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# OTTO FOLIN

*1867—1934*

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*A Biographical Memoir by*

PHILLIP ANDERSON SHAFFER



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To honor Otto Folin on the twenty-fifth anniversary of his appointment as professor of biological chemistry in Harvard University, after two years as associate professor, a group of his colleagues early in 1934 arranged for the painting of his portrait by an artist of distinction and for its presentation at a gala dinner to be held in the fall to celebrate the event.

The death of Folin on October 25 changed that occasion to a memorial meeting held on November 23, at which addresses were made by several of his associates. The portrait by Pollak-Ottendorf was unveiled at that time and now hangs in the library of the Harvard Medical School. It shows in the background a simple instrument and two flasks, an emblem of the colorimetric methods Folin introduced in biochemistry and medicine. This portrait has come to be recognized by historians [1] as an emblem of progress in a field of which Folin was a pioneer. The portrait is reproduced with this memoir.

The address by Henry A. Christian, later printed in Science,[2] is a glowing tribute by a friend of many years and excerpts from it constitute a fitting introduction to this memoir.

"My part . . . is to speak of Dr. Folin from a dual point of view, that of an internist acknowledging the significant value to clinical medicine and surgery of his biochemical investigations, and that of a member of the medical faculty . . . paying a tribute to him as a stimulating teacher and leader, above all as a wise, helpful and beloved colleague.

"I speak with the authority of one who daily in my care of patients now for many years has utilized the methods that Dr. Folin perfected both for a better understanding of what ails sick humanity and as a guide to their therapeutic management; and of one who for twenty-seven years sat with him in faculty and committee meetings, somewhat bedeviled his peace and comfort when I used the laboratory over his head and above all in personal contacts learned to know the quality of his mind and the character of his personality.

"You, my younger colleagues and students, scarce can vision medicine without the methods of blood analysis perfected by Folin and his pupils and those inspired by Folin's own accomplishments, so completely have these micromethods of quantitative analysis become a factor integrated into the web and woof of the fabric of clinical medical and surgical lore.

"Not all of the methods have been the product of Folin's own hand or originated in Folin's laboratory, but it has been, however, from his own ingenious methods and the wisdom of his approach to important biochemical problems that has grown the whole range of the microchemical analyses of the blood and other body fluids which are daily in use in hundreds of hospitals and thousands of doctors' offices the world over. He was the recognized leader in this phase of clinical laboratory technique, and some of his own methods are perhaps always in use - - - (somewhere in the world).

"Folin is now a fine tradition in the Harvard Medical School, not alone to the faculty, but to the members of twenty-six classes of medical students - - -; his personality, his character, his widely critical attitude toward men and their investigations, his friendly helpfulness to others, the restraint of his spoken word not failing in clarity, his modesty, his sense of humor and other qualities have endeared him to us. Long will the memory of him remain a potent factor in our individual activities."

Tributes by other friends and associates will be cited as we attempt to unfold the growth of this modest, unassuming but determined personality who left warm and generous memories among those about him and whose research so interested and stimulated a number of his contemporaries that together he and they may be said to have inaugurated a new branch of biochemistry, now called quantitative clinical chemistry. A comment by one of his contemporaries whose interest in methods paralleled that of Folin and gave rise to many controversies between them and also to their friendship, is the following paragraph in a letter written by Stanley R. Benedict soon after Folin's death.

"One of the qualities which so impressed me in Folin, so rare among scientific workers, was the fact that he was able to drop out personalities when it came to a matter of difference of scientific opinion. I have known no one with whom it was possible to have such strenuous differences of opinion or view-

point in scientific work and have this not interfere one iota in the close personal friendship which lasted over more than twenty-five years."

Otto Knut Olof Folin was born on April 4, 1867, in the village of Åsheda, province of Småland, in southern Sweden. His ancestors were small landowners in that region. Of the eight children of his grandparents, Jonas and Maria Folin, landowners in comfortable circumstances, two were sons: Jonas became a minister and Nils Magnus, the father of Otto, was a tanner in the village of Åsheda.

About the year 1850 Nils Magnus Folin married Eva Olson, daughter of a small landowner, when she was sixteen years of age. Thirteen children were born to these parents; first twelve sons of whom Otto was the youngest, and last a daughter, Gertrud. Five of the sons died in infancy and three in early manhood. Only the eldest, Wilhelm, who died at the age of 18, and Otto had more than elementary schooling. Among their relatives were several ministers and a district judge, but only his mother seems to have been an inspiration to her youngest son.

It is said that the father, Nils Folin, had a good mind but was a poor manager and his business as a tanner did not prosper. Support of the family depended more and more upon the mother; fortunately she was a woman of ability and courage. Faced with the heavy toll of illness and death among her children she decided when still quite young to become a nurse. At intervals she attended three annual sessions in Gothenburg to take courses for licensure in nursing and midwifery—concerned meanwhile about the young children she had to leave at home. She was at that time quite poor and it took heroic courage to carry through her plans. . . . She became licensed as the official midwife of a large district, was interested in her work and was much liked in the community. In her country practice she found means of supporting her constantly increasing family. Because she was on twenty-four hour duty, the home life was somewhat casual, although Otto remembered that his mother at home was efficient

and cheerful. She had a wonderful constitution, a cheerful disposition and a wholesome philosophy of life which enabled her to carry on until her children were self-supporting. The boys lived at home and attended the local school until confirmed in the Lutheran Church at the age of fourteen and then were sent to a neighboring town to be apprenticed in various trades.

