aim of the Committee on Improved Teaching was to undertake a program to help the staff to become better teachers by the use of new teaching techniques as well as old ones that had proved successful. During the fall of 1969 weekly sessions of an education seminar were held for the staff and graduate students. Faculty members took turns making presentations on various aspects of teaching but with emphasis on techniques.

Second APS Fall Meeting

In September 1970 the Bloomington group again hosted the Fall Meeting of the American Physiological Society. The last day was assigned to the Indianapolis group. Symposia, demonstrations, and so forth were set up in Indianapolis on September 3, 1970, in connection with the Bloomington APS meeting. A local committee included Drs. Selkurt, Friedman, Higgins, Kohlstead (Eli Lilly), Nash, and Lloyd, with Dr. Friedman organiz-

ing a symposium on transcapillary exchange, Dr. Nash on regulation of electrolytes, Dr. Armstrong on transport, and Dr. Lloyd on control of the pulmonary circulation. Eli Lilly Company gave substantial financial assistance and other help such as providing bus transportation for the tours. Overall, the second APS Fall Meeting using the combined format again proved to be a success.

Medical Education

Student Evaluation. The results of the Flextest (which replaced the Indiana State Boards) showed graduating seniors from the Department of Physiology to be performing fairly well but not as well as those from other basic science departments. The National Boards revealed the need for remedial work in physiology to be required for a number of students.

Curriculum change. In the spring of 1973, the freshman medical class was divided into four groups, each of which was to spend one and a half hours per week for 12 weeks in the following: 1) a physician's office, 2) community hospitals, and 3) participating in the "buddy system" with junior and senior medical students in the university hospitals. It was hoped that this program would provide "clinical experience" for the freshman, and it proved to be moderately successful. At the end of the academic year (May 1973), about 200 medical students, 129 dental students, and 82 students in allied health (about 50% occupational therapists and 50% physical therapists) were enrolled at Indianapolis.

Applied Physiology

By 1974 the student laboratory experience under a newly organized applied physiology section was being evaluated and reshaped under a more uniform format. Specifically, there were to be three or four laboratories on exercise with impedance cardiography, renal experiment, cardiac tamponade, or hemorrhagic shock in dog and an acid-base experiment as an adjunct to the acid-base lectures. Greater flexibility allowed dedicated students to do individual library projects or supervised research in a staff member's laboratory. Emphasis was placed on the importance of doing both human non-invasive experiments and multiorgan animal experiments.

Joining the faculty in the fall of 1975 were Dr. Nira Ben-Jonathan (Illinois, 1972), replacing Dr. Beck, and Dr. H. Glenn Bohlen (Bowman Gray, 1973), replacing Dr. Abel, who took the chair at South Carolina in 1975. The ongoing fall course, allied health, progressed well, under the leadership of Dr. Judy.

Correlated Teaching with Neurobiology

In September 1975 the first-year medical schedule had changed to the trimester system (11 weeks per term). Physiology was paired with neurobiology in the third term. An attempt was made to achieve better coordination between physiology and neurobiology but met with little success. Second year and beyond students interested in research would be supported financially from faculty research grants.

Despite the lack of a training grant, there were twenty graduate students in our program as of October, 1978: thirteen in the Ph.D. program, six in the M.S. program, and one undecided as to the program of choice. Four students received the Ph.D. degree and four received M.S. degrees.

Status of Allied Health Physiology

Dr. Gerald Zimmerman (Ohio State, 1973), who had recently joined the department, reported that all was well in the allied health physiology course. He replaced Dr. Beck, who had gone to the University of South Carolina. Cardiopulmonary resuscitation was now offered in the course, having become a part of the medical school curriculum.

Status of Dental Physiology

Dr. Nunn reported that there were 133 students in the dental physiology course in 1978. The enrollment in dental physiology had remained stable since 1958, the date of the first physiology class at the medical center, and in 1980 the number accepted was 139.

Status of Medical Physiology

The situation has been quite different for the medical physiology course, growing from about 160 in 1958 to some 200 in the 1960's and 1970's. The development of the regional centers has had a profound influence on enrollment: the regional centers increased the total entering class to 305 first-year students while the enrollment at Indianapolis was returned to the original 160. The Bloomington program in the 1960's pioneered a two-year schedule for its regional program. Since 1981-82 all centers have shared a two-year program, though on a pilot basis in the newer centers such as Ft. Wayne.

Critique of the Trimester System

A study group of Dr. W. V. Judy (Physiology), Dr. R. B. Peterson (Anatomy), Dr. R. S. Rosenthal (Microbiology), and Dr. R. A. Harris (Biochemistry) found that teaching of the first-year curriculum, now being taught in trimester mode, seemed to be meeting with more success. Under the current system courses proceed from the molecular basis to a more functional basis during the first year: biochemistry, microbiology, gross anatomy, histology, physiology, to neurobiology, with Emergency medicine presented in the third trimester. Each phase runs for 11 weeks. Many faculty members felt that this was not an improvement, but it was pointed out that three major courses at a time in a bi-semester mode as compared to two at a time for the tri-semester mode meant less competition between courses at any one time with the same amount of material covered.

Performance based on National Board Scores indicated a steady improvement with the trimester system. However, other methods of evaluation may also be needed, e.g., a yearly poll conducted by the clinical faculty to see how well the students have been prepared to clinical courses.

Historical Articles

Historical Articles Section Editor: Orr E. Reynolds, APS; Associate Editors: Horace Davenport, Department of Physiology, University of Michigan; Ralph Kellogg, Department of Physiology, University of California, San Francisco; Arthur B. Otis, Department of Physiology, University of Florida; Executive Editor: M.C. Shelesnyak, APS.

Retirement of Chairman E. E. Selkurt

In July, 1980, Dr. Selkurt formally retired as chairman but remained as Distinguished Professor of Physiology (Figure 2). He had been president of the American Physiology Society in 1976 and president of the Association for Chairman of Departments of Physiology in 1971-72. Dr. L. K. Knoebel took over as acting chairman. After an extensive search for a replacement Dr. Rodney A. Rhoades, Associate Professor in the Physiology Department, was appointed chairman on July 1, 1981. Dr. Rhoades obtained his Ph.D. at Ohio State University in 1966 and spent 11 years at Pennsylvania State University before coming to Indianapolis in 1977. In 1981 Karen Viewegh was appointed as administrative assistant to Dr. Rhoades. She has kept an eye on all departmental research grants and periodic monthly budget updates, including estimates of consistent expected costs and approximate monies remaining in the respective accounts. Figure 3 shows the members of the Department of Physiology in 1981.

Renewed Problem of Space

Following the selection of the new chairman the problem of additional space, arose. The problem was resolved when the Department of Pharmacology moved into the recently completed fourth and fifth floors of the southeast wing of the original Medical Sciences building. Thus the Department of Physiology was able to take over a part of the recently vacated third floor space in the south wing, including two departmental offices, chairman's office, two laboratory spaces, and plus some additional space in the inner wing. In the north wing, physiology took over two rooms that had been shared with pharmacology. Another room was converted into an instrumentation room, and the former departmental office became a computer terminal room. The expanded space significantly improved the efficiency of operation of the department, especially for research. Moreover, new space was provided for the possible expansion of the faculty size. As of August 1982 there were fourteen full-time faculty members as well as four adjunct appointments: Dr. Richard Pflanzner (Biology), Dr. Shirley Mueller (Neurology), Dr. Robert Hogan (Radiology),



Figure 2

Dr. Ewald E. Selkurt in his laboratory (1979) with technician, Mary Ann Neel and graduate student, Gregory M. Hockel.

and Dr. Daniel Peavy. In October 1981, Dr. S. A. Kempson (University of London, 1975) was selected to replace Dr. William Judy, who moved to Methodist Hospital.

An interesting development begun in 1981 was the promotion of a new International Sports Science Center located on the IUPUI campus, its activities to include education, research and service, aside from practical aspects of sports competition (track, tennis, swimming, cycling). Dean Beering, now president of Purdue University, has designated research money and asked various departments for faculty who might be interested in aspects of exercise physiology, ranging from very basic studies in endocrinology, renal, cardiovascular, respiration, neurophysiology, and so forth to applied problems in stress testing.

This brings the departmental history through the fiscal year 1982-1983. Future developments and changes in the department can begin at this point. Much is expected to happen in the future, particularly in the light of the changing national picture and how it will effect training and research in Physiology.

Figure 3

The Department of Physiology faculty, graduate students, technicians and secretaries, in 1981.



Row 1: Bill Selig, Pam Carmines, Mike Rubin, Kim Hankins, Dr. Nira Ben-Jonathan, Ann Hollingsworth, Dr. Mark Heiman, Dr. Ching-Tiang Hing. Row 2: Aziz Alam, Maximo Panitch, Nancy Wagner, Mary Ann Neill, Lydia Arbogast, Dr. Ewald Selkurt, Anthony Zhao, Dr. George Tanner. Row 3: Stanley Stump, Dr. Glenn Bohlen, Dr. Richard Meiss, Walley Hopkins, John McCutchan, Dr. Zafar Iqbal, Dr. David Filler, Dr. William Judy. Row 4: Dr. L. Ken Knoebel, Dr. Arthur Nunn, Scot Harper, Joe Haeberle, Dr. Carl Rothe, Joan Lafuze, Dr. Rodney Rhoades, Mike Hoefer.

Health Benefits of Animal Research

The Mouse in Biomedical Research

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It is the function of society to determine whether the gain from animal research with its attendant benefit to man and the environment is justified.

At a time when the use of animals in experimental research is being seriously debated, it is important to step back and review what has been learned and what may lay ahead in the field of biomedical research and medicine. Scientists as well as the lay public are bound by ethical and moral principles to treat living creatures with respect and protect their environment. By the manner in which we address relevant biological questions, so shall we be judged by our peers and the public. By the manner in which we manage our environment, so will be judged by future generations.

The use of animals in experimental research is by its very nature an encroachment on those precepts respecting animal life. Therefore, it is incumbent on those scientists using animals to determine carefully and critically 1) if animals are needed to answer important scientific questions and 2) if deemed necessary that such research animals are then maintained in a protective environment and subjected only to minimal discomfort consistent with the experimental design.

Throughout history mankind has suffered from terrible diseases. Over the ages plagues have decimated large segments of the population. Hundreds of millions of people died from the "Black Plagues" caused by *Yersinia pestis*, a bacterium transmitted from rodents to humans via fleas or by infectious droplets from pneumonic victims. In the early 1900's, during the influenza virus pandemic, bacterial meningitis and pneumonia associated with *Haemophilus influenzae* caused countless deaths. As late as 1950, whooping cough caused by *Bordetella pertussis* killed over 1,000 American children. The threat of bacterial disease is not a thing of the past as witnessed by the recent experience in the United States with Legionnaires Disease caused by a newly recognized bacteria, *Legionella pneumophila*.

Parasitic diseases are a major cause of human death and suffering. Schistosomiasis, a blood fluke affecting millions of people, is fortunately of limited public health importance in the United States. But, due to foreign

travel, geographic boundaries have become blurred. Malaria, Chagas' disease, trichinosis, and amebic dysentery are not names of the past in many parts of the world.

Viral diseases including polioencephalomyelitis, German measles, hepatitis, rabies, the hemorrhagic fevers, and viruses transmitted by arthropod vectors, such as yellow fever, continue to ravage human populations in underdeveloped countries. To date, only smallpox has been eradicated worldwide.

Polio, a most cruel disease of children and young adults, is now all but eliminated in the United States. But without basic research performed on mice in the 1930's, followed by a long list of scientific achievements by dedicated scientists, an effective vaccine could not have been developed.

The mouse has played a fundamental role as an experimental animal in many of the infectious diseases just discussed. Mice were and, in many cases, still are an essential part of diagnostic medical microbiology. They are important in viral isolation studies of diseases caused by arthropod-borne vectors and in rabies virus diagnosis. Mice are still a significant investigative model for understanding basic host mechanisms of disease, and for studying susceptibility and resistance to infectious agents (15). A basic understanding of host-parasitic relationships is critical if we are to achieve better diagnostic, preventive medical approaches, and therapeutic applications to these problems.

In addition, there is a long list of diseases of humans that are usually thought to be noninfectious but cause immense suffering and in many cases contribute to mortality. Arthritis, diabetes mellitus, central nervous system disorders, immune deficiency diseases, metabolic disorders, hematologic dyscrasias, and cancer are diseases that are all too familiar. The use of mice has provided us with a valuable method to elucidate fundamental biological phenomena associated with these diseases. Through creative basic and applied research studies, we have developed effective therapeutic measures for infectious and noninfectious diseases.

Abnormalities of development are frequently associated with genetic dysfunction. Down's syndrome is a tragic example of a chromosome disorder resulting in a child with a "mongoloid" appearance, mental retardation, cardiac abnormalities, and an increased likelihood of developing leukemia. Through plant and animal investigations we have gained an understanding of genetic disease that has enabled use to diagnose and, in some cases, treat genetic disorders. Genetic counseling of prospective parents is now commonplace. The use of mice in studying basic genetics at the classical and molecular levels has resulted in development of organ transplantation as well as effective methods to modify the course of immunologic disease. With advances in molecular biology it is now possible to think in terms of modifying genetic errors of metabolism by gene manipulation.

Alternate methods of animal research are not new to science and are not a result of public pressure per se. Experiments conducted outside the living animal body (in vitro) as contrasted with in vivo, or within the living body, are essential for understanding biology. Scientists

have carefully attempted to simplify experimental systems to minimize extraneous interactive events. Biochemists, physicists, physiologists, microbiologists, and physicians frequently perform *in vitro* experiments. Major advances in cell biology are now possible by use of tissue culture systems developed over 30 years ago. Virology is dependent on this system for isolation, propagation, and study of most viruses. The understanding of how individual cells and their subcellular components function, and the biology of cell membranes and how cells react to certain chemicals can be studied effectively in these *in vitro* systems.

However, at times, it is important to understand how the body functions as a whole; how abnormal proteins affect the entire body producing a disease or syndrome; how an entire organ functions, not just one of its cell types; how the body accepts or rejects foreign tissue or an organ from another animal; how the body fights infection; and how the body deals with cancer.

The question is not whether animals are necessary to understand biology or whether alternate methods of research are more appropriate, but rather how can we best further our knowledge to reduce human suffering and improve the human condition. Judicious and careful use of experimental animals still remains an important research method. As science becomes more advanced, *in vitro* procedures tend to replace *in vivo* procedures. Rabbits are no longer used to confirm human pregnancy, since a chemical procedure is now available. Guinea pigs are no longer used to isolate the tubercle bacillus, since culture methods are now available. In a majority of instances tissue culture procedures have replaced the widespread use of mice to isolate viruses in diagnostic virology laboratories. However, in arbovirus research, mice still appear to be more sensitive than tissue culture methods.

Even the type of mouse used in research changes through the years. Random-mated or outbred mice are used in large quantities, but special inbred mice with unique genetic backgrounds have become essential animal models of human biology. Cells from these unique animals used in *in vitro* experiments further attest to the importance of a combined and thoughtful approach to both *in vitro* and *in vivo* biomedical research.

The use of mice in biomedical research has resulted in tremendous advances in biology and medicine. As in the past, mice continue to play a major role in answering questions relating to infectious and noninfectious diseases. Through the pioneering work of Strong, Little, Snell, Green, and others using inbred mice, the fundamentals of animal genetics began to unfold. Classical or Mendelian genetics paved the way for genetics research at the molecular level.

The mouse is *the* research animal used in immunobiology. Our understanding of tissue histocompatibility and subsequent advances in organ transplantation was in large measure the result of early work done by C. C. Little and the Nobel Laureate Dr. George Snell at the Jackson Laboratory, Bar Harbor, Maine. Careful search for phenodeviants, or mutants in inbred mice, has resulted in a wide assortment of genetic animal models of human disease. These mice have diseases affecting almost all organ systems and tissues mimicking a wide range of human disease from diabetes to cancer. These include neurologic mutants causing hydrocephalus, cerebellar dysfunction, and myelin abnormalities; neuronal cell

disorganization, retinal dystrophies, corneal and lens abnormalities; hematologic dyscrasias including various anemias and clotting disorders; muscular dystrophies; reproductive abnormalities; immunodeficient diseases; endocrine and metabolic dysfunctions; and an extremely wide range of malignant and benign neoplastic tumors. *Genetic Variants and Strains of the Laboratory Mouse*, edited by Margaret C. Green, 1981 (83), lists hundreds of strains and mutants attesting to the rich biological material available through research on mice.

The gain from animal research in the past has indeed benefited mankind and our environment. The future use of mice in research should result in similar advances.

History

Mus musculus is the formal scientific name of the house mouse; a lowly animal despised by the ancients for eating their grain, feared by elephants in the literature of childhood, and loved by all ages through the talents of Walt Disney.

The history of mice in science, however, is marked by great achievements in our understanding of the laws of nature, biology, and medicine. Joseph Priestly (1733-1804) used the mouse in experiments which indicated that growing plants could turn fatal gases (CO₂) into life supporting air (O₂). Mice were prized by pet fanciers for their unique colors and following Mendel's observations became a natural animal to study heredity.

William E. Castle (1867-1962), an early pioneer in mammalian genetics, studied the effects of heredity and variability on evolution (31). The term "genetics" was coined by Bateson in 1908.

Recognition that breeding of siblings would produce an "inbred" mouse with unique characteristics was pursued by Leonell Strong at Cold Spring Harbor in 1919, along with C. C. Little, who is credited with developing the first inbred mouse strain designated DBA. A long list of strains, still very important in current research, can be traced to these innovative investigators: A, CBA, C3H, C57BL/6, C57BL/10. E. C. MacDowell at the same period developed the strains C58 and BALB/c (130).

Dr. Clara Lynch, about the same period, brought into the United States from Lausanne, 10 pairs of white mice. At the Rockefeller Institute she propagated this small group of mice, which later became the progenitors for all our major current inbred and outbred white or "Swiss" mice used in biomedical research. By the 1920's, with the research of L. C. Dunn and Dobrovolskaia-Zavadskia, and with additional New Zealand strains developed at Mill Hill, England, the majority of progenitor strains were established (130).

In the early 1920's, genetic anomalies such as rodless retina (110), a middle ear abnormality, shaker (123), and hypopituitary dwarfism (183) were beginning to be recognized. C. C. Little began inbreeding mice in 1909 with coat color genes dilute (*d*), brown (*b*), and nonagouti (*a*), which finally evolved into DBA strains. By 1916 C. C. Little recognized that cancer in mice was to a marked extent influenced by hereditary factors.

In 1929 C. C. Little established the Roscoe B. Jackson Memorial Laboratory (now called the Jackson Laboratory) in Bar Harbor, Maine, as a center for mammalian genetics. This institution, through the intervening years, has developed into a world-class laboratory currently housing over 1,000,000 mice daily, consisting of over 700 inbred strains, congenics, mutants, and hybrids. Through

research using inbred mice at the Jackson Laboratory and throughout the world, fundamental biological questions were asked. It became apparent that developmental biology of mice and humans were extremely similar. A workable experimental animal system, the inbred mouse, was defined to study heredity and its influence on disease processes ranging from infectious diseases to cancer within strains. The study of mutations with their single gene effect has made the mouse the premier mammalian animal model for understanding this aspect of genetics and developmental biology.

The sorting out and understanding of what genes do, where they are located (loci), on which chromosome, and in what relationship to other loci (linkage) slowly evolved. In 1935, 11 loci were defined principally on the basis of coat and eye color. With increasing identification of linkage groups, techniques to identify individual mouse chromosomes, and with knowledge of translocations that involved linkage groups, the assignment of individual genes to a chromosome became reality. By 1979 over 400 loci were identified (157). The mouse gene linkage map is slowly emerging, and it is now estimated that over 30,000 structural genes are present in the mouse genome.

Starting in the 1930's and continuing through his illustrious career, Dr. George D. Snell studied the transfer of tissue resistance genes to another inbred strain. He developed over 160 *congenic resistant* strains, i.e., mice that are genetically identical except for a small piece of chromosome carrying genes determining graft acceptance or rejection. These congenic mouse strains were used to study genetic factors that influence whether normal or malignant tissue would be accepted or rejected by a recipient host. This led to the concept of a major histocompatibility complex now found to be present in animals and humans. Understanding of this fundamental genetic mechanism has led to advances in basic immunology and in organ and tissue transplantation. This achievement resulted in Dr. George Snell being awarded the Nobel Prize in Medicine, 1980. More congenic strains were also developed by other scientists for studying loci controlling expression of many other genetic traits. These mice have enabled scientists to study in detail gene products such as enzymes and alloantigens.

The ability to localize polymorphic genes to individual chromosomes was established through the observation that different strains of mice had variants of certain enzymes. Dr. Donald Bailey (14) and later Dr. B. A. Taylor (196) developed recombinant inbred (RI) strains of mice by brother-sister matings of F2's produced from two different inbred strains. By establishing these RI strains and examining these enzyme variants, knowledge of the location and order of additional loci on mouse chromosomes rapidly advanced.

Today over 1,000 loci have been identified. Over 370 mutant genes are listed in catalogues of mouse stocks. Approximately 1,000 different strains, congenics, hybrids, and mutants plus many outbred stocks are now available. Over 20 million mice are used annually in biomedical research in the United States, with over 50 million used worldwide. The mouse has been carefully developed through the 19th and 20th centuries to be one of man's best friends. Mickey and Minnie Mouse of Walt Disney fame entertained us, but their real life cousins have provided us with knowledge that will lessen human suffering and truly improve the human condition.

Of Mice and Men is hopefully a prologue to a better world.

The following sections are intended to provide an overview of research areas that have benefited from the use of mice.

Bacteriology

The history of microbiology is intimately interwoven with experimental studies utilizing mice. Studies in 1853 by Theirsch showed the infectiveness of cholera discharges. Transmission of anthrax was demonstrated by Davaine (1863, 1864) (48). Koch (114) proved that a bacterium caused a recognizable disease, anthrax, and Koch's postulates were outlined. In 1880 Koch also observed that different types of mice showed dissimilar responses to infection, i.e., resistance vs. susceptibility. In 1884 Nicolaier (134) produced tetanus in mice and recognized that a toxin might be associated with a bacterium. Pasteur used mice in studying both rabies and anthrax. Mice were at the forefront of experiments dealing with the nature of infectious disease and immunization.

As inbred strains of mice became available, strain differences and their responses to infectious agents were detected and the mechanism of these phenomena were studied. These observations also helped to identify immunodeficiency diseases and their role in host-mediated defenses against infection. The athymic mouse with its depletion of T-cell lymphocytes represents an important immunodeficient model recognized initially in athymic mice housed in conventional animal facilities. Nonpathogenic bacteria were able to cause death in these immunodeficient mice. Relatively normal survival, however, could be achieved if these athymic mice were maintained in microbiologically clean environments.

The mouse is an excellent research animal to study the pathogenesis of bacterial diseases. A model for human antimicrobial-associated diarrhea (AAD) was developed using *Clostridium difficile* as a causative organism (138). Transmissible murine colonic hyperplasia in mice (TMCH) (18, 19) caused by *C. freundii*, strain 4280, may prove of some importance in understanding neonatal enterocolitis. It has been shown that the LPS^d allele (211) controls the host response to bacterial lipopolysaccharides, important in the host defense mechanism against salmonella. The cellular and humoral responses to bacterial infection have been studied extensively in mice, as well as effectiveness of therapeutic regimes.

Virology

Viruses are infectious particles that require living cells in order to replicate. Viruses were only visualized once technological advances were made in electron microscopy. But well before viruses could be seen and cultivated in cell tissue culture systems (in vitro), mice were used to isolate and study this extremely important group of agents infective to both humans and animals.

Since the literature in this field is so large, no attempt will be made to review this subject in detail. Large numbers of outbred mice of different stocks began to be used in virological research in the late 1920's through the 1940's. Andervont (5, 6) pioneered the use of mice in virology working with herpes simplex virus (HSV) along with Theiler (197), who worked with yellow fever virus (YFV). The list rapidly grew and included rabies virus,

the encephalitic togaviruses, and influenza virus. During the 1940's extending into the 1950's the Rockefeller Foundation arbovirus group made outstanding advances in our understanding of viral diseases (198). Through inoculation of infectious material into brains of infant mice, they were able to isolate a wide range of viral antigens, antigenically type them, and develop sensitive diagnostic procedures to detect infection in humans. The yellow fever vaccine, which saved countless lives, grew out of this work by Theiler and others.

Although in vitro tissue culture technology in virology began with Enders et al. (63), the importance of mice in virology did not cease. Mice, as animal models of viral disease, made important contributions to our understanding of diseases including Herpesviruses (65), viral exanthemas (ectromelia) (67), viral infections leading to immune complex disease (lymphocytic choriomeningitis virus) (7, 28), viral interference phenomenon, and the role of genetic backgrounds in host-parasite interactions (15, 22, 43, 45, 80, 81, 89, 90, 105).

Research on mice clearly indicated that host as well as agent played a major role in viral infections. The genotype, age (85, 121), and sex as well as dose, route, and virulence of the agent could markedly influence the final outcome of infection (71, 182).

Progress in viral cellular and basic molecular immunology involved a change from outbred or random-bred mice to the more sophisticated inbred strains. The use of congenic mice (genotype identical to control strains at all except one locus or small chromosome segment) and the recombinant inbred mice developed by Bailey (14) provided the tools to explore the effects of immunosuppression and immunoreconstitution, immune-mediated injury and recovery, humoral immunity, and cell-mediated immunity. The role of the histocompatibility complex in cell-mediated host response was further studied.

Mice have been used extensively in the study of viral immunity and have played, either directly or indirectly, a part in the development of vaccines (127, 167) against diseases such as influenza (20), poliomyelitis, yellow fever (188, 197), eastern/western/Venezuelan encephalitis (2, 27), and rabies (10-12, 100, 109).

The use of mice in current virology research is extensive and uses both outbred mice and special inbreds, mutants, congenics, hybrids, and recombinant inbred strains. These studies include molecular virology, immunology, pathology, and therapeutic trials (32, 36, 120, 142).

Genetics

Genetic diseases in experimental mammals provide disease conditions that are reproducible, definable, and available on inbred strains with relatively homogeneous genetic backgrounds. Animal models of genetic disease may provide genetic markers for presymptomatic analysis of diseases. They provide genes that are valuable not only as models of human genetic disease but also for our understanding of normal biological processes. They permit comparative gene mapping of animal models and human with genetic "tags."

The understanding of genetically related human diseases is complicated because of genetic heterogeneity in each individual and between individuals in a large population. Genes contribute to the way humans or animals respond to disease or to metabolic malfunctions. Understanding of the underlying causes of a disease,

which may involve genetic regulation, and of basic biological processes is critical to effective treatment. Mutant genes that cause in experimental mammals diseases which have similarities to human diseases can be propagated and maintained for study. This means that human-like disease conditions may be made available in animals for different types of analysis, e.g., such as biochemical and morphological. Studies can be repeated and confirmed. By maintaining these mutant genes in inbred mice it is also possible to control the heterogeneity of the genetic background. The mice within an inbred strain are genetically more than 99% identical to one another. This means that the affected and control mice probably differ only by the mutant gene being analyzed and that differences between them can be attributed to that gene.

