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LOUIS BARKHOUSE FLEXNER

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A Biographical Memoir by

JAMES M. SPRAGUE

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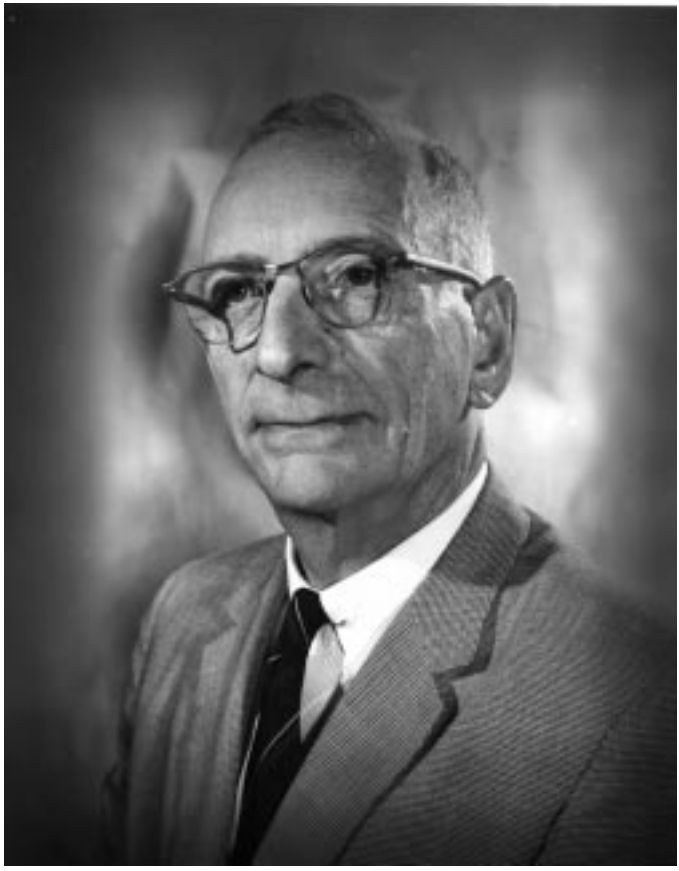

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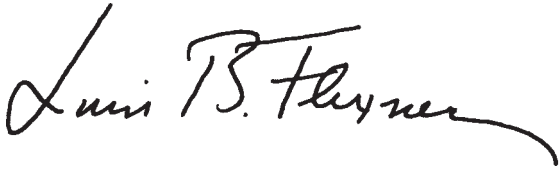

LOUIS BARKHOUSE FLEXNER

January 7, 1902—March 29, 1996

BY JAMES M. SPRAGUE

LOUIS FLEXNER WAS A major scientific figure, in direct line with his distinguished uncles Abraham and Simon. He was a pioneer in the field of neurochemistry and made notable contributions also to the physiology of the cerebrospinal fluid and meninges, to the function of the placenta, and to the biochemistry of development. Through his organization and direction of the Institute of Neurological Sciences at the University of Pennsylvania, he had a major role in the development of neuroscience in this country.

Louis Barkhouse Flexner was born in Louisville, Kentucky, on January 7, 1902, to Ida Barkhouse and Washington Flexner. He grew up in Louisville and Chicago in a home of modest but adequate means and in a family that was congenial, cooperative, industrious, and interested in literature and sports and to a smaller degree in music. Education for the children was emphasized, with nothing spared in its support. He came from a family famous for its contributions to medicine. Uncle Simon Flexner was a professor of pathology at the University of Pennsylvania School of Medicine and later became director of the Rockefeller Institute for Medical Research in New York City. Uncle Abraham Flexner conducted the definitive study on the organization of U.S. medical schools and wrote a book that revolutionized U.S.

medical education. Abraham was responsible for the organization of the Institute for Advanced Study in Princeton and became its first director. Simon Flexner's influence was felt early by Louis, who, at age seven, won a Louisville newspaper's writing contest on "How I Intend to Earn My Living" with an essay on his intention to cure epilepsy.

Louis received his undergraduate education at the University of Chicago (B.S., 1923) after which he received his medical education at Johns Hopkins University (M.D., 1927). Flexner's rating at Hopkins placed him with Keffer Hartline (who was later to receive a Nobel Prize in physiology) at the bottom of the class of 1927, something of which both men were inordinately proud! This rating did not, of course, reflect on their intellects or abilities. Medical school proved to be difficult for Louis. He was confused by the volume of material, the variety of subject matter, and the unsettled state of numerous important areas. Essential support and stimulus from his earliest days came from Lewis H. Weed, director of the Department of Anatomy and dean of the school.

