Edited by
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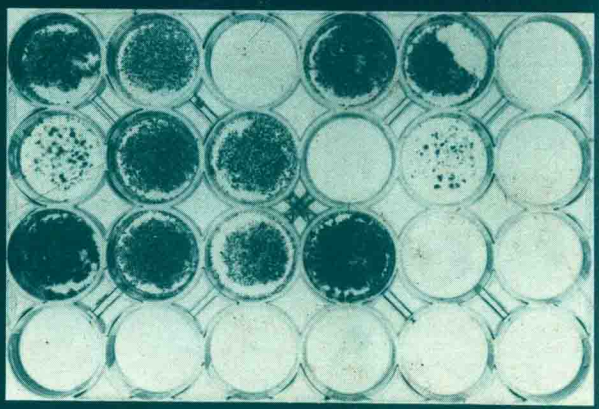

Carbohydrate Metabolism in Cultured Cells

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Foreword

It is perhaps obvious to any student of Biology that the discovery of chemical processes in whole organisms has usually preceded the elucidation of the component steps. However, it is perhaps less obvious that the unravelling of the sequences in which those chemical steps occur in living matter, of the precise mechanisms involved, and of the manner in which they are regulated, would have been achieved neither by the study of intact plants and animals nor even of extracts derived from them. Our ability to understand the nature and regulation of metabolism rests on two main premises: the postulate that life processes can indeed be validly investigated with individual cells and cell-free extracts, and the thesis that there is an essential "unity in biochemistry" (as Kluyver put it, 60 years ago) that enables events in one organism to be legitimately studied in another.

Of particular utility in this latter respect has been the use of cultures of single-celled organisms, growing in defined media—especially prokaryotes, such as *Escherichia coli,* and eukaryotes, such as *Neurospora* and *Saccharomyces* sp., to which both biochemical and genetical techniques could be applied. It was, of course, Pasteur's observations of bacterial fermentations that first overthrew the belief that oxygen was essential for all energy-yielding processes: his recognition that "*la fermentation. . . . . c'est la vie sans air*" laid the foundations of our knowledge of glycolysis. Investigations of mutants of *Neurospora* led Beadle and Tatum to develop the concept that any one enzyme was specified by its appropriate and designated gene; the code in which this specification was written was subsequently deciphered largely with the use of *E. coli.* And our knowledge of the manner in which carbohydrates are taken up by cells, and of the manner in which the operation of multitudinous metabolic processes are coordinated one with another so as to maintain the constancy of the

interior milieu, rests also on work with *E. coli*, done largely in the laboratories of Monod in France and of Umbarger in the U.S.A. some 30 years ago.

Yet it is now also clear that Monod's epigram, that what applied to *E.coli* applies also to *E.lephants*, cannot be wholly true. We now know that, although the genetic code is indeed universal, the manner in which it is expressed in eukaryotic cells differs significantly and is more complex than the manner in which this expression occurs in prokaryotes. Eukaryotic cells furthermore contain membrane-bounded compartments that not only separate different cellular components but also segregate different cellular events; these are not found in prokaryotes. The component cells of plants and animals communicate with their neighbors in the tissues and organs of which they form a part, and with distant parts of the organism via chemical messengers: again, prokaryotes do not manifest to any great extent these biological capabilities. The study of intermediary metabolism in eukaryotic cells and of its regulation will thus inevitably reveal complexities that cannot be deduced from our knowledge of these processes, elucidated with single-celled free-living microorganisms.

The present book attempts to focus on this gap, and to bridge it, by bringing together up-to-date reviews of information obtained from "classical" studies with microorganisms with information obtained from plant and animal cells treated as if they were microorganisms. Expertise in these different areas is usually not common to cell physiologists: it is rare for animal or plant biochemists to be familiar with the potential inherent in microbial methodology, and (vice-versa) few microbiologists have any deep understanding of the problems as well as the promise of growing plant or animal cells in culture. I greatly welcome this move to bring together practitioners in these apparently disparate fields: the history of Biochemistry surely teaches us that major progress is made only when the methodology of one discipline is brought to bear on another.

Hans Kornberg

Preface

A book devoted to carbohydrate metabolism demands an explanation since any casual, but observant, biochemist knows that all that has to be said on carbohydrate metabolism is available in biochemistry textbooks. The less casual observer will know that there is more to carbohydrate metabolism than even the most comprehensive biochemistry text can convey or the most complete metabolic map describe. In this book I have elicited the help of various expert authors to bring to fruition a seed that was planted some twenty years ago when I began my work on carbohydrate metabolism (*Escherichia coli*), in the laboratory of Hans Kornberg in Leicester. For a few years I deserted the charms of carbohydrate metabolism for the more ephemeral delights of interferon research, but soon realized the error of my ways and returned, albeit in animal cells, to the study of carbohydrate metabolism.

