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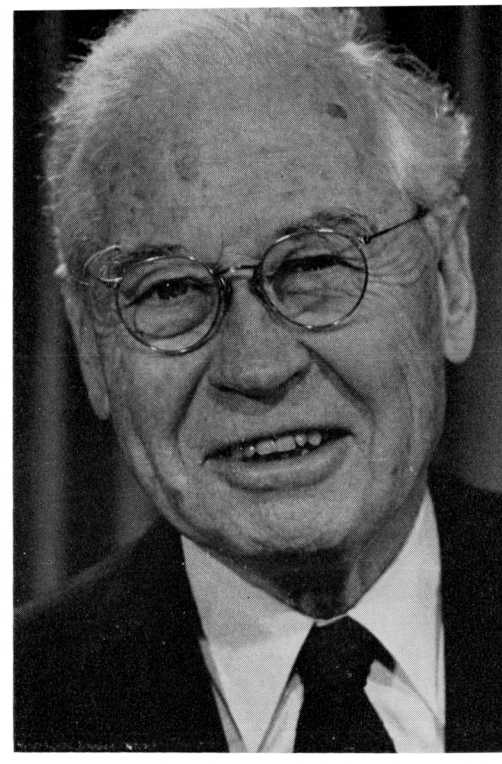
The Clinical-Chemical Interface of Medical Science: Its Development in this Century*



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When I seriously sat down to prepare my remarks to you this evening, I was aghast to see the title that I had given Dr. Sunderman. Who am I to presume to speak on the "Development of the Clinical-Chemical Interface of Medical-Science in this Century?" It's a damn good title, but I'm certainly not qualified to live up to it. What I'd like to hear is a panel of friends such as Donald Van Slyke, Otto Folin, William Mansfield Clark and John P. Peters gathered here to discuss the subject. So I invited them—and they accepted—or at least let's imagine that they have. Each in his way made technological and conceptual developments that have led to the present high state of the medical sciences. But each became involved in applying chemistry to clinical problems through a fortuitous state of circumstances.

Let me briefly introduce them to you.

First is Donald Van Slyke, born in 1883 of Dutch descent, who left us in 1971. He worked hard in the lab and played tennis throughout his life. He was unbeatable at either.

Second is Otto Folin, born in Sweden in 1867, who was head of biochemistry at Harvard from 1907 until his death in 1934. He invented many quantitative methods useful in clinical practice and was a much-loved teacher.

Third is William Mansfield Clark who lived from 1884 to 1964 and spent most of

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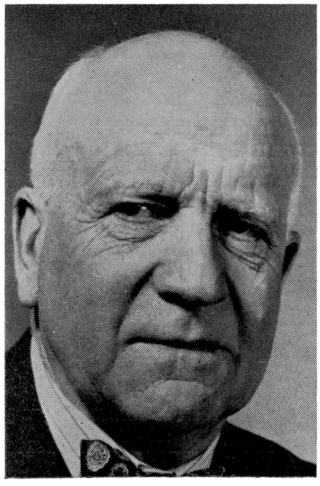
his life as head of physiological chemistry at Johns Hopkins. His contributions were in acid-base and oxidation-reduction biochemistry. He was a perfectionist in his research and was highly respected by his students.

Finally, there was John P. Peters, always called Jack, born in 1887, who was Professor of Medicine at Yale from 1921 until his death in 1955. A scholar in medicine, who was feared and revered by his residents and never compromised on principle, Jack teamed up with Van to write the classic two volume work *Quantitative Clinical Chemistry.*

We'll try to get a word from each of them.



Donald D. Van Slyke

1883–1971

DONALD VAN SLYKE

Van Slyke had taken a Ph.D. in 1907 in organic chemistry under Moses Gomberg who discovered organic free radicals. He had expected to follow in his father's footsteps and become an agricultural chemist. However, through a chance conversation between his father and P. A. Levene at a meeting of the American Chemical Society, he became an assistant to Levene at the newly-formed Rockefeller Institute for Medical Research. Seven years later, he was selected by Dr. Simon Flexner, the director, to head the chemistry division of the Hospital of the Rockefeller Institute. Here he had intimate contact with a small group of clinical investigators and the responsibility of applying chemistry to the solution of selected clinical problems and here he remained until he reached the retirement age of 65—after which he had a second productive career at the Brookhaven National Laboratory, dying while still active at age 88. He never really aged.