From Weed, he received an orientation that largely determined his subsequent activities and career. Soon after Flexner entered medical school, Weed started him on a problem in experimental embryology concerning the central nervous system, which was carried through to publication. For many years thereafter, his major interest centered on the development of the nerve cell. Weed also gave him a point of view towards medical education and the organization of a school of medicine.

On graduation from medical school, Flexner was not, however, certain that he wanted a career in anatomy. Thus, he welcomed an opportunity to work with the famous biochemist Leonor Michaelis, then at Hopkins, who was beginning his studies on oxidation-reduction potentials (1928,

1929). Although he had valuable time with Michaelis for about two years, he recognized his inadequacies in physical chemistry and mathematics and decided against continuing the collaboration with him. Still unsure of the kind of career he wanted, Flexner took an internship at the University of Chicago Clinics. This experience was decisive. Although offered the possibility of spending a year in Vienna with Sigumd Freud, he refused. After eight months his request to be relieved of clinical duties was accepted; he spent the remaining part of the year working on the development of the human cerebral cortex with G. W. Bartelmez at the Department of Anatomy of the University of Chicago. At the beginning of the next year he was back at Hopkins on the staff with Dr. Weed, where he spent the next nine years (1930-39) in the Department of Anatomy doing research on the cerebral-spinal fluid and establishing himself as an investigator of real promise. He absorbed from chairman and mentor Professor Weed a philosophy of medical education to which he adhered for the rest of his life. The keystone of this philosophy was the absence of spoon-feeding and the recognition that an instructor's main contribution was to teach students how to educate themselves.

Flexner's continuing interest in problems of mammalian development was broadened and stimulated by several experiences. First among these was a year spent with Sir Joseph Barcroft in the Physiological Laboratories at Cambridge (England). Barcroft at that time (1933-34) was essentially beginning his work in the field of fetal physiology. Here Flexner also came under the influence of Joseph Needham, author of *Chemical Embryology,* and an American, Donald Barron, who moved to physiology at Yale University in 1939 and became a lifelong friend.

In 1941 Flexner was offered a research position as a staff member in the Department of Embryology of the Carnegie

Institution of Washington. His studies there on the biochemistry of development received wide recognition, including a Harvey Lecture (1951-52). The environment and the support and insight of laboratory director George W. Corner and Vannevar Bush, president of the Carnegie Institution of Washington, proved ideal for the rapid development of his interest in this area. He remained with the Carnegie for a decade, having spent four of these years during the Second World War on a full-time basis as technical aide to the Committee on Aviation Medicine of the National Research Council's Committee on Medical Research.

In 1951 he was offered the chair of anatomy at the University of Pennsylvania. Before accepting the post he went to Philadelphia several times to meet the members of the department and sound out their feelings about having him as the new head. He satisfied himself that he would meet a welcome reception—a good example of his consideration of other people's feelings.

This switch to the University of Pennsylvania occurred for two reasons. First, he desired to get back into medical education both at the department and at the school level. Second, he wanted to see whether he might develop a department of anatomy whose faculty was working at the frontiers of biological knowledge in what appeared to him to be two of the important fields of biology: (1) structure and function of the central nervous system and (2) the central problems of embryology, that of cellular differentiation.

With customary vigor he quickly set up a research laboratory with his wife Josefa as co-investigator and research associate and began to expand the horizons of a good but traditional anatomy department. He foresaw that three fields of medical research would become prominent in the future and would be appropriate for an anatomy department:

(1) cell differentiation and development, (2) ultrastructure and imaging, and (3) neuroscicnce. He began to build a department around these three disciplines.

Flexner understood that neuroscience research must be multidisciplinary in order to grow; in 1953 he undertook the organization of the Institute of Neurological Sciences (now known as the Mahoney Institute) by adding neuroscience staff to the department and encouraging other university departments to do likewise. From a beginning nucleus in 1954 of six members, including William Chambers, Eliot Stellar, John Brobeck, Per-Olaf Therman, Louis Flexner, and James Sprague, the Institute now includes over 130 neuroscientists distributed among 24 basic science and clinical departments throughout the university. The Institute has been an enormous success nationally and locally; its members helped shape the development of the departments of anatomy, psychology, biology, neurology, and psychiatry at the University of Pennsylvania.

Such is the power of a new and dynamic concept conceived and brought to term at the right time. This development was supported initially by a small grant from the National Institute of General Medical Sciences, part of the National Institutes of Health. The general support of NIGMS director Fred Stone was of great importance to us in securing the first training grant awarded by the National Institutes of Health. The Mahoney Institute led the nation in recognizing the impending explosive growth in the brain sciences, and celebrated its fortieth anniversary in 1994 at the annual meeting of the Society for Neurosciences.

