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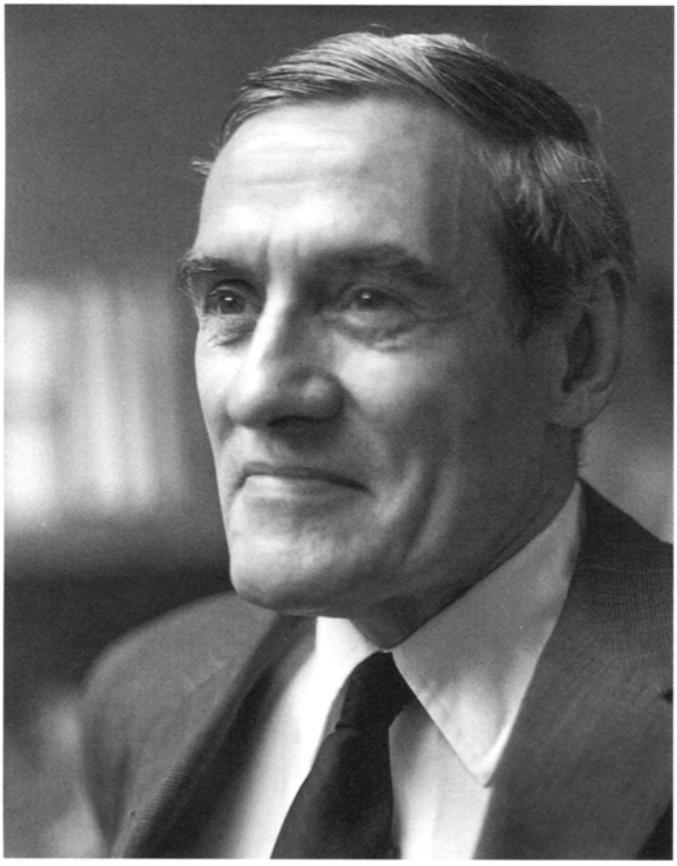

Martin G. Larrabee

BORN:

Boston, Massachusetts
January 25, 1910

EDUCATION:

Harvard College, B.A. (1932)
University of Pennsylvania, Ph.D. (1937)

APPOINTMENTS:

University of Pennsylvania (1934)
Cornell Medical College (1940)
University of Pennsylvania (1941)
Johns Hopkins University (1949)

HONORS AND AWARDS:

National Academy of Sciences, U.S.A. (1969)

Martin Larrabee did pioneering work on the electrophysiology and metabolism of sympathetic ganglia throughout his career. As part of his thesis work on the cat stellate ganglion with Detlev Bronk, he discovered the phenomenon of "prolonged facilitation," which David Lloyd later demonstrated in monosynaptic spinal reflexes and called "post-tetanic potentiation."

The History of Neuroscience in Autobiography, Volume 2

Martin G. Larrabee

After being imposed on the world in Boston, Massachusetts, on January 25, 1910, by parents of old New England stock, I grew up in a five-story, brick, row house that my grandfather had built in that city.

My father was a physician whose recreational activities were mostly photography, hiking, and exploring and trail building in the White Mountains of New Hampshire. He participated in some of the earliest winter climbing around Mount Washington and edited the Appalachian Mountain Club's *White Mountain Guide* for nearly 20 years. In a unique activity, for many years up to his death in 1935, he joined a group of professional men on Sundays year-round at the Appalachian Mountain Club's "Woodlot" on a private estate in Dover, just outside of Boston. Among other activities, they felled trees, cut, split, and corded the logs, built a shack with a fireplace and stove, and constructed bridges, all for the sheer joy of physical effort and agreeable company. I frequently accompanied him there during my teenage years. My pleasant contacts with this group of older men, every one of whom I liked and respected, no doubt strengthened my ideals and affected my general attitude toward life. There and through innumerable enjoyable hikes with my father, I developed a lifelong love of woods and mountains, to which I have always escaped for vacations, either in the United States or in Europe. By extraordinary good fortune, at breakfast in a mountain hut in New Hampshire in 1936, I first met Barbara Belcher, who later become my wife of 53 happy years. In the 1970s I led the development of 30 miles of hiking trails in a state park and along a large reservoir near Baltimore.

In childhood I played with some excellent educational toys. One was Meccanno, a British construction set that included a great variety of gears among its many ingenious parts. For example, I built a differential gear system so that I could understand what was in that bulge in the rear axle of the auto ahead. I also had a Canadian electrical set, from which I wound a motor that actually ran and could be reversed or have its speed controlled by switches and rheostats that were made in such a way that one could see exactly how they worked. And I had a small shop next to my bedroom, where I took lessons in woodworking and built many radios. Perhaps this was a precursor to my later tendency to construct many of my own research gadgets and modify them as they developed under my own hands, rather than submitting complete designs to the laboratory shop.

I prepared for college at Newton Country Day School, to which I commuted by trolley for 8 years. There I was drawn to mathematics and physics. I found they were subjects one could get hold of—there was a clear distinction between right and wrong—so that you usually knew where you stood when you had finished a problem. Also, at nearby Massachusetts Institute of Technology, there were fascinating Saturday morning physics lectures directed at high-school students, with lots of exciting demonstrations.

Harvard College

I graduated from Harvard College with a B.A. in physics in 1932, after once considering changing to engineering. While in college I spent one summer at the physiological optics lab at Dartmouth College, assisting ongoing studies of the effects of size differences between the retinal images in the two eyes and designing lenses to correct this disparity. Then, during my junior year, I saw on a bulletin board an announcement of a then bounteous scholarship of $300 in biophysics at the Johnson Foundation at the University of Pennsylvania. Almost simultaneously my father showed me a report of the foundation's program in the *Journal of the American Medical Association.* So I wrote to the director, Detlev Bronk, who suggested that I spend the following summer, 1931, at the Marine Biological Laboratory in Woods Hole, Massachusetts, working with Keffer Hartline. That was the very summer in which Hartline first recorded from single fibers from the eye of the horseshoe crab (Hartline and Graham, 1932) and thus began the work that eventually earned him a trip to Stockholm, a dance with the Queen of Sweden, and other matters.

At Woods Hole that summer or the next I also worked with Baldwin Lucké, a pathologist from the University of Pennsylvania, studying the osmotic properties of Arbacia eggs by measuring the time course of volume changes after transfer to various solutions. The procedure involved microscopic measurements of the diameter of individual eggs, repeated until statistically respectable data were accumulated. This seemed rather tedious, so I made my first contribution to science by suggesting a diffraction method that would measure the average diameter of hundreds or thousands of eggs at a time. The following summer I assisted Lucké in using this method, and it resulted in my first coauthorship (Lucké et al., 1935).

Through these experiences I decided that science was fascinating, rewarding, and fun, and that its practitioners were friendly, stimulating, and thoroughly honest.

University of Pennsylvania, the Johnson Foundation

In the fall of 1932 I arrived at the Johnson Foundation, which was also the Biophysics Department in the graduate school at the University of Pennsylvania. I spent lab rotations on frog muscle physiology with Emil Bozler,

on effects of X-radiation on the metabolism of fern spores with Raymond Zirkle, and on blood pressure responses to pulsatile perfusion of the carotid sinus of the cat with Bronk.

