ANNUAL REVIEW OF BIOCHEMISTRY

ANNUAL REVIEW OF BIOCHEMISTRY

VOLUME 60, 1991

CHARLES C. RICHARDSON, *Editor*
Harvard Medical School

JOHN N. ABELSON, *Associate Editor*
California Institute of Technology

ALTON MEISTER, *Associate Editor*
Cornell University Medical College

CHRISTOPHER T. WALSH, *Associate Editor*
Harvard Medical School

ANNUAL REVIEWS INC. 4139 EL CAMINO WAY P.O. BOX 10139 PALO ALTO, CALIFORNIA 94303-0897

ANNUAL REVIEWS INC.
Palo Alto, California, USA

International Standard Serial Number: 0066–4154
International Standard Book Number: 0–8243–0860-3
Library of Congress Catalog Card Number: 32-25093

♾ The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48-1984.

Typesetting by Kachina Typesetting Inc., Tempe, Arizona; John Olson, President
Typesetting Coordinator, Janis Hoffman

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA

Annual Review of Biochemistry
Volume 60, 1991

CONTENTS

(*continued*)

Annu. Rev. Biochem. 1991. 60:1–37

50 YEARS OF BIOLOGICAL RESEARCH—FROM OXIDATIVE PHOSPHORYLATION TO ENERGY REQUIRING TRANSPORT REGULATION

Herman M. Kalckar *

Department of Biochemistry, Harvard University, Cambridge, Massachusetts 02138

KEY WORDS: ATP, molecular enzyme defects in infants, galactose chemotactic mutants, energized down-regulation.

CONTENTS

* The Editorial Committee was saddened to learn that Dr. Kalckar died at the age of 83 on May 17, 1991.

My School and Family

This autobiography, requested by the Editorial Committee of the *Annual Review of Biochemistry*, has, I hope, mobilized my wit sufficiently to make the accidental reader dwell for more than a few moments and to raise curiosity about my intellectual pursuits in Copenhagen, where I grew up.

Copenhagen at that time was often referred to as "The Athens of the North." Although I was an erratic pupil in school and occasionally felt miserable, I have many interesting and rewarding memories from the old-fashioned school I attended, which, according to modern standards, especially for athletic activities, must be considered very modest. Its name was "Østre Borgerdyd Skole," which literally meant "the school for civic merits in the Eastern (Østre) borough of Copenhagen." I suppose that "merits" were meant to be applied to a larger part of the world than that quiet, attractive part of the Danish capital in which the school was located (the three Kalckar sons, of which I was the middle one, lived in easy walking distance from the school).

The Athenian flavor of "Borgerdyd Skolen" could not help making itself felt. The retired "Rector" (headmaster), J. L. Heiberg, was a world-renowned scholar in Greek, having translated Archimedes from Latin to Greek with great precision before the original Greek inscriptions were discovered. Our physics teacher, H. C. Christiansen, from the heather-covered, austere region of Western Jutland, was a formidable and passionately devoted teacher in mathematical physics. He had written a very concentrated, and in many ways very fine, textbook in physics, in which calculus played a prominent role. We were a little scared of him, because his temper would flare up if we tried to cut corners in our preparations and tried instead to memorize the text and formulas from his book. Christiansen insisted in no uncertain terms that physics and calculus, vector analysis, etc can only be grasped by using pencil and paper over and over again in order to find the right polarity in the fine network of a creative scientific argument. I dare say that we got the Ørsted-Ampere rules right.

I have allocated so much space to this alert teacher because I happened later to choose biological research and teaching as my occupation. Scientific arguments in pursuit of "truth," or I prefer the term "high fidelity," require a special kind of alertness.

Although our education in biology in school was largely systematic and somewhat static, it was brightened greatly by some extraordinary demonstrations in human physiology by a world-famous "Jutlandian" zoo-physiologist, August Krogh, professor at the University of Copenhagen and Nobel Laureate of 1923 for his description of capillary blood flow and its regulation. Krogh was, to my knowledge, the only physiologist in the 1920s who took an active interest in introducing the principles of human physiology to Danish

high school boys. Krogh had constructed the microtonometer for measuring oxygen tension in small samples of blood as well as a respiratory spirometer for metabolic measurements. The spirometer and the ergometer bicycle were very popular at these school demonstrations.

