Methods in Cell Biology

Edited by DAVID M. PRESCOTT

VOLUME VI

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DAVID M. PRESCOTT

DEPARTMENT OF MOLECULAR, CELLULAR AND
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PREFACE

In the ten years since the inception of the multivolume series *Methods in Cell Physiology*, research on the cell has expanded and added major new directions. In contemporary research, analyses of cell structure and function commonly require polytechnic approaches involving methodologies of biochemistry, genetics, cytology, biophysics, as well as physiology. The range of techniques and methods in cell research has expanded steadily, and now the title *Methods in Cell Physiology* no longer seems adequate or accurate. For this reason the series of volumes known as *Methods in Cell Physiology* will now continue under the title *Methods in Cell Biology*.

Volume VI of this series continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

CONTENTS OF PREVIOUS VOLUMES

Volume I

Volume II

Volume III

Volume IV

Volume V

CONTENTS

Chapter 1

Cultivation of Cells in Protein- and Lipid-Free Synthetic Media

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I. Significance of Culturing Cells in Synthetic Media

Protein-free, chemically defined synthetic media are of great use in analyzing the chemical nature of cultured cells and of the metabolites excreted from them as well as in examining the effect of certain substances on cells, especially in cases where the substances may first interact with serum proteins in the medium. A number of studies have been carried out along these lines, as will be discussed later. However, a very limited number of kinds of cell lines have been grown indefinitely in protein-free media.

II. Composition of Chemically Defined Synthetic Media

Many mixtures of synthetic media have been reported. Table I lists the names of mixtures and authors of synthetic media, so far as known to us, which have been designed for the cultivation of mammalian cells. Different kinds of cells naturally have different nutritional requirements. In addition, our knowledge of cell metabolism is very limited. These are among the reasons why descriptions of so many mixtures have been published and not every kind of cell has as yet been serially grown in such media, especially in the primary culture.

Some of these mixtures were designed theoretically, all others empirically. Our work on synthetic media stemmed from our question of whether high molecular weight substances in media might be essential for cells as a nutritional source. By the use of primary culture of rat ascites hepatoma AH-130 cells, we estimated the consumption of serum proteins in the medium by the cells following cultivation. However, little decrease was detected in the amount of proteins. The addition of ^{131}I-labeled serum proteins also revealed little incorporation of the proteins into cells. These findings showed that proteins presumably do not serve cells as nutritional substances. We tried to replace serum proteins in the medium with other high molecular weight substances, especially with so-called plasma expanders, which were developed during the Second World War; alginic acid, dextran, and polyvinylpyrrolidone (PVP). All of them were more or less effective. However, the highest efficiency of substitution was obtained with 0.1% PVP (K-90, average molecular weight 700,000; Badische Anilin- und Soda-Fabrik, West Germany), which replaced approximately 99.5% by volume of serum proteins added to the medium in the optimal concentration (Katsuta *et al.*, 1959a). This result was introduced into the cultivation of L-929 cells in protein-free media. When transferred to medium consisting of 0.05% PVP, 0.4% lactalbumin hydrolyzate (NBCo, U.S.A.), and 0.08% yeast extract (Difco Lab., U.S.A.) with no supplement of serum, the proliferation of L-929 cells was readily initiated and has continued up to the present (Katsuta *et al.*, 1959b). This subline was designated as L·P1. In parallel to this cultivation, other trials were made with other mixtures of protein-free media consisting of (1) PVP and lactalbumin hydrolyzate and (2) lactalbumin hydrolyzate alone. Continuous cell growth in these media was eventually obtained; the sublines grown in (1) and (2) were designated as L·P2 and L·P4, respectively (Katsuta *et al.*, 1961).

Since L-929 cells were demonstrated, as above, to be capable of grow-