Transmission through Sympathetic Ganglia

After this, Bronk adopted me as his personal research assistant and set me studying transmission through the stellate sympathetic ganglion of the cat. The procedures allowed either maintenance of normal circulation under artificial respiration or an artificial perfusion system which I later developed using an idea that came to me while sitting on a boulder in the middle of a New Hampshire stream. My doctoral thesis on these preparations established the groundwork for several papers, publication of which was long delayed while the manuscripts travelled afar in Det Bronk's briefcase during World War II. Although my degree was awarded in 1937, two of the three resulting papers were not published until some 10 years later!

A major finding in my thesis research was a phase of increase in the number of ganglion cells responding to a volley of presynaptic impulses, which lasted many seconds or minutes after a series of conditioning volleys over the same presynaptic axons. This increase did not occur after conditioning volleys over axons other than those used for testing and was not produced by volleys sent into the ganglion antidromically over the postsynaptic fibers. Therefore we concluded that activity caused long-lasting changes in the presynaptic endings, such that they delivered a stronger stimulus to the postsynaptic cells (Larrabee and Bronk, 1938, 1947). We termed the phenomenon "prolonged facilitation." After our reports, David Lloyd at the Rockefeller Institute demonstrated the same phenomenon in monosynaptic reflex arcs in the spinal cord and resoundingly renamed it "post-tetanic potentiation" (Lloyd, 1949). This is rather a misnomer, for the phenomenon can be demonstrated after a single stimulus under suitable conditions, although Lloyd was unable do so in his preparations. These findings changed the thinking of some leading reflex physiologists, who had hypothesized that enduring after-effects on reflex responses resulted from the late arrival of impulses from closed chains of neurons, around which impulses had been set reverberating by the conditioning activity. There ensued an extensive literature applying the new name to superficially similar phenomena elsewhere in the nervous system, sometimes uncritically without demonstrating its presynaptic localization.

A second finding on the stellate ganglion dispelled another contemporary notion, that synaptic transmission was always significantly more sensitive to ischemia than was simple conduction along axons, so that this characteristic could be used to distinguish synaptic from nonsynaptic pathways. The stellate ganglion that we were studying contained not only a transynaptic route to the inferior cardiac nerve, but also many axons that passed through

uninterruptedly to form the cervical sympathetic trunk. Thus these two kinds of pathways could readily be submitted to identical conditions of ischemia. The result was that conduction over many axons failed before the last of the synaptic pathways were blocked (Bronk et al., 1948).

Thus the relatively simple anatomy of sympathetic ganglia and their ready access for well-controlled experiments began to establish them as useful tools for understanding basic cellular physiology in more complex situations, such as those in the central nervous system. At the same time W. Feldberg and his associates, in studying the pharmacological properties of these ganglia, were initiating the whole field of chemical transmission at neuron–neuron synapses.

Some of my observations on ganglionic transmission at that time were made on responses in single fibers dissected from the inferior cardiac postganglionic nerve. These dissections were very difficult because the postganglionic fibers in this nerve are unmyelinated and thus belong to the smallest and most delicate class of nerve fibers. For some reason I added glucose to the dissecting medium; this was fortunate, because years later it was found that these small nerve fibers are especially sensitive to lack of this substance (Larrabee and Bronk, 1952). However, when I attempted to resume such recording after it was interrupted by work related to World War II, I could not again accomplish it.

During my graduate student days I contributed to the general techniques of electrophysiology by discovering serious distortions caused by hysteresis in the varying magnetic field of the then-popular Matthews Mirror Oscillograph. This resulted in its replacement by constant-field mirror oscillographs until cathode ray devices became generally available. I also revealed a treacherous tendency of stimulating current to spread to unintended nerves, caused by badly arranged systems, which led to the practice of placing an isolating transformer close to the preparation. I clearly remember the day that Ian Gaylor (from Scotland) and I discovered this flaw, for it had caused a serious error in my doctoral dissertation, which happened to be in the hands of the referees at that very moment.

With a distinct feeling of discomfort I recall that in those days we made our dissections in a small electrically shielded enclosure that was heated to body temperature and humidified to saturation. We dashed in and out of this enclosure to take recordings via amplifiers that were located outside. The recordings were made on seemingly endless strips of photographic paper, which sometimes reminded me of Laocoon and the serpents when I was hassling them through the dark-room developing tanks.

Being Det Bronk's research assistant, my laboratory experiences were broadened by requests for collaboration from other departments, especially pharmacology. There were experiments on the cerebral cortex of cats with mescaline, also some with picrotoxin (a convulsant that produced large, intermittent discharges over the sympathetic nervous system), and recording

of convulsant activity in the cerebral cortex caused by cortical stimulation. I also assisted Robert Pitts, a fellow of the Rockefeller Foundation, who came equipped with a Horsely-Clark stereotaxic instrument, in recording the responses of the sympathetic nervous system to stimulation of the hypothalamus (Bronk et al., 1940; Pitts et al., 1941).

Cornell Medical School

In 1940 Det Bronk accepted the chairmanship of the Department of Physiology and Biophysics at the Cornell Medical School in New York City and took along his close associates, including Keffer Hartline, Arthur Rawson (mechanical engineer), John Hervey (electrical engineer), Frank Brink, Philip Davies, Glen Millikan, and me. During the first semester we taught neurophysiology to a class that had already studied other aspects of physiology. In the second semester we conveniently reversed the order of topics and started the new class with neurophysiology. After that we went back to Penn, thus avoiding topics we didn't know much about.

Respiratory Reflexes

That year at Cornell I had the pleasure of working with Clint Knowlton, a Commonwealth Fund postdoctoral fellow. We recorded the responses to inflation of the lungs of cats in single afferent fibers ascending the vagus nerve and in single efferent fibers descending to the diaphragm in the phrenic nerve. In addition to the well-known, slowly adapting receptors in the lungs and their reflex inhibition of inspiration, we discovered a second set of pulmonary afferents that were activated only on deeper-than-normal inspiration and rapidly adapted when the inspiration was maintained. Their reflex action promoted, rather than inhibited, inspiration. We concluded that this reflex would augment any deep inspiration, as in preparing for a sneeze or cough, and dubbed it the "autogenous pulmonary reflex" (Larrabee and Knowlton, 1946; Knowlton and Larrabee, 1946).[1]

Back to the Johnson Foundation at Penn—the War Years

Det Bronk led us all back to the Johnson Foundation at Penn in the summer of 1941, and soon the lab became deeply involved in research related to World War II. The following are some activities of which I was aware at the time.

[1] An incident with a ridiculous aftermath occurred when Knowlton purchased a toy printing press at Macy's department store in order to run off form cards to help sort our data from 99 single afferent fibers. Several years later his promotion in the Air Force was delayed when a security check revealed that he had owned a printing press while living in New York City in 1941!

Det, as Special Consultant to the Secretary of War, served as Coordinator of Research in the Office of the Air Surgeon General and was away much of the time in Washington and other parts of the world. Among other things, he was involved in organizing and recruiting for the Aviation Physiologists program, which instructed air crews in the physiological problems of high altitudes. He was also a member or chairman of numerous committees concerned with research in the war effort (Brink, 1979).

