THE CELL MEMBRANE

Its Role in Interaction with the Outside World

Edited by
Edgar Haber

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A Volume in Honor of Professor Herman Kalckar on His Seventy-fifth Birthday

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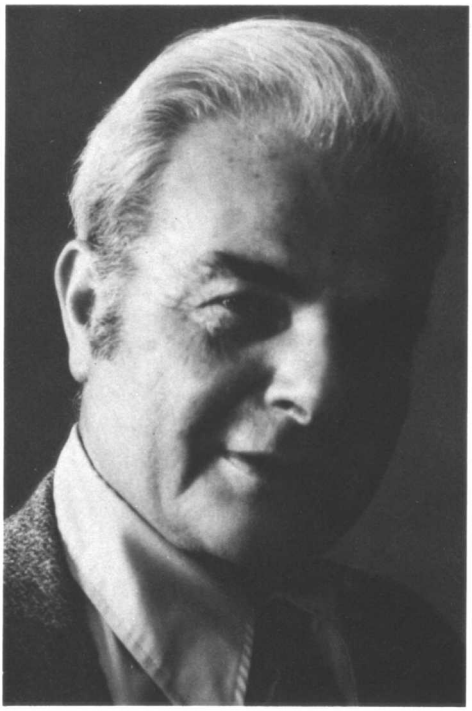

Herman M. Kalckar

PREFACE

The contents of this book reflect a symposium held in honor of Professor Herman Kalckar's seventy-fifth birthday. His impact on the history of biochemistry is reflected by the diversity of the contributions of his former students and friends. Speakers came from Asia, Europe, and the United States to discuss both procaryotes and eukaryotes. The unifying theme was the cell membrane, both its organization and its function. Ektobiology, a topic that has held the attention of Professor Kalckar for many years, was clearly defined as a central topic in biology. This subject deals with the key structure whereby the cell interacts with the outside world and which, in a sense, defines the boundary between what is the cell and what is not.

Topics discussed include the biogenesis of membrane proteins, sugars and lipids, the role of membrane components in osmoregulation, and mechanisms of nutrient transport. Of great interest is the system for surface recognition evolved in vertebrates, exemplified by the HLA system of man. Neoplasia causes changes in the cell membrane that may be of significant future potential in the diagnosis and treatment of malignancies as well as in the understanding of the process of transformation. The changes in glycosphingolipids and carbohydrate antigens in relation to oncogenesis are detailed. I should like to recognize Doctors Kurt J. Isselbacher, Phillips W. Robbins, Victor Ginsburg, and Hiroshi Nikaido for their assistance in organizing the symposium. Ms. Jean Brumbaugh deserves special thanks for putting this book together.

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HERMAN KALCKAR: AN APPRECIATION

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A Brief Biographical Sketch

Herman Moritz Kalckar was born of Ludvig Kalckar and Bertha Melchior in Copenhagen, Denmark, on March 26, 1908. He received his M.D. degree from the University of Copenhagen and later served as Associate Professor in the University's Institute of Medical Physiology. In January 1939 he left for the United States where he travelled under the auspices of the Rockefeller Foundation. He then worked successively at the Public Health Research Institute of New York and at the National Institutes of Health in Bethesda. In 1958 he was appointed Professor of Biology at Johns Hopkins University, and in 1961 as Professor of Biological Chemistry at Harvard Medical School and Wellcome Biochemist and Chief of Biochemical Research at the Massachusetts General Hospital. At present he is Emeritus Professor at Harvard University and Distinguished Research Professor of Biochemisty at Boston University.

Professor Kalckar is the recipient of many honors including membership in the National Academy of Sciences, and the American Academy of Arts and Sciences and foreign membership in the Royal Danish Academy. He has been a Harvey Lecturer and a Fogarty Scholar of the National Institutes of Health and recipient of honorary degrees from Washington University, the University of Chicago and the University of Copenhagen.

It is not my intent to write even a short biography of Herman Kalckar, but simply to point to a few highlights of his career, particularly the early and formative years during which a revolution in fundamental biochemistry was revealed, and the period when he had a major influence on the development of a relationship between

medicine and biochemistry with the discovery of the mechanism of galactosemia. His interest in Ektobiology that characterized the Massachusetts General Hospital-Harvard years are the subject of this volume. The most recent decade is discussed here in a chapter by C. William Christopher.

Oxidative Phosphorylation

Herman Kalckar's career was shaped by notable biochemists, many of whom became his close personal friends. During the Copenhagen days, his mentors were Ejnar Lundsgaard and Fritz Lipman. Seminal to the beginning of Kalckar's research was the major discovery by Lundsgaard that lactic acid formation alone could not explain the source of the energetics needed for muscle contraction. As a consequence of showing that iodoacetate-treated muscle could carry out a considerable amount of mechanical work anaerobically without any lactic acid being formed, the Meyerhof-Hill hypothesis was shattered. Phosphocreatine was shown to be essential by Lundsgaard's demonstration that there was a linear relationship between the amount of phosphate released from phosphocreatine and the amount of mechanical work performed by the muscle. Kalckar, working with kidney tissues, began to appreciate the respiration as a critical factor in active phosphorylation. He varied the nature of the electron donors and demonstrated that glutamic acid, dicarboxylic and tricarboxylic acids markedly increase Qo_2 as well as phosphorylation. Thus, the concept of oxidative phosphorylation was born. In 1937, Kalckar was able to show that in thoroughly dialyzed kidney extracts or washed kidney mince where glucose was absent, malate underwent oxidation and phosphopyruvate accumulated. Thus one of the main pathways of gluconeogenesis from pyruvate and CO_2 underwent its initial definition.

