I am honored to write the prefatory chapter for the inaugural volume of the Annual Review of Pathology: Mechanisms of Disease. This publishing venture signals that pathology takes its rightful place alongside the other biomedical sciences.

I thought it may be of interest to some to delineate how a circuitous path led me into a career in experimental pathology and to give some of the flavor of a past era in experimental approaches. Thus, my title.
EARLY DAYS

South Africa: An Education

I was born in Johannesburg, Republic of South Africa. My forebears immigrated to South Africa in the mid-to-late nineteenth century from Poland and Lithuania. I was educated at the King Edward VII High School. This was a public school run along the lines of the private (i.e., “public”) schools of Britain. Despite its semifeudal atmosphere, it provided an excellent classical education, including four years of Latin—which I have unfortunately mostly forgotten, except for a few “tags,” which I trot out on occasion to make an impression. There was, however, an excellent course in Modern Poetry, which provided a basis for much enjoyment in later years. The teacher was, not inappropriately, named Keats. Perhaps my greatest triumph at school was to score the winning, and only, try (goal) in a rugby match played against a rival school. (Years later, when I thought I might try out for the Harvard rugby team, my best friend promptly broke his leg at the first practice, and so, discretion being the better part of valor, and age telling, that was the end of athletic glory for both of us.)

Throughout school the teaching was in English, but we also had to learn Afrikaans, the other official language. (Regrettably, no black African language was required in those times.) I thoroughly enjoyed Afrikaans, in which I became quite fluent. It is a “modern” language with few irregular verbs and rules and with a rich literature descriptive of the landscapes, peoples, and flora and fauna of southern Africa.

During my high school years, I helped start an evening school for adult blacks. The headmaster, whom I had regarded as a conservative fellow, gave us students permission to use the school facilities even though, I am sure, this would not have met with approval from the governmental authorities if they had been aware of our venture. He was upset when one evening we had an influx of students and were short of space and so used his office as a classroom. I was called in to see him the next morning, but there was only an expression of mild displeasure and no retribution. On average we had approximately one hundred students who attended every session. In setting up this school, we consulted with Alan Paton, of *Cry the Beloved Country*, and my visit to see him left an indelible impression on me.

I entered the Medical School of the University of the Witwatersrand directly from high school, which is the British custom. I was interested in biology and not particularly interested in medicine, but as zoology or botany did not enthral me, I chose to study medicine as a portal to the biological and biomedical sciences. After two years of medical school, an inspired program, created by the renowned anatomist and anthropologist Professor Raymond Dart, the head of the Anatomy Department and the discoverer of the first *Australopithecine* (the Taung child skull of *Australopithecus africanus*), enabled scientifically inclined students to drop out of medical school for a year or two and pursue a science degree.

Another influential member of the Department was Joseph Gillman, officially a histology lecturer, though he had wide-ranging interests in other fields. Gruff, irascible, and iconoclastic, Gillman inspired students constantly to question dogma and orthodoxy. I remember the shock when I was ushered into a large laboratory occupied by approximately eight students and was told that I was expected to produce a thesis in a year or two. We could pretty well do what we liked, courses were few, and the interests of the students were diverse. I decided to work in cytogenetics, and my thesis was on the chromosomes of a delightful little animal—the bush baby, or night ape (*Galago senegalensis moholi*). The work was done under the direction of my fellow students Sydney Brenner and Philip Tobias. Both were interested in the evolution of chromosome shape and number, and though only one year ahead of me, they knew more about cytogenetics than anyone on the faculty. Today, Brenner

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is a renowned molecular biologist and Nobel Laureate, and Tobias has an international reputation as a foremost anthropologist. In those days the tissue-culture techniques for conducting cytogenetics had not been developed. To get useful spreads of chromosomes, one had to macerate the tissue in acetic acid and squash it between two glass slides—sometimes, in frustration, we would jump on the preparation, which was padded with books. The illustrations were made with a camera lucida. Some chromosome numbers published in that era were wrong because of the primitive techniques available. In addition to our laboratory studies, many of us spent enjoyable weekends camping in the countryside and hunting for fossils in caves and other rich sites under the direction of the eminent anthropologists Raymond Dart and Robert Broom and other paleoanthropologists on the faculty, as well as under the leadership of students such as Sydney Brenner and Phillip Tobias.

One of the highlights of the science program was the compulsory course History and Philosophy of Science taught by Brian Farrell, later of Oxford, who was a logical positivist. We became aficionados of L. Wittgenstein, B. Russell, J. Dewey, S.K. Langer, and A.N. Whitehead, and my readings by these authors, as well as by biologists J. Needham and C.H. Waddington, were important influences in my thinking about science, although I must admit that I was defeated by Wittgenstein’s *Tractatus Logico-Philosophicus*.

As South Africa was rather remote from scientific centers, the students imposed a voluntary levy on themselves to import luminaries from abroad. I remember particularly well the stimulating visit of C.H. Waddington, the eminent British embryologist, geneticist, and philosopher of science. One of our professors was a Marxist and a Lyssenkoist, who also favored the eccentric (to my mind) therapeutic theories of the Russian physician A.D. Speransky; consequently, our philosophical discussions were at times quite rowdy yet good tempered.

Professor Dart had established on the roof of the medical school a colony of baboons, which was the first primate study center in Africa. I helped Joe Gillman take blood from these beasts—not an easy job. Our research and philosophical discussions in the laboratory beneath the colony were carried on with the symphonic accompaniment of the raucous calls of the baboons, of which Sydney Brenner gave a great imitation.

Interest in the history, philosophy, and nature of science has remained with me, and I find it curious that many of the students and faculty I encounter in the United States seem singularly disinterested in such matters. I would think that in these days when issues regarding science and society are at the forefront, some background thinking by scientists on the nature and limits of science would be useful.

After one or two years in the science program, most graduates resumed their medical studies, many now fired with the ambition of making their careers in basic science or academic medicine. The medical curriculum at that time was a classical one based on the Scottish model. The courses in the first year were botany, zoology, organic and inorganic chemistry, and physics. The classical organic chemistry laboratories (spot tests and so on) placed me in good stead in later years in developing cytochemical techniques. Dissecting dogfish, frogs, and rats in zoology and making meticulous drawings were quite enjoyable. Botany was not enthralling—a matter of plant identification and classification; there was no mention of evolution or adaptation. We had fun, however, in dumping huge specimens of various species that we had dug up in the veldt, and which took several students to move, at our professor’s door; sometimes he was pleased, other times not. He was a patient man. The teaching of physics was dull; rolling balls up and down inclined planes on a sunny afternoon was tiresome. The second year consisted of gross anatomy, histology and embryology, physiology, and biochemistry (then known as physiological chemistry).
The year was greatly enlivened by the unforgettable laboratory teaching and inspiring lectures by Raymond Dart. During some of his lectures on evolution, the lecture hall would be filled with stuffed crocodiles, apes, large mammals, skulls, and skeletons so that there was scarcely room for the students as we stumbled over all this paraphernalia into our seats. The third year was devoted to bacteriology, surgical anatomy (a course taught by surgeons in which anatomy sprung alive and became relevant to clinical work), pathology, and pharmacology.

I did not find the Pathology Department at that time particularly inspiring, as it was very classical and descriptive in approach, although diagnostically sound. Nevertheless the subject matter interested me, and I began to read some papers and books on experimental pathology, although one of the professors tried to discourage me by saying that “work on animals was not relevant to the human.”