Although I was perhaps the most restless of three, very different, brothers, the relatively modest Kalckar home where I grew up was an oasis for me. Here most of my interest in the humanistic disciplines developed and thrived. My mother, Bertha Rosalie, born Melchior, read French (Flaubert, Proust, etc) and German (Goethe, Heine, Lessing, etc) and spoke both languages. English came home at a slower rate (see below). Both my parents insisted that I treat the Danish language with care, reverence, and love. We belonged to a middle-class, Jewish-Danish family—Danish for several generations. We were mainly free-thinkers; however, my mother was attached to the Jewish faith and thought that it behooved us to acquire some religious education under the auspices of the main synagogue in Copenhagen and learn a minimum of Hebrew. My father, Ludvig Kalckar, took interest in reading Jewish and Yiddish humorists. All of us, however, responded most to Danish humor: cartoons, limericks, prose, and especially the theater.

My father, born in 1860, attended the world premiere of Ibsen's "A Doll's House" at the Royal Theater in November 1879. He wrote an enthusiastic report about the event. Although he was a businessman by necessity rather than by choice, my father carried on his profession as a consultant and broker most conscientiously. During World War I, he could easily have earned 10 to 20 times more than he did, had he not stubbornly refused to deal with stocks. He said in his quiet, unassuming way that he did not care to earn money on sunken ships and lost lives, and he "meant business." He always insisted, in his shy fashion, that this was a personal issue, and that he enjoyed seeing his relatives and friends socially, speculators and nonspeculators alike. So in spite of my father's late evening hours in his office, we did not grow rich. My mother was very patient and understood his principles. During the war she helped the International Red Cross in its relief efforts for prisoners of war and their families.

In contrast to its experience in World War II, Denmark was rather lucky during and after the World War I, almost too lucky in terms of gluttony, be it profiteering at the stock market or in the wild export of milk products.

We were very interested in the history of Denmark's relations with its neighbors, especially Germany. Germany under the Kaiser was not felt to be a trustworthy neighbor. Like so many other Danes, neither of my parents liked the Kaiser. My father had an extensive library on the history of Slesvig and Holstein, the disputed border provinces annexed by Bismarck in 1864. In 1918–1920, the popular, nationalistic Danish King Christian X wanted both provinces returned to Denmark. My Francophile mother fully supported this

idea. In contrast, my father felt that restraint was more important than blind nationalism. He supported the return of North Slesvig, but felt unable to make a judgment in regard to the southern provinces. Fortunately, Denmark was blessed with a democratic statesman, Danish by birth and by sentiment, yet very familiar with the populations of both North and South Slesvig. His name was Hans Peter Hansen-Nørremølle, and he and his equally wise friend, H. V. Claussen, approached people unfailingly as human beings, regardless of whether they were from North or South Slesvig.

The verdict of their fair and patient inquiry was that Denmark should concentrate its efforts on North Slesvig, where the pro-Danes were in the majority, and not on South Slesvig, where the majority of the population remained pro-German. The liberal Danish government at that time respected this genuinely democratic recommendation, although it was not universally popular at the time. In general, the liberal Danes at that time showed great and truly democratic foresight. Imagine the abysmal conditions in Denmark under the Nazi occupation 1940–1945 if it had annexed the Slesvig-Holstein provinces. The real Nazi terror in Denmark started only three years after the invasion. I may be wrong in my analysis of these factors; after all why should the brave Norwegians have been exposed over five years to Nazi Terror? Both countries were lucky to have their staunch Kings at that time, Christian X and the heroic Haakon VII of Norway.

