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Biochemical Adaptation

PREFACE

One of the great accomplishments of biochemistry and molecular biology has been the elucidation of many of the major unifying principles and mechanisms that serve as the foundations of all living systems. Common mechanisms of energy transformation, catalysis, and the coding and processing of genetic information testify to the unity of life at the molecular level. While no one can deny these triumphs of reductionist approaches to biology, these insights into unifying principles of biochemical design in living systems seem to offer relatively few direct answers to a question of central importance to many biologists: How to account for the mechanisms underlying the immense diversity of organisms? What are the fundamental ways in which the basis biochemical structures and functions of living systems are adaptively modified to allow organisms to exploit the full range of natural environments and to maintain the radically different modes of life we see in nature?

The question of how a set of common mechanisms are extended into uncommon and diverse contexts is not new. Decades ago a similar gap existed between the fields of comparative anatomy and physiology. This gap was bridged by the concept of adaptation, and it is our belief that the concept of adaptation can be extended to the molecular level to effect a bridge between the observations of universal molecular mechanisms, on the one hand, and extreme biological diversity, on the other hand. Thus, the focus of our book is on the ways in which the ubiquitous molecular structures of organisms are modified to permit organisms to thrive in such diverse environments as the polar regions, deserts, and the deep sea, and to achieve modes of living that may involve major changes in type and quantity of nutrients available and in the oxygen that is present to support respiration.

In developing the central theme of biochemical adaptation we have selected examples for study that strike us as providing especially clear illustrations of the fundamental strategies of adaptation at the biochemical level. Our scope of treatment is not encyclopedic. Instead, we have focused on topics for which there either are numerous data, which allow a detailed analysis to be achieved, or where the basic

phenomenology is so interesting that, despite a lack of large numbers of data, it seemed to us worthwhile to draw questions of potential interest to the readers' attention. Our hope is that the examples we have chosen will be exciting and will provide the reader with an impetus to examine other, less well-studied problems in biochemical adaptation.

Our indebtedness to those whose efforts have helped make this book possible is a pleasure to acknowledge. At the top of the list are the students and postdoctoral scholars who have given us the type of stimulation that has kept our enthusiasm for this writing project, and for research per se, at a high pitch. P.W.H. wishes to express particular thanks to the students and postdoctoral fellows contributing to the current fermentations in his laboratory: H. Abe, J. Ballantyne, M. A. Castellini, G. P. Dobson, J. F. Dunn, B. Emmett, R. Foreman, C. J. French, U. Hoeger, T. P. Mommsen, B. J. Murphy, W. Parkhouse, E. A. Shoubridge, and R. Suarez. Earlier students who have continued to influence the thinking and work in P.W.H.'s laboratory include J. Baldwin, H. Behrisch, J.H.A. Fields, H. Guderley, M. Guppy, T. P. Moon, T. Mustafa, T. Owen, J. Storey, and K. B. Storey. In addition, P.W.H. wishes to acknowledge his numerous colleagues around the world who have made the entire enterprise all the more exciting and who on occasion have combined the adventures of intellect with the adventures of scientific expedition.

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Finally, we wish to emphasize that the task of preparing a book transcends the generation of ideas. The manipulation of manuscripts and figures, while the primary responsibility of the authors, could

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LIST OF ABBREVIATIONS

Common Metabolites

AMP, ADP, ATP	adenosine 5′-mono-, -di-, -triphosphate
cAMP	3′,5′-cyclic AMP
ArgP	arginine phosphate
CMP, CDP, CTP	cytidine 5′-mono-, -di-, -triphosphate
CoA	coenzyme A
Cr, CrP	creatine, creatine phosphate
DG	diglyceride
DHAP	dihydroxyacetone phosphate
DNA	deoxyribonucleic acid
2,3 DPG	2,3 diphosphoglyceric acid
FAD^+, FADH	flavin adenine dinucleotide, and its reduced form
F6P	fructose 6-phosphate
F1,6BP	fructose 1,6-bisphosphate
F2,6BP	fructose 2,6-bisphosphate
G3P	glyceraldehyde 3-phosphate
G6P	glucose 6-phosphate
GMP, GDP, GTP	guanosine 5′-mono-, -di-, -triphosphate
imid	imidazole
IMP, IDP, ITP	inosine 5′-mono-, -di-, -triphosphate
KGA	ketoglutarate
MG	monoglyceride
NAD^+, NADH	nicotinamide adenine dinucleotide, and its reduced form
$NADP^+$, NADPH	nicotinamide adenine dinucleotide phosphate, and its reduced form
P5C	pyrroline-5-carboxylate
PEP	phosphoenolpyruvate
PGA	phosphoglycerate
Pi	inorganic phosphate
PPi	inorganic pyrophosphate
TG	triglyceride
UMP, UDP, UTP	uridine 5′-mono-, -di-, -triphosphate