The text we used was so old-fashioned that the micrographs were round, as in nineteenth-century fashion. Remarkably, we attended autopsies or organ demonstrations almost every day during the school year, which was the duration of the course in Pathology, and there was an admirable museum available. The specimens were well preserved in natural color, and the relevant clinical history, microscopic slides, X rays, autopsy findings, etc. accompanied each specimen. One could cover a case or two a day and thus learn a good deal of pathology and medicine. If accompanied by a friend, each could take a turn in acting as an interlocutor. The museum was several stories tall and a spiral stairway was lined with shelves of specimens; thus one started at the top and spiraled one’s way down through the realms of pathology as the days went by. In later years when I came to the United States, I learned that such museums were rather rare, were an expensive proposition to develop and maintain, and were regarded as somewhat out-of-date. I think, however, that they are useful teaching aids and regret their so-called obsolescence.

In pharmacology we had to learn how to write and read prescriptions written in Latin and how to dispense the medicines. Some of the graduates would likely practice in rural areas and would have to do their own dispensing, as no pharmacists would be available. I learned to grind powders and roll pills and dispense them in small round boxes. I made up and bottled all sorts of tinctures and elixirs, wrapping and sealing the bottles neatly in white paper. My father, who was a pharmacist, thought my skills in dispensing were acceptable but not “highly elegant.”

A course in forensic pathology was intriguing but often a cause for queasiness. At the final exam each student was allotted a case to solve. I had a case of accidental poisoning. The remainder of the third year as well as the entire fourth, fifth, and sixth years were devoted to walking the wards—clerking in all aspects of clinical medicine and surgery as well as in the so-called subspecialties of obstetrics (I delivered more than one hundred babies), otorhinolaryngology, dermatology, and so on. The best times were spent “hanging out” in what we called the casualty (also known as the emergency room in the United States) at night, on a voluntary basis, hoping to get a good case, which we could clerk and follow through the night and thereafter. As we got to know the staff, we were allowed to perform procedures, culminating, if one showed enough skill, in the performance of quite major surgeries, such as appendectomies (under supervision of course). Overall, the clinical training was excellent, and upon graduation we were equipped to handle most of what faced us as interns. However, the basic sciences were at times somewhat short-changed.

Although the faculty in general were not particularly activist in regard to the disparities and inequities existing between the South African whites and nonwhites, there were notable exceptions, such as Raymond Dart, Joseph Gillman, and his brother Teddy (Theodore). The student body was, by post-1950 South African standards, liberal and tolerant in outlook, with the exception of a hard
core who opposed the relatively progressive attitude of the school allowing the presence of nonwhite students, who had been accepted since 1940, when Dart was Dean. However, the nonwhites could clerk patients only in the segregated nonwhite hospitals but not in the all-white hospitals. There were protests from some supporters of strict segregation against the presence of nonwhite students in the anatomy dissecting hall and at autopsy demonstrations, where, in both cases, cadavers of white women were on view. In the anatomy hall the protestors wanted the nonwhite students segregated to a separate room. Their request went nowhere. Professor Dart, the Department Chairman, offered instead to segregate the protestors. I cannot remember whether his offer was accepted. Unfortunately, nonwhite students were excluded by the Department of Pathology from the autopsy demonstrations when white female cadavers were visible. This led to protests from a considerable number of the student body.

The relatively liberal attitude of most students was conditioned to some extent by the presence of large numbers of ex-service men and women, many of whom had returned from World War II filled with idealism for a better South Africa. (South Africa had had a purely volunteer army because of a population that was divided in its support for the war against fascism. Many of those younger medical students who had not yet joined the armed services had been persuaded by officialdom that they would be of greater use if they first completed all or part of their medical training, particularly if they had close relatives already in the services; I was was one of them.)

Because of the influx of ex-service folk, our class was very large—220 students overall—but the administration and faculty dealt well with the logistical problems implicit in accommodating such a large group. An interesting consequence of the size of the class was that greater reliance was placed on the use of the nonwhite, segregated hospitals for clinical work, and so many of us were exposed to diseases, such as malnutrition, tuberculosis, syphilis, rickets, and pediatric gastroenteritis, that were rarely seen in the hospitals reserved for whites. Several of us realized that socioeconomic conditions, racism, and politics could determine the health and welfare of populations.

Thus we found inspiration in the teachings and practice of Sidney Kark, a graduate of our school, who propounded a multidisciplinary and multilevel approach to health care that comprised medical, social, economic, cultural, and political facets. He established a health care center at Pholela, in rural Zululand, which we attended during summer vacation. This center became a model for a national health system of primary care based on nonracial health centers, surprisingly supported by some far-seeing administrators in the government of Prime Minister Jan Smuts. (These concepts became the inspiration for Jack Geiger, a visiting medical student from the United States, later of Tufts University Medical School, to found the movement that led to the establishment of more than 800 health clinics in the United States.) Kark also started a course in social medicine, not under academic auspices, but at the request of, and partly organized by, some of my interested classmates. A cadre of students, including myself, were enthusiastic about this exciting, comprehensive approach to the delivery of health care, and some were desirous of making a career in the fields of social medicine and public health as conceived by Kark.

For my own part, I could not make up my mind between a career in academic medicine and this novel and intriguing alternative pathway. However, with the downfall of the Smuts government in 1948, and the advent of the Nationalists with their stringent concepts of Apartheid, the Karkian revolution in health care in South Africa virtually died, and many of my involved friends and colleagues emigrated for both political and professional reasons. Some carried the ideas of Kark abroad and promulgated them in diverse countries such as the United States, Israel, and the United Kingdom. As mentioned below,
I spent some time working in a clinic in an African township while I tried to decide my future career plans.

As the ironclad Apartheid rules came into effect, it became increasingly difficult for non-white students to be admitted to medical school. An onerous and drawn-out process had to be undertaken to gain permission for each student. The majority of the student body, as well as some of the faculty, participated in resistance meetings and demonstrations, and our student representative organizations were active in protest. These had some impact, both locally and globally, but were quite disruptive of the normal academic routine and atmosphere, yet they were obviously necessary.

I graduated M.B.B.Ch. [i.e., Bachelor of Medicine and Bachelor of Surgery (Ch., Chirurgia in Latin, from the Greek Cheirourgia)] in 1950, and after internships in both medicine, at an all-white hospital, and surgery, at a nonwhite hospital, I felt the need to put my hands to work utilizing the theoretical and practical knowledge that I hoped I had learned. I worked for a while as a general practitioner in a clinic in an African township, and it was most satisfying practicing the many skills I had acquired in my training, including minor surgery, obstetrics (sometimes delivering babies in poorly-lit shanties), subspecialties such as dermatology, as well as general medicine. As a student I had spent a rotation at the clinic, so I knew the ropes. Eighty thousand Africans were packed into a few square miles, and poverty was rampant. It was somewhat dangerous for a white person to walk the streets, but wearing a white coat and carrying a stethoscope afforded protection. As in the hospital in which I had been a surgical intern, trauma was a major component of the cases we saw, especially on weekends, when there was intermittent chaos in the township owing to the drinking of potent and often illicit, or toxic, alcoholic brews. These concoctions, dispensed in bars known as shebeens, no doubt assuaged the loneliness and boredom of poor migrant workers, separated from their families left behind in rural areas, but led to the exacerbation of intertribal animosities with consequent ferocious fights, gang warfare, or random acts of violence. In addition to clubs and knives, a favorite weapon was a sharpened bicycle spoke inserted expertly into vital areas.