My Two Great Teachers

Before discussing my debut in research on biological phosphorylations in 1935, it seems fitting to pay my special respects to my two great teachers, Ejnar Lundsgaard and Fritz Lipmann. Fritz Lipmann's memorial words about Ejnar Lundsgaard in 1968 are so beautifully chosen that I have selected excerpts from them:

> Word has come from Copenhagen of the death, during the last days of 1968, of Ejnar Lundsgaard. In these times of rush and short recall, I am particularly keen to refresh the memory of the scientific community about his truly great discovery which is now slipping into the background. Personally and scientifically, I encountered Lundsgaard during my period of maturation and I owe him much. When I moved to Copenhagen in 1932 and stayed until 1939, we saw each other a great deal. In the fall of 1967, his friends and colleagues went to Copenhagen to celebrate the 40th anniversary of the discovery of what he called the a-lactacid contraction of iodoacetate-poisoned muscle. When this startling news reached us in Meyerhof's laboratory, it was very upsetting to our group, which looked upon glycolytic lactic acid as the link between metabolic energy generation and muscle contraction.
>
> The interest in iodinated organic compounds which might have metabolic actions similar to thyroxin induced Lundsgaard to study iodinated acetic acid. Its injection into animals, however, yielded a rather unexpected effect: for a few minutes the animal behaved quite normally, but suddenly it turned over and its muscles became rigid. Such rigor was dependent on prior muscle activity, since denervated or curarized muscles did not respond.

But when rigor developed, the expected burst of lactic acid was missing. Lundsgaard elegantly solved the puzzle. He showed that: (i) iodoacetate inhibited glycolysis; and (ii) poisoned muscle performed a limited number of normal contractions at the expense of dephosphorylation of creatine phosphate. then newly found in need of a function. The rigor mortis-like condition developed when the limited supply of creatine phosphate was exhausted.

Lundsgaard had discovered that the muscle machine can be driven by phosphate-bond energy, and he shrewdly realized that this type of energy was "nearer," as he expressed it, to the conversion of metabolic energy into mechanical energy than lactic acid. He was right, because it soon developed that the glycolytic reaction is a feeder of phosphate-bond energy and not of acid. On the way, Lundsgaard also provided an enormously useful tool for studying enzyme mechanisms. Iodoacetate has become one of the standard reagents for SH-blocking in enzymes. Thus, iodoacetate inhibits glycolysis because it blocks the functional SH in phosphoglyceraldehyde dehydrogenase.

In the middle thirties, Lundsgaard became professor of physiology at Copenhagen University and trained many biochemists and physicians. Herman Kalckar was one of his graduate students. And, even though I did not formally work with him, I consider myself his pupil. My subsequent work was profoundly influenced by his discoveries, which changed our concepts of metabolic energy transformation.

First Descriptions of Oxidative Phosphorylations in Tissue

During my studies in physiology and pathology at the University of Copenhagen, my interest was captivated by a few revolutionary developments in cell physiology that forced us all to reinterpret the role of lactic acid in mammalian cells. Otto Warburg found that tumor cells were unable to suppress their high generation of lactic acid formation even if they were supplied with excess oxygen. Ejnar Lundsgaard discovered that muscle contractions could occur without the formation of lactic acid, and Fritz Lipmann was able to interpret the Pasteur reaction. Lundsgaard found that frog muscles that have been exposed to iodoacetate are only able to perform a limited number of twitches; the phosphocreatine splits into phosphate and creatine. Lipmann was the first to believe in Lundsgaard's discovery as well as his interpretation. A few years later, in 1933, Lipmann was invited to Copenhagen. I was indeed lucky to have the good fortune of having Lundsgaard and Lipmann become my mentors.

In 1934 Lundsgaard became chief of the physiology department. His time was therefore more restricted, and so Fritz Lipmann became my foremost mentor. Lipmann encouraged me to study the newer literature on carbohydrate and phosphate metabolism in isolated mammalian tissue extracts or tissue particle preparations. For example, Hans Krebs seemed to have had much luck in disclosing different types of respiratory metabolism in tissue particle preparations from a variety of organs, especially pigeon breast muscle. About the same time, he formulated the tricarboxylic cycle, later called the Krebs tricarboxylic cycle.

For a variety of reasons, I elected to study the metabolism of phosphate and carbohydrate in extracts of kidney cortex. One might argue that pigeon breast muscle, bristling with enzymes catalyzing the Krebs cycle, would be superior. In order to obtain this tissue, however, I would have had to train myself to cut off the heads of pigeons, and I greatly preferred to avoid killing animals, probably from a lack of courage. In any case I was lucky to obtain fresh mammalian kidney from the perfusion experiments on anesthetized cats and rabbits, conducted at least once a week in the physiology department.

