OTTO FOLIN AND DONALD D. VAN SLYKE: PIONEERS OF CLINICAL CHEMISTRY (1)

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With the start of the 20th century, clinical chemistry emerged into its own space on the mosaic of medical practice. The pattern of its future growth and development took shape during the first two decades of the new century, the United States leading the way with the decisive breakthrough. Until then the United States had played no role in the growth or development of clinical chemistry. Afterward, the nation quickly achieved leadership which it never relinquished.

Two names dominated this period, as their papers filled the pages of the *Journal of Biological Chemistry* and other publications: Otto Knut Folin (1867-1934) and Donald Dexter Van Slyke (1883-1971). Their systematic explorations on blood and urine set the style and shaped the parameters for clinical chemistry for the remainder of the century as they developed practical and clinically applicable methods of analysis. On the basis of a new approach to methodology—analysis of small volumes of biological fluids—they determined normal ranges, correlated variations with pathological conditions, and elucidated metabolic pathways in health and disease. Neither Folin nor Van Slyke held medical degrees; yet their research and teaching of biochemistry and clinical chemistry demonstrated that chemists could make great contributions to advances in medical diagnosis and the treatment of disease.

The Flexner Report and Medical School Reform

Folin and Van Slyke received a valuable assist in the development of clinical chemistry from an unlikely and unexpected source, the "Flexner Report." Abraham Flexner (1866-1959), a former Louisville, Kentucky high school teacher and educator, had published his first book, *The American College* (1908), in which he severely criticized some of the current educational practices. The book drew the attention of Dr. Henry S. Pritchett, president of the recently established Carnegie Foundation for the Advancement of Teaching, and he asked Flexner to make a study of American medical schools. Flexner asked whether he was being mistaken for his brother Simon, director of the recently formed Rockefeller Institute for Medical Research in New York. Pritchett replied that he wanted an educator's evaluation, not that of a medical practitioner. Published in 1910, the report exposed the disgraceful practices of the American (and Canadian) medical school systems and made specific recommendations for correcting the deficiencies. The report had a far-reaching effect on the practice of science in the laboratories of medical schools and hospitals and on the research and teaching of biochemistry.

Flexner specifically referred to "clinical chemists" and "clinical chemistry." Concerning the laboratories connected with the university hospital, he wrote (2):

> To suffice for clinical investigation the laboratory staff must be so extended as to place, at the immediate service of the clinician, the experimental pathologist, experimental physiologist, and clinical chemist in position to bring all the resources of their several departments to bear on the solution of concrete clinical problems. Of these branches, experimental pathology and physiology have already won recognition; the next step in progress seems to lie in the field

> of clinical chemistry, thus far quite undeveloped in America.

His emphasis on the use of laboratory sciences in the training of medical students and in the teaching of specialties contributed to the favorable environment for the rapid growth of clinical chemistry. Flexner's views complemented and reinforced what Folin had said two years earlier to the Harvey Society. Folin reminded the medical profession of the large variety of clinical material available for biochemical investigations in the large city hospitals. If these hospitals are to become centers for biochemical research, as they should, according to Folin, they must provide laboratory facilities, personnel, and independence to the trained chemist to work on the many biochemical problems seeking answers (3).

Otto Folin

Otto Folin was 15 years old when, at his mother's urging, he left home in Sweden in 1882 to join his older brother in Minnesota. What followed is the familiar American success story. Immigrant boy arrives penniless and in debt, works as unskilled laborer, learns English and the new customs, acquires an education, and becomes a professor at Harvard (4). Folin graduated from the University of Minnesota and entered the University of Chicago for graduate study in 1892, the year it opened. At Chicago, he completed his doctoral work on urethanes under Julius Stieglitz (1867-1937) in 1896. Then, acting on the advice of the eminent physiologist, Jacques Loeb (1859-1924), Folin decided to take additional training in Europe in the newly emerging field of physiological chemistry, which barely existed in the United States. Six months of the first year, 1896-97, was spent in the laboratory of Olof Hammarsten (1841-1932) at the University of Uppsala, not far from Folin's childhood home. Here he examined the properties and composition of a hydrolysis product of a glycoprotein, mucin, from submaxillary glands. A short paper on this subject was published in *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* in 1897 (5). It was Folin's first contribution to biochemistry.

