METHODS IN CELL BIOLOGY

EDITED BY
DAVID M. PRESCOTT

VOLUME XIII

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Contributors to This Volume

Michael Balls

Dennis Brown

Peter J. Bruns

D. E. Buetow

Harold I. Calvin

R. Farookhi

Norman Fleming

Lester Goldstein

Paul V. C. Hough

Aloys Hüttermann

Christine Ko

Hiroshi Miyamoto

Eduardo Orias

Wolf Prensky

David M. Prescott

Leif Rasmussen

A. L. Rosner

Bruce K. Schrier

Per O. Seglen

Jerry Shay

C. Sonnenschein

A. M. Soto

Walter E. Stumpf

George Veomett

Gertrud Wendlberger

Samuel H. Wilson

Erik Zeuthen

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Edited by

DAVID M. PRESCOTT

DEPARTMENT OF MOLECULAR, CELLULAR AND
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CONTENTS

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

MICHAEL BALLS, Department of Human Morphology, The Medical School, University of Nottingham, Nottingham, England (213)

DENNIS BROWN,[1] School of Biological Sciences, University of East Anglia, Norwich, England (213)

PETER J. BRUNS, Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York (247)

D. E. BUETOW, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois (283)

HAROLD I. CALVIN, International Institute for the Study of Human Reproduction, Columbia University College of Physicians and Surgeons, New York, New York (85)

R. FAROOKHI, Tufts University School of Medicine, Tufts Cancer Research Center, Boston, Massachusetts (195)

NORMAN FLEMING,[2] School of Biological Sciences, University of East Anglia, Norwich, England (213)

LESTER GOLDSTEIN, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado (239)

PAUL V. C. HOUGH,[3] Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado (1)

ALOYS HÜTTERMANN, Forstbotanisches Institut der University Göttingen, Göttingen, West Germany (153)

CHRISTINE KO, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado (239)

HIROSHI MIYAMOTO,[4] The Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark (15)

EDUARDO ORIAS, Section of Biochemistry and Molecular Biology, University of California at Santa Barbara, Santa Barbara, California (247)

WOLF PRENSKY, Molecular Cytology Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York (121)

DAVID M. PRESCOTT, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado (1,7)

LEIF RASMUSSEN, The Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark (15)

A. L. ROSNER,[5] Tufts University School of Medicine, Tufts Cancer Research Center, Boston, Massachusetts (195)

BRUCE K. SCHRIER, Behavioral Biology Branch, National Institute of Child Health and Human Development, and Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland (105)

[1] *Present address:* Institut d'Histologie et d'Embryologie, École de Médecine, Université de Genève, Geneva, Switzerland.

[2] *Present address:* Anatomisches Institut, Universität Zürich, Zürich, Switzerland.

[3] *Present address:* Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York.

[4] *Present address:* Department of Physiology, Kinki University School of Medicine, Nishiyama, Sayama-cho, Minamikawachi-gun, Osaka, Japan.

[5] *Present address:* Department of Pathology, Beth Israel Hospital, Boston, Massachusetts.

PER O. SEGLEN, Norsk Hydro's Institute for Cancer Research, Department of Tissue Culture, The Norwegian Radium Hospital, Montebello, Oslo, Norway (29)

JERRY SHAY, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado (1)

C. SONNENSCHEIN, Tufts University School of Medicine, Tufts Cancer Research Center, Boston, Massachusetts (195)

A. M. SOTO, Tufts University School of Medicine, Tufts Cancer Research Center, Boston, Massachusetts (195)

WALTER E. STUMPF, Departments of Anatomy and Pharmacology, University of North Carolina, Chapel Hill, North Carolina (171)

GEORGE VEOMETT, Department of Molecular, Cellular and Developmental Biology University of Colorado, Boulder, Colorado (1,7)

GERTRUD WENDLBERGER, Forstbotanisches Institut der University Göttingen, Göttingen, West Germany (153)

