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HERMAN MORITZ KALCKAR

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A Biographical Memoir by

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HERMAN MORITZ KALCKAR died in Cambridge, Massachusetts, on May 17, 1991, at the age of eighty-three. His scientific career spanned much of the period of development of modern biochemistry, to which he made contributions of central importance.

Kalckar was born in Copenhagen on March 26, 1908, into a family he described in an autobiographical essay as having been middle-class Jewish-Danish for many generations. Kalckar's broad interests in literature and the arts had their origins in his early family life. His mother, Bertha Rosalie Melchior Kalckar, read widely in French and German as well as Danish literature. His father, Ludvig Kalckar, a businessman, was devoted to music and the theater. Ludvig Kalckar attended the world premiere of Ibsen's "A Doll's House" at the Royal Theater in Copenhagen in November 1879 and later wrote an enthusiastic review of it. Herman Kalckar traced some of his own enthusiasm for music, and in particular for Mozart, to his father's example.

Herman's younger brother, Fritz Kalckar, was a gifted physicist and a colleague and protégé of Niels Bohr. The death of Fritz in 1938 at twenty-eight years of age was a devastating blow for the entire family.

Kalckar received his early schooling in the Ostre Borgerdyd Skole, located within an easy walk of his home in Copenhagen. The headmaster, J. L. Heiberg, was a Greek scholar of international repute, and Kalckar paid tribute to the "Athenian flavor" of the school. He felt a special gratitude to the physics teacher, H. C. Christiansen, whom he recalled many years later as a formidable and passionately devoted teacher.

Kalckar completed his studies for a degree in medicine at the University of Copenhagen in 1933 and then began his scientific career in 1934 as a candidate for the Ph.D. degree in the Department of Physiology under the direction of Ejnar Lundsgaard. Lundsgaard had earlier made the important finding that frog muscles poisoned with iodoacetate and therefore unable to carry out glycolysis (the splitting of glucose to lactic acid) are nevertheless capable of carrying out a limited number of contractions. Lundsgaard later showed that these "nonlactic" contractions were at the expense of the dephosphorylation of creatine phosphate, which had been discovered and characterized only a few years earlier by Cyrus Fiske at the Harvard Medical School.

In 1932 Fritz Lipmann, unable to work in Nazi Germany, moved to Copenhagen, where he was closely associated with Lundsgaard. Lipmann became one of Kalckar's mentors and a close friend, a relationship that was to be lifelong. Lipmann was already deeply interested not only in Lundsgaard's work on the role of phosphate esters in muscle contraction but also in the biological functions of phosphorylation reactions more generally. In his masterly and highly influential review in 1941 Lipmann was to emphasize the central role of adenosine triphosphate (ATP) as an "energy-rich" phosphate ester, the breakdown of which to adenosine diphosphate (ADP) and inorganic phosphate (Pi) drives not only muscle contraction but also a host of other energy-requiring processes. Because the cell has a limited supply of ATP,

the ADP formed by its breakdown must be continuously rephosphorylated to ATP. In muscle this may be done by use of creatine phosphate, a cellular reserve of "energy-rich phosphate" that is present, however, only in limited amounts. At that time the only primary source of energy known to biochemists for the rephosphorylation of ADP to ATP was the splitting of glucose to lactic acid in muscle or in yeast to alcohol and carbon dioxide. The brilliant achievements of Otto Warburg (which both Kalckar and Lipmann greatly admired) had revealed the reactions by which ADP is phosphorylated to ATP during glycolysis.

Glycolysis, however, is a process that can occur anaerobically in the absence of molecular oxygen. Classic investigations by Pasteur had made it clear that aerobic metabolism of glucose by yeast is vastly more energy efficient than the anaerobic process. How is energy captured by the oxidation of sugars and other foodstuffs linked to the reduction of molecular oxygen? This was the question confronted by Kalckar as he began the investigations in the period 1937-39 that led him to the demonstration that cell-free extracts of kidney cortex catalyze oxidative phosphorylation—that is, the formation of ATP in reactions strictly dependent on the reduction of oxygen and independent of glycolysis. An important technical point in these experiments was the use of sodium fluoride to inhibit interfering phosphatases that otherwise would break down ATP and other phosphate esters almost as soon as they were formed.

As is now well known, aerobic nonphotosynthetic organisms, including ourselves, derive vastly more metabolic energy from oxidative phosphorylation than from any other source. It is estimated that the complete utilization of glucose in muscle leads to the production of about seventeen times more ATP via oxidative phosphorylation than is produced in anaerobic glycolysis. Oxidative phosphorylation is

therefore a process of central bioenergetic importance. It became (and still is) the object of intensive studies in many laboratories throughout the world. Localized in the inner membrane of the mitochondrion, oxidative phosphorylation proved extraordinarily resistant to biochemical dissection. Two decades were to pass before real insight was gained into the mechanism of oxidative phosphorylation with the development by Peter Mitchell of the chemiosmotic theory.

