MODERN TRENDS IN PHYSIOLOGY AND BIOCHEMISTRY

WOODS HOLE LECTURES DEDICATED TO THE MEMORY OF LEONOR MICHAELIS

Edited by

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1952

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK, N. Y.

125 East 23rd Street
New York 10, N. Y.

Library of Congress Catalog Card Number: 51-14025

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

The Department of Physiology of the Marine Biological Laboratory decided to commemorate the seventy-fifth birthday of Professor Leonor Michaelis by dedicating the Physiology Course of 1950 to him, who had once been an instructor of the course. It was also decided to publish the lectures given on that occasion. The invitation to Professor Michaelis to be a guest of the Laboratory for the Summer of 1950 was sent in September of 1949. He accepted the invitation promising to take active part in the discussions which follow both lectures and seminars. Unfortunately his death one month later converted the celebration into a memorial tribute. This book, containing some of the lectures of the staff and those of the guests who were invited to join us, is in honor of the memory of a great scientist, our colleague, Professor Leonor Michaelis.

In 1898, when Jacques Loeb started the Physiology Course of the Marine Biological Laboratory, he brought to it his fundamental philosophic principles as well as his scientific method. Since those early years the teaching staff has changed many times but the guiding principles have been retained. Physiology, the science concerned with the study of the mechanism of life, must go deeper and deeper into the physical and chemical phenomena which in their integration make up the vital processes. Michaelis, with Loeb, pioneered in this science and introduced into biology the now well-known physico-chemical methods. As structural chemistry advanced and analytical methods for the detection and measurement of minute quantities were developed, physiology advanced in the study of the intricate mechanisms of the interactions of molecular entities present in living systems. This approach, sometimes called molecular biology, has always been the aim of the Woods Hole staff. However, the staff has emphasized that at the molecular level, the chemical and physical reactions, essentially similar in all living cells, show definite differences from cell to cell. These differences are produced by the regulatory mechanisms which vary not only among different species but also within the same species at different stages of its ontogenic development.

The subjects discussed in the twenty chapters of this book may be arranged in six groups:

I. The physiology of the cell is treated in five chapters: the morphology of the cytoplasm and the functions of its different components as isolated by various methods; the physicochemical as well as the physiological properties of the nucleus and the interrelations between cytoplasm and nucleus; the molecular structure of the cell membrane and the rates of penetration across the membrane as studied in the erythrocyte; and finally, the mechanism of cellular division.

II. The properties of muscle, now the center of much attention thanks to Albert Szent-Györgyi's foresight, are discussed in three chapters. Here are presented new methods for the purification of some of the protein components of muscle, the application of thermodynamic principles to muscle contraction, and the transport of material across the muscle and nerve membranes.

III. The nerve fibers of the squid and other marine animals are a favorite research tool of the nerve physiologist, and Woods Hole is naturally an active center for the study of nerve physiology. Five chapters are devoted to this problem. The chemical, enzymatic mechanisms, the electrical phenomena, and the physicochemical changes in the ultrastructure of nerve are studied in relation to nerve activity and with the aim of explaining this process. Another chapter discusses the transmission processes at nerve-muscle junctions. Finally the problem of nerve activity is presented from the comparative-evolutionary point of view.

IV. Since most of the energy necessary for the performance of vital activities is produced by enzyme reactions, a knowledge of these reactions is of primary interest to the physiologist. Some aspects of this problem are discussed in five chapters. As a special tribute to Michaelis, who discovered the fundamental principles of these particular topics, there is one chapter with the experimental demonstration of the Michaelis enzyme substrate complex, and another with a discussion of the mechanism of enzymatic oxidation-reductions. The third chapter discusses the physicochemical properties of chymotrypsin, a protein which seems to fulfill the criteria of purity. In the fourth chapter models of metallo-proteins are presented in a rigorous study of copper and calcium proteins. The last chapter discusses the oxidative pathways of carbohydrate metabolism.

