PER ASPERA AD ASTRA: THE ROAD TO METABOLIC MAPPING AND IMAGING OF LOCAL FUNCTIONAL ACTIVITY IN THE NERVOUS SYSTEM

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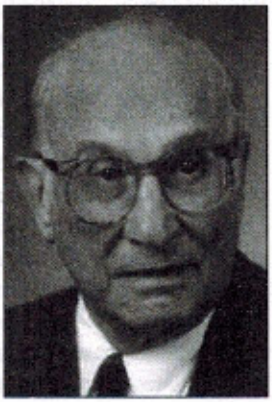
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As far back as I can remember, I aspired to becoming a research scientist in the biomedical field. I believe my interest in biology began when my brother, six years older than I and my only sibling, set up a balanced aquarium, and I was fascinated by it. I avidly studied the anatomy, classifications, habitats, and diseases of fish and aquatic plants. My reading then expanded into biology and science in general. Like many at that time, I was influenced by Paul De Kruif's Microbe Hunters, Donald C. Peattie's Green Laurels, and Eric Nordenskiöld's History of Biology. I spent hours studying Chemistry in Medicine, a book distributed freely by the Chemical Rubber Company, in which each chapter described the history of a specific medical or biological discovery, e.g., vitamins and the cause and cure of pellagra, the development of germ theory, and the contributions of Pasteur, Koch, Metchnikoff, and others.

We were then in the depths of the Great Depression, and family finances were far too inadequate to support college educations for both my brother and me. Fortunately, the Philadelphia Board of Education provided two scholarships to each high school graduating class, one to the University of Pennsylvania and the other to Temple University, and the Mayor's office of the city offered several scholarships on the basis of a competitive examination. My brother won a Mayor's Scholarship to Penn, and I graduated first in my class and won the Board of Education scholarship to Penn.

THE COLLEGE YEARS

I entered college in September, 1939, just after World War II had begun in Europe. My intention was to study zoology, a choice consistent with my previous interests but also influenced by the fact that my brother had majored in zoology before me. I had had access to his textbooks, and, while still in high school, eagerly studied his zoology texts. Jobs for zoologists were scarce during the depression, and so I also considered medicine or veterinary medicine, rationalizing that they too represented biology even though applied. Penn then had no special

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premedical curriculum. Premedical students pursued a standard liberal arts and sciences curriculum and chose as electives courses that were prerequisites for medical school admission. Prescribed courses in the first two years included English literature and composition, foreign language, mathematics; biology, chemistry, and social sciences. Between September, 1940, and June, 1941, I enrolled in a particularly memorable two-semester course in modern European history, the first semester covering the period from the Congress of Vienna in 1815 to the onset of World War I, and the second from 1914 to the last day of the course. A syllabus listing the titles of all the lectures was distributed in advance and included the last lecture entitled "Europe – Subject to change without notice." We found this title amusingly ambiguous because it was during World War II when Nazi armies were rampaging throughout Europe, and it was unclear whether it was Europe or the subject of the lecture that was "subject to change without notice." On the morning of this lecture newspapers headlined the invasion of Crete by the Germans. The lecture that morning was on the battle of Crete, an extraordinarily scholarly and erudite review of the history of Crete and its strategic importance to the combatants.

Majors were chosen at the end of the sophomore year. I wanted to choose zoology, but my brother argued that demands for zoologists were limited and admission to medical school doubtful because of quotas limiting the admissions of some minorities at most medical schools at that time. He recommended chemistry because of better prospects for employment. We compromised; I would major in zoology but would also take as electives all chemistry courses required of chemistry majors. This was a fortunate decision because not only did I enjoy chemistry, but the background in chemistry later proved to be critical in my biological research.

My first research experience was in my third year at Penn when I enrolled in a graduate/undergraduate course in general physiology taught by Lewis V. Heilbrunn, who passionately tried to define all biological phenomena in terms of physical and chemical mechanisms. Heilbrunn's course extended over two semesters and included both lectures and laboratory work. His lectures, like his textbook (1), paid particular emphasis to the role of calcium in biological processes and its influence on cell membrane integrity and intracellular protoplasmic viscosity. His strong interest in calcium was probably derived from studies done by his students, D. Mazia and J. M. Clark (2), who found that electrical, osmotic, mechanical, or ultraviolet stimulation of Elodea cells caused almost instant formation of calcium oxalate crystals in the vacuoles. The vacuoles were known to contain oxalic acid, and it was reasoned that the stimulations must have raised the intracellular ionic Ca^{++} concentrations, probably by release from bound sites. These and many other observations led Heilbrunn to propose that Ca^{++} release was a critical component in the processes of excitation, conduction, muscular contraction, blood-clotting, secretion, etc., ideas that were certainly very far ahead of their time.