Developmental analysis of an animal model of a human disease makes it possible to determine what happens metabolically before the actual disease symptoms appear. Not only does this understanding of a disease's progress help design therapy but also allows early detection and treatment. Because so many genes have been mapped in the mouse (145) it is usually possible to identify the presence of a disease-causing gene early by "tagging" it with a closely linked marker gene. One example of this is the diabetes gene (40), which is closely linked to a coat color gene called misty (*m*) (221, 222). By making appropriate matings it was possible to link the *db* gene with the *m* gene. Thus it is possible to follow the development of diabetes from shortly after birth simply by analyzing those mice with the misty coat color. Analysis of the *db* gene in mice has led to a better understanding of the complex nature of hormonal abnormalities on cellular metabolism.

Mutants that are kept and studied because they make good genetic markers upon analysis often turn out to be models of human diseases. An example is the beige (*bg*) gene (26, 137). This locus was identified originally because the beige mutant causes a light coat color in mice. It has since been shown that the beige mouse is a good animal model for the Chédiak-Higashi syndrome in human beings (209). This disease is characterized by abnormal giant lysosomes in all granule-containing cells. The effect is marked by increased susceptibility to infections and cell dysfunction or damage in affected cells. It also affects granules in hair, causing the beige color. This model has increased our understanding of gene produced enzymes by contrasting normal mice with those with specific enzyme deficiencies. A second example, the staggerer (*sg*) mutation, was discovered in 1955 as a recessive inherited trait that caused behavioral abnormalities, suggesting defects in the cerebellum (176). Morphological studies showed that a deficiency of granule cells in this mutant leads to abnormalities in Purkinje cells. Because of its potential in the study of normal processes of the mammalian brain, staggerer has been maintained since then in the Jackson Laboratory's Genetic Resource. In 1982 it was discovered that staggerer is invaluable in the analysis of a critical molecule in brain development, the neural adhesion molecule (59, 60).

Comparative gene mapping between human beings and experimental mammals has made it possible to identify with more certainty animal models of human genetic diseases (144, 145, 157). Although serious comparative mapping began only around 1977, it is a rapidly growing field and is already showing us that many seg-

ments of the genomes of other organisms are conserved in human beings. Disease-causing genes in mice and human beings, which have very similar or identical basic defects, may produce similar but not completely identical symptoms. Linkage of the gene in each species to a second gene which can be shown unequivocally to be homologous between the species is strong evidence that the disease genes are homologous also.

Many genetic diseases in human beings are not directly detectable with prenatal testing. However, it is often possible to identify the presence of such a gene in a fetus by looking for a closely linked detectable marker. In the past these were limited to biochemical markers, but with the new DNA technology it is possible to screen for polymorphic differences in the DNA itself. If a gene has a mouse homologue, knowledge of the genetic markers close to the mouse gene often speed the search for similar markers in human beings.

We also know through comparative mapping that the X chromosome is highly conserved in all mammals (136). Therefore genes on the X chromosome can be especially good models for human X-linked diseases. One example is hypophosphatemia in the mouse and vitamin D-resistant rickets in human beings (62). Another is the androgen receptor locus that causes a disease called testicular feminization in both species (124, 128, 218).

X-Linked Genetic Homologies Between Mice and Humans

The X chromosome is conserved in all mammalian species (136) and is involved in sex determination. Females have two chromosomes (XX); males have an X chromosome and a Y chromosome (XY). The presence of a single X chromosome in males permits the expression of a "recessive" trait, which might not be expressed in a heterozygous condition in females with XX chromosomes.

Of particular interest is the placement of similar genes on the X chromosome of many mammalian species. This linkage conservation is extremely important in studying X-linked known human diseases that may also occur in animals, specifically the mouse (33, 35, 72). This conservation also permits observation and analysis of evolutionary changes within a single chromosome. Homologous enzyme loci, X-linked in both mice and humans, are α -galactosidase A (116), glucose-6-phosphate dehydrogenase (141), hypoxanthine phosphoribosyl transferase (34), ornithine transcarbamylase (50), phosphoglycerate kinase (117), phosphorylase kinase (102), and steroid sulfatase (76). Mouse mutations, which are X linked, also represent similar diseases that occur in humans. An example is testicular feminization (125), which in both humans and mice causes an external female appearance, small undescended testes, and a reduced sensitivity to androgens. Both conditions appear to have a defect in androgen receptors on target cells (128, 218).

A problem associated with a copper binding defect is associated with X-linked mottled (*Mo*) locus in the mouse (64, 103). An X-linked condition in humans, Menke's kinky hair syndrome (a progressive brain disease) (150), may also have a defect in copper metabolism.

There are an X-linked immunodeficiency (*xid*) (164), an X-linked histocompatibility locus (*H-X*) (13), and a sex-linked anemia (SLA) (66) in the mouse, and there is

an X-linked blood group (*Xg*) in humans. There are also X-linked neurological and skeletal mutations in both mouse and man (83).

Molecular Biology

Molecular biology provides a body of knowledge and a practical method by which we can acquire an understanding of the genetic basis of the developmental and physiological regulation of an organism. This understanding will include knowledge of the nucleotide sequence of DNA in the chromosomes and of the complex structures that DNA forms as it interacts with the proteins of the cell during phases of cell division and differentiation. Molecular biology will also tell us how transcription of DNA is controlled to produce the various specialized molecules a cell must acquire if it is to serve, for example, as a muscle cell or a nerve cell. The mechanisms controlling transcription of a gene will involve the identification of specialized cellular signals that interact with the DNA to determine when during development and in which cells genes are to be expressed.

It is estimated that a complex organism like a human being or a mouse requires the expression of ~50,000 genes. The expression of each gene must be coordinated in time and in space with all other genes. There is much experimental evidence to indicate that uncoordination, or loss of expression, of even a single gene can cause severe disease. It therefore follows that any rigorous attack on diseases of humans, whatever organ they affect, must include a molecular biological approach if an understanding of the cause of the disease is to be achieved.

Among experimental organisms amenable to analysis by molecular biologists, the mouse stands at a pivotal position. With a few exceptions, virtually all basic information on genetic mechanisms obtained from the study of simpler organisms such as yeast or *Drosophila* has been tested for relevance to the biology of the human being by first being analyzed in the mouse. This is because mice and humans share many similar genetic and physiological traits and because in mice many of the genetically determined traits have been established experimentally in highly refined breeds. For example, breeding experiments first performed with corn and then later at the molecular level with *Drosophila* indicated that mutations could be caused by pieces of DNA which possessed properties enabling them to move from place to place in the genome. These segments of DNA, called transposons, can sometimes be translocated into the DNA of another gene, resulting in abnormal gene expression. Evidence now exists to suggest that diseases in humans, including cancer, are associated with similar movements of DNA segments (113). One of these human cancers, Burkitt's lymphoma, appears to be caused by movement or translocation of a segment of chromosome 8 to chromosome 14, resulting in the abnormal expression of immunoglobulin genes and a transformed cell. The movement of these genomic fragments may be associated with the presence of a gene, such as *c-myc*, which is analogous to an avian myelocytomatosis virus oncogene (156). Cellular oncogenes may move in a manner analogous to the transposons present in corn and *Drosophila*. The special role of mice in this story is that a selected inbred strain of mice can be induced to acquire a lymphoma which is a close copy of the Burkitt's lymphoma.

Hence much of the molecular knowledge we now have of the human cancer is available because of the existence of a similar disease in the mouse (23, 112).

The structure of transposons in lower organisms and movable viral sequences in mammalian organisms is being exploited in an additional more directed program. It now appears possible that viruses and other movable DNA sequences will be able to be introduced into embryonic mice with the subsequent integration of the DNA into the host DNA causing, on occasion, abnormal gene expression. These experiments will allow the molecular biologist to discover the regions of DNA that are important for regulation of the gene and in addition provide an animal in which the consequence of a particular DNA insertion on the development and physiology of the animal can be studied. These experiments require 1) a mammalian embryo that can be experimentally manipulated, 2) an animal for which a detailed knowledge of the genetic map is available, and 3) the ability to identify the genetic origin of the introduced gene. That these conditions are favorably met by the mouse is illustrated by the recent production of an embryonic lethal mutation in mice caused by the introduction of a retrovirus into the collagen gene (166). This mutation results in a block in transcription of the collagen gene and an arrest in the development of the embryo. It is likely that this experimental approach will cause mutations which are counterparts of many of the diseases of humans.

Immunology

Immunoglobulin structure has been studied extensively in the mouse, partially due to the availability of various neoplastic plasma cell tumors derived from mice (147). These immunoglobulins can be produced in large quantities providing scientists with homogenous immunoglobulins. The hybridoma technology based on the work of Kohler and Milstein (115) has provided an extensive range of homogeneous mouse antibodies. These scientists fused, in culture, malignant myeloma cells with spleen cells from a mouse previously immunized against sheep red blood cells. A cloned fused myeloma cell then produced a population of cells containing a set of genes specific for the production of sheep red blood cell antibody. This experiment set the stage for the large-scale production of monoclonal antibodies (148).

This hybridoma technique is used to produce very specific antibodies against various components of cells, tissues, or related antigens. The applications of monoclonal technology are extremely diverse, ranging from typing of cell surface antigenic determinants to therapeutic applications in the treatment of human cancer (129, 155, 179).

Understanding the basic molecular structure of immunoglobulins and their functional components is critical to further advances in immunology. An immunoglobulin is comprised of four-chain units each folding into three-dimensional subregions called domains. The C domain comprises the majority of the functional immunoglobulin molecule (65-75%). The multidomained (Ig) molecule can be further fragmented by proteolytic enzymes into two Fab and one F_C fragment. Using BALB/c mice, Rudikoff et al. (158) crystallized a pepsin Fab fragment. This along with other studies allowed the molecular comparison of the V region domains of mouse and human V_K and V_H domains to determine

the degree of similarity or conservation of this molecular arrangement over millions of years of evolution (140). The results indicated an almost perfect alignment of the framework regions of these domains, indicating a remarkable similarity of mouse and human immunoglobulin at these regions (149). This molecular evidence along with functional physiological studies of mice and humans clearly indicate that basic biological systems have in many cases been conserved through evolution. These facts provide the basis for comparative biology and animal experimentation.

Genes are the controlling elements in cells that guide the production of enzymes, proteins, immunoglobulins, coat color, and physical appearance. In eukaryotic cells, until very recently the concept was that there existed a one-to-one relationship between one gene and one polypeptide chain. With the advent of DNA cloning and sequencing, it now appears that the structural gene is composed of discontinuous DNA portions composed of regions which in part code for amino acid sequences (exons) and intervening regions which do not code (introns) along with flanking sequences. The transcription copies of mRNA thus contain exons and introns. Subsequently, the mRNA is cleaved by enzymes eliminating the noncoding regions or introns leaving only a chain of mRNA coding elements, exons (106).

Immunoglobulin genes appear to be different in that the gene element is arranged in different sequences in undifferentiated cells vs. those cells that have been antigenically stimulated and differentiated. This process of rearrangement of DNA in immunoglobulin-producing cells appears to be an important mechanism in providing the unique diversity of immunoglobulin essential in host-defense interaction (53, 101). Following DNA sequence rearrangement, normal transcription then takes place.

Inbred strains of mice have been and are critical to understanding gene structure and function. Different inbred strains of mice have been demonstrated to differ in their ability to respond to specific antigens such as dextran, suggesting that an idotype of a specific responder mouse strain is linked to an allotype (24). The idiotypes or antibody molecules with specific determinants can be studied by using congenic mice, which vary from control strains at only one locus and with RI strains developed by Bailey (14). Both these animal models provide the tools for studying gene polymorphism (215). Our understanding of mRNA transcription, production of a polypeptide chain, folding of these chains, glycosylation, assembly of the immunoglobulin molecule, secretion from the cell, immunoglobulin transport, and action at a target site has been possible only through the availability of mouse plasmacytomas, hybridoma technology, inbred strains of mice, special congenics, and recombinant inbred mice. The role of the mouse in basic molecular immunology will continue to be paramount in furthering our knowledge in this area of biomedical research.

Immune Response Disorders

The development of inbred strains of mice and their maintenance for long periods provided the opportunity to detect naturally occurring mutations at the Jackson Laboratory, Bar Harbor, Maine. An extensive search for clinically recognizable mutations among nearly one million mice housed daily has been extremely productive.

Among hundreds of mutations examined and characterized over the years, there were observed a few remarkable mutations associated with abnormal immune responses. These included the MRL/MpJ-*lpr* strain (132), the "motheaten" mouse (49, 52, 171, 172), and "wasted," a mutant with both immune response disorders and brain pathology similar to a syndrome ataxia telangiectasia in humans (173). The MRL strain has an autosomal recessive mutant gene, *lpr* (lymphoproliferation), which is associated with mouse T-cell lymphoproliferation and early onset autoimmune disease. A second model disease from an RI strain BXSB develops B-cell lymphoproliferation, an autoimmune disease that is accelerated in males (133).

"Motheaten" mice have impaired humoral and cellular immunity. They produce autoantibodies to thymic cells and DNA, develop splenomegaly, lymphadenopathy, thymic involution, pneumonitis, and immune complex disease affecting skin and kidneys. The patchy hair loss is the basis for the name "motheaten" (171).

New Zealand Black (NZB) and the hybrid New Zealand Black/New Zealand White, F1 (B/W), were the first animal models of autoimmune disease (29, 98). NZB mice develop Coombs positive hemolytic anemia with its attendant complications. Autoimmune complexes are found in glomerular deposits. The role of retrovirus is unclear in this disorder (46, 47). The B/W New Zealand mice have a disease similar to systemic lupus erythematosus (SLE) of humans. Investigative work has indicated that these affected mice may have premature maturation of their immune system (78) and that feedback regulatory systems may not operate properly (30).

Other strains with varying types of immune response disorders include the Palmerston North mouse (213), the Snell-Bagg dwarf mouse, which suggests a role for pituitary hormone in immune regulation (56), and the Ames dwarf mouse, which has a severe T-cell mediated immune deficiency (55).

For an informative review of this subject see Talal (195).

Hematology

The red blood cells that carry O₂ to the tissues and the white blood cells that destroy invasive particles are derived from a common precursor, the stem cell. Stem cells have no identifiable distinguishing features. They are primitive cells with no known specialized membrane characteristics, size, or protein components. They comprise only a small proportion, about one cell in every 100,000 cells, of the mammalian bone marrow.

In diseases of the blood-forming elements such as aplastic anemia or leukemia, an understanding of the stem cell, or progenitor cell, is basic to developing successful treatments. A method had to be found to separate stem cells of the mouse from other cells in bone marrow and spleen so in vitro and in vivo experiments could be performed on identifiable stem cell populations.

A quantitative method to assess numbers of stem cells was developed (201) whereby cells from normal mouse spleen or bone marrow were injected intravenously into lethally irradiated histocompatible host mice. Following lethal irradiation these injected normal transplanted stem cells multiply and replace the host's destroyed stem cells, preventing death. Within the recipient's spleen, visible "colonies" of cell populations derived from clones of these transplanted stem cells can then be detected.

Results of this assay are expressed as a number of spleen colony-forming units or CFU-S.

The development of this technique for assessing numbers of stem cells was based on two critical factors: 1) the growing knowledge of transplantation genetics and 2) the availability of inbred strains of mice.

Even before this assay was perfected, Dr. Elizabeth Russell, of the Jackson Laboratory, performed the first successful "cure" of a blood disorder by bone marrow transplantation in *W*-anemic mice (162). This pioneering experiment preceded and opened up the entire field of bone marrow transplantation in human beings. Additional advances in bone marrow transplantation in mice were made by the early work of Bernstein (21).

Further assays were necessary to study stem cells. It was found that after experimental treatment with bone marrow transplantation, if the host mouse is allowed to survive, it acquires the characteristic blood phenotype of the donor. The time between marrow transplantation and change in the host blood phenotype is a measure of the comparative number and hardiness of the donor stem cells. These assays have been used to monitor stem cell (51) numbers during the development of in vitro culture methods. Stem cells from the mouse can be grown in culture flasks for up to 20 weeks, and the following questions have been investigated: What other cell types are necessary to support stem cells? Which drugs destroy cancerous stem cells? Are there drugs that foster normal stem cell replication? Do mice with inherited blood disorders have normal stem cells? The same culture conditions have been adapted to human blood cells (77), and the same types of questions are now being asked. The assay for human stem cells was developed first for the mouse (57, 58) and involves the explanting of stem cells from one culture environment to another. The assay environment supports differentiation of the stem cell progeny into red and white blood cells. If no differentiated cells appear, we conclude that the stem cells are either defective or lost. The elusive stem cell can now be studied in vitro in both the mouse and the human being. Problems still exist, however, because answers acquired in vitro may not be the same as those acquired in the animal. For this reason, questions posed for cells in vitro must also be asked in vivo. The inbred mouse is essential for these studies.

In addition to the role of the inbred mouse in the development of blood assay systems, the mouse with inherited anemias is equally as important to researchers. There are at least 17 different mutations in the mouse that cause anemias (159). The defects include a lack of stem cells, malabsorption of iron, deficiencies of red blood cell cytoskeletal proteins, deletion of globin genes, loss of iron transfer capabilities, a faulty environment that halts red blood cell differentiation, and nondisjunction during mitosis. Some of the inherited anemias in the mouse, while exhibiting rather different symptoms from a hereditary anemia in the human, do share some characteristics. For example, both Hertwig's anemia in the mouse (97) and Fanconi's anemia in the human have chromosome abnormalities albeit different (194). The basic biochemical lesion causing the defect is probably also different in the two diseases but the end result is similar—a loss of defective cells leading to anemia. α -Thalassemias, inherited conditions caused by deficiencies in α -globulin production in the mouse (160, 161), are very similar in the mouse and human. There is an indica-

tion that both of these conditions may be caused by a deletion of the adult α -globin genes (216). Mice with deletions such as α - and a recently discovered β -thalassemia in the mouse (181) are good subjects for experimental transfer of normal genes to try to cure these conditions. Gene transfer techniques that may ultimately be used to cure humans are being developed in the mouse. A new type of spherocytic anemia in the human (3) appears to mimic an inherited spherocytic anemia in the mouse (108). In both, there is red blood cell destruction caused by lack of a cytoskeletal protein. Scientists are learning a great deal about the assembly and function of the cytoskeletal proteins. Knowledge about cytoskeletal assembly garnered in the mouse may help us understand the various hereditary hemolytic anemias in the human. Research on the basis of human and mouse inherited diseases proceed hand in hand. Wherever possible, advances in one field are quickly applied to the other.

Oncology

Virtually every organ and tissue of mice, as in humans, is subjected to neoplastic processes. Attention of scientists to this incredible array of mouse tumors, both benign and malignant, was slow in recognition but rapid when its potential was realized. There are tumors of the young, of the old, of males, of females, of the hematopoietic system, and of the liver, lung, bone, and endocrine organs.

The mouse, without question, has contributed immensely to our knowledge of oncology, and the factors that contribute to initiation, promotion, autonomy, and at times remission of cancer.

The early pioneering work of Little, Snell, Strong, and many others carefully documented the role the genotype played in modulating the frequency and type of neoplasia in the mouse. The H-2 complex, which specifies cell surface antigens and in turn histocompatibility, was also found to affect tumor cell surface antigens. Tumor immunology found its basis in exploiting these animal model systems.

Our knowledge of viral oncology is, in large part, based on information gained in the mouse, but mention must be made of a long list of agents and systems in chickens, hamsters, cats, cattle, and nonhuman primates.

Mouse leukemia virus (MuLV) is associated with lymphomas/leukemias. Moloney murine sarcoma virus (M-MuSV) is associated with mesodermal tumors, and mouse mammary tumor virus (MuMTV) associated with epithelial mammary or breast tumors in mice. There are a large number of oncogenic viruses or strains of viruses in mammals, fish, and invertebrates. The MuLV is divided into three groups: ecotropic viruses, which replicate only in mouse cells; xenotropic viruses, which are restricted in mouse cells but yet will grow in cells from other species; and amphotropic viruses, which replicate in both types of cells (86, 93, 122). The presence of oncogenic virus in the genome of mouse cells permits vertical transmission of this agent from mother to offspring. Dependent on the genetic background of the mice, tumor expression of this virus may occur at varying ages. Ecotropic virus may be expressed late in life with neurological disease (75). Mouse mammary tumor virus (MuMTV) is excreted in mothers' milk and is associated with development of mammary tumors in susceptible mouse strains (99).

Molecular biologists have used mouse models to gain insights into the structure and function of gene elements and oncogenic viruses. There is active research in investigating the genetic control of these viruses, their expression, and the molecular events leading to cell transformation or cancer.

Because the mouse is susceptible to development of cancer, it is an ideal system to test potential cancer treatments. Hybridoma technology has provided homogeneous antibodies (monoclonal antibodies) that can be directed against specific antigenic cell surface determinants or cancer cells (see section on Immunology). These antibodies may affect the tumor through the antibody-complement system or through a cell-mediated process. These monoclonal antibodies may also be "tagged" with compounds highly toxic to tumor cells, thus acting as a target-directed carrier of the anticancer compound and increasing its effectiveness. Trials of cancer chemotherapeutic agents of various types are under investigation.

Lymphoproliferative Diseases

Lymphoproliferative diseases are a major form of human disease comprising three major groups: nonneoplastic lymphocytic proliferation, non-Hodgkin lymphoma and related leukemia, and Hodgkin lymphoma. The study of lymphocytic proliferative disease has been immensely aided by availability of inbred mice with these diseases (132, 133). Pattengale and Frith (143) discuss the similarity of the mouse non-Hodgkin neoplastic diseases with those of humans, and have suggested a modified classification of these tumors.

Neoplastic lymphocytic proliferation in the mouse and human can be broadly divided into three types: B-cells with easily identified cell surface and/or cell cytoplasmic Ig; T-cells with easily detectable surface (THy 1); and non-B-, non-T-cells lacking both easily detectable markers. The Hodgkin-type neoplasms include cells of the histiocytic/monocyte series, i.e., histiocytic sarcoma. The older terminology of reticulum cell sarcoma is being questioned. These tumor cells may be transformed immature B lymphocytes rather than histiocytes (139).

Hodgkin's disease in humans is clinically and histologically an extremely variable entity affecting lymphoid tissue. Although the SJL/J mouse is proposed as a possible animal model for human Hodgkin's disease (119, 178), it is not clear that it is an acceptable model at this time (74). Non-Hodgkin's disease mouse models are, however, similar to the human disease, both clinically and histologically (143). This important model of human lymphoid cancer holds great promise for future research. The role that genetics plays in modulating this form of cancer is under intense study. The availability of this mouse animal system should provide researchers with the opportunity to study underlying mechanism of cancer biology and test therapeutic regimes.

The retroviruses or type C oncornaviruses are RNA viruses that contain the RNA-dependent DNA polymerase (reverse transcriptase) (210). Mouse leukemia was first induced with such an agent (86).

Mice appear to have within their genome information necessary for the synthesis of similar oncornaviruses to the Gross agent (1). These genes are transmitted vertically to their offspring and can be activated by a variety of stimuli. The role of these endogenous viruses that have xenotropism properties is unclear in tumorigenesis. A long list of type C viruses has been isolated from mouse

hemopoietic tumors, and these may induce neoplasia (87, 154).

The replication of ecotropic murine retroviruses and their ability to produce neoplasia is regulated by a number of genes, one of which is Fv-1 (187). The type of neoplasia usually induced in the lymphatic series is of the T-cell type (177); but the Abelson virus induces a B-cell lymphoma (180).

Pattengale and Frith (143) examined histologically and by cell surface immunologic markers 88 spontaneous tumors in BALB/c STC-rl mice. Sixty percent were B-cell lymphomas, a group that according to Dunn's classification would have been "reticulum-cell sarcomas," 17% B-cell lymphomas of other types, and 23% presumable T-cell lymphomas. This observation is of importance in evaluating the mouse as a spontaneous disease model of human lymphoma.

A listing of hemopoietic tumors by mouse strain, frequency, and reference can be found in Furmanski and Rich (74).

Chemical Carcinogenesis

Chemical carcinogenesis is of two parts: 1) those experiments designed to determine if a chemical has carcinogenic potential in animals and thus dangerous to humans and 2) those experiments that utilize mutagens and carcinogens to study the basic mechanism of carcinogenesis. The mouse is one of the most valuable species used in these testing programs (see section on Radiobiology and also reviews under section on Pharmacology).

Radiobiology

The atomic age, ushered in at the end of World War II, stimulated intense investigation into the effects of radiation on humans and animals. Centers such as the Oakridge National Laboratory for Radiological Research in Tennessee and Argonne National Laboratory for Radiobiology in Illinois were pioneers in testing vast numbers of mice for effects of radiation.

Early investigators primarily studied high-dose radiation levels from 300-500 rad (near lethal dose for mice) up through 5,000 rad, a dose that causes bone marrow associated death within 8-20 days. If intestinal injury overrides bone marrow death, animals may die within 4-6 days.

This early work using outbred and inbred strains of mice clearly demonstrated that some animals were more radiosensitive than others: survival of cells and animals was dose dependent; but if the dose was split over time, an increased dose was required to achieve the same effect. It was also determined that survival was related to age at which exposure was received (203, 207).

The survival shortening following nonlethal radiation was at first thought to affect the aging process by setting the "aging clock" forward (96). This concept was shown to be incorrect. Shortening of survival was due primarily to an increased early incidence of cancer (189, 212). To a lesser extent decreased survival was also related to an increase in degenerative diseases.

Radiation is a complete carcinogen, able at high doses to induce cancer in almost any organ that under normal conditions is subjected to cancer. However, because unique cancers are not induced by radiation, i.e., cancers appearing in animals not submitted to the known doses of radiation, it is difficult to determine if a particular

tumor was radiation induced or of spontaneous origin. It is clear, however, that in a population of mice exposed equally to radiation, there is a marked overall increase in tumors alone than expected normally.

Tissue radiosensitivity to tumor development was investigated and three classes were noted: very sensitive—thymic and ovary; moderately sensitive—myelopoietic tissues, lung, endocrine organs, breast, and uterus; low sensitivity—bone, skin, CNS, liver, kidney, gastrointestinal tract, pancreas, stomach, and urinary tract (188, 204-206).

The action by which radiation causes cancer is of primary concern to molecular biologists and radiation biologists. The process of ionization involves transfer of energy to a molecule or atom and the "knocking out" of an electron(s). In addition, excitational energy is dissipated to orbital electrons of the atom. These events cause disruption or alteration of chemical bonds within cells causing profound biological changes. In addition, reactive free radicals are formed such as hydrogen peroxide, which react with molecules and also affect biological activity (111).