By the time Kalckar left for the United States as a Rockefeller Research Fellow in January 1939, his work on oxidative phosphorylation was already well recognized and of interest to a number of investigators, among them Robert Robison at the Lister Institute in London and Carl and Gerty Cori at Washington University in St. Louis. He stopped to visit each of these laboratories and in the latter demonstrated the methods that were needed to detect oxidative phosphorylation. By adequate aeration and the use of Warburg vessels, the Coris were able to confirm the phenomenon.

At the end of February of 1939, he had arrived at the California Institute of Technology, which was then, as today, a center of important research. He came in contact with Henry Borsook, Max Delbruck, Norman Horowitz, Thomas H. Morgan, and Alfred Sturtevant among others. In discussions with Charles Coryell, he was encouraged to write about energetic coupling via phosphorylation in biological syntheses. Linus Pauling effected the publication of this work in Chemical Reviews in 1941. This was a monumental paper

that had an exceptional impact on the field of biochemistry.

Hereditary Human Galactosemia

Galactosemia is a disease of infants characterized by severe vomiting, diarrhea, and jaundice. If ingestion of mother's or cow's milk is not stopped, permanent changes including cataracts, cirrhosis of the liver, and mental retardation occur. The consequences of the disease may be effectively prevented if milk (containing galactose) is omitted from the diet and other foods substituted. It is of particular interest that in the absence of lactose or galactose, normal development and growth is not impaired. With renewed lactose administration, galactosemia and galactosuria resume. How does the organism manage to grow normally without the ability to synthesize essential galactosyl compounds, such as galactolipids of the brain?

In 1956, Kalckar, working with Isselbacher and Anderson, designed a sensitive and specific methods that pinpointed the enzymatic defect. It had been demonstrated by other investigators earlier that the administration of galactose to the affected children gave rise to an accumulation of alpha-galactose-1-phosphate in red cells. This excluded a galactokinase defect as the basis of the disease. Kalckar and his coworkers did, however, identify a clear deficiency of galactose-1-phosphate uridylyl transferase. It is the conversion of Gal-1-P to UDP-Gal that is blocked, resulting in the accumulation of toxic level of of Gal-1-P. Since the conversion of UDP-Glucose to UDP-Gal is unimpaired, there is adequate substrate for the synthesis of essential galactose containing compounds from glucose compounds.

Aside from the interest in defining the mechanism responsible for a disease, this discovery had wider importance. It was a graphic demonstration to physicians of the value of biochemical investigation in understanding pathophysiology and a catalyst to further research on inborn errors of metabolism, of which many have now been defined. On a more personal level, it was the beginning of the distinguished research career, largely based on the biochemical investigation of disease, of the young postdoctoral fellow participating in the research, Kurt Isselbacher.

These vignettes are but a narrow window on the many contributions to science by Herman Kalckar. I hope that a biographer takes up the challenge of revealing Kalckar's entire career, which will certainly be an inspiration to all of us. As is clear in Christopher's chapter, it is a career that is still in full development.

THE CELL MEMBRANE OF PROCARYOTES

OUTER MEMBRANE PERMEABILITY OF GRAM-NEGATIVE BACTERIA IN THEIR INTERACTION WITH THE OUTSIDE WORLD

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ABSTRACT

Gram-negative bacteria including Escherichia coli and Salmonella typhimurium produce outer membranes that serve as an effective permeability barrier. In order to shut out noxious substances as much as possible, which include the bile salts in the case of enteric organisms, yet allow the rapid influx of nutrients, these bacteria have evolved porins, proteins with channels that are ideally suited for this purpose. Furthermore, the enteric organisms contain multiple species of porins, and have developed an elaborate mechanism of regulating the production of different species of porins so that their chances of survival would be maximized under different environmental conditions, providing us with a remarkable example of ecological adaptation at the molecular level. These porins are also of interest because analogous proteins occur in the outer membranes of organelles of eukaryotic cells, such as mitochondria.

INTRODUCTION

Kalckar (1965) has always emphasized that the phenomena we encounter in the world of higher animals and plants, for example, the phenotypes of Escherichia coli mutants defective in their galactose metabolisms, were very instructive in our understanding of the potential and real consequences of such defects in humans. This idea has always sustained me in my studies of the ways the bacteria adapted to various environments, especially during the years in which the "relevance" of basic research has been questioned, sometimes rather severely, by the society in general and by the administrations in power.