The laboratory portion of the course consisted of some prescribed experiments followed by an original research project. My research project in the first semester was to determine if protoplasmic flow in the pseudopod of the amoeba obeys Poiseuille's Law. The results showed that it does not. My project in the second semester was to fractionate cells and localize enzymes to the subcellular components. This was several years before isolation of mitochondria by Hogeboom et al. (3). The cell we chose was the unfertilized frog egg because of its low cost, availability, and uniformity. The cells were homogenized and fractionated by centrifugation into cytosol, lipids, yolk, and pigment fractions. We localized lipases to the lipid fraction, dipeptidases to the cytosol, and a few other enzymes that I no longer remember. The results do not seem very interesting now, but the experience whetted my taste for research. Consequently, in my final year, 1942-43, I chose as an elective course, "Undergraduate Research

in Zoology," that enabled me to continue research with Heilbrunn. The States had by then entered the war, and Heilbrunn had obtained a grant from the US Army to study effects of heat on biological systems, probably because of heat-related casualties suffered by the British 8th Army in North Africa. His entire group worked on various aspects of the problem. My assignment was to determine the heat sensitivities of nerve and muscle. Electrodes were applied to both the nerve and muscle of the rat sciatic nerve–gastrocnemius preparation, and muscle contractions were monitored by a lever and pen-writing assembly. Either the muscle, nerve, or both were immersed in Ringer's solution at 41°C, and the nerve and muscle were electrically stimulated alternately until the muscle stopped contracting. When nerve alone was heated, the muscle responded to either nerve or muscle stimulation for relatively long periods. When only the muscle was heated, muscle contractions in response to nerve stimulation ceased very quickly but continued to be evoked for much longer periods by direct stimulation of the muscle. We concluded that it was the myoneural junction that was most susceptible to heat, and this observation was considered sufficiently interesting to publish. While I was drafting a manuscript, Paul LeFevre, then one of Heilbrunn's graduate students and later well-known for his work on red cells, called my attention to a publication by Claude Bernard in Charles Richet's Dictionnaire de Physiologie in 1870, describing essentially the same experiments and conclusions, except that he had used oil instead of Ringer's solution. Because similar experiments with the same conclusions had already been published, we decided not to publish ours, an attitude hardly in fashion today. Apropos differences in attitudes between then and now, Heilbrunn once remarked that "anyone publishing an average of more than two full papers per year is not doing good work or his own work." Today such a publication rate would probably be insufficient to gain promotion, tenure, or grants.

Clockwise from the left: Seymour Kaufman, Seymour Kety, Yvonne Posternal, and Jean Sokoloff dining at the L'Osteau de Baumanière, in Les Baux, Provence, France, following the 1st CINP Congress in Rome, Italy, in 1958

My experience with Heilbrunn led me to consider graduate studies with him. He was willing to accept me as a graduate student but recommended against it because his graduate students were being drafted into the military before they achieved their degrees. He advised me to apply to medical school instead because medical students were being deferred from the draft until completion of their studies. He also remarked that an MD degree did not necessarily spoil everyone for scientific research. I, therefore, applied to Penn's medical school, and Heilbrunn wrote a letter of recommendation to the admissions committee, strong enough for them to admit me to the class beginning in March, 1943.

MEDICAL SCHOOL AND INTERNSHIP

Medical schools during the war adopted an accelerated program to speed up the supply of physicians to the armed forces. The semester system and vacations were abandoned, and the

four-year curriculum compressed into approximately three years. Three months after I entered, the military took over medical, dental, and veterinary schools. Most of the students, including me, were inducted into the Army Specialized Training Program (ASTP) with the rank of Private First Class; a few joined the Navy V-12 Program. ASTP members at Penn were barracked and given some military training, e.g., saluting, marching, assembling for reveille each morning, and marching to breakfast, but the university retained control of curricular and educational affairs.

Gross Anatomy was our first course and was overlapped soon afterward by Histology and Neuroanatomy. At first, I found the atmosphere stifling. Compared with the excitement in Heilbrunn's laboratory, the rigidity of the curriculum and the treatment of first-year students reminded me of grade school. I could not develop much enthusiasm for subjects that required memorization of huge amounts of descriptive information. Nevertheless, I persisted, studied hard, and survived this dull period until eventually we progressed to physiology, biochemistry, and pharmacology, subjects more to my liking. These courses were well taught by excellent teachers who emphasized the research that unearthed the facts being presented. All these courses included hands-on laboratory experiments. Pharmacology was probably the best taught course, mainly because of an outstanding trio of teachers, Carl Schmidt, Julius Comroe, and Seymour Kety. They emphasized physiological mechanisms, and the physiology of each system was comprehensively reviewed before specific actions of drugs were examined. Their lectures were also directed at mechanisms and rational approaches to diagnosis and therapy. Experimental methods and findings were described and critically evaluated. Healthy skepticism permeated the course, an attitude reflected in Schmidt's opening lecture of the course in which he cautioned us never to be seduced by the dictum, "Post hoc, ergo propter hoc."

The clinical courses interested me less than the basic sciences. Neurology was fun because with knowledge of neuroanatomy and neuropathology one might deduce the location and nature of a lesion. Surgery, gynecology, and obstetrics were dull, and pediatrics required dealing with crying children who were difficult to examine. I liked internal medicine, particularly metabolic and endocrine disorders which involved physiological chemistry. In general, my medical school experience did little to divert my interest from basic science to the practice of medicine, but my focus did change from cellular to mammalian physiology and biochemistry.