Frank Brink was Civilian Consultant to the Secretary of War assigned to the Air Surgeon General's Office and spent much of his time writing reports in that office at the Pentagon.

Hartline worked on vision problems, helping to persuade the U.S. Navy to change ship deck lighting from blue to adaptation-preserving red, developing head-worn infrared binoculars for landing aircraft and driving vehicles in the dark, studying the visual distortion caused by thick plastic enclosures over cockpits in fighter planes, determining the optimum degree of magnification for hand-held binoculars, and various other advisory and experimental projects for the armed services. These efforts were assisted by Lorus Milne and Irving Wagner.

Glen Millikan, in cooperation with the Coleman Instrument Co., developed the oximeter to determine the oxygen saturation of the blood in high-altitude flyers by measuring the color of blood with light transmitted through the ear. A modification of this device that fits over a finger is now widely used in medicine, especially in operating rooms.

John Pappenheimer, who joined the Johnson Foundation for the war years, measured accumulation of dangerous concentrations of carbon monoxide in military tanks during maneuvers and firing. He also investigated possible use of the "oxygen candle," a disagreeable looking solid chemical that, when ignited, liberated more oxygen than it consumed. This is apparently the same material that caused a brief fire on the Russian space station in early 1997.

John Lilly developed the electrical capacitance manometer, with displacements so small that tracings of pulsatile blood pressure could be obtained through intraarterial needles. He also helped in many other projects.

Many tests of psychomotor performance and susceptibility to the bends were conducted in altitude chambers at the Medical School, first in a two-man chamber improvised out of a commercial candy-cooker by our mechanical engineer, Arthur Rawson, and later in a standard Air Force multiperson refrigerated chamber. I arrived at the altitude lab one day to find three physiology professors inside the big chamber screaming over the intercom, "Its going up! Its going up!" Meanwhile a high school student at the controls outside was staring at the rate-of-climb indicator and replying, "No it isn't! No it isn't!" The indicator was indeed reading zero, but only because the rate of climb was so fast that the needle had completed a 360° turn! Moreover, the altitude was getting dangerous. So I pushed the operator aside and halted the ascent, thus making a significant contribution to physiology.

In the altitude chambers, Robert Hodes and I tested the effects of ingestion of various chemicals on tolerance to anoxia, including ammonium chloride, nicotinic acid and its amide (suggested by Royal Calder, a physician of Houston, Texas), and methylene blue (suggested by Matilda Brooks).

Julius Comroe of the Pharmacology Department, John Lilly, Robert Hodes, and I undertook a major project, involving many medical student volunteers, to help validate a procedure proposed by the U.S. Air Force to select crews resistant to the "bends." The latter are severe pains caused by gas bubbles in the tissues that were incapacitating some high-altitude flyers.

In collaboration with the Princeton Branch of the Fire Control Division of the Frankford Arsenal, I converted my office into a shooting gallery (no shots were fired), to compare various methods of determining the angle of lead necessary to hit a moving tank. I projected a picture of a beautiful California valley onto a long-paper kymograph, across which brown-paper tanks would travel at any desired speed. The subjects used binoculars with reticles of various design to estimate the angle traversed by the target tank between timer clicks that were separated by intervals equal to the time-of-flight of a projectile.

Toward the end of the war, in an operating room at the army hospital in Framingham, Massachusetts, Hodes and I attempted to measure regeneration across nerve sutures by standard eletrophysiogical techniques. This was rather unsuccessful because of severe problems caused by the stimulation artifact when recording from nerves in continuity. Hodes and I also collaborated with William German, a neurologist at the Philadelphia Naval Hospital in evaluating peripheral nerve injuries by percutaneously stimulating the nerves above the injury and recording the action potentials thus evoked in muscles below the injury. We extended this method to all the major nerves in the extremities and obtained some elegant recovery curves by reexamining war victims over a period of several months. We also determined conduction velocities, in both injured and uninjured nerves, by measuring the difference in latency when stimulating at two different distances above the muscle. We were surprised to find that the normal velocities were considerably slower in nerves to the intrinsic muscles of the hand and foot than in those to the more proximal muscles that moved these same extremities. This was the first application of the method of nerve-evoked electromyograms to such a wide variety of nerves, and it resulted in my only substantial scientific paper from the war work (Hodes et al., 1948). From these experiences I developed a deep respect for the diagnostic skills of a neurologist in evaluating a cooperative patient without the aid of gadgetry. However, we could help in cases of suspected malingering or hysterical paralysis by definitively demonstrating the integrity of the peripheral nerve–muscle system.

In concluding this admittedly incomplete description of the Johnson Foundation's contributions to the war effort, I must recognize the essential contributions of our engineers, Arthur Rawson and John Hervey, who were

involved in almost every project and without whose support we could never have achieved as much. I should also cite the staff of our excellent machine shop under Rawson, especially Victor Legallais.

After the War

Mostly Effects of Anesthetics on Sympathetic Ganglia

As life returned to normal after the war, and the prewar work was written up and published, I again ventured briefly into respiratory reflexes, this time with Hodes (Larrabee and Hodes, 1948). Then I resumed the study of sympathetic ganglia, which I found suitable for a great variety of investigations and became my prime object of study for the rest of my career. Many sympathetic ganglia can be studied *in situ,* and some problems can even be investigated in waking, unanesthetized animals (e.g., Larrabee, 1968). Alternatively, some ganglia can be perfused or excised with relatively little damage and bathed with artificial solutions, thus exposing them to well-controlled environmental conditions. Moreover, the ganglion cells are at rest unless stimulated, and activity can be induced by naturally conducted nerve impulses initiated by electrical stimulation of the preganglionic nerve some distance away. This permits observation of effects of activity on metabolic processes without concern about abnormal applications of current or of electrode products to the cells under study, which occur in some preparations of excised brain tissue.

And now began the pleasure of associating with postdoctoral fellows from other countries. The first was Jean Posternak from Geneva. He and his wife, Yvonne, became good friends, and my wife, Barbara, and I later visited them many times in Switzerland.

Jean and I briefly studied the effects of a natural release from the adrenal glands, after brief clamping of the aorta, that could completely block transmission through sympathetic ganglia (Posternak and Larrabee, 1950). We then initiated an investigation of the effects of many anesthetics on synaptic transmission through the ganglia and on conduction along associated nerve trunks. We quantitatively defined the degree of selective action of an anesthetic on synaptic transmission as the ratio of (a) the concentration required to reduce the maximal compound action potential of the preganglionic nerve trunk by 50% to (b) the concentration required for similar action on the postsynaptic response. Both the blocking potency, as measured by the molecular concentration needed, and the degree of selective action tended to increase systematically with molecular weight, reaching a 10-fold selectivity with pentobarbital, which was the heaviest agent we tested (Larrabee and Posternak, 1952).