My studies on phosphorylation and respiration in kidney cortex extracts turned out to be rewarding. In the presence of oxygen (aeration in Warburg manometer vessels), inorganic phosphate was captured and esterified to various substrates such as glucose or glycerol. A special gift of 5'adenylic acid (5'AMP) from the late Dr. Pawel Ostern of Lwow, Poland gave me additional valuable insight. The kidney extracts converted the 5'AMP to adenosine triphosphate (ATP). When phosphatase activity was arrested by the addition of sodium fluoride, the P/O ratio approached 1. Under anaerobic conditions, no phosphorylation was detectable,[1] even in preparations well "spiked" with fluoride. I also noted that addition of dicarboxylic acids, like fumarate or succinate, stimulated oxygen consumption as well as phosphorylation. I never managed to get P/O ratios higher than 1.5, however, and then only if succinate was added to the cortex preparation (1).

Oxidative phosphorylation remained at about the same order of magnitude when I merely added fumarate or succinate to the extract, i.e. in the absence of glucose. This observation seemed very puzzling, and it induced me to pursue this particular aspect. I found that fumarate (or malate) in the absence of glucose not only stimulated respiration, but also gave rise to an accumulation of a phosphoric ester with properties different from those of hexose phosphate or glycerophosphate. What was the nature of this phosphoric ester? In pursuing this question, I once more received encouragement and advice from Lipmann.

In 1934–1935, work by Gustav Embden opened up new perspectives regarding phosphoric esters and their role in glycolysis; this new panorama was further expanded with the discovery of *phosphoenolpyruvic acid* (PEP) by Lohmann & Meyerhof. It was customary at that time to use the stability of phosphoric esters in acid or alkali as a first criterion of the nature of the different esters. The properties of the phosphoric ester formed in my fumarate experiments did not correspond to those of ATP. Since PEP was able to serve as a phosphate donor, and even able to catalyze the conversion of ADP to ATP, PEP attracted my special interest. PEP was known to be chemically

[1]The awareness that respiration stabilizes P-ester levels was expressed in 1932 not only by Lundsgaard but independently by Engelhardt in his work on nucleoated avian erothrocytes, to which he added redox dyes.

Figure 1 V. A. Belitser, H. M. Kalckar, June 1960, Kiev

dephosphorylated rapidly at room temperature in alkaline iodine and even more rapidly by mercuric chloride. I used these highly specific criteria and found that the ester formed from fumarate or malate fulfilled all of these criteria. PEP seemed indeed to be formed in the cortex preparations from oxidation of malate in the presence of inorganic phosphate.

This type of generation of PEP could scarcely have originated from hexose phosphate, since the presence of sodium fluoride barred not only phosphatase activity but, as shown by Lohmann & Meyerhof, enolase activity as well; in this way the conversion of 2-phosphoglycerate to PEP was arrested. I therefore believed that with fumarate or malate in the experiments, PEP must have originated from one of the dicarboxylic acids, including oxaloacetate, all members of the Szent-Gyorgyi-Krebs di-tricarboxylic acid pathway. I published my new observations in *Nature* (3).

Lipmann's response to my discovery of PEP generation from dicarboxylic acids in respiring kidney cortex dispersions was unreservedly positive. As I mentioned, in spite of my repeated efforts, I never succeeded in obtaining P/O ratios significantly above 1.5. The further development in this direction we owe to V. A. Belitser in the USSR; in an extensive paper on oxidative phosphorylation he showed that pigeon breast muscle and rabbit heart muscle,

in the presence of oxygen, are able to bring about phosphorylation of creatine to phosphocreatine. Belitser & Tsybakova showed that in the presence of arsenous acid and fluoride, the oxidation of succinate to fumarate did in fact generate P/O ratios as high as 3–4. The paper appeared shortly before the outbreak of World War II in 1939 (2). Much later, in June 1960, I had the privilege to meet Belitser in Kiev (see photo, p. 7), when the National Academy of Sciences sent me to the USSR Academy (Akademi NAYK) at the beginning of the scholarly exchange between the two academies.