When it came the turn of her youngest son, the mother contrived to give him three years of schooling in the home of the Rev. Mr. Lange who conducted a private school for boys. There Otto was drilled in elementary mathematics and in German. Following his confirmation in the church he was apprenticed for only a few months. Then at the age of fifteen Otto was sent to the "great advantages of America," where two of his brothers and an aunt with her husband had already settled among groups of their countrymen in Minnesota.

The ticket for the voyage (in 1882) was provided by his brother Axel who lived at Stillwater, a lumber town on the St. Croix River. There our young immigrant began the struggle to support himself, working at the log-boom while dreaming of ways to continue his schooling and learning English. At that early age he appears to have acquired a settled determination to become an educated man and to seek a career of service in some field that might yield satisfaction and perhaps a measure of distinction in life. One discernible source of that ambition was the example of his mother.

To gain a knowledge of English more quickly young Otto soon went to a less-graded country school near the farm of his aunt and her husband with whom he lived and worked as a farm hand for a year or two. With that meager preparation he returned to Stillwater, living now and again with his brother while passing grade after grade in the grammar and high schools of that town. In six years he was able to meet the requirements of the combined eight-year course. During the school terms he found jobs in the town to provide board and sometimes lodging. His favorite job, then and later, seems to have been as night clerk in hotels, where he could study at night. Summers he spent in the harvest fields or on the St. Croix log

boom. In later years Axel told his niece, "Otto was never idle. He studied early and late . . . even while sawing logs he had a book open before him." At the age of 21 he graduated from high school in the class of 1888.

In the fall of that year Folin secured employment in Minneapolis which enabled him to enroll as a student at the University of Minnesota. Four years later he graduated in the class of 1892, receiving the B.S. degree. Evidence indicates that his scholastic record was good, that he "did more work in chemistry than most students in the scientific course" and "showed marked ability in English." In November 1890 he became a citizen of the United States. During his senior year he was an editor of the student paper "Ariel", and was known among fellow students as an advocate of democratic policies in student affairs.

When the University of Chicago opened in the autumn of 1892, Otto Folin was one of the Minnesota graduates of that year accepted for graduate studies. He chose chemistry as his major and physiology as his minor subject; a choice which may be regarded as a first indication of his intent to become a physiological chemist. He was fortunate in having gifted teachers to guide his basic preparation for a career in that subject; J. U. Neff was the professor of chemistry and Jacques Loeb the professor of physiology. Julius Stieglitz, then a junior member of Neff's staff, was Folin's faculty advisor and later directed his research for the dissertation.

During the first years of this period Folin's efforts were devoted to preliminary courses in several branches of chemistry and related subjects required of graduate candidates. About this time Stieglitz had undertaken with Lengfeld and others what proved to be a prolonged investigation of certain molecular rearrangements that occur when organic compounds containing halogen attached to nitrogen (brom-acet-amides) were treated with sodium methylate, yielding urethanes instead of the expected hydroxyl amines. The primary interest in the problem concerned the reaction mechanisms, a matter of theoretical chemistry. When Folin was ready to begin his research, the

topic assigned to him was a study of the products of this reaction with a series of such compounds. The data recorded in his thesis, "On Urethanes", published in the American Chemical Journal (1897), give evidence of extended experience in organic preparations, in the conduct of these reactions and analysis of the products, but include little consideration of the mechanisms involved or discussion of the conclusions that might be drawn.

The reason for these omissions in Folin's thesis became evident to this biographer on reading the memoir of Stieglitz written by W. A. Noyes for the National Academy of Sciences. Noyes cites (page 293) the research on this problem (with Lengfeld) as the "first work of importance done by Stieglitz at Chicago", and states that a logical concept of the mechanism of such reactions was possible only in the light of the idea of electronic bonds advanced by J. J. Thomson in 1897; and was first appreciated by Stieglitz (and Noyes independently) in 1901,—four years after Folin's thesis was written. So it appears that Folin had the not uncommon experience of drawing a thesis topic the implications of which he was unable to grasp because the approach to its theoretical aspects had not yet been discovered. Furthermore it may be admitted, as suggested by one of his friends who read a draft of this memoir, "theoretical chemistry was not Folin's dish."

After completing the writing of his thesis in August 1896 Folin went to Europe to begin his acquaintance with physiological chemistry, to which field he had decided to devote his life. The first year he spent in the laboratory of Olof Hammarsten at the University of Upsala, not far from the scene of his childhood. Here he examined the properties and composition of an hydrolysis product of a glyco-protein, mucin, from submaxillary glands, a so-called "animal gum." A short paper bearing this title, published in Hoppe-Seyler's Zeitschrift in 1897 was his first contribution to biochemistry.

During the summer of that year he worked in Salkowski's laboratory in Berlin, where he took up an analytical problem which was to hold his interest at intervals throughout his life,

and also be the subject of his last paper in 1934: "on the determination of uric acid."

