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Annu. Rev. Pharmacol. Toxicol. 1995. 35:1–27

A RESEARCH TRAIL OVER HALF A CENTURY

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KEY WORDS: vascular pharmacology, peripheral adrenergic mechanisms, receptor theory, photorelaxation, EDRF, NO

ABSTRACT

The author describes his major research activities from the time of his PhD thesis work (1937–1940) on properties of erythrocyte membranes to the present. His involvement in research on circulatory shock during World War II led to a continuing interest in the physiology and pharmacology of smooth muscle and cardiac muscle. From 1956 to 1978, his main areas of research were photorelaxation of blood vessels, factors influencing contractility of cardiac muscle, peripheral adrenergic mechanisms, and receptor theory. The major findings of his and his collaborators in these areas are described. He then recounts how an accidental finding in an experiment in 1978 on preparations of rabbit aorta eventually led to the discovery of endothelium dependent relaxation and the endothelium-derived relaxing factor (EDRF); and how additional findings led him to propose in 1986 that EDRF is nitric oxide.

EARLY EDUCATION

I was born in the lovely coastal city of Charleston, South Carolina, in 1916 and lived there until I was thirteen. In Charleston I first became enamored of "natural history" when I attended nature study classes and field trips to nearby beaches, marshes, and woods sponsored by the Charleston Museum. I became an avid shell collector and bird watcher (that was before the term "birder" was coined), and I still enjoy these hobbies. In 1929 my family moved from Charleston to Orangeburg, South Carolina, an inland, rural town of about 8,000 inhabitants, where my mother had grown up and still had some family. The

0362-1642/95/0415-0001$05.00

reason for the move was that Furchgott's department store in Charleston, which had been started by my grandfather and was being run by my father and his two brothers, was unable to survive in the midst of the Depression, and my father decided to open a women's clothing store in Orangeburg. So, I spent my high school years in Orangeburg, enjoying small-town life and competing with my first cousin Edwin Moseley for the highest grades in our class. He won.

Within the first couple of years of high school, I knew that I wanted to be a scientist. My parents were encouraging. They gave me chemistry sets and a small microscope as presents. I liked to read popular books about scientists, although there were not many available at that time. My father subscribed to the Sunday *New York Times,* in which there was often a column on science that I found very exciting.

During the four years that I was in high school, my older brother Arthur was at the University of North Carolina at Chapel Hill majoring in mechanical engineering. I wanted to attend college there also, but that was not possible when I finished high school in 1933 because tuition for me, as an out-of-state resident, was more than my father could afford at that time. So I spent my freshman year at the University of South Carolina, where my tuition was much less. However, by the summer of 1934, my father moved his business from Orangeburg to Goldsboro, North Carolina, where he felt that the local economy was better than in Orangeburg. So now, as a resident of North Carolina, I was able to register at the University at Chapel Hill as a sophomore majoring in chemistry.

At Chapel Hill I had a number of excellent teachers in chemistry. During my junior and senior years, I had a small amount of financial support from an NYA job (NYA being the initials of the National Youth Administration, which was set up by the federal government to help students during the Depression). In that job, I was a research lab assistant to a junior faculty member working on the physical chemistry of solutions of cellulose. I had decided early in my college years that I would go on to graduate work in some branch of chemistry. My preference by the time I was a senior was physical organic chemistry. I sent letters to dozens of chemistry departments applying for a graduate fellowship or teaching assistantship. I had an excellent academic record, but by graduation time I still had not received a definite offer of a position for graduate training. I was almost resigned to taking a job in the chemical industry, when around the middle of June while I was in Florence, South Carolina, where my parents now lived, an unexpected offer of a teaching assistantship came to me from the Department of Physiological Chemistry at Northwestern University Medical School in Chicago. I was to be a graduate student of Dr. Henry Bull, who had recently come to Northwestern, and whose research interests were physical chemical aspects of biochemistry. At Northwestern they needed some-

one who had already taken a course in biochemistry to fill an open teaching assistantship. Fortunately, I had taken a short course in biochemistry at Chapel Hill.

NORTHWESTERN AND COLD SPRING HARBOR (1937–1940)

Before I went to Chicago, I worked for two summer months in 1937 for Eastern Airlines at the Philadelphia airport—a job that my older brother Arthur, who was employed by that airline, helped me obtain. The job allowed me to save some money and also allowed me free air travel to Chicago, which helped a lot, since my stipend as a teaching assistant at Northwestern was only $50 a month for a nine-month academic year. (It did go up to $60 a month by my third year.) When I arrived in Chicago, it had already been arranged for me to share a room with two more-advanced graduate students. One of them, Herman Chinn, and I continued to room together during the course of my graduate work. During the Second World War, Herman did pioneering work on the testing of antihistamines as antimotion sickness agents on troop transport ships. Living in Chicago was quite a change from living in the Carolinas. When I would walk to work in the winter from our rooming house, which was about a mile from the medical school, the chill wind whipping in from Lake Michigan along Chicago Avenue was quite an experience for a southern boy.

My course work at Northwestern was partly at the medical school on Chicago Avenue and partly at the Evanston campus to which I would travel via the El. At the Evanston campus, my courses were mainly in physical chemistry, under Dr. Malcolm Dole, who was also on my PhD advisory committee. At the Chicago campus, I had to take physiology and bacteriology (along with medical students), Henry Bull's course on physical chemistry in biochemistry, and some assorted graduate courses in physiology and biochemistry. Bull had me enroll immediately after coming to Northwestern in the second half of the physiology course for medical students (the first half was given to these students in the second semester of their freshman year). I had to struggle to keep up. The only biology course that I had taken in my undergraduate work was botany, and that didn't help much. By the time I took the first half of the physiology course, in the second half of the year, along with first-year medical students, I was already experienced and sailed through without difficulty. The course was under the direction of Dr. Andrew Ivy, who had built up a sizeable physiology department faculty for those times. In contrast, the biochemistry faculty consisted of only the chairman (Dr. Chester Farmer), Dr. Bull, and two part-time lecturers.

My laboratory work with Bull began with the preparation of purified egg albumin. He was studying the physical chemical changes that occurred in this

protein after various methods of denaturation had been employed. He had begun to involve me in some of his studies when the summer of 1938 came along, and that turned out to be a special summer for me. Bull had been invited to present a paper on his work at the sixth Cold Spring Harbor Symposium on Quantitative Biology, which was to take place at the Cold Spring Harbor Biological Laboratory of the Long Island Biological Association. The theme of the symposium, which was to run for five weeks in a leisurely fashion, was the structure and function of proteins. Bull had obtained permission from the director of the Cold Spring Harbor Laboratory, Dr. Eric Ponder, for me to attend the symposium, while earning my room and board by running the slide projector at the lectures. Fortunately I was able to get a ride to New York City with one of the first-year medical students at Northwestern, and then after spending a day at the 1938 World's Fair outside of New York City, I went on to the Cold Spring Harbor Laboratory. The symposium was very exciting, and I met many distinguished scientists. Ponder and a physician-scientist, Harold Abramson, arranged to have me assist in a research project at the laboratory for the rest of the summer after the symposium was over. The project was on the electrophoretic mobility of rabbit erythrocytes and ghosts, measured with the use of a microelectrophoresis cell and light- and dark-field microscopy (1).