Experiments in mice have indicated that by reducing O₂ tension of cells, there is an increase in resistance to radiation, supporting the free radical hypotheses. The administration of thiols, which may act by scavaging free radicals, also appear to be beneficial. These effects are seen only if done at the time of exposure but not following exposure.

Treatment of radiation sickness is limited to bone marrow transplantation and supportive therapy. Our knowledge of transplantation genetics, learned through using inbred strains of mice, is critical to this approach.

The later scientific literature from the 1960's and 1970's to the present is oriented to long-term effect of low-dose levels of radiation. Because effects of low-dose radiation, mutagens, and some potential chemical carcinogens are difficult to evaluate in long-lived mammals, the mouse, with defined genotypes, is the animal of choice for such studies.

Radiation biology, along with the study of mutagens and chemical carcinogens, are very powerful tools for the molecular biologist. These approaches, together with other methods that perturbate genes, gene expression, and gene cell regulation, further our knowledge of cell biology. Understanding the basic mechanism relating to cell division and growth is a primary goal of cancer biologists.

Pharmacology

Pharmacological screening and testing is a fundamental procedure in evaluating new compounds for possible physiological effects and potential toxic or unwanted side effects. The mouse is used extensively in the pharmaceutical industry. Metabolic pathways in many cases are similar among animal species and humans (104), but caution must be used in extrapolating data directly. This field is extensive and reviews should be consulted (17, 153, 200).

Behavior Genetics

The mouse is without doubt one of the principal research animals used to understand genetically related aspects of mammalian behavior. The availability of inbred strains, mutants, congenics, recombinant inbreds, with differing neuroanatomy, biochemistry, and neuro-

physiology have provided scientists with the biological means to study this extremely complex and elusive discipline. It is not within the range of this monograph to detail the extensive research in this area. Reference to the bibliographic reports entitled *Behavioral Studies Using Genetically Defined Mice—A Bibliography* (184-186) lists by behavioral categories 1,916 individual references. These categories include activity, aggression, audiogenic, seizures, communication, emotionality, feeding, learning, maternal, memory, psychomotor, regulation, reproduction, biorhythms, sensation, and social.

Treatment effects on behavior were also listed and included age, early development; age, maturity, and senescence; alcohol, central nervous system; manipulations and measurements.

Other behaviorally relevant areas listed included mutations of coat color; neurological and others; neonatal and teratogenic; population size; pharmacologic agents; and genetic selection.

As an example of mice used in behavioral research, convulsive disorders will be discussed in some detail.

Convulsive Disorders

In humans epilepsy is a major medical problem. Over one million people in the United States have recurrent attacks of epilepsy or convulsions yearly. Over 10 million people seek medical advice at least once in their lives related to convulsion disorders.

Convulsions may be secondary to a medical illness and not a recurrent or lasting problem. Status epilepticus, recurrent seizures every few minutes, may, however, be life threatening.

Recurrent convulsions that occur over long periods may be associated with a primary or acquired neurological disorder such as a brain tumor. Idiopathic epilepsy is a convulsion due to an unknown cause.

A convulsion or seizure is the result of disorderly, rapidly discharging neurons resulting in disturbances of sensation, loss of consciousness, and convulsive movement, or one or all of these signs.

Idiopathic seizures or convulsions therefore have as their basis unknown causes, but these may be the result of subtle biochemical changes, subtle cellular or membrane defects, subtle structural developmental abnormalities, or a small region of focal discharges due to unknown causes or an undetected fibroglial scar of unknown etiology.

Seizures are classified into generalized (other than grand mal), generalized (grand mal); petit mal or minor epilepsy; common focal seizure patterns (psychomotor) epilepsy; localized motor seizures; somatic, visual, and other seizures; and psychic phenomena.

Biochemical studies indicate increased sensitivity of epileptic brain foci to neurotransmitters such as acetylcholine. Hypotheses include a deficiency of γ -aminobutyric acid (GABA), an inhibitory transmitter (175), and changes in metabolic kinetics.

Mental retardation is rarely associated with idiopathic epilepsy, but if it does occur it may signal the presence of an underlying cerebral disease.

Evocation of seizures (reflex epilepsy) occurs in one in every 15 patients with seizures. The evoking stimuli may be a flickering light, touching a particular part of the body, making certain defined movement, or hearing certain sounds. This last stimuli is classified as audiogenic seizures (AS).

Mice have been extensively used in behavior research (73, 184-186) and, in particular, audiogenic seizure research (41, 73, 165). This form of epilepsy in the mouse closely resembles the human disease without an auditory stimulus, and these mice are undistinguishable from normal mice. Two types of AS susceptibility exist in mice: 1) occurring spontaneously as an inherited trait, and 2) a susceptibility that can be acquired through physical and/or biochemical procedures. The inherited form is a form of idiopathic epilepsy, since no specific biochemical or structural abnormality has been detected.

The susceptibility of mice to audiogenic stimuli is dependent on age and genetic background. Hall (88) reported a marked difference for AS susceptibility between C57 and DBA mice. Since that report intensive genetic studies have contributed information to suggest that inherited susceptibility to AS is influenced by the actions of at least one major gene, a number of minor modifying genes, and a variety of environmental factors (168).

Anatomical studies in mouse models suggest that the neocortex is not primarily involved in AS. Willcott and Lu (219), however, reported that the primary pathways of AS included the central nucleus of the inferior colliculus, deep superior colliculus, and adjacent tegmentum.

Cerebellar involvement is also suggested by evidence in mice that neuronal gangliosides and glycosphingolipids are less concentrated in susceptible mice at 21 days of age (169 and 170). Willcott and Urban (219) produced lesions in the anterior cerebellar cortex and found an increased evidence and severity of AS in these lesioned mice. This and other studies suggest that the cerebellum may play an important role in AS.

In mouse models, AS neurophysiological, biochemical, and endocrine studies can be carried out combined with detailed knowledge by the phenotype and neuropathology. Although a complete understanding of the underlying defect or defects in AS has not been established, a number of basic biological phenomena have been investigated. These biochemical, physiological, and neuropathological studies will continually help understand the basic mechanism responsible for some types of convulsive disorders in humans.

Convulsions in mice may also occur in association with single gene mutation. Noebels (135) tabulated a number of these mutant mouse genes, suggesting that in many they can be attributed to metabolic defects.

Mutations affecting cerebellum and having neurological phenotypes evidenced in part by convulsions include jimpy, myelin synthesis deficiency, myelin deficiency, trembler, and wobbler-lethal (175). Mutations affecting the cerebellum have been extensively studied. These include staggerer, weaver, and lurcher (175).

Aging

As the poet Robert Burns observed, there are many instructive parallels between the lives of mice and men. Not the least of these is in their aging patterns. Their key physiological systems decline with age after the peak of functional capacities at maturity, and this decline leads to disease. Mice age 30 times more rapidly than humans, a major difference between mice and human beings, which greatly enhances the value of mice for research in aging (39).

Mice have other major advantages for biomedical research on aging: both their genetic composition and

their environmental conditions can be precisely and reproducibly defined. Since aging appears to be complex, this definition is vital to interpreting experimental data. Studies of aging in the immune system illustrate this problem. The human and mouse immune systems (79) appear to work in the same fashion, and mouse models have provided most of the exciting recent advances in immunology.

The immune system declines with age in functional ability in both mice and humans (126). This is dramatically shown by the shrinking of an essential tissue, the thymus, which begins in early maturity. The functions of the thymus decline with age, and methods of restoring them are being developed using the mouse model. Changes with age appear to affect both the thymus cells that perform immune responses and those that regulate such responses in other cell types. Hormone production by the thymus also diminishes with age, as does the production of other substances that are essential for effective immune functions. These discoveries, almost entirely dependent on the mouse model, may lead to effective treatments for improving immune response in elderly people, reducing their vulnerability to infectious diseases and possibly cancer.

A fundamental question in biology is whether all our cells age at similar rates (91). By use of mice it is possible to transplant cells and tissues between genetically identical animals of different ages. Such experiments have shown that some cells and tissue types are capable of functioning longer than mice live. Stem cells from old mice function as well as those from young mice after transplantation into young recipients. Extremely sensitive tests of long-term stem cell repopulating abilities have been developed and do not detect any effect of age (92). Recent experiments suggest that stem cells from old mice have the same long-term functional capacity as those from unborn fetal mice. These findings show that all body cells are not affected equally by the aging process and suggest that stem cells do not age at all.

If aging is not controlled within each tissue that changes with age, it may be affected by communications between the tissues. This, to some extent, implicates the neurological and endocrine systems, as these allow tissues to communicate by means of nerve fibers and circulating hormones. Studies of changes with age in these systems are much more efficient using well-defined mouse models. Recently a striking example of interactions in the aging of these systems has been brought to light. One of the organs that ages most rapidly is the ovary, causing menopause in middle-aged women. The ovaries in mice are also exhausted after about the same fraction of life span. While this exhaustion would seem to result from the loss of eggs or ova, recent studies have shown that the pituitary gland is also involved (70). This gland acts as a monitor between the nervous system and all the glands that produce circulating hormones. It produces hormones necessary for the ovary to function. However, in old animals it fails to produce these hormones, so that even a transplanted young ovary fails to function in an old mouse. If ovaries from a young mouse are removed, the mouse allowed to age, and then a young ovary transplanted into the aged mouse, the young ovary will function. The pituitary appears to lose its ability to stimulate the ovary as a result of hormones produced in the ovary over time. Thus aging of the ovary and pitu-

itary is a feedback system, in which ovarian function causes pituitary aging. The aged pituitary then contributes to the loss of ovarian function with age.

Without the genetic control afforded by the mouse model, the elegant transplantation experiments necessary to make the advances discussed would not have been possible. Without the mouse's rapid aging rate, they would have taken too long. But perhaps the most promising development is an increasing recognition that changes with age and the tests to determine accurately the aging state may now be used to evaluate treatments in the mouse that may alter the aging process in these systems (152).

Progress in biomedical science becomes possible once important health problems are adequately defined so that treatments may be reliably evaluated. For example, the protection afforded by immunization was developed by Jenner in 1798 (107) before physicians knew what they were immunizing against or how the immune system worked. This history suggests that progress can be made in improving the health of older people without having to understand all facets of the aging process. However, we must reliably measure how systems decline with age. Well-defined mouse systems will allow us to develop a series of assays for changes with age in a variety of physiological systems. In mice the changes will happen relatively quickly, and the systems can be precisely defined. Each individual strain of mouse has its own set of vulnerable systems and will provide a convenient model for people in whom those systems malfunction with age. Treatments to affect those systems can be reliably and objectively evaluated using appropriate types of mice. As effective treatments are discovered, they not only will suggest treatments useful in human beings but will lead to a better understanding of the aging process. As aging is better understood through advances in cell biology and molecular biology, more effective treatments will be developed and evaluated using the mouse model. This concept is attracting great interest and gives hope for major health improvements in the future as a direct result of using the mouse in biomedical research on aging.

Deafness

The mouse has been used extensively as an experimental model to study human deafness. Ehret (61) described the mouse auditory system as a typical mammalian system without the specialization of some animals (e.g., the bat). Webster and Webster (214) have studied CBA mice after auditory deprivation and found abnormalities in the central nervous system. This finding may be applicable to humans, in that many children experience this type of hearing loss due to infections in childhood. The C57BL/6 inbred mouse has been shown to lose hearing with age. The pattern of this hearing loss closely parallels that seen in humans. The C57BL/6 mouse is therefore an excellent experimental model for human presbycusis and can be used to study the effect of environmental factors on the rapidity of hearing loss with age (95). As in the case of humans, many genotypes of mice experience hearing loss following excessive exposure to noise (94). The pattern of hearing loss due to noise exposure is exactly parallel in the human and mouse, in that higher frequencies are affected before low-frequency hearing. The mouse otocyst is the only mammalian otocyst to have been cultured *in vitro*. Van de

Water and Ruben (208) have observed the differentiation of the mouse inner ear in organ culture.

It has recently been shown that the LP/J inbred mouse developed bony lesions in the middle ear which are similar to human otosclerosis (37). This is the first known occurrence of otosclerosis in a nonhuman. Otosclerosis affects approximately 15 million Americans, and it is the major cause of hereditary conductive hearing loss in the United States. This experimental model may help provide the means to identify the cause and cure of this common disease.

Muscular Dystrophy

Present-day researchers studying the etiology of the muscular dysgeneses and/or neuromuscular disorders have at their disposal three animal model systems: the mouse, chicken, and hamster. Of these, the mouse is the animal of preference as it more nearly mimics the human dystrophies, especially the neuromuscular ones (174). The genetics of the avian model are not well known, and there is no information on the genetic locus in the hamster.

A voluminous literature is available on the biochemical alterations in muscle utilizing the mouse strain (190). In addition, there has been a great deal of work on the possible neurogenic aspects of dystrophy using the mouse as the experimental animal (4, 25).

The mouse was the first animal model available to study the genetic dystrophies, and early biochemical and physiological studies were done on this animal.

It is becoming more apparent, however, that these diseases may not result from a simple muscle or nerve defect (54, 69, 192, 193, 202). In all animal model systems and particularly the human, Duchenne and CMD dystrophies it is apparent from the literature that these disorders are prenatal in their origin and are genetic in transmission.

To further clarify the genetic nature of these disorders, two criteria of any animal model system must be met: 1) that it closely resemble the human disease and 2) that the genetic locus (or loci) be known. Only the mouse presently fits these criteria, and of particular importance is the fact that mice can be bred for the pure homozygous condition. This ensures researchers performing studies of in utero ages that they are dealing with a definable condition.

There is a paucity of literature on the in utero development of the dystrophies, and this applies to all animal model systems. Two papers in particular that stand out in this regard are the works of Platzer (146) and Banker et al. (16). Both of these papers deal with the murine system.

If we are to significantly further our understanding of these diseases, researchers will have to have at their disposal a model system wherein the genetic background is well understood and where homozygous offspring can be guaranteed. Only the mouse affords both of these.

It is also noteworthy that the Jackson Laboratory has a strain of mouse wherein a normal muscle population does not develop (*dy/dy*). This is an essential model system to study factors relating to control of myogenesis in an in vitro system and affords the researcher a model system to further our understanding of normal maturation of muscle populations and the role of nerves in this process.

The Athymic (Nude) Mouse

The athymic (nude) mouse is a special hairless mouse. The recessive gene (*nu*) that causes the absence of hair also causes a developmental defect in the thymus causing animals homozygous with this gene *nu/nu* to be athymic. The absence of a thymus results in a deficiency of immune responses that depend on thymus-derived lymphocytes (T-cells). Bone marrow-derived lymphocytes (B-cells) are present and appear to have the capacity to function normally in the athymic mouse. To the immunologist, the athymic mouse offers a unique opportunity to study B-cell functions in the absence of T-cells, to study the role of the thymus in development of the immune system and other physiological processes, and to study restoration of immune function through transplantation of thymic tissues or individual cells.

Rejection of foreign tissues requires the presence of functioning thymus-dependent immune responses. The athymic mouse is unable to reject tissues from other mice or from a wide variety of mammals, birds, reptiles, or amphibians, thus allowing these tissues to live in this foreign environment. The ability of the athymic mouse to accept tissue from a variety of sources makes it possible to study development or function of isolated tissues in vivo. Human tumors transplanted into the nude mouse preserve many of their original characteristics. This has led to the use of human tumor-athymic mouse model 1) to study cancer biology and 2) to screen selected cancer chemotherapeutic agents prior to clinical trials in humans. The athymic mouse has also been an important animal model in the study of viral, bacterial, and parasitological nude infections (8). This extraordinary susceptibility in nude mice has been used to develop animal models of leprosy, malaria, tuberculosis, and trypanosomiasis. These diseases can be studied in vivo without the complicating effects of T-cell-mediated immunity found in normal animals. The athymic mouse has also been used to study pneumocytosis and candidiasis. These diseases often cause morbidity and mortality among individuals undergoing cancer chemotherapy or therapy to prevent rejection of a transplanted kidney. The athymic mouse is, again, an important animal model for these diseases. For additional information on the athymic nude mouse refer to refs. 42, 151, 163.

Animal Models—General

The mouse has been and is being used extensively in a far-ranging list of disciplines and systems. In this brief discussion it is not possible to list or describe the hundreds of genetically defined diseases or genetic variants of mice that bear closely on human health and disease. But a selected listing may be descriptive: neurological mutants; eye diseases affecting the cornea, lens, and retina; musculoskeletal disorders including various forms of muscular dystrophy; abnormal bone growth and vitamin D-resistant rickets; hematologic dyscrasias including anemias and clotting dysfunctions; variances in metabolism including defects in copper-binding capacity, taurine utilization, creatinine synthesis; lipid, cholesterol, and carbohydrate synthesis, and utilization in obese mice; enzymes including β -glucuronidase, catalase, 8-levulinatase, and esterases; endocrine mutants including diabetic mice; reproductive variants of both males and females including testicular feminization, infertility and sterility of various forms, and X-linked disorders; ab-

normalities of skin, collagen, and pigmentation; genetically influenced noninfectious diseases such as amyloidosis, dystrophic calcification, necrotizing polyarteritis, glomerulonephritis/nephrosis, obstructive uropathy, polycystic kidneys, lipid storage diseases; megacolon, odontogenic hamartoma, osteoarthritis, and osteoporosis; periodontal disease; and peptic ulcers (44, 68, 82, 83).

Freezing of Mouse Embryos—Cryopreservation

The dream of immortality has always been a focal point of myth and legend, of science fiction, of controversy. Preserving semen in a frozen state has been successful in a wide range of mammalian species, including man. For the last thirty years bull semen has been collected, frozen, and used to inseminate cows throughout the world. The impact on improved milk production has been dramatic through careful application of basic science to animal husbandry.

Embryo transfer in cattle involves insemination of high-quality semen into a high-quality milk-producing cow hormonally treated to superovulate, waiting a few days for conception and early blastocyst development, flushing the oviducts, and collection of these blastocysts or early embryos. Single embryos are then transferred into recipient host pseudopregnant cows allowing them to be surrogate mothers for potentially superior embryos. This technique, when further refined to include embryo freezing on a commercial scale, will provide an incredible advance in animal husbandry by allowing large numbers of high productive calves to be born to average producing cows (9, 223). This represents a further advance over semen preservation and insemination.

Human embryo transfer has recently been successfully completed. The technique of mouse embryo freezing (38, 131) has been perfected at the Jackson Laboratory by Dr. L. Mobraaten.

Over 700 genetically different stocks of mice are maintained at the Jackson Laboratory. These stocks include inbred strains; special genetic stocks such as congenic lines, recombinant-inbred strains, and multiple marker linkage stocks; mutant stocks; translocation stocks; and inversion stocks. Since the founding of the Jackson Laboratory in 1929 investigators have made stocks available to the scientific community at large. Today, the Animal Resources program of the Laboratory raises and distributes over two million mice each year. New scientifically valuable mutations are frequently found among these mice. Many of these mutations do not generate an immediate interest or once having been extensively studied fall into disuse. However, experience has demonstrated that many such mutations become the object of great scientific interest due to new discoveries and research trends. It is therefore important to preserve a large pool of genetic diversity to maximize progress of future research. Frozen embryo storage provides a reasonable means to preserve this diversity and is insurance against the accidental loss of stocks currently in wide use. It offers a possible additional advantage in that it may allow control to be exercised over the genetic drift that occurs in inbred strains.

A program to freeze embryos from stocks maintained at the Jackson Laboratory for long-term preservation in a repository was started in late 1978. At the present time over 300,000 embryos from about 350 different stocks of

mice have been frozen. Briefly, eight-cell embryos are aseptically collected on *day 3* (appearance of the vaginal plug is taken as *day 1*) from the oviducts of donors mated after gonadotropin-induced ovulation. Embryos are microscopically selected and placed in phosphate-buffered saline (PBS) to which a cryoprotectant (dimethyl sulfoxide) is added. The media containing the embryos is then "seeded" with an ice crystal to force the phase change from liquid to solid at the freezing point of the solution (-6°C). The special cryovials containing the embryos are then slowly cooled at the rate of 0.5°C per minute by means of a programmable freezing rate controller until the temperature reaches -80°C . At that point the vials are placed in liquid nitrogen storage containers for permanent storage at -130 to -196°C (217).

Embryos are recovered by slow thawing at the rate of $4-8^{\circ}\text{C}$ per minute. After thawing they are placed in a culture medium and allowed to incubate at 37°C for approximately 24 h. After culture the embryos, which are then at the blastocyst stage, are surgically transferred to a pseudopregnant female recipient for development to term. Pseudopregnant recipients are obtained by mating hybrid female mice in proestrus to a sterile male 3 days before embryo transfer.

The ability to freeze mouse embryos at a point in time, while continuing to breed its relatives generation after generation, and then revitalize the frozen embryos allows careful comparison of the initial frozen mouse and its progeny with its related descendants.

Application of this technique to other laboratory animals should also be rewarding. Transfer of this technology to animal husbandry will have a major impact on food production and may aid in preservation of some endangered species. The protection and preservation of special stocks of mice is now possible.

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Man's Best Friend or Man?

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The recent surge in concern for the "welfare" of animals has sparked a series of events that may sound the death knell for significant sectors of the biomedical research community. Spin-offs could involve research of all kinds dealing with any living species as the experimental subject. Less than a year ago, Congress enacted legislation restricting funds for animal use; the Department of Defense prohibited the use of animals for any research related to studies pertaining to wound ballistics. More recently, Massachusetts legislated against the use of animals obtained from pounds for research. There is growing concern on both sides of the issue of whether it is morally right to subject any living creature to experimentation. Opponents of animal research are even marching in protest!

On July 29, 1983, Dr. John Beary, the Acting Assistant Secretary of Defense for Health Affairs, forwarded a memorandum to the Armed Service's Secretaries, the President of the Uniformed Services University of the Health Sciences, and Directors of the Defense Agencies advising them that, "Effective immediately, the use of all animals for ballistic research within the Department of Defense is suspended." The subsequent debate over the Defense Department appropriations bill included the statement agreed upon by both House and Senate that, "No funds be appropriated pursuant to this act shall be used to purchase animals or otherwise fund the use of animals for the purpose of training students or other personnel in surgical or other medical treatment of wounds produced by any type of weapon."

The Department of Defense eventually relaxed its absolute suppression of animal research related to ballistics, and early this year rephrased the restrictive measures so that they apply only to cats and dogs. At the same time two reports appeared in *Nature*: one stating that 13.5 million cats and dogs are killed each year in the United States for the simple reason that no one wants them (1); the other stating that in England the Royal Society for the Prevention of Cruelty to Animals kills 100,000 unwanted cats and dogs each year or 10 times the number of these animals used annually by laboratories (2).

Does the military really need to shoot live animals in order to train its medical students, residents, and even "fully trained" (Board eligible or certified) surgeons the proper treatment of war wounds? In the opinion of those within the latter three categories, the answer is an emphatic yes. We agree. A recent report by the American College of Surgeons' Committee on Trauma indicated

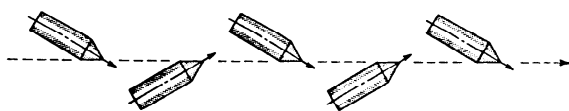
that of those taking Part I of the American Board of Surgery's examination, only 25% evidenced any significant experience in the management of a victim of trauma. When one considers the appalling loss of life each year in the United States as a result of firearms (30,000), it becomes apparent that there is an urgent need for training in missile injuries not only for the military surgeon in combat abroad but also in peacetime at home.

A second concern is for research. Are there unanswered questions in wound ballistics? Is it necessary to shoot live animals in order to answer such questions? The answer to both questions is again yes. Before addressing these questions regarding teaching and research, a definition of the term ballistics is in order.

Ballistics is the science of the motion of a projectile during its travel through the barrel of a firearm, during its subsequent trajectory through air, and during its final complicated motion after striking the target. Wound ballistics is a special case of the latter when the target is animal tissue. Knowledge of wound ballistics is crucial to the treatment of victims of missile injuries. For example a 22-caliber (0.22 inches in diameter) bullet fired from a handgun will produce a wound of entrance that is indistinguishable from that caused by a high-velocity military rifle (M-16) of similar caliber (223 or 0.223 inches in diameter) and weight (55 grains or 3.58 grams). This bullet may pass through the body and produce a wound of exit. Conversely the fired round may dissipate all of its kinetic energy within the victim and there may be no exit wound. In the case of the latter event the wound of entrance may appear identical to the wound of entrance due to the handgun. We know from experience, however, that the likelihood of massive internal damage will be associated with the bullet fired from the rifle, whereas the internal injuries resulting from the handgun round may be negligible (3).

The difference in the destruction of tissue by these two similarly sized bullets derives from the relation between kinetic energy of the missile (KE) and the product of the weight (W) of the missile and the square of its velocity (V^2). More precisely $KE = W V^2$ entrance — $W V^2$ exit. Obviously the kinetic energy of a missile varies linearly with its weight but exponentially with its velocity. The muzzle velocity of the handgun round is less than 1,000 feet per second, whereas that of the rifle has 10 times the kinetic energy of that leaving the handgun, although the two bullets are similar in weight.

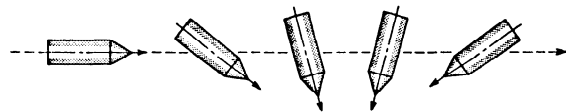
As muzzle velocity of a missile approaches and exceeds 3,000 feet per second, additional ballistic phenomena occur and cause a change in angle of incidence of the missile on impacting the target. This results in considerably more tissue damage than that caused by the bullet striking the victim pointed end first. These phenomena are yaw, tumbling, precession, and nutation. They are exhibited schematically in Figures 1-4. Yaw is a deviation of a bullet in its longitudinal axis from the straight line of flight. Tumbling is the action of forward rotation around the center of the mass. Precession is a circular yaw. Nutation is the rotational movement of the missile in small circles forming a larger circle in a rosette pattern in flight forward.



YAWING IS THE DEVIATION OF A BULLET IN ITS LONGITUDINAL AXIS FROM THE STRAIGHT LINE OF FLIGHT

Figure 1

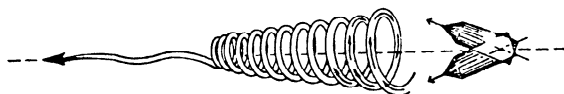
Schematic diagram of bullet yaw.



TUMBLING IS THE ACTION OF FORWARD ROTATION AROUND THE CENTER OF MASS

Figure 2

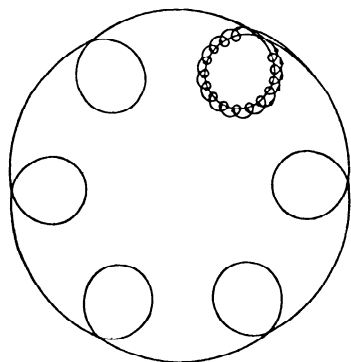
Schematic diagram of bullet tumbling.



Precession is a circular yawing around the center of mass in a spiral fashion.

Figure 3

Schematic diagram of bullet precession.



Nutation is the rotational movement in small circles forward in a rosette pattern.