The war ended in August, 1945. I graduated from medical school in March, 1946, and entered internship at the Philadelphia General Hospital, a city hospital with 2500 beds. The army released us into the reserves to complete our internships and pass licensing board examinations before recalling us to active duty as medical officers. It was a rotating internship in which I rotated through internal medicine, general and orthopedic surgery, gynecology and obstetrics, clinical laboratory medicine, neurology, psychiatry, and tuberculosis services. My first service was in psychiatry. Treatments then in vogue were insulin-shock for schizophrenia and electroconvulsive therapy for schizophrenia, depression, and manic-depressive psychosis. Paresis was treated with fever therapy, induced by malaria in whites and intravenous typhoid vaccine administration in Afro-Americans; penicillin was then not yet readily available. We saw many patients with alcoholic hallucinosis, delirium tremens, hysterical paralyses, amnesia, and drug intoxications. Patients also received psychotherapy. I found mental diseases to be intriguing, but psychoanalysis, then popular in American psychiatry, ignored physical and

chemical mechanisms and offered explanations based on abstract entities, such as id, ego, superego, and unconscious mind, which lacked physical or biochemical structure or properties. Psychiatry challenged anyone who sought explanations on the basis of physical and chemical mechanisms.

Internships during the war were also condensed into an accelerated program of nine months' duration, obliging us to complete all the required rotations within that period. When the war ended, however, the accelerated program was terminated, and normal one-year internships were reinstituted. In order to return to the prewar schedule, our internship was extended to 15 months, but the extensions were limited to a single service, and mine was in psychiatry. Thus, throughout my internship I served one month in neurology and seven months in psychiatry.

THE ARMY YEARS

My internship ended in June, 1947, and the army allowed me two months to pass board examinations before recalling me to active duty. My first assignment was at the Medical Field Services School, Fort Sam Houston, Texas, to receive four weeks of training in basic military medicine. After that, because of my experience in neuropsychiatry during internship, I was ordained a neuropsychiatrist and transferred to the Station Hospital, Camp Lee, Virginia. The medical facility at Camp Lee was a 150-bed hospital that provided a range of medical services to the military personnel and their dependents. Few of my patients were neurological; most of the neurological patients suffered from head, spine, or peripheral nerve injuries, subarachnoid hemorrhages due to cerebral aneurysms, occasionally brain tumors, strokes, and multiple sclerosis, and one case of myotonia congenita. Psychiatric patients, however, were many and varied and included a wide range of psychiatric disorders, diagnosed according to a manual prepared by the Menningers. Those requiring hospitalization included schizophrenia, manic-depressive disorders, psychotic depression, alcoholic hallucinosis, delirium tremens, etc. Most of them, however, were outpatients suffering from alcoholism, personality disorders, anxiety neurosis, hypochondriasis, psychosomatic disease, and conversion reactions (e.g., hysterical paralyses and amnesia). A frequent condition seen in young recruits away from home for the first time was diagnosed as acute situational maladjustment. Character disorders were common, particularly one then called constitutional psychopathic inferiority, now one of the sociopathic personality disorders. As a physician I was obliged to help my patients, but all I could offer was a type of psychotherapy that I considered consistent with the best teaching of the time and within my limits of competence. Accordingly, I practiced a diluted version of psychoanalysis which was then the most popular approach to psychiatric treatment. Some patients did improve, particularly the conversion reactions. There was one patient in her thirties who had suffered from many systemic symptoms for years, but the medical service could find no organic basis, made the diagnosis of psychosomatic disease, and referred her to the neuropsychiatric clinic. After about six months of psychotherapy she declared that she was feeling better than she ever had in eleven years. I wondered what changes, if any, psychotherapy could have elicited in her brain to dispel the psychosomatic symptoms. For me, mind and brain were inextricably linked, a linkage irrelevant to psychiatry at that time. This case and a few others like it aroused my interest in physiological and biochemical mechanisms in the brain in mental disease.

RETURN TO THE UNIVERSITY OF PENNSYLVANIA

In 1948 Kety and Schmidt, my former teachers at Penn, published their nitrous oxide method for measuring cerebral blood flow (CBF) and metabolism in conscious human subjects (4). This method offered a means to examine brain functions in psychiatric disorders. My army service ended in August, 1949, and shortly thereafter I visited Kety who had transferred from Schmidt's department of pharmacology in the medical school to a new department of physiology and pharmacology, chaired by Julius Comroe in the graduate school of medicine. The timing was opportune. He had just learned that his National Institutes of Health (NIH) grant application, which included a salaried position for a still unnamed fellow, had been approved for funding. Remembering me favorably from medical school, he was willing to appoint me to that position.