At that time we never saw cases of myocardial infarction, peptic ulcer, or appendicitis in the black population, although these were common in the white population. Blacks suffered from hypertension, diabetes, strokes, tuberculosis, venereal diseases, and malnutrition. Now, as I understand it, the emerging black middle class exhibits the diseases of Western “civilization.” At the clinic I was under the tutelage of Mervyn Susser and Zena Stein, husband and wife, former prominent student leaders in opposition to Apartheid, and later luminaries in the fields of social medicine and public health and Chairpersons and Professors at Columbia University Medical School, respectively. They revolutionized clinical practice by introducing an appointment system—virtually unheard of until then for black Africans—and by keeping accurate vital records, which led to several significant publications in public health, particularly in regard to maternity care.

The clinic was supported financially in part by an annual “Rag,” a carnival-like event in which the university students virtually took over the city for a day or two, with parades, floats, dances, and other rather wild activities. As students, Sydney Brenner and I were one year appointed as the official cameramen and made a movie that had several shots taken at weird angles, particularly by Syd—shades of The Cabinet of Dr. Caligari.

However, despite my enjoyment of clinical work, I gradually came to realize from my reading of the current literature that pathology and the nascent science of cell biology were what interested me. I saw pathology as a bridge between basic and clinical science. Furthermore, the political climate strengthened my belief that I should seek a career abroad.
London: Training in Pathology

I then went to London and became a trainee, first at the Central laboratories, Lewisham Hospital, which serviced a large area; then at the Department of Pathology at the Postgraduate Medical School, Hammersmith Hospital; and lastly at the Royal Cancer Hospital (now the Royal Marsden). I trained in morbid anatomy (histopathology) and all branches of laboratory medicine—hematology, bacteriology, and clinical chemistry. My Chief at the Royal Cancer Hospital was Rupert Willis, who had a marvelous biological and embryological approach to the evolution of tumors; his books are classics. Unfortunately, he became ill and Dr. Skelton took his place. Knowing of my interest in cytogenetics, he proposed that I study the recently discovered Barr body (sex chromatin). As is now well known, in the cells of the female one of the X chromosomes remains condensed during interphase and can be seen as a small clump of heterochromatin. This is not found in males. Our idea was to see if some of the diverse tissues found in teratomas from males showed such sexual dimorphism. To this end we obtained, from the London Hospital, which kept specimens for years, a series of teratomas found in the testes of horses gelded around the turn of the century. Unfortunately, the material was not sufficiently well preserved for our study. The famous cytogeneticist P.C. Koller had his laboratory close by, and as he was one of the heroes of my days in science in South Africa, it was a pleasure to visit with him.

I was impressed that the pathologists at the Royal Cancer Hospital made rounds on patients with the surgeons and that when a biopsy arrived for diagnosis we would go upstairs to the wards to examine the patient. In fact, some of the senior pathologists had patients directly under their care. It was also impressive that after surgery the surgeons would come to the pathology laboratories to go over the specimens with the pathologists, and some of the “old-timer” surgeons were pretty good gross pathologists. I was disappointed later when I came to the United States to find that the pathology laboratories were in the bowels of the basement and we rarely saw a patient.

One of my teachers at Hammersmith Hospital was A.G. Everson Pearse, who stimulated my interest in cytochemistry. He was a pioneer in the field and later the author of an authoritative textbook. The pathology staff quite frequently applied cytochemical methods in diagnostic work and research, which convinced me of the potential usefulness of this technology.

As it was rather difficult in those days to obtain a foothold in an academic department in the United Kingdom, particularly for someone from the “colonies,” I decided, on the kindly advice of Lord Cohen of Birkenhead, President of the Royal College of Physicians and the premier physician in England, to try and obtain an academic position in the United States. During my interview with Lord Cohen I was offered a glass or two of sherry. I never before, nor since, have had the chance to imbibe at an interview. (As an aside, Lord Cohen’s hobby was said to be crocheting doilies, which he was reputed to do while sitting in the House of Lords. When I visited him in his office in Liverpool, where he was Chairman of Medicine, I thought that he may have been crocheting, but his rooms were very dark and illuminated by a single light over his desk, so I was not sure.)

Boston: Nascent Research and Teaching

Shortly after my rather bizarre interview with Lord Cohen, I was surprised to see an advertisement in The Lancet for a residency in pathology at Massachusetts General Hospital—a most unusual occurrence. I applied forthwith, but I was rejected because another young pathologist [W.M. (“Whitey”) Thurlbeck, I believe] from southern Africa had already been accepted to the program, and the Chief, Benjamin Castleman, did not think it was fair to have two residents from the same part of the world when positions
for foreign candidates were scarce. However, Castleman kindly forwarded my application to Arthur T. Hertig, who was the Chairman of Pathology at Harvard Medical School. Hertig was looking for someone to replace Guido Majno to teach Medical Sciences 201ab, the seminal, historic, and comprehensive year-long course in all the basic sciences, including General Pathology, required of all the graduate (Ph.D.) students at Harvard Medical School and taught by junior faculty members assigned from each basic science department. The course, funded by the Commonwealth Fund, was an early and influential experiment in integrated teaching, which was later applied to the curricula for medical students. Hertig thought I would be suitable to replace Dr. Majno as I had a degree in Physiological Chemistry and knew some pathology and therefore could presumably hold my own with the basic scientists. He hired me sight unseen.

As I also wanted to complete my residency training, I became, in 1955, an assistant resident pathologist at Beth Israel Hospital, afternoon and nights, and taught Medical Science 201ab in the mornings as a research fellow at the Central Pathology Department at the medical school. Carrying out these double duties was quite a task, especially as my scientific background was somewhat outdated—for instance, I had never seen a Warburg apparatus, which was then an example of big-time technology in biochemistry. To my amazement, no one had ever verified then or since that I hold any degrees whatsoever.

I learned a great deal trying to keep up with students and faculty. The course was the sole teaching responsibility of the young faculty representing each basic science, and we were expected to participate in all the activities, helping to teach each other’s sections and laboratories. For instance, a biochemist helped me mount hands-on experiments, which the students conducted on chemical carcinogenesis in the liver or on aminonucleoside nephrosis, all studied with a multidisciplinary approach. For me, it was a formative experience and a lot of fun. I got to know the faculty, which led to good friendships and collaborations in the future. In learning the principles and concepts of general pathology, I was greatly indebted to my predecessor, friend, guide, and colleague, Guido Majno, who also introduced me to Lord Florey’s great book, *Lectures in General Pathology* (1). I could not believe my good luck in having so fortuitously landed in such a hive of research and teaching.

Having completed my residency, I moved to the Central Pathology Department situated on the quadrangle at the medical school—a department responsible solely for teaching and research. Upon my arrival I found that a laboratory was not available, so I took refuge in a small room (10 feet by 20 feet) in the Department of Microbiology. My friendly neighbor was Dr. Albert Coons, who was then developing his now ubiquitous immunofluorescence techniques and who, in many discussions, reinforced my interest in cytochemistry, immunocytochemistry, and structure-function relationships.

