Cell Culture Methods for Molecular and Cell Biology

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

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

Methods for Serum-Free Culture of Cells of the Endocrine System

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Address all Inquiries to the Publisher
Alan R. Liss, Inc., 150 Fifth Avenue, New York, NY 10011

Library of Congress Cataloging in Publication Data

Main entry under title:

Methods for serum-free culture of cells of the endocrine system.
(Cell culture methods for molecular and cell biology; v. 2)
Includes index.
1. Cell culture. 2. Culture media (Biology)
3. Endocrine glands. I. Barnes, David W. (David William), 1949- . II. Sirbasku, David A. (David Andrew), 1941- . III. Sato, Gordon. IV. Title: Serum-free culture of cells of the endocrine system. V. Series.
QH585.M464 1984 591.1'42'0724 84-7202
ISBN 0-8451-3801-4

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SERUM-FREE CULTURE OF NEURONAL CELLS

Preface

The growth in vitro of a variety of endocrine-responsive cells can be achieved using serum-containing culture medium. However, results of studies using these conventional culture systems often have been less than satisfactory. If in vitro experiments in the areas of biochemical and molecular endocrinology are to be meaningful, the culture medium must be as defined as is possible in order to eliminate substances that might interfere with hormone responses, as well as to eliminate endogenous hormones that would mask or otherwise prevent the identification of expected cellular responses. Further, nontarget cells must be eliminated from the cultures to prevent measurement of secondary or unrelated effects.

The serum-free approaches to endocrine cell cultures described here are workable solutions to these problems, and do provide for identification of cellular responses that are not identifiable in serum-containing culture medium. For example, the growth-promoting effects of estrogens described in this volume are observed in serum-free, defined media, while these same responses may not be easily demonstrable in serum-containing medium. The effect described in this volume of ACTH on normal adrenal cell steroid biosynthesis is another area of biochemical endocrinology that is more easily characterized under serum-free conditions. Methods are detailed for the establishment of fibroblast-free cultures of normal human prostate cells through serum-free techniques that will be valuable to many researchers interested in function and growth regulation of this major site of endocrine-related cancer in man. Although defined media for many cell types have important components in common (i.e., transferrin, insulin, and many nutrients), it is possible to design restrictive serum-free media that make possible the growth of only selected cell types from primary cultures. Other methods described further demonstrate the importance of the substratum and matrix composition in chemically defined media for

endocrine target cells and the use of serum-free assay conditions to identify new growth factors for human and rat normal and malignant mammary cells.

Also detailed are the preparation and use of chemically defined media specifically developed for the culture of fibroblast-free monolayers of normal functional endocrine target cells from breast, ovary, testes, and adrenals and serum-free organ culture of mammary tissue. The methods for establishing these cultures from tissues are presented, and when applied along with the general approaches to formulation of serum-free, defined media described in Volume 1 of this series, suggest ways to approach the culture of other endocrine target cells not described in these volumes.

The methods given here are intended for those wishing immediate directions for application of a previously formulated medium for a given cell type and for those investigators with the need for developing new types of defined media, or having the need for modification to suit a new cell type. We hope that, in addition to the immediate usefulness of the methods presented, that they will also provide a working base for new applications.

David W. Barnes
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Growth of GH_3, a Rat Pituitary Cell Line, in Serum-Free, Hormone-Supplemented Medium

Izumi Hayashi

GH_3 is a functional rat pituitary cell line [Yasumura et al., 1966] originally developed from a transplantable pituitary tumor MtT/W5 [Takemoto et al., 1962] carried in female Wistar/Furth rats. A cloned cell line, GH_3 secretes both prolactin and growth hormone into culture medium [Tashjian et al., 1968, 1970]. Using serum obtained from thyroidectomized cows, Samuels et al. [1973] demonstrated the dependence of GH_3 cells on thyroid hormone for growth. The other growth-promoting factors for GH_3 that were supplied by the hypothyroid cow serum were eventually identified, and now GH_3 cells can be maintained in a completely defined culture medium supplemented with hormones and purified growth factors. There are a number of approaches one can take to derive the complete elucidation of growth requirements for a given cell line [Bottenstein et al., 1979; Barnes and Sato, 1980]. This chapter describes the process that was taken to identify the growth factors for GH_3, as one such example.

DERIVATION OF THE SERUM-FREE MEDIUM

Studies in Thyroid Hormone-Depleted Medium

The effect of 3,3,5-triiodothyronine (T_3), the biologically active form of thyroid hormone, on the growth of GH_3 cells can be clearly demonstrated by using either hypothyroid serum obtained from propyl thiouracil-treated rats, or serum extracted with activated charcoal (Fig. 1). Initially, the basal medium

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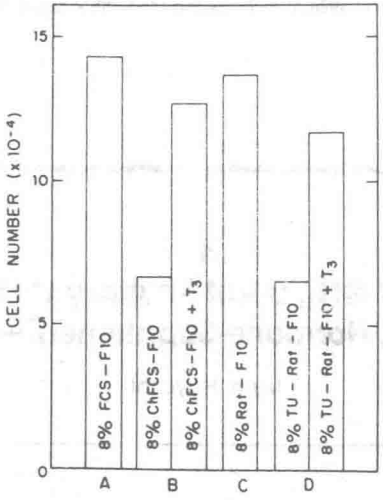

Fig. 1. Effect of T_3 on the growth of GH_3 cells in thyroid hormone-depleted media. Medium F10 supplemented with 8% FCS (A), 8% ChFCS (B) with or without T_3 (1×10^{-9} M), 8% rat serum obtained from a normal Wistar-Furth rat (C), 8% hypothyroid rat serum (TU) obtained from a propyl thiouracil-treated rat (D). The inoculum was 3.5×10^4 cells per 35-mm plate. The cells were counted on day 4.

used to demonstrate the effect of T_3 consisted of Dulbecco's modified Eagle's medium (DME) or Ham's F-10 medium (F10) supplemented with charcoal-extracted fetal calf serum (ChFCS) or calf serum (ChCS). When T_3 is added at 1×10^{-9} M to ChFCS, growth equivalent to that in rich medium (DME supplemented with 12.5% horse serum [HS] and 2.5% FCS) is obtained (Fig. 2). However, unexpectedly, the effect is not observed when T_3 is added to ChCS, indicating the requirement of GH_3 cells for substance(s) other than T_3. Since FCS seemed to serve as a better indicator serum, this serum was chosen over calf serum for the medium in which to further investigate the growth requirements for GH_3 cells. Since charcoal extraction is an empirical method used for the removal of steroids, thyroid hormones, and other aromatic compounds, ChFCS was subjected to a repeated treatment with activated charcoal (2ChFCS) to minimize the background. The effect of T_3 at varying concentrations was examined in medium supplemented with either 8% FCS, ChFCS, or 2ChFCS. The result is shown in Figure 3. The optimal dose for T_3 is 1×10^{-9} M in all serum supplements. Although the basal cell number is lower for cultures supplemented with 2ChFCS than with ChFCS, the addition of T_3 at higher concentrations did not enhance the growth of cells