I had come specifically to study cerebral blood flow and metabolism, but I acquired a much broader experience in physiology. The grant that provided my salary was on the use of Kety's $^{24}Na^{+}$ clearance method (5) to study peripheral circulation, and I, therefore, divided my time between studies on the cerebral and the peripheral circulations. Working with the $^{24}Na^{+}$ clearance method introduced me to radioisotopic techniques and forced me to learn about radioactivity, a subject neglected in my college physics courses. Also, the clearance method was based on the design and mathematical analysis of a kinetic model. Physiological modeling was new and fascinating to me, but my mathematical skills were limited, and I was forced to extend my knowledge of mathematics. Once again, I was a student and studied every night until early morning hours. I did not feel deprived because with an annual income of $2500 other activities were very limited. My wife, who had been a navy nurse during the war, was enrolled at Penn under the G.I. Bill of Rights and could not help financially. By 1952 my salary reached $4000, but then our son was born, further straining our financial resources.

Richard Wechsler was Kety's first postdoctoral fellow, and I was his second. We overlapped for about a year, and he taught me the procedure of the N_2O method. Both of us learned from Kety the principles of inert gas exchange between blood and tissues (6) on which the N_2O method was based. Additional new fellows arrived, and we became a highly interactive group that worked closely together, constantly argued about the rationale and interpretation of results of our experiments, and regularly discussed and exchanged information about publications in our fields of interest and in physiology in general. We worked together as a team in all the projects within the overall research program with no detectable rivalry among us and no prior decisions or concerns about authorship on publications. Authorship evolved by natural selection; each of us gravitated in our reading and thinking to specialized areas of interest so that it became obvious who was best qualified to write the first draft of the manuscript. Whoever authored the first draft became first author. It was several years later that I became aware of rivalries and conflicts among coworkers with regard to authorships, but never in Kety's group.

In addition to research-related discussions, there were also discussions of broader scope among ourselves and with Kety. We usually gathered for lunch where we discussed news, politics, foreign affairs, as well as science. Kety and I often argued about psychiatry. Although I too was skeptical about psychiatric theory and practice at that time, my previous experience in caring for psychiatric patients had made me more tolerant. Kety, however, was a critical

"hard-nosed" physiologist who considered psychiatry to be unscientific. I felt compelled to defend it and argued that it was not psychiatry but psychiatrists that were at fault. That was not an original idea. I had read an article by Iago Galdston (7) in which he compared Freud's impact on psychiatry with the inauguration of the Eiffel Tower at the Paris Exposition in 1889. At its opening a powerful lantern at the top of the tower was directed downward, producing a giant circle of bright light on the ground below. It was night, and those outside the circle were in the dark and could see nothing, but those within the circle of light were so blinded by its brilliance that they also could see nothing. Apparently, Kety and I were both very persuasive in our arguments because I, subsequently, gravitated more deeply into basic science while Kety's interests drifted toward psychiatry in his later studies on the genetics of schizophrenia.

The department of physiology and pharmacology provided a superb environment for training. Contact between fellows and staff was continuous and close, and expectations and standards of performance were high. Seminars were held every Saturday morning, and all staff members, including fellows, took turns presenting their work. There were no exceptions; if one had nothing to present, that itself might be revealing. There was little tolerance for pomposity or verbal gymnastics, and it was unwise to be glib. Every statement might be challenged, and every method or conclusion questioned or criticized. Presenters were stretched to the limits of their knowledge of the subject. One dared not make rash statements that could not be backed up by facts or reason. We learned to be just as critical of our own work as of that of others. In science it was more important never to be wrong than ever to be right. Scientific literature should never be polluted with bad or trivial science. How different today when publications often appear to have been written more to contribute to one's bibliography than to scientific knowledge.

My first research project was on the effects of hyperthyroidism on cerebral blood flow and oxygen consumption ($CMRO_2$) in man. The N_2O method had revealed decreases in $CMRO_2$ in many clinical conditions, but none yet with increased $CMRO_2$. It seemed likely that $CMRO_2$ was increased in hyperthyroidism because it is accompanied by large increases in total body metabolic rate and marked anxiety. We, therefore, designed a long-term study in which patients with Graves' Disease would be studied before and after treatment and also compared with normal subjects of comparable age. The first few experiments showed that, contrary to expectations, $CMRO_2$ was normal in hyperthyroidism. While our study was still in progress, an abstract appeared reporting this same finding. We had been scooped. Kety's comment was, "It must not have been such a great idea. Somebody else thought of it too." It reflected an attitude that valued uniqueness more than speed. The study was eventually completed and published (8), but by then I had become intrigued with the question of why the brain failed to participate in the total body's increased metabolic rate in hyperthyroidism. What was different about the brain's metabolism? Inasmuch as the literature revealed no mechanisms for the stimulation of metabolic rate by thyroid hormones in responsive tissues, such as liver, muscle, and kidney, how could one explain their lack of effect in brain? There was evidence in the literature that thyroid hormones might have actions specifically related to protein metabolism. Mature brain was known to derive almost all its energy from oxidative glucose metabolism, and the testis, the only organ other than brain known to have a respiratory quotient of 1.0, indicating only carbohydrate oxidation, had also been reported to have its rate of O_2 consumption unaffected by thyroid hormones. Perhaps, this was only a coincidence, but it also suggested that thyroid hormones might be acting primarily on protein metabolism and that effects on energy metabolism were only secondary. Effects on energy metabolism might, therefore, not be apparent in tissues in which protein turnover was low compared to that of

carbohydrate. Also, if thyroid hormones acted on protein turnover, was it on protein synthesis, protein catabolism, or both? Thyroid hormones were essential for body growth and brain maturation and had been reported to stimulate O_2 consumption in developing brain when protein synthesis is undoubtedly active, but not in mature brain in which rates of protein synthesis were believed to be low. These considerations led us to speculate that the effects of thyroid hormones on energy metabolism were secondary to an effect on protein synthesis.