Common Enzymes

CPK	creatine phosphokinase
CS	citrate synthase
FBPase	fructose 1,6-bisphosphatase
α-GPDH	alphaglycerophosphate dehydrogenase

HK	hexokinase
IDH	isocitrate dehydrogenase
KGDH	ketoglutarate dehydrogenase
LDH	lactate dehydrogenase
MDH	malate dehydrogenase
ODH	octopine dehydrogenase
PDH	pyruvate dehydrogenase
PEPCK	phosphoenolpyruvate carboxykinase
PFK	phosphofructokinase
PGK	phosphoglycerate kinase
PK	pyruvate kinase

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CONTENTS

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CHAPTER ONE

Biochemical Adaptation: Basic Mechanisms and Strategies

The Paradigm of Adaptation

When scientists attempt to take a broad view of their field of inquiry and discern the dominant conceptual themes running through their discipline, they frequently speak of the "paradigms" of the field. Such paradigms are the world-views or conceptual frameworks within which most, if not all, of the detailed questions of investigation are phrased (Kuhn, 1970). In the chapters that follow we treat varied facets of what is probably the most encompassing and general paradigm in biology, a conceptual framework that finds expression at all levels of biological organization, ranging from the molecular level to the population level. This is the concept of "adaptation," the modification of the characteristics of organisms that facilitates an enhanced ability to survive and reproduce in a particular environment.

In the study of biology, adaptation is usually a dominant theme, whether explicitly stated or not. Often we begin our study of biology with a consideration of the vast diversity of anatomical forms present in the biosphere. In the study of fishes, for example, we note a myriad of body forms, some of which are designed for rapid cruising and capture of swiftly swimming prey, and other forms which are designed for minimal locomotory activity and a prey-capture strategy involving such passive approaches as the "float-and-wait" behaviors of many deep-sea fishes. We also find that some of these deep-living fishes have lures for attracting prey. Yet other fishes have anatomical specializations allowing both air- and water-breathing under different environmental conditions. Such highly conspicuous characteristics at the physiological, morphological, behavioral, and ecological levels of biological organization are typically the types of phenomena that constitute the literature dealing with organismal adaptations.

Our treatment of adaptations, in contrast, focuses on a different level of biological organization, namely, the biochemical attributes of organisms that are responsible for such critical capacities as the generation of adequate amounts and types of metabolic function, the

transport of gases between the cells and the environment, the maintenance of a proper solute microenvironment (pH and osmotic conditions) for macromolecular function, and the abilities to exploit the particular types of energy resources available to the organism. These types of biochemical adaptations can be regarded as "interiorized" phenomena, to distinguish them from other, generally more familiar biochemical adaptations that are important in the interfacing of the organism with its environment. Such "exteriorized" biochemical adaptations include cryptic coloration, bioluminescence, chemical signaling, chemical defense, chemical predation, and antigen sharing in the molecular mimicry of certain parasites. These exteriorized biochemical adaptations appear formally analogous to the conspicuous morphological, behavioral, etc. adaptations mentioned above. In contrast, the interiorized adaptations which serve as the primary focus of our analysis generally can be discerned only when the organism is biochemically "dissected." As we emphasize throughout our analysis, the very success with which organisms have adapted their enzyme systems, membranes, respiratory pigments, etc. for function in diverse environments may lull us into thinking that the interior biochemical machineries of different species are similar, if not identical. This, as we show, is a misconception. The interiorized biochemical adaptations we examine display the same wealth of diversity found in more apparent, exterior features of organisms.

To appreciate the characteristics of this diversity, and its function in enhancing the adaptiveness of organisms for their particular environments, we must first consider the set of biochemical structures and functions that are at once absolutely essential for all living systems known and highly sensitive to perturbation by the physical and chemical features of the environment. The adaptations treated in the subsequent chapters of this volume will be found to serve the following key functions:

1. The preservation of the structural integrity of macromolecules (e.g., enzymes, contractile proteins, and nucleic acids) and macromolecular ensembles (e.g., membranes and ribosomes) for function in specific environments.
2. The provision of adequate supplies of a) the energy currency of the cell adenosine triphosphate (ATP), b) reducing power to drive biosynthetic steps, and c) metabolite intermediates for the synthesis of storage compounds (glycogen, fats, etc.) and for the synthesis of nucleic acids and proteins.

3. The maintenance of mechanisms for regulation of metabolic rates and directions of metabolic flow in response to the organisms' needs and changes in these needs as the environment varies.

These, indeed, are the common and fundamental requirements of all living systems, and they must be realized in all environments, under all conditions. How these fundamental requirements are realized by different organisms under widely varying environmental conditions is, therefore, at the heart of biochemical adaptation and is the primary focus of the chapters to follow.