V. The study of the action of foreign agents such as drugs being also the domain of physiology, a chapter devoted to the mechanism of drug action is included.

VI. The staff of Woods Hole has been keenly aware of the integrity of the evolutionary process of life which takes place in the structure as well as in the function of living systems. A special chapter is devoted to a discussion of the best known examples of biochemical evolution.

Throughout all the chapters, whatever the particular subject matter, will be observed the cardinal principle of the Woods Hole Physiology Course—explanation of life phenomena at the molecular level.

I wish to express my thanks to the publishers for the preparation of the indexes and for their wholehearted cooperation.

E. S. Guzman Barron

Memorial Lecture

Read at the
Annual Meeting of the Marine Biological Laboratory, Woods Hole, Massachusetts, August 22, 1950

Leonor Michaelis, 1875–1949

By E. S. GUZMAN BARRON

The Marine Biological Laboratory mourns the death of Professor Michaelis. The loss is more deeply felt as his death came just two months after the Department of Physiology had decided to celebrate his seventy-fifth birthday by dedicating to him the Physiology Course of 1950. He was very touched by this tribute and had accepted our invitation to spend the summer with us. Students and staff expected to profit from his genius and his wisdom. A member of the corporation since 1928, Professor Michaelis was an instructor of the Physiology Course for ten years.

Born in Berlin, Germany, on January 18, 1875, Leonor Michaelis graduated from the Medical School in Berlin in 1897. That his life was to be devoted to the pursuit of knowledge was demonstrated early. While still a student he published a paper on the histology of milk secretion which was rewarded with a prize from the Medical School, the only recognition that was to be awarded to him on time. In Professor Hertwig's laboratory young Michaelis worked on embryological problems. His paper on the cytology of the fertilization of the ovum of the amphibian *Triton taeniatus*, as well as his doctor's thesis on the determination of the direction of the first cleavage in the frog's egg, reveals rare insight and clarity of conception.

It was natural that Michaelis' early scientific steps were directed toward microscopic anatomy, since medical thinking at that time was strongly influenced by the rapid advances made with the aid of the microscope. Perhaps he would have directed himself toward pathology if, "by some strange coincidence" as he says it, he had not met Paul Ehrlich, who at that time was looking for a young histologist. Ehrlich offered him a one-year position as his private assistant in the State Institute for Serological Testing and Research. It was here that Michaelis studied the chemistry of staining, and it was here that the vital staining of mitochondria with Janus green originated. There is no doubt that this one year at Ehrlich's laboratory exerted a decisive influence on the direction of his investigations. The whole field of physicochemical sciences as applied to biology and medicine was here opened to him. With his powerful and quick intellect he visualized the unlimited applications of physics and chemistry to biological phenomena. It must be recalled that the science of biochemistry at the start of the century, when Michaelis entered Ehrlich's laboratory, was still in its infancy, mostly sheltered in physiological and pathological laboratories; and the *Journal of Biological Chemistry*, *Biochemical Journal*, and *Biochemische Zeitschrift* did not appear until six years later. Here in front of him was a field of vast possibilities, and he with no financial facilities, and no formal, classical training to enter it. Indeed Ehrlich himself had advised him to enter clinical medicine, which he had to do, becoming an assistant to Professor Litten in a department of one of the municipal hospitals in Berlin from 1900 to 1904. A herculean task was this, taking care of the sick and at the same time preparing himself with the solid foundations required for fundamental research in quantitative biology. With no teachers to guide him, armed only with his genius and his tenacious perseverence, he studied chemistry, as he once told me, from a dictionary, and mastered the branches of mathematics necessary to become familiar with the solution of physicochemical problems. And while thus schooling himself for the task ahead, he still found time to continue his investigations in histology and staining.

In 1903 he was made a *Privadozent* at the University of Berlin with a thesis on immunological protein precipitins. By combining enzyme techniques he observed that short digestion of serum albumin with pepsin changed profoundly the immunological properties. He was also the first to show that aqueous extracts of normal livers could be used for the Wassermann test, and that the complement fixation test in the Wasserman reaction could be replaced by a direct precipitation test with extracts of syphilitic fetus of high potency.