Testing this hypothesis required biochemical experiments on the effects of thyroid hormones on protein synthesis. Because there was then no practical method for studying protein synthesis in vivo, in vitro biochemical experiments would be required. I had a fair amount of book knowledge of biochemistry but little biochemical laboratory experience. I, therefore, tried to persuade biochemical colleagues to undertake such studies. One such biochemist was B.D. Polis, one of the only two PhD students Otto Meyerhof had had in the US. He encouraged me to undertake the biochemical studies under his supervision, but I was then too busy with studies of cerebral blood flow and metabolism and peripheral blood flow to do so.

THE YEARS OF THE NATIONAL INSTITUTE OF MENTAL HEALTH

In 1951, about two years after I had joined him, Kety left Penn to become scientific director of the intramural research programs of both the National Institute of Mental Health (NIMH) and the National Institute of Neurological Diseases and Blindness (NINDB). Because I was then his most senior fellow, I became responsible for continuing his research projects, and he returned to Penn several times per month to discuss the work. The department had two major research interests, pulmonary function and cerebral circulation and metabolism. After Kety's departure, the group working on brain shrank because the magnet was gone. We were reduced to a graduate student, technician, and me, and feeling quite isolated I began to explore opportunities elsewhere. When Kety became aware of this, he offered me a position at NIH. My past experience qualified me for appointment in either the basic or clinical research programs in the intramural research program of the NIMH. I chose basic research.

My appointment was in the section on cerebral metabolism of the laboratory of neurochemistry. Kety was section chief and also acting laboratory chief until a permanent one was recruited. There were two other sections in the laboratory, lipid chemistry under Roscoe Brady and physical chemistry under Alex Rich. Biochemistry was well represented throughout the intramural program, and we organized a biochemical journal club. In the first round of meetings each of us described our previous research before coming to NIH, and I presented my work on hyperthyroidism and the hypothesis that many physiological effects of thyroid hormones could be explained by a stimulation of protein synthesis or turnover. Seymour Kaufman, a superbly trained biochemist in the laboratory of cellular pharmacology, was present at the session and told me afterward that he had arrived at a similar hypothesis from an entirely different perspective. Testing the hypothesis would require biochemical experiments, but he offered to collaborate with me, provide the biochemical expertise, and supervise and train me in biochemistry. I accepted, and thus began my career in biochemistry. With Kety's encouragement we initiated experiments in 1955 to develop and characterize an in vitro assay system for protein synthesis that could be used to examine the effects of thyroid hormones. Because both of us were then also involved in other projects, in my case studies of cerebral circulation and metabolism, progress was slow, but we eventually developed an effective assay that demonstrated that thyroid hormones did, indeed, stimulate protein synthesis (9,10). This experience and the apparent capability of biochemistry to obtain definitive solutions seduced

me away from physiology, and in 1959, when my projects on cerebral blood flow and metabolism were essentially completed, I turned my efforts fully to biochemical research. My main research project was still on mechanisms of thyroid hormone actions, but my interests broadened to include the relationships between biochemical processes and physiological functions in the nervous system.

When I first arrived at NIMH, my plan had been to study cerebral blood flow and metabolism in normal subjects and patients with mental or neurological dysfunctions. Our first studies were on normal aging and dementia (11) and the effects of LSD in normal subjects and schizophrenic patients (12). These studies were done with the N_2O method which determines average blood flow and metabolism in the brain as a whole. The method was adequate to show reductions in clinical conditions associated with depressed consciousness but failed to detect changes in cerebral energy metabolism during normal physiological alterations in mental function or psychiatric disorders. We found no changes in average $CMRO_2$ in the whole brain during mental exercise, slow-wave sleep, sedation or tranquilization, schizophrenia, mild alcoholic inebriation, or LSD intoxication. There were at least three possible explanations for the negative results: 1) mental functions not associated with altered levels of consciousness are unrelated to energy consuming processes in brain; 2) local increases and decreases in metabolic rates occur throughout the brain without altering average metabolic rate in the brain as a whole; 3) specific mental functions are localized to regions too small to be detectable in measurements of the total brain's metabolic rate. A method that determined local cerebral metabolic rates in conscious animals was clearly needed.