During these travels I myself delved in a number of the directions represented in this book and began to learn the lessons of cross-fertilization and hybrid vigor. When I was approached with a view to producing a book on carbohydrate metabolism, I felt that something new had to be said if it were worth saying at all. I hope that this book, in which two main themes are explored, the regulation of carbohydrate metabolism and the use of cultured cells as an experimental system, does say something new, besides underlining established fact, and identifies areas worthy of further exploration and enlightenment.

In the first chapter Herman Kalckar and Donna Ullrey describe their studies on the regulation of hexose transport in fibroblasts and discuss in detail the experimental procedures used. Their chapter also illuminates one of the dominant subthemes running through the book, namely the use of well-characterized mutants to dissect metabolic pathways, in this case the regulation of transport. In the next chapter, Pelin Faik and I paint a broad canvas of the utilization of

carbohydrates by animal cells, and again develop the idea of the use of biochemical mutants. We also emphasize the use of well-defined culture systems for exploring the regulation of differentiation and gene expression in animal cells. The use of well-defined mutants is further emphasized by Immo Scheffler's chapter on respiration-deficient mammalian cells, which illustrates how biochemical genetics are beginning to unravel the complexities of mitochondrial biogenesis. Wallace McKeehan presents convincing evidence for his hypothesis that glutamine metabolism is of major importance in the energy metabolism of mammals and that the series of reactions leading to glutamine breakdown, glutaminolysis, is a metabolic pathway deserving further attention and investigation.

These opening chapters on carbohydrate metabolism in animal cells are followed by chapters on plant cells and the African trypanosomes. Gagik Stepan-Sarkissian and Michael Fowler describe uptake and metabolism of the wide range of carbon sources that plant cells in culture will grow on. As they point out, this is a relatively unexplored facet of plant cell culture that no doubt will become of more importance as more cells find industrial uses. Alan Fairlamb and Fred Opperdoes cover the methods of cultivation of trypanosomes, the substrates used, and end products of their metabolism. They draw particular attention to that fascinating organelle, the glycosome, which contains many of the glycolytic enzymes, and to the possibilities of glycolysis as a target for chemotherapy against the trypanosomes, which cause such devastating illness.

Tony Romano continues with a description of the transport systems for sugars in baker's yeast and filamentous fungi. His chapter leads into the elegant contribution by Juana Gancedo on carbohydrate metabolism in yeast. She emphasizes the methodological approaches, presents an overview of carbohydrate metabolism, and ends with an interesting and informative discussion of regulatory mechanisms. This is followed by a monumental treatment of carbon metabolism in filamentous fungi by William McCullough, Clive Roberts, Stephen Osmani, and Mike Scrutton. This is an exhaustive treatment of special note since there have been no comparable reviews of this topic published recently. They manage to cover all aspects in great detail, including the breakdown of compounds, the enzymes involved, and the synthesis of carbohydrates.

Finally, there are three chapters on bacteria. Active transport of sugars into the Enterobacteriaceae falls naturally into two parts: that involving phosphorylation of the sugar, and this is covered by Pieter Postma; and that involving the transport of unmodified sugar (active transport), which is covered by Peter Henderson. Both chapters present an exhaustive account of the state of the art and show the elegant methods of molecular biology and recombinant DNA now available for dissecting metabolic pathways. Finally, Ron Cooper develops the theme of convergent pathways of sugar catabolism in bacteria and shows how one can divide metabolism into branch lines and main trunk pathways.

It is hoped that the reader will first dive into those sections with which he or she is most familiar before swimming into less certain waters. However, the reader is encouraged to take the plunge and is certain of finding many familiar rocks on which to cling. Any good qualities that the book may have are entirely due to the authors; the faults must lie with me.

Finally, it gives me great pleasure to be able to thank those who have helped bring this book to completion. Special thanks must go to the staff of the Wellcome Trust, and especially to Wendy Jacobs and Sue Parkes for their cheerful forbearance on the word processor, to Siân Spry for keeping my filing trays clear, and to Derek Metcalfe for oiling the wheels. Without them this book would never have been finished. Finally, grateful thanks to my wife Pelin, without whom this book would never even have been started.

Michael J. Morgan

London

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