My first laboratory was a rather modest affair. I had, as mentioned, little space and no equipment or grants. With no seed money (lavish or otherwise) in those days, I was obliged to scrounge equipment from my colleagues and hone my scavenging skills. For instance, I recovered from the garbage dump, which was located behind our building, an old, but rather elegant, brass and asbestos oven. I fixed and calibrated the primitive thermostat (a copper coil) and used the oven for 20 years.

My first few grant applications were rejected as I had a short publication track record and I lacked formal postdoctoral training. I guess I am essentially self-taught. Eventually, a pathology study section had some faith in me and I obtained modest funding. To supplement my income, I was also an Associate in Pathology at the then Peter Bent Brigham Hospital, under Gustave Dammin, signing out autopsies and walking a well-worn path between the Brigham and my laboratory on the quadrangle at the medical school.

Electron microscopy was then in its bloom as an exciting, new, and sexy technology for
application in biology. Shortly after my arrival, Don W. Fawcett had been appointed as Chair of the Anatomy Department, so I asked him if I could become one of his trainees in electron microscopy. Unfortunately his roster was full, but he allowed me to become an unofficial trainee with free run of the department. Through the goodwill and friendship of the members of the department—Jean-Paul Revel, Susumo Ito, and Elizabeth Hay—I learned a great deal about electron microscopy. Later, Revel, Ito, and I became collaborators on several projects.

Fawcett assigned me to study the virally caused Lucké carcinoma, which occurs in the kidneys of the frog (*Rana pipiens*)—no doubt because I was a pathologist. As it was winter I had to palpate the kidneys of hundreds of dormant frogs, which were held in a cage under the ice in a lake. I only found three or so tumors, but they did not fix properly, nor embed well in methacrylate, which caused all sorts of distortions in the tissues—so-called explosions. I tried a variety of maneuvers such as polymerizing under UV light in the cold, at room temperature, on top of the manifold of Sus Ito’s car, and on my furnace at home, all to no avail. I eventually ran out of tumor material. Such were the trials and tribulations of the early days in electron microscopy.

However, I was able to redeem part of the project some months later by using a different embedding medium that I imported from Switzerland (Vestopal-W, invented by E. Kellenberger, in whose laboratory I later worked). I showed that the proximal tubules of summer frogs, which were known to have secretory function, had high levels of enzymes supporting oxidative phosphorylation, which were markedly depleted in winter- and summer-starved frogs. There was no decrease in the latter two cases in the number of mitochondria, but marked disarray in organization of mitochondrial cristae (2, 3).

The proximal tubules of *Necturus maculosus*, like those of other species, reactively absorb many substances. Unlike other species however, the majority do not secrete spontaneously into their proximal tubular urine. Their proximal tubular cells had an anaerobic pattern similar to that of the winter- and summer-starved frogs, and their mitochondria in the proximal tubules, although numerous, had few cristae. In contrast, in both *Rana pipiens* and *Necturus maculosus*, oxidative enzymes and normal-appearing mitochondria were present in the distal tubules of both species. We concluded that secretion in the proximal tubule is accompanied by high oxidative phosphorylation, whereas reabsorptive processes are accompanied by glycolysis (4).

My early interest in cytochemistry as a tool for studying structure-function was heightened when, in an early paper, we were able to illuminate an ongoing discrepancy and dispute regarding the site of ammonia production in the kidney via the activity of glutaminase I, an enzyme for which we devised a novel cytochemical method. We found that there were species differences in the localization of the enzyme that could resolve discrepancies between biochemical data in one species and physiological data in another (5).

My first successful ultrastructural paper was one of the earliest studies of the fine structure of the osteoclast. It was rather foolhardy for beginners to attempt to cut bone, as diamond knives were not then available, so we used glass knives to cut thin sections of the relatively soft callus of healing experimental fractures (6).

Progress and promotion were slow. I was an instructor for four years. Arthur Hertig thought I would never get promoted unless I stopped my research and wrote and published instead. So in the final year of my four-year instructorship (a record tenure at that rank according to Hertig), I squeezed out some publications, which are mentioned above, and was on my way as an Assistant Professor—but I was on my way in more than one sense.
Geneva: Cholinesterases, Skiing, and Wine

In 1961 I had to leave the United States because my temporary visa had expired. Despite the best efforts of Harvard and several senators, I was required to spend two years abroad before re-entering the United States as an immigrant. Harvard kindly gave me a leave of absence, but without pay. Consequently, I spent two years in Geneva, Switzerland, at the Ecole de Medicine as a Collaborateur Scientifique. I worked in the Institutes of Histology and Embryology, Physiology, and Biophysics, under Professors Ch. Rouiller, J.M. Posternak and E. Kellenberger, respectively. My assigned project was to localize at the ultrastructural-level cholinesterase activity in heart muscle. Much biochemistry and physiology had been done in Posternak’s department on these enzymes. I designed novel techniques utilizing specific substrates for localizing cholinesterases at the light (7) and ultrastructural levels (8) and found that they were present in the longitudinal elements of the sarcoplasmic reticulum and in the A-band, but not elsewhere. Previous work using a nonspecific substrate had localized cholinesterases to the M-band. We deemed this activity an organophosphate-resistant, nonspecific esterase (9). My light microscopic method for localizing cholinesterases (7) became popular with neurobiologists and is much cited, as is the ultrastructural method (8).

My new laboratory had a beautiful view of the Jura Mountains, and all the benchwork and the hood were handcrafted, but for some inexplicable reason there was no hot water, which had to be fetched in containers from down the corridor. This omission was later rectified. The electron microscopy was done in Kellenberger’s laboratory. This was a fascinating place: As his laboratory was in the forefront of phage and bacterial structure and genetics, a succession of eminent scientists visited, including pioneers in molecular biology and genetics.

Kellenberger had built one of the earliest electron microscopes, and newcomers to the laboratory, like me, were assigned to use it. The resolution obtainable was that of a very bad light microscope or worse. After a day or two of struggling with this monster the joke played on the newcomer was revealed—nobody used the microscope because it was useless. Thus was the rite of passage passed.

Another embarrassment was when I was asked to give a demonstration to Roullier’s group of my lead stain, which I had just developed (10). Nothing worked—it was a disconcerting disaster. I blamed the water in Geneva.

A peculiarity of Rouillier’s laboratory was that after a paper was written it was locked away for a few months. Then it was reread and if Roullier and the author still liked it, it was sent off to a journal. In the meantime, if someone else had published on the same topic, then too bad! It did not seem to matter. I found this attitude quite admirable, but rather frustrating. While in Geneva I learned to ski (badly) and polished my skills as an oenophile. Roullier spoke limited English and my French was worse, so we began each day by reading Hemmingway’s The Old Man and the Sea in our respective language to each another—a pleasant, if limited, way to learn. A compensation was that Roullier gave me tutelage in wines, in which he was expert. It was in Roullier’s laboratory that I met Lelio Orci, who later succeeded him as chairperson.

Boston Regained

Despite the gratifying offer of a permanent position in Geneva, I decided to return to the hurly-burly of Harvard and American science, rejoining the Central Pathology Department in 1963. I gained tenure in 1965 and succeeded my predecessors Arthur T. Hertig and Benjamin Castleman as the Shattuck Professor of Pathological Anatomy in 1972. This is the oldest full-time professorship of pathology in the United States, founded in 1847.
(funded then by the fees paid by the students) and endowed in 1853 by George Shattuck, Professor of Medicine.