How to study local cerebral energy metabolism in unanesthetized man or animals was then not obvious, but Kety (6), in his classical analysis of the principles of inert gas exchange between blood and tissues, had derived an equation that suggested a means to measure local blood flow throughout the brain. CBF was, of course, not metabolic rate, but it was believed to be adjusted to energy metabolism. Kety and two neurophysiologists at the NIMH, William Landau and Walter Freygang, had already initiated development of such a method, and I joined them when I arrived. The outcome was the [^{131}I]trifluoroiodomethane ([^{131}I]CF_3I) method (13-16), which determined local CBF simultaneously in all regions of the brain in conscious, behaving animals. Localization was achieved by a unique quantitative autoradiographic technique that not only measured but also provided visual images of the local isotope concentrations and, therefore, also the relative rates of local CBF throughout the brain. Applications of this method proved that local CBF does indeed change with local functional activity. For example, retinal stimulation with light increased CBF in structures in the visual pathways of the cat, and the autoradiographs provided the first example of the imaging of functional activity in the brain (14,16). This method was later adapted to measure regional CBF in the human brain with PET and with $H_2{}^{18}O$ as the tracer instead of [^{131}I]CF_3I.

Changes in local CBF elicited by altered functional activity were believed to be secondary to changes in local energy metabolism, but CBF is also strongly affected by systemic factors, e.g., arterial pCO_2, pO_2, and pH. One would, therefore, expect local energy metabolism to be more directly and specifically linked to local functional activity than blood flow. In 1955–56 I attempted to devise a method to determine local cerebral glucose utilization ($lCMR_{glc}$) with [^{14}C]glucose that took advantage of the spatial localization made possible by quantitative autoradiography. It soon became apparent, however, that rapid loss of labeled products of [^{14}C]glucose metabolism, mainly $^{14}CO_2$, that could not be quantified would render such a method extremely difficult if not impossible, and I abandoned the project.

In 1957 I was preparing a chapter on energy metabolism in the central nervous system for the Handbook of Physiology. While discussing some issues in the chapter with Don Tower of the NINDB, I learned from him about 2-deoxyglucose (2-DG), a glucose analog which in pharmacological doses produced a comatose state like that of hypoglycemic coma. Sols and Crane (17) had shown that hexokinase, the enzyme that phosphorylates glucose to glucose-6-phosphate (G-6-P), could also phosphorylate 2-DG to 2-DG-6-phosphate (DG-6-P). The next step in glycolysis is isomerization of G-6-P to fructose-6-phosphate (F-6-P), but DG-6-P, lacking the hydroxyl group on its second carbon, cannot be isomerized and, therefore, not metabolized further down the glycolytic pathway. DG-6-P, therefore, accumulates to levels that competitively inhibit G-6-P isomerization to F-6-P, thus blocking glycolysis at this step and causing coma. DG-6-P could accumulate to such levels because it is a poor substrate for enzymes in brain that might metabolize it and because of negligible glucose-6-phosphatase activity in brain. When I learned that phosphorylated products of 2-DG were retained in the cells, it occurred to me that radioactive 2-DG in tracer amounts combined with quantitative autoradiography might be used to measure $lCMR_{glc}$, and I filed the idea away for future work.

In 1964 Martin Reivich and Jane Jehle joined our laboratory and adapted the autoradiographic [^{131}I]CF_3I method for measuring local CBF for use with [^{14}C]antipyrine (18), later replaced by the more diffusible tracer [^{14}C]iodoantipyrine (19). This made quantitative autoradiography with ^{14}C available, and the idea of measuring $lCMR_{glc}$ with 2-[^{14}C]DG was resurrected but again left for future research. Reivich returned to Penn in 1966, but he asked me if I were still interested in 2-[^{14}C]DG and would I be willing to consider collaborating with him on the development of a method to measure $lCMR_{glc}$. I accepted, provided that the initial experiments be done in his laboratory because mine was then occupied with other projects. Early experiments in his laboratory with brain slices incubated in vitro showed proportionate uptakes of 2-[^{14}C]DG and glucose from the medium. This supported the idea of using 2-[^{14}C]DG to measure $lCMR_{glc}$ in vivo and encouraged us to design a kinetic model that was essentially like that of the local CBF method, but modified to include a compartment for the metabolism of the tracer. We derived an equation with which to calculate $lCMR_{glc}$, but it also required simultaneous measurement of local blood flow and other factors difficult to determine. This early attempt was reported in 1971 (20). The model and equation were not wrong but required information difficult if not impossible to obtain, and the project stagnated.

In 1968-1969 I spent a sabbatical year in Jean Roche's laboratory of general and comparative biochemistry at the Collège de France in Paris. There I worked with J. Nunez and J. Pommier studying peroxidase-catalyzed iodination of tyrosine residues in serum albumin, a model system for the peroxidase-catalyzed iodination of thyroglobulin in the pathway of thyroid hormone biosynthesis. I was intrigued by the complex non-Michaelian kinetics exhibited by the reaction (21). While working on this problem I became facile with enzyme kinetics which encouraged me to consider an alternative model for the 2-[^{14}C]DG method, one based more on principles of enzyme kinetics than on blood flow and tissue-blood exchange. When I returned to my own laboratory late in 1969, I found the project on actions of thyroid hormones in shambles. Rather than resurrect it, I turned my full attention to developing and evaluating such a model. The first animal experiment was done in rats in February, 1971, by C. Kennedy, M. Des Rosiers, J. Jehle, and myself. We were later joined by C. Patlak, K. Pettigrew, O. Sakurada, and M. Shinohara, all of whom played important roles. The method was presented in 1974 at the annual meeting of the American Society for Neurochemistry. Detailed descrip-

tions of the theory, procedure, and results of numerous applications of the method in various physiological and pharmacological states followed later (22-24).