The following year was devoted to studies in Kossel's laboratory at Marburg where he was again assigned an investigation of another hydrolysis product of protein present in "Witte's peptone." There he met A. P. Mathews but was a year ahead of Walter Jones and P. A. Levene, three of his American contemporaries who studied in Kossel's laboratory about that time. Hilding Berglund, a fellow countryman of Folin and later a co-worker in his laboratory at Harvard, refers to this period in the following paragraph of an obituary article on Folin published in a Swedish journal.[3]

"During his stay in Marburg, Folin started getting eager. He had applied his knowledge of organic chemistry to biological problems and used his German in thorough discussions on long Sunday hikes with other young doctors from Kossel's laboratory. His interests in the intermediary stages of protein metabolism were started here, and he never quite lost sight of this problem, although it was somewhat in the background due to other interests during the last ten years. There he also discovered a new practise in colorimetry used in the brewing industry, which was not without connection with the appearance of his first colorimetric method, that on the determination of creatinine."

Three papers reporting the data from these laboratory studies in Europe appeared promptly in Hoppe-Seyler's Zeitschrift; and he returned to Chicago, where he was awarded the Ph.D. degree in 1898.

Ready and anxious to begin an academic career in physiological chemistry, there seemed to be no opening for him. That subject was not then represented at the University of Chicago; in only a few universities and medical schools in this country had the need for its cultivation been recognized—usually by its assignment to instructors in physiology or pharmacology or medical chemistry. Only Yale had a department of physiological chemistry, established in 1882 by Chittenden. So Folin accepted employment as chemist in a commercial laboratory, to await a different kind of opportunity which arrived a year later.

It is appropriate at this point to note two durable personal associations Folin formed at Chicago and to record the memories of one of his friends from that period.

Laura Churchill Grant, a young girl of Canadian parentage whose family lived in St. Paul, Minnesota, after graduation at Vassar College entered the University of Chicago as a graduate student in mathematics and economics. She received the M.A. degree in economics in 1896. She and Folin were married three years later.

Dr. Leo Loeb was a friend of the Folins from their days in Chicago and on request has written me his memories of that relationship. The following excerpts portray Folin's personality.

"When I think of these many years during which we were friends, there are certain points that impressed me.

"Foremost perhaps was his imperturbable equanimity. He always showed an even temperament: I have never seen him discouraged. He tended a little towards the optimistic side in all his attitudes and judgments. He was rather quiet, somewhat slow and deliberate in speech. He was thoughtful and thorough in sizing up situations, and attempted to see various aspects of a question; he wanted to be just. I never saw him excited or wrought up. He looked at persons and events in a quiet, friendly, rather hopeful way. He saw the little conflicts which constitute so great a part of life, in particular of social life, the tricks played by one on the other in the pursuit of the competitive struggle. Yet he accepted it all with a kindly, humorous spirit, without bitterness, without sarcasm. To a certain extent he even extracted some enjoyment out of these games, which he watched, without exactly participating in them.

"His thoughts were essentially practical and realistic, but at the same time he was interested in the general principles underlying the surface appearances. He was very appreciative of good work of others and very encouraging in the expression of his appreciation; I had occasion to experience it in my own case. Yet he could be critical. And he did not hestitate to express his criticisms. So far as political-social questions are concerned, he was a liberal and a democrat, but not a radical; he was essentially independent in all he did and thought; but he always sympathized with those who were underprivileged and he wished that their condition might be improved by legitimate means.

"He was a devoted husband and father: he was physically strong and tried to take all possible burdens from his wife who lacked his physical strength and power of resistance.

"On the whole, he had a happy life and he was appreciative of what men and life had given him; and others appreciated him. He accomplished much in his life work as a teacher and investigator."

In the summer of 1899, Folin was offered an assistant professorship of chemistry at West Virginia University. With this position in sight, he and Laura Grant were married on September 11, 1899 and moved to Morgantown, West Virginia. In that small institution, perched on a hill overlooking the Monongahela River, Folin gave during one year a course in quantitative analysis and another in elementary physiological chemistry. The writer of this memoir had the good fortune to be a student in both courses. The text used in the second course, the first Dr. Folin had given, was a laboratory manual by F. G. Novy; Hammarsten's book (translated by Mandel) was used "for reference." Many of the exercises in Novy's volume were omitted; the margins of my copy bear notes "no microscope," "apparatus not available"; but the omissions were more than compensated by the spirit of inquiry aroused in several of the students who voluntarily worked overtime on small tasks they were encouraged to think of as "research problems."

In the spring of 1900 Folin was offered a new position as research biochemist at the McLean Hospital in the suburbs of Boston. Learning that he might bring an assistant he offered the writer that post, an appointment held for three memorable and profitable years, the memories of which will color the following account of the beginning of Folin's career at McLean.

Dr. Edward Cowles, Medical Superintendent of McLean Hospital (for the Insane) at Waverley, Massachusetts, decided to establish a research laboratory for physiological chemistry, hoping that studies by that approach might in time contribute to better understanding of mental diseases. Folin was invited to plan, equip and conduct that laboratory and to formulate his own program. He accepted promptly because he sensed it would provide opportunities to carry out the kind of studies

he thought would interest him most and which he felt qualified to undertake. That hope and expectation were realized; furthermore the environment and facilities were exceptionally fortunate and agreeable.