About this time I began to measure the oxygen uptake of sympathetic ganglia, exploiting the oxygen electrode that Philip Davies had developed for rapidly measuring tissue oxygen concentration (Davies and Brink, 1942;

Davies, 1962). Phil also devised ingenious procedures to prevent the electrode from becoming obstructed by nonreactive substances, such as proteins attracted by the electric field, and from movement of the solution to which it was exposed. He applied it to the cerebral cortex of cats, measuring oxygen concentration and metabolism with impressive spatial resolution (Rémond et al., 1946).

Jean Posternak and I thrust a sharpened version of the oxygen electrode into the stellate ganglion of the cat and estimated the rate of oxygen consumption from the rate of fall of concentration when the blood flow or an artificial perfusion was suddenly stopped. We demonstrated increases in oxygen consumption during activity induced by stimulating either the preganglionic or postganglionic nerves, and were even able to detect an acceleration after as few as two volleys of impulses (Larrabee and Bronk, 1952).

Leaving the Johnson Foundation

Here we come to the end of the Johnson Foundation years, and I cannot leave them without reflecting on what a wonderful experience it was. Det Bronk took care of the finances before the days of federal largess, frugally spending the income from the endowment. The latter was in memory of Eldridge Reeves Johnson, who is credited with building the first talking machine when it was invented by Thomas Edison. Det also obtained grants from various sources, including the Supreme Council of the Scottish Rites Masons, the National Foundation for Infantile Paralysis, and the American Philosophical Society. We were a close-knit, highly cooperative group, with few responsibilities other than the conduct of the best research of which we were capable. We lunched together in the department library, where we were joined by several research-oriented physicians from the medical school and hospital, in which we were located. Lunch conversations ranged from serious discussions of each other's research problems and triumphs to trivial pursuits, such as calculating the time required for a nerve impulse to reach the moon. Electrophysiological experiments were not started until after lunch, due to electrical disturbances in the morning from diathermy machines on the floor below, but frequently continued far into the night after a nucleus had supper together at a nearby restaurant. Again, the engineering support by Arthur Rawson and John Hervey was most important to our work. Art's policy of keeping the excellent machine shop always open to our tinkering was most helpful, even though someone occasionally strained his kindly Quaker disposition almost to the breaking point. Many of the world's best known neurophysiologists were Bronk's friends and visited the laboratory, sometimes followed by an evening at the Bronks' home for further discussions. Among those that I recall were Herbert Gasser, head of the Rockefeller Institute, Ralph Gerard from Chicago, E.D. (later Lord) Adrian from Cambridge, England, and J.C. (later Sir John) Eccles

from Australia. In an informal way, a number of us would frequently drift out to the Bronks' country home on Sunday afternoons. Helen Bronk, a saint-on-earth in many ways, would cheerfully feed supper to all who wanted to stay, without previous knowledge of the number, without kitchen help except for any wives who happened to be present, and before the days of microwaves and deep-freezers. In the stimulating and friendly environment of the Johnson Foundation, we were a harmonious, secure group that laughed a lot. I wish I had memory and space for more of the humor.

Johns Hopkins University and the New Department of Biophysics

On January 1, 1949, Bronk became president of Johns Hopkins University. There he had found an endowment for a biophysics department that had not yet been formed, so he led his team southward to fill the gap. Included were all participants in the earlier migrations to Cornell Medical School and back, except for Arthur Rawson, who had already taken a position at the Army's Camp Detrick laboratories in Frederick, Maryland. Also included were graduate students Francis Carlson, Ted MacNichol, Lloyd Beidler, Clarence Connelly, and Paul Hurlbut; all except Beidler stayed as departmental members after receiving their degrees. Keffer Hartline was appointed chairman of the new Thomas C. Jenkins Department of Biophysics. Until our new building was completed a year or two later, most of the group was lodged in rooms loaned by the Biology Department in the Faculty of Arts and Sciences on Hopkins' Homewood Campus. However, I was provided generous laboratory space by Abner McGehee Harvey in the Department of Medicine at the Medical School and Hospital complex in East Baltimore. There I was in frequent contact with Vernon Mountcastle in Physiology, Stephen Kuffler in Ophthalmology, and John Magladery in Neurology. Also, I was especially helped in getting oriented by Joseph Lillienthal and Kenneth Zierler in the Department of Medicine.

More on Anesthetics in Sympathetic Ganglia

At this time the leading theory of anesthetic action was that these agents primarily depressed oxidative metabolism and that the suppression of function was a secondary effect of the metabolic disturbance. This theory was supported by some observations on oxygen consumption by excised brain tissue, but it seemed to me that the concentrations employed might be excessive and that better controlled experiments could be made on sympathetic ganglia, where we had by then developed techniques for measuring oxygen uptake and synaptic transmission simultaneously. Moreover, Duncan Holaday and I had shown in naturally circulated cat ganglia that transmission was affected at surgical levels of anesthesia with ether and chloroform,

so that the sensitivities of sympathetic synapses to at least some anesthetics were similar to those in the central nervous system (Larrabee and Holaday, 1952). Joined by Juan Garcia Ramos from Mexico City and later by Edith Bulbring from Oxford, England, we found on superior cervical ganglia excised from rabbits that seven different anesthetics all suppressed the transynaptic response by as much as 50% at concentrations that had no detectable effect on oxygen consumption. In contrast, two typical metabolic poisons, cyanide and azide, both affected oxygen metabolism at least as much as they did synaptic transmission (Larrabee et al., 1952). Thus the hypothesis of a primary effect of anesthetics on metabolism was not supported.[2]

The Superior Cervical Ganglion of the Rat

Searching for a laboratory animal more convenient than cats and rabbits, I tried various smaller species and found that superior cervical ganglia excised from rats survived well in a typical oxygenated, bicarbonate-buffered, physiological solution. Therefore I adopted this preparation for many subsequent investigations.

Effects of Lack of Oxygen and Lack of Glucose

Ramos, in a second and very thorough series of experiments, compared the survival of transmission through excised rat ganglia under various conditions of anoxia and aglycemia, separately and combined. He first showed that transmission survived about as long when both substances were supplied to an excised preparation as when the ganglia were left *in situ* with the natural blood supply after cutting the preganglionic nerve. Failure in the absence of glucose was accelerated by repetitive stimulation and delayed by preexposure to an elevated glucose concentration. The failure was also delayed by reducing the temperature of preparations stimulated infrequently, but was essentially independent of temperature when stimulated at frequencies above 1 per second. These and other results were reported in detail at a Cold Spring Harbor Symposium (Larrabee and Bronk, 1952).

Later we found that oxygen uptake was not greatly affected at the time of transmission failure caused by withdrawal of glucose, so energy metabolism must have shifted smoothly to endogenous substrates (Larrabee et al., 1957). William Stekiel, in his doctoral dissertation, subsequently showed that ammonia output was increased more than six-fold in the absence of

[2] Edith Bulbring somewhat facetiously explained that there are, in ascending order, physiological concentrations, pharmacological concentrations, biochemical concentrations, and biophysical concentrations. In other words, each discipline except the first tends to raise the concentration until something interesting happens, with little concern about the relevant level in the intact organism. I have always tried to avoid that pitfall.

glucose, indicating utilization of amino acids and/or proteins (Larrabee et al., 1957). F.J. Brinley, as part of his doctoral dissertation, found that glucose-free metabolism not only maintained the normal high internal potassium of the ganglion cells almost as well as did metabolism in the presence of glucose (Brinley, 1967), but also restored it after considerable potassium had been lost during temporary withdrawal of oxygen (Larrabee and Klingman, 1962). Thus the oxidation of endogenous substrates sufficed for some but not all functions. We speculated that protein used in the absence of glucose might be essential for conduction or transmission; in that case the eventual physiological failure would result from an active, rather than a passive, process.