We then turned our attention to determining if glucose utilization was linked to functional activity in neural as it is in other tissues, and if the 2-DG method could localize changes in neuronal activity on the basis of altered $lCMR_{glc}$. To do so, we carried out what we called "recovery experiments" in which increases or reductions in functional activity were experimentally induced in selected neural pathways, and the 2-[^{14}C]DG method was used to look for changes in $lCMR_{glc}$ in stations of the pathways. The results were unequivocal; $lCMR_{glc}$ was clearly linked to local functional activity, and the effects on $lCMR_{glc}$ were so pronounced that they often could be visualized directly in the autoradiograms without the need for quantification. A particularly dramatic demonstration was the visualization of the nature, extent, and distribution of the ocular dominance columns and the loci of representation of the blind spots of the visual fields in the striate cortex of the monkey (25,26).

The quantitative 2-[^{14}C]DG method required laborious manual densitometric analyses of the autoradiograms which yielded massive amounts of data. Quantification was, therefore, limited to a relatively few selected structures, and valuable information in the autoradiograms was being lost. A more convenient means to combine the quantitative capability of the method with the spatial resolution of the autoradiography was needed. We, therefore, assembled a computerized image-processing system that scanned and digitized the autoradiograms, computed $lCMR_{glc}$ for each pixel, and reconstructed the autoradiographic images in pseudo-color on a monitor with the metabolic rates quantitatively encoded in a calibrated color-scale that was simultaneously displayed (27). This technique provided color-coded quantitative maps of the distribution of $lCMR_{glc}$ throughout the nervous system with often dramatic visual displays of the locations and magnitudes of the changes evoked by alterations in functional activity. This technique was first presented in 1978 at the meeting of the Society of Neuroscience in St. Louis, MO, by C. Jarvis, who used it to identify structures in the monkey nervous system metabolically activated by visually cued unilateral arm movements contralateral to a visually deprived hemisphere. Its reception was sensational. The issue of Chemical & Engineering News reporting on the meeting featured on its cover a color-coded autoradiogram of the striate cortex showing the reduced metabolism in the deprived hemisphere with the caption, "Visualizing Brain Chemistry in Action."

After the 2-[^{14}C]DG method successfully developed, Reivich suggested its adaptation for use in man. Autoradiography was obviously not practical for human use, and a less invasive technique for measuring local isotope concentrations in brain was needed. David Kuhl at Penn had developed a section-scanner that measured local concentrations of γ-emitting isotopes in sections of human brain by external scintillation counting, and Reivich enlisted his collaboration. To use it, however, a γ-emitting isotope had to be inserted into the deoxyglucose molecule which contains only hydrogen, oxygen, and carbon. Hydrogen has no γ-emitting isotopes, and the half-lives of ^{15}O and ^{11}C are 2 and 20 minutes, respectively, too short at that time to synthesize deoxyglucose labeled with either of them. An alternative possibility was fluorinated deoxyglucose. Fluorine is so small an atom that when introduced into appropriate positions in the molecules of metabolic substrates, they often retain the biochemical properties of the natural compound. Furthermore, ^{18}F, a positron-emitting isotope, has a half-life of 110 minutes. Alfred Wolf, a radiochemist at Brookhaven National Laboratory, was brought into the project, and his team developed a synthesis for 2-[^{18}F]fluoro-2-deoxy-d-glucose (2-[^{18}F]FDG). Experiments were, however, first carried out with 2-[^{14}C]FDG to establish that

2-FDG retained the biochemical properties of deoxyglucose. The 2-[^{18}F]FDG adaptation of the DG method was then developed and used for the first time in man with Kuhl's Mark IV Section Scanner (28), and, subsequently, with positron-emission tomography (PET) by Kuhl's colleagues, Michael Phelps and Edward Hoffman, pioneers in the development of PET scanners (29). PET offered better spatial resolution and accuracy than single photon tomography, and 2-[^{18}F]FDG is now widely used with PET in studies of the human brain and heart in health and disease and for the detection, localization, and staging of neoplasms in the whole body.

It is especially gratifying that this odyssey that began with basic physiological and biochemical research ultimately ended with the establishment of a new and useful field in neuroscience, the metabolic mapping and imaging of local functional activity in the nervous system of animals and humans.