Figure 4

Schematic diagram of bullet nutation.

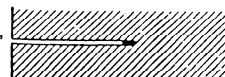
Additional ballistic properties include the phenomena of cavitation and secondary missiles. Cavitation, or cavity formation along the bullet track, results from the momentary acceleration of tissue in a direction forward and laterally away from the track of the bullet or other missile. A transient water vapor-filled cavity develops around the bullet and its track. The cavity may be many times the diameter of the bullet. If velocity of the missile is low (less than 1,000 feet per second), cavitation is negligible as seen schematically in Figure 5A. As the velocity and the kinetic energy of the missile increase, the tissues are compressed and accelerated away from the bullet and its track, forming within a few microseconds a cavity (Figure 5B) which enlarges at sub-atmospheric pressure as the bullet passes. Within a few microseconds, the cavity begins to collapse as a result of atmospheric pressure and tissue recoil. The cavity reforms and collapses several more times at rapidly diminishing amplitude until all energy has been dissipated. This alternating stretch and compression of the tissues adds substantially to the tissue damage in a wound from a high-velocity missile. An additional threat is that clothing and other contaminating debris may be sucked into the entrance and exit wounds due to the sub-atmospheric pressure of the cavity.

Secondary missiles may result if a bullet strikes tissue such as bone or any dense object near the victim, im-

parting kinetic energy to the tissue or object and converting it to an additional missile. The latter has all and perhaps even more tissue destructive potential than the original bullet or primary missile. A gunshot wound of the face may result in a secondary missile, e.g., tooth, being driven into the brain. Metallic objects such as coins, belt buckles, dog tags, or even a steel helmet can be sources of secondary missiles. In addition, stones, glass, and other dense material near a victim can be converted to secondary missiles if struck by a bullet. The most common secondary missile is bone (Figure 5C).

These ballistic phenomena are important to the forensic pathologist and law-enforcement official as well. The successful prosecution of felonious assault by gun often depends on the assiduous evaluation of the ballistics surrounding the wounding or killing of the victim. For example, the wound of exit usually exceeds in diameter the wound of entrance depending on the relative thickness of the target (Figure 5E). This information thus defines the direction of fire by the assailant. If the width or depth of the target is sufficiently great, entrance and exit wounds may have similar diameters (Figure 5, D and F), perhaps misleading those in attendance to overlook the extensive internal damage resulting from cavitation. Bullets impacting a target at high velocity and with an altered angle of incidence may cause an irregular cavity and a variably sized exit wound (Figure 5G). Bullets traveling at ultrahigh velocities (~5,000 feet per second)

A LOW VELOCITY, NO CAVITATION, ENTRANCE AND EXIT SMALL.



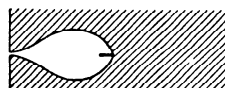
B HIGHER VELOCITY, FORMATION OF CAVITY. ARROWS SHOW DIRECTION AND MAGNITUDE OF ACCELERATION OF TISSUE.



C VELOCITY AS IN B, BUT DEFORMATION OF BULLET AND CREATION OF SECONDARY MISSILES UPON PENETRATING BONE.



D VERY HIGH VELOCITY, LARGE CAVITY, AND SMALL ENTRANCE. EXIT MAY BE SMALL.



E VERY HIGH VELOCITY, THIN TARGET, LARGE, RAGGED EXIT.



F VELOCITY, CALIBER, AND THICKNESS OF TISSUE SUCH THAT CAVITATION OCCURS DEEP INSIDE AND ENTRANCE AND EXIT ARE SMALL.



G ASYMMETRIC CAVITATION AS BULLET BEGINS TO DEFORM AND TUMBLE.



H WOUND PREDICTED FOR ULTRA-HIGH-VELOCITY, SMALL-CALIBER PROJECTILES NOW UNDER DEVELOPMENT. NO EXIT WOUND. FRAGMENTATION OF BULLET.



Figure 5

Schematic diagram of ballistic effects of bullets of differing velocities upon tissues of varying density and thickness.

induce large saucerized defects in experimental animals (Figure 5H), resembling an avulsive-type injury characteristic of a shark bite.

Careful questioning of the participants in and observers of the wounding incident along with a knowledge of wound ballistics are required of the attending physician and the medical legal authority. Proper identification of the weapon, bullet, and distance between assailant and victim can have significant impact on the diagnosis, treatment, and even prognosis of the gunshot wound victim.

When one considers the fact that over half (51%) of US servicemen who died in Viet Nam succumbed to small arms (albeit high velocity) weaponry, then the significance of education and research related to wound ballistics assumes proper proportion. Are there unanswered questions in wound ballistics and, if so, is animal experimentation necessary to address these questions? Is education in wound ballistics dependent on live animals for demonstration, teaching, and learning experiences? Must dogs and cats be used for research and training in ballistics? The answer to the first question is yes. As in any field, the more questions answered, the more questions raised. For example, the degree of tissue injury or extent of irreversible damage resulting from a gunshot wound remains ill defined. The definition of nonviability, or that amount of tissue which requires debridement to effect recovery, remains imprecise at best and is often left to unscientific guesswork. If skeletal muscle contracts when stimulated, bleeds when incised, or simply looks dead (cyanosis, desiccation, etc.), then it is excised. These criteria for nonviability are indications for debridement; they also are several hundred years old. One wonders why in this age of "high technology" more precise parameters cannot be developed. In fact, a number of seemingly promising avenues have been explored, but so far to no avail. Thus vital dye staining, liquid crystallography, and thermistor probe temperature recording have all been tried in an attempt to define more specifically the extent of tissue nonviability surrounding the tract of the missile. None have found useful application, nor have they proven superior to the traditional clinical assessment stated previously. Our knowledge of infectivity of bullets, plumbism, ideal time and technique of wound closure, etc. is still largely based on anecdotal data with few controlled clinical trials to support what is now standard practice. There may well be more art than science in the management of gunshot wounds.

In educating the various echelons of personnel caring for the victim of a gunshot wound, live animals will be necessary. The question is often heard, "Why not use simulated human tissue for demonstration as well as experimental purposes in studies of wound ballistics?" The answer is simple; simulated human tissue such as clay, soap, or gelatin blocks afford nothing more than simulation and as such impart little practical knowledge of benefit to the physician in attendance. Devitalization of tissue cannot be simulated any better than can be simulated hemorrhage or obstruction of the airway. Research and education in wound ballistics can be conducted on humans only to a limited extent. Man's "dearest friend" is the logical compromise between himself and a block of clay. Why are dogs the logical choice for wound ballistic studies and instruction? There are several reasons.

Dogs are relatively inexpensive, easy to handle, readily available, and of a size more comparable to humans (cats are really too small for research and demonstrations in wound ballistics). Perhaps of greater importance is the fact that dogs have served as the preferred large animal experimental-teaching model for many decades. There are probably more canine data relating to such diverse fields as cardiovascular physiology, sepsis, stress ulceration, wound healing, and trauma than from any other large animal. Thus the data base is much larger than that for goats, sheep, or pigs. The latter have been suggested as alternatives to cats and dogs for wound ballistic studies and are mandated by the Department of Defense. These animals are more expensive and more difficult to handle, and relatively little is known about them in comparison with the dog.

The canine trauma model was the choice for the American College of Surgeons' Committee on Trauma when it sponsored and developed the Advanced Trauma Life Support (ATLS) course. This two-day symposium on management of a victim of trauma concentrates on the first hour after injury and appropriate evacuation from field to hospital. Its counterpart in medicine is the Advanced Cardiac Life Support (ACLS) course. While ACLS is open to all interested "students," only physicians or doctors of osteopathy are allowed to matriculate through ATLS. The reason is simple. A significant proportion of the course is devoted to the "dog lab," where students learn basic techniques in resuscitation of a victim of trauma. These are invasive and considered surgical skills. The instructors all must be surgeons. The ATLS program has been enthusiastically delivered and received throughout the United States and Canada. Increasing numbers of hospitals require their Emergency Room staff and Trauma Surgeons to become certified in ATLS by the American College of Surgeons. Increasing numbers of residency training programs require their surgical residents to be ATLS certified. The Uniformed Services University of the Health Sciences—Edward A. Hebert School of Medicine requires ATLS training for all its students as a prerequisite to graduation. Other medical schools probably will follow. If the use of dogs is prohibited in these programs, then research and education in wound ballistics will surely suffer.

Most of those involved in such experimentation and instruction are "fond of dogs" if not actual "dog lovers." It is a prime responsibility of those conducting such programs to minimize fear, discomfort, and pain of the experimental animal in such studies. In the programs referred to above, no dog is abused beyond what would be associated with a percutaneous venipuncture to inject a rapidly anesthetizing dose of pentobarbital. The dog feels no pain thereafter and is killed at the termination of the experiment or the instruction period. The goal is to improve our intellectual and technical skills in the management of human beings who, for whatever reasons, sustain a gunshot wound in combat abroad or in peacetime at home. There are no satisfactory alternatives.

Is it not proper that knowledge and experience in wound ballistics be developed and that they be available to the hundreds of thousands of human beings injured by firearms each year in this country? Is it less worthy to use, compassionately, unwanted dogs for such purposes than to kill them in the pounds by the hundreds of thousands?

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From *Air Forces Times* (9 April 1984)

Colon, Honduras—As a 1981 graduate of the Uniformed Services University of the Health Sciences School of Medicine presently serving in Honduras with US Army Special Forces, I feel entitled (in fact, obligated) to hold an opinion on the current controversy regarding the wounding of live animals in combat wound training.

Having been both an instructor and student for live-animal training, let me preface any discussion of the issue by stating emphatically that animals are well cared for prior to wounding. They are fully and competently anesthetized during wounding and, if reawakened after wounding, receive attentive care in proper sterile medical fashion from students who are highly concerned and motivated. In those courses not requiring postoperative wound care, animals are chemically euthanized prior to regaining consciousness. Several difficult and highly instructive procedures usually are performed to gain maximum teaching benefit from the sacrifice of an animal.

The major issue at point is the need for training in live-wounded tissue for those most likely to face future combat medical challenges. There can be no reasonable scientific doubt that the nature of high-velocity (assault rifle) bullet wounding is dramatically different from low-velocity (pistol, light rifle) wounding. The squared increase of force with respect to velocity produces wounds of devastating effect. This difference is substantial and significant and definitely not easily understood or treated without prior exposure to the local and remote effects of wounding and their sequelae (after effects). Anecdotal medical history provides examples of this fact being rediscovered by each generation of physicians involved in armed conflict, usually after having unsuccessfully and inappropriately treated wounded young soldiers with techniques more applicable to low-velocity (civilian) wounds. Training and practice in the care of high-velocity wounds is essential to provide an experienced supply of physicians, physician's assistants and Ranger and Special Forces medics who are competent, capable, and confident in the treatment of such casualties in situations where large numbers of casualties may occur rapidly.

The need for live-wound training has long been recognized; even in today's modern laboratory no satisfactory substitute has been found. In the early 20th century the basic groundwork of scientific wound ballistics was laid. The wounding of cadavers (human and animal), the use of soap blocks or gelatin and other methods were tried both in Europe and America and found grossly unsuitable. Live-animal training provides the surest safeguard of the American soldier's life if wounded. It must be recognized that anything less than high-velocity wound experience for medical care providers represents a com-

promise in the quality of care to be provided on the battlefield.

In a basic, though, perhaps unfairly emotional view: should the first live-wounded tissue a medic or young physician see be that of an American soldier, son, husband, or father (or even daughter, wife, mother) in the woods, in the dark, in the rain? Our lack of foresight and preparedness in the training of medical personnel may diminish the chances of that young American's survival, to the greater loss of our society. The academic sacrifice of animals is not to be taken lightly and should not pass without question.

As to the suggestion that American medical personnel be sent to world trouble spots for wound training, I can only hope that this was advanced in "tongue-in-cheek" fashion. In my present duty I have treated only two gunshot-wounded Central American soldiers but would have hated to tell either of these patients to "bleed a bit more slowly, I'm learning as I go along."

High-velocity bullet-wound training is an essential and integral part of military medical training. The current disagreements between intelligent and sensitive persons on both sides of the issue may be correctly resolved if we remember the lifesaving responsibilities of the medical personnel to be trained.

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His interest in government began as a newspaper reporter in Ohio. He also reported local and state government activities in Kentucky and as a managing editor of the *Louisville Courier-Journal & Times* directed the news coverage of the affairs of government in Indiana.

The University of Kentucky Medical Center selected Samuels in 1965 to direct its Division of State and Local Services, which is responsible for the institution's public and governmental affairs programs.

In 1970 Samuels began managing national governmental affairs programs as Executive Director of the American Society of Allied Health Professions and then as Executive Director of the American Association of Blood Banks and the National Society for Medical Research. He is a founder of two national public interest organizations, the Coalition of Health Funding and the National Commission for Health Certifying Agencies.

Samuels also serves both Federal and private agencies as a consultant in the areas of public and governmental affairs and health manpower education. The American Society of Association Executives has awarded him the designation of Certified Association Executive (CAE), a recognition that has been granted to less than 1,000 association executives.

He is a graduate of the University of Kentucky with a degree in journalism and of Georgetown University in political science.

Congressional Amendment Proposed By APS Would Punish Lab Intruders

The American Physiological Society has proposed a Congressional amendment to the Animal Welfare Act that would make it a Federal offense to break into a federally funded research facility and destroy or steal research materials, equipment, or animals.

The proposal, approved by the APS Council in August, was presented by former Society President Walter C. Randall in September at the public hearing on HR. 5725, the "Improved Standards for Laboratory Animals Act." Rep. George E. Brown, Jr. (D-CA), sponsor of the bill, conducted the hearing as chairman of the House Agriculture Subcommittee on Operations, Research, and Foreign Agriculture.

The Society's proposed amendment was supported in testimony presented by the Association of American Medical Colleges and the National Society for Medical Research.

"The Society urges the Congress," Dr. Randall said, "to add provisions to the Animal Welfare Act authorizing Federal prosecution of those persons involved either directly or indirectly in the interference with federally funded research by the destruction and/or theft of equipment, animals, or data materials as well as the prosecution of those persons who obtain such stolen equipment, animals, or data materials."

The Society also urged that anyone convicted of such offenses should be liable for punitive damages as well as for the replacement costs and the cost for repeating experiments.

The Subcommittee invited 22 organizations and individuals to present views on the proposed bill that would add further restrictions to the Animal Welfare Act regarding the use of laboratory animals. The bill is a companion bill to S.657, which was introduced by Sen. Robert Dole (R-KS) during the first session of the 98th Congress. Neither bill is expected to be acted upon by the Congress during this session, but both bills are expected to be reintroduced when the 99th Congress convenes next January.

The complete text of Dr. Randall's testimony and the text of the Society's proposed amendment follow.

APS Testimony on HR. 5725

"My name is Walter Randall and I am here to present the American Physiological Society's views concerning HR. 5725, the 'Improved Standards for Laboratory Animals Act.'"

"Fourteen months ago I presented the views of the Society at the Senate hearing on S. 657, which is the

companion bill to HR. 5725. I am pleased to report that most of the recommendations and many of the concerns the Society expressed at that time have been incorporated as changes in the House version of this Congressional proposal to amend the laboratory animal sections of the Animal Welfare Act. The Society applauds Mr. George Brown, sponsor of HR. 5725, for his inclusion of our recommendations and for his sensitive understanding of our concerns.

"The American Physiological Society, which I recently served as national president, represents more than 6,200 physiologists who use laboratory animals in their work as researchers and teachers. Because physiology is the study of how living beings function, it is understandable that physiologists are the largest users of live animal models for research with more than half of the total numbers of animals required being used in the areas of cardiovascular, neurophysiological, endocrinological, and respiratory research.

"The need for laboratory animals in research is crucial and will continue inasmuch as there are no nonanimal adjunct methods available that can replace them. May I reemphasize that the spectacular advances in medicine during the last 20 years have been vitally dependent upon animal models, and this dependency will continue for the foreseeable future.

"On the other hand, the Subcommittee should know that there is a decline in the numbers of dogs, cats, and frogs used by physiologists for educational purposes. According to a Society survey only 66% of the departments of physiology in the nation's colleges and universities are now using those animals for teaching, compared with 90% in 1979.

"There is an obvious and growing use of computer and cell culture models as supplements to animal experiments in teaching. However, what must be understood is that such computer models cannot be used for much basic live animal research, and the validity of the computer models used in teaching depends entirely upon the research measurements gained from live animal experiments.

"I cite this difference in the use of animals in research with the use of animals in education because it is evidence that when proven and reliable nonanimal adjunct methods are available, they are used voluntarily by responsible physiologists as a means to conserve a vital resource.

"There also is conclusive evidence from Federal records that in other areas of research and teaching the use of live animal models decreases whenever reliable adjunct methods become available.

"Because of the evidence showing both a definite trend of decrease in the numbers of laboratory animals being used and the growing use of reliable and proven adjunct methods, the American Physiological Society questions seriously whether laboratory animal standards as provided by the Animal Welfare Act are a legislative issue. It is the opinion of the Society that it now is a regulatory issue and that the provisions for change in the Act can be initiated by the Secretary of Agriculture.

"Many of the amendments proposed in HR. 5725 already are being addressed by the regulatory bodies within the National Institutes of Health, the Public Health

Service, the Office of Technology Assessment, and by the Interagency Research Animal Committee. The actions proposed by these groups very well could and should accomplish most of what now is being proposed in HR. 5725.

"What the Society believes to be a primary need at this time to ensure compliance with standards set for laboratory animals is for the Congress to strengthen the role and authorities of the Animal and Plant Health Inspection Service (APHIS), the agency within the US Department of Agriculture responsible for enforcement of the Animal Welfare Act.

"For the Congress to add additional areas of responsibility for APHIS without advancing its authorities and resources will surely lead to greater frustrations for everyone concerned with the welfare of laboratory animals. Such an action certainly will not satisfy the intent of the Congress nor will it serve the best interests of the public, science, and animal welfare.

"The judgment of the Society is that the logical approach to ensuring proper care and treatment of laboratory animals lies within the regulatory framework of the Animal Welfare Act, currently the only Federal legislative authority governing the use of animals. For this reason alone the Society has opposed other proposals that would place similar legislative authorities in other departments of the Federal Government because such actions could lead to a divergence of Federal standards and regulations, thus creating much confusion to the researchers and increasing both the institutional and Federal costs in monitoring the nation's biomedical research program.

"If the Congress has conclusive evidence that the standards for laboratory animals require additional legislative restrictions, the Society would then support the concept of amending the Animal Welfare Act.

"The Society would like to offer to the Subcommittee an amendment to the Animal Welfare Act that was proposed at the annual meeting of the membership last month and has been unanimously endorsed by the Society's governing board.

"The basis for this proposed amendment is the recent criminal events at more than a dozen Federally supported research institutions where laboratories were trashed, equipment vandalized, research data destroyed, and animals stolen. Such actions have caused the loss of untold millions of Federal dollars and a waste of incalculable numbers of scientific man-hours of work. Each of the projects that were interrupted by such actions will have to be restarted with the expense being borne by the Federal Government. It is especially ironic that these actions also double the animal usage for the research.

"The Society urges the Congress to add provisions to the Animal Welfare Act authorizing Federal prosecution of those persons involved either directly or indirectly in the interference with federally funded research by the destruction and/or theft of equipment, animals, or data materials as well as the prosecution of those persons who obtain such stolen equipment, animals, or data materials.

"Those who are convicted of such offenses should be held liable for both punitive damages and the cost of replacing materials, data, equipment, animals, or records that may have been damaged or cannot be returned as well as the cost for repeating the experiments that have been interrupted or invalidated.

"Most federally supported research institutions are looking to the Congress to provide the support needed to halt the increasing number of incidents of attack that go beyond the limits of civil demonstrations. The scientific community will work closely with the Congress to develop adequate provisions that would stem this unnecessary burden for the researchers and this inexcusable waste of monies the Congress appropriates for biomedical research and the needless loss of animal lives.

"The American Physiological Society appreciates this opportunity to express its views of HR. 5725 and the opportunity to submit to the Subcommittee its recommended amendment to the Animal Welfare Act. In addition to this statement, the Society also has submitted some proposed language changes for HR. 5725. It would be my pleasure to respond to the members of the Subcommittee who may have questions."

Amendment Proposed by APS

Findings

The Congress further finds that the welfare of animals as well as productive use of Federal research funds require regulation to prevent unauthorized possession, alteration, destruction or transporting of research records, test data, research materials, equipment and/or research animals."

Amendment

"SEC. 28(a) It shall be unlawful for any person —

"(1) to break and enter into any Federally supported research facility with intent to destroy, alter, duplicate or obtain unauthorized possession of records, data, materials, equipment or animals.

"(2) by theft or deception knowingly to obtain control which is unauthorized or to exert control which is unauthorized over records, data, material, equipment or animals of any research facility for the purpose of depriving the rightful owner or research facility of the records, material, data, equipment or animals or for the purpose of using, concealing, abandoning or destroying such records, material, data, equipment or animals.

"(3) to possess or use records, material, data, equipment or animals or in any way to copy or reproduce records or data of a research facility knowing or reasonably believing such records, materials, data, equipment or animals to have been obtained by theft or deception or without authorization of the research facility.

"(b) Any person who violates any provision of this section shall be fined not more than \$5,000 or imprisoned for not more than one year, or both, for each such violation; and the United States District Court or the United States Magistrate, as the case may be, shall determine the reasonable cost of replacing materials, data, equipment or animals, and records which may have been damaged or cannot be returned and the reasonable cost of repeating any experimentation which may have been interrupted or invalidated in consequence of a violation of this section; and any persons convicted of such violation shall be ordered jointly and severally to make restitution to the research facility in the full amount of the reasonable cost so determined."

Career Opportunities in Physiology

Initiation of a Career in Pharmaceutical Industries

As a Ph.D. Physiologist

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In the process of finding positions in the pharmaceutical industry, I have become aware of several factors that are considered in the selection of scientists. I hope my discussion of these observations will alert interested physiologists to the opportunities and requirements of industrial employment.

Most pharmacology departments require postdoctoral as well as graduate training for entry level doctoral positions. As in academia, the physiologist gains an advantage when such experience is obtained in recognized research laboratories. The younger investigator whose training is still in progress may enhance his marketability for a pharmaceutical position by selecting research projects that evaluate some pathophysiological state (such as hypertension) or may be applied to the drug discovery process. Students may also choose elective courses in pharmacology to enhance their academic credentials.

Scientists can benefit from a procedure known as networking by which useful professional contacts are made and maintained. A good start can be made in your own institution by interacting with members of other laboratories and by following the careers of those colleagues. Personal contacts can also be established at scientific meetings. The chances of meeting investigators at conventions can be improved by presenting your work. The acquaintances resulting from such an effort can provide you with valuable information regarding the role of a physiologist in an industrial setting and may be helpful in locating employment prospects.

It is important that a scientist who has experienced only an academic environment learn from industrial investigators the consequences of selecting a position in pharmaceutical research. In general, some of the advantages that are recognized are the slightly higher salaries and the relatively greater availability of research funds. The industrial scientist must also expect increased

accountability to higher corporate levels and additional managerial responsibility. These factors vary throughout the industry and the interested candidate would be well advised to assess each company individually.

A physiologist must also recognize that a particular company must be evaluated in terms of the direction of its research efforts. The individual should assess his personal career goals and determine whether they can be best achieved in that or any other pharmaceutical concern.

Once a scientist has located a potential job via personal communications, advertisements, or a professional placement service, a well-written resume and a cover letter highlighting the candidate's suitability may open the door to a personal interview. An invitation for an on-site visit is most often accompanied by a request for a seminar. The oral presentation should be viewed as an opportunity to demonstrate not only scientific knowledge but also communication skills. A carefully prepared talk can create a very favorable impression.

An interview can serve as a learning experience for the candidate, especially if he comes prepared to ask questions as well as to answer them. In particular, the scientist should get a clear idea of the research goals of the company, anticipated responsibilities, and opportunities for professional development.

The above reflections are intended to serve as broad guidelines for a physiologist beginning a career in pharmaceutical research. The individual will discover that industrial positions offer unique challenges for the creative scientist.

Personal Viewpoints of a Physiologist

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One of the most important factors in successful career placement for physiologists, be it in academia or industry, is professional recognition by peers. Recognition is gained through prestigious professional publications and associations with well-known laboratories in the field. The recognition has become so indispensable nowadays that, without it, the chances of getting a good job would be very small, simply because of the scarcity of good jobs and disproportionate availability of qualified candidates. These statements are based purely on personal job hunting experiences. Through these experiences, I became able to really appreciate the importance of the recognition that I earned over the years since my first major publication and the subsequent ones produced at the University of Texas, Yale, and then Oxford. So when the time came for me to find a permanent position last summer, a few phone calls to my colleagues and prospective employers who knew my work well were sufficient to obtain interviews, most of which led to job offers from both academia and industry.

Presented at a Career Opportunities in Physiology Symposium entitled "Initiation of a Career in Pharmaceutical Industries" at the 68th Annual Federation of American Societies for Experimental Biology (FASEB) Meetings, St. Louis, MO, 3 April 1984 (sponsored by the American Physiological Society).

Given these new offers, my problem was not of finding a job but of choosing the one that would best suit me in the long run. In many ways, making such a decision can be infinitely more difficult and complicated than finding the job itself, particularly in a situation where all the offers are equally attractive. Because of the difficult position I was in, I pondered many questions while considering the choice of a job in industry and I present some of my opinions or biases on these questions. Since these opinions are based on a very short history with the industry, they may be overly subjective and premature and therefore should not be taken too seriously nor be generalized to apply to the whole pharmaceutical industry.

My first question is, Why would the pharmaceutical industry be interested in hiring cellular physiologists in the first place? Traditionally, pharmaceutical companies discover new drugs through screening, a practice that requires little physiological expertise or understanding of the basic biological mechanisms involved. Rather, it is hard work and, to a certain extent, pure luck that seem to matter (1). However, after decades of exploitation of the available drug resources, the number of drugs that can be discovered by the traditional means has diminished rapidly, making the search for new drugs increasingly difficult and financially unattractive because of the amount of labor and risks involved. To remain competitive, far-sighted establishments are looking for better alternatives, alternatives that lie in understanding the basic biological mechanisms governing the specific responses, so that keys that are modeled after these mechanisms or the biological molecules involved can be created to unlock the secrets for breakthrough. To find these biological keys, extensive basic research must be done at all levels. As a result, pharmaceutical companies are looking for biological scientists with desirable professional backgrounds, and these naturally include cellular physiologists.

Given this new opportunity, questions arise as to whether the physiologist would be expected to devote full time to basic research and, if so, would be given full freedom to develop research interests, directions, and goals. My experience so far indicates that the answer would be positive. Inasmuch as the resources of the pharmaceutical industry are always directed to specific aims, their recruitment efforts are likewise geared toward scientists who are not only very competent in their professions but, more importantly, share similar research interests, directions, and goals with the company. Once the scientist is hired, it is expected that his research will continue along the same lines as previously developed.

