GROWTH, NUTRITION, AND METABOLISM OF CELLS IN CULTURE

EDITED BY

George H. Rothblat and Vincent J. Cristofalo

VOLUME I

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PREFACE

The use of cell and tissue culture as a tool for the study of a wide variety of fundamental biological problems has grown rapidly over the last two decades. Early in this period one of the major uses of cells in culture was for virus research. These studies have yielded considerable information about the basic molecular biology of viruses, as well as of the mechanisms of viral pathogenesis, infectivity, and immunity.

Concurrently, it was recognized that cells in culture could be used as an experimental system for studying the cell under controlled environmental conditions, and a steady stream of research has been directed at describing and understanding details of the biochemistry and physiology of cells in culture and their relationship to the *in vitro* environment. As a result of this basic work, new areas of research are now emerging in which problems which were heretofore unapproachable can be studied with cell cultures. For example, the methodology for the cultivation of normal human cells has become almost routine so that now the nature of a wide variety of human diseases and inborn metabolic errors can be elucidated at the molecular level. Techniques for the cultivation of differentiated functional systems, such as muscle and nerve, are now available. The cultivation of cells from poikilothermic vertebrates, invertebrates, and plants has added another dimension to the use of cell cultures in biological research.

The potential of cell culture can be fully realized only if the vast and complex literature which has accumulated can be critically evaluated and summarized. Therein lies the scope and purpose of these volumes. We have attempted to bring together in this two-volume treatise a comprehensive series of reviews that summarize the current status of knowledge of the growth, nutrition, and metabolism of various types of cell cultures. The chapters are both detailed and comprehensive enough

for the specialist and broad enough to provide a general background for the nonspecialist.

Volume I is comprised of contributions that describe the uptake, synthesis, and degradation of biologically important compounds, particularly the major components usually present in tissue culture medium. Volume II deals with specialized mammalian, plant, and invertebrate cell systems and techniques. In these chapters the culturing of specific classes of cells, including the establishment of their special nutritional requirements and metabolic features, are discussed.

We hope that these two volumes will meet the needs of investigators who routinely use cell culture techniques, as well as those of students and individuals in associated areas of cell and molecular biology. If they do, it is because of the efforts of the authors who contributed their chapters with care and enthusiasm. We wish to thank them all. In addition we wish to give our special thanks to Dr. H. Koprowski of the Wistar Institute whose interest and enthusiasm encouraged us to undertake this task.

GEORGE ROTHBLAT
VINCENT J. CRISTOFALO

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Paul M. Kraemer

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GENERAL INTRODUCTION

John Paul

In few areas of science is it as easy as in the field of tissue culture research to discern how the structure of present knowledge is based on the individual contributions of a large number of investigators. The goal has always been obvious to thinking biologists: the precise control of the environment in which cells from multicellular organisms grow so that meaningful quantitative experiments can be done. The questions raised by the behavior of these cells have such profound significance, relating, as they do, to the physical nature of all plants and animals, including man himself, that the field has attracted some of the best scientific minds of this century. Among the Nobel Prize winners who have done research with tissue culture at one time or another are Carrel, Warburg, Lippman, Enders, Crick, Watson, and Medawar. Some of these scientists stayed on in the field to make contributions to it, while others, emulating the man who is generally given credit for initiating modern tissue culture, made their contributions and then turned to other things.

This founder of modern tissue culture was Ross Grenville Harrison, whose paper "Observation on the Living Developing Nerve Fiber" appeared in the *Proceedings of the 23rd Meeting of the Society for Experimental Biology and Medicine*, reported in *The Anatomical Record* of June 1, 1907. In this paper he described how he took fragments of the medullary tube from frog embryos and implanted them in a lymph clot. He observed these cultures frequently and was able to show that, as nerve cells developed, they formed fibers which grew out into the lymph clot. This not only resolved a dispute about the origin of nerve fibers but it demonstrated, in a most dramatic way, that cells could survive and develop in tissue culture and could be used effectively to tackle biological questions.

Harrison's experiments were not the first attempts to maintain tissues *in vitro*. In the 1880s chick embryos had been successfully maintained in saline by Roux (1885), and Arnold (1887) had studied amphibian lymphocytes in culture. Hence, successful whole embryo culture and culture of hemopoietic cells preceded Harrison's experiments by some twenty years. His work is, however, rightly considered the point of departure for modern tissue culture because it created so much excitement and interest that the technique was taken up by many scientists immediately thereafter. From then until now contribution upon contribution has been steadily added to the impressive edifice which is the technology of modern cell and tissue culture.