While Flexner was chair, the other branches of the department also developed outstanding research and teaching programs with a faculty that included Howard Holtzer, John Marshall, James Lash, John Liu, Gabriel de la Haba, Donald Scott, Andrew Nemeth, Frank Pepe, Jean Piatt,

Roy Williams, Culver Williams, Mary Jane Hogue, Michael Harty, and Robert Johnson. Their work attracted many excellent graduate and medical students and postdoctoral fellows. What emerged during the Flexner era (1951-67) was the picture of anatomy as a broad, comprehensive, and very active discipline in the School of Medicine and the university.

The significance of Flexner's own research in the biochemistry of memory, done in close collaboration with his wife, was recognized by his election to the National Academy of Sciences (1964), American Academy of Arts and Sciences, and the American Philosophical Society. He was also a member of the American Society of Biological Chemists and the American Association of Anatomists, which he served as secretary-treasurer from 1956 to 1964. The Democratic National Committee named him to a newly created Advisory Council on Technology in 1959. Flexner served on numerous scientific boards, including those of the U.S. Public Health Service, United Cerebral Palsy Association, National Council to Combat Blindness, National Research Council, National Paraplegic Society, and the National Foundation for Infantile Paralysis, where he joined the original Committee on Research. He received the Weinstein Award in 1957 in recognition of his work on the development of the central nervous system. In 1974 he was awarded an honorary degree (Doctor of Laws) by the University of Pennsylvania for his "unremitting pursuit of the highest ideals of scholarship and his uncompromising standards of research and teaching." Another Penn tribute was the establishment of the Louis B. Flexner Lectureship, one of the signal gatherings of neuroscience investigators each year, sponsored by the Mahoney Institute of Neurological Sciences.

The Flexners met when Josefa was a research associate in the Biochemistry Department of Johns Hopkins University

from 1930 to 1931. A Catalan by birth and daughter of a distinguished Spanish family, Josefa studied at the University of Barcelona and at the University of Madrid, where she received her doctorate in pharmacy in 1927. She also spent a year studying pharmacology in England before coming to the United States for a year. Unrest and the civil war interrupted her career in Spain. Louis and Josefa were married in a village in the Pyrenees Mountains sixty years ago, and worked together until his death in 1996. She was known as Pepita and was indeed a very important colleague in his research and proved to be a lively and intellectually stimulating person to his laboratory visitors.

Louis served as chair of the Department of Anatomy from 1951 to 1967. His retirement was a simple process, which consisted of moving his office and laboratory across the hall, where he continued his research on memory and his teaching of medical students into his early nineties.

Flexner's first paper, published with J. Berkson in the *Journal of General Physiology* in 1928, was titled "The rate of reaction between enzyme and substrate" and was a forerunner of his scientific interests for the rest of his life. His mentor in anatomy, Prof. Lewis Weed, suggested the meninges and cerebrospinal fluid as a promising area for him.

Flexner began with the development of meninges in amphibia (1929), later joining Weed in the study of cerebrospinal fluid and intracranial pressure in the cat, macaque, and chimpanzee. From 1933 to 1937 Flexner made several important discoveries, among which was the proof that the production of cerebrospinal fluid was a process of secretion by the choroid plexuses. This work culminated in two important reviews, "Some problems of the origin, circulation, and absorption of the cerebrospinal fluid" (1933) and "The chemistry and nature of the cerebrospinal fluid" (1934).

Flexner spent the next two years at Cambridge Univer-

sity, working in the laboratory of Prof. Joseph Barcroft, where he began studies of fetal development, including physiology and biochemistry of the placenta and uterus. Returning to Baltimore, Flexner initiated a series of studies on placental permeability in the rat, guinea pig, goat, rabbit, pig, cat, and human, using radioactive substances as tracers. He published his findings in a long series of papers titled "Biochemical and physiological differentiation during morphogenesis" between 1937 and 1960. These studies initially concentrated on the transfer of substances in the placenta, but they were extended to the developing kidney, liver, and cerebral cortex in collaboration with many colleagues—Isador E. Gersh, R. Stiehler, Arthur Grollman, Richard Roberts, H. Pohl, Alfred Gellhorn, William Straus, Walter Wilde, Dean Cowey, Gilbert Vosburgh, Leslie Hallerman, Nathaniel Proctor, Virginia Peters, Max Hamburgh, A. Hughes, and especially his wife Josefa Flexner. His productivity was marked by brilliant insight, broad understanding of the material and technical ingenuity. His findings were summarized and discussed in *Genetic Neurology* in 1950, in a Harvey Lecture in 1952, and in the *Biochemistry of the Developing Nervous System* in 1955.