REFERENCES

1. Heilbrunn LV (1938) An Outline of General Physiology. Philadelphia: WB Saunders
2. Mazia D, Clark JM (1936) Free calcium in the action of stimulating agents on Elodea cells. Biological Bulletin 71: 306-323
3. Hogeboom GH, Schneider WC, Palade GH (1948) Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. J Biol Chem 172:619-635
4. Kety SS, Schmidt CF (1948) The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure, and normal values. J Clin Invest 27: 476-483
5. Kety SS (1949) The measurement of regional circulation by local clearance of radioactive sodium. Am Heart J 38:321-328
6. Kety SS (1951) The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol Rev 3:1-41
7. Galdston I (1950) Psychiatry without Freud. AMA Arch Neurol Psychiat 66:69-81
8. Sokoloff L, Wechsler RL, Mangold R, Balls K, Kety SS (1953) Cerebral blood flow and oxygen consumption in hyperthyroidism before and after treatment. J Clin Invest 32:202-208
9. Sokoloff L, Kaufman S (1959) The effects of thyroxine on amino acid incorporation into protein. Science 129:569-570
10. Sokoloff L, Kaufman S (1961) Thyroxine stimulation of amino acid incorporation into protein. J Biol Chem 236:795-803
11. Dastur DK, Lane MH, Hansen DB, Kety SS, Butler RN, Perlin S, Sokoloff L (1963) Effects of aging on cerebral circulation and metabolism in man. In JE Birren, RN Butler, SW Greenhouse, L Sokoloff, MR Yarrow eds Human Aging. A Biological and Behavioral Study. Public Health Service Publ. No. 986, 59-76. Washington, D.C.: US GPO
12. Sokoloff L, Perlin S, Kornetsky C, Kety SS (1957) The effects of d-lysergic acid diethylamide on cerebral circulation and over-all metabolism. Ann NY Acad Sci 66:468-477
13. Landau WH, Freygang WH, Rowland LP, Sokoloff L, Kety SS (1955) The local circulation of the living brain: values in the unanesthetized and anesthetized cat. Trans Am Neurol Assoc 80:125-129
14. Freygang WH, Sokoloff L (1958) Quantitative measurements of regional circulation in the central nervous system by the use of radioactive inert gas. Adv Biol Med Physics 6:263-279
15. Kety SS (1960) Measurement of local blood flow by the exchange of an inert, diffusible substance. In HD Brunner ed Methods in Medical Research (vol VIII). Chicago: Year Book Publishers, pp.228-236
16. Sokoloff L (1961) Local cerebral circulation at rest and during altered cerebral activity induced by anesthesia or visual stimulation. In SS Kety, J Elkes eds The Regional Chemistry, Physiology and Pharmacology of the Nervous System. Oxford: Pergamon Press, pp. 107-117
17. Sols A, Crane RK (1954) Substrate specificity of brain hexokinase. J. Biol. Chem. 210:581-595
18. Reivich M, Jehle J, Sokoloff L, Kety SS (1969) Measurement of regional cerebral blood flow with antipyrine-^{14}C in awake cats. J Appl Physiol 27:296-300
19. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L (1978) Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine. Am J Physiol 234:H59-H66.
20. Reivich M, Sano N, Sokoloff L (1971) Development of an autoradiographic method for the determination of regional glucose consumption. In RW Ross-Russell ed Brain and Blood Flow. London: Pitman, pp. 397-400
21. Pommier J, Sokoloff L, Nunez J (1973) Enzymatic iodination of protein. Kinetics of iodine formation and protein iodination catalyzed by horse-radish peroxidase. Eur J Biochem 38:497-506
22. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897-816

23. Sokoloff, L (1981) Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. J Cereb Blood Flow Metab 1:7-36
24. Sokoloff L (1982) The radioactive deoxyglucose method. Theory, procedure, and applications for the measurement of local glucose utilization in the central nervous system. In BW Agranoff, Aprison MH eds Advances in Neurochemistry (vol. 4) New York: Plenum Publishing Corp, pp. 1-82
25. Kennedy C, Des Rosiers M H, Jehle JW, Reivich M, Sharp F, Sokoloff L (1975) Mapping of functional neural pathways by autoradiographic survey of local metabolic rate with [^{14}C]deoxyglucose. Science 187:850-853
26. Kennedy C, Des Rosiers MH, Sakurada O, Shinohara M, Reivich M, Jehle JW, Sokoloff L (1976) Metabolic mapping of the primary visual system of the monkey by means of the autoradiographic [^{14}C]deoxyglucose technique Proc Natl Acad Sci USA 73:4230-4234
27. Goochee C, Rasband W, Sokoloff L (1980) Computerized densitometry and color coding of [^{14}C]deoxyglucose autoradiographs. Ann Neurol 7:359-370
28. Reivich M, Kuhl D, Wolf A, Greenberg J, Phelps M, Ido T, Casella V, Fowler J, Hoffman E, Alavi A, Som P, Sokoloff L (1979) The [^{18}F]fluoro-deoxyglucose method for the measurement of local cerebral glucose utilization in man. Circulation Res 44:127-137
29. Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with [^{18}F]2-fluoro-2-deoxy-d-glucose: validation of method. Ann Neurol 6:371-388