Upon my return from Geneva, the direction of my research was partly determined in a serendipitous fashion. One of the most prestigious awards at Harvard Medical School are the annual Dunham Lectures, given to only foreign scientists, many of whom are, or become, Nobel Laureates. The week of the lectureships was, in the 1960s, celebrated in grand style and was considered one of the highlights of the year. It was traditional for a member of the junior faculty to “look after” the Dunham Lecturer, to act as chauffeur, general factotum, and to ensure that he or she made all appointments on time and did not get lost. I was assigned to be Lord Florey’s shadow in 1965 when he was the Dunham Lecturer, and this turned out to be a most enjoyable and illuminating experience. Florey was the co-winner of the Nobel Prize in 1945, together with Fleming and Chain, for the discovery, purification, and therapeutic application of penicillin. We had many conversations about science. One of Lord Florey’s main interests at that time, in fact the topic of one of his lectures, was the nature of the ultrastructural bases for the permeability of the microcirculation to macromolecules. In fact, so engrossing was the topic that, in driving my distinguished guest to the grand banquet held in his honor, I got so thoroughly lost we arrived somewhat late, much to the annoyance of the Chairman of the committee, a most distinguished biochemist and, I believe, ex-marine, who reprimanded me in military style—one of the low points of my career. Lord Florey was unfazed.

The tracers suitable for ultrastructural studies were, at that time, essentially limited to large molecules such as colloidal carbon and ferritin, the latter of which Florey had been using. He urged me to develop smaller tracers to study the passage of small molecules across the endothelium, which, as indicated below, I attempted to do.

**RESEARCH OVERVIEW**

I was a relatively late starter in a serious research career, but I believe the two threads running though most of my research (namely, the study of the structural components of cells and their functions and the analysis of how disease states change structure and function) were entwined early. Although to do this I frequently had to pioneer the invention and development of several technologies, I usually perceived these efforts as a necessary means of addressing problems and not as ends in themselves. My papers, I believe, reveal a meld of disciplines including cell biology, experimental pathology, biochemistry, and physiology, perhaps reflecting my lack of specialization in any one field. Such eclecticism is probably not possible in these days of hyperspecialization.

In summarizing some selective areas of research in which I have been engaged, I must admit that I have, not infrequently, merely indulged my curiosity, and sometimes my involvement in a topic has been accidental. On occasion, though, there has been a logical extension of a train of thought. I do not, for obvious reasons, provide a comprehensive review, but instead delineate a topic and provide a minimum of references that summarize our work. To those who have made important contributions to the area I deal with, I have referred to their papers on a highly selective basis; to those omitted, I apologize.

**ULTRASTRUCTURAL TRACERS**

**Horseradish Peroxidase and the Diaminobenzidine Reaction**

Werner Strauss (11) had used horseradish peroxidase (HRP) to study endocytic electron uptake at the light microscopic level, but benzidine, the electron donor in the peroxidase reaction used at that time to reveal the site of the enzyme, did not yield a sufficiently electron-opaque reaction product suitable for electron microscopy. HRP had the prospect of being a suitable tracer molecule: It is not
highly charged, it is suitably small, and its peroxydatic activity could enhance sensitivity. I thought that a suitable substrate (electron donor) could be formulated, one that would yield an insoluble reaction product that would reduce osmium tetroxide and bind the reduced osmium. Modified benzidine, with additional amino groups, seemed to be a suitable candidate; 3,3′-diaminobenzidine (DAB) was the answer. Luckily, before I embarked on an organic synthesis, for which I was ill prepared, I found that DAB was commercially available as a reagent for detecting vanadium in milk. In the DAB-peroxidase reaction, HRP oxidizes DAB in the presence of H₂O₂ and converts it into an insoluble brown polymer; this polymer causes reduction of added osmium tetroxide, and the reduced osmium forms an insoluble electron-opaque precipitate at the site of the HRP. Thus, when HRP is injected into the bloodstream, the pathways that it takes to reach the tissues can be followed by fixing tissues at various times after injection and by performing the DAB reaction. Furthermore, HRP can be cross-linked to antibodies and other proteins for localization studies in vivo and in vitro.

It amuses me to think that the discovery of DAB and its usefulness in detecting peroxydatic activity may well be attributed to Sherlock Holmes (see A Study in Scarlet). In that story, Dr. Watson first meets Holmes when he visits the chemistry laboratory at St. Bartholomew’s Hospital, London, and Holmes gleefully shows Watson his new reagent and method for detecting blood stains, no doubt because of the peroxydatic activity of the hemoglobin in red blood cells. Holmes’ reaction yielded a brown precipitate, as does DAB: “[A] brownish dust was precipitated to the bottom of the glass jar.” The forensic methods for detecting blood in the 1889s, the guaic, or the preferable benzidine reactions, yielded blue or blue-green colors, respectively.

In the first paper introducing this technique (12), we studied the passage of HRP through the glomerulus into the urine; as HRP is smaller than albumen, it readily crosses the glomerular basement membrane, passes through the epithelial foot processes, and through the slit diaphragm, into the urinary space, where a considerable portion is absorbed by endocytotic uptake from the glomerular filtrate by the proximal tubular cells. This paper, which validated the methodology, is one of the most quoted in the biomedical literature, having been cited (according to The Institute for Scientific Information, Philadelphia) some 7000 times.

**THE BLOOD-BRAIN BARRIER AND OTHERS**

In another classic paper Thomas Reese and I (13) used the DAB-HRP method to establish for the first time that the cerebral endothelial cells form the structural basis for the so-called blood-brain barrier. The concept of a barrier between cerebral blood vessels and brain tissues had existed since the work of Paul Ehrlich in the late nineteenth century. He found that intravenously injected dyes failed to stain certain areas of the brain, whereas non-neural tissues were stained. It was not clear, however, until the high resolution obtainable with the electron microscope, as to whether the structural barrier was at the level of the endothelium, the basement membrane, astrocytes, or glial cells. We found that extensive tight junctions (zonulae occludentes) between the endothelial cells, and a paucity of endothelial vesicles, effectively prevented HRP from crossing the endothelium. This paper concentrated the attention of physiologists, morphologists, and pharmacologists on the cerebral endothelium as the site of the blood-brain barrier and also led to the construction of in vitro models utilizing endothelial cells in culture (14).

Eveline Schneeberger and I also defined the alveolar-capillary barrier (blood-air barrier) as residing at the tight junctions between the alveolar epithelial cells (15). Similarly, Elio Raviola and I defined a putative...
blood-thymus barrier, limited to the cortex, but not found in the medulla, but the mechanisms appeared complex and included impermeable cortical capillaries plus other factors (16).

**VASCULAR PERMEABILITY**

In contrast, in a paper published the same year, I found that HRP passed across the endothelium of the heart and skeletal muscle microcirculation apparently through incomplete junctions, or maculae (17), with a possible contribution from endothelial vesicles (18). We postulated that the incomplete junctions represented the small pores Pappenheimer (19; see also Reference 17) thought underlay the semipermeable properties of microcirculatory endothelia to macromolecules smaller than albumin. I think it is unreasonable to expect to find pores corresponding exactly in shape and size to those proposed by the physiologists. The theoretical pores are symmetrical, water-filled, equivalent pores, and it seems unlikely that a living endothelium would exhibit these exactly—it behaves as if it has such theoretical pores. Our paper (17) has been frequently quoted, but it must be admitted, however, that the relative roles of junctions (17) and/or Palade vesicles (18) in microcirculatory permeability are still a matter of some controversy.

