MIAMI WINTER SYMPOSIA
VOLUME 3

The Molecular Basis of Biological Transport

edited by
J. F. Woessner, Jr.
F. Huijing

Academic Press

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DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF MIAMI SCHOOL OF MEDICINE
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The Molecular Basis of Biological Transport

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3. *J. F. Woessner, Jr., and F. Huijing, editors:* THE MOLECULAR BASIS OF BIOLOGICAL TRANSPORT, 1972

4. *J. Schultz and B. F. Cameron, editors:* THE MOLECULAR BASIS OF ELECTRON TRANSPORT, 1972

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PREFACE

In January, 1969, the Department of Biochemistry of the University of Miami School of Medicine and the university-affiliated Papanicolaou Cancer Research Institute joined in presenting two symposia on biochemical topics. These symposia have begun to develop as a tradition over the years and have attracted national interest.

In 1970, the two symposia were entitled "Homologies in Enzymes and Metabolic Pathways" and "Metabolic Alterations in Cancer." The full report of this meeting was published as the first volume of a continuing series under the title "Miami Winter Symposia."

In 1971, the value of the series was enhanced by including all discussions as well as the full text of the reports. This year it was decided to publish the results of the two symposia separately. This step was taken to prevent the volumes from becoming unwieldy and to permit a wider scope in the selection of topics for the two symposia.

This volume, the third in the series, contains the proceedings of the symposium held in Miami on January 10-11, 1972. The fourth volume, to be published simultaneously, will deal with the other symposium entitled "The Molecular Basis of Electron Transport." Associated with the symposia is an opening lecture, named in honor of the University of Miami's distinguished Visiting Professor, Professor Feodor Lynen. The first Lynen Lecture was given in 1970 by Dr. George Wald. The second lecture was by Dr. Arthur Kornberg. This year's Lynen Lecture was delivered by Dr. Harland G. Wood. This lecture forms the opening of this volume.

The symposia are organized so as to ensure publication as rapidly as possible. The speakers are strictly enjoined to present their finished manuscripts at the time of the meeting; the discussants edit their comments before the symposia adjourn. We thank them, the speakers, and the many local helpers, faculty and administrative staff, who have made this venture possible. Particular thanks are due to the organizers and coordinators of the present symposium: W. J. Whelan, K. Savard, B. J. Catley, and Olga F. Lopez.

We also acknowledge with gratitude the financial assistance of the Howard Hughes Medical Institute, the Departments of Anesthesiology and Dermatology of the University of Miami, Coulter Electronics, Inc., and M.C.B. Manufacturing Chemists.

In 1973 the topics of the Miami Winter Symposia will be "Protein Phosphorylation in Control Mechanisms" and "The Role of Cyclic Nucleotides in Carcinogenesis." Dr. Earl W. Sutherland, Nobel Laureate, has accepted the invitation to be the fourth Lynen Lecturer. These Symposia are scheduled for January 15-19, 1973.

J. F. Woessner, Jr.
F. Huijing

The Molecular Basis of Biological Transport

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THE THIRD FEODOR LYNEN LECTURE:

MY LIFE AND CARBON DIOXIDE FIXATION

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The Invitation to present the Third Feodor Lynen Lecture is a great honor and is of special significance to me because Feodor Lynen is one of my most cherished friends. George Wald and Arthur Kornberg have used this occasion to reminisce about their scientific experiences and since I have made no contributions to the subject of this symposium, "The Molecular Basis of Biological Transport", I clearly have no obligation to speak on the subject. Therefore, I likewise will take the occasion to look back over my life and my career as a scientist.

My grandfather, Peter, and my grandmother, Emiline Wood, came to Minnesota by oxcart and settled near Delavan where they homesteaded on a land grant from Abraham Lincoln, apparently because they got stuck in a slough and decided that if they ever got out they were going to stop traveling. Lots of good decisions are made that way. They lived in a sod house the first winter. My father, William Wood, was the second youngest of sixteen children, nine boys and seven girls. My mother, Inez Goff, was raised on a farm near Mapleton, Minnesota, not far from Delavan. Grandfather John Goff and Grandmother Ann August Goff had a very modern farm with a large round barn, in which I loved to play as a child. My ancestors apparently were largely English and some Holland Dutch. I was the third born of a family of five boys and one girl. The Woods were quite good in athletics, in fact, all five boys were captains of the Mankato (Minn.) High School football team during their senior year, and William Wood had one or more sons on the team each year for fifteen years.

Athletes, then as today, were enticed to colleges. We all went to college and four of the sons, Chester, myself, Earl and Wilbur earned Ph.D. or M.D. degrees and the fifth, Delbert, a degree in law. Louise, the daughter, lost the rough draft of her Ph.D. thesis in an automobile accident and didn't get her degree. She seems to have progressed quite well without it and is now Executive Secretary of the Girl Scouts of America. I often wonder how my parents managed all this. We remain a closely knit family with frequent gatherings of the clan and the brothers meet in Minnesota to hunt deer each year.