By the end of the summer, I had become very interested in the physical chemistry of the red blood cell membrane. When I returned to Northwestern in the fall of 1938, Bull approved continuation of my research on red blood cells as a PhD thesis project. In particular, I was fascinated by the unexplained phenomenon of the transformation of mammalian red blood cells suspended in unbuffered isotonic saline from discs to perfect spheres when a small drop of the suspension was placed between slide and coverglass. I discovered that the disc-sphere transformation depended on two factors. The first was a rise in pH to over 9.0 in the unbuffered suspension, as a result of the alkaline nature of the glass surfaces (pH was measured with a semi-micro glass electrode that I constructed). The second factor was the removal from the suspension of the red blood cells by adsorption onto the glass surfaces of the slide and coverglass of a substance in the suspension that prevented sphering produced by elevation of pH of the suspension. I demonstrated that this substance, which I termed the antisphering factor, was serum albumin, which could not be effectively removed from the red cells simply by multiple washing and centrifuging (2). In addition to the work on shape changes in erythrocytes, my PhD thesis work involved additional studies on the electrophoresis of the cells under various conditions and on other aspects of the physical chemistry of erythrocyte membranes.

In the summer of 1939 at the invitation of Ponder, with whom I had extensive correspondence during the year and who had become in effect the major advisor for my PhD thesis research, I returned to Cold Spring Harbor to

continue research on red blood cells. To earn my room and board, I waited on tables in the communal dining room. I also was able to attend the symposium talks of that year, which were on biological oxidations. There, I first became aware of the new developments in oxidative energy metabolism and the importance of high-energy phosphate compounds. Among the many outstanding biochemists in attendance were L Michaelis, Fritz Lipmann, and Carl Cori. Ponder and his young wife Ruth were very hospitable to me. I was much impressed with his skill in applying mathematics in his research, his facility in scientific writing, and his large record collection of classical music.

I was able to complete and defend my thesis in time to receive the PhD degree in June of 1940. Earlier that spring I had attended the annual meeting of the Federation of American Societies for Experimental Biology (FASEB) in New Orleans. Fortunately I had been asked by Henry Tauber, an Austrian biochemist working for a pharmaceutical firm in Chicago, to share the driving in his car on the round trip to New Orleans as well as his room in a run-down hotel in New Orleans. Thus, I was able to attend this meeting at very little expense. One reason Tauber had invited me on the trip was that I had actually taught him to drive in the summer of 1936 in Florence, South Carolina, where he, an Austrian refugee, happened to be doing research on cancer in a small private hospital, and I happened to be visiting with my parents. At the FASEB meeting in New Orleans, where gatherings of participants were still called smokers and even a fancy meal was not more than two dollars, I had some interviews about possible postdoctoral jobs. One of the interviews was with Dr. Ephraim Shorr, an associate professor of Medicine at Cornell University Medical College in New York City, whom I had met when he was a speaker at the Cold Spring Harbor Symposium the summer before. A few weeks later Shorr offered me a postdoctoral position in his laboratory. Although I was hoping to get a position that would allow me to continue work on the physical chemistry of proteins or cell membranes, none came through, so I accepted the position with Shorr, with the understanding that I would begin in September.

The reason for waiting until September to begin work at Cornell was because I wanted to spend one more summer at the Cold Spring Harbor Biological Laboratory. This time, however, I went as an invited speaker to the symposium, whose theme that summer was the permeability of cell membranes. My talk was entitled "Observations on the structure of red cell ghosts." At that symposium, there were again a number of established distinguished scientists like KS Cole, Robert Chambers, and FO Schmitt and, in addition, a number of bright young scientists like Hans Neurath, who had also been at the 1938 symposium; Hugh Davson, who with Danielli had developed the lipid bilayer membrane model; and Benjamin Zweifach, with whom I was to later collaborate in research. While at Cold Spring Harbor, I also had a chance to finish

up some research on red blood cells with Ponder and to write two manuscripts for publication on our work. (It seems that writing manuscripts came much more easily then than it does now.)

CORNELL UNIVERSITY MEDICAL COLLEGE (1940–1949)

I worked in Ephraim Shorr's laboratory at Cornell University Medical College for nine years. When I arrived, Sam Barker, a young research associate, was there to instruct me in the methods and procedures they were using to study tissue metabolism (largely using Warburg manometers) and the turnover of rather ill-defined tissue organic phosphate fractions from canine cardiac muscle during incubations in vitro. For such studies the lab was one of the first to use radioactive phosphate, which we obtained from the cyclotron laboratory at Berkeley. Barker left toward the end of my first year at Cornell, and I was then responsible for running the laboratory for Shorr. Shorr himself would sometimes take part in preparing tissue for the Warburg experiments; he was quite capable in the laboratory in addition to being a busy and excellent clinician. (He was in charge of the endocrine section of the Department of Medicine of the Cornell–New York Hospital Center.) He was also a very cultured New Yorker.

During my first two years at Cornell, my major project was phosphate exchange and turnover, using radioactive phosphate and slices of dog left ventricular muscle. I presented a paper on that work at the annual FASEB meeting in Boston in 1942 (the last such meeting before their cancellation for the remainder of World War II) and published a full paper on the work in the *Journal of Biological Chemistry* in 1943. The methods and equipment we used in that work have long been superseded, but we did manage with chemical and some early enzymatic methods to show the extremely fast turnover of creatinine phosphate and the terminal phosphate of ATP in resting cardiac muscle (3). It should be remembered that at that time there were no automated radioactivity counters to give automatic printouts of counts per minute. One placed the Geiger-Müller counter tubular probe into the solution to be assayed and sat nearby with a stopwatch in hand while watching the counts mounting on a readout meter.

The 1943 paper was my first full publication after three years of work at Cornell. One likely reason for sparse output was that the United States had entered World War II in December of 1941, and Shorr, like many others, began to undertake research that had more relevance to the war effort. With government and other support, he shifted the major research in the lab to circulatory shock—first concentrating on changes in tissue energy metabolism resulting from hypoxia associated with hemorrhagic shock and then mainly on factors

that might account for *irreversible* shock, the condition in which restoration of blood volume is no longer able to raise pressure and sustain life in the animal subjected to maintained low blood pressure as a result of controlled hemorrhage. To help in this new line of research, Shorr recruited Benjamin Zweifach, then a bright young physiologist who had trained with Robert Chambers and had developed a beautiful method for observing the circulation in part of the mesentery (the mesoappendix area) of the anesthetized rat. The thin mesoappendix area, irrigated with temperature-controlled physiological salt solution, was spread out over a special stage that allowed the blood flow through the vessels to be observed directly by microscope. Changes in vessel diameters and flow in response to the topical application of vasoactive agents, such as epinephrine, could be visually graded. Also, changes due to intravenous injections of agents via the rat tail vein could be evaluated.

In brief, the rat mesoappendix test, conducted by Zweifach and technicians whom he trained, produced evidence by 1944 of two vasoactive factors in circulatory shock. The first factor appeared in the plasma of dogs in the early reversible (by transfusion) stage of hemorrhage. Intravenous injections of this plasma increased the sensitivity of the small arterioles and precapillary sphincters to topically applied epinephrine in the mesoappendix test. This factor was termed VEM (for vasoexcitatory material). As the irreversible stage of circulatory shock developed, VEM activity disappeared from the plasma, and a new factor appeared that markedly decreased the sensitivity to epinephrine in the mesoappendix test. This factor was termed VDM (for vasodepressor material). We developed evidence, in part from in vitro experiments with tissue slices, that hypoxic kidney was the probable source of VEM and that hypoxic liver was the probable source of VDM. By late 1945, these exciting developments led to a lead article in the journal *Science* by Shorr, Zweifach, and me (4).

