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haemoglobin, isoenzymes and tissue differentiation

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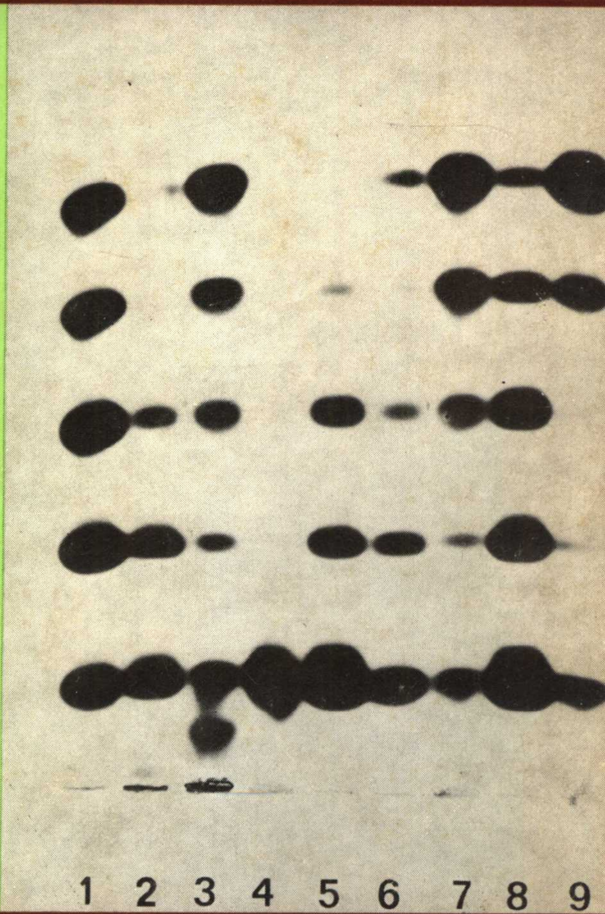

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HAEMOGLOBIN, ISOENZYMES AND TISSUE DIFFERENTIATION

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Editors' preface

The aim of the publication of this series of monographs, known under the collective title of '*Frontiers of Biology*', is to present coherent and up-to-date views of the fundamental concepts which dominate modern biology.

Biology in its widest sense has made very great advances during the past decade, and the rate of progress has been steadily accelerating. Undoubtedly important factors in this acceleration have been the effective use by biologists of new techniques, including electron microscopy, isotopic labels, and a great variety of physical and chemical techniques, especially those with varying degrees of automation. In addition, scientists with partly physical or chemical backgrounds have become interested in the great variety of problems presented by living organisms. Most significant, however, increasing interest in and understanding of the biology of the cell, especially in regard to the molecular events involved in genetic phenomena and in metabolism and its control, have led to the recognition of patterns common to all forms of life from the bacteria to man. These factors and unifying concepts have led to a situation in which the sharp boundaries between the various classical biological disciplines are rapidly disappearing.

Thus, while scientists are becoming increasingly specialized in their techniques, to an increasing extent they need an intellectual and conceptual approach on a wide and non-specialized basis. It is with these considerations and needs in mind that this series of monographs, '*Frontiers of Biology*' has been conceived.

The advances in various areas of biology, including microbiology, biochemistry, genetics, cytology, and cell structure and function in general will be presented by authors who have themselves contributed significantly to these developments. They will have, in this series, the opportunity of bringing together, from diverse sources, theories and experimental data, and of integrating these into a more general conceptual framework. It is unavoidable, and probably even desirable, that the special bias of the individual authors will become evident in their contributions. Scope will also be given for presentation of new and challenging ideas and hypotheses for which complete evidence is at present lacking. However, the main emphasis will be on fairly complete and objective presentation of the more important and more rapidly advancing aspects of biology. The level will be advanced, directed primarily to the needs of the graduate student and research worker.

Most monographs in this series will be in the range of 200–300 pages, but on occasion a collective work of major importance may be included somewhat exceeding this figure. The intent of the publishers is to bring out these books promptly and in fairly quick succession.

It is on the basis of all these various considerations that we welcome the opportunity of supporting the publication of the series '*Frontiers of Biology*' by North-Holland Publishing Company.

E. L. TATUM
A. NEUBERGER, *Editors*

Authors' preface

One of the most interesting and significant developments in present day biology is provided by the realization of the widespread occurrence of isoenzymes (or isozymes) and multiple molecular forms of specific proteins. In the last decade, this phenomenon has developed from an item of biochemical curiosity to the status of an extremely powerful and pervasive influence in the analysis of complex biological manifestations.

This book provides a contemporary compilation of recent results in this field, and extensive comment on the role of protein multiplicity in biology. A full account of the phylogenetic divergence and ontogenetic progressions of haemoglobin and isoenzymes in higher organisms is presented, and special reference is made to the relevance of these phenomena to the central biological problem of tissue differentiation. The book develops the theme that detailed study of the cellular phenotype in this manner may serve as a theoretically satisfying, yet eminently utilitarian, approach to the complex biological problems of growth and development, evolution and tissue differentiation.