There are also many other questions to be considered, such as, will a physiologist like myself who is accustomed to the pace and atmosphere of the academic institutions experience great difficulty adjusting to the supervised managed industrial setting? Initially, this bothered me the most, but as I became familiar with the system, it quickly diminished. As I understand, the supervisory system has little to do with the day-to-day research activities of the individual scientist and does not have direct jurisdiction over the decisions made in

the laboratory. Instead, the immediate supervisor is there to advise and guide the collective research effort of the group and be the spokesman for their ideas and concerns to the upper management.

What about relationships with fellow scientists in the company? So far, I have experienced very friendly and open relationships with fellow scientists, partly because there is little competition between scientists within the company and an absence of anxiety over job security. At the professional level, however, a physiologist, particularly a cellular electrophysiologist, does have some problem in relating the work to that of others and consequently is somewhat isolated, but continued interchange can improve this situation. As far as communication outside the company is concerned, scientists are encouraged to publish in professional journals once the work is cleared of possible legal complications, to attend related professional meetings, and to undertake joint projects with scientists outside. Thus it is very unlikely that a physiologist would lead an isolated professional existence in industry.

Finally, what are the advantages for working in the pharmaceutical industry? This question, like the others already addressed, very much depends on the particular company and the nature of the job. It is generally true, however, that there are distinct financial advantages, such as generous laboratory set-up money, long-term research support, and professional research services. At a time when stable grant support and good career-oriented jobs are becoming scarce, these are important factors for choosing a purely research-oriented position in industry. Additionally, there are no teaching responsibilities and very few administrative duties. These advantages translate directly to a substantial net gain of research time and increased productivity. Furthermore, given the very large resources of scientific expertise and talent readily available in the research and development division of the pharmaceutical company, there is always a better chance to discover something new that may eventually mature to useful therapeutic agents.

Even though a pure research position at a pharmaceutical company may not appeal equally to everyone even with all the advantages mentioned above, it does represent an attractive opportunity that many academic environments cannot provide.

1. The drug industry moves from discovery to designs. *The Economist, Science & Technology*. Jan. 7, 1984.

Announcing **CAREER OPPORTUNITIES IN PHYSIOLOGY**

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resource for Physiologists in Training

Career Opportunities in Physiology

Careers in Pharmaceutical Industry for Physiologists

Personal Viewpoints

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Within the past few years, the pharmaceutical industry has expanded its interests and efforts in the area of understanding basic mechanisms that regulate physiological processes. In particular, the cardiovascular field has seen expansion at all levels of research, including the in vivo, electrophysiological, and molecular-biochemical areas. This expansion and realization on the part of the industry of the importance of understanding cellular physiological mechanisms for ultimate development of therapeutics has opened the door for academically oriented research individuals. A research career in industry now offers an attractive alternative to an academic research career.

My presentation is divided into two major sections along guidelines offered by the Careers Opportunity Committee. In the first section, my personal background and approach to research are provided to give the reader some idea as to the type of in vitro mechanistic research that can be done in an industrial setting. The second deals with my personal viewpoints on some pitfalls to avoid when considering an industrial position, the advantages of an industrial vs. an academic position, and some insight into obtaining an industrial research position.

Personal Background

I obtained my Ph.D. from the Department of Physiology at the University of Cincinnati College of Medicine in 1979 under the tutelage of Dr. Joseph DiSalvo. I then emigrated to the Department of Pharmacology at the University of Texas Health Science Center at Dallas where I was a postdoctoral fellow in the laboratory of Dr. James T. Stull. Following this two-year postdoctoral fellowship, I obtained my current position, which is supervisor of the Cardiovascular Biochemical/In Vitro Unit at Wyeth Laboratories, Inc. My research throughout my career has focused on the intracellular regulation of smooth muscle contractile activity by Ca^{2+} -calmodulin and the cyclic nucleotides (mostly cAMP). Specifically, my work has focused on myosin light chain and cAMP-dependent protein kinase, the two effectors of these second messengers. In my research I employ several models of contractility, including standard intact

muscle preparations and enzyme and purified contractile protein systems. Thus a knowledge of standard biochemical techniques is essential for this research approach.

Three avenues of research are pursued by my research group. They are 1) defining the importance of these systems in normal physiological and pharmacological function in smooth muscle, 2) examination as a possible source of aberration in cardiovascular pathophysiology (i.e., hypertension), and 3) development of pharmacological modulators as an approach to specific therapeutic cardiovascular agents. This latter avenue is almost unique to investigators in industrial positions and thus offers an advantage over what is available to most academicians. Within this research program, an approximately equal blend of basic mechanistic research and drug development is realized.

Personal Viewpoints

There are several pitfalls and considerations that one should be aware of before pursuing a position in the pharmaceutical industry. This is especially true for the in vitro mechanistic physiologist: since the relative merit of your research may not be readily apparent to potential superiors and upper management personnel, commitment for support of your research program by the company should be readily apparent. This support should be made in terms of scientific as well as technical and equipment budgeting. Thus a clear proposal of your research plans and needs is essential before seeking a position.

Likewise, the temporal commitment by the company for your position should be apparent. The period of support for your type of research (3, 5, 10 years) is a very important consideration.

Screening responsibilities and allocation of basic research efforts are two other important considerations that should be addressed prior to acceptance of an industrial position. The research-oriented individual must be cautious so as not to be overburdened by repetitious in vitro screening responsibilities. Meeting, travel, and publication policies of the company are also considerations; however, most pharmaceutical companies encourage their scientists to present and publish their research findings when proprietary and patent rights are not an issue. Finally, the personal growth and promotion potential for the scientist beyond the entry level is a consideration in choosing the right company.

Some obvious advantages that an industrial position offers over an academic position are listed in Table 1. Although written and oral communication skills are important to the industrial researchers, the lack of grantsmanship, especially time spent writing grants, is an enormous advantage over academia offered by indus-

Table 1

Advantages of an Industrial vs. Academic Research Position

1. Lack of grantsmanship
2. No teaching load
3. Pay/benefits
4. Excitement of drug development

Table 2

Selling an in Vitro Physiologist to Industry

1. Pharmacological experience—Research; postdoctorate (academic or industrial)
2. Demonstration of excellence and productivity as a researcher in your field
3. Unique approaches to research—ideas and techniques
4. Compatible areas of research interest

try. Similarly, a near immediate start-up with equipment and technical support, as well as continued financial support, are part of most industrial positions.

Another advantage for the research-oriented individual is a lack of teaching load in industry. Thus a greater percentage of time can be devoted to research. Also, pay and benefits are generally greater in industry compared with academia. This is usually true not only during the initial phase of your career but also in subsequent raises and promotions. Finally, the excitement of drug discovery and the opportunity through interactions with medicinal chemists and/or clinicians to design specific pharmacological modulators with potential, ultimately therapeutic usefulness is a unique advantage of the industrial physiology position.

The final area covered deals with the practicalities of obtaining an industrial position (Table 2). What can a graduate or postdoctoral student with a Ph.D. in physiology do to garner an industrial position? Of prime importance is practical pharmacological experience, preferably in the laboratory of a recognized leader in your particular field. This can be gained by incorporation of pharmacologically related problems into your research repertoire or by doing postdoctoral work in a Department of Pharmacology. This objective can be achieved in either an academic or industrial setting, the latter offering the opportunity to gain first-hand knowledge and experience in industrial research.

As with an academic position, the pharmaceutical industry is interested in obtaining top-notch researchers. You are judged in this area, for the most part, as you would be judged by an academician. Thus a demonstration of excellence as a researcher in your field of expertise is essential. Your publication record (number and journals published in), grant awards, personal references, and postdoctoral experiences all are used in this assessment. Of further interest to most companies are unique approaches to research that you may offer. These can be initiated and nurtured during postdoctoral work. It should also be pointed out that a compatible area of research interest is a major factor, especially for the in vitro mechanistic physiologist. Trends and timing of state-of-the-art research cannot be overlooked.

In summary, I would emphasize that the pharmaceutical industry appears to be expanding in its support of physiologically related mechanistic research. A person trained in physiology offers the advantage of application of mechanistic homeostasis to all levels of research. The criteria for obtaining an industrial research position are not very different from those for an academic position: hard work, excellence, and productivity as a researcher are paramount. Thus a good and productive postdoctoral tenure, preferably in the laboratory of a recognized leader in your field, is essential.

What a Director of Pharmacology Looks for in a Physiologist

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This report focuses on both director's and candidates' viewpoints for physiologists exploring career opportunities in the pharmaceutical industry. In our organization a director works closely with senior scientific staff and personnel department representatives to select candidates. Collectively, this experience forms the basis of the director's (employer's) points of view outlined below.

At Warner-Lambert/Parke Davis, 15% of the Ph.D. level scientists within the Pharmacology Department have been trained formally in physiology departments; their subspecialties are predominantly in the cardiovascular and neurology areas. The opinions and discussions with graduate physiologists who have elected to continue their careers in our Pharmacology Department are the basis for the physiologists' (employees') points of view. It is with this background that I will discuss career opportunities for physiologists in the pharmaceutical industry.

Director's (Employer's) Points of View

The key characteristics of successful candidates in our organization are listed in Table 1. In seeking highly trained competent scientists we look for graduates from academic centers of excellence to be specialists or subspecialists in specific areas of research; candidates usually have a minimum of two years of postdoctoral experience. Since not all pharmaceutical organizations have programs in the various subspecialties in physiology, candidates should identify companies with appropriate programs as targets for potential employment.

To be successful, a candidate must be interested in new drug discovery and development. The industrial setting is not incompatible with, nor does it preclude, the individual from conducting the highest quality mechanistic-based research, a point often misunderstood. In the pharmaceutical industry it is also essential that multidisciplinary teams work together toward the achievement of their research goals. If a candidate is unwilling or incapable of this approach, industry may not be the place to go. Innovativeness and creativity, characteristics not always identified as team processes, are nonetheless important qualities used in identifying

Table 1

Key Characteristics of Successful Candidates

- Training in academic center(s) of excellence, Ph.D., postdoctoral experience
- Career objectives—goal-oriented individuals who are interested in new drug discovery
- A team person (multidisciplinary approach)
- Communication skills—oral and written
- Leadership skills—effectively interacting at all levels within the organization

Table 2
Dimensions Used for Evaluating Candidates

Technical proficiency
Oral communication
Innovativeness (creativity)
Job motivation
Career ambition
Judgement
Initiative
Planning and organization
Tenacity (persistence)
Behavioral flexibility
Rapport building
Controlled demeanor
Impact (image)

successful candidates. The capability to recognize problems and generate imaginative and creative solutions in work-related situations are highly desirable traits, and an appropriate balance of activities (team, departmental, and individual) is attainable and works well in the pharmaceutical industry.

Candidates should have excellent oral and written communicative skills. Their research seminar (part of the interview process), publications, and presentations at scientific meetings provide the potential employer with an excellent opportunity to evaluate these skills. Candidates without strength in these areas or unwilling to develop them will probably not do well in the pharmaceutical industry (or in academia)! Finally, candidates must have or show the potential to develop strong interpersonal skills. They must provide leadership to their scientific unit and interact effectively at all levels within the research organization. A more comprehensive list of dimensions used in our organization for evaluating candidates is outlined in Table 2. While the immediate supervisor will not have any difficulty in judging the scientific qualifications of the candidate, he or she may lack the skills and experience for selecting the best candidate(s). The director, personnel department, immediate supervisors, peer-group scientists, and others provide valuable input into candidate selection by expressing their views on characteristics such as those outlined in Table 2.

Employees' Points of View

Recently hired physiologists in our department responded to a short questionnaire on who should or should not pursue careers in the pharmaceutical industry; candidates should pay particular attention to these profiles (Table 3). Although the number of respondents is low and limited to employees in our organization, I believe they are representative of candidate physiologists' viewpoints.

Table 3
Who Should Seek Out a Career
in the Pharmaceutical Industry?

Someone who has an interest in new drug discovery (goal-oriented research)
Someone who is able to accept the "team" approach to problem solving
Career goal is broad-based goal-oriented (applied) research
Disease/treatment orientation
Someone who wishes to avoid intense competition for research resources of academia
Someone who likes writing and open discussions with colleagues
Someone willing and able to meet specific deadlines
Someone willing to interact with individuals with diverse backgrounds
People with strong (diverse) scientific backgrounds and willingness to apply this knowledge
Organized and flexible—willingness to expand interests and knowledge

Given the information contained in this overview, I would summarize by providing the following recommendations to physiologists seeking employment in industry.

1) Get as much information as possible about career opportunities in the pharmaceutical industry from experienced people. This can be obtained in a variety of ways, both informally (as in discussions at scientific meetings, e.g., the Federation Meetings or personal contacts) or more formally via interviews.

2) Make certain that you research the company—most organizations are delighted to send you publication booklets, annual reports, etc. Read the employees' publications; go to their presentations at scientific meetings. Industrial pharmacologists often participate in university seminar programs; this affords another excellent opportunity to gain insights about the company.

3) Match your training to company research interests; identify and select appropriate companies to pursue.

The real "take-home" message that I can give you is to make an informed decision regarding the potential of a career in the pharmaceutical industry. Dr. David Bohr expressed this view in somewhat different terms (1):

Choosing a job might not be as crucial as those two other biggies—selecting the right spouse and picking the right profession—but it's close. In most cases, these choices are made on the basis of personal biases, but better selections can be made by being maximally informed of all options."

1. Bohr, David F. How to get a job in physiology. In: *Career Opportunities in Physiology*, edited by D. J. Ramsay. Bethesda, MD: Am. Physiol. Soc., 1984, p. 27-28.

Microcirculation, *New Handbook of Physiology*

Preparation of the new edition of the *Handbook of Physiology* on the cardiovascular system has provided an opportunity for consolidation of essential concepts and new developments of microvascular physiology. Each chapter in *Microcirculation*, edited by Eugene M. Renkin and C. Charles Michel, introduces the scope and principles of the topic it describes and offers more experienced investigators a critical assessment of the status of current ideas and techniques. It is also hoped that this volume will help cardiovascular physiologists to correlate phenomena at the macrocirculatory and microcirculatory levels.

The volume begins with a historical review of the contributions of Poiseuille to our understanding of microvascular flow. This is followed by two chapters on the structure of the microcirculation, a chapter on endothelial cell biology, and one on microvascular growth and adaptation. The next two chapters are devoted to microcirculatory dynamics of blood and lymph. Six chapters on material transport in and around the microcirculation cover the mechanics and thermodynamics of transport, movement of fluid, movements of small solutes and of macromolecules, transport in the interstitium, and transport modeling. A chapter on control of the microcirculation and exchange forms a bridge between these chapters and the rest of the volume. The next eight chapters describe microcirculation and exchange in selected organs and organ systems: liver and spleen, heart, gastrointestinal system, lungs, synovial joints, adipose tissue, brain, and eye. Finally, there are chapters on capillary portal circulation and on disseminated intravascular coagulation.

This volume completes the section *The Cardiovascular System*, which presents a comprehensive view of the functions of the heart, vascular smooth muscle, peripheral circulation and organ blood flow, and microcirculation and blood-tissue exchange.

Members of the Society may order *Microcirculation* or the other volumes in the section at special prices, when ordering from the APS Business Office, 9650 Rockville Pike, Bethesda, MD 20814.

Volume	List Price (US)	APS Member Price
I. <i>The Heart</i>	\$130.00	\$104.00
II. <i>Vascular Smooth Muscle</i>	95.00	76.00
III. <i>Peripheral Circulation and Organ Blood Flow</i>	245.00	196.00
IV. <i>Microcirculation</i>	275.00	220.00

Workshop on Computer-Based Education

On Sunday, April 1, 1984 a *Workshop on Computer-Based Education* was held in St. Louis preceding the 68th Annual meeting of FASEB. The Workshop was organized by Dr. Joel Michael, member of the APS Education Committee, and was staffed by Drs. D. Kim, H. Modell, R. Rakowski, J. Randall, C. Rothe, A. Rovick, and L. Thomas, Jr.

An audience of 50 biomedical scientists representing many disciplines (physiology, pharmacology, nutrition, histology, cell biology, pathology, and biomedical engineering) and many different institutions (colleges, universities, medical schools) participated in the all-day session. The focus of the Workshop was on the preparation of educational exercises (lessons or simulations) for eventual use on computers. No prior background in computers or computer-based education was assumed.

The morning session began with three lectures aimed at providing a broad background in computer-based education (CBE) (J. Michael), CBE applications in physiology (H. Modell), and computer hardware and software issues (L. Thomas). The focus then turned to specific applications, with presentations on the writing of computer lessons (A. Rovick) and the use of simulations in teaching exercises (J. Randall).

In the afternoon the audience was divided into small working groups, each of which had been assigned a topic to be worked on. Each group attempted to organize the educational content for a CBE exercise. As an aid in this, demonstrations of existing CBE exercises were conducted by the staff. The organization for the afternoon was deliberately kept flexible, and groups and individuals had ample opportunity to interact with one another.

The afternoon finished with a general group discussion of problems in the CBE area. Three problems were identified as being particularly important. It is significant that a similar list of issues was generated by a CBE Workshop at the 1983 FASEB meeting (*The Physiologist* 26: 323-325, 1983).

1) The rapid advance of computer technology has resulted in many, frequently incompatible machines competing for the same market. This has limited the "transportability" of existing CBE materials; users of different systems cannot easily share or exchange materials. As a result there is a serious problem of duplication of effort with individuals and institutions developing similar teaching programs. While it was recognized that "ideas" are clearly portable (in ways that actual computer programs may not be), the avenues for effective communication of such ideas remain limited.

2) The resources available to support work in CBE are too limited. Few institutions have committed funds for such activity, and much current work is "supported" from departmental and/or individual resources. Many new CBE materials are developed by individuals on their own time out of personal interest. Furthermore, there is almost no external funding available to support advances in this field.

3) In the long run, the most serious problem may well be the lack of professional academic rewards for faculty working in this area. Promotion, tenure, salary incre-

ments, etc. are awarded predominantly for productivity in the research laboratory not for efforts to develop innovative teaching techniques and materials. There is essentially no external grant support for CBE activities, and there are few refereed high-quality journals in which one can publish. Thus two of the measures by which rewards are apportioned do not accrue to CBE practitioners. This is a particular problem for junior faculty members who feel, quite correctly, that they must devote their major efforts to climbing the academic ladder. It is clear that CBE still lacks academic validity and credibility.

In spite of these readily identified problems, there was certainly widespread enthusiasm for the potential of CBE in biomedical teaching. There was also a clear sense that ongoing efforts would be continuing and that new contributors were continuing to be recruited into the field.

Although all of the returns are not yet in, informal conversations between the Workshop staff and participants as well as those evaluation forms already received suggest that the experience was viewed as a very positive one by participants.

There was a consensus that further efforts at training in CBE would be welcomed; a large number of applicants could not be accommodated at this Workshop (more than 120 application forms were received). There was also strong support for attempts to find additional avenues for communications about CBE, including the inclusion of symposia and workshops in future APS and FASEB meetings and the completion of the first phase of the FASEB CBE cataloging effort and its continued development.

Joel Michael
Department of Physiology
Rush Medical College
Chicago, Illinois 60612

Availability of Zinc, LSRO Report

An Expert Scientific Working Group of the Life Sciences Research Office has completed a study of serum zinc and related variables collected during the Second National Health and Nutrition Examination Survey, 1976-1980. Data analyses and interpretation of the results by the Expert Scientific Working Group form the basis for the report entitled "Assessment of the Zinc Nutritional Status of the US Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976-1980." The report, sponsored by the Center for Food Safety and Applied Nutrition, Food and Drug Administration, provides a description of the methodology used to collect data on serum zinc; reference data for serum zinc values in the United States; and a discussion of the differences in serum zinc values due to time of sample collection, fasting status of the subject, sex, age, race, pregnancy, use of oral contraceptive agents, and poverty status. The prevalence, and possible adverse effects, of low serum zinc values are evaluated. Other topics addressed in the report are the utility of serum zinc for the assessment of zinc nutritional status, and recommendations for future surveys of zinc nutritional status. *Copies of report:* FASEB Special Publications Office, 9650 Rockville Pike, Bethesda, MD 20814 (\$12.00 postpaid).

Sustaining Associate Members

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Cooper Appointed Vice Chairman of Board of Upjohn Company

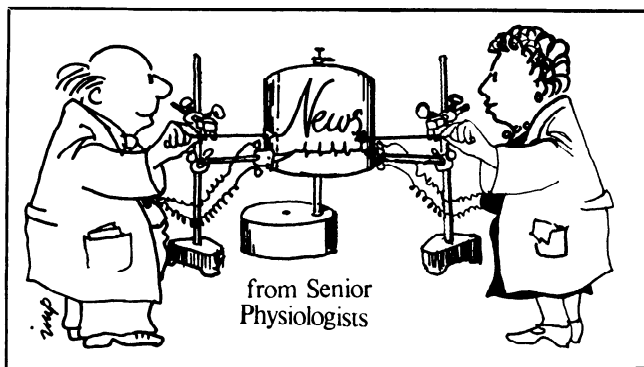
Effective October 1, 1984, **Theodore Cooper**, APS member and member of the APS Financial Development Committee, became vice chairman of the board of The Upjohn Company. As vice chairman, Cooper will retain his responsibilities for worldwide pharmaceutical research and development, medical affairs, regulatory affairs, pharmaceutical quality control, and financial. In addition, he will be responsible for corporate legal administration.

Glenn Advances at Ciba-Geigy

APS member **Thomas Glenn** of Berkeley Heights has been appointed senior vice president of research and a member of the pharmaceuticals division management committee for Ciba-Geigy Pharmaceuticals of Summit, New Jersey. He most recently served as executive director of biology research, the position he has filled since he joined the company in 1982. Prior to joining the company, he was the codirector of the clinical pharmacology research unit, University of South Alabama College Medical Center, as well as chairman of the Department of Pharmacology at the University of South Alabama College of Medicine.

Wellcome Visiting Professorships

Announcement of Wellcome Visiting Professorships in the Basic Medical Sciences for the academic year 1984-85 was made by the Federation of American Societies for Experimental Biology and The Burroughs Wellcome Fund. These Visiting Professorships are designed to stimulate interest in the basic sciences and to recognize eminent scientists in Physiology, Biological Chemistry, Pharmacology, Pathology, Nutrition, Immunology, and Cell Biology and are offered on an annual basis. The constituent societies and the Executive Committee of FASEB chose the Medical College of Ohio at Toledo to host APS member **Robert M. Berne** and the University of Missouri College of Economics to host APS member **Alfred E. Harper**.



Earl H. Wood to E. B. Brown:

Although officially retired for 2 years and with no laboratory facilities at my disposal, I have been nearly as busy as ever. Ada and I spent 6 months in Kiel, Germany, May–Nov. 1983, supported by a Humbolt Award for senior US Scientists from West German Government. I worked at the Kinder Klinik of the University of Kiel with Prof. Paul Heintzen, who is an expert in computer-based dynamic angiocardigraphic technics. They are interested in the Dynamic Spatial Reconstructor (DSR) system developed in our laboratory here in Rochester. Because of technological roadblocks gradually being overcome here in Rochester, the system is still ahead of its time in Europe. However, there is a remote possibility I may return to Kiel in 1985 to expedite introduction of such an updated system at that time. We enjoyed very much our stay in Kiel and attendance at meetings in Europe and in Moscow for IUPS 5th International Symposium on Gravitational Physiology. Since return have been involved with the APS ad hoc Committee on Edward Taub case as chairman, also on APS Finance Committee, and on FASEB Building Development Committee. In March I gave two talks at Oral Roberts University. Dr. Marion Ledbetter, Pediatric Cardiologist there and one of my former Fellows, inveigled me into this as a stopover on the way to St. Louis and then to Washington, D.C. to a 2-day symposium organized by NIH at National Academy of Science on Animals vs. Human Welfare Problems. In any event we are still busy and enjoying life.

Mayo Foundation and Mayo Medical School
Rochester, MN 55901

John R. Brobeck to E. B.:

For the past two years the School of Medicine has generously continued my employment on a full-time basis even though officially I am a Professor Emeritus. In each spring semester I teach an undergraduate course in "Mammalian Physiology" for 80–120 students, using Arthur Guyton's intermediate text, *Human Physiology*. It is less rigorous and more fun than the medical course was. Throughout the year I have some modest responsibilities as a go-between for Deans of the health schools, the Vice President for Health Affairs, and the office of our Provost, relative to appointments and promotions. But research has gone by the board; I could not persuade the authorities at NIH that what I wanted to learn about control of food intake was important enough for support.

Dept. of Physiology G4
University of Pennsylvania
Philadelphia, PA 19104

Harry Adler to Bob Alexander:

After a career divided between the US Air Force and the private practice of medicine, during which I published some 70 papers on colon physiology and aviation medicine plus a monograph *Dysbarism*, my writing is now confined to a novel (sales = zero) and regular letters to the President and my congressman expressing my righteous indignation. I am also busy ruining a perfectly good house with half a dozen "do-it-yourself" kits and am proving that you should never plant a larger garden than your wife can take care of.

122 Ware Blvd.
San Antonio, TX 78221

D. Bruce Dill to Bob:

I have an appointment as research professor, University of Nevada, Las Vegas, and after closing our Boulder City laboratory in 1980 maintain a small office at home with a desk, typewriter, three filing cabinets, a bookcase, and telephone. I have a small residue of funds that makes possible the help of two part-time students, one a typist.

My most recent paper was published in 1983, and for the last year I have been writing a small book based on our desert studies, *The Hot Life of Man and Beast*, which Charles C. Thomas has agreed to publish.

My 93rd birthday, on April 22, was celebrated at the home of our long-time friend Marguerite Knickerbocker in the picturesque nearby desert town of Nelson. Participating were my daughter Betty, my son-in-law S. M. Horvath, Director of the Institute of Environmental Stress, Santa Barbara, my grandson Mike Horvath and his wife Claudia, and my great-grandson Devin Horvath, 18 months.

That evening at home in Boulder City, three mothers whose sons and a daughter were my laboratory assistants in 1966 came in. I had about 40 such assistants from 1966 to 1980. All were honor students in science and mathematics at the Boulder City High School. I called myself their surrogate grandfather. They and their parents were and are my good friends. Friendships with these talented young men and young women and with their parents have been a continual source of pride and happiness.

303 Wyoming St.
Boulder City, NV 89005

Otto Edholm to Bob:

Thank you for your letter which arrived on my 75th birthday. Although I have not been too well lately, when I get *The Physiologist* I turn to news of Seniors and feel I am in touch with old friends and colleagues. May I thank you and the Society for your interest in Senior Citizens.