In 1938, I was an active participant at the International Congress of Physiology in Zürich. I presented some of my recent findings on the oxidative phosphorylation of glycerol and the generation of PEP from dicarboxylic acids (3). My text and communication were presented in German, since I did not yet trust my ability to lecture in English. However, Professor R. A. Peters from the University of Oxford, who was chairman of the session, and Dorothy and Joseph Needham, University of Cambridge, were actively interested in some of the metabolic aspects of my topic, and they asked questions. I therefore had to switch to English, and enjoyed a very reassuring dialogue. I can still see and hear the very tall Joseph Needham rising and introducing himself to the audience: "Joseph Needham, Caius ("Kee-ees") College, Cambridge—can I ask a few questions?" My English apparently sufficed.

Plans for Leaving Denmark

While I was trying to finish my PhD thesis, we lost my younger brother Fritz, just six months after his exciting journey to California in 1937. He died suddenly in a "status epilepticus," which, according to his California friends, he had been close to a couple of times in 1937. His fatal attack happened barely a year before the effective treatment of epilepsy became available in the United States and Denmark. My mother never got over this loss. The Bohr family, who also loved Fritz dearly, rendered deep personal comfort. We were touched by Niels Bohr's speech at the modest cremation ceremony and by his request to bring with him young Aage Bohr, who had enjoyed a warm friendship with Fritz. This all happened in January 1938. My PhD thesis became dedicated to the memory of Fritz Kalckar.

Although I admittedly was depressed during 1938, I had much encouragement and inspiration as well. Lundsgaard and Lipmann were formidable mentors and generous personalities. Funding for my research came from three sources: the University of Copenhagen, financed by the Ministry of Education; the Carlsberg Foundation; and the Rockefeller Foundation.

My personal salary for teaching, during the last years as assistant professor, came from the University of Copenhagen. The medical students who came to my afternoon lectures in physiology numbered 30 to 40, only about 20–30% of those who showed up at the professor's morning lectures. Yet I felt that I

had the cream of the crop of medical students and that we generated a sophisticated dialogue. Our texts were Lundsgaard's excellent textbook interwoven occasionally with the British classic, "Starling's."

In the late 1930s the defense for a "Dr. Med." degree was a highly ceremonial event, whether at Danish, Swedish, or Norwegian universities. In my case, the ceremony took place in the old original section of "København" under the auspices of the almost 500-year-old university, in the middle of January 1939. My formal defense of my thesis was not as formidable as Professor Lundsgaard's concentrated and stylish opposition, but once he was over the critique of the language (mainly my casual Danish text and some of the tables), the scientific dialogue proceeded very well indeed. Lundsgaard agreed with Lipmann that the thesis revealed several new and important features regarding a relatively new field, metabolic biology, barely 20 years old.

During 1938 I came to know a most interesting scholarly chemist and biochemist, Kaj Linderstrøm-Lang at the Carlsberg Laboratory. Lang attracted scores of bright young biochemists, especially from Great Britain and the United States. Among the latter I came to know one of Lang's favorite American scholars, Rollin Hotchkiss from the Rockefeller Institute. By 1937 he and Lang had already developed protein chemistry along fundamentally new lines.

Through Lipmann, Lang had developed a strong interest in the bioenergetics of protein synthesis and wanted to learn more about phosphorylation systems. Lang fully agreed with Lipmann that the postulate that peptidases catalyzed synthesis as well as hydrolysis of peptides in proteins was not the answer to the problem of cellular protein synthesis. I was mainly a listener to these fascinating discussions and cherished the ideas that transphosphorylations and ATP might well be involved in cellular protein synthesis. Lipmann's visionary ideas began to take shape in 1938 via his bold experiment in which he generated ATP from his "homemade" acetylphosphate, catalyzed by a bacterial enzyme. This was performed in early 1939, after I had left Denmark, and only six months before the outbreak of World War II. It was Lipmann's last performance in Denmark during his last months there.