One can say Van Slyke saw clinical chemistry develop; he contributed personally to much of it. (As his first assistant from 1921–1926, I had the privilege of observing what was happening at the Clinical-Chemical Interface and of participating, at times, to a minor extent).

This was the period when the chemical description of the body fluids in terms of mass and composition in health and disease was under investigation. Methods had to be devised for quantitative determination of individual constituents. Since existing chemical methods usually required large samples of blood, they had to be modified and adapted to accommodate what were then regarded as small samples—meaning one cubic centimeter of blood or other fluid.

Quantitative measurement of the end products of the chemical reactions involved were limited to gravimetric, titrimetric, colorimetric and gasometric procedures. In his time, Van Slyke made use of all four—although he is best known for his ingenious use of gasometric techniques.

Each of our guests was young at the start of this century. Three, at least—Van Slyke, Folin and Clark—were well equipped to advance the chemical knowledge of the day. The fourth, Peters, the M.D. of the four, had the foresight to visualize a promising future for the use of sound quantitative chemical knowledge in the solution of clinical problems and the ability to make it stick.

What can they be said to have left us? First and foremost their students. I'll wager that every one of you medical scientists in

this audience has come under the influence of one of these students—or if not directly —then one of their student's students.

What is taken for granted today was a completely blank wall in the early 1900's. Let me enumerate some of the things, which today you would regard as old hat but for which they had to break ground:

1. The inorganic composition of the extra and intracellular fluids of the body.
2. The transport of oxygen and CO_2 and the control of salt, water and acid-base balance.
3. The metabolism of fats, carbohydrates and proteins by the several tissues.

Subjects unknown to them in their early days were:

1. The role played by vitamins and hormones in metabolic control.
2. That proteins were chemical individuals.
3. The composition and mode of action of enzymes.
4. Bioenergetics and biochemgenetics—two subjects still under active investigation.
5. Organelles of cells—their structure composition and functions.
6. Membranes of all kinds and how transport is effected through them.
7. Immunochemistry.
8. How the nervous system acts to achieve a coordinated desirable metabolic result.

Included in this abbreviated list are some topics that are essentially settled and others that are still under investigation and obscure.

For a moment, let us transport ourselves back to the early 1900's. What kind of questions would we first attempt to answer? Remember, we'd have no isotopes, no chromatography, no sophisticated spectro-photometers—not even any glass electrodes.

I expect we would do about what Van Slyke and Folin did, particularly if we had been exposed to Emil Fischer's pioneer work on amino-acids and proteins: we would try to study the fate of proteins in the body. Folin adapted the Nessler reaction for the determination of ammonia following Kjeldahl digestion of proteins to the Dubosq colorimeter. Van Slyke took advantage of the reaction of nitrous acid with aliphatic amino acids with the evolution of gaseous nitrogen and devised a glass apparatus for its measurement. Both Folin and Van Slyke used their respective methods to study the digestion of proteins, the absorption of amino-acids and their metabolism in the tissues. Both greatly advanced our knowledge. Folin was led to believe that all tissues were equally able to deaminate amino acids, whereas Van Slyke showed that the liver is the primary site of amino acid metabolism and urea formation.

When Van Slyke left Levene's laboratory for the Hospital of the Rockefeller Institute, he took his responsibility for aiding in the study of disease through chemistry very seriously. Since F. M. Allen was then applying the so-called starvation diet to the treatment of diabetes, Van Slyke, with Glen Cullen, undertook to devise a method for the early detection of acidosis,—an inevitable lethal concomitant of diabetes in the preinsulin days. This led to his simple and adequately accurate gasometric method for plasma bicarbonate concentrations.