During the war years, I was not solely involved in research on tissue metabolism and circulatory shock. I had originally been given a deferment from the military draft because I have vision in only one eye (the result of a detached retina in my right eye caused by a blow received while playing intramural football in college). In 1943 Eugene DuBois, chairman of the Department of Physiology at Cornell, arranged that I join his department as an instructor in order to replace a staff member who had left for military service. Although I was teaching in physiology, mainly in laboratory exercises and conferences for students, I still spent most of my time in research in Shorr's lab. About halfway through the war, my draft deferment for my impaired vision was canceled, but by then I was involved in teaching medical students and in research funded by the US Office of Scientific Research and Development, so I was given a new deferment on the basis of my work. By then my work also included an excursion into infrared spectroscopy, especially of steroidal hormones and their metabolites. The absorption spectra were laboriously obtained

on a homemade, manually operated double-beam infrared spectrophotometer (one of the few in existence then). My assistant in the work was Harris Rosenkrantz. We eventually published three papers on the work in the *Journal of Biological Chemistry.*

The work on VEM and VDM continued after the war ended. Shorr wrote several review-type articles between 1946 and 1950 on the subject of the possible role of these factors not only in circulatory shock but also in hypertension. He sometimes included me as a coauthor, although I had rather little to do with the writing. I had attempted to isolate the VEM-like material that accumulated in incubation fluid when kidney slices were incubated anaerobically. I was able to concentrate it somewhat, and it appeared to be a labile dialyzable peptide, but I failed miserably at isolating it. On the other hand, Abraham Mazur, a professor of biochemistry at the City College of New York who worked part time with us, successfully purified a VDM-like material from liver and showed that it was ferritin. He and Shorr published this finding in 1950. (Ferritin or not, we might now wonder whether VDM could somehow be related to nitric oxide!)

Unfortunately, the only bioassay procedure for detecting VEM and VDM activity involved the subjective evaluation of changes in sensitivity to epinephrine in the rat mesoappendix test. Intravenous injections of solutions containing high levels of impure VEM or purified ferritin did not affect blood pressure in experimental animals. Attempts to develop an in vitro bioassay system also failed. These failures tempered my enthusiasm and, I think, that of Zweifach for the significance of VEM and VDM in the regulation of circulation but not that of Shorr, who even gave a Harvey Society Lecture on the subject in the early 1950s. However, the failed attempts to develop an in vitro bioassay for VEM and VDM were important for me, for they introduced me to the pharmacology of smooth muscle, a subject that has been a major interest of mine ever since.

Two of the isolated smooth muscle preparations that I unsuccessfully tested for bioassay of VEM and VDM were a helically cut strip of rabbit aorta, which responded with contraction to epinephrine, and a longitudinal segment of rabbit duodenum, which exhibited spontaneous rhythmic contractions that were inhibited by epinephrine and stimulated by acetylcholine. At that time contractions of such smooth muscle preparations mounted in organ baths were recorded with isotonic levers on kymographs. One day in the course of making tests on segments of rabbit duodenum mounted in oxygenated Krebs solution, I was surprised to see that during the first hours of the experiment, contraction amplitude did not stabilize as usual but declined gradually and markedly, even though the rhythmic frequency remained unchanged. I suspected that my technician had forgotten to add glucose to the Krebs solution, so I added the glucose, which quickly increased contraction amplitude to the normal level.

This finding led to a simple procedure for finding out which sugars and fatty acids could be utilized for energy in contractions of intestinal smooth muscle under aerobic and anaerobic conditions and to analyze the sites of action of metabolic inhibitors (5).

By 1949 I had decided that I would like to move into a regular position in a preclinical department and become independent in my research. I received two interesting offers at the assistant professorship level: one in physiology at Duke and one in pharmacology at Washington University School of Medicine. After visiting both places, I decided to accept the offer from Washington University, in part because the new chairman there, Oliver Lowry, was someone I had known in the Enzyme Club in New York City and in part because I was becoming interested in pharmacology as a discipline. This interest was partly the result of studies I had begun on the effects of drugs and other agents on smooth muscle preparations in vitro but largely the result of my close friendship with Walter Riker, who was then a junior member in the Department of Pharmacology at Cornell at the beginning of a distinguished career. His enthusiasm for research in pharmacology was contagious. After these many years, we are still close friends.

In the summer of 1949 my family and I drove from New York to St. Louis. My wife, Lenore, a native New Yorker, said she felt like she was going west in a covered wagon. By that time we had two daughters, ages four and one. Later we had a third daughter born in St. Louis. It might be noted here that none of my daughters became scientists; instead, they all went into art (like my younger brother, Max). It might also be noted here that my wife Lenore died in 1983 and that I now have a new wife, Margaret (Maggie). I have been very fortunate in having wives who encouraged my work, even though it often reduced the time I could give to family matters.

WASHINGTON UNIVERSITY (1949–1956)

My seven years in the Department of Pharmacology at Washington University were enjoyable ones. Oliver (Ollie) Lowry had been appointed chairman of that department a year or so before I came, replacing Carl Cori, who was then able to concentrate on heading the Department of Biochemistry. Lowry was already well recognized for his ingenuous methods involving enzymology, spectrometry, and fluorometry in the quantitative analysis of important enzymes, substrates, and products in extremely small amounts on tissue. He was very helpful in introducing me to enzymatic-spectroscopic methods (as developed by Kalckar) for analysis of ATP, ADP, and AMP. As the new chairman, Lowry inherited two faculty members, Helen Graham and Edward Hunter, and recruited two new ones, namely me and Morris (Morrie) Friedkin, who had just returned from a postdoctoral year in Kalckar's laboratory in Denmark. I

had never taken a course in pharmacology as a student, much less taught one, so I spent a lot of time during my first year in St. Louis reading the second edition of *The Pharmacological Basis of Therapeutics* by Goodman and Gilman in order to keep ahead of the medical students. I also had to get used to teaching the student laboratory exercises in the course. In addition to retaining a number of classical physiological pharmacology experiments, Lowry had introduced some new ones on drug metabolism and pharmacokinetics. Later, when I set up my own department in Brooklyn, I adopted for the pharmacology course there much of the lecture, laboratory, and conference program that I had participated in at St. Louis.

Lowry's department was a stimulating place for research. Over the years, the departmental staff grew steadily. Lowry attracted outstanding postdoctoral fellows, such as Eli Robbins and Jack Strominger. We often joined the members of Carl Cori's biochemistry department for seminars and journal club meetings.

My first research project at Washington University was a continuation of the work I had begun at Cornell on energy metabolism and function of rabbit intestinal smooth muscle. I was able to obtain a small grant to support my research on smooth muscle and to hire a technician, Marilyn (Wales) McCaman. Three papers on our work appeared in the *American Journal of Physiology* between 1951 and 1952. Marilyn became my first graduate student. By the middle of 1951, my favorite in vitro smooth muscle preparation shifted from the rabbit duodenum to the rabbit thoracic aorta. I found that the helical (spiral) strip of that vessel, properly cut and mounted in organ chambers for isotonic recording, gave reproducible contractions to epinephrine and norepinephrine after equilibration in oxygenated Krebs bicarbonate solution. At first I had planned to study the effects of disturbances in energy metabolism on these contractions, but I became much more interested in using the aortic strip for studies on drug-receptor interactions.

In 1953 I published a paper entitled "Reactions of strips of rabbit aorta to epinephrine, isoproterenol, sodium nitrite and other drugs" (6). Among the other drugs was acetylcholine. I found that it only produced contractions, whether it was added to resting strips or strips precontracted with some other agent. That was a paradoxical response, since acetylcholine was known to be a very potent vasodilator in vivo. Little did I suspect then what I was able to show many years later—namely, that relaxation of arteries by acetylcholine is strictly endothelium dependent and that my method of preparing the strips inadvertently resulted in the mechanical removal of all the endothelial cells.

