Cell Culture Methods for Molecular and Cell Biology

David W. Barnes, David A. Sirbasku, and Gordon H. Sato, *Editors*

Volume 1: Methods for Preparation of Media, Supplements, and Substrata for Serum-Free Animal Cell Culture

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Alan R. Liss, Inc., 150 Fifth Avenue, New York, NY 10011

Library of Congress Cataloging in Publication Data

Main entry under title:

Methods for preparation of media, supplements, and substrata for serum-free animal cell culture.
(Cell culture methods for molecular and cell biology; v. 1)
Includes index.
1. Culture media (Biology) 2. Cell culture.
I. Barnes, David W. (David William), 1949-
II. Sirbasku, David A. (David Andrew), 1941-
III. Sato, Gordon. IV. Title: Serum-free animal cell culture. V. Series.
QH585.M46 1984 591'.07'24 84-7203
ISBN 0-8451-3800-6

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Volume 2: Methods for Serum-Free Culture of Cells of the Endocrine System

Volume 3: Methods for Serum-Free Culture of Epithelial and Fibroblastic Cells

SERUM-FREE CULTURE OF EPITHELIAL CELLS

SERUM-FREE CULTURE OF LYMPHOID CELLS

Preface

For several of the past decades, experimental approaches to the study of cellular physiology were limited to either studies in the whole animal, or short-term studies with isolated cells or slices from various tissues. On the one hand, animal studies have provided our basic understanding of organ system physiology, but on the other hand this approach was limited because the in vivo measurements represented the net effect of many homeostatic changes that occurred during the experiments. In short, what was needed was a new experimental approach to cellular physiology in which isolated, functionally differentiated cells could be maintained in culture under conditions that allowed direct manipulations of the environment and measurement of the resulting changes in the function of a single cell type. The maintenance of cells in vitro has proven to be a major scientific undertaking of great importance, and it is now universally accepted that in vitro methods are indispensible to the study of cellular physiology.

Cell culture methods have been evolving rapidly. The first researchers in the field recognized that to establish even short-term cultures of animal cells in vitro, a basic requirement was the design of chemically defined synthetic media that contained the necessary mixtures of nutrients, vitamins, ions, and other essential metabolites, all of which were then to be maintained at the proper pH for life functions to be expressed. Pioneers such as Ted Puck, Charity Waymouth, Harry Eagle, Richard Ham, and others, led the efforts to provide all the workers of this field with formulations useful for specific cell types or purposes, and other formulations of synthetic media that were useful with a wide variety of cell types.

As these types of studies progressed, attention began to turn toward the problem of the most undefined component used in tissue culture media, namely the animal serum or plasma supplement. For a variety of cells in culture, it was widely accepted that the addition of this fluid was

essential for growth. Serum obviously was growth-stimulatory for some cell types; what was less obvious was that the serum supplement was toxic to other cell types or inhibitory to some physiological responses of interest. In nearly all cases, serum is the source of both growth-promoting and growth-inhibiting substances and, taken as a whole, serum-supplemented media greatly favor proliferation of mesenchymal-origin cells (i.e., fibroblasts) over growth of other cell types. Problems of apparent cellular "dedifferentiation" or fibroblast overgrowth were encountered by many researchers, using conventional culture techniques, and this led to a widely held view that cells which expressed differentiated functions could not be maintained in culture. This position was in part proven incorrect by workers demonstrating that functionally differentiated tumor cell types could be maintained in culture and that these cells continued expression of tissue-specific functions. But even with these initial successes, the problems remained of how to establish highly differentiated normal cells in culture selectively and how to support their proliferation in the absence of the undefined serum supplement.

In these four volumes, a different and unifying approach to cell culture is described. The concepts basic to this approach evolved from ideas contributed by investigators in areas of physiology and endocrinology, as well as those in fields more traditionally associated with cell culture methodology, and spring from attempts to replace serum in culture medium with purified components chosen to serve for cells the role usually provided by serum components. The methods are based on the experimentally established concepts that the function of the serum supplement is to supply hormones, growth factors, cell attachment proteins, extracellular enzymes and enzyme inhibitors, metal ion transport proteins and other binding proteins, lipoproteins, essential fatty acids, and other specific nutritional requirements for growth and tissue-specific expression by cells in culture. By supplementing media with appropriate mixtures of these defined substances, various differentiated cell types can be maintained in culture and be shown to express tissue-specific functions not previously identified in vitro.

In an attempt to satisfy the needs of both the novice and seasoned investigators wishing to utilize serum-free culture methods, we have organized the series beginning with this volume, which contains a description of the general methods of preparation of many of the common media components, followed by three other volumes, each dealing with detailed formulations for specific cell types. In this first volume, methods are presented for establishing the basal nutrient requirements of various cells and preparation of serum-free media, as well as complete methods

of preparation of several of the most common polypeptide growth factors used in defined media. Also, in this first volume, several important methods are described for the preparation of cell attachment proteins and extracellular matrix, which have proven to be essential components of many formulations of defined media. Careful evaluation of the properties and functions of the components described in Volume 1 will greatly aid the reader in the process of selecting which are likely to be important additions to the serum-free media desired. Volume 1 is organized to act as a companion to any of the other three, allowing the researcher to select one or more additional specialized volume of interest.

Beyond this general volume, the other three incorporate useful examples of how to apply these methods to individual problems. For example, in the second volume, entitled "Methods For Serum-Free Culture of Cells of the Endocrine System," methods are described for specific formulations effective with adrenal, pituitary, testicular, ovary, prostate, and mammary cells. The section on mammary cell culture is especially useful, since it is one of the most complete available on serum-free methods for cells from this organ.

Volume 3 of this series, "Methods for Serum-Free Culture of Epithelial and Fibroblastic Cells," describes much of what is currently known about the growth of these important cell types in a defined environment. This volume addresses the issues of culturing both normal and malignant epithelial cells, and the conditions that must be met to maximize expression of tissue-specific differentiated functions. Methods also described in Volume 3 deal directly with the uses of defined media to characterize the phenotypic and genotypic changes in untransformed and transformed fibroblasts. This field of research has received new interest with the discovery of oncogenes using in vitro transformation systems, and formulations of serum-free defined media for normal and transformed cells may become even more important with applications in these new directions.

Volume 4, the final of this series, is entitled "Methods for Serum-Free Culture of Neuronal and Lymphoid Cells." The first part of the volume focuses on applications to neuronal-origin cells that have been difficult to maintain in conventional serum-containing media. In addition, the preparation of neurotrophic factors is discussed in detail. The second part of Volume 4 addresses the methods of preparation of several of the important lymphokines, details growth conditions for lymphocytes, and also describes approaches to the growth of hybridomas and production of monoclonal antibody in serum-free, defined media. The latter method promises wide applications and is a broadly useful technical advance.

It has been our goal to provide a collection of culture methods that are

detailed and useful at the laboratory bench. For this reason, less emphasis has been placed in this series on the conceptual importance and implications of the various aspects of the serum-free systems described. More thorough treatments of these aspects can be found in several recent publications: "Growth of Cells in Hormonally Defined Media" (Cold Spring Harbor Press, 1982) and "The Use of Serum-Free and Hormonally Defined Media" (J. Mather, ed., Plenum Press, 1984). The editors thank the contributors for sharing their detailed methods and for their helpful comments which have greatly improved these volumes. We hope that the information provided here will serve as a catalyst for future advances and that these volumes will inspire new applications moving well beyond our present-day technology.

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