To this date many important features of oxidative phosphorylation remain imperfectly understood, but Kalckar's work opened the way to its systematic exploration.

At about the same time as Kalckar's experiments V. A. Belitzer, working in virtual isolation in the Soviet Union, made similar observations on oxidative phosphorylation in experiments on preparations derived from pigeon breast muscle. Although Belitzer's work did not become known in western Europe until a considerable time after Kalckar's first publications, it was characteristic of Kalckar that he was always generous in acknowledging Belitzer's contribution. During his trip to the Soviet Union in 1960 Kalckar looked up Belitzer in Kiev and took some pains to make arrangements to be photographed with him.

Kalckar's early experiments on oxidative phosphorylation also provided evidence for the production of phosphoenolpyruvate from fumaric or malic acids, observations that later provided an important clue to the mechanisms involved in the formation of glucose from noncarbohydrate sources in animal tissues.

Kalckar later wrote an interesting historical account of the origins of the concept of oxidative phosphorylation and his early experimental work on this theme (1974).

In 1939, having completed his work for the Ph.D. degree, Kalckar was appointed a Rockefeller research fellow for a year of postdoctoral study at the California Institute of Tech-

nology. On his trip across the United States he stopped at the famous laboratory of Gerty and Carl Cori at Washington University in St. Louis, then one of the few centers of the "new biochemistry" in the United States. There he found Sidney Colowick, then a graduate student, attempting to duplicate Kalckar's experiments on oxidative phosphorylation, without success. Colowick, untrained in the methods introduced by Warburg and followed by Kalckar, had been simply incubating tissue extracts in test tubes without providing for the efficient diffusion of oxygen into them. Thirty-five years later Colowick summarized Kalckar's helpful advice: "'Shake it!' said Dr. K., and everything was OK!"

Kalckar's stay in California allowed him to take the famous microbiology course in Pacific Grove taught by C. B. van Niel, whose insight into the underlying unity of the biochemistry of living organisms and charismatic personality made a deep impression on Kalckar, as on so many others. This experience may have planted a seed that led later to Kalckar's interest in microbial molecular biology.

During his stay in Pasadena, with the encouragement of Linus Pauling, Kalckar undertook the preparation of a comprehensive review of bioenergetics with emphasis on the role of phosphate esters in energy transduction. Its publication (1941) did much to advance the new ideas that he and Lipmann were pursuing.

In 1940 Kalckar accepted an appointment as research fellow in Cori's Department of Pharmacology at Washington University. The invasion of Denmark by the Nazis in the spring of 1940 had made it impossible for Kalckar and his wife, Vibeke, to return to Copenhagen, and Kalckar was fortunate to have the opportunity to spend the next three years in a stimulating and productive environment with congenial colleagues. He joined forces with Sidney Colowick. Their work led them to the discovery in muscle extracts of

a remarkable enzyme, named myokinase by them, but now more precisely called adenylate kinase, that catalyzes the following readily reversible reaction:

$$\text{ATP} + \text{AMP} \longleftrightarrow 2\ \text{ADP} \qquad (1)$$

Many biological processes lead to the production of adenosine monophosphate (AMP), which, however, cannot be phosphorylated to ATP during oxidative phosphorylation or glycolysis, processes that are specific for ADP as phosphate acceptor. In the absence of reaction (1), which "rescues" AMP by converting it to ADP, all of the adenine nucleotides of the cell would be irreversibly converted to AMP. Later experiments by other workers showed that mutations that block the activity of adenylate kinase are lethal to cells of *Escherichia coli*.

In 1943 Kalckar was appointed research associate at the Public Health Institute of the city of New York. One of the attractions of the post was a laboratory equipped with a new ultraviolet spectrophotometer, then still a rather rare instrument. Kalckar developed spectrophotometric methods for the study of the metabolism of nucleosides and nucleotides, the building blocks of RNA and DNA. It had earlier been reported by Klein that the enzymic hydrolysis of nucleosides is stimulated by the addition of phosphate or arsenate. Coming from the Cori laboratory, the center of work on glycogen phosphorylase, Kalckar realized the possible significance of the role of phosphate and soon made the important discovery that the phosphorolytic cleavage of nucleosides is similar to that of glycogen.

$$\text{ribose-hypoxanthine} + \text{Pi} \longleftrightarrow \text{ribose-1-P} + \text{hypoxanthine} \qquad (2)$$

The reaction is readily reversible. Indeed, the equilibrium position lies to the left of Equation (2) as written, favoring the synthesis of the nucleoside rather than its breakdown. As the first demonstration of the enzymic synthesis of a nucleoside, this work attracted considerable attention. It must be recalled that in 1945, when this work was done, very little was known of the metabolism of nucleic acids or indeed of their functions in living cells. Later work was to show that nucleoside phosphorylases function as "salvage enzymes" in the degradation rather than the synthesis of building blocks of nucleic acid.