In 1954 I published a paper on the use of dibenamine in differentiating receptors in the aortic strip, and in 1955, a review in *Pharmacological Reviews* on the pharmacology of vascular smooth muscle (7). In that review, I tried to develop receptor theory as a logical base for interpreting the responses of

vascular smooth muscle to many neurotransmitters, hormones, and drugs. In order to derive equations to account for the very slow onset and offset kinetics of competitive antagonists as compared to the fast kinetics of agonists, I developed a biophase model in which the agents moved between an aqueous extracellular phase and a lipid membrane phase containing the receptors. Although I paid homage in my review to AJ Clark for his pioneering work in developing receptor theory, I took issue with his hypothesis that response of a tissue to an agonist is proportional to the fraction of receptors occupied by the agonist. Our results with dibenamine, which behaved as an irreversible competitive blocking agent of adrenergic α-receptors, had indicated that with a strong agonist like epinephrine, one could still achieve over half of the maximum contraction when only a small fraction of receptors was still active. This was the beginning of my interest in the concept of "receptors reserve" or "spare receptors." [A year later, RP Stephenson published his classic paper on the subject in which he introduced the concepts of efficacy, full agonist, and partial agonist (7a).]

In my 1955 review, I also briefly reported on a newly discovered phenomenon—that strips of rabbit aorta undergo reversible relaxation when exposed to light of proper wavelength and intensity. The accidental discovery of photorelaxation came from the observation in one experiment that the active contractile tone of two strips in one pair of organ chambers fluctuated with time, whereas the tone of two strips in another pair of chambers remained steady. The two strips showing fluctuations did so synchronously. Those two strips, but not the other two, were in organ chambers near a window through which they were exposed to skylight. Suspecting that the fluctuations in tone were due to fluctuations in light intensity on the strips near the window (it was a cloudy-bright day), I closed the shade on the window, and both strips increased in tone. I opened the shade, and both decreased in tone. From that point on, we never allowed our strips to be exposed to direct skylight. (The usual overhead fluorescent lights do not produce photorelaxation.) I extended some of the studies of the characteristics of photorelaxation that I had begun in St. Louis when I moved to Brooklyn.

In addition to working on in vitro smooth muscle preparations at Washington University, I began what became many years of research on the pharmacology of an in vitro cardiac muscle preparation: the isolated electrically driven left atrium of the guinea pig. To begin that work, I had the assistance of a very able technician, Taisija De Gubareff. Using chemical and enzymatic methods for analysis of creatinine phosphate, ATP, ADP, and AMP, we showed that neither development of *experimental failure* in vitro (a steady loss of contractile force over hours) nor recovery from failure on addition of a cardiac glycoside was due to changes in the concentration of these high-energy phosphates (8). We also reported on the effects of an-

aerobiosis and of several positive and negative inotropic agents. We collaborated with my good friend William Sleator of the Department of Physiology to study the changes in cellular action potentials (measured with intracellular microelectrodes) associated with the changes in contractility of the guinea pig atrium in response to epinephrine and acetylcholine (9) and a number of other inotropic agents.

SUNY MEDICAL CENTER IN BROOKLYN (1956 TO PRESENT)

In 1956 I accepted the position of chairman of the new Department of Pharmacology at the State University of New York (SUNY) College of Medicine at New York City (actually in Brooklyn, and later changed in name to SUNY Downstate Medical Center and more recently to SUNY Health Science Center at Brooklyn). The department had previously been part of a joint physiology and pharmacology department headed by Chandler Brooks, but with the opening of a new, relatively huge (for the time) basic science building for the medical school on Clarkson Avenue (directly across from Kings County Hospital) and with good financial support from the State University, there was ample room and resources for a separate department. Brooks, who headed the search committee that selected me and who helped me a great deal in getting the new department underway, stayed on as chairman of the Department of Physiology and dean of the newly organized graduate school. From his former joint department, I inherited Julius Belford as an associate professor and Bernard Mirkin as an assistant professor. For additional faculty, I recruited Kwang Soo Lee as an associate professor and Leonard Procita, Lowell Greenbaum, Walter Wosilait, and Arthur Zimmerman as assistant professors or instructors, all in time for them to teach our first course for medical students. The following year CY Kao joined the staff. Also during the first year, we accepted our first graduate students, namely Maurice Feinstein, who worked with me, and Arnold Schwartz, who worked with Lee. During that year I didn't do much bench work in the research lab, since most of my time was spent organizing the department and learning how to be a chairman. (I never became a well-organized administrator and was always poor at delegating authority.)

In Brooklyn I continued research on photorelaxation of blood vessels, factors influencing contractility of cardiac muscle, peripheral adrenergic mechanisms, and receptor theory and mechanisms. Then, about twenty-three years after moving to Brooklyn, the research in my laboratory largely shifted to endothelium-dependent relaxation of blood vessels. For convenience I shall divide the

discussion of research in Brooklyn into subsections corresponding to the five areas that I have listed; however, the division is somewhat arbitrary, for certain research projects could well be placed in two or more of these areas.

Photorelaxation of Blood Vessels

To facilitate research on photorelaxation, I acquired new equipment in Brooklyn that allowed more quantitative studies of the phenomenon. Helping with this research were Eugene Greenblatt, my first postdoctoral fellow, and Stuart Ehrreich, my third graduate student. Among other things, we were able to obtain an accurate action spectrum (with a peak at 310 nm) for the photorelaxation (10). Later we observed that addition of sodium nitrite to the bathing medium greatly sensitized the rabbit aortic strip to photorelaxation and shifted the peak of the action spectrum to about 355 nm. Ehrreich and I found that many other smooth muscle preparations (from stomach, intestine, and uterus) that did not ordinarily relax in response to radiation did so in the presence of inorganic nitrite. Percy Lindgren, a visiting faculty member from Stockholm, also worked with us for a while on photosensitization by nitrite.

Many years later in the early 1980s, after the discovery of endothelium-derived relaxing factor (EDRF), I again began research on photorelaxation. Although photorelaxation did not depend on the presence of endothelium on the strip or ring of rabbit aorta, we found many similarities between it and endothelium-dependent relaxation (as produced by acetylcholine or A23187). Not only were photorelaxation and endothelium-dependent relaxation causally dependent on the elevation of cyclic guanosine monophosphate (cGMP) as a result of stimulation of guanylate cyclase, but both were inhibited by hemoglobin and by methylene blue (11). This work was carried out with Desingarao Jothianandan, who has been a most helpful research associate in my lab over the past fourteen years. Then, after EDRF was identified in 1986 as nitric oxide (see below), Kazuki Matsunaga (a postdoctoral fellow) and I reinvestigated the potentiation of photorelaxation by sodium nitrite. Using a cleverly designed perfusion-bioassay-type apparatus, Matsunaga clearly demonstrated that the potentiation was due to the photoactivated release of NO from nitrite (12). It is tempting to hypothesize that light (in the absence of added nitrite) produces relaxation of vascular smooth muscle by photoactivating the release of NO from some endogenous compound, but we have as yet been unable to demonstrate such a release.