At that time physiological chemistry was a novelty in Boston. Soon after his arrival at McLean in October 1900, Folin went to the libraries in Cambridge and Boston, and to consult a few friends and new acquaintances especially at the Harvard Medical School on Boylston Street. Frequently his young assistant accompanied him. One of these early visits was to A. P. Mathews, then assistant to Professor Bowditch. In search of a volume located in the pathology library, Mathews took his visitors to call on Dr. Councilman. When Folin was introduced as the physiological chemist at McLean Hospital, Dr. Councilman asked in his brusque manner, "So, do you know Pfaff?" Folin had to admit that he had not yet made the acquaintance of Dr. Pfaff, a reply that dampened and almost closed the interview. For Pfaff since 1898 had been the instructor in both pharmacology and physiological chemistry at the Harvard Medical School, with a laboratory at the Massachusetts General Hospital. He was perhaps the only avowed representative of either of these subjects around Boston at the time of this incident. Four or five years later Alsberg (in 1904) and L. J. Henderson (in 1905) were appointed instructors in biochemistry at the Harvard Medical School.[4]

During Folin's first months at McLean while the laboratory tables were being installed and the apparatus secured—most of it imported from Germany—the "chief's" time was devoted to reading about metabolism, the chemical composition of urine, and the claims (mainly in French medical journals) of the presence in urine of insane patients of toxic substances thought to be concerned with their mental states. The first experiment undertaken, as the writer recalls, was an attempt to test the toxicity of normal and other urines, and of their known constituents separately by injection in rabbits. The only marked toxicity observed was that due to potassium and ammonium salts, effects already known. Folin was skeptical of the idea then harbored

by some French writers and by Halliburton and Mott in England, that "toxins" perhaps related to choline or other nitrogenous bases derived from nervous tissue could be a factor in mental disturbance; yet that idea was probably in Dr. Cowles' mind in creating the chemical laboratory, and the possibility was not wholly rejected by Folin. Finding no evidence for it in the preliminary experiments Folin gave up that approach.

He decided instead to study the protein metabolism of normal vs. mentally disturbed individuals by measuring as accurately and as completely as possible all of the known nitrogenous and other products excreted in the urine, hoping thereby first to learn the normal range of variation in the partition of the total nitrogen among the known products and residual fraction and then to consider possible abnormal variations. Although the primary purpose was ostensibly a search for abnormal features, they were presumably unrecognizable except by contrast with the normal patterns which were then unknown. To establish norms would alone be an important undertaking and progress could in any case be made toward that.

The first essential would be to devise more and better quantitative methods before any worthwhile surveys could be started. These were the considerations that led to Folin's interest in devising suitable quantitative methods for urine and blood analysis, an interest which held his attention for the rest of his life. The methods he developed enabled Folin, and following him, many others with even better methods to explore normal and abnormal features of metabolism with consequences not then foreseen.

In the only autobiographic note left by Folin he describes the sequence of his work in a simpler way. Among old papers found in his desk at the medical school after his death was the following signed document in his own handwriting (dated April 9, 1924), a copy of which was sent to the writer by Professor Harry C. Trimble.

"When I was appointed chemist to the McLean Hospital in 1900 it became my duty to do chemical research on problems bearing on mental diseases. As the pathologist wanted all the brain material I took to the field of metabolism.

"It was hopeless to try to find deviations from the normal in the metabolism of the insane without far more exact knowledge of the human waste products than was available. My immediate and comprehensive problem became, therefore, the chemistry of urine. I realized that by thus interpreting my duty to the hospital I could do work of more general interest. I probably also followed my taste, for I enjoyed the mere puzzle aspect which is always present when one tries to devise a new method.

"My papers on the Laws Governing the Chemical Composition of Urine and a Theory of Protein Metabolism (1904) will probably be considered my best; but the data for those papers came easily and naturally by the help of the new methods for the determination of urea, ammonia and creatinine which I had devised during the preceding three years.

"My later studies—Protein Metabolism from the Standpoint of Blood and Tissue Analysis—in the main represent attempts to pursue experimentally theoretical concepts which I had developed on the basis of urine analysis. My elucidation of amino acid absorption does not stand out well, partly because it was presented in the form of a series of short papers, and partly because Van Slyke soon came into the field with a method of his own. My "best work" in the field of blood and tissue analysis, aside from the methods developed, should be the work on uric acid, now in press. It will be the starting point for much new metabolism literature."

The first years at McLean were mainly devoted to devising and testing methods for determinations of urea, ammonia, uric acid, creatinine and creatin, sulfates and urine acidity. Each of course was a different problem presenting difficulties not at once fully recognized, requiring change and improvement as experience in their use revealed the defects. The first of Folin's colorimetric methods was that for creatinine, the application of a color reaction of that substance with picric acid noted by Jaffé many years earlier. Although other color reactions had been used long before to estimate biological products, such as Nessler's reagent for ammonia in water analysis, Folin's method for creatinine, using a more delicate and precise instrument for color comparison—the Duboscq colorimeter pictured in his portrait, is commonly regarded as the introduction of colorimetry into modern biochemical procedures. This method for creatinine

and his later similar adaptation of the Nessler reaction for ammonia in blood and urine are still standard procedures.

At intervals some fourteen papers describing methods he devised during this period were published.

In the meantime the methods were used in studies of metabolism of normal individuals and selected hospital patients, each on uniform diets of known composition; the data being the amounts of the above metabolic products excreted in the urine, the complete collection of which was carefully guarded.