**GLOMERULAR PERMEABILITY AND THE SLIT DIAPHRAGM: THE “ZIPPER”**

Following up on our initial studies on glomerular permeability to HRP (12), we utilized a variety of peroxidatic tracers of different sizes (HRP, myeloperoxidase, catalase), as well as endogenous plasma albumin and IgG (localized with specific antibodies labeled with HRP). Several of these studies were collaborations with Manjeri Venkatachalam and my dear friend, the late Ramzi Cotran. We concluded, in agreement with Caulfield and Farquar (20), that the glomerular basement membrane (GBM) was the major barrier to the passage of proteins into the urine. However, Graham Ryan and I emphasized that this concept pertained to conditions of normal blood flow (see below) (21, 22). Richard Rodewald and I studied in detail the fine structure of the slit diaphragm and found it consisted of a meshwork of struts and pores, somewhat resembling a zipper, by which name the structure has become known. The pores, we calculated, were of sufficient size, shape, and density to constitute a barrier to the passage of molecules the size of albumin or larger (23). In addition, we proposed that the slit pore also constituted a regulator of hydraulic flow (23).

In what has been referred to as a unique experiment (24), Graham Ryan and I restricted blood flow to the glomerulus and observed the distribution of plasma albumin tagged with HRP. Under normal-flow conditions, albumin was restricted by the GBM. Under low-flow conditions, albumin as well as larger molecules, such as IgG, passed into the urine (21, 22). When normal flow was restored, molecules were once again restricted by the GBM. We interpreted our results in terms of changes in molecular sieving, perhaps associated with concentration polarization and/or charge modulations in the GBM. However, Smithies (24) has lately adduced a novel hypothesis: that transport across the GBM is by diffusion and that in the above experiments the difference in concentration of albumin on the two sides of the GBM disappears because diffusion is no longer accompanied by the wash-out of Bowman’s space that normally occurs as a result of flow of liquid.

In recent years, exciting new evidence for the role of the slit diaphragm in glomerular permeability is the remarkable discovery by Tryggsvason and associates (25, 26) that a unique protein, nephrin, directly constitutes at least part of the structure of the slit diaphragm and, cogently, abnormal nephrin or its absence results in proteinuria and slit-pore defects (25, 26). This exciting work has opened a new chapter in the pathophysiology of glomerular filtration and nephrology.
It is gratifying to me that after many years of relative obscurity our earlier findings and hypotheses regarding the slit diaphragm now have considerable relevance. It is clear, however, that many issues remain unresolved. I am sure that in the near future the approaches of molecular physiology will unravel the mysteries of glomerular filtration.

FURTHER STUDIES ON THE RENAL GLOMERULUS

Over the years we maintained our ongoing interest in glomerular pathophysiology, including the development of methods for isolating and culturing glomerular cells—studies initiated in my laboratory by Jeffrey Kreisberg and Richard Hoover. We showed that cells of apparent mesangial origin were contractile, as had been postulated, but not proven, for many years. This function has been related to control of glomerular filtration via decrease in capillary surface area.

Jonathan Diamond and I developed a new model for studying focal and segmental glomerular sclerosis, in which an acute phase of proteinuria was followed by a latent phase, then a chronic phase, thus mimicking many aspects of human disease. In collaboration with Barry Brenner’s group, we showed that the acute nephrotic phase is accompanied by severe morphologic injury and renal insufficiency. The nephrotic syndrome resolves, but it is accompanied by glomerular capillary hypertension, leading to recurrent proteinuria and glomerular sclerosis, which was alleviated by angiotensin 1–converting enzyme inhibitors. We postulated that glomerular hypertension led to glomerular sclerosis and renal failure after an episode of acute glomerular injury, in accordance with the bounteous experimental and clinical evidence produced by Brenner’s group (see Reference 28).

GAP JUNCTIONS

Another electron-opaque tracer I introduced was the use of colloidal lanthanum. Using this tracer, Jean-Paul Revel and I succeeded in revealing the fine structure of electrotonic (so-called gap, close, or nexus) junctions. These junctions are the structural correlate of the electrophysiologically defined electrical synapses that occur in excitable tissues. We showed that these junctions, consisting of subunits in hexagonal arrays, also are formed by cells in nonexcitable tissues. We now know that they are basic to cell-cell communication. Viewed in light of more recent biochemical electron diffraction and crystallographic studies by others, our original model of this junction appears to have been quite perceptive.

It is amusing that in our original paper we did not use the rather unsuitable, oxymoronic term gap junction (there is no real gap), although the introduction of that term has almost invariably been referenced to our original paper. We did, however, later use the term in an abstract and in seminars and at meetings, so we are partly to blame for following the fashion. Our original paper is also a “citation classic.”

REACTIVE OXYGEN SPECIES

When activated in phagocytosis polymorphonuclear leukocytes undergo a respiratory burst, generating reactive oxygen species such as H₂O₂, superoxide; some of which are bacteriocidal. In the 1970s, the exact sites where these reactive oxygen species are generated were, from biochemical data, somewhat controversial. The ultrastructural cytochemical approaches pursued by Richard Briggs, John Robinson, Manfred Karnovsky, and myself have been summarized. We designed two ultrastructural cytochemical methods for detecting H₂O₂ and found that it was generated at the site of contact with the bacterium at the surface membrane. As the bacterium is internalized by phagocytosis, the membrane of the phagosome (derived from the surface) is the site of H₂O₂ generation. Subsequently, we showed by a novel technique the similar generation sites of superoxide, a precursor of H₂O₂. We verified, at the ultrastructural level,
the delivery of myeloperoxidase from cytoplasmic granules into the phagosome, which contains powerful mechanisms for bacteriocidal action. Marla Steinbeck, A.U. Khan, and I later demonstrated by devising a chemical trap method that leukocytes upon activation also generate singlet oxygen (32). The significance of this finding was not initially apparent as singlet oxygen is short lived. However, recently, Babior and associates (33) have shown that when antibodies are bound to the leukocyte cell surface, ozone (a potent and long-lived cidal agent) is generated from singlet oxygen. Together with Abul Abbas (35) we mapped the inherent distribution of surface immunoglobulin on murine-B lymphocytes, thereby utilizing monovalent antibodies labeled with fluorescein. We then treated the cell surface with monovalent antibodies to fluorescein labeled with ferritin. The cell-surface distribution of the complexes was revealed by rapid freezing of pre-fixed cells and freeze-etching. We found that the surface Ig was in small clusters and interconnected networks and was compared with the expected random (Poisson) distribution. Surprisingly, we also found that the distribution was nonrandom to a high degree of statistical significance, suggesting organization in a specific manner. The functional significance of this microclustering was not elucidated. Perhaps it contributes to the efficiency of antibody binding. A parallel study on Ia-antigen distribution on murine-B cells showed a similar nonrandom distribution of microclusters of the antigen.