Factors Influencing Contractility of Cardiac Muscle

My first graduate student in Brooklyn, Maurice Feinstein, did his PhD thesis research on the effects of experimental congestive heart failure, asphyxia, and ouabain on high-energy phosphates and creatine content of the guinea pig heart. My second graduate student, Albert Grossman, who began work in 1957,

did his thesis research on the effects of frequency of stimulation, extracellular calcium concentration, and various drugs on calcium exchange and contractility of the guinea pig left atrium. Grossman and I published three papers based on his thesis research (see 13), which was one of the first attempts to determine the rates of exchange of calcium (using ^{45}Ca) between extracellular fluid and assorted intracellular "pools" of calcium in cardiac muscle under various conditions affecting contractility. We showed that the positive inotropic effects of norepinephrine and strophanthin-K were correlated with an increase in rate of exchange of calcium in an intracellular pool associated with the contractile process and that the negative inotropic effects of acetylcholine and adenosine were correlated with a decrease in rate of exchange in that pool.

While working at Washington University, I had become interested in effects of frequency of contraction on contractile force in the isolated guinea pig left atrium and other cardiac preparations. Why did force increase with frequency over a wide range (the *positive staircase* effect)? We found that the positive inotropic agents norepinephrine, cardiac glycosides, and excess extracellular calcium could all increase force at all frequencies to essentially the same maximum, thus eliminating a force-frequency relationship. We also began work with ryanodine, which produced a negative inotropic effect on the guinea pig atrium and actually changed the force-frequency effect from a positive to negative staircase (mimicking the normal staircase in frog heart). Sleator, De Gubareff, and I had shown that the decrease in force with ryanodine (unlike that with acetylcholine or adenosine) was not associated with a decrease in duration of the action potential. Grossman's thesis work demonstrated that the increase in force with frequency did correlate with an increase in rate of calcium exchange in the intracellular pool associated with contraction but that the initial negative inotropic action of ryanodine was not accompanied by a significant decrease in rate of calcium exchange in that pool.

About five years after Grossman had finished his work, another graduate student, Peter Wolf, continued work on the effects of ryanodine on contractility of the isolated guinea pig left atrium. He showed that longer exposures to relatively high concentrations of ryanodine did interfere with both influx and efflux of ^{45}Ca. We developed a hypothetical model to explain the effects of ryanodine on the force-frequency pattern and on the normally strong postrest (PR) beats. In this model, we postulated that the myocardial cells had two different calcium pools in the sarcoplasmic reticulum, both of which released calcium with each action potential but only one of which gained calcium from the other during rest intervals and was more sensitive to ryanodine. We never published this hypothetical model, but it fits fairly well with more recent work of others on the reactions of ryanodine with receptors involved with calcium transport in the sarcoplasmic reticulum.

Peripheral Adrenergic Mechanisms

In writing the 1955 review on "The Pharmacology of Vascular Smooth Muscle," I had become interested in the mechanisms by which sympathetic postganglionic denervation and certain drugs such as cocaine markedly potentiate the response of effector organs to epinephrine and norepinephrine yet markedly reduce the response to the sympathomimetic tyramine. My second postdoctoral fellow, Sadashiv (Sada) Kirpekar, was assigned to work in this area. He proved to be a gifted investigator, and we published a number of papers together on work carried out between 1959 and 1962. In one paper (14), with the running heading "The Cocaine Paradox," we presented evidence that in aortic strips of rabbit and isolated electrically driven atria from guinea pig and cat, cocaine potentiated responses to norepinephrine and inhibited those to tyramine by blocking one and the same site on adrenergic nerve terminals. Blockade of this site inhibited the neuronal uptake of norepinephrine from the region of the adrenergic receptors, thus potentiating its action; however, blockade of the site also inhibited uptake of tyramine, whose sympathomimetic action depends on release of norepinephrine from neuronal storage sites, thus inhibiting its action. The site, which we called the transfer site, later became known as the uptake-1 (U1) site. In the same paper we showed that reserpine, which depleted neuronal storage granules of norepinephrine, did not interfere with activity of the uptake site. In addition to Kirpekar, Peter Cervoni came in as a postdoctoral fellow to work on peripheral adrenergic mechanisms. Both he and Kirpekar later became faculty members in the department; Kirpekar stayed on and became a stellar figure in the field of adrenergic mechanisms before his untimely death in 1983.

In 1960 I was invited to present a paper on some of my studies on receptors for sympathomimetic amines at a CIBA Foundation conference on Adrenergic Mechanisms held at CIBA House in London. It was the occasion for my first trip abroad and was very exciting. Among the many distinguished pharmacologists at the conference were Sir Henry Dale, Sir John Gaddum, and JH Burn. At that time Burn was pushing his cholinergic-link hypothesis for norepinephrine release at adrenergic nerve terminals. I felt strongly that he had misinterpreted the experimental results that had led to the hypothesis, and in the discussion sessions I presented our own results with isolated atria, which indicated that there were nicotinic cholinergic receptors on adrenergic nerve terminals that when stimulated by nicotine or acetylcholine triggered a transient release of norepinephrine but that played no role in release of norepinephrine on electrical stimulation of the nerve. We had a somewhat heated discussion on the matter during the conference, and even though he and I remained at odds about his hypothesis, he was a most hospitable guide for my wife and me when we visited Oxford after the conference.

In 1962–63 I spent a sabbatical year in the Department of Physiology of the University of Geneva, where Jean Posternak was chairman. Although I did some research and teaching there, I spent most of my time writing papers on research that my colleagues and I had completed during the preceding few years and on a review of receptor mechanisms (see below). I also visited a number of laboratories in Europe where outstanding research on adrenergic mechanisms was in progress. Among these were the laboratories of US von Euler in Stockholm, E Muscholl in Mainz, and John Gillespie in Glasgow. A special reason for visiting Gillespie in Glasgow was that Kirpekar was there working with him on a two-year fellowship. One of the interesting research problems on which Kirpekar began to work with Gillespie was to find an explanation for the increase in output of norepinephrine from the perfused cat spleen in response to nerve stimulation after pretreatment with alpha-adrenergic blocking agents such as phenoxybenzamine. The mechanism of this paradoxical increase in adrenergic transmitter release in neuroeffector systems after alpha-adrenergic receptor blockade was eventually solved several years later by Kirpekar and other investigators independently. The solution was that alpha receptors reside on adrenergic terminals, which when stimulated by norepinephrine, inhibit release of more norepinephrine in response to nerve stimulation (a negative feedback). Blockade of these presynaptic inhibitory receptors (later classified as α_2-type) by antagonists prevented them from mediating the inhibitory action of released norepinephrine and thus potentiated the output of the transmitter in response to nerve stimulation.

Between 1965 and 1970 I was fortunate to have several very competent coworkers in research on peripheral adrenergic mechanisms. In addition to Kirpekar, there were Pedro Sanchez Garcia (a visiting research associate who later became a leading pharmacologist in his native Spain), Jerome Levin (a postdoctoral fellow), and Arun Wakade (a graduate student who later became a faculty member). With Sanchez Garcia we investigated the mechanism by which bretyllium (a drug of some clinical interest at the time) induced its positive inotropic effect in isolated guinea pig left atrium (15). Wakade investigated the metabolic requirements for uptake and storage of norepinephrine in adrenergic nerve terminals of the left atrium, and Levin compared the relative importance of neuronal uptake and catechol-O-methyltransferase in modulating responses of the rabbit aorta to catecholamines. Kirpekar and I continued to collaborate and frequently published together on studies on the perfused cat spleen, which had now become his favorite neuroeffector system for studies on adrenergic mechanisms.