It has been known for a long time that Gram-positive bacteria and Gram-negative bacteria are quite different in the organization of their surface layers, and that much higher amounts of lipids and proteins were found in the "cell wall" of Gram-negative bacteria (Salton, 1964). Kellenberger and Ryter (1958) also noted clearly the presence of a triple-layered, membrane-like structure on the surface of E. coli cells. However, to my knowledge, Bladen and Mergenhagen (1964) were the first to coin the word "outer membrane" for the extra membrane structure that was found in a Gram-negative species. Glauert and Thornley (1969) also contributed significantly to our understanding by emphasizing, in their review, the ubiquitous presence of this membrane layer in all Gram-negative bacteria so far examined, and its absence in all Gram-positive species.

STRUCTURE AND PROPERTIES OF THE LIPID BILAYER REGION

In spite of our knowledge on the presence of this extra membrane layer, its functions are poorly understood. We were led to the study of the structure-function relationship of this unusual membrane through our interest in the lipopolysaccharide (LPS), a characteristic component of the outer membrane. Toward the end of the 1960's both Westphal and his associates in Germany and the Roantree-Stocker group at Stanford found that mutants of E. coli and Salmonella producing severely truncated LPS molecules were strikingly more sensitive than the wild type organisms toward various antibiotics and other inhibitors (Schlecht and Westphal, 1969; Roantree et al., 1969). The inspection of the structures of the agents to which the mutants became hypersensitive suggested to me that they were mostly hydrophobic or large molecules. Assay of the rate of diffusion of a hydrophobic semisynthetic penicillin, nafcillin, indeed confirmed that the cells of wild type S. typhimurium were completely impermeable to this drug, but those mutants with defective LPS had an outer membrane permeable to this agent (Nikaido, 1976). These experiments, then, showed that the outer membrane of E. coli or S. typhimurium is normally a very effective barrier against the penetration by hydrophobic molecules. This was surprising because lipid bilayers are usually quite permeable to any hydrophobic molecules, and the search for the basis of this low permeability led to the discovery that the bilayer in the bacterial outer membrane is unusual in its asymmetric construction, containing only LPS in its outer leaflet and only phospholipids in the inner leaflet (Smit et al., 1975; Kamio and Nikaido, 1976). We do not know why the leaflet composed entirely of LPS would inhibit the diffusion of hydrophobic molecules, but we can certainly speculate about it. Unlike phospholipids, which contain only two hydrocarbon chains linked to the glycerol backbone, LPS has seven fatty acid chains connected to the glucosaminyl-glycosamine backbone (Qureshi et al., 1982). If, as is commonly believed, the diffusion of hydrophobic molecules involves

the pushing aside of the lipid molecules to create a cavity for the solute, such an act will be much more difficult with the LPS monolayer than with the phospholipid bilayer. Furthermore, the fatty acid residues in LPS are all saturated. This will reduce the fluidity of the hydrocarbon domain of LPS, as indeed found by the insertion of spin-labeled probe into LPS bilayers (Nikaido et al., 1977). It is well known that bilayers with less fluid hydrocarbon regions show lower permeability to hydrophobic solutes (de Gier et al., 1971). Regardless of the precise molecular mechanism of this low permeability, clearly it is an ecological advantage, or necessity, for many Gram-negative organisms to have an outer membrane with these properties. For example, E. coli and other enteric organisms normally live in the upper part of the intestinal tract of higher animals, where one encounters high concentrations of bile salts, powerful detergents that would dissolve the cytoplasmic membrane of these bacteria if the outer membrane barrier were absent. The classical selective media for enteric bacteria use this barrier property, in order to inhibit the growth of Gram-positive and some other Gram-negative bacteria by the inclusion of hydrophobic inhibitors such as dyes, bile salts, and other detergents. This concept also explains the fact that only a few Gram-positive species are found in the intestinal tract, and that either they are unusual in their resistance to bile salts (enterococci) or found predominantly in the lower part of the tract (clostridia).

PORINS AND PROPERTIES OF THE PORIN CHANNEL

The finding of the low permeability of the lipid bilayer matrix still did not explain how various nutrients crossed the outer membrane, because most of the nutrients used by bacteria are very hydrophilic, and the lipid matrix would be even less permeable for these solutes. When we examined the permeability of intact outer membrane by studying the influx of radioactive solutes into the enlarged periplasmic space, i.e., the space between the outer and cytoplasmic membranes, of plasmolyzed E. coli and S. typhimurium cells, we found that solutes with less than about 600 molecular weight were able to cross the outer membrane extremely rapidly in the time scale of our experimental set-up (Decad and Nikaido, 1976). What then produces this apparently nonspecific permeability? We have studied this problem through a reconstitution approach. The first few tries involving the reconstitution of LPS/phospholipid liposomes produced no encouraging results, but then Taiji Nakae in the laboratory had the first promising results by reconstituting vesicles with phospholipids, LPS, and a crude mixture of outer membrane proteins (Nakae, 1975). The results were not the strongest, in the sense that even when 200 μg protein was added to 1 μmol phospholipids only 75 percent of the [^{14}C]-sucrose diffused out of the vesicles during gel filtration. However, Nakae quickly found