During the summer of 1897 he worked in the Berlin laboratory of Ernst Leopold Salkowski (1844-1923). There, his first contact with hospital patients led to an interest in the urinary end products of nitrogen metabolism. He improved a quantitative method for urinary uric acid and published it in 1897 as sole author (6). The analysis of uric acid remained a lifelong interest. Six months of the next year, 1897-98, was spent with

Figure 1. Otto Folin. Portrait by Emil Pollak-Ottendorf, 1934 (National Library of Medicine, Bethesda, MD)

Albrecht Kossel (1853-1927) in Marburg, where he applied his knowledge of organic chemistry to biological problems and where his interests in the intermediary stages of protein metabolism had their beginning. Two more papers appeared in *Hoppe-Seyler's Zeitschrift* (7, 8). It was at Marburg that he discovered the new technique of colorimetry used in the brewing industry and the color comparator invented by Jules Duboscq (1817-1886). This instrument was to become the basis of his major contributions to developing simple, reliable, and convenient colorimetric methods for clinical chemistry. On his return to Chicago in 1898 he was awarded the Ph.D.

There were no academic positions available in physiological chemistry in the US. In the few universities and medical schools where this subject was being taught, it was assigned to instructors in physiology, pharmacology, or medical chemistry. Only Yale had a department of physiological chemistry, established in 1882 by Russell Chittenden (1856-1943) (9). Consequently, Folin accepted a position as chemist in a private, commercial laboratory in Chicago, specializing in analysis of water, food, and medical products and in toxicology. A teaching opportunity came in the summer of 1899. He accepted an assistant professorship of chemistry at

West Virginia University, where he offered courses in quantitative analysis and elementary physiological chemistry.

Metabolic Studies at McLean Hospital

In 1900 Folin received an offer from the McLean Hospital in Waverley, Massachusetts, a suburb of Boston. Edward Cowles (1837-1919), the medical superintendent of this private psychiatric hospital, had established a laboratory for physiological chemistry in 1889, one of the first of its kind in the US to support research. His objective was to advance the understanding of mental diseases by searching for a connection between abnormal mental states and urinary excretion, especially of urea and uric acid. Cowles believed, as did others, in a correlation between insanity and chemical toxins produced by faulty metabolism and poor nutrition. He expected to find evidence of this in the patient's urine. Research conducted by resident physicians was begun in 1891-92. Blood changes in hemoglobin, red and white blood cell counts, differential count of white cells, and specific gravity were also studied. When larger laboratory facilities were built in 1895, Cowles planned a special research department to be run by a professional biochemist. Folin was asked to plan, equip, and develop his own program of research toward achieving Cowles' objective: uncovering an association between mental status and urinary excretion (10).

Nineteenth-century clinical chemistry involved chiefly the examination of the urine. This was understandable; its collection offered no technical difficulties or risks, and the quantities of fluid available allowed utilization of the gross methods of gravimetric and volumetric analysis already in use and requiring large volumes of specimen. The objective at that time was to isolate the particular substance in pure form, then weigh it or titrate it.

Finding no evidence of toxicity in urine of insane patients, as was claimed by some French writers, nor of qualitative differences, Folin turned to the study of protein metabolism of normal versus mentally disturbed individuals by measuring as accurately and completely as possible all of the known nitrogenous and other products excreted in the urine of patients fed a standard diet. He would thereby establish the normal range of the nitrogenous fractions and then consider whether differences were due to an abnormal metabolism in mental disease. Normal patterns were then unknown. To establish norms would by itself be an important undertaking; but first, he had to devise additional and improved quantitative methods before any survey could be initiated. This was to lead to his lifelong interest in quantitative methods for nitrogenous end products in urine.