In early 1971 I began my second sabbatical leave, this time at the relatively new medical school of the University of California at San Diego (UCSD) (located in La Jolla). I became a visiting professor in Steve Mayer's Pharmacology Division of the Department of Medicine. One reason for this choice of

a sabbatical site was that I wanted to learn the method for analysis of cyclic AMP that Mayer had developed (this was before the development of radioimmunoassays for cyclic nucleotides). The other reasons were that La Jolla was a very attractive location and that two old friends, Morris Friedkin and Benjamin Zweifach, had recently joined the faculty at UCSD. I did apply Mayer's method for analysis of cyclic AMP in some experiments on the relaxation of strips of stomach muscle by isoproterenol. However, I did not do much research at La Jolla, partly because a fair amount of my time that year was devoted to duties as president of the American Society for Pharmacology and Experimental Therapeutics (ASPET).

On returning from La Jolla to Brooklyn in 1972, I continued research on the role of receptors located on prejunctional terminals (varicosities) of adrenergic nerves. I collaborated with Kirpekar in an attempt to characterize the prejunctional α-adrenergic receptors on the nerve terminals in cat spleen by comparing the potencies of a series of sympathomimetic amines for inhibiting the release or norepinephrine evoked by nerve stimulation. At the same time, one of my graduate students, Odd Steinsland, was conducting an exciting thesis project on cholinergic receptors on prejunctional adrenergic nerve terminals in the isolated, perfused central ear artery of the rabbit (a modified de la Lande preparation). With the use of various muscarinic agonists and antagonists, he first pharmacologically characterized the prejunctional receptor through which acetylcholine produces a marked inhibition of norepinephrine release (monitored by both the degree of vasoconstriction and [^{3}H]norepinephrine release) (16). He then went on to study the release of norepinephrine from the adrenergic neurons in the ear artery by cholinergic agonists acting on prejunctional nicotinic receptors. At the same time I was continuing studies, with the assistance of Taruna Wakade, on the pharmacology of cholinergic nicotinic receptors on adrenergic prejunctional terminals in the guinea pig left atrium (17). We found that an essentially irreversible blockade of nicotine-induced release of norepinephrine could be produced by a short temporary exposure to phenoxybenzamine if the exposure was made in the presence of a stimulating dose of nicotine (an example of a so-called metaphilic effect). A few years later, Mohammed T Khan, working with me as a postdoctoral fellow, demonstrated a similar phenomenon of enhanced blockade of catecholamine release by phenoxybenzamine in the perfused cat adrenal medulla when the exposure to the drug was made during the stimulation of release by nicotine, acetylcholine, or nerve stimulation.

Receptor Theory and Mechanisms

When I came to Brooklyn in 1956, receptor theory and mechanisms was not yet a popular area of research. When I first gave a course on this subject to

graduate students in 1957–58, the literature on the subject was relatively sparse: papers by Clark, Gaddum, Schild, Ariëns, Stephenson, Nickerson, and me. Studies on receptor mechanisms at that time mainly involved pharmacological procedures in which one obtained concentration-response data for an agonist acting on an isolated tissue under different conditions, such as in the absence and presence of an antagonist, and then attempted to analyze the data with equations derived for some theoretical model in which response was assumed to be some function of the fraction of receptors occupied by the agonist. I became interested in developing suitable theory (occupation theory) and in vitro procedures for differentiating and characterizing receptors. In particular, I concentrated on receptors for adrenergic and cholinergic agents, using as test tissues the rabbit aortic strip, duodenal segment, and stomach fundus muscle, and the guinea pig electrically driven left atrium and tracheal ring.

In 1963 toward the end of my sabbatical year at the University of Geneva, I completed a review of "Receptor Mechanisms" for Volume 4 of the *Annual Review of Pharmacology*. In it I took the opportunity to analyze and criticize some aspects of Paton's ingenuous rate theory of drug action on receptors and to stress the importance of Stephenson's ideas on efficacy and spare receptors. In 1965 at a symposium on receptor mechanisms at Chelsea College in London, I presented a paper on the use of β-haloalkylamines as irreversible receptor antagonists in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes (18). Using a slightly modified form of Stephenson's equations and introducing a term, ε, for intrinsic efficacy, I derived a simple equation that predicted that a plot of $1/[A]$ against $1/[A']$ (where [A] and [A′], respectively, are concentrations of the agonist that give equal responses of the effector system before and after irreversible inactivation of a fraction of the receptors) should be linear, with a slope equal to $1/q$ (where q is the fraction of receptors still active) and an intercept on the ordinate axis equal to (slope $- 1)/K_A$ (where K_A is the dissociation constant of the agonist-receptor complex). Thus, from double reciprocal plots of this type, one could determine both the fraction of receptors still active and the dissociation constant K_A of an agonist. For different agonists acting on the same receptor, one could calculate from the K_A values the fractional occupation of each to obtain the same standard response before receptor inactivation and thus obtain relative efficacies. Using this approach, Paula (Bursztyn) Goldberg (a graduate student) and I compared the dissociation constants and relative efficacies of agonists acting on muscarinic cholinergic receptors of isolated strips of rabbit stomach fundus muscle (19); later John Besse (a postdoctoral fellow) and I compared the dissociation constants and relative efficacies of agonists acting on α_1-adrenergic receptors of rabbit aorta (20). In the 1966 paper (18), I also developed a hypothetical model for a multistep system from receptor-agonist complex to measured response and used this model in an

attempt to explain how there could be different efficacies for different agonists acting on the same receptor. In light of what is now known about receptor signaling pathways through G-proteins, it is probably better to admit that the pharmacological procedure that we developed for obtaining agonist-receptor dissociation constants can only give approximate relative values. Nevertheless, the procedure has proven useful in a number of studies.

In 1972 I published a review entitled "The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory" (21). In it I attempted to formulate the methods and necessary conditions for the classification and differentiation of receptors by pharmacological procedures designed to give accurate dissociation constants of competitive antagonists acting on a given receptor, and accurate relative potencies and, if possible, dissociation constants of agonists acting on the same receptor. In particular, I attempted to point out pitfalls in such procedures and how to avoid them. For example, I derived theoretical equations to illustrate how removal of the agonist from the region of the receptor by active uptake or enzymatic destruction could markedly alter the slope of a Schild plot for competitive antagonism from the theoretical slope of 1. Later, Aaron Jurkiewicz (a visiting research associate from Sao Paulo), Niede Jurkiewicz, and I successfully used these theoretical equations to analyze propranolol antagonism of isoproterenol in guinea pig tracheal strips before and after blockade of agonist removal by active uptake (22).

Another interesting aspect of receptor mechanisms was the phenomenon of receptor desensitization, as manifested by a fairly rapid reversible loss of response of an effector to an agonist during exposure to the agonist. I studied agonist-induced desensitization in the case of muscarinic cholinergic receptors mediating negative inotropic and chronotropic responses in isolated guinea pig atria (with a graduate student, Edward Laski) and in the case of nicotinic cholinergic receptors on adrenergic nerve terminals mediating release of norepinephrine from those terminals in guinea pig atria and in the perfused central ear artery of the rabbit (with Taruna Wakade and Odd Steinsland) (17, 23).

In 1977 I organized a three-session symposium on receptors for the annual FASEB meeting. By then binding of radioligands (usually ^{3}H-labeled competitive antagonists) had been used for several years for quantifying specific receptors in membranes from homogenized cells and for determining the dissociation constants of competitive antagonists and agonists for those receptors. Most of the papers at the symposium were reports of studies with radioligands (e.g. RJ Lefkowitz on both α- and β-adrenergic receptors; P Seeman on dopamine receptors; S Snyder and colleagues on serotonin receptors and opiate receptors). My paper at the symposium (24) was partly a discussion of how pharmacological procedures for differentiating and characterizing receptors based on occupation theory were still very useful in conjunction with the

exciting new developments in receptor research being made with specific radioligands. I showed that dissociation constants of several agonists and antagonists for muscarinic receptors on gastrointestinal smooth muscle as determined with pharmacological procedures in my laboratory were in quite good agreement with the dissociation constants determined by Yamamura & Snyder (24a) with the radioligand [^{3}H]quinuclidinyl benzilate. Later in my laboratory, one of my graduate students, Jay Stollak, used both pharmacological and radioligand binding procedures for characterizing both adrenergic α_1-receptors and serotonin receptors in smooth muscle of rabbit thoracic aorta.