The method proved clinically useful in all sorts of disease states involving acid-base abnormalities and led Van Slyke, in 1921, to his classic characterization of acid-base abnormalities which has stood the test of time.

H. "Tell us about your early lab days, Van."

Van Slyke speaks: "First of all, I never set out to be a clinical chemist. I was fascinated by amino acid and protein chemistry and their metabolism in the body. During my seven years in Levene's laboratory, I had the good fortune to literally dream up

a gasometric method for measuring amino acid nitrogen. It was quite accurate and with it Gus Meyer and I were able to show that the liver is the primary organ for amino acid metabolism and urea formation.

"After I transferred to the Hospital of the Rockefeller Institute to head its department of chemistry, I was responsible for chemical studies on the diseases under investigation at the Hospital. At various times these were diabetes, pneumonia and nephritis. However, I never gave up my interest in and work on what might be called basic biochemical attacks on physiological problems such as transport of oxygen and CO_2, control of acid-base balance, the role of the kidney in ammonia formation and clearance of urea from the blood.

"Nor did I ever completely stop work on amino acids. In 1920 while determining the hexone bases in a gelatin hydrolysate, I encountered a discrepancy which led, many years later, to the identification of a new amino acid,—hydroxylysine. This amino-acid has recently proved to be of great importance in membrane structure through its linkage with mucopolysaccharides.

"Although we tried to complete, to the best of our methodology, each problem that we undertook before undertaking another, we made some oversights. One of these was our failure to recognize the presence of carbonic anhydrase and of carbamino CO_2 in red blood cells. Both of these were within our reach during our experiments on CO_2 transport by and distribution in blood, but we missed recognizing them. You were responsible for that, Baird."

H. "Yes, Van. A little knowledge of thermodynamics is a dangerous thing."

Van Slyke. "I must confess some satisfaction over having the manometric gasometric apparatus so readily adaptable to the determination of a large number of substances other than those already in the gaseous state such as oxygen, CO_2, CO and nitrogen. With the able help of Folch, Sendroy and other fellows, gasometric methods were developed for amino-nitrogen, urea, lipids, sugar, lactic acid, chloride potassium and calcium. These proved useful until they were supplanted by modern technology.

"After I went to Brookhaven, I was able to improve considerably the determination of the isotope C^{14} through adapting the manometric apparatus for its measurement of $C^{14}O_2$ in the gaseous state. In keeping with the times, I also enjoyed, at Brookhaven, microing the manometric apparatus and methods for determinations on samples in the microliter range instead of the milliliter range.

"Of course, I didn't do these things myself. Throughout the 42 years at the Institute and my 22 years at Brookhaven, I had a constant procession of young associates (over 70 in all) in the laboratory who stayed with me for a few years and then moved on,—usually to professorships in the academic world. Some had M.D. or Ph.D. degrees when they arrived; a few received their Ph.D.'s while with me. Though I never was officially a professor, I feel that I've done my share of teaching at the clinical-chemical interface and would like to be remembered for that quite as much as for the publications from my laboratory."

H. "Well, Van, if I were to ask you what gave you the greatest pleasure among the many contributions you have made, what would you say?"

Van Slyke. "Working out the behavior of blood as a physical-chemical system from the properties of its constituents, particularly hemoglobin. This required the determination of the concentrations of water and inorganic constituents extra-cellularly and intra-cellularly, the concentrations and distributions of oxygen and carbon-dioxide in free and combined form and the titration curves of oxy and reduced hemoglobin.

From three known physical-chemical relations (electroneutrality, osmotic equilibrium and the Gibbs-Donnan Law), we were able to account for the changes that occur in blood when its pH is changed or its degree of oxygenation is altered.

"Had it not been for the need for a more accurate method to measure blood gases in these studies, the manometric blood gas apparatus might not have been developed.

"It also made me think through what was meant by buffer value in theoretical terms. This was my best theoretical contribution."

H. "Thank you, Van."

Otto Folin

1867–1934

OTTO FOLIN

My next distinguished panelist is Otto Folin. His entry into a career of medical chemistry differed greatly from that of Van Slyke,—though strangely enough their contributions and interests often supplemented each others.