When Folin began his detailed studies of nitrogen metabolites in urine, there was no commercial source of purified chemicals, water, standards, calibrated glassware, or instruments designed for use in the clinical chemistry laboratory. Procedures for testing of urine—mostly qualitative, some quantitative—filled major portions of books on clinical diagnosis by laboratory methods. Practical quantitative chemical analysis of blood was virtually nonexistent or was described only briefly. The development of blood chemistry was hampered by a shortage of blood for experimental and diagnostic purposes, as a result of the gradual abandonment of bloodletting as a therapy late in the 19th century. Large volumes of blood were required for chemical analysis and there was no well developed or convenient technique for drawing the large amounts needed. Furthermore, the plasma proteins (and red cells) interfered markedly with the methods; consequently, blood was rarely tested. Hematological procedures, on the other hand, namely, blood counts, hemoglobin, and white cell differential, were readily supplied by finger stick.

The first years at McLean were spent mainly in devising and testing methods for the determination of nitrogenous constituents in urine, most of which were known qualitatively, *e.g.*, urea, ammonia, uric acid, creatinine, and creatine. Previously, quantitative methods for these constituents were frequently laborious or complicated and, as in the case of urea, were nonspecific; or in the case of uric acid, they required relatively large amounts of specimen. Folin's first colorimetric method was developed for urinary creatinine in a reaction with picrate ion in alkaline medium at room temperature to form a red color. Color comparison was made with an artificial standard—N/2 potassium bichromate—after correlation with pure creatinine had been established. Although other color reactions had been used long before this to estimate biological products, *e.g.*, Nessler's reagent for ammonia in water analysis, Folin's use of the Duboscq colorimeter for color comparison in the quantitative analysis of creatinine in urine in 1904 ushered in the modern era of clinical chemistry (11). The color reaction, discovered by Max Jaffe (1841-1911) in 1886 (12), is the longest continuously used colorimetric procedure for blood or urine analysis; and until 1936, when the reaction with 3,5-dinitrobenzoic acid was described (13), it was the only method for creatinine.

Folin's studies at the McLean Hospital revealed no metabolic evidence related to mental disease, but in the course of his work he had developed methods for biochemical research that promised to deliver significant results of a more general physiological interest and importance. While at McLean, a personal misfortune struck Folin. In the spring of 1903 a benign tumor was removed from his left parotid gland. During the surgery it was necessary to cut the facial nerve. This procedure permanently altered Folin's appearance.

Colorimetric Methods for Blood Analysis

Folin's simple colorimetric method for the quantitative estimation of urine creatinine in 1904 was the breakthrough that opened up the possibilities of this rapid, simple, and inexpensive technique for analysis. It gave great impetus to the development of additional methods for quantitative analysis of other nonprotein nitrogen products in urine. The increased sensitivity of colorimetric procedures allowed use of smaller samples and resulted in greater accuracy than was previously possible with the older gravimetric and volumetric (titrimetric) methods. What followed in laboratories in the US and abroad was the use of Folin's small-sample, reliable methods for the design of research protocols to study the composition of urine from normal individuals and patients with various disorders. However, the analysis of urine had limited clinical usefulness. It gave information primarily about the excretion of abnormal amounts of urine constituents.

Folin then turned to refining analytical methods to make them applicable to the same constituents in blood, but in much smaller samples than were required by other methods. He recognized that, since blood plasma reflected the condition of the extracellular fluids as a whole, blood analysis was a better guide to metabolic reactions and clinical evaluation of nephritis than was urinalysis. It was much more important to know what metabolic products the kidneys failed to excrete and accumulated at harmful levels in the blood, than it was to know what and how much was excreted in the urine (14). In 1914, ten years after introducing the alkaline picrate colorimetric reaction for creatinine in urine, Folin described the first satisfactory method for determining creatinine in blood. He published the first extensive data with this reaction for normal individuals and in various pathological states (15). Folin followed with colorimetric methods for urea, uric acid, creatine, ammonia, and nonprotein nitrogen in blood. These analyses served as a tool for quick and reliable assessment of the retention of the ordinary nitrogenous waste products caused by failing kidney function. Their practical value as an aid in diagnosis and determination of operative risk represented an important advance for medicine and surgery. Other investigators in America and Europe followed Folin's lead and modified his procedures or developed their own practical colorimetric methods.