In 1904, a paper about some iron-containing inclusions in tumors of the urinary bladder aroused the interest of Professor von Leyden, who appointed him research assistant for the newly created Institute for Cancer Research at the University Clinic. He worked here on experimental cancer, and once was sent to Jena to bring the ultramicroscope newly built by Siedenhopf and Zsigmondy at the Zeiss optical works. With this instrument von Leyden had great hopes of finding the cause of cancer; Michaelis had great hopes of utilizing the instrument for studies of colloidal chemistry (which he indeed succeeded in doing).

In 1905, Michaelis received the title of Professor. What could a Professor do with no salary, no laboratory, no funds to support his work? Fully realizing the hopelessness of this situation, he accepted the position of bacteriologist at the municipal hospital "am Urban" in Berlin, which brought him in contact with Peter Rona, the chemist of the hospital. The close friendship and association with Rona lasted throughout his seventeen-year stay at the hospital. It was here that the full powers of Michaelis came to glorious eclosion. In his humble research room, with barely space enough, as Albert Szent-Györgyi has said to me, for one man to walk through, and with scant equipment, Michaelis accomplished, either alone or in collaboration with his friend Peter Rona and a number of young scientists from abroad and from Germany, the series of remarkable investigations on the ionic environment, on weak electrolytes, on adsorption, on proteins, on enzymes—chapters which are now the secure foundation of quantitative biology.

Simultaneously with Sörensen, Michaelis foresaw the influence of the hydrogen ion concentration on biological phenomena and proceeded to develop the theory and practice of hydrogen ions. The hydrogen electrode he built for exact measurements. The theory of buffers, or, as he called them, "hydrogen ion regulators," was lucidly presented. The theories of the dissociation of amphoteric electrolytes, and of the isoelectric points of amino acids and proteins were developed quantitatively, after Hardy had introduced in a qualitative way the concept of the isoelectric point. He developed the method of electrophoresis in such a way as to keep the pH constant during the flow of current. The isoelectric points of casein, hemoglobin, serum albumin, serum globulin, native and denatured globin, gelatin, and edestin were thus determined. He was the first to show that the charge of a colloid, enzyme, or even of whole bacteria was not a property inherent in the substance alone but was dependent upon the pH and that there is an isoelectric point characteristic of the substance.

He was devoting his attention at the same time to the field of enzymes. Mindful of the influence of ionic environment, he studied the effect of the hydrogen ion concentration on enzyme activity, and although Sörensen published first similar studies, Michaelis proceeded to extend Sörensen's observations by showing that the dependence of enzyme activity on pH was of the same nature as the dependence of the dissociation of a weak acid on pH. By comparing the enzymatic activity at constant pH but varied concentrations of substrate he presented lucidly and quantitatively the concept of affinity constant. Thus the theory of enzyme-substrate combination, postulated by Henry in 1903, was established on a firm basis ten years later, in 1913. At a time when the protein nature of enzymes was as yet unknown, Michaelis guided by his genius foresaw it; with daring and his elegant simplicity he put forth the theory of enzyme-substrate complex formation. Now the Michaelis enzyme-substrate complex is one of the solid foundations of enzymology.

In a study of the effect of adsorption on enzyme activity he gave evidence of site specificity on the enzyme surface. And in his studies on the nature of enzyme inhibitors he distinguished the competitive inhibitors from those which influence the rate of splitting of the enzyme-substrate into the split products and free enzyme.

Studies on adsorption, on bacterial agglutination, on blood sugar, on buffers, and on pH indicators crowded those years of intense production.