LIPID DOMAINS

Over the years, our laboratory has been interested in the mechanisms underlying various cell-surface phenomena that relate to physiological and pathological processes (discussed above). In collaboration with Richard Klausner, Alan Kleinfeld, and Eliezar Dawidowicz, we used free fatty acids (FFAs) as one class of probe to perturb structure and function at the cell surface. FFAs produced profound biological effects when incorporated into surface membranes. These effects included alteration of membrane-bound enzymatic activity, platelet aggregation, lymphocyte...
mitogenesis, surface receptor capping, and cell-substrate and cell-cell adhesion (see References 36, 37). These phenomena required explanation in terms of membrane structure. Thus, the interaction of FFAs with cell-surface membranes and lipid bilayers was studied by monitoring the emission polarization changes of the fluorescent probes 1,6-diphenyl-1,3,5,-hexatriene (DPH) and 8-anilino-1-naphthalene sulfonate (ANS) (38). The differential polarization effects of cis-unsaturated FFA versus trans-unsaturated and saturated FFA, when incorporated into plasma membranes as well as into mixed-phase vesicles, were similar. Using solution theory in mixed-phase vesicles, we understood the shift in the transition midpoint temperature to mean that cis-unsaturated FFA preferentially partitioned into fluid domains, whereas trans-unsaturated FFA and saturated FFA preferentially partitioned into solid-like domains.

The fluorescent lifetime of DPH in membranes and vesicles was measured using the phase-modulation technique. The results were analyzed in terms of site heterogeneity (38) and indicated that although DPH decay in single-phase vesicles was monoexponential, a considerable degree of heterogeneity was observed in mixed-phase vesicles and also in plasma membranes (with the exception of the erythrocyte membrane). These results, together with the FFA effects, were interpreted in terms of lipid domains (relatively fluid and gel-like) existing in membranes (38).

We theorized that the insertion of various FFA into specific domains caused perturbation of domain structure with consequent effects on the conformation, structure, and function of proteins embedded in those domains. In general, our working hypotheses were that cis-unsaturated FFA, or subtracted cholesterol, further fluidized gel-like domains and vice-versa for trans-unsaturated FFA, saturated FFA, and for added cholesterol. Many questions regarding the precise mechanisms involved remain unanswered. Nevertheless, the concept of membrane lipid domains initially put forward remains viable, perhaps in more modern terms, such as that of lipid rafts.

HEPARIN AND VASCULAR SMOOTH MUSCLE PROLIFERATION

In recent years, we became interested in the proliferation of vascular smooth muscle (VSMC) that occurs after vascular injury, particularly in arteries, for instance, following angioplasty, stent placement, or organ transplantation, and which is of considerable clinical import in these situations. Our studies are summarized in Reference 39. Many of these studies were performed by Elazer Edelman, Richard Hoover, and John Castellot. We initially surmised that an insult to the endothelium would lead to activation of the clotting cascade and the generation of thrombin, which is a mitogen. Thus inhibition of thrombin generation by administration of the antithrombin-III binding agent, heparin, should inhibit proliferation of VSMC. Some-what to our surprise this turned out to be the case, initially in vivo after air drying or balloon injury of the aorta or carotid arteries [as shown by me and Alexander Clowes (40)]. (In proofing the paper we both failed to notice that our names were misspelled. This is embarrassing, as the paper has been cited often.) The inhibition of growth was also observed in vitro (see Reference 39). However, these were serendipitous findings, as removal of the antithrombin-III binding capacity of the heparin by a variety of means caused retention of the antiproliferative but lost the anticoagulative activities. This antiproliferative activity was novel for heparin, as other glycosaminoglycans were virtually inactive. Heparin is a structurally heterogeneous, highly charged (O- and N-sulfated and carboxylated) glycosaminoglycan consisting of varying repeating disaccharide units of alternating D-glucosamine and uronic acid sugars, including L-iduronic acid. The structural specificity of the latter lends special antiproliferative properties to the molecule.
The heparin antiproliferative activity was size dependent (hexamers were the minimum), and O-sulfation (but not N-sulfation) was required (whereas the anticoagulant activity required both). There are inhibitory effects early in the cell cycle and inhibition of expression of early response genes. The final block in the cell cycle was in early to late G1 and was fully reversible when the heparin or its derivatives were removed.

With John Castellot and Richard Hoover, we showed that the growth of glomerular mesangial cells, which resemble VSMC, was also inhibited by heparin (41). Furthermore, the chronic phase of our glomerular nephritis model referred to above (28), in which there is mesangial cell proliferation, was prevented by administration of a nonanticoagulant, antiproliferative heparin derivative, as well as by whole heparin (42), and proteinuria was markedly diminished.

We demonstrated that endothelial cells produce heparin-like molecules (heparan sulfates) that are antiproliferative for VSMC and render them unresponsive to growth factors (see Reference 39). Similarly, glomerular epithelial cells produce heparin-like molecules antiproliferative for mesangial cells (idem). We surmised (39) that in endothelial or glomerular epithelial cell injury, VSMC or mesangial cells, respectively, are released from nonresponsiveness to growth factors, as the heparin-like molecules are no longer secreted from the adjacent heparin-like molecule producers, the endothelial, or glomerular epithelial cells. Such modeling may also apply to smooth muscle proliferation occurring at other sites such as in the pulmonary vasculature and in bronchoproliferative diseases.

The mechanisms underlying the antiproliferative activity of heparin and similar molecules are complex and not clear. Heparin has anti-inflammatory effects; for instance, it blocks the adhesion of leukocytes to endothelia and their invasion into the artery or other tissues to provide a source of growth factors for VSMC proliferation. It does not appear to be effective by directly binding to growth factors nor by blocking binding to their cell receptors. Laurel Pukac of my laboratory found that serum and platelet-derived growth factor BB activate a heparin-sensitive pathway involving protein kinase C and raf-1 (43). Suppression of C-dependent mitogen-activated protein kinase activation appears to be a key element in the antimitogenic actions of heparin. In contrast, epidermal growth factor does not stimulate these kinases and acts through a heparin-insensitive pathway. However, no sole inhibitory mechanism has been unequivocally established, although several other signal transduction mechanisms have been suggested. For instance, in both VSMC and mesangial cells, heparin-sensitive, Ca\(^{2+}\)/calmodulin-dependent protein kinase has been demonstrated (44). Furthermore, the heparin-induced block in G1 to S phase, which we described above, is claimed to be caused by p27(kip1)-mediated inhibition of cyclin-dependent kinase 2 (44a).

**CARDIAC TRANSPLANTATION AND GRAFT ARTERIOSCLEROSIS**

VSMC proliferation is one of the characteristics of progressive graft arteriosclerosis, which is a leading cause of morbidity and mortality in organ transplants after the first postoperative year. David Adams and I developed the Lewis-to-F344 heterotopic rat cardiac transplant model to study these phenomena (45). These strains have minor histocompatibility differences, and the donor (Lewis) hearts, attached to the blood supply in the inguinal region, develop chronic rejection characterized by progressive graft arteriosclerosis and chronic inflammation in the myocardium. We showed that graft arteriosclerosis developed in well-defined stages: (a) early adherence of monocytes and T cells to the endothelial surface, proceeding to (b) subintimal infiltration of monocyte/macrophages and T cells, and, finally, (c) a diffuse arterial concentric intimal thickening, predominately consisting of proliferating VSMC. This appeared...
to be an ideal model for studying the effects of a nonanticoagulant heparin on the development of the lesion and the inhibition of chronic rejection and graft arteriosclerosis. A large amount of nonanticoagulant heparin was required for administration over a period of months and this was prepared for us by the Institut Choay of Paris, France. Unfortunately, the large bottle of the white, fluffy powder that we received disappeared from our cold room overnight, obviously stolen by persons unknown, no doubt for nefarious purposes. We were thus unable to conduct this long-term experiment, so we investigated the roles of activated macrophages in the pathogenesis of graft arteriosclerosis.