At this point the investigations were interrupted temporarily by Folin's first illness. In June 1903 he entered the Massachusetts General Hospital where Dr. S. J. Mixter removed a tumor of the left parotid gland and found it necessary to cut the facial nerve, altering Folin's appearance permanently. His concern was greatly moderated by the opinions of pathologists, including especially that of Dr. Leo Loeb, that the tumor was benign. There was no recurrence, and until late in life Folin's health was good.

In 1904 a long paper giving results of the metabolism experiments appeared in two installments in the American Journal of Insanity (to please Dr. Cowles)—where it reposed almost unnoticed, perhaps because of its forbidding contents. It recites at length reviews of literature, plan and methods with 67 tables of data, followed by cautious though speculative interpretations largely negative as regards relation to mental states. The summary states that "From a constructive, positive point of view it must be admitted that (the experiments) teach very little that is tangible concerning mental diseases, except for the suggestion—that general paralysis may be associated—with some demonstrable metabolism disorders." (An example of the exception had been reported in a paper, "On Phosphate Metabolism" in the American Journal of Physiology in 1902.) This statement brought to a close Folin's attempt to discover metabolic evidence related to mental states. But in the course of this study he had secured methods and a plan of experiments that promised significant results of more general physiological

interest, as he explains in the last paragraph of the summary of this paper.

"From a general physiological point of view . . . the data should have considerable value . . . to throw light on the laws of the normal secretion of urine. . . . In the continuation of this work I expect to use the diet (here) described and in addition another—low-nitrogen diet—which I believe will be more adapted to bring out any metabolism characteristic that may occur . . ."

The next papers in 1905 were those Folin referred to as perhaps his "best." They are still cited in texts and monographs as the "classic work of Folin" on principles of intermediary metabolism; and as he wrote "my later studies represent attempts to pursue experimentally concepts which I had developed on the basis of urine analysis."

The genesis of the data and arguments presented in these papers, as explained in a footnote in the second one, is worth recording here.

"For the opportunity to examine the urines given in table 1 (the starting point of all subsequent experiments recorded in this paper), I am indebted, on the one hand to Professor Bowditch, and on the other hand, to Dr. Ernest van Someren of Venice. Professor Bowditch kindly brought Dr. van Someren on a visit to this laboratory, and the latter while here consented to remain the guest of the McLean Hospital long enough to permit the collection of a series of consecutive twenty-four-hour quantities of urine. Dr. van Someren is known to many readers of this journal through his close associations with Mr. Horace Fletcher, a popular writer on the value of the thorough mastication of all kinds of food."

As Horace Fletcher stimulated Chittenden to undertake one of his well known researches, so did van Someren furnish the clue that gave added emphasis and new direction to the development of Folin's investigations. The level of van Someren's protein metabolism was found by Folin to be about a third of what was then considered "normal," a fact which led him to study the influence of low-protein diets on the urinary products of other normal subjects.

From the data on normal—high and low—protein diets

Folin deduced the "laws" governing the composition of urine and on these he built his "theory" of protein metabolism. His data showed a clear distinction between those products in urine independent of the amount of ingested protein (creatinine) and those dependent upon it (urea, etc.), a distinction between "endogenous" and "exogenous" metabolism, a conception which became generally accepted and was a guiding principle for several decades.

The prestige resulting from these papers, together with the spreading popularity of his methods of chemical analysis, doubtless led to the selection of Folin for appointment in 1907 as associate professor of biological chemistry and in 1909 as Hamilton Kuhn Professor at Harvard.

Before surveying briefly the continuation of Folin's researches at Harvard, it may be of interest to glimpse the environment of the laboratory when he entered it. One of his first graduate students and later assistant professor on his staff, Walter R. Bloor, has written his memories of that period at this author's request. The following quotations are excerpts from Dr. Bloor's letter.

"I came to Harvard primarily because I had heard of Folin as that new brand of chemist, the biological variety, and my training seemed to steer me into biological chemistry. Coming to Harvard Medical School I found the department in charge of Alsberg and Henderson. They said that a new man, Folin, had just been added to the staff, that he was upstairs and interested in research—a term which sounded very large and out of reach of my limited background. However, Alsberg took me up and introduced me and I was asked to come in anytime I felt like it. Alsberg got me going on preparations and Henderson tried to interest me in pH. I felt that I would like to know something about this research business, so I came to talk a good deal with Folin. Before long I was fortunately made first assistant.

"Folin was much interested in the medical students' laboratory and spent the whole time there when the class was in session. He did not circulate a lot but took one student or a group and worked intensively with them, doing a piece of research with the exercise they were working on. There were some distinguished students in those first groups; George Minot, W. W. Palmer, F. R. Rackemann. Joslin was around often. Henderson continued to lecture to students. He and Folin couldn't

agree on urine acidity; Folin was for titration as the best measure and Henderson was for pH. Dr. Emerson, a pediatrician, often came to work with Folin on fat in feces, and was enthusiastic about cabbage juice for babies—before vitamins were thought of. Joseph Pratt worked in Christian's laboratory and came to see Folin often. Langley Porter and another clinician were working in the laboratory.

"When I returned to Harvard (1914) there were a good many graduate students; Sumner, Doisy, McElroy, Youngbird, Pettibone. Fisk had graduated in medicine, was then an assistant. Rappleye, then a medical student, did a piece of original work, mostly biochemical, every year in school. Richard Bell and Richard Lyman (both physicians) each paid the other his salary and were active workers in the laboratory. Bell worked with Doisy and Briggs. (Lyman was a joint author with Folin in a number of papers of clinical interest). Folin used to bake the family bread on Saturday night and Sunday morning. His lunch was rolls and butter with coffee he boiled on the Kjeldahl still."