In so doing, the book analyses the focal problems and research approaches to tissue differentiation, discusses the genetic involvements of growth and evolutionary divergence, considers the physiological rationale for the common presence of multiple forms of specific biological proteins, the currently favoured models for eucaryotic protein synthesis, the turnover characteristics of specific proteins during growth, maturation and senescence, new experimental approaches to the study of degradation mechanisms and the subcellular microlocalization of specific proteins, and hormonal influences in higher organisms.

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C.J. MASTERS
R.S. HOLMES

Acknowledgement

Several illustrations and diagrams in this volume have been obtained from other publications. Some of the original figures have been slightly modified. In all cases reference is made to the original publications in the figure caption. The full sources can be found in the reference lists at the end of each chapter. The permission for the reproduction of this material is gratefully acknowledged.

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CHAPTER 1

Introduction

1.1 Tissue differentiation

The topic of tissue differentiation enjoys a position of considerable prominence in contemporary biological thought. It stands recognized as one of the most central of biological problems – a challenging pursuance of great complexity, fraught with technical and conceptual difficulties; yet a quest that seems worthy of the candle, offering the promise of eventual benefit commensurate with the investigative effort.

The scope of the problem and the extent of the involvements may best be discerned, perhaps, if differentiation is viewed in broad perspective as the sum of those processes giving rise to the progression from a common ancestral cell to a series of specialized cells and tissues. Such a definition involves both the establishment of tissue identity, which is so dramatically evidenced by the development of multicellular organisms, and also the individuality of tissues arising in the course of evolutionary divergence. Obviously, then, many major streams of active biological research will be implicated and accommodated amongst these diverse and wide-ranging phenomena.

To name some of the main areas which have been closely identified with the study of tissue differentation, we may list the following:

> Genetics (including gene variation); the synthesis of proteins and other cellular macromolecules; membrane function; metabolism and its regulation; turnover; compartmentation; hormonal influences; organelle biogenesis; tissue function; and morphogenesis.

To biologists familiar with the extent and detailed content of some of these individual aspects, a comprehensive consideration of the interplay between

subject matter, an organized and centrally oriented approach is necessary if such complex systems during tissue differentiation must seem at least formidable, if not forbidding. Some comfort may be taken, however, from the knowledge that rapid, even spectacular, progress has been made in many of these related disciplines in recent years. Also, it is apparent that an increasing number of biologists are focussing their research efforts on the central problem. Consequently, it may be reasonable to expect that the deeper mysteries of tissue differentiation may soon be ripe for revelation.

Despite the magnitude of the problem, some solace may also be found in the potential for benefits which may derive from such research efforts. Just as the normality of tissue differentiation presupposes a continuum encompassing a myriad of coordinated processes, so abnormalities of differentiaion within individual human beings may be conceived as giving rise to imbalances and disorders at many stages of development, and these many possibilities of deviation from normality as being related to the causation of disease. Abnormal differentiation has been aligned with many of the major diseases of mankind in the past, but to further stress the magnitude of involvement of this correlation, it may be useful to cite a quantitative parameter such as the estimated loss of life years due to birth defects in humans. It may be surprising to many biologists to note just how large this vital statistic looms by comparison with the other well-publicized, and perhaps better known, major causes of mortality (fig. 1.1).

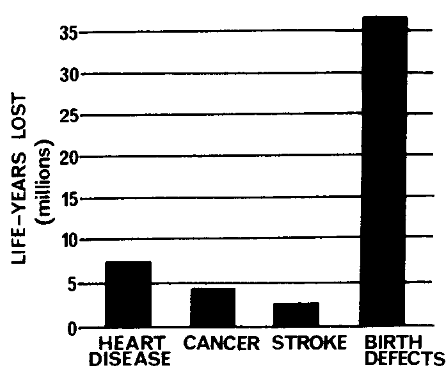

Fig. 1.1. A comparison of the estimated loss of life years annually in the United States due to major diseases.

1.2 Research approaches to differentiation

While it may be readily granted, then, that tissue differentiation is an eminently worthy subject for research efforts, nevertheless it should be apparent that, in view of the complexity and wide-ranging nature of the subject matter, an organized and centrally oriented approach is necessary if intricacy is not to lead to perplexity. This fact is attended to by the past history of the subject. In spite of a great deal of investigative effort, most scientists would acknowledge that little overall progress occurred over hundreds of years, and that the reason for this lack of progress may be traced back to the fact that many of the disparate lines of research aimed at this problem were peripherally oriented rather than focally directed.

In this age of molecular biology, of course, investigations at the molecular level would be favoured by many as most likely to lead towards a basic explanation of the phenomena of tissue differentiation, but in seeking such a fundamental, pivotal approach, two broad possibilities remain. We may attempt either to analyze the problem by considering a molecular approach to the simplest examples of differentiation, or we may accept the desirability of studying complex organisms, and choose to examine differentiation *via* the behaviour of a simple molecular parameter. Within either of these options, there will obviously still be gradations of focal relevance, and these merit some further discussion.