I went to Macalester College in St. Paul, Minnesota, as did my sister and my brothers, except Chester. There, I met Mildred Davis from Foley, Minnesota and we were married at the beginning of our junior year. This was quite unusual in those days and it required a meeting with the President of the College for permission to continue in school. We were married in September, 1929, the year of the stock market crash and the start of the Depression. To meet our financial needs, I had decided to drop athletics and get a job but changed my mind when a well-to-do alumnus gave me $50.00 to help pay tuition, which in those days was $87.50 per semester. I was also given a job as assistant trainer, worked for my board in the dormitory by making salads, worked in the chemistry stockroom, corrected exam papers in chemistry and represented a clothing store on campus. My wife, Milly, worked in the library and helped correct the papers. We lived quite happily in a single room in a private home and had our meals in the dormitories. Marriage proved to be a good decision, my grades improved and we both graduated in 1931 with no debts.

My Ph.D. training was in microbiology at Iowa State University in Ames, Iowa under Professor C.H. Werkman. Small things change one's life. Professor O.T. Walters of Macalester was helping me fill out my applications for fellowships, which were all being made in departments of chemistry, since I wanted to be a chemist. During one of my visits to his office, he took a book from a shelf and said, "Why don't you apply here?". The book was "Bacteriology, for Students in General and Household Science" by R.E. Buchanan, Chairman of the Bacteriology Department at Iowa State College. This resulted in a

successful application in microbiology. The fellowship paid $50.00 a month for 9 months, thus $450 plus tuition switched my career from chemistry to bacteriology. That first year, we paid $32.50 a month rent for an attic apartment, leaving $17.50 plus some savings from summer work for food. Later, I got a job firing furnace in an apartment house and this reduced the rent to $15.00 and in addition the fellowship was increased to $540 per year. Our first daughter was born during those graduate student days. We lived a relatively happy life without a car and had little money for entertainment but we had plenty of friends in the same or worse circumstances.

Microorganisms have proved a fertile area for the study of biochemistry and I was fortunate, through no foresight on my part, to receive my graduate training in microbiology. In 1931, when I began my Ph.D. training, the biochemistry of intermediary metabolism was still in its infancy and had not as yet taken root in the USA. Professor Werkman was particularly influenced by the investigations of Prof. A.J. Kluyver and his co-workers of Delft, Holland who were attempting to deduce the pathways of metabolism of a variety of bacteria by determining quantitatively the products of fermentations. Professor Werkman had been trained as an immunologist and was just entering the field of carbohydrate metabolism when I arrived. Although I was associated with him for eleven years, I never could diagnose his formula for success. It seemed to me that the assignments to his students were either vague, broad or impossible, but somehow they often ended in success. My assignment was to study the fermentation by the propionic acid bacteria. Professor Werkman handed me C.B. van Niel's Ph.D. thesis (1), which was a magnificent study of the propionic acid bacteria, and said, "Read this and everything else that has ever been published on these bacteria and then get to work". I did what I was told, but it seemed that van Niel had done most everything that needed doing. There was one controversy, however; van Neil (1) maintained that succinate was not formed from the fermented carbohydrates but was a product of the aspartate present in the yeast extract used in the medium. On the other hand, A.I. Virtanen (2,3) had found that, under certain conditions, succinate and acetate were the only products formed by suspensions of propionibacteria from glucose. At that time, it was considered that the for-

mation of succinate occurred as follows:

$$\text{Glucose} \rightarrow 2 \text{ pyruvate} \rightarrow 2 \text{ acetate} + 2 \text{ } C_1 \text{ compounds}$$
$$\downarrow$$
$$\text{succinate}$$

Since no C_1-compounds such as CO_2 or formate were found as products, Virtanen (2) proposed the succinate was formed by 4- and 2-carbon cleavage:

$$\text{Glucose} \rightarrow \text{succinate} + \text{acetate}$$

Thus there was a clear difference of opinion between van Niel and Virtanen and it seemed possible we could settle this difference if we studied the fermentation using a more defined source of nitrogen for the growth of the organisms. At that time we did not know about the vitamin requirements of the propionic acid bacteria and thus were unable to obtain growth on a defined medium. Nevertheless, we were able to show that succinate is formed from glucose since we obtained as much as 5.2 g of succinate per liter from a medium containing only 4.0 g of yeast extract (4). Furthermore, the carbon utilized by fermentation of glucose was not accounted for by that occurring in the products, unless succinate was included as one of the products. In addition, the oxidized products did not equal the reduced products, as required in an anaerobic fermentation, unless succinate was considered a product from the glucose.

Later with Edward Tatum and W.H. Peterson, while on a postdoctoral fellowship at the University of Wisconsin, we showed for the first time that vitamin B_1 is required for growth of a microorganism (5). We were then able to grow the propionic acid bacteria with ammonium sulfate as a nitrogen source (6) but by that time the question of the source of succinate had been settled.

The most important discovery made during my studies for the Ph.D. degree was that the propionic acid bacteria utilize carbon dioxide, but this finding was not included as a part of my thesis. This came about as follows. In addition to balance studies on the fermentation of glucose, I decided to set up experiments with glycerol as a carbon source. We used $CaCO_3$ to neutralize the acid produced by the fermentation and to maintain a neutral pH. The