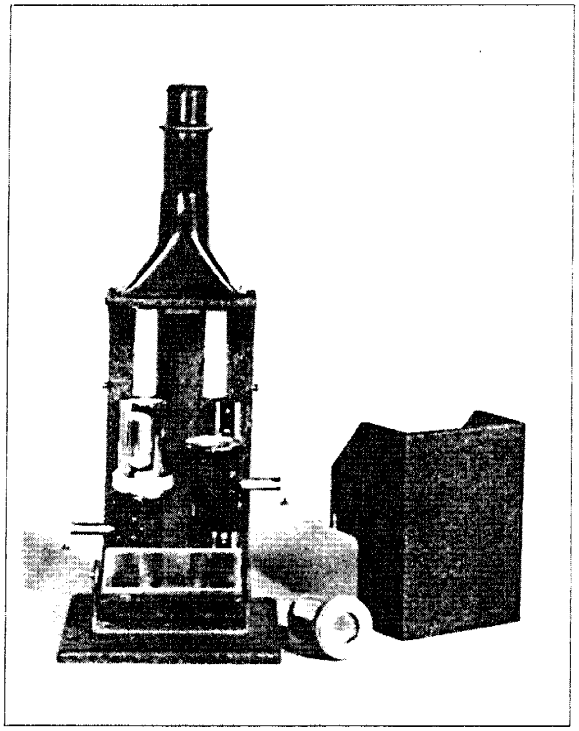

Figure 2. Duboscq-type Visual Colorimeter (front view), Bausch & Lomb, Rochester, NY, ca. 1950. Note reflecting mirror, light shield, fixed plungers, and glass bottom of sample cup.

Folin Joins Harvard Medical School

The publication of several papers on a new theory of intermediary metabolism of ingested protein, along with the growing popularity of his methods of chemical analysis, brought Folin to the attention of the biochemical profession. It led, no doubt, to his appointment in 1907 as associate professor of biological chemistry, and in 1909, as Hamilton Kuhn Professor of biological chemistry and head of the department at Harvard Medical School, the first nonphysician on the faculty. He remained at Harvard until his death in 1934, teaching biochemistry to first-year medical students and building his department into a center of graduate study and research with a strong emphasis on analytical methods and clinical applications. Two of his graduate students later won

Nobel Prizes: James B. Sumner (1887-1955) in 1926 for the first crystallization of an enzyme, urease; and Edward A. Doisy (1893-1986), with Henrik Dam, in 1943 for the isolation and synthesis of Vitamin K. A third member of the department, George H. Hitchings, a teaching fellow for the 1929-30 academic year, would share a Nobel Prize in Medicine or Physiology in 1988 for his part in the discovery of important principles for creating a rational method of designing new compounds that selectively operate against various disease states. Other students were Walter Ray Bloor (1877-1966), who developed methods for the determination of cholesterol and other blood lipids; Philip A. Shaffer (1881-1960); and Cyrus H. Fiske (1890-1978), who, with Yellapragada SubbaRow (1895-1948), discovered phosphocreatine in 1927 and developed a popular method for serum phosphorus.

Folin's best known collaborator was Hsien Wu (1893-1959). In 1919 they published "A System of Blood Analysis (16)." Using tungstic acid as the protein precipitant, they combined a number of different analytical procedures into a widely used, simplified, and compact system of blood analysis on a protein-free filtrate. Prior removal of the blood proteins is necessary because they interfere with most added chemical reagents. This was a welcome response to the rapidly increasing number of chemical blood analyses being performed in hospitals and a landmark development in clinical chemistry. The tungstic acid protein-free filtrate was adopted worldwide and remained in use until the introduction of "automated" analysis in the late 1950s, when deproteinization was accomplished by dialysis and, in subsequent years, by other innovative technologies and methods that did not require prior removal of proteins.