In 1938, Dr. Warren Weaver, Director of the Rockefeller Foundation, visited Copenhagen. My candidacy for a research fellowship was being discussed. I was appointed a Rockefeller Research Fellow in January 1939, shortly after my defense of my PhD degree at the University of Copenhagen. Now came the important problem: Which laboratory in the United States would stimulate and widen my horizon in general biology and bioenergetics? Linderstrøm-Lang was very familiar with Cal Tech's Departments of Chemistry, with Linus Pauling and Charles Coryell, and Biology, with the great geneticists T. H. Morgan, A. Sturtevant, and Calvin Bridges. Bridges had

once shown my brother the *Drosophila* laboratory. Lang also knew the biochemist there, Henry Borsook, who was interested in thermodynamics and the bioenergetics of peptide formation. Borsook still operated on the old-fashioned notion of reversal of peptidase activity in order to reclaim polypeptides and knew as yet nothing about phosphorylations. I followed Lang's recommendation and decided on Cal Tech.

California Institute of Technology and Writing a Review on ATP

In January 1939 I visited London for the first time. My purpose was to visit Dr. Robert Robison, an outstanding biochemist and the Editor of the *Biochemical Journal*. He was instrumental in publishing my fumarate-PEP paper (4) in the journal. Although I had decided to spend most of the year 1939–1940 at Cal.Tech's Biology Department, I wanted to make a brief stop at Washington University in St. Louis, where Carl and Gerty Cori worked and where in 1936 they had discovered the first phosphorolytic fission with isolation of glucose-1-phosphate, generated from glycogen and catalyzed by a muscle enzyme, which they now called phosphorylase. I arrived in the bleak and sooty city of St. Louis in February 1939, and was well received by the Coris. Unexpectedly, they were actively interested in my articles on oxidative phosphorylation but were unable to repeat the experiments. Gerty wanted me to make a few demonstrations, but Carl cut it short and simply asked a few questions, and so did I. I saw that the Coris used the old-fashioned Meyerhof extract technique, using test tubes. I explained to them that kidney cortex extracts show a very high oxygen consumption, and in order to see oxidative phosphorylation I always aerated the cortex extracts by shaking them in Warburg vessels.

A bright young researcher in the Cori department, Dr. Sidney Colowick, witnessed my discussion with the Coris. He was asked to compare my technique with theirs. Colowick soon confirmed that the simple shaking technique was indeed needed in order to observe oxidative phosphorylation in the cortex preparations. The Coris wrote to me with the good news and with their best "thanks." This response may well have prepared the way for Carl Cori's later generous invitation to come to their lab when my first-year fellowship was running out.

On March 1, 1939 I arrived in Pasadena. I was received by a young student who soon became my good friend, David Bonner, originally from Salt Lake City. My other friend there was Max Delbrück, whom I had met briefly in Copenhagen at Bohr's Institute. I should later share many interesting reunions with Delbrück.

Thanks to the Bonner brothers, James and the younger David, I managed to find a bungalow shortly after the arrival of my wife Vibeke. We all agreed

that the Cal Tech faculty club, "Atheneum," was nice but too expensive as a domicile for a research fellow. The Bonners were also most helpful in introducing me to a superb auto mechanic, José Plannes (of Basque origin); José was THE auto mechanic all the young Cal Tech scholars turned to then. José helped me superbly too. A Ford coupe, vintage 1934, was for sale and I bought it. It held up, not only on desert roads but in the mountains as well. After I received my driver's license, I had the nerve in early April to drive Dr. Hugo Theorell from Stockholm the whole way up to Mt. Wilson observatory, which he wanted to see. The snow was just beginning to melt.

Through Theorell I met Linus Pauling, who later came to play a very positive role in my career. I gave seminars and began to write an extensive review, much inspired by one of Dr. Linus Pauling's former coworkers, Dr. Charles Coryell, who was very interested in the energetics of phosphoryl donors like phosphocreatine and ATP. Pauling's ideas about resonance had greatly influenced Coryell's thinking, and I incorporated some of this into the review. I did not know then about the intriguing review that Lipmann had started, for which he was collecting thermal data. Lipmann's review was to have a great impact in biochemical circles.