Homeostasis and Adaptation

The concept of homeostasis, which can be traced back over one hundred years to the work and writings of Claude Bernard, was first formalized into a coherent theory by W. B. Cannon early in this century. The theory simply states that, in the face of external perturbations, organisms harness mechanisms for the preservation or maintenance of an almost-constant internal state. It is evident that an end result of many of the above adaptive strategies is indeed the maintenance of homeostasis. In metabolic terms, the concept requires that both the direction and rate of metabolic reactions be adaptively regulated; glucose homeostasis, for example, requires the regulation of both gluconeogenesis and glycolysis (oppositely directed pathways).

Enantiostasis and Adaptation

Although homeostasis is commonly observed in many organisms, it is also evident that in many adaptational strategies, the maintenance of homeostasis is not achieved; indeed, it may not even be attempted. The phospholipid compositions of membranes of cold- and warm-adapted species are different; the solute composition of euryhaline invertebrates depends upon the external medium; and blood and intracellular pH varies with temperature. In considering this problem, Mangum and Towle (1977) point out that function, not state, is being preserved in many of these organisms. Membrane fluidity is adjusted with temperature in order to preserve membrane-based enzyme, hormone, and transport functions; solute levels are adjusted to conserve enzyme structure, function, and regulation; blood pH is adjusted as temperature changes in order to preserve protein function. The outcome of adaptation in all such cases is not homeostasis (the same state)

but is better termed enantiostatis (conserved function), a concept first formalized by Mangum and Towle (1977).

The Basic Mechanisms of Biochemical Adaptation

In analyzing the paradigm of adaptation at the biochemical level, we shall work within the framework provided by a short list of basic adaptation mechanisms. We hasten to stress at the outset of this discussion that the basic types of mechanism discussed below, and throughout this book, are not invariably clear and distinct from each other, and in some events it may be difficult to discern which particular basic mechanism is, in fact, involved in an organism's response to its environment. On balance, however, we feel that it is heuristically useful to give the reader a skeleton to flesh out in the chapters which follow, realizing that the "bones" are not disjoint, but are closely connected.

We shall frame much of the subsequent analysis in this volume in terms of the following three mechanisms or "strategies":

1. *Adjustments in the macromolecular components of the cell or body fluids.* Two distinct classes of adjustments are possible. First, the quantities (concentrations) of given types of macromolecules, e.g., enzymes, may be altered in adaptive ways. Second, new types of macromolecules, e. g., new isozyme* or allozyme forms, may be

* The terms "isozyme" and "allozyme" will appear frequently in this volume, so it is essential that the reader understand this terminology referring to enzyme variants. "Isozyme" is a generic term used to refer to different variants of a given type of enzyme. These variants can arise from two different genetic bases. First, two or more gene loci for a particular type of enzyme (or enzyme subunit) may be present in the genome of the organism. Rigorously, the expression, "multiple-gene-locus isozymes" would be preferable for use in discussing these isozymes, but we shall generally use only the word "isozyme" when discussing this type of enzyme variant. Second, in diploid organisms allelic enzyme variants may exist when a given enzyme (subunit)-coding locus is polymorphic. These allelic isozymes are commonly termed "allozymes."

Whereas the above terminology is adequate for discussing enzyme variants in a single species, interspecific comparisons necessitate additional expressions. In the discussions to follow, we will employ the expression "interspecific homologue" to refer to the "same" enzyme found in different species, where "same" generally refers to the enzyme coded by a gene locus common to all species being examined. For instance, the glycolytic enzyme lactate dehydrogenase (LDH. EC 1.1.1.27; NAD: lactate oxidoreductase) is coded by at least two, and typically three, separate gene loci in vertebrates. The LDH

added to the system and may replace previously existing macromolecules which no longer are well suited to function in the altered environment. For simplicity, one could refer to these two strategies as quantitative" and "qualitative," respectively.

2. *Adjustments in the microenvironment within which macromolecules function.* Adaptations of this type, which again can have both quantitative and qualitative attributes (e.g., total osmotic concentration may vary as may the types of solutes used to adjust osmolarity), are to be viewed as adjustments that confer on macromolecules the proper structural and functional characteristics. Microenvironmental adaptations will be seen to play a vital and complementary role vis-à-vis macromolecular adaptations.
3. *Adjustments in the outputs of macromolecular systems, especially enzymes, without changes in the amounts or types of machinery present.* Adaptations of this class can be viewed as differential rates of use of macromolecular systems pre-existing in the cells according to the local needs in time and space of the organism for the particular type of metabolic activity. These adaptations thus involve the important phenomenon of *metabolic regulation*: the appropriate increases and decreases of enzymic activity in response to the organism's requirements for such activities as locomotion, anaerobiosis, hibernation/estivation, and growth. These regulatory phenomena will, of course, depend heavily in many cases on changes in the compositions and concentrations of low molecular weight effectors (activators and inhibitors of enzymes, for instance) in the cells.