Modern chemical analysis of small quantities of blood would have been impossible without the colorimetric methods developed by Folin and others. These procedures entailed some sacrifice of accuracy since the product to be analyzed was not isolated and the reaction occurred in a complex chemical milieu—even if the proteins were removed before the analysis. The methods were far from specific and the results obtained were often falsely high because of nonspecific substances which gave the same color reaction as the substance analyzed. In spite of the shortcomings of these early methods, however, the analyses were of distinct clinical use in the diagnosis of diabetes, uremia, gout, and other diseases.

Folin's new methods, based on visual colorimetry and small volumes of specimen, were a stimulus to the growth of clinical chemistry. This activity coincided with the beginning of the institutional reform of biochemistry during the first two decades of the 20th century. The professional prestige of biochemists was largely advanced by their success in developing diagnostic tests for the practicing physician.

Folin helped found the American Society of Biological Chemists in 1906 and served as its third president in 1909. After the establishment of the *Journal of Biological Chemistry* in 1905, he submitted most of his papers there. He joined the editorial committee in 1919, serving as chairman for many years.

In 1908 Folin proposed that American hospitals employ clinical chemists to advance "our ability to differentiate between the physiologic and the pathologic (17)." He cautioned that although hospitals should become involved in biochemical research, clinicians can neither do nor direct chemical work. Systematic biochemical research requires the "ingenuity, resourcefulness and critical judgment of the trained chemist (17)."

Much of the early work in methods and applications in clinical chemistry was published in the *Journal of Biological Chemistry*. To a large extent, during the first quarter of the century, biochemistry *was* clinical chemistry. The *Journal of Laboratory and Clinical Medicine*, founded in 1915, served as another major outlet. After the clinical chemists formed the American Association of Clinical Chemists in 1948, this organization began to publish *Clinical Chemistry* in 1955. It had been preceded in 1949 by *Scandinavian Journal of Clinical & Laboratory Investigation* and followed in 1956 by an international journal, *Clinica Chimica Acta*, based in the Netherlands.

Donald Dexter Van Slyke and the Rockefeller Hospital

Donald D. Van Slyke received his Ph.D. from the University of Michigan in 1907 in organic chemistry under Moses Gomberg (1866-1947), the discoverer of organic free radicals. Van Slyke, expecting to follow his father's career as an agricultural chemist, had actually been offered a job with the Bureau of Chemistry in Washington. The elder Van Slyke [Lucius Lincoln (1859-1931), Ph.D. University of Michigan, 1882 (Prescott)] was a chief chemist at the Geneva Agricultural Experiment Station in New York. A chance encounter between Lucius Van Slyke and Phoebus Aaron Theodor Levene (1869-1940) at an American Chemical Society meeting

Figure 3. Donald D. Van Slyke (National Library of Medicine, Bethesda, MD)

in 1907, however, led to a job offer from Levene at the newly formed Rockefeller Institute for Medical Research in New York (18). By 1913 the administrators of the Rockefeller Institute recognized that internal medicine was moving rapidly ahead along chemical lines. To guide this advance they believed that the hospital of the institute should now have an experienced chemist in a senior position to conduct his own research while serving as a general advisor to physicians on chemical problems. The chemist would have to develop an interest in medical problems and be temperamentally able to cooperate with physicians, for whom the patients came first. After studying the chemistry of proteins and amino acids and their analysis at the institute for seven years, Van Slyke was selected in 1914 to develop a department of chemistry in the hospital, related to clinical chemistry (19).

Although Van Slyke had no experience in clinical work, Simon Flexner (1863-1946), the director of the institute, was impressed by his training in organic chemistry and his publications in biochemistry. Through an arrangement by Levene, who had collaborated with Emil Fischer, Van Slyke spent a year in Berlin in 1911 working with Fischer (1852-1919) and Emil Abderhalden (1877-1950), publishing a paper with each (20, 21). Uncertain as to whether he was capable of the clinical work, Van Slyke agreed to the new responsibility for one year, provided he could return to Levene's laboratory if it was not to his liking. Van Slyke found that the young doctors were all just about his age, and they welcomed him and his assistant, Glenn Cullen (1890-1940) into their group. He found medicine fascinating and remained in the position for the remainder of his tenure at Rockefeller. There he applied chemistry to the solution of clinical problems related to diseases under investigation at the hospital (22). Because the Rockefeller Hospital was a research facility, the researcher served as the chief and the physician or surgeon as the assistant. As a service chief, Van Slyke had free access to blood and urine specimens. He taught himself kidney physiology and disease and soon found himself in charge of a ward of patients with Bright's disease (23). He went on hospital rounds and instructed the resident staff on special diets or other preparations required for the patients under study. He also instructed them on what to look for in the patients and then to report from a clinical point of view. This arrangement was an unusual privilege. In most institutions at that time clinicians dominated the partnership with biochemists and infrequently acknowledged any help from them in the study of disease (24).