In 1946 Kalckar returned to Copenhagen, where a new laboratory was set up for him with the support of Ejnar Lundsgaard and with financial backing from American as well as Danish sources. The principal theme of research at the new "Cytofysiologisk Institute" was the metabolism of nucleosides and nucleotides. Kalckar attracted gifted young collaborators, such as Hans Klenow, Morris Friedkin, and Walter McNutt, and the laboratory became a leading center for work in this field.

In 1952 Kalckar began his studies on the metabolism of galactose in microbial and animal tissues. This became a principal pursuit after his move to the National Institutes of Health in 1952, first as a visiting scientist and later with a permanent appointment at the National Institute of Arthritis and Metabolic Diseases.

In mammals the utilization of galactose, a component of milk sugar and therefore a major constituent in the diet of infants, begins with its phosphorylation to galactose-1-P, which then must be converted to glucose-1-P, the further metabolism of which occurs by well-known reactions. The pioneering work of Luis Leloir led to the discovery of the central role of uridine diphosphate derivatives in the interconversion of galactose and glucose, sugars that are epimers, that is,

differing in configuration only at a single carbon atom (C-4). Leloir showed that the two sugars are interconverted in the form of their uridine diphosphate derivatives as in Equation (4), catalyzed by an epimerase, and suggested that the synthesis of uridine diphosphate galactose (galactose-1-P-P-uridine) might take place by reaction (3):

$$\text{galactose-1-P} + \text{glucose-1-P-P-uridine} \leftrightarrow \text{galactose-1-P-P-uridine} + \text{glucose-1-P} \qquad (3)$$

$$\text{galactose-1-P-P-uridine} \xleftrightarrow{\text{NAD}} \text{glucose-1-P-P-uridine} \qquad (4)$$

In the sum of (3) and (4) the uridine-linked forms of the sugars cancel out, and the overall reaction is:

$$\text{galactose-1-P} \longleftrightarrow \text{glucose-1-P} \qquad (3 + 4)$$

In 1953 Kalckar and his collaborators reported direct evidence that the synthesis of uridine diphosphate galactose does in fact occur in extracts of the yeast *Saccharomyces fragilis* by reaction (3), catalyzed by the enzyme galactose-1-P uridylyl transferase. They also found an alternative reaction for the synthesis of uridine diphosphate galactose in yeast:

$$\text{galactose-1-P} + \text{P-P-P-uridine} \leftrightarrow \text{galactose-1-P-P-uridine} + \text{P-P} \qquad (5)$$

It is important to note that reaction (5) does not occur in human tissues, in which reaction (3), catalyzed by the uridylyl transferase, is an essential step in the utilization of galactose as an energy source.

Kalckar devised a method to determine the levels of the

uridylyl transferase catalyzing reaction (3) in lysates of red blood cells, employing his favorite spectrophotometric approach. At just this time Kurt Isselbacher was also at NIH, carrying out research in collaboration with Gordon Tomkins and Julius Axelrod. As part of his clinical duties, Isselbacher was treating a child whom he diagnosed as suffering from galactosemia, a severe inherited disorder characterized by the inability to break down galactose, which leads to accumulation of high levels of galactose in blood and tissues. Isselbacher sought out Kalckar, and a collaboration was begun that soon led to the finding that the enzyme defect in the most serious form of human galactosemia is in the uridylyl transferase that catalyzes reaction (3). This in turn led to the development of a simple test for the presence or absence of this enzyme in red blood cells that is now widely used to screen newborn infants for galactosemia, a disease that can be effectively treated by removal of milk and other sources of galactose from the diet. The development of this test was of great practical consequence since early diagnosis is vital to prevent severe mental retardation and other developmental defects.

In 1958 Kalckar accepted a professorship in the Department of Biology of Johns Hopkins University. This year also marked the publication of his highly original proposal that the contamination of foodstuffs from the fallout following atmospheric tests of nuclear weapons could be measured by the analysis of the content of strontium-90 in the milk-teeth of young children. As he pointed out, measurement of radiation from deciduous incisor teeth would reveal the levels of isotope ingested about seven years previous to shedding of the teeth, when the calcified structure of the teeth had been deposited. By this proposal Kalckar hoped to focus attention in a dramatic way on the pollution of the environment by tests of nuclear weapons. The idea attracted

considerable attention, and extensive collections of milk-teeth were in fact made, particularly by a group of researchers at Washington University in St. Louis. It was learned that milk-teeth from children born in 1956 contained about ten times more strontium-90 than teeth from children born in 1950. Fortunately, after the ban on atmospheric testing the levels fell once again to lower levels.