Charles Edwards, who was the first graduate student to do his thesis research in my laboratory, adapted the glucose oxidase method to measure glucose depletion in 25-μl batches of solution that had bathed a rat ganglion. Anesthetics, surprisingly, increased glucose uptake considerably, accompanied by an increase in lactate output that accounted for most of the extra glucose (1955).

Pseudorabies Virus and a Puzzling Discharge of Impulses

In the early 1950s I was joined by John Dempsher, fresh out of medical school with fellowships from the U.S. Public Health Service and the National Foundation for Infantile Paralysis. He first tried the effects of various bacterial toxins on the rat ganglion, without interesting results. Then, at the First International Poliomyelitis Congress in New York City, I happened to sit at a banquet next to Albert Sabin, of polio vaccine fame. He told me that in attempting to infect the central nervous system with pseudorabies virus inoculated into the eyes of mice, he had sectioned the whole head and noticed typical lesions in the superior cervical ganglion. He suggested we investigate the effects of this virus in our rat preparations. Frederick Bang in Hopkins' Department of Epidemiology offered to grow the virus for us, and David Bodian in Anatomy offered to do the histology. We found, about 2 days after inoculating the virus into the vitreous humor of one eye of a rat, that virus had appeared in the ipsilateral superior cervical ganglion, that many or all ganglionic neurons were histologically abnormal, and that remarkable spontaneous discharges of nerve impulses appeared in both the preganglionic and postganglionic nerves. These discharges involved many cells, lasted about 1 second, and were separated by silent periods of 1 or more seconds. At the earliest stage of infection spontaneous activity appeared only in the postganglionic nerve, at the latest stages only in the preganglionic nerve. The spontaneous discharges continued in the same patterns after the ganglia were excised. They never occurred in the ganglion contralateral to the infection and never in uninoculated animals. Since many different cells obviously fired in each burst, there was evidently

some kind of communication from cell to cell. However, the mechanism remains a mystery because there are no known synaptic connections to accomplish this (Dempsher et al., 1955).

The Move to Homewood

In 1953 Bronk left Hopkins to become president of the Rockefeller Institute in New York City, taking along Hartline, Hervey, Brink, Connelly, and Hurlbut. I was invited to join them, but chose to remain at Hopkins. I moved my laboratory from the medical area to commodious space that then became available in the Biophysics Department at Hopkins' Homewood Campus. I also obtained a research grant from the National Institutes of Health that became effective September 1, 1954, for studies on "Metabolism and Function in Sympathetic Neurons." This was funded continuously for some 43 years, at least through November 30, 1997, and is the only funding outside of Hopkins that I ever sought.

Metabolism at Rest and in Activity

I now began a study of the changes in ganglionic metabolism that accompany the shift from the resting to the active state, which in our experiments was caused by naturally conducted nerve impulses that were initiated by stimulating the preganglionic nerve at some distance from the ganglion.

I built a respirometer for oxygen uptake, after the design of Carlson et al. (1950), in which sterile bathing fluid continuously flowed slowly past the ganglion while the concentration of the remaining oxygen was measured downstream with an oxygen electrode. According to suggestions of Philip Davies, the electrode tip was coated with collodion, which stabilized it in the bicarbonate buffer system being used and rendered it insensitive to movement of the solution. To enable ready sterilization, the respirometer chamber was made of glass, which gave me the fun of learning glass-blowing on a rather small scale. The preganglionic nerve was pulled into a side arm with its own flow of solution, which permitted stimulation without danger of electrode products affecting the measured oxygen uptake. The postganglionic nerve was drawn into a channel in the plug that closed the top of the respirometer, in order to record the postganglionic response. With this I measured the resting rate of oxygen uptake and its increase during stimulation. The activity increment was readily measured during stimulation as infrequently as once per second, increased with frequency, and reached an upper limit about 40% above the resting level at 15 per second (Larrabee, 1958).

Paul Horowicz, then a graduate student, and Michel Dolivo from Lausanne, Switzerland, measured glucose uptake and lactate output by chemical methods (Horowicz and Larrabee, 1958; Dolivo and Larrabee, 1958). In

ganglia at rest, the difference between these two rates provided enough substrate to account for about 92% of the oxygen taken up. Another 3% was accounted for by ammonia production, measured by William Stekiel, under the assumption that the ammonia was liberated on conversion of glutamine to glutamate and that the latter was all oxidized to CO_2. These results were in reasonable agreement with the contemporary understanding that glucose is the major substrate for energy production in neural tissues, although Stekiel's ammonia measurements suggested a small contribution from amino acids or proteins.

By 1957 the Tracerlab CE-14 low-background system for beta counting had become available, with anticoincidence circuitry for reducing cosmic ray counts. The background counting rate was only about 1 count every 2 minutes, enabling us to measure $^{14}CO_2$ output from our small tissue samples, which had fresh weights of only about 1 mg. For this I built a system in which slowly flowing, humidified 5% CO_2–95% O_2 picked up the $^{14}CO_2$ from the incubation medium and, after dehumidification, carried it through a chamber sealed to a Geiger counter. Thus $^{14}CO_2$ output was measured continuously. (Twenty years later this apparatus was still in use in my laboratory.) Using glucose labeled with ^{14}C, we could now measure incorporation of glucose carbon into CO_2 and, using paper chromatography, into various products in the tissue and in the bathing medium, without confusion from products of other, unlabeled substrates that would be included in chemical measurements.

The results on resting ganglia were that 64% of the glucose carbon went to CO_2 and 24% to lactate in the bathing fluid, while 12% was incorporated into tissue constituents, thus accounting for all of the carbon in the glucose. However, the $^{14}CO_2$ output was only 86% of the oxygen uptake, raising a possibility of some oxidation of other substrates, which would have to be endogenous because no substrates other than glucose were provided in the bathing solution (Larrabee and Klingman, 1962). Moreover, there were greater problems concerning the extra metabolism that was associated with activity. When the preganglionic nerve was stimulated about five times per second, glucose uptake by the ganglion rose about 55% above the resting rate, but the chemically measured lactate output rose enough to account for much of the extra glucose. Only about 24% of the extra glucose remained after allowing for the extra lactate produced, and this could account for only about one-quarter of the extra oxygen consumed during the activity (Larrabee and Klingman, 1962). In contrast, experiments with [^{14}C]glucose soon showed that much more than the 24%, in fact about half of the carbon in the extra glucose, was actually converted to CO_2 (Horowicz and Larrabee, 1962a,b). I believe this was the first unequivocal evidence for increased oxidation of glucose in neural tissue during activity. However, the increase in CO_2 output from glucose was only about 67% of the increase in oxygen uptake. A reasonable interpretation of all these findings is that extra glucose

is indeed metabolized to CO_2 for energy production during activity, but that some endogenous substrate or substrates, possibly including glycogen, are also used, both for production of lactate and for oxidation to CO_2. Stekiel had found no increase in ammonia production during activity, so proteins or amino acids seem unlikely candidates (Larrabee et al., 1957).

