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Otto Folin's Medical Legacy

Ed. note: This concludes a series of three articles on Folin, the last of which appeared in Clin Chem **29:***1852 (1983).*

At its July 1, 1898, convocation the University of Chicago finally conferred the doctoral degree on Otto Folin. Only a few days previously he had returned from Europe after an 18-month absence. There he had studied physiological chemistry in three avant-garde laboratories. In 1896 he had completed his doctoral dissertation in organic chemistry but had gone abroad prior to the convocation for two reasons. He wanted to take advantage of an immediate opportunity for training in the new discipline of biochemistry, the "wild and wooly west of science," as he described it, and there was no place to get it in the U.S. except with Professor Chittenden at the Sheffield Scientific School in New Haven. He also wanted to visit his family in Sweden, whom he had last seen in 1882 when, as a boy of 15, he had immigrated to Minnesota with the help of a brother already there. Folin had become a dedicated American citizen.

Young Folin returned to Chicago broke, deeply in debt, and unemployed. On top of this desperate situation, he was in love. At age 31, he was understandably eager to visit his fiancée, Laura Grant, of St. Paul. Even so, he was too mentally disciplined to visit her before he had found a job, a "footing" in Chicago (*1–4*).

There were no openings for biochemists in medical schools because at the turn of the century few of them offered courses in biochemistry. In this horse-and-buggy era of the Spanish-American War, practically all colleges and proprietary schools of medicine admitted students with little more than a high school education. Chemical training, therefore, was minimal or not required. Not until 1910 did the Flexner Report (*Medical Education in the United States and Canada*) appear and awaken the medical world into acting on its gross educational deficiencies (*5*).

In Europe, physiologists in medical schools had, almost singlehandedly, promoted biochemistry as a scion of physiology. Folin had studied there under three noted professors: Hammarsten (Uppsala), Salkowski (Berlin), and Kossel (Marburg). At the University of Chicago, the eminent physiologist Jacques Loeb had encouraged Folin's trip to Europe, and hoped, though futilely, to hire him upon his return.

Folin's doctoral thesis was on the group of organic nitrogen compounds known as the "urethenes," the esters of carbamic acid. This work led to his later interest in proteins and their catabolic products. It was in Salkowski's laboratory in the Pathological Institute of the Charity Hospital, University of Berlin, that Folin found the principal focus of his career. There he made his first contact with hospital patients and discovered the fascination of working with an end-product of nitrogen metabolism in urine. It was thanks to Salkowski's astute choice of subjects for that abbreviated summer of 1897 that Folin's initial contribution to clinical biochemistry was on analysis for uric acid, a subject that intrigued him for the rest of his life. One of his last two papers, published posthumously in 1934, was on uric acid. This continuity was typical of many other research themes that he would undertake.

After struggling financially for a year, first at work in a basement laboratory in the home of a pioneer gastroenterologist, Fenton B. Turck (1857–1932), and then in a private "medical" laboratory, he married. He then spent a year teaching chemistry at West Virginia University, in Morgantown. Folin's career as a clinical biochemist began with his appointment in 1900 as a research chemist to the venerable McLean Hospital for the Insane, in Waverley, MA.

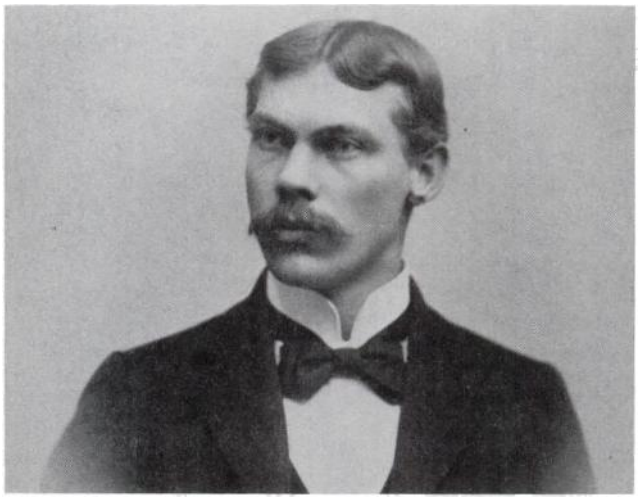

Fig. 1. Otto Folin in 1897, at Uppsala

The McLean Hospital is a division of the Massachusetts General Hospital. Privately run, it maintained about 180 patients. In hiring Folin, Superintendent Edward Cowles (1837–1919) was achieving the second step of a longstanding goal of his, to create a research laboratory comprising neuropathology, chemistry, and psychology. The laboratory of biochemistry that would be built for Otto Folin was, in effect, the first hospital clinical research or study center in the United States. The neuropathologist, August Hoch (1868–1919), was unquestionably instrumental in selecting patients for Folin's experiments and in paving the way clinically for his pioneering studies on urine.