Biochemistry was in its infancy. Accurate methods for blood constituents in small specimens were just becoming available. The concentration and distribution of many of the inorganic constituents of the body were not known. Proteins were not yet regarded as chemical entities; enzymes had not yet been isolated and characterized; the existence of hormones and vitamins was suspected, but they had not yet been clearly identified. Although Van Slyke began his career as an organic chemist, his interest in physiological function in health and disease resulted in an acceleration of new knowledge and the development of quantitative clinical chemistry. Van Slyke's design of accurate analytical methods for measuring gas and electrolyte equilibria in blood and the transport of blood gases furthered the understanding of respiratory physiology in heath and disease.

Institutional context was crucial to Van Slyke's success in integrating chemistry and clinical medicine, because the Rockefeller Hospital encouraged a cooperative attack on a problem from all sides—chemical, physiological, and clinical. Clinical problems provided opportunities to develop, extend, and improve analytical procedures; new techniques led to discoveries in the physiology of disease. Van Slyke's first clinical prob-

lem was one in diabetic acidosis. Severely ill diabetic patients, under the most efficient treatment (low-calorie diet) available in the pre-insulin days, sometimes developed acidosis which, by the time it had become clinically noticeable, progressed at a very rapid rate to a fatal coma. What was needed was a method for detecting earlier stages of the acidosis. Van Slyke began by defining acidosis in chemical terms, rather than in descriptive medical language. He devised an instrument and developed a simple, reliable gasometric method for measuring the carbon dioxide content of plasma or serum (25). This rapid and relatively simple test for quantifying blood acidity made it possible to anticipate and prevent the fatal acidosis. The instrumental method was soon adopted in most hospital laboratories in the US and Europe. It continued in general use well into the 1960s, when it was replaced by automated colorimetric methods for bicarbonate levels in plasma or serum. Van Slyke's technique for studying acidosis by using quantitative biochemical analysis dramatically increased understanding of the disease processes and provided a basis for rational treatment before the discovery of insulin. Van Slyke made fundamental contributions to the understanding of buffer action, acid-base balance, fluid and electrolyte equilibrium, and carbon dioxide transport by hemoglobin and oxyhemoglobin. He developed a method for determining clearance of urea from the blood and a rapid procedure for the determination of red cell, hemoglobin, and plasma protein concentration—under battlefield conditions—by measurement of specific gravity. His elegant yet precise and accurate analytical methods produced quantitative data that clarified the physiological and pathological states of humans.