9 Church St.
Hungerford, Berkshire RG17 0JG, UK

John F. Hall, Jr., to Bob:

Since my retirement from the Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base in 1972 after 32 years of service, I have remained fairly active, and both I and my wife are reasonably healthy. My chief interests are travel, photography, book collecting, visits with our families and relatives, and

reading. I especially enjoy each copy of *the Physiologist* and find many articles in it quite stimulating. I also carefully read *Science* each week, and while I find some of the recent articles on molecular biology, genetics, and immunology difficult going, I nevertheless manage to grasp the exciting and stimulating aspects that modern research in these areas almost weekly presents. Monoclonal antibodies, for example, do appear a most promising development in the search for cancer therapy. Occasionally I try to attend either a Spring or Fall Meeting of the APS, and these occasions are also stimulating. I wish to thank you and the Society for your kind greetings, especially since this year I observed my 75th birthday!

5989 Helenwood Dr.
Dayton, OH 45431

Robert E. Johnson to Bob:

Your committee does a valuable service by keeping us in touch with friends and colleagues whom we may not see often enough. My wife, Margaret, and I have now lived in rural Vermont for some four years after leaving Illinois in 1980 to be near our younger son, Charles, who is State Naturalist for Vermont. We are both well and active and will celebrate our fiftieth wedding anniversary in January 1985. My professional life is busy, although I don't teach any more and do miss the students. I keep a colony of *Terrapene carolina carolina*, trying to find out how these land turtles are able to live so long (over 150 years sometimes) and almost never develop any kind of cancer. I gave a paper on them at the XXIX IUPS Congress in Australia. I have written on nutrition, environmental stress, and the history of biogeography in the 19th century and edited a book written by an old friend and colleague, the late C. Frank Consolazio on *Nutrition and Performance* (Pergamon Press, 1983). I am able to visit the University of Vermont in Burlington every once in a while and have found congenial colleagues in the Departments of Physiology and Biophysics, Medicine, and the History of Medicine group. My wife and I enjoy ourselves both professionally and socially in this University community.

Horn of Moon Enterprises
RFD 1
Montpelier, VT 05602

Frederic T. Jung to Bob:

I live in a retirement home for men. It is wonderfully well administered; its location in downtown Evanston, with easy access to post office, shops, banks, and transportation has many advantages. I read *JAMA*, *Scientific American*, and *Science News*. I have not done any laboratory work since I left Northwestern University Medical School to join the headquarters staff of the American Medical Association in 1946. Since then my work has been mostly journalistic. My typewriter is constantly busy with correspondence and with reports to my family on history and keepsakes. I still contribute papers to programs of the Chicago Literary Club, of which Carlson, Cannon, Luckhardt, the two Dragstedts, Wartman, Gerard, von Bonin, and McCullough were such enthusiastic members. I continue my allegiance to science to the extent that I have opportunities to plead for the critical attitude of scientists in a world confused by superstition and pseudoscience. It is still necessary to

present scientific research as a "standard to which thoughtful men can repair." A word of caution to my younger colleagues would be to urge them to be extremely careful about even the simplest experimentation on human subjects, especially on themselves.

1555 Oak Ave.
Evanston, IL 60201

Leon C. Chesley to Roy Greep:

In response to your letter for my 76th birthday, I'm still active and feel as frisky as a colt. When I faced mandatory retirement at age 70, I fell victim to an act of criminal insanity: my laboratory was converted to two two-bed wards in a hospital rumored to imminently suffer a cut of 50% in beds. I keep busy writing chapters for books, reviews, and papers based on data accumulated over the years in lectures to medical students and various meetings and consultations. Except for working in the laboratory, I'm doing all that I ever did. The difference is no pay. I shouldn't presume to offer any words of wisdom; my own credo is "keep busy."

450 Clarkson Ave.
Brooklyn, NY 11203

J. Raymond Johnson to Roy:

Thank you for your birthday greetings and note. A little over two years ago, I started a letter to Hal Davis, but for some reason I didn't ever finish it or get it in the mail. After apologizing to Hal and to other members of the Senior Physiologists Committee, I proceeded to describe some of my activities and travels I had made with my wife Teri after my retirement in 1976. Even though I was beginning to feel the effects of old age by 1982, I could still manage to play nine holes of gold, climb our stairs that seemed to be ever increasing in length and steepness, and do most of my daily chores. In that letter to Hal, I also reminisced over some of my active years in physiology and recalled with pride my earlier associations with some of our outstanding APS members, both past and present.

During the past two years, life has changed a great deal for me. This is probably one of the reasons why I never finished the letter to Hal Davis. Teri, who had begun to suffer more from long existing osteoporosis, began to show definite signs of another much more serious malady. Before the end of 1982, she required so much care that someone had to be with her almost constantly. In order to have some free time for other responsibilities, I had a home health care service send someone in for four hours a day. For a little over two months in the spring of 1983, I placed Teri in a nursing home while I was recovering from a back injury. And now, over a year later, I am still scheduling more than 80% of my time to the care of a 79-year-old invalid with Alzheimer's disease. She is not exactly the same person I married and lived with for over fifty years, but she is still my wife and I love her very much. I intend to continue taking care of her in our own home as long as I am physically able to do so, rather than submit her to the loneliness and feeling of insecurity she would suffer in the environment of a nursing home.

Creighton University
Omaha, NE 68178

W. T. Liberson to Roy:

Thank you for thinking of me on my 80th birthday. Contrary to what the younger set expects from an octogenarian, my activities, interests, and thoughts are not much different from what they were when I was in my twenties. I still go to my lab of clinical neurophysiology between 8 and 9 A.M. and do a full day's work of patient care, research, and teaching. I still do my writing and reading on weekends, when I also edit colleague's papers. I am also still writing poetry and am preparing a second edition of *Eugene Onegin Revisited*.

I have always believed that neurological diseases could be treated in a more innovative way than has been done in traditional surgery. I am pleased to report that I recently joined a microsurgeon in Norfolk, Virginia, who has confirmed my fondest hopes. I believe that what Dr. Julia Terzis does so brilliantly in patients with brachial plexus injuries can be applied to several other neurological diseases. I firmly believe that rerouting nerve messages along interposition grafts as she does in catastrophic nerve injuries can improve the prognosis of hemiplegics, paraplegics, patients with muscular dystrophy, etc.—with the help of rehabilitation-minded clinical neurophysiologists, of course.

I still feel as strongly as ever that confirmed unexpected observations are more conducive to scientific progress than theories which are vulnerable to new discoveries and techniques. I believe that the limitations of theories have unfortunately detracted from the historical achievements of some of my mentors, such as Wedensky, Pavloff, Lapiques, and Richet. A case in point is my recent reassertion of the law of "3.5" that I observed and formulated 50 years ago. According to this law most of brain wave frequencies are multiples of 3.5 cycles per second, either on macro- or microlevels. I have not to date found any evidence to change my belief in this observation.

In regard to more general issues, no matter how much I hate the dictatorial aspects of communism which I escaped from in the twenties, I believe, because it is rooted in humanitarian ideas of the last century, that a skillful negotiator or a brilliant writer will someday be able to convince Russian pragmatists that the no. 1 enemy of Russian communism is the buildup of nuclear armaments and that the danger of nuclear war dictates a radical revision of Soviet revolutionary tactics. Once this has occurred, America will take care of its own nuclear strategists.

435 W. 57th St., Room 3L
New York, NY 10019

Herbert Pollack to Roy:

How thoughtful of you and the APS to "remember" my birthday. It was a very pleasant day with many telephone calls and cards. At my request everything was kept at a low key. However, next year when I celebrate my eightieth I will pull out the stops. The fringe benefits of 80 include life membership at the Cosmos Club without any more dues!

While I have been retired many times I have never stopped working. Currently I am consultant to the US Department of State in matters relating to health hazards from exposure to electromagnetic radiation. In the past 24 months I have served as consultant to Comsat, MCI, Pennsylvania Bell, New Jersey Bell, ITT,

Atlantic Satellite, Rochester Telephone, Natural Gas Pipeline of America, and several TV stations across the country. The Engineering Foundation has invited me to serve on two panels at their coming meeting. The *Southern Medical Journal* published an article of mine in the June 1983 number. To carry out my responsibilities I now use an IBM-PC computer. I tap the data banks including the National Medical Library from my desk and keep up to date in my field. I attend some meetings, but not as many as I used to. The computer saves me much of that time and effort, but I miss the personal contacts and the boardwalk in Atlantic City.

When I was first retired I missed a secretary and went to business school to learn typing. The Word Processor software in my computer has been the greatest thing since the self starter in the automobile. My letters and manuscripts are clean and professional looking. My regards to all of my friends who may read this chitchat. Let the younger ones take heart, senility does not necessarily begin at 65, or at least I may not be aware of it.

2700 Calvert St., NW
Washington, DC 20008

Donald F. Proctor to Arthur Otis:

Your letter of inquiry has reached me just as I passed my 71st birthday, an occasion I never expected to enjoy since I have a congenital cardiac condition. At the time I entered medical school 51 years ago, my life expectancy was said to be to the age of 21 years. Somehow, in spite of that dismal outlook, I continue to feel healthy and enthusiastic about life as a whole. We have just roughed out studios in our barn so that Janice has her own area for painting and I have a spot which I can freely litter with chips from my wood carving.

I am still working and generally at my office each morning. My work as Deputy Editor of the *American Review of Respiratory Disease* takes me to that office every afternoon. Owing to some reduction in the acuity of my eyesight I see only an occasional patient, usually a singer with voice problems. My book on breathing, speech, and song has attracted occasional favorable comment from teachers of singing. My latest book, on nasal physiology, seems to have provided more information than most people want to know. I do have two chapters coming out in the new *Handbook of Physiology*, the Macklem-Mead volume, and I have been involved in five scientific papers during the past year. As you know my major effort during what years remain to me is bent on the history of the physiology of breathing. So far I have more or less completed chapters on ancient history, Galen, and Harvey, and am now making progress on the exciting 17th century in England, about which R. G. Frank has already written so beautifully.

My only "words of wisdom" for younger colleagues would involve two points. Keep an active interest in some nonscientific phase of life. Mine has been focused on singing and sculpture. And it is amazing to find the quantity of work one can do if you make good use of the odd hour or two which may turn out to be free unexpectedly. I generally keep some jotting paper available for such occasions. I shall always look back upon the year in Rochester with you, Hermann, and Wallace as one of the happiest and most stimulating of my life.

Johns Hopkins University
Baltimore, MD 21205

Hiawatha Designs an Experiment

Maurice G. Kendall

Hiawatha, mighty hunter
He could shoot ten arrows upwards
Shoot them with such strength and swiftness
That the last had left the bowstring
Ere the first to earth descended.
This was commonly regarded
As a feat of skill and cunning.

One or two sarcastic spirits
Pointed out to him, however,
That it might be much more useful
If he sometimes hit the target.
Why not shoot a little straighter
And employ a smaller sample.

Hiawatha, who at college
Majored in applied statistics
Consequently felt entitled
To instruct his fellow men on
Any subject whatsoever,
Waxed exceedingly indignant,
Talked about the law of error,
Talked about truncated normals,
Talked of loss of information
Talked about his lack of bias
Pointed out that in the long run
Independent observations
Even though they missed the target
Had an average point of impact
Very near the spot he aimed at
(With the possible exception
Of a set of measure zero.)

This, they said, was rather doubtful.
Anyway, it didn't matter
What resulted in the long run.
Either he must hit the target
Much more often than at present
Or himself would have to pay for
All the arrows that he wasted.

Hiawatha, in a temper
Quoted parts of R. A. Fisher
Quoted Yates and quoted Finney
Quoted yards of Oscar Kempthorne
Quoted reams of Cox and Cochran
Quoted Anderson and Bancroft
Practically in extenso
Trying to impress upon them
That what actually mattered
Was to estimate the error.

One or two of them admitted
Such a thing might have its uses
Still, they said, he might do better
If he shot a little straighter.

Hiawatha, to convince them
Organized a shooting content
Laid out in the proper manner
Of designs experimental
Recommended in the textbooks

(Mainly used for tasting tea, but
Sometimes used in other cases)
Randomized his shooting order
In factorial arrangements
Used in the Theory of Galois
Field of ideal polynomials
Got a nicely balanced layout
And successfully confounded
Second-order interactions.

All the other tribal marksmen
Ignorant, benighted creatures,
Of experimental set-ups
Spent their time of preparation
Putting in a lot of practice
Merely shooting at a target.

Thus it happened in the content
That their scores were most impressive
With one solitary exception
This (I hate to have to say it)
Was the score of Hiawatha,
Who, as usual, shot his arrows
Shot them with great strength and swiftness
Managing to be unbiased
Not, however, with his salvo
Managing to hit the target.

There, they said to Hiawatha,
That is what we all expected.

Hiawatha, nothing daunted,
Called for pen and called for paper
Did analyses of variance
Finally produced the figures
Showing beyond peradventure
Everybody else was biased
And the variance components
Did not differ from each other
Or from Hiawatha's
(This last point, one should acknowledge
Might have been much more convincing
If he hadn't been compelled to
Estimate his own component
From experimental plots in
Which the values all were missing.
Still, they didn't understand it
So they couldn't raise objections,
This is what so often happens,
With analyses of variance.)

All the same, his fellow tribesmen
Ignorant, benighted heathens,
Took away his bow and arrows,
Said that though my Hiawatha
Was a brilliant statistician
He was useless as a bowman,
As for variance components
Several of the more outspoken
Made primeval observations
Hurtful to the finer feelings
Even of a statistician.

In a corner of the forest
Dwells alone my Hiawatha
Permanently cogitating
On the normal law of error
Wondering in idle moments
Whether an increased precision
Might perhaps be rather better
Even at the risk of bias
If thereby one, now and then, could
Register on the target.

The author, the late Sir Maurice George Kendall was a highly respected British statistician. Knighted for his many contributions, he was also President of the Royal Statistical Society, Honorary President of the International Statistical Institute, and a Fellow of the American Statistical Association among his many honors and associations. This poem was published in *The American Statistician* 13:5, December, 1959, and is reprinted here with the permission of that journal.

A New 'Physiology in Medicine' Series: Gastrointestinal Disorders

Editorial This issue of HOSPITAL PRACTICE contains the first of a series of seven articles that will appear monthly and deal with the physiology and pathophysiology of gastrointestinal disorders. These articles continue the "Physiology in Medicine" series jointly presented by this journal and the American Physiological Society.

It comes in the wake of a six-article series on dynamic imaging of the coronary circulation in health and disease. It was with that series that the American Physiological Society revived its "Physiology in Medicine" program specifically designed to provide practicing physicians with an ongoing correlation between basic physiologic concepts and developments and the problems confronted in clinical diagnosis and therapy. That program had previously been conducted as a joint effort with *The New England Journal of Medicine*.

In a sense the second series will have a more ambitious objective than the cardiac imaging unit, which focused almost exclusively on ischemic heart disease—a protean problem, to be sure. On the other hand, the gastrointestinal series will address a wide range of common and often perplexing clinical entities, focusing particularly on certain aspects of hepatic dysfunction, on the metabolic derangements leading to gallstone formation, on the pathophysiology of the secretory diarrheas, on the physiology of gastric acid secretion and the pathogenesis of peptic ulcer, and

on bilirubin metabolism and congenital jaundice.

It is an ambitious undertaking, and we feel most fortunate that our collaborators include some of the most outstanding contributors to and teachers of current physiologic and pathophysiologic concepts. A rundown of the series attests to this, as well as to the breadth of the content.

The first article in the series, written by Mortimer Levy and Marvin J. Wexler of McGill University, appears in this issue of HOSPITAL PRACTICE. Its subject is the physiology and pathophysiology of salt and water balance in liver disease. Levy and Wexler review currently proposed pathogenic mechanisms for inordinate renal salt and water avidity in liver disease. Specifically, they focus on two mechanisms currently invoked to account for salt and water accumulation in hepatic disease: the "overfilling" hypothesis, which proposes that a primary signal from the liver provides the stimulus for salt and water retention in hepatic disease; and the "underfilling" hypothesis, which proposes that abnormalities in hepatic perfusion referable to the cirrhotic process produce underfilling of the arterial tree, a reduction in effective circulating volume, and secondary salt and water retention by the kidney.

The second article in the series, by Steven Schenker and Anastacio M. Hoyumpa, Jr., of the University of Texas, San Antonio, reviews the physiology and pathophysiology of hepatic coma. Schenker and Hoyumpa will provide a summary of the means used to produce hepatic coma experimentally, of the contribution of the liver to hepatic encephalopathy, of brain pathology in hepatic encephalopathy, and of the roles of various toxins—for example, ammonia,

This editorial is by Thomas E. Andreoli, Professor and Chairman of the Department of Internal Medicine at the University of Texas Health Science Center, Houston. Dr. Andreoli serves as the editor of "Physiology in Medicine" for HOSPITAL PRACTICE and the American Physiological Society.

Editorial

mercaptans, and fatty acids—in the pathogenesis of hepatic encephalopathy. The article will also discuss the changes in brain neurotransmitters in hepatic encephalopathy and the ways in which these changes derange cerebral function.

In the third article of the series, David H. Van Thiel of the University of Pittsburgh will review the effects of ethyl alcohol upon gonadal function. It is, of course, well recognized that chronic alcoholism in men is accompanied by hypogonadism. Van Thiel will present an argument consistent with the view that it is the alcohol abuse per se, rather than the associated alcoholic liver disease, that is primarily responsible for hypogonadism. Van Thiel will also propose that the feminization seen in alcoholic men—for example, gynecomastia—requires the presence of both liver disease (most notably portosystemic shunting) and alcohol abuse.

The fourth article in the series, by Henry J. Binder of Yale University, will deal with the pathophysiology of the secretory diarrheas. Binder's article will review the derangements in ion secretion and reduced ion absorption that result in diarrhea. The article will also provide a summary of the normal mechanisms for salt and water absorption by the small intestine and describe the role of various secretagogues in the pathogenesis of diarrheas induced by infection and by various laxatives.

In the fifth article of the series, Bernard F. Smith and J. Thomas Lamont of Boston University will review the pathogenesis of gallstones. The article will provide a careful analysis of the role of bile acids in minimizing cholesterol deposition in the biliary tract, the abnormalities in lipid metabolism in individuals with gallstones, and therapeutic strategies that follow from

those pathophysiologic considerations.

The sixth article, by Jon I. Isenberg of the University of California, San Diego, will consider the physiology of gastric acid secretion and the pathogenesis of peptic ulcer disease. Isenberg will provide a frame of reference for understanding the mechanisms of hydrochloric acid secretion in the gut. He will also assess the role of normal protective factors that prevent autodigestion and the derangements of those factors in peptic ulcer disease. Contributions of various agents, both provocative and protective, in the pathogenesis and treatment of peptic ulcer disease will be reviewed.

Finally, the gastrointestinal series will conclude in January 1985 with an article by John L. Gollan of Brigham and Women's Hospital, Boston, on bilirubin metabolism and congenital jaundice. This presentation will summarize our knowledge of the processing of bilirubin by hepatocytes and will discuss in detail various congenital abnormalities of liver function that result in jaundice, among them Gilbert's disease, the Dubin-Johnson syndrome, and Rotor's syndrome.

The diversity that will be represented in this series is reflective of the diversity of the processes required for humans to fulfill one of the most basic of the requirements of all living organisms: the ingestion, transport, and metabolism of nutrients. As with the cardiac imaging articles, the goal of this series will be to shed light on such basic processes, in this instance the light of the dramatic achievements in understanding that have been made by physiologists and their closely allied scientific colleagues.

THOMAS E. ANDREOLI, M.D.

Announcements

NSF Seeks Nominations for Tenth Alan T. Waterman Award

The National Science Foundation Alan T. Waterman Award Committee has issued a call for nominations of candidates for the tenth annual award. Intended to give recognition to an outstanding young researcher in any field of science, mathematics or engineering and to encourage further high quality research, the award was established by the Congress in 1975 to mark the 25th anniversary of the National Science Foundation and to honor the first Director of the Foundation, Dr. Waterman. In addition to a medal, the recipient receives up to \$50,000 per year for up to three years of research or advanced study in the mathematical, physical, medical, biological, engineering, social or other sciences at the institution of the recipient's choice.

Deadline for nominations: December 31, 1984. **Information:** Mrs. Lois J. Hamaty, Executive Secretary for Alan T. Waterman Award Committee, National Science Foundation, 1800 G St., NW, Washington, DC 20550. Phone: (202) 357-7512.

Clinical Cytopathology for Pathologists 1985 Postgraduate Institute

The Twenty-sixth Postgraduate Institute for Pathologists in Clinical Cytopathology given by The Johns Hopkins University School of Medicine, consists of two courses, both of which must be taken: March-May 1985, Home Study Course A is provided each registrant for intensive personal study; and May 6 and 17, 1985, In-Residence Course B at the Johns Hopkins Medical Institutions, Baltimore, MD. This Institute is designed solely for pathologists who are certified (or qualified for certification) by the American Board of Pathology or its international equivalent. It provides an intensive refresher in all aspects of the field of Clinical Cytopathology with time devoted to newer developments and techniques, special problems, and recent applications including immunodiagnosis and needle aspiration. Topics are covered in lectures, explored in small informal conferences, and discussed over the microscope with the Faculty. Abundant self-instructional material is available to augment at individual pace. A loan set of slides with texts (Course A) will be sent to each participant within the United States and Canada for home-study during March and April before Course B in Baltimore. Prior

special arrangements to study Course A must be made by participants outside of the United States and Canada. Credit hours 152 in AMA Category I.

Registration deadlines: Application and completed pre-registration is advised before November 30, 1984; however, completed pre-registration must be accomplished before April 5, 1985, unless by special arrangement.

Information: John K. Frost, 604 Pathology Bldg., The Johns Hopkins Hospital, Baltimore, MD 21205.

Dynamic Aspects of Microtubule Biology

A Conference on Dynamic Aspects of Microtubule Biology, sponsored by the New York Academy of Sciences, will be held at the Barbizon-Plaza Hotel, New York City, December 3-6, 1984. **Theme:** This conference will focus on expression of genes for tubulins and MAPs, properties and functions of MAPs, microtubule assembly in vitro and in vivo, actions of drugs on microtubules, interactions of microtubule proteins with other cell constituents, and the molecular basis of microtubule function. The conference will feature invited lecturers and a limited number of poster presentations. **Chairman:** David Soifer, Ph.D. **Information:** Conference Department, The New York Academy of Sciences, 2 East 63rd St., New York, NY 10021.

Mathematical Modelling in Physiology and Clinical Medicine

The Second International Summer School on Mathematical Modelling in Physiology and Clinical Medicine will be held at the University of Padua, Padua, Italy, June 3-7, 1985. **Sponsors:** Centre for Bioengineering, University of Padua; Institute for Research in System Dynamics and Bioengineering—CNR, Padua; Research Centre for Measurement and Information in Medicine, The City University, London; and Royal Free Hospital and School of Medicine, London. **Aims:** The Summer School will make available recent advances in modelling methodology to clinicians and clinical scientists in Europe, appraising them with current modelling practice, and indicating through examples and case studies the applicability of such approaches to complex problems in physiology and medicine. **Scope:** The course will show what modelling theories and techniques exist, how they can be applied to real world problems, indicating the successes which can be achieved and, equally, the constraints which must be observed. It will also show how dynamic modelling can provide insight into the nature of physiological processes, can be used clinically for teaching diagnosis and patient management, and can also be a useful aid in relation to experimental design and laboratory organization.

Information: Prof. C. Cobelli, Dept. of Electrical Engineering and Electronics, University of Padua, Via Gradenigo, 6A, 35131 Padova, Italy, or Dr. E. R. Carson, Research Centre for Measurement and Information in Medicine, The City University, Northampton Square, London EC1V 0HB, UK.

Future Meetings

1985

FASEB Annual Meeting	April 21-26, Anaheim
Joint APS/The (British) Physiological Soc Mtg	Sept. 12-14, Cambridge (UK)
APS Fall Meeting	October 13-18
	Niagara Falls/SUNY, Buffalo

1986

FASEB Annual Meeting	April 13-18, St. Louis
IUPS Congress	July 12-20, Vancouver, Canada
APS Fall Meeting	Oct. 5-10, New Orleans

THE PHYSIOLOGY TEACHER

Some Comparative Aspects of the Renin-Angiotensin System

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Ann Arbor, Michigan 48109

Ever since Tigersted and Bergman's work of nearly a century ago (33), considerable attention has been directed at the renin-angiotensin system (RAS). Investigators have studied the biochemical, physiological, pharmacological, and even the evolutionary aspects of the system. However, essentially all of the information we have concerning the RAS has come from experiments with mammals. We can now characterize the function of this system as one of maintenance of salt and water balance. The end product of renin secretion, angiotensin II (ANG II), is a very potent hormone which, as will be seen later, partially controls many of those systems that regulate quantity of water and salt ingested and excreted.

Since this paper concerns comparative aspects of the renin-angiotensin system, the function of the RAS in as many different species as possible are considered. A paper which promises to consider "comparative physiology" implies that the author will follow the same sequence recommended to Alice by the Queen of Hearts: "Begin at the beginning, go on to the end, then stop." But I will begin by presenting a view of the functions of the RAS as defined in mammalian species, working backward to the earlier species in which the RAS is found. This chronology allows us to consider how the system arose and what its original function was; by tracing the RAS backward, it may be possible to discern where some of the changes occurred in the system and perhaps even why. I will try to present a view of what I believe to be now established, giving data relating to those effects in mammalian and nonmammalian species, and finally, I will offer some speculation concerning evolution of function—all from our limited knowledge of the effects of ANG II in animals other than mammals.

Many hormones control both salt and water; in periods of deficit, animals have a strong drive to ingest both. Conversely, during periods of surfeit, the body is programmed to eliminate these substances in greater quantities. Physiologists are concerned with those mechanisms by which both the deficit and surfeit are recognized and how this recognition changes rates of intake or excretion. We also know that change in the volume of the extracellular fluid compartment often leads to a change in blood pressure. Thus it is not accurate to characterize the RAS as a system solely concerned with salt and water balance; we must also acknowledge that the RAS is intimately concerned with control of blood pressure secondary to changes in the volume of the fluid compartments. The RAS also directly affects blood pressure through its action on the vasculature.

Figure 1 shows the biochemical steps involved in the production of the active peptide, ANG II. The kidney is the main site for the production of renin. The liver produces a substrate upon which renin acts in the blood to cause the liberation of a decapeptide, angiotensin I (ANG I). ANG I, in turn split by converting enzyme, an enzyme which cleaves two terminal amino acids to form an octapeptide, ANG II, the most active component of the system. Converting enzyme is found mainly in the lungs but also in other tissues. However, most of the conversion occurs in a single pass through the lungs. Currently, there are drugs that can block this system at two different points. Competitive antagonists, such as saralasin, are blockers at the level of the ANG II receptor; i.e., they bind to the receptor, preventing ANG II from binding. The second class of drugs are converting enzyme inhibitors, which prevent conversion of ANG I to ANG II. Either one of these drugs is able to decrease the action of the RAS, but they do so in slightly different ways.

Although Figure 1 implies that the kidney is the only source of renin, this may not be the case. There is growing evidence that the brain and some other tissues also produce renin and all of the requirements of a RAS; i.e., renin, substrate, and converting enzyme are all present in the brain tissue such that ANG II can be formed endogenously.