In my paper at the 1977 symposium (24), I also reviewed work that had been carried out in my laboratory on β-adrenergic receptors mediating relaxation of guinea pig tracheal smooth muscle. I presented an analysis of the concentration-response curves we had obtained using several different agonists and competitive antagonists that showed that this smooth muscle did not exclusively contain the β_2-type of the β-adrenergic receptor, as the dogma of that time would have it, but had an admixture of the β_1-type as well—usually as a small fraction of the total of β-receptors but, depending on the guinea pig used, sometimes much more. Because of this variability, the ratio of potencies of isoproterenol to norepinephrine ranged from about 600 (rarely, reflecting the β_2-type only) to about 12 (reflecting almost equal contributions of β_1- and β_2-types.)

Endothelium-Dependent Relaxation, EDRF, and Nitric Oxide

Having obtained pharmacological evidence that guinea pig tracheal smooth muscle sometimes has a sizeable fraction of the β_1-type adrenergic receptor along with the β_2-type (see above), I decided that we should reexamine the type of β receptor in the smooth muscle of rabbit thoracic aorta, which in our earlier studies had been classified as a typical β_2-type (22). The plan was first to redetermine, in a series of experiments, the relative potencies of isoproterenol, norepinephrine, and epinephrine in producing relaxation of preparations of rabbit aorta after irreversible blockade of α-adrenergic receptors by dibenamine pretreatment. To produce contraction after the pretreatment (so that relaxation by the catecholamines could be measured), we planned to use a muscarinic agonist such as carbachol or acetylcholine, as we had used in our earlier studies of both aortic and tracheal preparations. In the very first experiment of this planned series, on May 5, 1978, my technician did not follow directions correctly. Before any blockade of α_1-adrenergic receptors by dibenamine, he tested carbachol (for contraction) before rather than after washout of a previous test dose of norepinephrine. The response to the carbachol was not a contraction but a partial relaxation of the norepinephrine-induced contraction! Acetylcholine was tested. It too produced relaxation. This was the first time that I had ever observed relaxation of rabbit aorta in response to

muscarinic agonists over the many years that I had been using this blood vessel for in vitro studies.

The unexpected relaxation of rabbit aorta in vitro by muscarinic agonists was exciting, for it was in accord with the potent vasodilating action of these agonists in vivo, and it immediately changed the direction of research in my laboratory. Why had we failed to observe acetylcholine-induced relaxation of aortic preparations in the past, and why did it now become manifest? A detailed account of our investigation of this matter has recently been published (25), so it will suffice here to outline briefly the sequence of findings. First, we were aware that the kind of preparation of rabbit thoracic aorta that we were then using was the transverse ring, rather than the helical strip that we had used in all of the earlier experiments with muscarinic agonists. After several weeks of work, we recognized that in any kind of preparation (ring or strip), gentle rubbing of the intimal surface, whether intentionally or unintentionally (as had occurred in our standard method of preparing helical strips) eliminated the relaxing response to acetylcholine. Strips as well as rings gave excellent relaxation when care was taken not to rub the intimal surface. At first we had some difficulty in showing that the cause of the loss of the relaxation response to acetylcholine by intimal rubbing was a mechanical removal of the endothelial cells, but this was clearly shown to be the case when we modified a silver-staining procedure for en face viewing of the endothelial cells on our aortic preparations. Finally, after a number of months, we were able to demonstrate with a so-called sandwich preparation that endothelial cells of rabbit aorta in vitro, when stimulated by acetylcholine, release a nonprostanoid substance (or substances) that acts on the smooth muscle cells of the blood vessel to activate relaxation. In our first full paper on this research in 1980 (26), we could also report that the obligatory role of endothelial cells in relaxation by acetylcholine applied in the case of a variety of arteries from a number of mammalian species.

The coauthor on the 1980 paper (26) was John Zawadzki, who had begun working with me as a research assistant around the middle of 1978. Zawadzki was very skillful technically and contributed much to the early research. Later he went on to obtain his MS and PhD degrees with me. Between 1980 and 1985, I was fortunate in having several other very able coworkers in research on endothelium-dependent relaxation. Among these were Peter Cherry, a graduate student; Maria Helena Carvalho, a postdoctoral fellow from Sao Paulo; Desingarao Jothianandan, a nonfaculty research scientist in my department, who is still working with me; and William (Billy) Martin, a postdoctoral fellow whose PhD research under John Gillespie at Glasgow had been an investigation of the properties of the bovine retractor penis inhibitory factor. By the time Martin joined my laboratory in early 1983, we had already found that a number of other agents besides muscarinic agonists produced endothelium-dependent

relaxation of isolated arteries. Among these were the calcium ionophore A23187, substance P and other tachykinins, ATP and ADP, and bradykinin. The work on bradykinin, which was found to be an endothelium-dependent vasodilator of dog and human arteries but not rabbit and cat arteries (which it relaxed by stimulating the release of prostacyclin even in the absence of endothelium), was part of Peter Cherry's PhD thesis research, and in the published report (27) on this work, the term endothelium-derived relaxing factor, along with the abbreviation EDRF, was first used.

In 1980 I had speculated correctly that EDRF stimulates guanylate cyclase of vascular smooth muscle, resulting in an increase of cGMP, which causes relaxation (28). However, I had speculated incorrectly that EDRF is a short-lived hydroperoxide or free radical formed as an intermediate product in the oxidation of liberated arachadonic acid by the lipoxygenase pathway. In 1983 I wrote a review on the role of endothelium in responses of vascular smooth muscle (29). By that time only about eight laboratories worldwide had begun to publish research in this new field, so the task of writing a review then was relatively easy. Among the researchers whose work I cited were Ferid Murad, who with Robert Rapoport had first clearly demonstrated the causal role of cGMP in endothelium-dependent relaxation, and Paul Vanhoutte, who with Jo de Mey had made a number of important observations on endothelium-dependent responses in canine arteries and veins. My friends Murad and Vanhoutte went on to establish laboratories that were among the foremost in the development of the field.

Between 1983 and 1985, with Billy Martin working with us, we made a number of key observations in my laboratory. One was that hemoglobin (but not methemoglobin) and methylene blue were potent inhibitors of both the relaxation and the increases in cGMP produced by endothelium-dependent relaxing agents (30). From Martin I also learned first hand about the work he and others in Gillespie's laboratory in Glasgow had done in trying to identify the nonadrenergic, noncholinergic (NANC) neurotransmitter that elicited relaxation of the bovine retractor penis muscle and certain other smooth muscle preparations. Relaxation produced by NANC nerve stimulation and relaxation produced by a partly purified, acid-activated extract made from the bovine retractor penis were both inhibited by hemoglobin. As discussed in a paper (31) that I presented at a symposium on mechanisms of vasodilatation held in July 1986, I was struck by the similarities of the transient relaxations produced by Martin's acid-activated bovine retractor penis inhibitory factor (BRPIF) and the transient relaxations of rabbit aorta produced by acidified solutions of sodium nitrite. Also, earlier in 1986, Rubanyi & Vanhoutte (31a) and Gryglewski, Palmer & Moncada (31b) had reported that the superoxide anion (O_2^-) rapidly inactivates EDRF and that superoxide dismutase (SOD) protects against this inactivation. Realizing that acidification of $NaNO_2$ solutions pro-

duces HNO_2 (pK of 3.2), which then generates low concentrations of NO and NO_2 as a result of a reversible dismutation, and assuming that the free radical NO might rapidly react with the free radical O_2^-, I began to speculate on the possibility that the acid-activatable BRPIF is inorganic nitrite and that EDRF is nitric oxide. It was already well known from much earlier work in the laboratories of Murad and Louis Ignarro that nitrovasodilators (first considered by Murad to be NO generators) and NO itself produced relaxation of smooth muscle by activating the synthesis of cGMP.