Again, we stress that these general categories of adaptive responses are not always distinct, and in many cases an organism may respond to an environmental change via all three adaptive strategies. Within these limitations, however, the reader may find these three basic strategies a useful conceptual framework for understanding the responses made by organisms to different environments, and we thus shall consider each of these strategies in somewhat more detail below.

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subunit found in highest concentration in locomotory muscle, especially the white muscle of fishes, is the muscle-type or "M" type. In aerobic tissues like heart, the "H" type of subunit predominates. A third subunit type is restricted to the testes or the retina. In discussing muscle-type (M_4) LDH tetramers in different species, we will be referring to subunits coded by a single type of gene locus, but the amino acid compositions of the subunits will often differ markedly from species to species.

Adaptive Changes in the Enzymic Machinery

Enzymes play two major roles, those of catalysis and the regulation of metabolic function. In viewing adaptive changes in enzyme systems, we must keep both functions clearly in mind, for in many cases the precise regulation of catalytic rates is even more crucial than the supply of a high level of catalytic power per se. To provide a basis for understanding when and how enzyme-level adaptations come into play during adaptive responses by organisms, it is appropriate to consider certain of the basic types of enzymic adaptations to be discussed later in this volume. Adjustments in the concentrations and types of enzymes present in organisms frequently are necessary for the following reasons:

1. Changes occur in metabolic demands, both in total flux rates and in the types of pathways needed, frequently as a consequence of environmental change or of developmental stage of the organism.
2. Changes in the physical environment, i.e., in temperature and hydrostatic pressure, may strongly influence enzyme function and structure.
3. Changes in the chemical environment—to the extent that these changes affect the composition and concentration of the intra- and extracellular fluids—may dictate the needs for altered quantities and types of enzymes.

The problems raised by these changes in habitat or developmental stage may necessitate both the "quantitative" and the "qualitative" changes in enzyme systems mentioned earlier. For example, a decrease in habitat temperature for an ectothermic organism (one whose body temperature is established largely, if not entirely, by the ambient temperature) may greatly reduce total metabolic flux and may alter the regulatory abilities of key enzymes governing metabolic flow. To a certain extent, these perturbations of metabolism may be offset by increasing the concentrations of enzymes in the cell. However, in other cases more of the same enzyme may not be a sufficient cure for the problem; instead, new isozyme forms may be needed to restore adequate catalytic capacity and, in particular, regulatory ability. Similar considerations apply in the case of changes in hydrostatic pressure, which also may cause alterations in catalytic rate and regulatory capacity. Alterations in the external environment's chemical composition also may create problems for enzyme function, especially in situations where these external changes, e.g., in total osmotic concen-

tration, lead to changes in the solute composition/content of the cells or to large alterations in water activity. However, as we analyze the responses of organisms to changes in external osmotic concentration, we most commonly find that the macromolecular machinery of the cells is protected against the effects of external osmotic changes, either through preservation of the status quo of the cellular fluids, as may be achieved by regulation of ion pumping, or through judicious adjustments in the composition of the macromolecules' functional microenvironment.

Microenvironmental Adaptations

Studies of biochemical adaptation and molecular evolution have customarily placed their major emphasis on the macromolecular components of cells, the nucleic acids and proteins, and the large molecular ensembles such as membranes. Surprisingly little attention has been paid to the low molecular weight "micromolecules" which bathe the macromolecular systems and establish many of their most critical biological properties. In our analysis, we shall attempt to redress this neglect of micromolecules. Several points, many of which have been mostly overlooked in previous analyses of, e.g., osmotic regulatory strategies, will provide a focus for our analysis. One key point concerns the bases for selecting particular types of solutes for roles as intracellular osmotic effectors (osmolytes). We will suggest chemical principles that determine if a given solute, whether it be an inorganic ion or a small organic molecule, is a "fit" component of the biological solution. This analysis will lead us to another important conclusion: namely, the evolution of biological solutions, e.g., cytosols, reflects the establishment of a "hospitable" environment for macromolecules to function in. The defense of this "hospitable" environment in response to changes in the chemistry of the external environment will also be treated, and this analysis may provide new insights into the rationales for the diverse osmoregulatory strategies of both aquatic and terrestrial organisms.

The discussion of microenvironmental adaptations will also involve consideration of the lipid milieu in which many enzymes, notably membrane-associated enzymes, conduct their functions. Lipids, while not "micromolecules" in the sense employed in our analysis, share with the aqueous solution surrounding soluble enzymes many common attributes vis-à-vis the requirements for providing proteins with a "hospitable" environment for function. The pervasive changes in lipid composition noted in studies of temperature adaptation, and