Blood Pressure

The original observation of Tigerstedt and Bergman was that a crude extract of kidney, when injected into another animal, caused a rise in blood pressure. This is now so well documented that no controversy remains. The only remaining controversy concerns the mechanism of action. Certainly injection of renin causes an increased generation of ANG II. It is well documented that ANG II is a potent vasoconstrictor. Thus the direct action of ANG II on vascular smooth muscle is vasoconstriction, leading to increased total peripheral resistance (TPR) and increased blood pressure. In the mam-

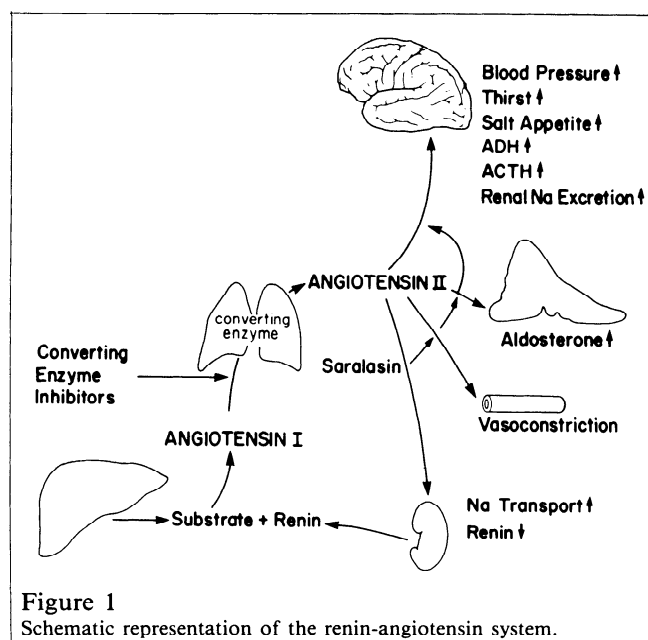


Figure 1
Schematic representation of the renin-angiotensin system.

mal all vascular beds tested respond in this way. However, there is another route of action. If ANG II is injected into the cerebral ventricles, it produces an increase in blood pressure which exceeds that if the same dose is injected intravenously (16). Data from experiments such as these indicate a central mode of action.

Antidiuretic Hormone

Although it is established that central injections of ANG II increase blood pressure through mechanisms other than antidiuretic hormone (ADH) release, we now also know that ADH is involved in the response. Bonjour and Malvin (4) showed that intravenous or intracarotid infusion of ANG II increased the circulating levels of ADH. Furthermore, Mouw et al. (22) demonstrated that infusions of very small doses of ANG II into the lateral ventricles result in substantial increases in ADH release. Though it appeared probable that this release was due to receptors located in the posterior pituitary itself, this was found not to be the case. If the posterior pituitary was incubated *in vitro* and ANG II was added to the medium, ADH release did not increase; in fact, it decreased (12). This was presumably due to the vasoconstriction caused by the high concentrations of ANG II in the medium. However, if an intact system was used, i.e., the posterior pituitary still connected to the hypothalamus by the stalk, and the experiment was repeated, a sixfold increase in ADH release occurred (12). These results are shown in Figure 2 and were interpreted to indicate that some hypothalamic nucleus, presumably the supraoptic or paraventricular, was receptive to ANG II and signaled the posterior pituitary to release ADH in response to ANG II stimulation.

However, questions arose concerning the physiological significance of this response. Is the response an important one? Can ANG II reach these receptor sites from the blood? And finally, can the brain itself either make ANG II from its own RAS or at least generate ANG II from some of the components that reach it via the blood? The data lead me to believe the answer to all of these questions is yes. However, some controversy is apparent in the literature. Some early studies suggested that circulating ANG II cannot stimulate the posterior pituitary to release ADH. These investigators, infusing ANG II at different concentrations, reported no significant increase in circulating levels of ADH (5, 30).

However, interpretation should be made with caution. ANG II is a potent vasoactive drug, and in most instances mean arterial blood pressure increased. We know that high-pressure receptors affect the rate of secretion of ADH; when pressure increases, ADH secretion is inhibited (30). Thus, in many experiments wherein ANG II was infused peripherally, one must consider that the negative effect of the increase in blood pressure on the secretory rate may mask any positive effect on ANG II itself.

A second difficulty in interpreting results of peripheral ANG II infusion is the fact that even if ADH release is stimulated immediately, the increased release is not immediately evident. Some finite time must pass before a statistically significant change in concentration is seen. These two points are well illustrated by the following studies.

Brooks and Claybaugh (5) infused ANG II intravenously in conscious dogs. Figure 3 shows their results.

Although ADH levels rose upon infusion, statistical significance was obtained only at 30 min following the start of infusion.

Mitchell et al. (21) looked at the problem differently. They recorded the spikes of magnocellular neurons of anesthetized rats. Figure 4 shows their results. If ANG II was infused, blood pressure rose and the rate of firing decreased. Similar results were obtained with phenylephrine. However, if the procedure was repeated in sino-aortic-denervated rats, ANG II increased blood pressure *and* the firing rate. Phenylephrine, on the other hand, increased only blood pressure. These data indicate that intravenously administered ANG II is an agonist to those neurons and that high-pressure receptors are inhibitory.

Additional evidence for the action of ANG II on ADH release was obtained by Yamaguchi et al. (34), who measured ADH levels in rats deprived of water. Before measurement, the animals received an intracerebro-

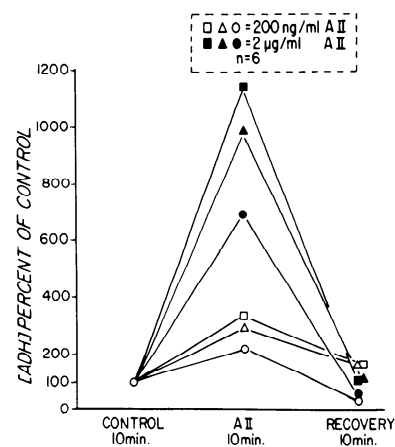


Figure 2
Effect of ANG II on the release of ADH from intact hypothalamohypophysial systems. From Ref. 12.

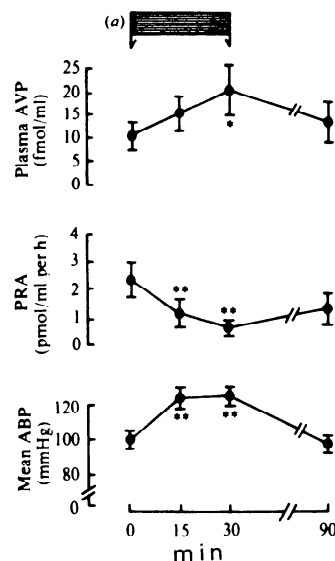


Figure 3
Effects of intravenous infusion of ANG II ($10 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) on levels of plasma arginine vasopressin (AVP), plasma renin activity (PRA), and arterial blood pressure (ABP). From Ref. 5.

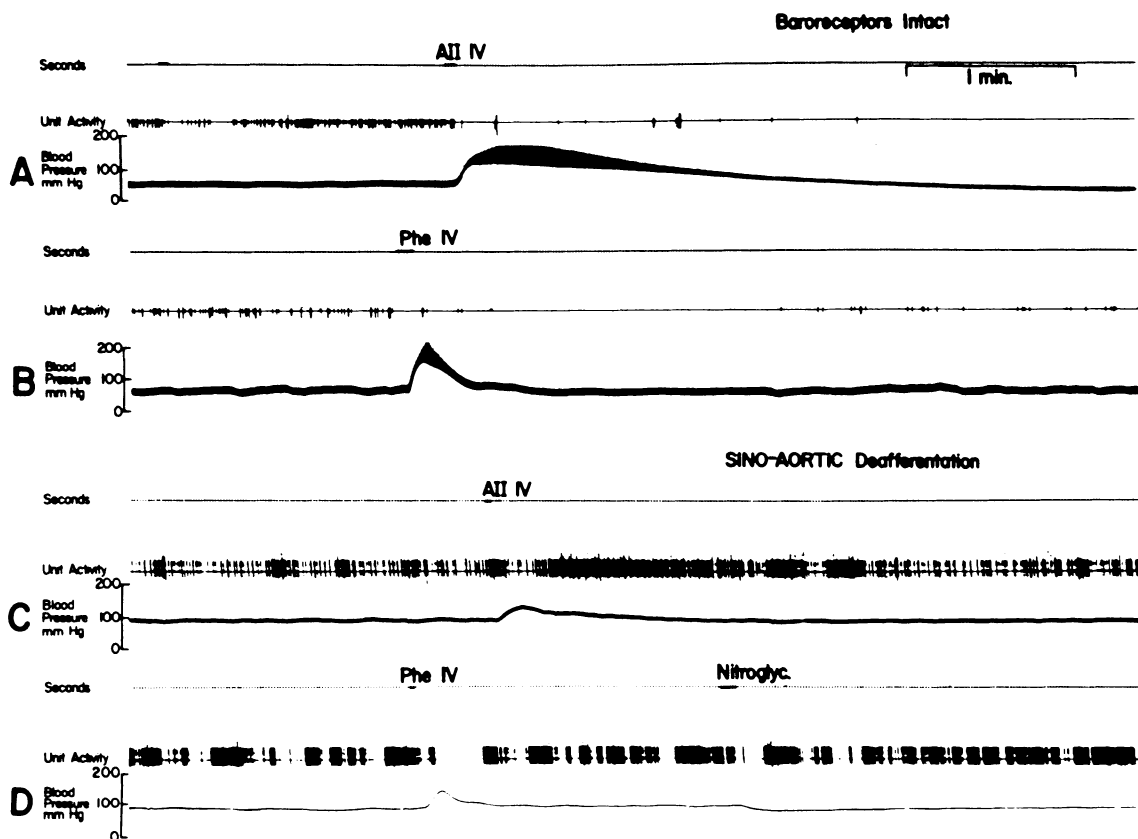


Figure 4

Effect of systemic ANG II on phenylephrine (PhE) in intact rats without high-pressure baroreceptors (e.g., sinoaortic deafferentation, SAD). A: In intact animal, ANG II administered to raise blood pressure by 50 mmHg caused inhibition of a magnocellular neuron. B: In intact animal, PhE titrated to raise blood pressure

by same amount also inhibited a magnocellular neuron. C: In SAD rat, ANG II (12 µg/kg) raised blood pressure to the same level (140 mmHg) without excitation of the magnocellular neuron. Nitroglycerine reduced blood pressure but did not affect the neuron's activity. From Ref. 21.

ventricular injection of the ANG II antagonist saralasin or the vehicle alone. Saralasin significantly lowered the ADH levels in plasma. The interpretation of these experiments is that inhibition of endogenous ANG II reduces the secretory rate of ADH. These data are very strong evidence for a physiological role of ANG II in regulating ADH secretion. Further support comes from the work of Sladek and associates (31). They were able to show that explants of the hypothalamic-pituitary axis increase the secretory rate of ADH in response to increased osmolality of the medium. However, if a converting enzyme inhibitor was first added to the medium, no change in secretory rate occurred. This supports the hypothesis that the brain not only has a functioning RAS but that endogenously generated brain ANG II is an intermediate messenger of increased osmolality.

Drinking Behavior

Many years ago, Fitzsimmons (9) clearly demonstrated that infusions of ANG II are dyspogenic. It does not matter whether ANG II is given intravenously or intraventricularly; both routes of administration result in a powerful dyspogenic response, although the doses required are different, being lowest when given in the cerebroventricular system. But is it physiologically important? I believe so. We have shown that rats that have been water-deprived for 36 hours show an attenuation of the drinking response when the competitive antagonist saralasin is infused intraventricularly (19). Figure 5 presents those results. All but two rats that

received saralasin had delayed and attenuated drinking. In addition, Barney et al. (2) have published data in which intraperitoneal injection of a converting enzyme inhibitor reduced drinking in rats deprived of water for 24, 36, or 48 hours. They concluded that ANG II accounts for part of the drinking response of water deprivation. Fregley and co-workers (11) presented similar data in which the converting enzyme inhibitor MK 421 also attenuated drinking due to dehydration but completely blocked drinking that occurred in response to isoproterenol injection. Isoproterenol is known to stimulate renal renin release; thus the blockade of the effect was thought to be due entirely to inhibition of the RAS.

These data offer strong support for a physiological role of circulating ANG II in the drinking response. Similar results were obtained by Atkinson et al. (1). Renal artery constriction of a uninephrectomized rat produced an increase in plasma renin activity, blood pressure, and drinking. However, if the experiments were replicated in rats infused with saralasin, the drinking response was attenuated, again suggesting that peripherally generated ANG II is dyspogenic.

Salt Appetite

Although we know that during periods of salt deficit animals have an increased salt appetite, the detector for this system remains unknown. However, it is clear that during periods of salt deprivation circulating levels of ANG II increase. One of the controllers of the renin secretory rate is the level of salt intake. As salt intake

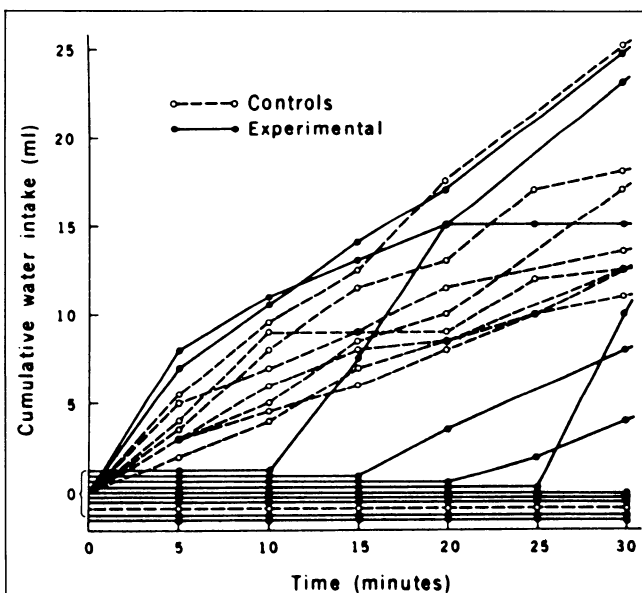


Figure 5

Inhibition of drinking by saralasin. Each line represents the cumulative water intake of one animal infused with artificial CSF with (experimental) or without (control) saralasin (P-113). Of 9 control animals, all drank immediately except one. Of 11 animals infused with P-113, 5 did not drink at all in 30 min and 4 drank only after a delay of 10 min or more. From Ref. 19.

declines, renin secretory rate increases, as does the circulating level of ANG II. It seems natural to hypothesize on this basis alone that ANG II can be a stimulator of salt appetite; in fact, this has been demonstrated. Epstein (8) showed development of an increased salt appetite in rats given continuous infusion of ANG II, an appetite specific for sodium. Furthermore, Fluharty and Epstein (10) have shown that ANG II may act synergistically with other hormones to increase salt appetite. In animals that were sodium replete, deoxycorticosterone acetate (DOCA) increased salt appetite. However, if ANG II was given simultaneously in doses too low to increase salt appetite when given alone, there was a very substantial increase in the salt appetite in the DOCA-treated animals. Thus it appears that ANG II acts synergistically with DOCA to increase the salt appetite. Epstein has shown that even aversive concentrations of NaCl will be consumed by rats given intravenous injections of renin. This indicates a very strong effect on the salt drive of an animal. It should be pointed out that these experiments all suggest a role in the brain for peripherally generated ANG II.

Aldosterone

Some years ago it was demonstrated that intravenous infusions of ANG II are potent stimulators of aldosterone release (18). Additionally, *in vitro* studies show that ANG II acts directly on the adrenal gland. However, aside from a direct effect on the adrenal, ANG II may also work centrally to alter aldosterone release by the adrenals. Brooks and Malvin (Figure 6) administered ANG II by intracerebroventricular infusion to anesthetized dogs and measured aldosterone levels (6), which actually decreased. More significantly, the release increased with infusion of either the converting enzyme inhibitor or the competitive antagonist to the RAS. In the dog, then, a central action of ANG II on aldosterone is apparently opposite to the prediction one would

make according to results from *in vitro* work. In contrast, Nicholls et al. (24), repeating these experiments on sheep, came to opposite conclusions. Intracerebroventricular injection of ANG II increased aldosterone release. These responses were not due to drug spillage into the venous system, since intravenous injection of the same doses were without effect. Furthermore, they were able to show that the intracerebroventricular effect could be blocked by dexamethasone treatment, indicating the mediator of the response was adrenocorticotropin. Thus the sheep appears to respond differently from the dog. The reason is unclear but perhaps has something to do with the fact that the sheep is a ruminant and herbivore, whereas the dog is neither. However, the discrepancy between these two sets of data still needs to be resolved.

Brain Renin-Angiotensin System

Although a wide range of effects of peripheral angiotensin has been worked out, the possibility of the existence of a brain RAS was in doubt for many years. To substantiate the existence of a brain RAS, demonstration must first be made that all the components for that system exist within the brain tissue itself. Early attempts to do so yielded success; workers were able to extract from the brain a protein capable of converting substrate into ANG I, and this was taken as evidence of the existence of an endogenous brain RAS. It was soon pointed out that the substance isolated could easily have been cathepsin D, a nonspecific protease. However, considerable refinements in the biochemical steps used to

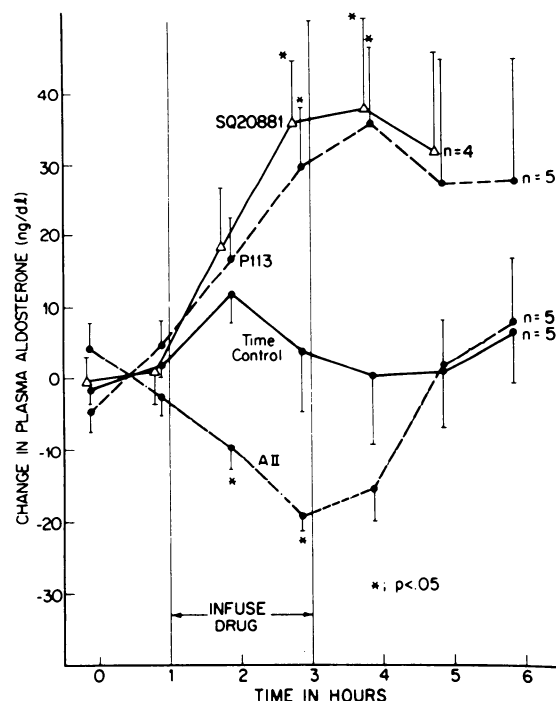


Figure 6

Effects of intraventricular infusion of ANG II, P-113, SQ-20881, and artificial CSF on plasma aldosterone concentration (ng/dl). Points \pm SE represent mean changes from averaged initial control values in each dog. * $P < 0.005$ when difference from time control group at each time was tested. Initial control values (in ng/dl) were as follows: for time group, 48.7 ± 14.8 ; ANG II group, 65.0 ± 9.1 ; P-113 group, 81.3 ± 10.9 ; and SQ-20881 group, 68.1 ± 7.7 . These values were not statistically significant from each other. From Ref. 6.

isolate compounds from the brain have occurred in recent years, making it possible to show that all of the components for the RAS exist in the brain. Through immunocytochemistry, many investigators have shown that the brain can bind antibodies specific to renin, ANG II, and converting enzyme. An excellent review of this topic has been given by Phillips (26). In addition, the cerebrospinal fluid of rat has been said to contain angiotensinogen (28). More specific experiments have demonstrated the existence of angiotensin-converting enzyme in some of the circumventricular organs of the rat, suggestive evidence of a brain RAS. Inagami (15), using affinity chromatography, has shown that although a major portion of the reninlike activity from extracts of brain tissue is due to nonspecific action of proteases, true renin can be separated. He has purified brain renin and also demonstrated the presence of an inactive prorenin. His immunohistochemical studies have localized renin to specific areas, indicating again that the brain is capable of forming renin and presumably the end product, ANG II. Other workers have demonstrated various components of the system in the brain. Additional evidence for the existence of brain RAS is provided by Basso et al. (3). They measured the angiotensinogen concentration and renin activity in cerebrum, cerebellum, hypothalamus, and brain stem in rats treated with DOCA and salt as well as in control animals. Animals so treated showed an increased concentration of angiotensinogen in these areas of the central nervous system; furthermore, the endogenous reninlike enzyme was also increased. These data are taken to support the presence of a brain RAS. Gregory and co-workers (13) did similar experiments in nephrectomized animals, showing significant elevations in angiotensinogen content in certain areas of the hypothalamus and midbrain following nephrectomy. More important, these areas were the same as those known to be involved in angiotensin-induced drinking and blood pressure elevations. Since it is unlikely that such a large molecule could cross the blood-brain barrier, these data lend support to the existence of a brain RAS. More recently, Phillips and Stenstrom (27) found ANG II in brain tissue from nephrectomized rats, using high-performance liquid chromatography. These data demonstrate convincingly the presence of a brain RAS.

Putting it all together, I believe that there does indeed exist a brain RAS and that many neurons in the central nervous system do respond to the octapeptide ANG II. Most of the areas concerned with the regulation of salt and water balance contain these receptors. The general scheme is one in which both peripherally and centrally generated ANG II can affect central receptors in many brain areas; the affected receptors control both the intake and output of salt and water.

Birds

The RAS has been studied in a few species of birds, and the evidence to date suggests that it has a function on birds as it does in mammals, except for its action on vascular smoother muscle. Nakamura, Nishimura, and Khosla (23) investigated this problem in the chicken. They were able to show that avian ANG II causes a biphasic response in the chicken, first depressor then pressor. This is shown in Figure 7. The pressor response could be blocked by reserpine. The authors conclude

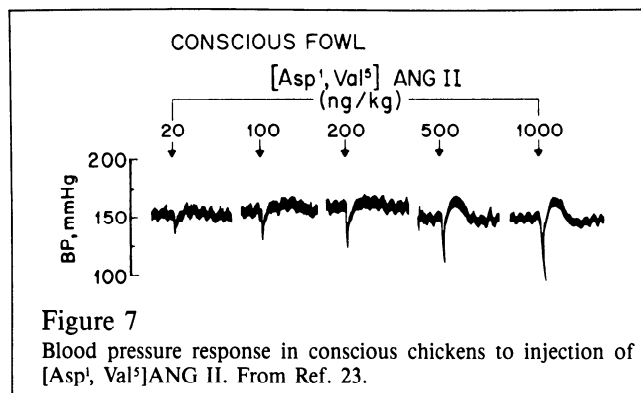


Figure 7

Blood pressure response in conscious chickens to injection of [Asp¹, Val⁵]ANG II. From Ref. 23.

that the vasopressor action of ANG II "may be primarily caused by release of catecholamines and . . . ANG II may exert a depressor action, possibly by acting directly on the vascular smooth muscle." This view is strengthened by the previous finding that ANG II is inactive on arterial strips from chicken, suggesting that at least in some species of birds angiotensin is either vasodepressor or inactive (32). Other actions of angiotensin have been tested in the chicken and pigeon. For example, it is a potent dyspnoeagen. Schwob and Johnson (29) have shown that this effect is probably physiological. They induced drinking in the chicken with the injection of ANG II in a dose-related fashion. It should be noted, however, that carnivorous birds do not appear to drink in response to ANG II (17).

Amphibians

Although amphibians also have been shown to have a RAS, the role of that system in amphibia is not well worked out. Injections of ANG II are vasoactive but do not initiate a drinking response. However, in the animals tested, drinking occurs only in times of severe dehydration and is not normally seen. Although some drinking responses in the frog can be attributed to severe dehydration, no drinking could be elicited on injecting ANG II (17). This may simply reflect a behavioral difference in replenishing the body's supplies of water. For a frog to increase water content of its body, it need only immerse itself in water; drinking is unnecessary. In amphibia then, ANG II may possibly serve the same role as in higher species, but through different action. It could act either by changing the permeability of the skin to water or by changing the water-seeking behavior of the animal. However, this is entirely speculative.

Teleost Fish

Although more work has been done with fish than in any of the other nonmammalian species, the major role of the RAS in fish remains unclear because of the paucity of data relating to many of the systems that have been worked out in mammals. Although we often speak of "fish" in a rather loose way, they may be classified into three rather arbitrary categories: stenohaline fish that are able to live only in a marine environment; stenohaline fish that can only live in a freshwater environment; and euryhaline fish, which are able to move out of salt water into fresh water, and vice versa. The latter are exemplified by the salmon and the eel. Add to this the fact that freshwater fish evolved over many millions of years, some as recently as about 50 million years ago, others perhaps as long ago as 600 million years. Thus the action of a hormone such as angiotensin might vary,

depending on the type of fish studied. In any event, a few things about the effects of angiotensin in different fish merit discussion.

Nishimura et al. (25) subjected the aglomerular toadfish to hemorrhage and reported an increase to plasma renin activity, suggesting the RAS in fish has something to do with maintenance of blood pressure. Churchill et al. (7) infused ANG II into another species of aglomerular fish, *Lophius americanus*. Figure 8 illustrates its vasoactive effect. Blood pressure increased during infusion and promptly returned to control values upon infusion termination.

The drinking function has also been the subject of study. Hirano et al. (14) showed that ANG II caused the euryhaline eel to drink, and since euryhaline fish must alter their drinking rates as they move between fresh water and salt water, this suggests the RAS may play a regulatory role. Our laboratory tested that hypothesis, using the euryhaline fish *Fundulus heteroclitus* (20). Figure 9 shows that not only did ANG II stimulate drinking but intramuscular injections of converting enzyme inhibitor also depressed the drinking rate. Since inhibition of endogenous ANG II formation reduced drinking, we conclude that the RAS is a physiological regulator of drinking in these fish.

Additional work was done with stenohaline marine and freshwater fish. We injected ANG II intramuscularly in the marine flounder and sculpin (Beasley, Shier,

Table 1

Percent Increase in Drinking
in Response to ANG II and Hemorrhage

	Flounder	Sculpin	Goldfish
ANG II	183+18*	270+26*	0
Hemorrhage	163+13*	640+20*	-14+22

Values are means \pm SE. * $P < 0.01$.

and Malvin, unpublished observations); both fish increased their drinking rates significantly when given ANG II or when subjected to hemorrhage. However, the freshwater goldfish did not increase drinking in response to either ANG II or hemorrhage (Table 1).

These data lead us to speculate on the origin of the ANG II drinking reflex. It has been well established that ANG II has potent blood pressure effects in almost all animals studied. Because of this, it is reasonable to assume that the RAS evolved in response to pressures which stressed homeostatic mechanisms regulating blood pressure; the RAS was primarily a vasoactive system. Only later did its other aspects evolve: as a regulator of thirst, salt appetite, ADH, and aldosterone secretion, and so forth. The alternate hypothesis is that the system evolved in response to the evolution of vertebrates living in seawater and having a blood osmolality of less than that of the surrounding sea. Such animals must drink continually to maintain salt and water balance. Sharks can live in a marine environment without drinking only because their blood has an osmolality close to that of seawater; however, it is worth noting that they lack a RAS. A functional RAS would be particularly important for animals living in areas of changing salinities, such as in or near estuaries. These animals would need a mechanism to change their drinking rates as they moved into water of different salinities. The RAS could have been the response to this environmental demand.