Similar questions concerning the extra substrates used during activity have arisen in recent studies of human brain by noninvasive PET and MRI techniques (Barinaga, 1997). However, I confess difficulty in comprehending how reliable metabolic rates can be deduced from such measurements of the concentrations of naturally present substances in a tissue with an intact and variable circulation of blood.

Phospholipids

My next foray was into phospholipids, stimulated by reports that acetylcholine in the presence of eserine increased the labeling of phosphatidyl inositol (PI) and phosphatidic acid in a variety of tissues (Hokin et al., 1960). However, the concentrations of acetylcholine used were even higher than those that I had earlier observed to depress rather than stimulate sympathetic ganglion cells. So I attempted to reproduce their results under more normal conditions in our ganglion preparations, where activity could be initiated by naturally conducted nerve impulses. In 1955 I was joined by Jack Klingman, who patiently bolstered my weak biochemistry, and by William Leicht, whose skillful hands conducted most of the experiments. We found that activity, generated by stimulating the preganglionic nerve, indeed increased the $^{32}P_i$ labeling of PI, but we were never able to confirm Hokin's finding of an effect on phosphatidic acid. The labeling of PI with ^{14}C from [U-^{14}C]glucose was also increased. There were no significant changes in labeling of phosphatidyl choline or phosphatidyl ethanolamine, or of any phospholipids in nerve trunks or in the nonsynaptic sensory ganglion of the vagus nerve. The effect on PI was prevented by blocking synaptic transmission with tubocurarine, and it could not be produced by sending impulses antidromically into the ganglion by stimulation of the postganglionic nerve. Therefore, we concluded that it was an effect of the synaptic transmitter acting on the ganglion cells and not simply a result of the discharge of impulses by these cells (Larrabee et al., 1963; Larrabee and Leicht, 1965).

I later extended the effect on PI to still more natural conditions by stimulating the preganglionic nerves of ganglia remaining *in situ* in anesthetized rats. I even showed that it was produced by normal sympathetic activity in the unanesthetized animal, where a control ganglion at rest was provided by preliminary cutting of the preganglionic nerve on one side (Larrabee, 1968). These were the first demonstrations of the effect on $^{32}P_i$ labeling of PI under physiological conditions. But nobody seemed to pay

much attention, perhaps because the reductionist philosophy of the time was satisfied with findings under simplified but highly artificial circumstances.

David Burt later showed that the PI effect was greatest in subcellular fractions containing synaptosomes and mitochondria (Burt and Larrabee, 1973). He also found that the labeling of PI with [^{3}H]inositol was increased during activity even more than that with $^{32}P_i$, and that there was a small but significant effect on labeling with [^{14}C]glycerol, but none with [^{14}C]acetate (Burt and Larrabee, 1976). Thus the increased turnover of the PI molecule during activity must involve most but possibly not all of its parts.

The Giant Axon of the Squid

Due to the interest in the giant axon of the squid that was generated by the ionic transfer experiments of Hodgkin and Huxley, F.J. Brinley and I investigated the labeling of lipids in this preparation. He incubated the axons with $^{32}P_i$ at the Physiology Department of the Medical School and brought them to me for analysis. In intact axons there was the expected labeling of phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidic acid. Most surprisingly, phosphatidyl choline, although apparently present in abundance, received no detectable ^{32}P label. Another finding, possible only with the giant axon, was that extruded axoplasm, when mixed with $^{32}P_i$, labeled the same lipids as did the intact axon (Larrabee and Brinley, 1968).

The Higher Inositides

Godfrey White from J.N.Hawthorn's laboratory in Birmingham, England, brought more biochemical knowledge, including reverse-phase paper chromatography for resolving the di- and triphosphatidyl inositides. Activity in the rat ganglia had no effect on $^{32}P_i$ labeling of either of these. We also examined the effects of two analogs of inositol, δ- and γ hexachlorocyclohexane. The latter is also known as "lindane," a highly toxic insecticide. The δ-hexane blocked axonal conduction and the transynaptic response at similar low concentrations, without reducing the $^{32}P_i$ labeling of any of the inositides. On the other hand, the γ-hexane had a striking selective action on synaptic transmission at a concentration of 0.07 mM, without effect on axonal conduction or on incorporation of $^{32}P_i$ into inositides. The results on transmission suggest that synaptic blockage may be an important component of the toxicity of the latter insecticide, for which the LD_{50} for rats is only 0.3 mmol/kg body weight (White and Larrabee, 1973).

Nerve Growth Factor and Its Antiserum

In 1965 Giovanni Toschi came to the lab from Rome, bearing a generous gift of nerve growth factor (NGF) from its discoverer, Rita Levi-Montalcini. He

also taught us to dissect the lumbar sympathetic chains from chicken embryos. Unfortunately we obtained no publishable results during Giovanni's relatively short stay, though his visit was educational for us and later led to many findings.

As our techniques with the nerve growth factor improved, Lester Partlow, for his doctoral dissertation, studied the effects the factor on sympathetic ganglia excised from chicken embryos. He demonstrated that the excessive growth of axons, which was caused by addition of the factor to the medium, was unaffected by profound inhibition of RNA synthesis by actinomycin-D or of protein synthesis by cycloheximide (Partlow and Larrabee, 1971). These findings dispelled a contemporary hypothesis that the growth effects were mediated by synthesis of a novel RNA and thence of a novel protein.

Partlow also found, in the absence of the inhibitors, that the rates of incorporation of labeled precursors into RNA and protein, which otherwise tended to decline 5–10 hours after excision, were better sustained if the nerve growth factor were added to the medium (Partlow and Larrabee, 1971). And Ted Brown, a talented undergraduate, found similar effects on $^{14}CO_2$ production from [1-^{14}C]glucose (Larrabee, 1969, 1972). These findings suggested a supportive, rather than a stimulative, action of the growth factor.

Brown, under Partlow's direction, also found that the growth of fibers induced by the growth factor around excised sympathetic ganglia was greatest at 14 days of embryonic age and that significant outgrowth could still be obtained until at least 19 days of age, whereas the growth response from dorsal root ganglia ceased abruptly after 15 days (Larrabee, 1972). He also found an axial gradient along the lumbar sympathetic chains in the development of the responsiveness, with the outgrowth becoming elicitable first in the more cephalad ganglia (unpublished).