Besides those named by Bloor, others who were close associates of Folin in his own researches were W. Denis in the series of papers on "protein metabolism from the standpoint of blood and tissue analysis," H. Wu on "system of blood analysis," H. Berglund, A. Svedberg, Trimble, Marenzi and Malmros in later years.

One of the major contributions by Folin was to foster and encourage an interest in biochemical research among all who came within his range of personal contact: medical students, graduates, physicians from far and near, and visitors who came for his counsel. Among those named above three are Nobel laureates, and others have attained distinction comparable to his own. That is not to say that Folin's influence is discernible in their accomplishments; he expected individuals to grow of themselves and made no attempt to train them.

Of the many papers listed in Folin's bibliography most of them are short descriptions of analytical methods and their revisions, to which he devoted much of his time, probably because he "enjoyed the puzzle aspect." Also he had to defend and improve his inventions, for he soon had many competitors. It would serve no purpose now, at a much more advanced stage

of this methodology, to consider Folin's procedures for urine and blood analysis, except to note again his preference for measurements by colorimetry which he adapted successfully for many different substances. With improvements a number of his colorimetric methods are still in wide use. Their popularity is attested by the demand for repeated editions of the laboratory manual he published in 1916, the fifth edition of which was published in 1934. This volume also set the pattern for many mimeographed laboratory directions in medical schools and hospital laboratories everywhere.

Apart from his methods Folin developed concepts of intermediary metabolism already referred to as his theory of protein metabolism, based first merely on urine analysis. In 1912 he turned to a study of these problems by quantitative analysis of blood and tissues. It had been shown long before that food protein is digested in the intestine rather completely to amino acids, yet by ordinary methods no considerable concentrations of these products were to be found in portal blood. In consequence it had been supposed that the amino acids were during absorption resynthesized to protein by the intestinal mucosa. Folin devised a simple method to determine fairly accurately the non-protein nitrogen of the blood, including urea and all other nitrogenous substances of small molecular weight such as free amino acids, ammonia, etc. It was this method, referred to as "blood n.p.n.," that was at once taken up by clinicians to measure the adequacy of kidney function.

With this method he was one of several to demonstrate that the free amino acids liberated from food protein in the intestine *are* present in portal and systemic blood, thereby disposing of the idea that resynthesis occurs in the mucosa. Next he undertook to follow the fate of absorbed amino acids when absorbed from blood by tissues.

With it Folin and Denis began a series of experiments to trace the fate of urea, and individual amino acids injected into the small intestine or intravenously in animals. About the same time D. D. Van Slyke, as Folin states in his brief autobiographic note quoted above, undertook similar experiments

which led to different conclusions. Folin concluded that all tissues absorb and deaminate the amino acids while Van Slyke believed from his data that the liver plays the dominant role in the deamination and conversion of the ammonia formed into urea. This issue was decided by Mann's results with animals after extirpation of the liver, when urea formation ceases. This conclusion was later confirmed and the enzymic mechanisms were discovered by which the liver performs this reaction.

In the course of this work Folin had hit upon phospho-tungstic-phospho molybdic acid complexes as sensitive color-producing reagents, applicable to a number of substances, phenols, tyrosin, uric acid, glucose, etc., under appropriate conditions. Also he found that tungstic acid was a simple and effective way to precipitate completely at about neutral reaction all of the proteins of blood without adsorbing the non-protein constituents. These tools were the basis for the "system of blood analysis" developed from 1920 on with the collaboration of Hsien Wu. In tungstic acid filtrates, sugar, non-protein-nitrogen, urea, uric acid, creatinine and creatin, amino acids, chlorides and other substances could be determined by the sort of micro-methods Folin was an expert in devising. As Berglund states in his appreciative article about Folin, "This is the work that made Folin's contribution to medicine so important. No hospital laboratory today is unaffected by Folin's work."

Many others had by then joined in improving and extending accurate and sensitive micromethods for blood and tissue analysis and their wide application in both experimental studies and in routine clinical diagnosis and therapy. In this development Folin and a number of his co-workers continued to participate.

So rapid was the growth of information from quantitative clinical chemistry and its spreading use in biochemistry, physiology and medicine, that special treatises began to appear, special courses of instruction were provided and even special professions of practitioners of clinical chemistry had arisen and become organized. The monograph of Peters and Van Slyke, published in 1931, second edition 1947; references in texts to "the pioneer work of Folin, Benedict, Van Slyke, Myers and others (who)

initiated the present phase of quantitative clinical chemistry," (Hawk, Oser, Summerson, 1947); and the tribute paid at the organization meeting (1949) of the American Association of Clinical Chemists, "it was largely the result of the pioneer work of Benedict, Bloor, Folin, Van Slyke, and others, beginning at the turn of this century, that the science of clinical chemistry evolved as we know it," attest the influence of Folin's early work.

Let us pause here to relate incidents reflecting other aspects of Folin's life during his later years.

It is not surprising that he retained fond memories of his boyhood in Sweden and high regard for the land of his birth. After his return to America in 1898, he did not revisit Europe until the summer of 1913, when he spent a short time in England, gave a paper at the International Congress of Medicine (published in abstract in *Lancet*), visited his relatives in Sweden and attended the International Physiological Congress held that year in Groningen.