In the few months before the symposium in July of 1986, Jothaniandan, MT Khan (who had recently returned to my lab), and I carried out a large number of experiments on rabbit aorta, comparing the characteristics of relaxation by EDRF (released by acetylcholine) and of acidified nitrite (as a source of NO). The characteristics (inhibition by hemoglobin, methylene blue, and superoxide generators, protection by SOD) were so similar that I had no hesitancy proposing at the symposium that EDRF is NO. I also proposed that the acid-activatable BRPIF that Martin and Gillespie found was inorganic nitrite and noted that our findings along with the findings of others on some of the properties of the relaxing factor released on stimulation of NANC nerves made it tempting to consider "the possibility that the smooth muscle inhibitory factor released on nerve stimulation is NO" (31).

At the 1986 symposium at which I proposed that EDRF is NO, Louis Ignarro, on the basis of his studies on isolated bovine pulmonary arteries, independently made the same proposal. (Unfortunately, our papers presented at that symposium were not published until 1988.) Using perfusion-bioassay procedures, our laboratory (32), shortly after those of Ignarro (32a) and Moncada (32b), showed that the characteristics of EDRF released into the perfusion stream from endothelial cells (sometimes on arteries, sometimes cultured cells in a column) were the same as those of NO with respect to rates of decay, inactivation by various agents, etc. In 1988 Moncada and coworkers (32c) went on to show that the source of endothelial NO is a guanidinium nitrogen of L-arginine, and that the constitutive enzyme involved (so-called nitric oxide synthase or NOS) is a calcium- and calmodulin-dependent oxygenase. Closely related to this finding were those of John Hibbs and of Michael Marletta and Dennis Stuehr, that a calcium-independent NOS could be induced in macrophages by endotoxin and certain cytokines. In the relatively short period since the proposal was made in 1986 that EDRF and the inhibitory factor released on NANC nerve stimulation are NO, research in the field of NO in biology has exploded in an unimagined manner—not only in cardiovascular physiology and pathology, peripheral neurobiology, and immunology, but all the way from molecular biology of the nitric oxide synthase isozymes to putative roles for NO in memory and central nervous system degenerative diseases.

In my own laboratory, work continues on isolated blood vessels. Several

years ago we showed that carbon monoxide, like NO, can produce endothelium-independent relaxation by stimulating the guanylate cyclase of the vascular smooth muscle, but with only about five thousandths of the potency of NO (33). We have been engaged in a number of studies on a variety of NO-donor drugs and an assortment of agents that either interfere with or potentiate release or actions of EDRF. Presently, we are trying to determine whether EDRF, when it is released abluminally from endothelial cells in the intact artery, is released simply as NO or as some complex of NO formed from newly synthesized NO and some other constituent. We are still having fun with our isolated vascular preparations, which we think we understand so well for a while but then suddenly realize that we do not.

CONCLUDING REMARKS

In 1982 I retired from the chair of the Department of Pharmacology at the SUNY Downstate Medical Center but continued as a professor. Julius Belford took over as acting chairman for two years, and then Stanley Friedman served well and valiantly as acting chairman until Robert Wong was appointed chairman in 1990. In 1989 I retired from my professorship (receiving emeritus status) so that I would be free of teaching duties and committee work related to the medical curriculum but could still continue research in the department. My retirement has also allowed me to spend about three and a half months each winter as an adjunct professor in the Department of Molecular and Cellular Pharmacology of the University of Miami School of Medicine. This arrangement was made possible by James Potter, the chairman of that department, and Casey Van Breemen, a professor conducting an outstanding research program on vascular smooth muscle. Van Breeman left Miami for Vancouver in 1993, but I still continue to go to Miami in the winter months, now sharing space in the laboratory of David Adams. Most of my time there I spend trying to catch up on the writing of manuscripts and on the reading of the burgeoning literature in our field of research—an impossible task these days! During the winter sojourns in Miami, I keep in touch with what is going on in my research laboratory in Brooklyn by means of an occasional visit, but mainly by frequent fax and telephone communications with my two or three coworkers there. I consider myself very fortunate in having this Brooklyn-Miami arrangement. Of course, an additional advantage for my wife Maggie and me is that the arrangement allows us to enjoy the very pleasant winter weather in Miami and some of the outdoor activities that it fosters (golf, for instance, in my case).

About the time of my retirement from the chair of the Department of Pharmacology in 1982, two departmental faculty members, Julius Belford and Stanley Friedman, and one former member, Ronald Rubin, organized a one-day symposium in my honor entitled "Receptors and mediators in the vascular

system." Speakers included Robert Lefkowitz, Perry Molinoff, Leon Goldberg, Philip Needleman, John Bevan, and Paul Vanhoutte. This was a special occasion for which I was very grateful to the organizers, the speakers, and the many present and former associates who attended the symposium. Whether it was deserved or not, the speakers all made me feel that I had contributed something through my work that had helped them in theirs.

From 1982 until the present writing, I have been the recipient of a number of honors and awards for research. Naturally, I have been very pleased to be the recipient; yet in thinking back about what aspects of my research have given me the greatest pleasure, I would not place the honors and awards first. I think that my greatest pleasure has come from each first demonstration in my laboratory that experiments designed to test a new hypothesis developed to explain some earlier, often puzzling or paradoxical finding, have given results consistent with the hypothesis. It is not just the immediate pleasure of obtaining such results but also the anticipated pleasure of discussing the results with others doing research in the same area—obviously an ego-supporting aspect.

I still enjoy doing bench work in the laboratory with my coworkers. The research is still rather "old-fashioned" pharmacological research. I was very lucky to stumble on unexpected results in 1978 that led to the finding of endothelium-dependent relaxation and EDRF, and eventually to NO; for if I had not, I would probably have continued concentrating on receptor theory and mechanisms and been left far behind by others in that field who have so brilliantly and successfully developed and used molecular biological and other advanced methodologies in their research.

Fairly often in the past several years when I have been a visiting lecturer at a medical school, I have met with a bright young researcher who enthusiastically tells me about his or her latest findings with cloned receptors. I follow the account and theoretical interpretations up to a point, but then I must admit that I cannot keep up with his or her ideas. I then think back to 1954 or 1955 when the distinguished pharmacologist Otto Loewi, who had been awarded the Nobel Prize for his discovery of chemical neurotransmission (acetylcholine in perfused frog heart) in the early 1920s, and who was now retired, visited Washington University Medical School to give a lecture. After his lecture, he visited with individual members of the pharmacology department. When he visited with me, I thought it would be a great opportunity to get his opinion about theories that I had been developing concerning receptor mechanisms in relation to our experimental findings. I began to outline my ideas and derive a few equations. He listened awhile and then politely explained that my work was too complicated for him to follow, but he wished me well with it. Sometimes, upon hearing the new generation of molecular biologists expound on their work on receptors, I think back to my meeting with Otto Loewi.

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