David Halstead, another doctoral candidate, followed the time course of various depressant effects of an antiserum to the nerve growth factor in the superior cervical ganglia of young mice. This antiserum had been produced in a horse and was given to us by the Wellcome Research Laboratories, courtesy of D. Caird Edwards. With a background in electrical engineering, Halstead exhibited unusual ingenuity and skill in developing procedures, such as routinely recording transmission through ganglia from mice 3 days of age and younger with body weights of 1.5 to 3 g. The transynaptic response was the most rapidly affected of all the attributes measured, falling to 79% of its control height in ganglia excised 12 hours after an injection of antiserum at 7 to 10 days of age, and to less than 5% after 2 days. By the second day, glucose metabolism to CO_2 had fallen to about 20% of the control, incorporation of labeled precursors into RNA and protein were down to about 40%, and the dry weights of the ganglia were about 50% of the control. Thus the antiserum affected the ganglia in several ways, raising a question about what was the primary event in their degeneration (Halstead and Larrabee, 1972).

Semiretirement

My tenure at Hopkins expired in 1975 because I had reached the age of 65, and no more graduate students came my way. Perhaps they feared I would not last to the completion of their thesis research. But I continued in the laboratory for more than 20 additional years, publishing another 15 research papers. Smaller but very adequate space was generously provided by the Biophysics Department and I volunteered most of my time, recompensed only very modestly from my shrinking but continuing NIH grant.

Carbohydrate Metabolism in Sympathetic Ganglia of Chicken Embryos

In the course of the experiments with the nerve growth factor, I became impressed with the sympathetic chain of chicken embryos as an admirable preparation for certain kinds of research. The eggs are relatively inexpensive, they come with their own built-in food supply, and can be delivered by the supplier at any desired age of development. Thus the laboratory incubator can contain a fine collection for age studies or provide eggs of a given age on any day between deliveries. So I abandoned the rat ganglia in favor of the chicken embryo.

I set out to describe the progressive changes in carbohydrate metabolism that occur during development, as the embryologists had done for morphological events. I used the lumbar sympathetic chains of the chicken, which can be excised as early as 6 days after laying, the day on which they have completed migration into their final position. At 8 days, the earliest age that I studied extensively, the carbon from labeled glucose was mostly released as lactate, with very little appearing in CO_2, so energy production was mostly glycolytic. It seemed probable that this was related to the poor blood supply, which was just beginning to develop in the ganglia and is not completed until the 12th day. By the 12th day the production of labeled lactate had declined by 80% and the output of $^{14}CO_2$ from [1-^{14}C]glucose had risen to a maximum. At this time the output from [6-^{14}C]glucose was only 20% of that from [1-^{14}C]glucose, indicating that much of the glucose was utilized via the pentose pathway.[3] As development continued, the C-1 output declined progressively to the level of the C-6 output, indicating subsidence of metabolism via the pentose pathway. Thus glucose metabolism, like morphological development, passed through several stages: the first was characterized by glycolysis to lactate, the second, by pentose pathway activity, and finally a conventional adult pattern of oxidation arose,

[3] For those unfamiliar with the pentose pathway, it may be characterized for present purposes as an alternative route for glucose metabolism that parallels the first few steps of the Embden–Meyerhoff glycolytic path. It is needed to form ribose for RNA synthesis and NADPH for fatty acid synthesis. At entry to this route, all carbon-1 of glucose is released to CO_2. It is frequently called the "pentose cycle" because some of its products are recycled into its entry.

presumably restricted mostly to the Embden–Myerhof pathway and the tricarboxylic acid cycle (Larrabee, 1972).

Later I repeated these experiments in more detail (Larrabee, 1985), and compared the results with some on dorsal root ganglia (Larrabee, 1987b). I showed, among other things, that the developmental rise and fall of activity in the pentose pathway was paralleled by a surge in lipid synthesis. Thus there must be an intensified formation of these products at this stage of development, peaking at about 11 days of embryonic age and supported by the pentose path's synthesis of NADPH, which is needed for chain elongation in the building of fatty acids (Larrabee, 1987a).

Evaluation of the Pentose Pathway

The foregoing experiments drew my attention to the pentose pathway, and I sought a way to determine how the metabolism of glucose was partitioned between this route and the more familiar Embden–Meyerhof glycolytic pathway. The then popular equations of Wood, Katz, and Landau (1963) were not applicable to our preparations because they depended on a frequently ignored assumption that the tissue was in an isotopic steady state, whereas our embryonic ganglia sometimes took as long as 15 hours for $^{14}CO_2$ output to rise to a steady value during exposure to [6-^{14}C]glucose and it seemed undesirable to work with the tissue so long after excision. On the other hand, our preparations showed a great difference between the time courses of $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose from the very beginning of incubation, and it occurred to me that this difference contained information that had not been available to other investigators. For example, when the output of $^{14}CO_2$ from [6-^{14}C]glucose rose more slowly than that from [1-^{14}C]glucose, it was evidence for activity in the pentose pathway, even if the final values were identical and investigators waiting for the steady state might conclude otherwise.

Accordingly, I laboriously derived equations describing the time course of $^{14}CO_2$ output from [1-^{14}C]-, [2-^{14}C]- and [6-^{14}C]glucose by the cyclic systems represented by the pentose pathway and the citric acid cycle, in terms of the time constants of pools that delayed the output of $^{14}CO_2$ and certain other parameters. These expressions could be fitted to the data on $^{14}CO_2$ output by adjusting the parameters, using a computer-driven, nonlinear, least-squares program. Thus, at the age of 65, I was finally introduced to the world of computers by someone else's graduate student, and trudged back and forth from my lab to the University's central computer, lugging a 10-inch stack of key-punched cards that had to be reloaded into the computer each time.

I applied this procedure to data from sensory ganglia of chicken embryos, obtained very satisfactory fits to time courses of the $^{14}CO_2$ outputs from variously labeled glucoses, and published the equations in a lengthy

and difficult paper (Larrabee, 1978). (I have not the least evidence that anybody except the referee ever read it, and I have some reason to believe that even he never finished it!) I soon found, however, that I had been incorrect in assuming that the pool of intermediates which delayed carbon-6 of glucose on its way to CO_2 was located somewhere in the pentose pathway: when I tried to identify this pool, the evidence indicated that no pool big enough existed in that path. Instead, paper chromatography of the ganglion tissue showed that the major differential incorporation of carbon-6 was into lipids, and in addition about half as much as this went into chromatographically immobile substances, possibly including proteins, lipoproteins, and nucleic acids (Larrabee, 1980).

Therefore, I adapted the time-course equations to a different model. In this model I abandoned the pool in the pentose path and assumed that the ganglia contained two different kinds of cells, one with all glucose entering the pentose system and the other completely lacking this pathway. This permitted an even better fit to the data and indicated that about 23% of the absorbed glucose entered the pentose pathway (Larrabee, 1980). Some support for the assumed difference between kinds of cells was later obtained, for the pentose path was less active in cultures containing only neuronal cells than in nonneuronal cultures (Larrabee, 1982). At least the new model showed that the time-course equations published in 1978 might have a usefulness beyond the particular faulty model for which they were originally developed.

Later I realized that rigorous upper and lower limits to the flux from glucose to the pentose pathway could be set from the rates of $^{14}CO_2$ output from [1-^{14}C]glucose and [6-^{14}C]glucose. Moreover, the range of uncertainty between these limits could be greatly narrowed by using time-course data, all without application of a specific model (Larrabee, 1989). The flux thus calculated for dorsal root ganglia in 10 μl of medium ranged from 12 to 36% of the glucose taken up, thus embracing the value of 23% that had been obtained in my 1980 paper with the more elaborate but assumption-laden equations. Moreover, the latter equations remained plausible, whereas they would have been invalidated if their result had fallen outside the new rigorously derived range.