In 1921 Michaelis received the title of *professor extraordinarius* of physical chemistry applied to medicine and biology at the University of Berlin. Again a professorship with no salary, no laboratory, no budget. Grim irony this "appointment" must have seemed to him! Hence he accepted in 1921 the position of consultant in an industrial concern for scientific apparatus where he was provided with laboratory facilities. The international reputation of Michaelis, however, was widespread. Less than one year later he was invited to the chair of biochemistry at the reorganized medical school of Nagoya. Three pleasant and fruitful years were spent in Japan, where he initiated, in collaboration with Fujita, his studies on membrane permeability. Struck by the selective permeability of cell membranes, he strove to construct a model which would show similar properties, and thus he made his dried collodium membranes permeable only to cations. Here again he gave proof of his rare insight, for he terminated these studies once he recognized that his models did not reproduce the properties of cellular membranes.

During his stay in Japan Michaelis received an invitation from Jacques Loeb for a lecture tour in the United States, to be given in the summer of 1924. On the very day of his departure from Japan he received the tragic news of Jacques Loeb's sudden death. The lectures, however, had all been properly arranged, and on that occasion Michaelis was asked to come to Johns Hopkins as a resident lecturer for three years, beginning in the spring of 1926 at the termination of his contract in Japan.

And thus, at Hopkins, started the third cycle of Michaelis' achievements. The field of oxidation-reduction processes and of heavy metal catalysis now received most of his attention. Here, as in the field of enzymes, his genius brought forth theories of fundamental importance for the understanding of the kinetics of biological oxidations. The simultaneous discovery with Elema, from Holland, of the two-step reduction of pyocyanine in acid solutions was the starting point of Michaelis' principle of monovalent oxidation-reductions and the intermediary formation of free radicals during the oxidation process of systems, which perform the reaction by transfer of two electrons. The presence of such free radicals, existing in equilibrium with their "parent substances" even in aqueous solutions, was a daring concept which appeared absurd to conservative chemists. Like his theory of enzyme-substrate formation, it was more the foresight of a genius than the result of a methodical and secure approach. The first paper on the theory of two-step oxidations had been rejected by both the *Journal of Biological Chemistry* and the *Journal of the American Chemical Society*. So indignant was one referee of the latter journal that in horror he wrote, "In short, a principle of modern scientific philosophy is violated." The paper was published in *Biochemische Zeitschrift*. Michaelis felt deeply this criticism. To answer the charges of lack of conclusive evidence and of too much reliance on potentiometric data he diligently started the study of quantum mechanics and of magneto-chemistry, attacking them with the same enthusiasm as when thirty years previously he had prepared himself in organic chemistry and mathematics. He simplified the method of the magnetic balance for the measurement of the paramagnetism of these intermediate compounds and left no room for doubt about the existence of free radicals. Thus the name of Michaelis has been incorporated in every modern textbook of organic chemistry.

Yet official recognition came always later. He was made a member of the National Academy of Science five years after his retirement as active member of the Rockefeller Institute, where he spent the last years of his life—from the end of his lectureship at Johns Hopkins until his death in October of 1949.

With the death of Michaelis we lose a man of an intellectual stature that is more and more rare in this age of narrow specialization. He had the priceless gift of transforming the most abstruse and complex concepts into simple and clear ideas; the books he wrote were models of clarity and conciseness. His textbook on embryology reached nine editions. *Hydrogen Ion Concentrations, Introduction to Mathematics for Biologists and Chemists, and Oxidation-Reduction Potentials* were all translated into various languages. His brilliant genius allowed him to perceive immediately the important points in any discussion, and his wide knowledge in the fields of biology, medicine, chemistry, physics, and mathematics was always at the service of those who went to him for advice.

The humanistic type of Gymnasium where Michaelis received his secondary education broadened his intellectual training and interests. With no difficulties in Latin or Greek he tackled the Japanese language when in Japan and became quite a connoisseur of the old Oriental civilizations. Music attracted him greatly. In his first semester at the University he even took a course in counterpoint with Professor Bellerman. His improvisations at the piano were always a delight to his listeners.

The Marine Biological Laboratory mourns deeply the loss of a great man of science and a scholar of broad interests, as well as a friend of many years' standing; and extends to his devoted wife, Hedwig Michaelis, the expression of our deep sorrow.

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