An important contribution in this period was the discovery by Flexner and colleagues (1960) that the lactic dehydrogenase (LDH) of the cerebral cortex of mouse and guinea pig consists of four components, two of which accounted largely for the increase in LDH activity that occurs when neuroblasts differentiate into neurons. This multiple form of enzymes was discovered independently by Flexner and by Markert and Moller (1959).

The last three reviews indicated Flexner's ongoing interest in the developing nervous system and pointed the way toward the later research on memory, for which he was best known. This line of research stemmed from a visit to an aging relative, whose long-term memory was intact but

whose short-term memory was severely deficient. An important step was the discovery that the developing brain synthesized protein at a remarkably high rate. A second crucial step came with the discovery by Yarmolinsky and de la Haba (1959) that the antibiotic puromycin inhibited protein synthesis in liver extract by 90%. When this substances was injected subcutaneously by Flexner and colleagues, protein synthesis in the mouse brain was inhibited by approximately 80% two to eight hours after injection. Disappointingly, learning and retention were unaffected. Flexner and collaborators next injected puromycin intracerebrally, the spread of which could be estimated by the use of fluorescin and the extent of inhibition of protein synthesis under these conditions was about 90%. When puromycin was injected so that it reached primarily the hippocampus and entorhinal cortex, short-term memory measured after one day was completely lost. Loss of long-term memory measured eleven to forty-three days later required a more extensive injection of the frontal, temporal, and ventricular areas. There was a clear dissociation between recent and long-term memory, based on anatomy, biochemistry, and behavior.

Continued experiments indicated that this loss of memory caused by puromycin was not due to its effect on protein synthesis but to peptydyl puromycin formation. This was shown by use of another antibiotic acetoxcycloheximide (AXM), an even more potent inhibitor of protein synthesis than puromycin. AXM, however, failed to affect memory when injected one or more days after learning (1966, 1967; Agranoff, 1982). These and other findings caused a shift in their experiments to the role of neurotransmitters and their receptors in the formation and expression of memory. These studies, which yielded many important findings, occupied the Flexners for the next thirty years.

During his fifties and sixties, Louis became an avid sailor; he purchased a modern version of the old Chesapeake bugeye

and named it *Josefa B* after his wife and colleague. Many of his friends, including the writer, had the pleasure of weekend cruises around the bays and anchorages of the Chesapeake Bay.

Louis Flexner was a man of many accomplishments, one of the more intangible of which was his leadership and the esprit de corps he infused in a faculty strong on ego and independence. Many are the students and postdoctoral fellows who trained in the department and who came back later to visit and said that their experience in the department was outstanding and unique. He built a strong department of talented people, and he took great care in selecting them, weighing not only their accomplishments but also, in so far as possible, their character. He once said, "I can admire the guy who is a great scientist, but at the same time I'll admire him a hell of a lot more if he has respect for other people." Before offering a position to a person outside the university, he would make a trip to the candidate's school to learn about his (or her) personality, talking to students, technicians, and peers. He had a strong character and was supportive and considerate of his staff, always having time to discuss research as well as personal problems. His advice was insightful and given to help the individual involved. The honoraria he received for lectures and service on committees and study sections was put into a separate account to help staff members purchase expensive equipment or animals or to attend meetings not fully covered in research grants. His forceful personality, high standards, and robust sense of humor made him a leading and colorful figure in the School of Medicine and the University of Pennsylvania.

Despite the high quality of his research and administrative accomplishments, Flexner's modesty was noteworthy, and at no point in his career did he seek prizes or solicit

special recognition. He was made uncomfortable by any exclusiveness, such as a journal club or symposium, which to be manageable was necessarily limited in membership; he preferred that everyone be invited. He failed to attend meetings of the National Academy of Sciences because several colleagues whom he considered worthy had not been elected. He thought they had been excluded unfairly. I was stimulated and felt privileged to be part of the development of the anatomy department and Institute of Neurological Sciences, which Louis Flexner took over and headed. He was very supportive of my own research and was a potent catalyst in developing a scholarly atmosphere of research and a spirit of democracy and scientific openness among faculty and students—a rare combination. All of us felt the euphoria of a shared enterprise of research and teaching at the cutting edge.

Louis died peacefully in his ninety-fourth year, only a few days after a massive stroke. He is survived by his wife, Josefa Barba, his sister Miriam Maderev of New Rochelle, and many nieces, nephews, and cousins.

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