Folin emigrated from Sweden to this country in 1882 when he was 15 years old. After undergraduate study at the University of Minnesota, he earned a Ph.D. in 1898 in organic chemistry while working under Professor Julius Stieglitz at the then young University of Chicago. After eight years as a research chemist at the McLean Hospital outside of Boston, Folin became Hamilton Kuhn Professor of Biological Chemistry and head of the department at the Harvard Medical School in 1907 where he remained until his death in 1934.

Folin's early interests were centered around protein metabolism and the development of quantitative methods applicable to such studies. The Dubosq Colorimeter, with which he had become familiar while in Germany in 1897, became his favorite instrument for quantitation. He is specially remembered for his methods and metabolic studies of nitrogen, urea, ammonia, uric acid, creatinine, sugar and a system of blood analysis developed with the late Professor Hsien Wu. He was a great and beloved teacher. In this connection, I cannot resist a digression.

Shortly before I left the University of Chicago for Harvard in 1935, I received a telephone call from Professor Stiegliz asking me to come to his office. Since I barely knew Professor Stiegliz, I wondered what I had done. When I got there, he said: "Sit down, Mr. Hastings. I hear you are going to Harvard. I want to tell you a story. A year or so after the University of Chicago opened in 1891, I was assigned a young graduate student. In time he came before the chemistry faculty for examination for his Ph.D. degree. The examination of my student was in the afternoon, following the examination of one of Professor Neff's students in the morning. Professor Neff's student had done brilliantly, answering all the questions put to him; but my student did miserably, answering hardly any of the questions. However, since I was young and this was my first graduate student, the faculty voted to pass him. Professor Neff's student was never heard from again, but my student was named Otto Folin. Good day, Professor Hastings."

H. "Professor Folin, what gave you the greatest pleasure of your contributions?"

Folin. "I suppose it was the night I delivered my Harvey Lecture on Blood Analysis in New York in 1920. Some years be-

fore, Hsien-Wu and I had introduced a number of methods for analyzing blood for clinically important constituents, among them a method for determining blood sugar. The method was based on the reduction of cupric ion to cuprous oxide in an alkaline copper tartrate reagent, and then the reaction of the cuprous oxide with phospho molybdic acid, ending up with a blue solution of phospho-molybdous acid which was proportional to the glucose present. Strict attention to temperature and time of heating was essential. My good friend, Stanley Benedict, professor of biological chemistry at Cornell Medical School, had taken exception in print to the accuracy of our blood sugar method. His point was that reoxidation of the cuprous copper occurred erratically and much too readily. He had illustrated this with data showing what happened when you heated the solution longer and shorter times than we had prescribed.

"On the night of the lecture, I had requested that he sit in the front row directly opposite the rostrum. I described the various methods that had been proposed for measuring blood sugar on one cc samples. Then I described our method and the criticisms that had been leveled at it by my friend Professor Benedict. Then, I said: 'I did not say to heat the solution for eight minutes or fifteen minutes, but exactly ten minutes.' And I took up the other points of criticism in like manner.

"Then I said: 'But I have devised a way to circumvent even those who do not choose to follow directions,' and I drew from my pocket a standard reaction tube, with the bottom portion containing the reactants drawn down so that it was connected with the upper portion by a four cm isthmus with only eight mm diameter. 'This cuts down reoxidation by air to an inconsequential amount. I find that Harvard medical students can now obtain accurate values for blood sugar, and I hope that Professor Benedict will also find it useful.' I presented this so-called Folin-Wu tube to him on the spot. I never heard any more criticism of the method from him."

H. "Thank you, Professor Folin."

William M. Clark

1884–1964

WILLIAM MANSFIELD CLARK

H. "Now, let's hear what Bill Clark might have to say. I'll let him speak for himself."

Clark. "Well, I never dreamed that I would spend most of my scientific life in a medical school or do research that would be of value to medicine. I wanted to be a first-rate physical chemist. That's why I went to Hopkins and worked on osmotic pressure measurements under Professor Morse. That's when I learned to be satisfied with nothing less than the maximum accuracy attainable. I also learned to be a pretty good glass-blower.