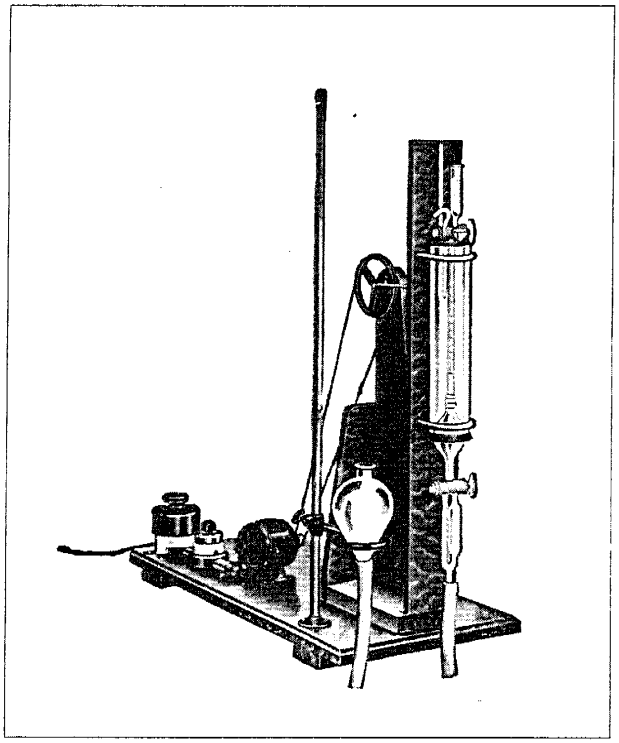

Figure 4. Van Slyke Volumetric Gas Analysis Apparatus with Water Jacket and Shaker

Van Slyke was as influential as a teacher in the hospital laboratory as Folin was in the medical school. Although Folin trained more professional biochemists, Van Slyke's wide-ranging investigations of disease states helped bridge the gap between biochemistry and internal medicine. A large number of the many individuals who passed through Van Slyke's laboratory went on to professorships of internal medicine or biochemistry or to other important posts in the US and abroad. Some of these were Vincent P. Dole, Franklin C. McLean, Christen Lundsgaard, John P. Peters, Michael Heidelberger, and A. Baird Hastings, who succeeded Folin in the chair of biochemistry at Harvard.

In 1914 the directors of the *Journal of Biological Chemistry* asked the Rockefeller Institute to take over its publication, with Van Slyke (age 31) to join the editorial board as managing editor. The nine-year-old *Journal* was the only publication in the US devoted solely to biochemistry. As such, it was to set the standards for publication of experimental data which would determine the direction and quality of biochemical research in this country. Toward the end of 1925 the ownership of the *Journal* and its management were transferred from the Rockefeller Institute to the American Society of Biological Chemists, and the editorial office was moved to Cornell University Medical College in New York City. Stanley R. Benedict (1884-1936) was appointed managing editor. Van Slyke, who remained on the editorial board until 1950, had also served as president of the society in 1921 and 1922.

Shortly after his retirement from the Rockefeller Institute in 1948, Van Slyke joined the newly formed Brookhaven National Laboratory of the Atomic Energy Commission in Upton, NY, as Assistant Director for Biology and Medicine. He remained at Brookhaven until his death at age 88.

Clinical chemists are especially indebted to Van Slyke for his collaboration with John P. Peters (1887-1955) of Yale University in the writing of the two-volume classic, *Quantitative Clinical Chemistry* (1931, 1932), which, for more than 30 years, was the authoritative source for clinical chemistry and even today re-

mains a valuable resource for the history of clinical chemical methods. A member of the National Academy of Sciences, Van Slyke was awarded the National Medal of Honor by President Lyndon B. Johnson in 1965.