I also published a critique of the classical Katz and Wood equations for evaluating pentose pathway activity (Wood et al., 1963), explicitly stating their implicit assumptions and rederiving their equations. By considering a tissue containing two kinds of cells, it was apparent that very misleading results could be obtained on nonhomogeneous tissues, even if each constituent obeyed all of Katz and Wood's assumptions (Larrabee, 1990).

Fluxes through Extracellular Pools of Lactate and Alanine

In the course of experiments on lactate production from [^{14}C]glucose, my attention was attracted to an unidentified radioactive peak on chromatograms

of media recovered at the end of an experiment. Its magnitude was sometimes comparable to that of lactate, and I was unaware of the release of any such sizeable product from glucose other than lactate and CO_2. This was finally identified as alanine (Larrabee, 1984).

The outputs and uptakes of both lactate and alanine were measured by tracer methods as functions of their concentrations in the bathing solution. This revealed a marked increase of output of each to the medium as its concentration was raised. The reason for the increase was apparently that much of the released material was normally reabsorbed via an uptake system that was saturable, so that uptake of the added material interfered with reuptake of that which was produced by the cells. The situation was greatly clarified by computer modeling, assuming that uptake varied with the extracellular concentration according to familiar Michaelis–Menton kinetics for both alanine and lactate, and that their outputs were affected in specific ways that were somewhat different from one another. Calculations for the model were made by numerical integration over the time course of incubation, thus allowing for progressive changes in concentrations and for reuptake of some of the released material. To enable these calculations, exchange rates between the extracellular space within the ganglion and the surrounding medium were shown to be directly proportional to the concentration differences, and the transfer coefficients for this exchange, as well as the volume of the extracellular space, were measured by wash-out experiments.

First, the model was used to predict the exchanges between the whole ganglion and the surrounding medium, and the best values for the various constants in the cellular exchange equations were found by least-squares fitting of the predictions to the observations. Some ingenuity was involved in this, because it was necessary to devise a way of fitting both the uptake and output data for several concentrations of a given substrate in a single computer run, and this involved telling the computer a little white lie due to the structure of the available program.

Once the best values of the constants had been chosen, it was possible to calculate the exchanges between the cells and the extracellular fluid, which cannot be measured directly, and thus determine the fluxes of lactate and alanine through their extracellular pools. It was estimated that as much as 45% of the glucose taken up was released to the extracellular fluid as lactate and alanine, but that over 65% of these released materials were reabsorbed before they could diffuse out of the tissue into the bathing solution (Larrabee, 1992). Thus the extracellular pools can be important routes for glucose metabolism and should be included as intermediates of carbohydrate metabolism. Whether the released materials were reabsorbed by the same cells that produced them or by different cells is unknown for our ganglion preparations. However, evidence for partial digestion of glucose by

one type of cell for subsequent utilization by other types has been reported for retina (Poitry-Yamate et al., 1995; Tsacopoulos and Magistretti, 1996).

Lactate Metabolism in Relation to Glucose Metabolism

My final investigations in this area asked how the source of CO_2, and hence of energy production, was partitioned between glucose and lactate when both were present. To this end, the two substrates were provided in various combinations of concentrations, with first one and then the other uniformly labeled with ^{14}C. Addition of each substrate was found to reduce the output of CO_2 from the other, but the sum of the outputs nevertheless always exceeded that from either alone, so energy production was always increased by combining the two (Larrabee, 1995).

Moreover, in a sense, lactate was a substrate preferred over glucose. For example, when both were present in the medium at a concentration of 5 mM, about 5 times as much CO_2 came from lactate as from glucose, and an excess from lactate persisted until its concentration was reduced below 2 mM (Larrabee, 1996).

By computer modeling, many details of the concentrations and flux rates of both exogenous and endogenous lactate were calculated for the sympathetic ganglia under study. This was done using the previously published equations for cellular lactate uptake and release as functions of the extracellular concentration and for the exchange rate between the extracellular fluid and the bathing solution.

Finally, a model for brain was constructed by replacing the function that had been used for transfer between the extracellular fluid and the bathing medium in the ganglion calculations by the transport properties of the blood–brain barrier of the adult rat (Cremer et al., 1979). This model predicted rates for consumption of blood lactate that agreed well with those reported by other investigators for dog brains *in situ* in the presence of a wide range of lactate concentrations, thus encouraging further calculations. Accordingly, it was estimated that, with a normal level of blood glucose (5 mM) and a typical resting value of blood lactate (1 mM), 6% of the CO_2 produced by the brain would be derived from blood lactate. If the lactate were raised to 5 mM, it would supply about 20% of the CO_2, and at 20 mM, a level commonly reached during intense exercise, it would supply more than half of the CO_2, about 60% (Larrabee, 1996), even in the presence of 5 mM glucose. This suggests that lactate may be a significant fuel for brain, especially during muscular activity, and deserves more experimental investigation at elevated concentrations than it has yet received.

In closing, it should be emphasized that these questions about use of lactate by the brain cannot be settled by chemical measurements of lactate in the arterial and venous blood of the brain under normal resting

conditions. This is because, under these conditions, the lactate concentrations may then be about 1 mM in both blood and brain, so that outflux of lactate formed in the brain may equal the influx from the blood and thus conceal the metabolism of blood lactate.

Epilogue

This account was written with the aid of my new personal computer during the spring of 1997 in my comfortable study in a congenial retirement community located in a beautiful valley outside of Baltimore. Barbara and I moved there three years earlier as her health was beginning to fail, before she died in 1996. I have made many friends in the community. Our son, David, lives nearby, and we enjoy a meal together weekly. My older son, Benjamin, born to my first wife, Sylvia, is also nearby in southern Pennsylvania.

In retrospect, I have had a happy and relatively easy life. I was well reared by my parents, sustained by Det Bronk's paternalism early in my career, and subsequently supported abundantly by a single research grant that enabled all the experiments I wanted to do. And, for more than 20 years after my tenure expired, I was generously provided with lab and office space by my University and its Biophysics Department.

I feel fortunate to have been invited to the 2nd International Neurochemical Symposium in Aarhus, Denmark, in 1958. This opened my provincial New England eyes to other parts of the world and led to numerous vacations in Europe, especially in the beautiful mountains of Switzerland. Later, Barbara and I also enjoyed many other trips to scientific meetings in far away places, including Brussels, Budapest, Milan, Strasbourg, Vienna, Hawaii, Japan, and Venezuela.

In writing this account I was interested to see the flow of research from project to project, as questions or opportunities that were revealed in one project led to the next. The flow was sort of an evolutionary process, punctuated by occasional events such as the development of the oxygen electrode by Philip Davies, suggestions received at a chance dinner with Albert Sabin, and the gift of nerve growth factor by Rita Levi-Montalcini. I enjoyed it all, never seemed to lack an exciting horizon, and find little that I would like to change.

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