From 1901 through 1907, Otto Folin made several extraordinary contributions to medicine and biochemistry that won him international recognition and opened the doors to

Fig. 2. Otto Folin using a colorimeter in his office, Building C-2, Harvard Medical School, *circa* 1932

modern clinical chemistry. He published a series of papers that brought fresh attention to the ancient practice of urinalysis. With the study of protein catabolism in mind, he began by "tooling up" through the introduction of new or modified analytical methods for ammonia, urea, uric acid, creatinine, creatine, and non-protein nitrogen (NPN); for total, ethereal, and inorganic sulfur; and for phosphate. These methods were not only the best then available for quantitative use, but were clinical, meaning that they were practicable for patients, rapidly performed, and required comparatively small sample volumes. Along with these sharpened methods he initiated colorimetry as early as 1902, using the Duboscq colorimeter to quantify the alkaline picrates of urinary creatinine and creatine. From that time on, he would steadily improve those methods, particularly of the nitrogen compounds, so that colorimetry and its facilitation of speedy volumetric analysis became a requisite feature of each of them.

Otto Folin, with the help of one assistant, next provided what were then the first reliable modern quantitative data on the chemical composition of urine. It should be noted that his early research was published in good part in the German *Zeitschrift für Physiologische Chemie,* simply because there was not yet an American counterpart. He would soon join a few others in establishing the *Journal of Biological Chemistry* (1905) and the American Society of Biological Chemists (1906).

Incidentally, Dr. Cowles' purpose in hiring Folin for the McLean Hospital was to determine whether research could uncover chemical differences between sanity and insanity. Folin soon found, however, that none of the numerous qualitative differences reported in the literature was valid. For this reason he turned to perfecting quantitative analysis as a possible means for detecting differences in degree rather than in kind. These studies were also not fruitful.

To study protein catabolism, Folin made use of two standardized *liquid* diets on his subjects, the patients, and colleagues at the hospital. One diet was considered balanced—at least in terms of fat, carbohydrate, and protein content (whole milk, cream, eggs, sugar, malted milk, salt, and water)—and was used in conjunction with determining the constant, "normal" urinary output of metabolites. The second diet was protein-free (low-nitrogen), consisting of arrowroot starch (carbohydrate), and cream (fat). Subjects were kept on these diets for a week or longer, and 24-h urine specimens were scrupulously collected. The specimens obtained after the first two days were then analyzed.

Folin found that the urea output varied with protein intake, whereas creatinine output was relatively constant. In the absence of protein intake there was a constant protein catabolism of about 20 grams per day, to which he applied the term "endogenous or tissue metabolism" to contrast with the "exogenous or intermediate metabolism" of dietary protein, terms that were used for at least three decades until the metabolic pool was elucidated with isotopes by Schoenheimer and his associates.

Folin wondered at the role of ammonia in the catabolism of protein to urea. As early as 1902 he attempted to measure NH_3 in animal blood. This was his first study in *blood* chemistry, a logical extension of his interests. He had also examined the urinary ketone bodies and measured the daily lipid output (total fat, neutral fat, fatty acid) in stools.

In this period there were few suppliers of chemicals and equipment in the United States. Folin literally had to create some of the tools for his work by purifying or synthesizing reagents, and by making some of his own equipment. His solid grounding in organic chemistry had prepared him well. For example, he could not go to the reagent shelf for "ACS-grade" creatinine. Instead he isolated it from urine, processed it to a high state of purity by solvent extraction, and verified it by nitrogen analysis. Creatine could then be prepared from the creatinine, and vice versa. A more unusual, but typical, example of Folin's ingenuity occurred a few years later. This was in response to the need for a practical container in which to collect 24-h urine specimens from women. To achieve this, he and an associate devised a divided bedpan and provided accompanying details for its shape, composition and cleaning. The division in the bedpan facilitated separate reception of urine and feces. The pan proved successful for use with both women and children (*6*).

Folin's attention was drawn to the organic acid components of urine when, in 1907, President Eliot, of Harvard University, asked him to become an associate professor and head the medical school's department of biological chemistry. He would be the first non-physician on the faculty. He accepted eagerly, although another attractive offer at the Rockefeller Institute had come his way.

Harvard brought new dimensions and challenges to Otto Folin's career. For 25 years he and his steadily growing staff taught biochemistry to first-year, second-semester medical students. He was a gifted teacher, in the forefront of his field, who never failed to prepare his lectures freshly and spiced them with humor. Once during a lecture on urinalysis the professor dipped a finger into a urine specimen and performed the ancient "taste test" for the presence of sugar. But the finger he put to his mouth was not the wet one. A couple of sharp-eyed students caught the deception. "We got even with him, however, because we put some white of egg in the urine specimen of one of our classmates. When the albumen was discovered on testing the urine, he was immediately rushed to the Brigham Hospital for all sorts of tests before the hoax was finally revealed" (*7*).