I was lucky in the summer of 1939 to be enrolled in a combined lab and lecture course taught by an unusual microbiologist, Dr. Cornelius B. van Niel, who, perhaps more than anybody else I had then encountered, sensed what he called "The Unity of Life." van Niel was Dutch and a graduate of Delft University, Biotechnological School, a highly distinguished school. There he wrote his PhD thesis about a new type of sugar fermentation catalyzed by certain bacteria that generated propionic acid and carbon dioxide. van Niel reminded me that a Danish cheese expert, Orla Jensen, had first sniffed the existence of such a type of fermentation when he found out that the gas in the "eyes" of Swiss cheese was pure carbon dioxide. I had not paid attention to these matters while in Denmark. Perhaps worse, I was ignorant of some important work at the Carlsberg Lab. O. Winge had made great basic contributions in yeast genetics by describing the existence of diploid yeast as well as monoploid. Clearly my fixation on the four "big L's" had made me overlook the great tradition of Danish genetics at that time, founded by Winge's great teacher and researcher, Wilhelm Johannsen.

Although the van Niel Lab was very near the Pacific coast (in Pacific Grove, California), we discussed rather sparingly the role of the microorganisms in the ocean, except algae and the chemosynthetic microorganisms. Photosynthesis was, of course, broadly covered and various postulates critically discussed. These discussions inspired me to expand my biochemistry review that I had started at Cal Tech.

The autumn of 1939 was unusually hot, even for Pasadena, in stark contrast to the climate of Pacific Grove. Fortunately, the hospitable Physics Professor,

C. C. Lauritsen, and his wife invited us over to their swimming pool. November was sunny and pleasant, as was December 1939. My wife and I were invited to a real traditional American Christmas dinner at the Morgan's (T. H. and Lilian) in the stylish old house built and designed by the founder of The Kerckhoff Institute for the Biological Sciences. The founder was of course Dr. Thomas Hunt Morgan himself, and the multidisciplined institute had largely served as my scientific home base. Morgan asked about Denmark and more specifically about Niels Bohr.

In early 1940 I said farewell to my friends at Cal Tech, and Linus Pauling generously offered to get my extensive review on bioenergetics published in *Chemical Review*, provided I could manage to send him a finished script by the end of 1940.

We sold our car and left California in January 1940 and travelled more or less comfortably by train to the Midwest. I sat up most of the night on the train to St. Louis writing page after page of the review for Linus Pauling. After a brief couple of days in colorful New Orleans, we continued north, arriving late February 1940 in the damp and smoky St. Louis. It was a depressing city, but there I had the benefit of working in a lab that had developed a new type of enzymology, which now had become my main interest.

I had partly lost my enthusiasm for pursuing P/O ratios in various tissue particle preparations. Yet Carl Cori and his able coworkers had managed to obtain good P/O ratios in heart tissue dispersions, keeping in mind my warning against using unshaken test tubes for trying to obtain P/O ratios in tissue dispersions. Admittedly, by now I would rather have learned enzymology "a la Cori." Nevertheless, Carl persuaded me first to participate in collaborative work with him and Sidney Colowick on P/O ratios. Later, Sidney and I tried to delve into enzymology on our own. Thus began some of my happiest months in research.

While writing these lines I realized that W. A. Engelhardt and M. N. Lyubimova discovered 50 years ago that myosin was endowed with ATP-ase activity (5). In my big composition entitled "The Nature of Energetic Coupling on Biological Synthesis" (6), I took the liberty to make a speculative sketch from the Engelhardt discovery on the possibility that phosphorylation and dephosphorylation of myosin are "synonymous" with contraction and relaxation of myosin.

More important than my speculative efforts of 1939 on the ATP-ase report, however, was Engelhardt's experimental design, which was based on a hypothesis that expressed much the same ideas, albeit with experimental data. The interesting article by Engelhardt came to light in the *Yale Journal of Biology & Medicine* 15:21–38 (1942–1943), thanks to Paul Talalay's translation from the Russian [Engelhardt, W. A. 1941. *Adv. Contemp. Biochem.* (USSR) 14:172–90; cf. Kalckar, H. M. 1969. *Biological Phosphorylations,*