In 1918 the University of Lund, Sweden, awarded him an honorary M.D. degree, and shortly thereafter tendered him a professorship. Mrs. Folin tells that "this honor touched his feelings more deeply than any other which he received." She has described also their last visit together in 1932.

"His health was definitely failing but he wanted to see more of Sweden than he had been able to earlier. Traveling was difficult for him because he suffered great discomfort in his feet, due to his circulatory condition. Dr. Andrea Andreen-Svedberg kindly secured a good car for us, and our daughter, Teresa, who had just graduated from Johns Hopkins Medical School, had time to drive her father and me. In this way we saw in a leisurely manner a great deal of southern Sweden and visited Otto's sister Gertrud in Traryd and his brother Gustaf and family in Almhult. We also went into Norway and Denmark. Dr. Svedberg, Dr. Malmros, Professor Liljestrand, Professor Berglund and many other friends made our stay in Sweden a very happy experience."

Some of Folin's personal characteristics and tastes have already been touched upon. One was his liking for golf, a game

which he and the writer first tried to teach one another on the course at McLean. There neither succeeded; but after Folin went to Harvard and moved his home from Avon Street, Cambridge to 133 Buckminster Road, Brookline, he became a member of the Chestnut Hill Golf Club and soon became an ardent and fairly good golfer, able to coach his son to become an expert. Over many years golf was a hobby and diversion, doubtless of great benefit in keeping him physically fit. He also credited to one of his cronies in golf his discovery of "permutit" for the extraction of ammonia from urine, one of his clever methods.

No one of his associates knew Folin better at close range in the later period of his life than Dr. Harry C. Trimble. From the article[5] he wrote in memory of his chief we quote the following paragraph.

"To those who had the privilege of personal association with Folin he was always a modest, friendly and unassuming leader. Finding his chief delights in his family circle, his department at Harvard Medical School, and in the game of golf at which he excelled, Folin's daily routine was simple and regular. Every morning of the school year he was early at his laboratory. There the young and struggling worker in biochemistry, whether from his own or other departments, or other universities, would always find the door unlatched. To all who entered, sympathetic, judicial discussion and encouraging counsel were available. A quaint and always kindly humor made of every interview an occasion. Tall, erect and spare of frame, possessed of a simple, innate dignity, he was always a striking figure, whether in his short white laboratory coat, in the lecture room, or at public meetings of scientific societies."

Improvement of techniques remained Folin's pride and joy; creatinine and uric acid held his interest to the end. His last paper sent to the *Journal of Biological Chemistry* on June 7, 1934 describes a "Simple process for preparation of a correct uric acid reagent (and some comments)." Its concluding comments are prophetic: "This is presumably my last paper on the preparation of the uric acid reagent and I hope that the method for the determination of uric acid described last year is also final." At the end he adds, "the determination of uric acid represents probably the most complex reaction that we have in

the whole field of practical colorimetry." He knew that he had not finished that problem; but he gave it his first and last efforts.

A touching glimpse of Otto Folin in his last days is the following characteristic letter written a few days before his death. The plan for a dinner in his honor, mentioned in the opening paragraph of this sketch, included an invitation to this writer to be a speaker about Folin as a biochemist. I wrote him a note to say I had gladly accepted the invitation and to ask suggestions about the tone and content of my remarks—on what I knew would be for him a painful occasion. The reply is written in a firm hand and in his normal style, betraying no evidence of the physical weakness he must have felt.

October 21, 1934.

Dear Shaffer,

Your nice little note came yesterday. First of all I must tell you that I have been and still am in more or less trouble. We left suddenly our summer place and Mrs. Folin took me straight to the Brigham Hospital about September first. I stayed there for ten days and since that time I have been confined to the house, most of the time in bed. I have a nasty combination of cystitis and orchitis. It is not painful and I have no temperature any more but I am supposed to rest and to drink so much water as to make it impractical to go to the school. It is getting to be very tedious and I don't believe now that I shall be in good order even by November 16.

Cannon came in and told me the other day about the arrangements for that date. He said that Christian would speak about my contributions to medicine and yourself about my contributions to biochemistry. I am rather sorry for both of you as well as for myself.

A light tone would be desirable and, of course, would be less trying on me, but I shouldn't overdo it before an audience of New Englanders. I should think that a concise sketch of the practical biochemistry as it was when you and I began our work at McLean is called for since that condition served to give a trend to my work on methods. My portrait includes a colorimeter and a couple of volumetric flasks, and it might therefore fit in pretty well to say something about the introduction of colorimetry into biochemistry. This is about all I can think

of at the moment, but if I think of anything else perhaps I will write you again. I wish it were over!

It was extremely nice and friendly of you to take on this work for your old friend; there is no one else that would be anywhere nearly as acceptable to me.

With kindest regards to Mrs. S.

Sincerely,
Otto Folin

Four days later he died of a heart attack.

"There is an intimate and touching resemblance between the countryside in Smaland around Malilla, and a little town, Kearsage, in a corner of New Hampshire, where Folin had his summer home and where his place of burial is. A resting place has been found in the stony field among the tall pines for one who was an outstanding scientist, a grave and upright man, beloved by everybody who came in contact with him." (Berglund.)