In 1961 Kalckar moved to the Harvard Medical School as professor of biological chemistry and head of the Biochemical Research Laboratory of Massachusetts General Hospital (MGH). He succeeded Fritz Lipmann in that position. Here he continued his studies on the metabolism of galactose in animal tissues with special attention to the epimerase that catalyzes reaction (4) above.

Kalckar now also became deeply interested in the role of the cell surface in sensory processes and in cell signaling and often referred to this field, then newly emerging, as "ektobiology." Winfried Boos, his young colleague at MGH, carried out an intensive study of the transport of galactose into cells of *E. coli*, which culminated in the isolation and detailed characterization of a specific galactose-binding protein that was shown to be an essential part of the transport system. Julius Adler and his colleagues at the University of Wisconsin discovered at about the same time that cells of *E. coli* can detect the presence of galactose in the medium and "chase" this sugar by a positive chemotactic response. Kalckar immediately suggested that the galactose-binding protein that could "recognize" galactose for transport might also be needed for the chemotactic response. The first tests of this notion, however, were disappointingly negative. Kalckar persisted in his idea, however, and further experiments revealed that an unexpected complexity of the transport system had rendered the first tests invalid. The final definitive experiments revealed that the binding protein plays a vital role

not only in the transport of galactose but also in chemotaxis, an outcome that gave Kalckar considerable satisfaction.

Kalckar was also greatly interested in the conversion of galactose to cell-surface lipopolysaccharides in bacteria, work vigorously pursued in his MGH laboratories by Hiroshi Nikaido. Nikaido's pioneering studies on the biosynthesis of lipopolysaccharide in *Salmonella* employed both genetic and biochemical approaches and did much to clarify the complex reaction sequences, particularly the role of lipid-linked intermediates.

Kalckar turned next to the problem of the regulation of the transport of sugars into mammalian cells and the importance of this process in tumor cells. This work was in part stimulated by a collaborative study in 1973 with Senitiroh Hakomori at the University of Washington in Seattle on carbohydrate utilization and the uptake of galactose in hamster cells transformed by polyoma virus.

In 1974 Kalckar retired as head of the Biochemical Research Laboratory but continued his research as visiting professor in the Huntington Laboratories at MGH until 1979. At that time he moved to the Department of Chemistry at Boston University as distinguished research professor, an appointment he greatly valued because it permitted him to continue his research interests in a new and stimulating environment to the very end of his life. The work on hexose transport and metabolism in normal and malignant cells continued to be the theme of much of the work in the laboratory at Boston University and the subject of many papers with his longtime collaborator, Donna Ullrey, the last of which was published only shortly before his death.

Kalckar's achievements in science brought him wide recognition, including election to the National Academy of Sciences, the Royal Danish Academy, and the American Acad-

emy of Arts and Sciences, as well as honorary degrees from Washington University, the University of Chicago, and the University of Copenhagen.

To his many friends, Kalckar's character and personality were as impressive as his scientific accomplishments. Throughout his entire career Kalckar won the affection and admiration of a large number of students and junior associates who were trained in his laboratory. The sweep of his intellect was very broad, his spirit was open and generous, and he had a wonderful sense of humor. Upon first acquaintance many found it difficult to follow the thread of his discourse, partly because he was apt to begin a new topic *in medias res* without explanatory preamble and partly because he often paid the listener the compliment of omitting from a chain of reasoning the links that seemed obvious. After one became accustomed to this style it enhanced the effect of his gentle, understated wit.

The same enlightened humanism that shaped Kalckar's tastes in music and the arts was evident in his view of world problems, as evidenced by his concern for the mounting threat of nuclear warfare and the dangers of continued testing of nuclear weapons, which he had dramatized by the milk-teeth collection project.

Kalckar's first marriage, to the musician Vibeke Meyer, ended in divorce in 1950. Three children, Sonja, Nina, and Niels, to whom he was deeply devoted, were born of his second marriage to the developmental biologist Barbara Wright. After dissolution of that marriage, Kalckar in 1968 married the interior designer Agnete Fridericia Laursen, who survives him. For the last twenty-three years of his life, Agnete's love and support were an essential part of Herman's life, and their home in Cambridge was a focus of warmth and hospitality for friends and colleagues.

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