In the laboratory he preferred to work with small groups and individuals. He often chided the students without malice with phrases such as "with all the mistakes you've already made, this one is inconsequential," and with expressions to the effect that if "you botched it up here, what would you do to your patients?"

Within a few years, Professor Folin turned his department into an American mecca of biochemistry, graduate study, and research. He attracted to his department outstanding future biochemists, among whom the most notable were Bloor, Doisy, Fiske, Shaffer, SubbaRow, Sumner, and Wu. Space does not permit a description of their grand contributions to biochemistry and medicine. Not listed among them is the woman, Dr. Willey Denis (*8*), of New Orleans, who, along with Hsien Wu, collaborated with Otto Folin to produce the major research that launched modern clinical biochemistry in the U.S. The early work of Folin and Denis preceded that of Stanley R. Benedict and Donald D. Van Slyke, but once all three were active, the new discipline became solidly rooted.

After the Flexner Report, medical schools rapidly made biochemistry a part of their curriculum. In contrast to the situation in European medical schools, these newly created departments developed independently from physiology. In a few years the medical schools not only provided the leadership in the general area of biological chemistry, but also that in the special area of blood chemistry. Thanks to the pioneer work of Folin and a few others, this new tool for diagnosis, treatment, and monitoring moved into the hospital laboratory.

Sometime after the arrival of Dr. Denis, in 1910, Professor Folin began developing the analytical techniques for *blood* nitrogen compounds in a "tooling up" process, just as he had done for urine. His objective remained the same: to determine the products of protein catabolism. In the four-year

period of 1912 through 1915 he published 50 papers and a monograph! Not only were the quantitative methods adapted to blood and tissue analysis, but also he:

- introduced phosphotungstate for uric acid determination and phosphomolybdate for assaying phenolic compounds
- demonstrated that the large intestine was a major source of NH_3 in portal blood
- showed that amino acids were absorbed as such from the small and large intestines, and not, as commonly thought at that time, first synthesized into protein prior to absorption
- found that chemically induced nephritis in cats led to nitrogen retention
- proved that nitrogenous digestion products were absorbed directly from the stomach
- established "normal" values for NPN, urea, and uric acid in blood

With his new phosphomolybdate reagent, Folin measured the tyrosine content of purified proteins and vanillin in vanilla extract, and he introduced a colorimetric method for epinephrine in extracts. He measured the decrease in blood uric acid in gouty patients being treated with a uricosuric drug. He introduced two protein-precipitating agents: metaphosphoric acid for blood and sulfosalicylic acid for urine. He extended the use of the Duboscq colorimeter into turbidimetry and improved the methods for determining ketone bodies in urine.

Hsien Wu (1893–1959) studied for his doctorate with Folin from 1917 to 1920, and in the process the two of them pushed blood analysis deeper and irrevocably into the daily practice of medicine. They created a remarkably simplified "system" of blood analysis based on the use of a tungstic acid (protein-free) filtrate suitable for determining the nonprotein nitrogen-containing constituents, to which improvements were made. Wu added the remarkable blood-sugar method. These methods would become universally used "bread and butter" laboratory tests for the next 40 years.

Although Otto Folin extensively studied the metabolic fate of injected sugars and uric acid, in his later years he was content to exploit his "knack for methods" and his fondness for blood chemistry by polishing his techniques. In 1923, he and Stanley Benedict became codirectors of the biochemical laboratory of, and later consultants to, the Metropolitan Life Insurance Co. of New York. They helped the insurance examiners by improving the qualitative and semiquantitative testing of urine, particularly for protein and sugar, and by introducing chemical testing of blood to supplement urinalysis.

These highlights of Folin's contribution to medicine leave out his considerable influence in the teaching of biochemistry in the medical schools of the United States, his professional work for the association and journal of biochemistry, the direction he gave to so many of his younger colleagues, and the many awards he won. Otto Folin was a dedicated scientist, a memorable teacher, a devoted family man, and a friend of many—a "workaholic" with a sharp but inoffensive sense of humor, humble and laconic.

After Folin's death at age 67, on October 25, 1934, P. A. Levene wrote Mrs. Folin: "You can understand the depth of my shock for only last March we had a long visit, reminiscing and discussing future plans. Our aims, ambitions and careers were identical and for a long time, we were two of a very small group. The group has grown large in number but nobody has taken the place of Dr. Folin in my life. . ."

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