Not very long after Harrison's experiments people began to think about carrying out biochemical studies with tissue in culture. Looking back, we can admire the courage and imagination of the early pioneers, but the magnitude of the problems of cellular biochemistry was not at all appreciated fifty years or so ago and quite naturally the questions asked were naive by today's standards. This implies no criticism of the early investigators; the questions we are asking today may well seem naive in another twenty or thirty years. The reason for remarking on it is simply that it enables us to recognize a rather clear watershed in studies on the nutrition and metabolism of cells in culture, which occurred in the early 1950s. In the early days of tissue culture the biochemical questions being asked were on the whole of a very general nature. Workers were concerned with trying to define the general nutritional requirements of animal cells and studying rather general parameters of metabolism, such as respiration. What distinguishes the modern era, starting in the mid-1950s, is the exploitation of cell and tissue culture techniques to answer specific problems, especially in the fields of virology and molecular and cellular biology. This transition was a result of different factors. First, there was the impact of the biochemical knowledge which had been accumulating with accelerating speed since the mid-1930s and which at last defined the true dimensions of the problems. Then there was a revolution in cell culture techniques, partly a result of improvements developed by cell culturists themselves and partly the result of a great invasion of the field by virologists. Finally, there was the emergence of the discipline of molecular biology: Cell cultures have now joined the T-even phages and *E. coli* in the molecular biologists' armamentarium.

The early development of cell culture nutrition is outlined in some detail by Dr. Waymouth (herself a pioneer of biochemical studies in tissue culture) in Chapter 2, Volume 1. The credit for foreseeing the importance of controlling the cellular environment and defining it in

chemically precise terms should probably be given to Lewis and Lewis (1911a,b), who in the years before World War I carried out experiments on the use of quite simple media for culturing tissues. The subject really got under way in the mid-1930s when Vogelaar and Erlichman (1933) and Baker (1936) produced media which are recognizable as precursors of today's media. A major advance was the introduction by Fischer (1941) of the idea of using dialyzed plasma as a basal medium to identify the small molecular components needed to supplement it. The medium which he and his colleagues (Fischer *et al.*, 1948) published has considerable similarities to some of those now commonly used, as has the medium published in the following year by White (1949).

At that time it was fashionable to recognize "synthetic" and "analytical" approaches to defining cellular nutrients. The former, of course, implied the arbitrary inclusion in mixtures of substances which had been demonstrated to be of some metabolic importance from general biochemical research; the latter implied the demonstration in tissue culture medium of nutrient substances. The distinction was always highly artificial and is never made now. It may be remarked, however, that the "synthetic" approach on the whole proved more fruitful.

In the decade following the publication of Fischer's and White's media many synthetic mixtures were published; finally, in 1955, Eagle published the first version of his medium. This was based on studies similar to Fischer's and was designed to contain only those small molecular components which were necessary to maintain the growth of some common cell lines. It came at a time when virologists were using tissue cultures intensively and when sophisticated biochemical studies were beginning to be undertaken; moreover, it proved to be a satisfactory general medium for most purposes. Hence, although many different media have been developed since that time, the appearance of Eagle's medium represents the culmination of the era of developing media for general purposes. A more recent trend has been the development of media for special purposes, and this will be discussed later.

One important general question of cell nutrition has, however, still not been answered. From the early studies of Fischer and later of Eagle, it became apparent that most cells had a requirement not only for small molecular species, but also for certain macromolecules present in serum. Although a few cells, in special circumstances, can grow without serum, virtually all animal cells, particularly primary cells, are dependent on the presence of factors in serum for growth. The role and nature of these serum factors have therefore been studied intensively. They have variously been identified as α-glycoproteins, fetuin, macroglobulins, and serum-bound small molecules. Until relatively recently serum was

thought to have a somewhat nonspecific effect in cell nutrition, but much new interest has been engendered by the observation that serum is in some way connected with the phenomenon of density control, i.e., the phenomenon which leads to a cessation of growth in cells when they have reached a certain density. Some aspects of this problem are discussed by Drs. Temin, Pierson, and Dulak in Chapter 3, Volume 1.