"My first job after getting my Ph.D. was as a research chemist in the dairy division of the Department of Agriculture where I was assigned to find out why there are holes in Swiss cheese. This led me to the study of the metabolism of molds and microorganisms and their ability to produce CO_2 and other acids. I needed better methods to measure pH and better understanding of acid-base equilibria in general. We

managed to design a rocking hydrogen electrode which could measure the pH of protein containing fluids in a CO_2 containing atmosphere and we synthesized a new series of acid-base indicators sulfaphthaleins, that covered a wide pH range. These were both widely used for years.

"In 1920 I put what was then known about acid-base chemistry, the methodology involved and the applications to biology, bacteriology and industry together in a book called the *Determination of Hydrogen Ions.* It must have met a need because it went through three editions and four reprintings.

"Since the hydrogen electrode can be thought of as measuring the potential between (atomic) hydrogen and hydrogen ions, it is a special case of an oxidation-reduction potential. This led me and my associates to a systematic study covering many years of reduction potentials of reversibly oxidizable organic compounds with and without biological origin and importance. The effect of pH changes and of coordination with ligands, which influenced the potentials, led to the concept of an oxidation continuum, culminating in what we now call the electron transport chain.

"I did part of this work at the Hygienic Laboratory (now the NIH) of the Public Health Service between 1920 and 1926 and most of the work at Johns Hopkins where I was professor of physiological chemistry from 1927 to 1952.

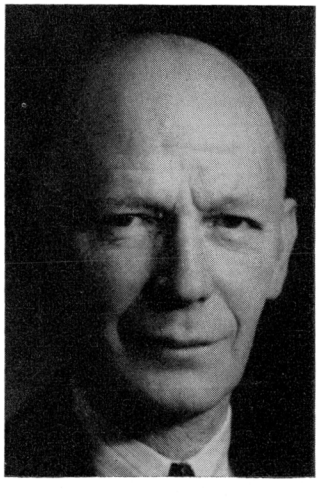
"Since I didn't know much about biochemistry as applied in medical schools, I took gross anatomy with the Hopkins medical students the first year I was there. This helped me, but it didn't seem to help the students learn what I considered they should know. However, we both learned together and by the time I retired, I had a great respect for medicine and the students had a good idea of what biochemistry could do for medicine.

"I never lost my love of glass-blowing, which I would resort to for relaxation when frustrated, or to golf where I could test the ravages of time on my accuracy. To have made three holes in one—one of them after I retired—was a special dividend in a happy life as a biochemist at the interface between medicine and chemistry."

H. "Professor Clark, what would you say were your major contributions to medicine?"

Clark. "My graduate students and my medical students. The former have contributed much to today's biochemical knowledge and the latter are practicing sound clinical medicine. My book, *Topics in Physical Chemistry,* has been of some help to medical investigators."

H. "Thank you, Bill."



John P. Peters

1889–1955

JOHN P. PETERS

H. "Now, Jack, tell us how you got into clinical chemistry. Was it in the year 1920–21 when you were with Van, in his laboratory at the Hospital of the Institute."

Peters. "Well, in a way and it had some exciting moments. Franklin McLean came breezing in from Peking and sold Van on taking on the job of proving whether L. J.

Henderson was right or wrong about the effect of oxygenating hemoglobin of different pH's on chloride and bicarbonate distribution in the blood. It meant that Van had to invent a more sensitive apparatus for O_2 and CO_2 determinations and that I was responsible for the equilibration of blood with gases of different CO_2 and O_2 tensions. The next year I went to Yale as an associate professor of medicine, and there I was to remain for the rest of my life. It was where our book *Quantitative Clinical Chemistry* was born.