SAMUEL H. WILSON, Behavioral Biology Branch, National Institute of Child Health and Human Development, and Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland (105)

ERIK ZEUTHEN, The Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark (15)

PREFACE

Volume XIII 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

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Chapter 1

Large-Scale Enucleation of Mammalian Cells

GEORGE VEOMETT, JERRY SHAY, PAUL V. C. HOUGH,[1] and DAVID M. PRESCOTT

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado

I. Introduction

Carter (1967) was the first to realize the potential use of cytochalasin B (CB), a metabolite from *Helminthosporium dematoideum*, for experimental enucleation of cultured mammalian cells. A major step in developing this potential was introduced by Prescott *et al.* (1972) and Wright and Hayflick (1973), who subjected cells to a centrifugal force in the presence of CB. Under these conditions the nucleus is forced into a cytoplasmic stalk which subsequently severs spontaneously.

Prescott *et al.* (1972) and Prescott and Kirkpatrick (1973) used glass and subsequently tissue culture–grade plastic cover slips as a substrate for cell growth. The cover slips are easily handled and can be placed sterilely into centrifuge tubes for the enucleation procedure. The number of enucleated cells obtained is limited by the surface area of the cover slips used for growth. Several other techniques have utilized the same principle of combined

[1] *Present address*: Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York.

centrifugation and CB treatment of tissue culture cells, but have varied the surface area for cellular growth. For example, Wright and Hayflick (1973) grow cells on the inner surface of centrifuge tube inserts, while Croce and Koprowski (1973) have described a technique in which the cells are grown directly on the inner surface of the centrifuge tubes. Follett (1974) has described a technique in which 35-mm tissue culture dishes used for cell growth are inverted in the wells of a centrifuge rotor and used for the enucleation procedure. Large numbers (approximately $0.5–1 \times 10^7$) of enucleated cells can be obtained by these procedures. The purity and viability of the cytoplasts (enucleated cells) using mass-enucleation procedures has been shown (Wright and Hayflick, 1973; Croce and Koprowski, 1973; Follett, 1974), but the karyoplasts (nucleated cellular fragments) have not been well characterized.

We describe here an alternate, relatively simple method for obtaining large numbers of enucleated cells and karyoplasts. This method utilizes 25-cm^2 tissue culture flasks both as substrate for cellular growth and as centrifuge tubes. The method is described for mouse L cells, but should be applicable to any cell that grows in monolayers.

II. Cells and Materials

Mouse L cells were grown in 25-cm^2 tissue culture flasks (Fisher or Falcon brand) in Ham's F12 medium supplemented with 10% fetal bovine serum and antibiotics.

The medium for enucleation consists of growth medium supplemented with 10 μg/ml of CB (Aldrich Chemical Co., Milwaukee, Wisconsin).

III. Techniques

A. Without Centrifuge Inserts

Cells are grown in the tissue culture flasks for at least 24 hours to allow the cells to attach firmly. The tissue culture medium is then removed, and medium containing CB is added to the flask until it is completely filled. The cap of the flask is then replaced and tightened.

The enucleation procedure is performed in a Sorvall RC-2B centrifuge, utilizing the GSA rotor, both of which are prewarmed to 37°C. Approxi-

mately 125 ml of water at 37°C is added to each rotor well; a filled flask is then added to each well. The rotor, with its cover off, is then spun at approximately 500 rpm, and water is continuously added to the rotor wells until all the wells contain the maximum volume of water, which is apparent when water is ejected from the rotor. The rotor is then stopped, the cover is replaced, and centrifugation is performed at 8500 rpm for 30 minutes.

The percentage of enucleation after one centrifugation is good, generally greater than 95% (Fig. 1). The karyoplasts form a pellet in the corner of the flask and are easily recovered. The trypan blue exclusion test indicates 80–90% viability. With this technique more than 90% of the karyoplasts can be recovered. Approximately $1–1.5 \times 10^6$ enucleated cells can be obtained from a single flask, or $6–9 \times 10^6$ from a single centrifugation run.