Even before reliable defined media for tissue culture cells were developed, general studies of the metabolism of tissue cultures were being conducted. Many of these had to do with carbohydrate metabolism. It was established quite early that glucose was the main source of energy for cells in culture (Lewis, 1922; Krontowski and Jazimirska-Krontowska, 1926; Krontowski and Bronstein, 1926; Warburg, 1930), and the quantitative requirements for oxygen were determined (Laser, 1933; Warburg and Kubowitz, 1927). In those early days, too, it was demonstrated that energy could be derived by deamination of amino acids (Holmes and Watchorn, 1927; Warburg and Kubowitz, 1927) and at about the same time the distinction between glycolytic and oxidative metabolism was made. Warburg (1930) reported that tumors almost invariably exhibited higher glycolysis than normal tissues. This claim was the subject of contention for many years and was satisfactorily resolved only when improved tissue culture technology made it possible to maintain cells in strictly controlled conditions. The reason for the difference is still not clear.

Two other general questions which excited interest in the early phases of cellular studies were whether respiration was essential for survival of vertebrate cells *in vitro*, and whether there was any special relationship of respiration to the cell cycle (in view of experiments which seemed to indicate that dependence on an intact electron transport system was absolute up to a certain stage of the cell cycle). It was rather clearly established that many cells in culture can go through a cell cycle in the presence of high concentrations of inhibitors of electron transport systems (Pomerat and Willmer, 1939) but the question of whether oxygen requirement is absolute in all conditions for prolonged maintenance of all eukaryotic cells is still open.

With the elucidation in detail of many metabolic pathways, most of these general questions assumed less importance and were replaced by more precise questions concerning the presence and amounts of different enzymes and inhibitors in specific kinds of cells. In Chapter 4, Volume 1, Dr. Gregg reviews our knowledge of energy metabolism in mammalian cells, and in Chapter 5, Volume 1, Dr. McLimans discusses the importance of the gaseous environment, while some special aspects of carbohydrate metabolism are also discussed in Chapters 10 and 11, Volume 1.

In the late 1940s increasing interest began to be taken in two specific fields of biochemical interest, nucleic acid and protein metabolism.

The earliest questions relating to protein metabolism arose in connection with studies on cell nutrition; they were concerned mainly with the importance of amino acids, serum proteins, and peptides in nutrition. Surprisingly, only in one of these areas have reasonably complete answers been obtained to date, in relation to the uptake of amino acids and their utilization in protein synthesis. This subject is reviewed by Dr. Patterson in Chapter 6, Volume 1.

Experiments on the behavior of nucleic acids in cells in culture were already carried out in the 1940s (Davidson, 1947; Davidson *et al.*, 1949). With the enunciation of Watson and Crick's theories concerning the nonconservative nature of DNA replication, important questions arose about DNA in mammalian cells. Did it behave during replication like the DNA of viruses and bacteria? Experiments by Graham and Siminovitch (1957) and Thomson *et al.* (1956, 1958), in which the metabolic stability of DNA and RNA was studied, provided a positive answer to this question. These experiments also demonstrated the potential use of cell cultures in tackling problems of this kind, and initiated the long series of studies in many laboratories which has provided us with an extensive understanding of the kinetics of synthesis of DNA and RNA in eukaryotic cells. A particularly important aspect of this field, which has attracted much attention recently, is the detailed metabolism of purines and pyrimidines, since, beside its own intrinsic interest, this knowledge has been turned to very good effect in the isolation and identification of mutants of cell lines which can be used in somatic cell genetic studies. This field is reviewed by Dr. Kelley in Chapter 7, Volume 1.

The role of lipids in cellular metabolism has also been the subject of intermittent interest since the early observations that cells in culture often accumulated lipid droplets. Few of the early studies were very revealing, and it was only with the relatively recent acquisition of detailed knowledge of lipid and steroid metabolism that the kinds of studies outlined by Dr. Spector and Dr. Rothblat in Chapters 8 and 9, Volume 1, became possible.

Culturing animal tissues in defined media did not by any means solve all the problems of studying their metabolism. It soon emerged that the patterns of metabolism in isolated cells were very sensitive to changes in the immediate environment, especially changes in oxygen tension, pH, glucose levels, and so on. It has emerged that for many studies it is necessary to maintain a rather constant environment, for example, by continuous perfusion, as discussed by Dr. Kruse in Chapter 2, Volume 2.