"The publishers, Williams and Wilkins, asked me to write a modest handbook for clinicians describing useful chemical methods and discussing their application to clinical problems. It was originally to be called "Quantitative Chemistry in Clinical Medicine." I soon found that it was going to be a bigger job than I could handle alone, and I asked Van Slyke to join me in writing it. He agreed and we made an outline and divided up the writing of the first draft of the chapters between us. We also agreed to exchange each chapter until it met the satisfaction of both of us. This may have improved the accuracy and completeness of the chapters, but it made publication of the book delayed until 1931; each chapter turned out to be a monograph. It also had grown to two volumes,—one of 1200 pages with 21 chapters called Interpretations dealing only with the physiological and clinical significance of these substances for which quantitative methods were available, and a second volume called Methods of about 1000 pages containing those methods, described in detail, that had been proven accurate and useful. Somewhere along the line, the title was shortened to *Quantitative Clinical Chemistry.*

"It must have met a need because the first edition sold out rather quickly here and abroad and the publishers soon asked us to prepare a second edition. God knows we tried, but medical research progressed faster than we could keep up with it,—at least to both our satisfactions. The upshot was that I brought out a second edition of Interpretations in 1946 without the chapters on hemoglobin and acid-base balance and the inorganic ions. Van kept working on the revision of these and the methods volume as long as he lived.

"Though our collaboration was a fortunate one, it was difficult because he was accustomed, as a chemist, to be content with nothing less than proven accuracy, whereas I was used to being confronted constantly with disease manifestations in patients,—no two of which were the same. It made it difficult to satisfy both of us at the same time on any one subject. However, the first edition seems to have been worth doing."

H. "Now, Jack, what do you think the development of biochemistry and other medical sciences has done for medicine and the treatment of disease in patients?"

Peters. "Well one thing is clear. It has made it more expensive. It has also made the medical school curriculum longer and more difficult. It has resulted in a generation of clinicians who not only have more understanding of the basis of many diseases, but it has given them the tools to improve diagnoses, to make prognoses more accurate and to follow the effects of therapeutic procedures. All the medical sciences have made this possible.

"But when this is said, there remains still the art of the physician to be reckoned with,—the need of the individual patient for that intuitive recognition by his physician of physical and mental symptoms and behavior born of clinical experience and compassion. We can correct abnormal mass and composition of body fluids; we can modify metabolism of fats, carbohydrates and proteins by diet, drugs and hormones; but we are still primarily dependent on the wisdom of the body to heal itself. It is the

wise physician who knows when to stop tinkering."

H. "Thank you, Jack."

In spite of the progress made in this century toward an accurate computerized description of man as an organized heterogeneous chemical system for the useful transfer of chemical energy, the whole remains much bigger than the sum of its parts. The medical scientist has magnificently enlarged the knowledge of man in health and disease. The physician still stands in the position of its interpreter in the interest of the patient. But let us each persevere to advance knowledge in that manner for which he is best fitted. Each sound contribution will find its rightful niche.

As the late, wise William H. Welch so prophetically said in an address in 1907 on the Interdependence of Medicine with Other Sciences of Nature, "It is well that the sciences of nature hold out attractions to so many different types of mind, for the edifice of science is built of material which must be drawn from many sources—the deeper we can lay the foundations and *penetrate into the nature of things,* the closer the workers are drawn together, the clearer becomes their community of purpose and the more significant to the welfare of mankind the upbuilding of natural knowledge."

So, ladies and gentlemen, you have heard snatches of what it was like to be groping at the clinical-chemical interface fifty or more years ago. I hope that my imagination and voice will be forgiven.

Was it a dawn that was breaking? Are we at high noon today? Or is today more likely another dawn?

I can think of no more fitting ending to this pleasant and profitable meeting of the Association of Clinical Scientists than to quote the Christmas thoughts from the Sanscrit of my friend and contemporary, the founding father of this organization, F. William Sunderman, Sr.:

Look to this Day! It is Life.
In its brief course lie all the
Verities and Realities of Existence.
Yesterday is but a Dream, and
Tomorrow is only a Vision.
But Today well lived
Makes every Yesterday a Dream of Happiness;
And every Tomorrow, a Vision of Hope.