In collaboration with Mary Russell, we showed that during development of lesions in rats on a normal diet, gene transcript levels in the donor hearts were significantly increased for the cytokine effectors associated with macrophage activation: interferon-γ, interleukin-6, monocyte chemoattractant protein-1 (MCP-1), macrophage colony stimulating factor, and the macrophage lectin Gal/GalNAc (46). These increased levels in gene transcripts were confined to the donor hearts and were not raised systemically in the tissues of the recipient rats, with the exception of interleukin-6. In several cases we confirmed cytochemically increased levels of the gene products in macrophages in the donor hearts.

We then inhibited the chronic inflammatory response in the donor hearts with a diet for the recipients deficient in essential fatty acids, which causes an increase in the ratio of Mead acid to arachidonate, which results in decreased prostaglandin and leukotriene synthesis and leukocyte functions. The diet deficient in essential fatty acids significantly decreased VSMC proliferation, graft arteriosclerosis, and the chronic inflammatory response (47). Gene transcript levels for MCP-1, interferon-γ, and CD4 were also significantly decreased compared with those levels in control hearts transplanted into hosts on a normal diet (48). We concluded that activated macrophage functions were major contributors to graft arteriosclerosis and VSMC proliferation.

**TEACHING**

I have always regarded teaching as an important component of my academic life. For almost 50 years I have taught general pathology to medical students, both as a lecturer and as a laboratory instructor. For 12 years I organized and ran the general pathology course. In the early days we had three mornings a week for a whole semester assigned to us and were able to perform experiments with the active participation of the students [e.g., aminonucleoside nephrosis, clearance of bacteria from the blood (with the collaboration of the microbiologists), anaphylactic shock, etc.]. We had lectures on the latest ongoing research in the laboratories of the faculty. Nowadays, unfortunately, owing to time constraints, the difficulties in obtaining permission for animal experiments, and the fashionable emphasis on clinical cases and “relevance,” the general pathology course is but a shadow of its former self. I, and many of the students and faculty, do not find this situation intellectually satisfying.

I have noticed over the years a rather strange phenomenon: The more senior the faculty, the less teaching they do. Professors may give one or two lectures a year and this is regarded as fulfilling their responsibility for teaching. Chairpersons are not infrequently too busy having to be entrepreneurial, or having to deal with the exigencies imposed by managed care, to participate significantly in teaching. This is unfortunate: Students are being denied wisdom and experience. In the best of all worlds, professors should profess—that is, teach—their discipline.

As there was, and still is, a large nonmedical population, consisting of Ph.D. graduate students, post-doctoral fellows, and faculty at our institution, I felt there was a gap in their education and experience at medical school resulting from a lack of exposure to mechanisms...
of disease and the concepts of pathogenesis. As a consequence, together with my friend and colleague, the late Ramzi Cotran, and with generous financial support from the Josiah Macy, Jr., Foundation, we organized an experimental course in molecular pathology and mechanisms of disease to service these needs. This course, which ran annually for five years, proved to be immensely popular, and even senior faculty from a variety of disciplines, such as physics, engineering, and biochemistry, attended. Similar courses were thereafter instituted at other schools. Several students told me that being exposed to the content and philosophy of the course had profound impact on their research interests and, in several instances, had changed the direction of their careers into investigating disease processes—a most gratifying result.

Because I believe that general and experimental pathology meld with other disciplines, as they are eclectic in the application of techniques and approach, I was pleased to chair the first interdepartmental, interdisciplinary Ph.D. program at our school, namely that in cell and developmental biology, for a period of approximately 17 years. In fact, I regard myself as a sort of hybrid cell biologist and experimental pathologist. This experience in the program, besides broadening my own perspectives, enabled me to bring Ph.D. graduate students in a variety of disciplines into the orbit of disease mechanisms and pathogenesis.

CODA

One thing I have learned over the years is that sometimes it takes a long time for the significance of one’s results and hypotheses to be fully recognized. This applies, for instance, to our work of several decades ago on the glomerular slit diaphragm (23), which now is recognized to be of considerable importance in glomerular filtration. The molecular nature of the structure, and its perturbation leading to proteinuria, is now being established (25, 26), reinforcing our original concepts that this structure plays a major role in filtration. Another instance is our concept of membrane lipid heterogeneity and lipid domains (38), which somehow never became popular when proposed in the 1970s, but which, in modern form, resonates, I like to think, in concepts such as that of lipid rafts and other membrane lipid heterogeneities. Patience is a virtue and sometimes a necessity. Occasionally, of course, we were plain wrong, as was later shown by us or others. An example is our initial reasoning as to the nature of the antiproliferative activity of heparin for VSMC. On the other hand, our papers on the blood-brain barrier (13), the gap junction (30), and the antiproliferative activity of heparin for VSMC (40) were fairly rapidly recognized for what they were worth, so one cannot complain.

I here provide an overview of the rather itinerant route by which I have evolved from the African veldt to the hallowed halls of Harvard. I have been extremely fortunate to have worked with talented collaborators and trainees, few of whom I have been able to mention, who have carried the load of work in many diverse areas of research. I am proud that more than two dozen have achieved prominent positions in academia and biomedical organizations. I am also grateful to have been amply supported by the National Institutes of Health. Overall, my rather hodge-podge career has been a lot of fun. However, I would add a cautionary note if any young pathologist should take my sort of career path as an exemplar. In this age of modern science—genomics, proteomics, and other “omics” of the future—I recommend a greater degree of specialization. Although I have enjoyed savoring many areas of pathology and cell biology, biomedical science today moves fast and is too complex for such an approach; one needs to concentrate more specifically in a discipline. However, to have as powerful an armamentarium as possible, I have always encouraged my students early in their careers to acquire expertise in the most basic of sciences—mathematics, physics, chemistry, molecular biology, biochemistry,
genetics, and the like—before plunging into disease-related areas. This approach may, I hope, enable students both to adapt to new techniques and concepts and to turn attention to a variety of emerging problems as well as provide them with the flexibility to move into newer areas of research.

As indicated, I was much influenced by Florey and by his book on general pathology (1). As Frank Fenner wrote (49), Florey viewed pathology with the eye of an experimental physiologist and not purely as a morphologist, which was the fashion of the time. He also had a strong background in biochemistry. I hope I am not being overly sententious when I say that today the pathologist should have the piercing eye of a modern biologist. Furthermore, work beyond the relatively easy task of data collection is needed, e.g., understanding the implications of the data, insightful synthesis, and conceptualization.

Those in pathology, no matter how basic their research, should, I believe, always try to keep in mind the unique concept that pathology embodies above all other sciences—the Concept of Pathogenesis. With this concept in mind, I believe that pathologists can make a singular contribution in their study of disease states.

Lastly, I can say the following regarding my own Odyssey:

_Heureux qui comme Ulysse, a fait un beau voyage._

Happy he who like Ulysses has made a glorious voyage.

Joachim du Bellay, 1559, *Les Regrets*

**LITERATURE CITED**


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