REFERENCES AND NOTES

1. This article, being submitted on the occasion of the 50th anniversary of the American Association for Clinical Chemistry, is intended to inform the members of the ACS Division of History of Chemistry about the early foundations of this discipline.
2. A. Flexner, *Medical Education in the United States and Canada. A Report to the Carnegie Foundation for the Advancement of Teaching*. Bulletin No. 4, Merrymount Press, Boston, 1910, 101-102.
3. O. Folin, "Chemical Problems in Hospital Practice," *The Harvey Lectures*, **1907-1908**, *3*, 187-198; also in *J. Am. Med. Assoc.*, **1908**, *50*, 1391-1394.
4. S. Meites, *Otto Folin: America's First Clinical Biochemist*, American Association for Clinical Chemistry, Inc., Washington, DC, 1989; see also P. A. Shaffer, "Otto Folin, 1867-1934," *Biogr. Mem. Natl. Acad. Sci. USA*, **1952**, *27*, 47-82.
5. O. Folin, "Zur Kenntniss des sogenannten thierischen Gummis," Z. *Physiolog. Chem.*, **1897**, *23*, 347-362.
6. O. Folin, "Eine Vereinfachung der Hopkinsche Methode zur Bestimmung der Harnsäure im Harn," Z. *Physiolog. Chem.*, **1897**, *24*, 224-245.
7. O. Folin, "Die Hopkins'sche Harnsäure-Bestimmung," Z. *Physiolog. Chem.*, **1898**, *25*, 65.
8. O. Folin, "Ueber die Spaltungsprodukte der Eiweisskörper. Erste Mittheilung. Ueber einige Bestandtheile von Witte's Pepton," Z. *Physiolog. Chem.*, **1898**, *25*, 152-163.
9. R. H. Chittenden, *The Development of Physiological Chemistry in the United States*, The Chemical Catalog Co., Inc., New York, 1930, 15-18; 27-28; 33-37.
10. G. S. Hall, "Laboratory of the McLean Hospital, Somerville, Mass.," *Am. J. Insanity*, **1895**, *51*, 358-364.
11. O. Folin, "Some Metabolism Studies. With Special Reference to Mental Disorders," *Am. J. Insanity*, **1904**, *60*, 699-732.
12. M. Jaffe, "Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins," Z. *Physiolog. Chem.*, **1886**, *10*, 391-400.
13. A. Bolliger, "The Colorimetric Determination of Creatinine in Urine and Blood with 3,5-Dinitrobenzoic Acid," *Med. J. Aust.*, **1936**, *2*, 818-821; see also S. R. Benedict and J. A. Behre, "Some Applications of a New Color Reaction for Creatinine," *J. Biol. Chem.*, **1936**, *114*, 515-532.
14. O. Folin and W. Denis, "Nitrogenous Waste Products in the Blood in Nephritis. Their Significance and the Methods for Their Determination," *Boston Med. Surg. J.*, **1913**, *169*, 467-468.
15. O. Folin and W. Denis, "On the Creatinine and Creatine Content of Blood," *J. Biol. Chem.*, **1914**, *17*, 487-491; see also O. Folin, "On the Determination of Creatinine and Creatine in Blood, Milk and Tissues," *J. Biol. Chem.*, **1914**, *17*, 475-481.
16. O. Folin and H. Wu, "A System of Blood Analysis," *J. Biol. Chem.*, **1919**, *38*, 81-110.
17. O. Folin, Ref. 3.
18. D. D. Van Slyke, "Oral History," National Library of Medicine, Bethesda, MD, 1971, 6-8.
19. G. W. Corner, *A History of the Rockefeller Institute, 1901-1953. Origins and Growth*, The Rockefeller Institute Press, New York, 1964, 274. Folin, while still at McLean Hospital, had been approached in 1906 to join the Rockefeller Institute but accepted the offer from Harvard instead (p 73).
20. E. Fischer and D. D. Van Slyke, "Ueber einige Verwandlung der *alpha*-Pyrrol-carbonsäure," *Ber. Dtsch. Chem. Ges.*, **1911**, *44*, 3166-3177.
21. E. Abderhalden and D. D. Van Slyke, "Die Bestimmung des Aminostickstoffs in einigen Polypeptiden nach der Methode von Van Slyke," Z. *Physiolog. Chem.*, **1911**, *74*, 505-508.
22. Ref. 18, p 18; see also D. D. Van Slyke, "Acceptance of the Kober Medal Award," *Trans. Assoc. Am. Physicians*, **1942**, *57*, 42-43.
23. Ref. 18, p 19.
24. E. C. Dodds, "American Impressions," *Middlesex Hosp. J.*, **1932**, *32*, 49-62; see also R. E. Kohler, *From Medical Chemistry to Biochemistry. The Making of a Biomedical Discipline*, Cambridge University Press, New York, 1982, 242-243.
25. D. D. Van Slyke and G. E. Cullen, "Studies of Acidosis. I. The Bicarbonate Concentration of the Blood Plasma; its Significance, and its Determination as a Measure of Acidosis," *J. Biol. Chem.*, **1917**, *30*, 289-346; see also D. D. Van Slyke, "Studies of Acidosis. II. A Method for the Determination of Carbon Dioxide and Carbonates in Solution," *J. Biol. Chem.*, **1917**, *30*, 347-368.

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