Since freshwater fish have a RAS and yet are unable to regulate drinking rates, the assumption would be that the reflex was lost when the marine species migrated to fresh water. Of course, this is quite speculative and is based on very few data. What is certain is that more information is required concerning the physiological role of the RAS in stenohaline and euryhaline fish.

I am indebted to Roberta de Boer for her help with this manuscript.

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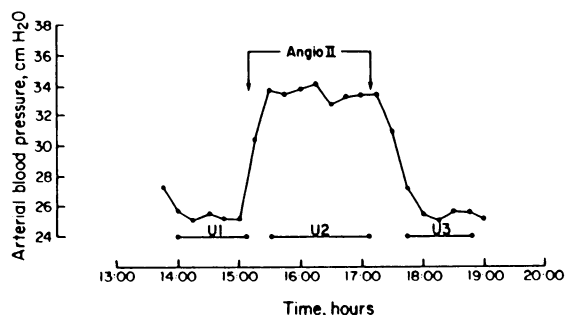


Figure 8

Pressor response in a goosefish to intravenous infusions of ANG II. Lines connect blood pressure reading made every 10-15 min. From Ref. 7.

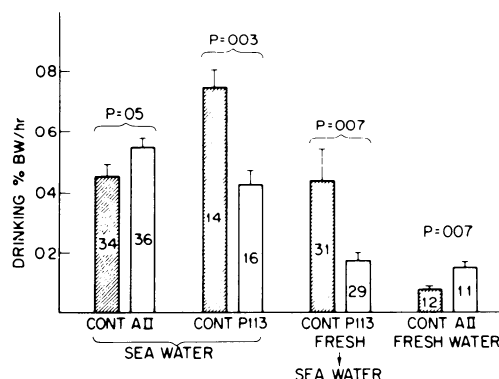


Figure 9

Drinking rates in control fish and in those injected with either ANG or P-113. Each bar represents mean drinking rates for that group \pm SE. Number within each bar denotes number of fish used for each group. From Ref. 20.

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National Repository for Cryobiology

The National Science Foundation (NSF) is providing a rare opportunity to impact future comparative physiological research on life systems naturally tolerant of low-temperature environments. NSF has asked the Society for Cryobiology to register by letter our interests in having access to polar life systems for experimental purposes. NSF is evaluating plans to establish a national repository for the cryogenic maintenance of biologics obtained from the Antarctic. The repository would 1) collect, curate, and dispense (without charge) samples, obtained from cooperating programs, to investigators requesting specific research samples; and 2) coordinate and support a nationwide graduate training program in studies focusing on polar species.

Polar environments offer a variety of challenges to resident life forms. Our perception is that their adaptations are indicative of life under constant stress, near the limits imposed by evolutionary constraints. A sampling of systems of interest is as follows: endolithic flora—species inhabiting the subsurface (intergrain) layers of rocks; microbial flora—probable ice nucleating species and survival and growth at constant subzero temperatures; invertebrate fauna—ice nucleating producing insects, krill, and other marine species; plants—extreme

frost hardiness among grasses, mosses, and algae as well as extremes of photoperiod and nutrient availability; diving mammals—hyperbaria, regional heterothermy, and hypoxia, wound healing at or near 0°C, cold detention, and permanently arteriosclerotic; penguins (selected species)—2- to 3-month fasts while active and regional heterothermy; fish—macromolecular antifreeze production.

This opportunity is in many ways unprecedented. If established, the repository will provide bioscience researchers with access to life systems best described as containing the “blueprints of adaptation.” The NSF has asked that we conduct a survey over the next month and identify a sampling of biomedical scientists having an interest in incorporating any polar system in some facet of his/her research program. If you have an interest, please write a *brief* note to the address below concerning the following: 1) the specific biologic material of interest (e.g., tissue or organ type); 2) the estimated amount of material (e.g., grams, kilograms); 3) the expected duration of access (e.g., 1, 2, 3 years).

Address: Dr. John G. Baust, Luyet Professor of Cryobiology, Institute of Low Temperature Biology, University of Houston-University Park, Houston, TX 77004.
Phone: (713) 749-1752.

Evolution of acid-base concept (1917-1984)

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Teaching in acid-base physiology has evolved along an irregular path, but continuing gains in understanding in this field have set the stage for more logical and consistent approaches. In this discussion, two areas are analyzed in which improved understanding impinges on conventional teaching. In the first portion, the accuracy and usefulness of a simpler rationale is evaluated in comparison with more complex approaches frequently taught in physiology courses. In the second part, certain problems in terminology are reviewed, giving emphasis to the very significant effects that the choice of words can have on the students' concepts.

Frames of Reference

Most emphasis in first-year physiology teaching has been given to relationships observed in whole blood. The temptation to do so is strong because our understanding is so thorough, thanks particularly to the elegant studies of Van Slyke, Wu, and McLean (15). You will recall that in blood there are two major types of buffering reactions—acceptance of hydrogen ions by blood proteins, primarily hemoglobin, and buffering in blood water, which depends on the reaction with bicarbonate. Furthermore, the change in plasma pH is a measure of the buffering by the proteins regardless of whether acidity is induced by metabolic or by respiratory variation (Figure 1). These relationships are usually represented, taught, and learned in physiology courses with the help of various three-dimensional diagrams.

But now let us move to the fourth year of medical school when students arrive on the wards: the patient in question is one with metabolic acidosis. In this case, the students will learn from the resident physician that bicarbonate should be administered and, furthermore, that the dose can be calculated by multiplying the measured decrease in the extracellular concentration of bicarbonate (milliequivalents per liter) by some fraction (usually given as 0.5) of body weight (kilograms). The students will probably be surprised that the resident pays little or no attention to the pH-bicarbonate diagrams which they studied so intensively during the first year. In the clinic, emphasis is given immediately to individual

changes in PCO_2 and bicarbonate. These are the determinant factors both for diagnosis and therapy. Of course, change in pH is the primary index of disorder or of hazard to the patient, but change in pH does not supply the most directly useful information needed to nail down the diagnosis or to plan therapy.

Whole body vs. whole blood

The important point is that the clinician is concerned with the whole body, whereas with the benefit of hindsight we can now see that conventional teaching in physiology probably focused too closely on the reactions in isolated blood. Differences in reactivity between the whole and its parts have been emphasized by the studies of Schwartz and Brown and their associates (3, 4). These authors demonstrated that the change in bicarbonate that occurs in the whole body in response to a given change in PCO_2 is only half that which occurs in isolated blood. Here we are dealing with quantitative differences in response, with summed effects of the reactions of CO_2 and carbonic acid with cell buffers. On the one hand, it is the reaction with protein buffers in erythrocytes to produce a gain in bicarbonate in plasma; on the other, it is the reaction with buffers in all cells to produce a gain in bicarbonate in extracellular fluids. Note that these differences apply only to respiratory effects. In the next section we turn to effects of metabolic change; here although the differences between whole blood and whole body are even more pronounced and are of a greater quantitative significance, the problem has not attracted the same attention, and the mechanisms of the buffering is not understood to this day.

Characteristics of Tissue Buffering in Metabolic Disorder

In tissues other than blood, buffering of metabolic change is measured by change in extracellular *bicarbonate* rather than by change in extracellular *pH*.

The early studies of Palmer and Van Slyke (11) demonstrated that the amount of bicarbonate required to normalize the depressed extracellular concentration could be estimated by multiplying the abnormal decrease (milliequivalents per liter) by the volume of total body water (liters). This is essentially (and is probably the original basis for) the clinical rationale as cited above. It follows that if buffering in total body water and in the extracellular fluids is proportional to the extracellular decrease in bicarbonate, then buffering in the tissue or intracellular fluids must be proportional also.

In difference to the erythrocyte in the whole body there is no close correlation between the acceptance of hydrogen ions and change in extracellular pH. In fact, studies using animals have shown wide dissociations. Pitts et al. (9, 14) estimated "tissue" buffering (i.e., other than blood and extracellular fluid) contributions in respiratory and in metabolic acidosis. These studies showed, for comparable decreases in pH, that approximately five times more hydrogen ions were accepted by tissue Na-H exchanges in metabolic than in respiratory acidosis. Furthermore, in this laboratory (8), we were unable to detect any change in the intracellular-extracellular distribution of the buffering of infused HCl,

and thus any change in the size of the tissue contribution, in the face of wide alterations of PCO_2 and pH.

Model Experiment

Independence from change in extracellular pH in the case of metabolic tissue buffering can be brought into focus by referring to results of an experiment which can be carried out in a teaching laboratory. If HCl, at a dose of 6 meq/kg, is infused into an animal, approximately half will be buffered in blood and extracellular fluids as the extracellular bicarbonate is reduced by half (Ref. 5, chapt. 5). The other half (3 meq/kg) is buffered in the intracellular fluids of the tissues. As a second experimental step, the pH can be returned to normal by hyperventilation, lowering the PCO_2 from 40 to 20 mmHg (8). The primary point to be made here is that when the pH is normalized in this way, the tissue buffers *do not* unload their newly acquired hydrogen ions. If they did, enough of the exogenous acid would be released back into the extracellular fluids to neutralize the remaining half of the extracellular bicarbonate. This does not happen; bicarbonate falls only slightly. Every clinician knows that full respiratory compensation for metabolic acidosis does not cause the bicarbonate to drop to zero. Thus under these conditions tissue buffering is achieved when the extracellular pH is normal.

A particular merit of the experiment is that a very useful number is brought to light, an estimate of the size of the tissue contribution. As noted for the example cited above, the number is a large one: 3 meq/kg of acid will neutralize 12 meq of extracellular bicarbonate.

Independence of metabolic tissue buffering from large changes in pH is an essential feature of acid-base physiology. Without such independence there would be very large intracellular-extracellular shifts of acid (and thus precarious fluctuations in bicarbonate) during such physiological events as hyperventilation, exercise, or breath holding (6).

The Mechanism

Specific properties of tissue buffering must then be recognized. Recall that in the erythrocyte the measure of buffering by hemoglobin is the change in pH regardless of whether induced by respiratory or by metabolic acidosis. The apparent indifference of tissue buffering to change in extracellular pH stands out in contrast and is indicative not only of dissimilarity between tissues and erythrocytes but also of difference between respiratory and metabolic phenomena (Figure 1).

We are left to answer the question as to the nature of the signal in metabolic disease. It is puzzling to have a buffering reaction that is unresponsive to decrease in ambient pH; but the story does not end here. The puzzle is compounded by investigative studies (16) that have failed to reveal a decrease in intracellular bicarbonate, at least in muscle, despite the large extracellular decrease. As we have pointed out earlier (8), if we assume an absence of a CO_2 gradient across relatively permeable cell membranes, we can then accept, as specified by the Henderson-Hasselbalch equation, that changes in the bicarbonate gradient (decrease outside but not inside) must be associated with change in the hydrogen ion gradient (H_i/H_o). Thus it may be that it is the decrease in the gradient, rather than in the external concentration, that affects the uptake of hydrogen ions by tissue cells in metabolic acidosis.

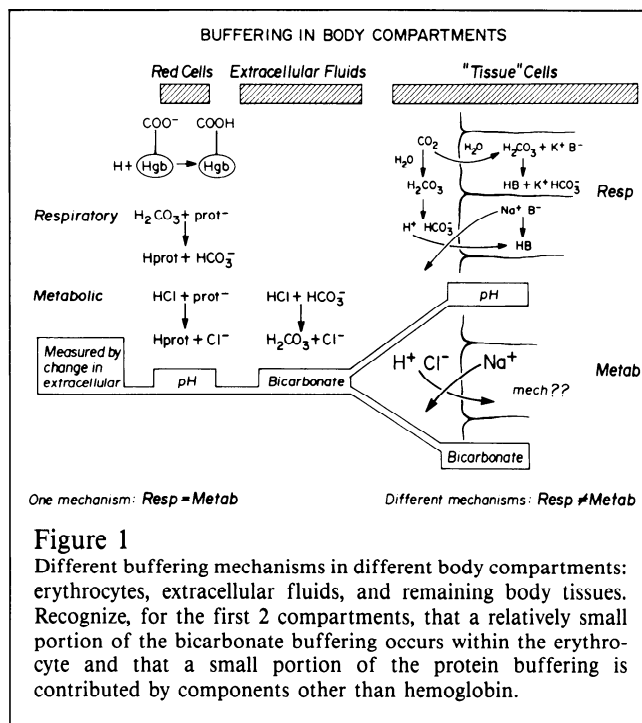


Figure 1

Different buffering mechanisms in different body compartments: erythrocytes, extracellular fluids, and remaining body tissues. Recognize, for the first 2 compartments, that a relatively small portion of the bicarbonate buffering occurs within the erythrocyte and that a small portion of the protein buffering is contributed by components other than hemoglobin.

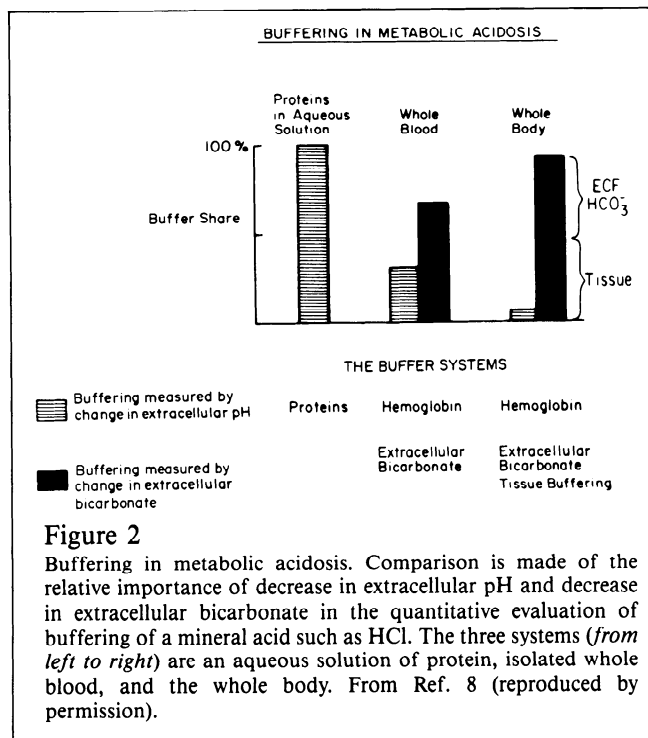
In Summary

The blood model that includes the two mechanisms, buffering by proteins and by bicarbonate, does not suffice for the whole body. Analysis must be expanded to include recognition of the special characteristics of tissue buffering, a third compartment and a third mechanism.

Significance with Respect to Clinical Rationale

At this point one might wonder how it is possible that successful medicine has been practiced despite the lack of emphasis given to the special characteristics of buffering in tissues other than blood. The answer is that the critical relationship to the bicarbonate change in metabolic disorders is taken into account by the time-honored clinical rationale discussed earlier. As shown for metabolic acidosis in the whole body in Figure 2, the bicarbonate-dependent extracellular and tissue components together account for 96-97% of the buffering, leaving only 1-3% to be contributed by the hemoglobin. This decisive dominance attests to the importance of the bicarbonate measurement. To be sure the measured value for the bicarbonate must be corrected for an abnormal PCO_2 ; but this correction is a small one, amounting in the whole body to little more than 1 meq/l for each change of 10 mmHg in the PCO_2 (4).

The corrected bicarbonate (as just described) is recommended as the most useful measure of the metabolic disorder, whereas the recorded change in PCO_2 (without correction) supplies the measure of the respiratory abnormality. The one of the two with the larger percentage deviation from normal is designated by the data to be the primary event; the smaller is then assigned as the compensatory response. This rationale is more easily remembered and applied at the bedside than is graphical analysis, which localizes respiratory or metabolic acidosis or alkalosis to specific quadrants on a complex diagram. As a second advantage, the clinician has immediately in hand the numbers needed for quantitative appraisal with respect to both the diagnosis and



therapy. Thus despite the existence of a third compartment and a different buffering mechanism, the relatively simple two-dimensional approach is available and is recommended.

Interpretation of "mixed" disturbances requires an additional level of expertise, the teaching of which is probably better reserved for the clinical years. For example, a normal PCO_2 in the presence of a reduced bicarbonate suggests superimposition of respiratory acidosis on metabolic acidosis.

Words and Concepts

Increase in our understanding of the buffering reactions has proceeded as it should over the last 67 years, expanding toward the more comprehensive—from the part to the whole. On the other hand, analysis in terms of the roles played by the individual electrolytes has shown a trend in the reverse direction, focusing down upon a single ion (H^+) and thus giving diminished emphasis to all others.

Two Sides of the Same Coin

It was considered appropriate in 1923 to entitle a paper in clinical research, "The metabolism of fixed base . . ." (7); and as chance would have it, it was in the same year that Lowry (10) and Bronsted (1) published their definitions of acid and base. Different usages in these and in other papers set the stage for terminological conflict. For most of the clinicians at that time, the word base referred to the cation (e.g., sodium), whereas by the Lowry-Bronsted definition, base refers to the proton acceptor and thus to the anion (e.g., bicarbonate). The doctors and the chemists went their separate ways for three decades. Clinical research, however, did move ahead. It was during this time that much of the foundation of our present understanding of acid-base physiology was laid down. Moreover, the principal investigative tool was the "balance study," a procedure dependent on careful measurements of inputs and outputs of the "fixed" ions (e.g., Na^+ , K^+ , Cl^-).

Certainly it was a suboptimal arrangement to have the clinicians and the chemists speaking different languages. This point was recognized in editorials published in the fifties (2, 12), and changes were instituted. Chloride and sodium were denied nominal roles as acids and bases, and formulations were put forth giving emphasis to the metabolism of hydrogen ions. "Conservation of sodium" became "secretion of hydrogen ions," and the descriptive term " H^+ balance" was introduced.

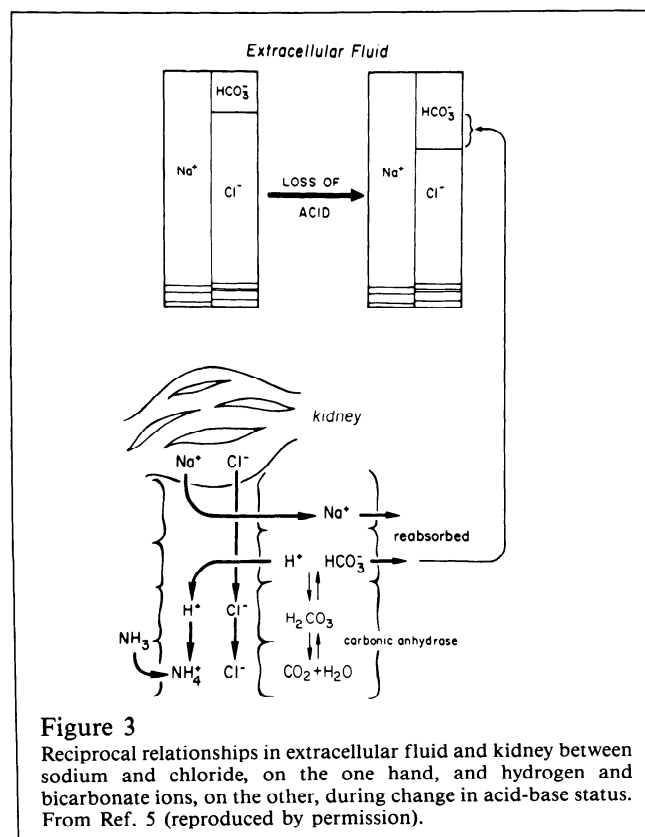
At this point, one might wonder how it could be that relatively successful medicine was practiced for the first 30-year period, when most emphasis was given to the balances of sodium and chloride, and yet also for a succeeding three decades, when attention has been directed to the metabolism of hydrogen and bicarbonate ions. Of course, the answer is that the two approaches are often opposite sides of the same coin. As represented in Figure 3, under many circumstances relative imbalances of sodium and chloride are closely reciprocal to those of hydrogen and bicarbonate.

Troubles with " H^+ Balance"

The term " H^+ balance" has obvious unitarian appeal and the ring of pertinence. Hydrogen ion concentration and excretion must relate to, or dictate changes in, acid-base balance. Nevertheless, there are serious drawbacks.

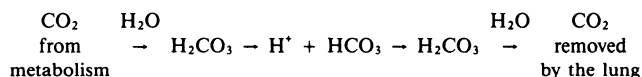
At the start, there is the problem of terminology. It is correct to speak of secretion of hydrogen ions into the lumen of the renal tubule; but once there, essentially all are bound to buffer anions and thus are no longer in the ionized form. The student must be educated to identify excretion of undissociated weak (i.e., titratable) acids, or ammonium, as " H^+ excretion."

Of more concern is that all the emphasis on hydrogen ions distracts attention from changes in the other components that are also vital to the constitution of the complete system. Metabolic alkalosis cannot be cured by



infusing hydrogen ions as lactic acid, since under normal conditions the organic material would quickly be oxidized to CO_2 and water. The student must be educated to know that vomiting entails loss of hydrochloric acid, and thus therapy requires replenishment of chloride as the physiological nonmetabolizable anion. Sodium and chloride are the two predominant electrolytes in the extracellular fluids, and their roles can be recognized without affront to physical chemistry.

Another worrisome outcome has been the use of the " H^+ balance" term in relation to respiratory events. One can find in acid-base chapters of medical texts references to loss of 10,000–20,000 meq of H^+ (or H_2CO_3 of "acid") by the lung each day. It then becomes a short step for the student to conclude that respiratory alkalosis is caused by an increased rate of loss of such quantities of H^+ by way of the lung (Figure 4). The error may be attributed to the practice of writing the following equation on one line.



In reality, CO_2 derives from metabolism and is removed as such by the lung. The reactions with water (and also the transport as bicarbonate) are on reversible side paths (Ref. 5, p. 122). If, with hyperventilation, the equilibrium of the last half of the equation is shifted to the right, there will be a minute decrease in the concentration of hydrogen ions but not a release of quantity to the outside.

Distinctively Different Reactions

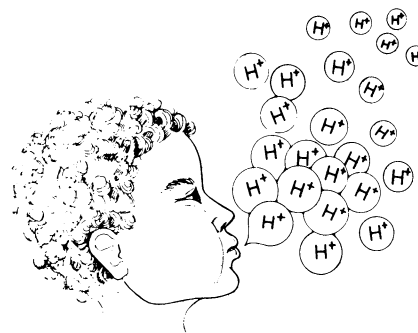
Differences between metabolic and respiratory effects in the whole body have been described with reference first to buffering and second to the balance concept; and it must now appear that analyses based on the word "acidosis," or on terms only of hydrogen metabolism or concentration, may be too general. Tissue buffering in metabolic acidosis is measured by change in bicarbonate (Figures 1 and 2) and is achieved quite independently of change in pH; however, respiratory buffering is associated with only modest changes in bicarbonate but very large changes in pH. Whereas the balance concept is appropriate only for metabolic change, the "Slyke" term ($d\text{HCO}_3^-/d\text{pH}$), in the case of the whole body, is appropriate only for respiratory buffering.

Recommendation

Certainly, a first order of business is to close the conceptual gap between first- and fourth-year teaching. More attention must be given in the first year to the whole body and specifically to the characteristics of tissue buffering in metabolic acidosis. The unsolved problems of mechanism pose a most interesting challenge to research. Although the tissue buffering is the largest component in whole-body buffering, investigators have yet to unravel details of the mechanism that accepts hydrogen ions in response to decrease in the concentration of extracellular bicarbonate.

There is also need for more precise quantitation with respect to the distribution of the buffering. As a start we have the data of Schwartz, Orning, and Porter (13). These authors observed that the distribution of buffering of HCl, between intracellular and extracellular compartments, remains relatively constant as the bi-

Figure 4



carbonate is reduced from 25 to 8 meq/l. On the other hand, further study is required to define the relative distributions in the alkaline range where there is the very large increase in extracellular bicarbonate. Additional quantitative information will enable us to move directly to the relationships in the whole body and to utilize computer analysis of all buffering processes simultaneously.

With regard to terminology and old and new approaches, we should hold to the middle ground, utilizing advantages of both. The newer is correct in terms of physicochemical concept, while the older in certain respects provides a more useful emphasis with reference to the clinical problem. At the start of the course at Johns Hopkins School of Medicine, we are apt to ask the students, "How is an excess of HCl buffered in the body?" A frequent response is to write the following equation on the board.



At the end of the course, we ask more challenging "CO₂ questions" (5) such as "Can you 'blow off' acid infused as HCl?" or "Can you 'blow off' CO₂ from extracellular bicarbonate?" If the student should place too much reliance on the one equation, he is likely to be on uncertain ground on both counts. In the case of the first question, the infused hydrogen ions from HCl will be buffered but not removed. Metabolic acidosis will obtain and persist until the excess H^+ and Cl^- ions are removed by the kidney (see Figure 3). In the case of the second, requirements for electroneutrality do not permit reduction in the bicarbonate without compensatory adjustment of other ions. With respect to these two questions, the equation appears detached, almost hanging in space. It needs to be tethered to the real world, which in this case is the closely integrated electrolyte system of the whole body.

In the author's view, we should make more use of the word "acid"; and when feasible, we should identify the acid in question—whether hydrochloric, phosphoric, or lactic. Surely the proton acceptor, or anion to be, is of physiological significance. Such expansion adds complexity, but oversimplification may only set the stage for confusion. It is gratifying that increase in theoretical understanding of the characteristics of tissue buffering clarifies the clinical rationale which has evolved on an empirical basis.

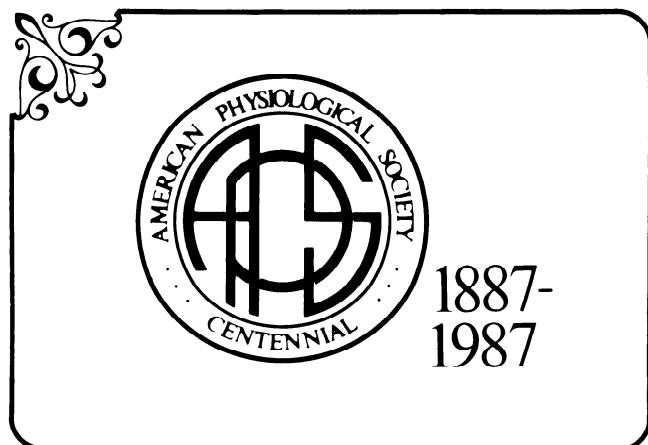
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Congress of International Society for Heart Research

The XII World Congress of the International Society for Heart Research will be held in Melbourne, Australia, February 9-13, 1986. *Information:* The Secretary, XII Congress, ISHR, Section of Cardiovascular Sciences, Department of International Medicine, Baylor College of Medicine, Houston, TX 77030.



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