The major drawback of this technique is flask breakage, which is about 10%. Fisher flasks tend to crack and shear at the neck, whereas Falcon flasks shear along the side seams. In both cases, flask breakage is minimized by balancing the rotor as described.

B. With Acrylic Inserts

Acrylic inserts were constructed for the GSA rotor and were designed to accommodate Falcon bent-neck tissue culture flasks. The design of these inserts is shown in Fig. 2. They fit very tightly into the rotor wells and may have to be custom-trimmed for some rotors. Approximately 125 ml of water and a filled flask are added to each assembly used. The assemblies are balanced by weight, the tops are screwed on tightly, and centrifugation is performed in the GSA rotor in a Sorvall RC-2B centrifuge at 37°C for 20 minutes at 10,000–11,000 rpm. The results of the enucleation are similar to those obtained by the technique described in Section III,A, i.e., generally greater than 95% enucleation. However, karyoplast recovery is poorer. The reasons for poorer karyoplast recovery are unknown, but may be related to the alterations in centrifugal force and angle at which the flasks are held during centrifugation.

The inserts greatly reduce the breakage of Falcon flasks; only 1–2% of the flasks fail under these conditions, generally by leaking at the neck. The straight-necked Fisher flasks are unsuitable for use with this technique.

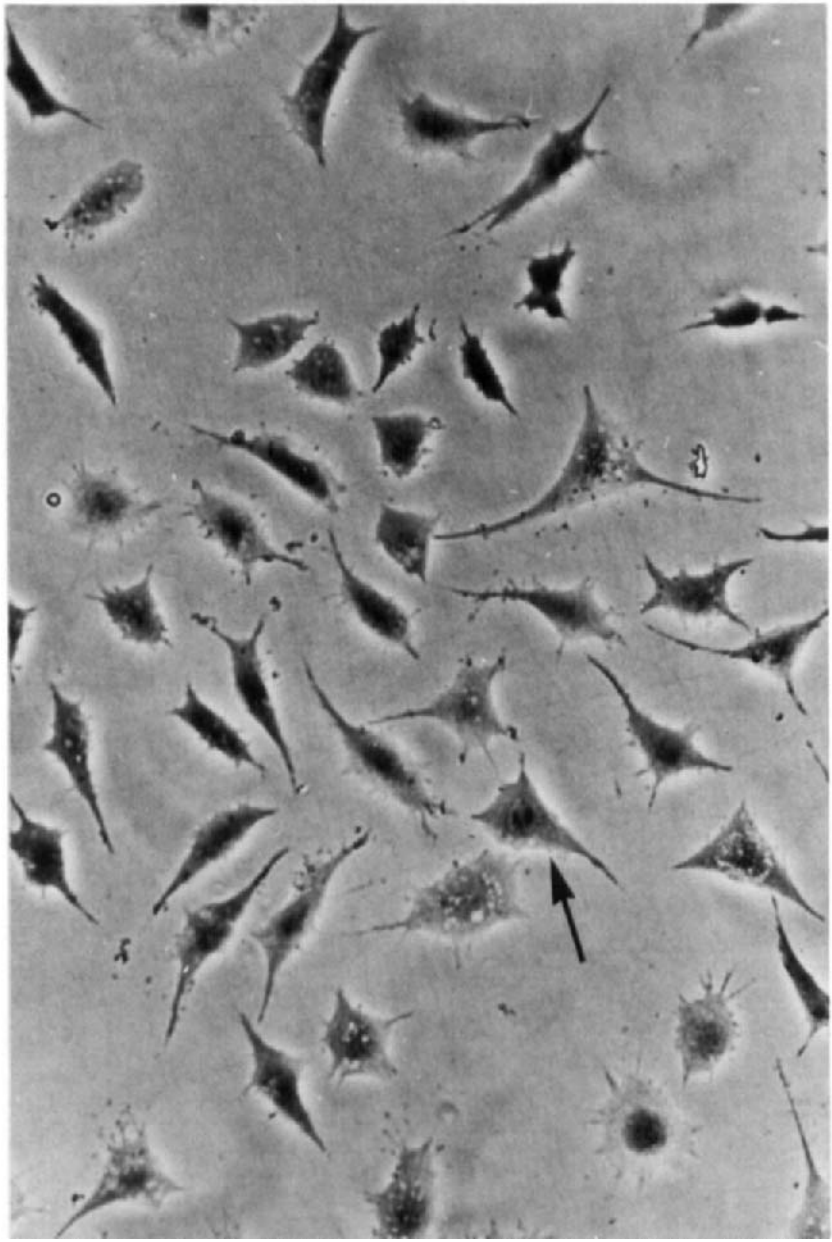

FIG. 1. Phase-contrast micrograph of enucleates from T-flask method of enucleation. (Arrow indicates nucleated cell for reference.) After fixation and staining, this preparation was shown to be 97% enucleated.

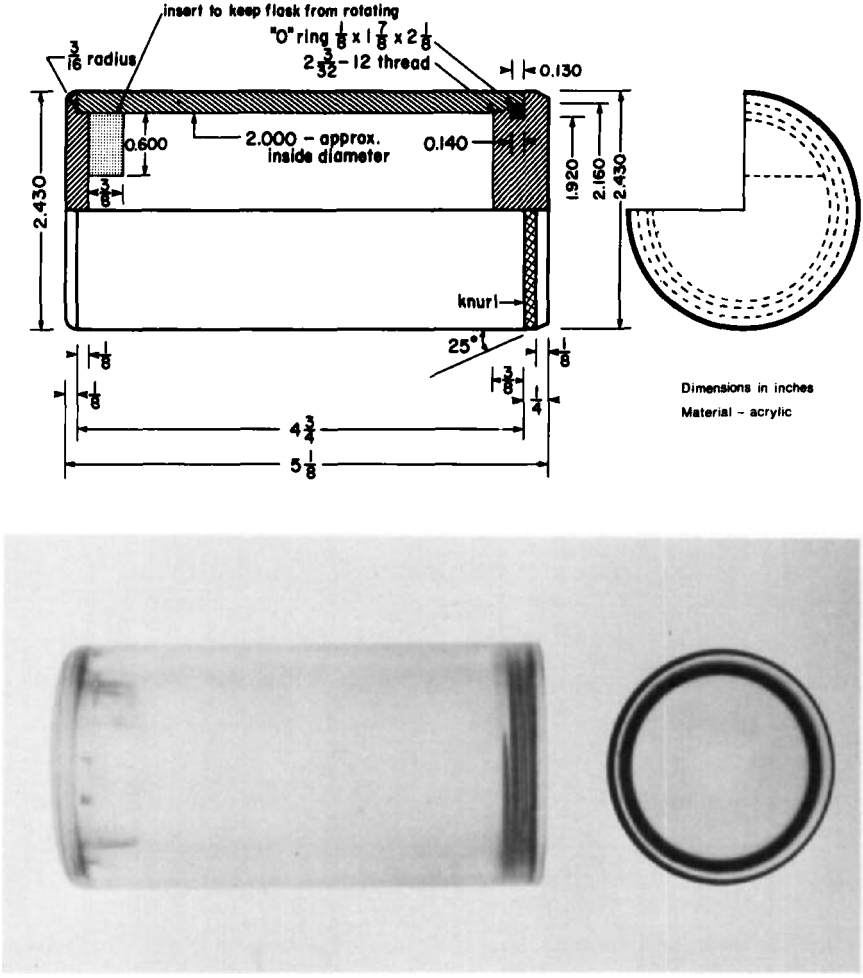

FIG. 2. Diagram and photograph of acrylic inserts for Sorvall model GSA rotor for use with T-flask enucleation procedure.

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We wish to acknowledge the assistance of E. Nettesheim in the design and construction of the acrylic inserts.