In the case of the first alternative, for example, it may be said that, while many of the most significant discoveries in molecular biology have emanated from research on micro-organisms and lower forms of life, such an approach in the case of differentiation may lead to a contradiction in terms. It is evident, for example, that the use of non-differentiating micro-organisms for the study of differentiation may never give rise to a full appreciation of the involvement of the subject. Many aspects of tissue identity in higher organisms are not represented in lower forms of life; and with those phenomena which are common to all levels of organization (for example, differential gene function), it becomes increasingly apparent that the processes are orders of magnitude more complex in mammals than in bacteria, and far removed from the processes typical of invertebrates.

Consequently, while there is no gainsaying that there is value in the comparative approach, and lessons to be learnt from the study of the lower forms of life, it should also be recognized that the focal value of such approaches may relate more to the way in which individual molecules adapt to evolutionary pressures, rather than to basic similarities in morphogenesis or gene function. We would propose, then, that in regard to the present subject matter, there is much to be said for the concept that "the proper study of mankind is man", and that the most relevant material for the study of tissue differentiation is that characteristic of mammals and similar highly developed organisms.

In adopting the approach of studying differentiative parameters in higher organisms, though, we are still left with the problem of which is the most appropriate level for research. While the general consensus of opinion would point to macromolecules as the most suitable objects of study in this context, there may well be a marked division between the protagonists of nucleic acids and proteins as materials offering the most promise for such an approach. To many scientists, conscious of the dramatic advances in genetics of the last few decades, and the deep impression which has been created in other branches of biology by this knowledge, the level of nucleic acids might seem the appropriate, logical contender for such a rationalization in an approach to tissue differentiation. Due reflection and careful consideration, however, would seem to indicate that this is not the case. On the contrary, it may be recalled that a basic concept of ontogeny and its attendant tissue differentiation is that most cells in an animal retain the same content and composition of DNA, despite the remarkable diversification of cell types which may be apparent in the adult. Neither can it be said that our techniques of RNA analysis are yet equal to the task of relating individual functions to fractions in the same way that is possible with proteins. Greengard (1970) has neatly summarized this situation in the following quotation:

> "At present our only test as to whether a given gene is functioning or not (in higher organisms) is the detection of the end product, the specific protein. It is highly questionable to what extent measurements of the synthesis of RNA fractions ... provide biologically significant information about gene expression. For example, we have no way of isolating, recognizing and quantifying mRNA molecules corresponding to a specific protein. Nor do we know how soon will the existence of a mRNA species result in the appearance of a protein, since the half life of the RNA, and the rate of the complex process of protein synthesis vary unpredictably. Thus, when observing increased RNA synthesis we cannot tell which, if any, protein structures are being transcribed, and whether these proteins are to appear within the next hour, day or week of development.
>
> On the other hand, observations of developmental changes in enzyme patterns and the quantitation of individual enzymes reflect firm biological realities. Further advances with this approach are far from being limited by our inability to analyze the individual steps involved in enzyme synthesis. A limitation probably operates in the reverse direction – more physiological information is required; matching of the enzymes with the physiological stimuli that promote their formation during development will provide the missing information for defining a system in which the molecular mechanism of gene expression can be studied in a biologically meaningful manner."

As a consequence of these considerations, then, the basic theme emerges that one of the most valuable, practical and centrally relevant means of studying the complex phenomenon of tissue differentiation, is by means of an observation of the properties of single proteins during development and evolution. This proposal, which forms the main connective thread of the treatment of differentiation in this book, recognizes the fact that the distinctions between tissues in highly developed animals may be attributed largely, if not entirely, to different enzymatic and protein compositions; and that not only may each cell be characterized by this distinctive assortment of proteins, but also each stage in differentiation. It is also predicted from this approach that epigenetics will form an intrinsic and fundamental aspect of this subject matter.

1.3 Haemoglobin and differentiation

Pride of place amongst those single proteins which have been most widely used in studies of differentiation must be accorded to haemoglobin. A massive volume of literature describes the research efforts which have been directed to both the evolutionary and ontogenetic rôles of this protein.

The advantages offered by this system may be conceived at both the molecular and the cellular level. The high concentration of this compound occurring in a conveniently accessible location has greatly facilitated comparative compositional studies, for example, and these have now proceeded to the stage where our knowledge of amino acid sequences and tertiary and quaternary structures is more detailed than with any other protein, and enables a satisfying conception of structure-function relationships (Chap. 2). Similarly, at the level of synthesis, the dramatic shifts in haemoglobin types, the ready availability during ontogeny of normal and abnormal synthesizing systems for study, and the possibility of the delineation of genetic involvement in these systems have greatly advanced our understanding of developmental processes (Chap. 4).

At the cellular level, of course the erythrocyte by virtue of its simplicity and uniformity again offers special advantages in such studies. In particular, if one acknowledges the deficiencies of micro-organisms in studies of tissue differentiation, the red blood cell is presented as a logical starting point for the molecular investigation of differentiation in higher organisms. Our present understanding of this subject is heavily beholden to the early, pioneering studies and subsequent investigations on haemoglobin, and it is