Otto and Laura Folin had three chillren. Joanna, who died in 1912; Grant, now a businessman in Detroit; and Teresa, M.D. Johns Hopkins, now married to Dr. Jonathan E. Rhoads, Associate Professor of Surgery at the University of Pennsylvania.

Though Otto Folin lived to see only one grandchild, there are at present (1949) nine, the youngest of whom bears the name Otto Folin.

A little known activity of Folin was his connection with the Metropolitan Life Insurance Company, related to the writer by Mrs. Folin. Folin and S. R. Benedict were appointed members of a committee to draw up a plan to reorganize the biochemical laboratory; shortly thereafter Folin was asked to become Director of that Laboratory. Although the offer was very advantageous financially he did not accept because he preferred to remain at Harvard. But he and Benedict shared responsibility for its conduct until Dr. Blatherwick became the director in 1928, when Folin and Benedict were given the title "Consulting Biochemists," an appointment Folin held until his death. At that time Dr. A. S. Knight was the Medical Director of this company. The writer suspects that this influence of Folin and Benedict may have been one of the seeds that later bore fruit

in the general support of medical research by a number of insurance companies.

Folin was an active contributor to the *Journal of Biological Chemistry* in which most of his papers were published and of which he was one of the collaborators from its first volume, issued in 1905. He was a charter member of the American Society of Biological Chemists founded in 1906 and in 1909 was its third president. A regular attendant at its meetings, often enlivened by his discussions and quiet humor, he was greatly respected by its members. When the Journal was transferred to the Society in 1919, Folin was elected to its Editorial Committee, of which he was chairman for many years. A brief obituary appeared in the Journal and a resolution in his memory was recorded by the Society.

He was a member also of the Physiological Society, the Society for Pharmacology and Experimental Therapeutics, American Chemical Society, and Society for Experimental Biology and Medicine. He was elected a member of the National Academy of Sciences in 1916. In 1932 he was made a member of Kaiserlich Deutche Akademie der Naturforscher.

He held the following honorary degrees: Sc.D. Washington University, 1915; Sc.D. University of Chicago, 1916; M.D. University of Lund, Sweden, 1918. Among the honors conferred on Folin the following tribute from the minutes of the Faculty of Medicine, Harvard University, December 7, 1934 should be included.

"We, members of the Faculty of Medicine of Harvard University, in meeting assembled, do herewith record our sorrow at the death of our colleague, Otto Folin, Hamilton Kuhn Professor of Biological Chemistry.

"Since 1907 Professor Folin has been a member of this Faculty, sharing in its deliberations and contributing to its discussions with the wisdom of a well-trained mind. Quietly, forcibly, with clarity of expression and touch of humor, was it his custom to share with us his opinions on matters under consideration by this Faculty; his words always carried weight; we valued his counsels; we knew the unselfishness of the man who spoke and the inherent modesty of one who never sought self-aggrandizement.

"Professor Folin, himself a recognized authority in his chosen field of biochemical investigation, was the ideal leader of an important department in this Medical School. Here he taught to class after class of medical students the facts and methods of a science that is of great importance to medicine and to them he gave the example of accuracy in methods of obtaining data to be used in the study and care of patients. To him came graduate pupils to be trained in investigation that they, in turn, might become teachers of students and seekers after greater knowledge of the chemical relationships of health and disease in man and animals. His pupils spread widely his influence into many laboratories for the betterment of mankind.

"We, his colleagues, pay tribute to the quality of the man and the significant importance of his contributions to biochemistry, particularly the development and application of methods of micro-analysis of body fluids and tissues. We recognize his leadership. We revere the memory of our colleague. Long will his influence be felt in the Harvard Medical School.

"What the Medical School is today is a mosaic of the lives of those who in the years since its founding have been of it; all of the brilliantly colored bits that make up this picture are attributes of the work and character of the men who have been members of its Faculty. Professor Folin has added gold and purple to the mosaic that we cherish.

"To Professor Folin's wife and children we tender our deep-felt sympathy in their sadness and sorrow."

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Professor H. C. Trimble provided a copy of a translation of Professor Berglund's article by Dr. Muus; a copy of the autobiographic manuscript by Otto Folin quoted in the text; and gave much other assistance including a careful preparation of Folin's bibliography.

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## KEY TO ABBREVIATIONS USED IN BIBLIOGRAPHY

Amer. Jour. Insan. = American Journal of Insanity
Amer. Jour. Physiol. = American Journal of Physiology
Arch. Int. Med. = Archives of Internal Medicine
Biochem. Jour. = Biochemical Journal
British Med. Jour. = British Medical Journal
Cleveland Med. Jour. = Cleveland Medical Journal
Jour. Amer. Chem. Soc. = Journal of the American Chemical Society
Jour. Amer. Med. Assoc. = Journal of the American Medical Association
Jour. Biol. Chem. = Journal of Biological Chemistry
Jour. Exper. Med. = Journal of Experimental Medicine
Jour. Ind. Eng. Chem. = Journal of Industrial and Engineering Chemistry
Jour. Phar. = Journal of Pharmacy
New Eng. Med. Jour. = New England Medical Journal
N. Y. Med. Jour. = New York Medical Journal
Physiol. Rev. = Physiological Reviews
Zeit. Physiol. Chem. = Hoppe-Seyler's Zeitschrift für Physiologische Chemie

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