METHODS IN CANCER RESEARCH

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VOLUME V

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Preface

When Volume IV of "Methods in Cancer Research" was completed it was anticipated that this treatise had reached a point where additions would not be necessary for several years. However, so many important new techniques apply to the cancer problem that at the urging of a number of colleagues two more volumes are being added.

Advances in methods pertaining to cell hybridization, organ culture, cell membranes, and methods for growing new cells are presented in this volume. Special methods particularly relating to lymphocytes which are of importance both to human neoplasia and animal model systems are presented from biochemical, morphological, and tissue culture aspects. The continuing growth of methods in molecular biology included relate to sequential analysis of proteins and RNA, methods for the study of DNA of tumor viruses, and additional aspects of enzymology of the nucleus.

As pointed out earlier, much more incisive methods are required for cancer research. However, more powerful methods are being developed with each passing year and the "critical mass" required for the solutions of this difficult problem may be envisioned in the not too distant future.

I wish to express my sincere appreciation to all of the contributors to this and previous volumes as well as to the many helpful individuals at Academic Press. In addition it is a special satisfaction to honor the memory of Kurt Jacoby with this volume.

HARRIS BUSCH

Houston, Texas
April, 1970

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This volume is dedicated to the memory of Kurt Jacoby who was both friend and advisor to many American scientists. His stimulation, encouragement, and kindly consideration of suggestions of many young authors were important in the growth of biochemistry, molecular biology, and other branches of science.

Kurt Jacoby provided a warm and interested personality, a search for excellence, and a transcendent humanitarianism that made an association with him considerably more than a business relationship. His sense of the future made it possible for the editor and many other scholars to develop works that fill gaps and provide stimulation to other colleagues in science. It is hoped that his principles and interests will long be continued.

Contents

BIOLOGICAL METHODS

Contents of Other Volumes

VOLUME I

Morphology

Transplantation and Metastasis

Carcinogenesis

VOLUME II

Immunology and Special Products

Cell Fractionation

Enzymes

VOLUME III

Molecular Biology

Sources of Antitumor Agents

VOLUME IV

Carcinogenesis

Biology

Therapy

Molecular Biology

CHAPTER I

ORGAN CULTURE METHODS

GERALD C. EASTY

I. Introduction

When cells are grown *in vitro* under conditions where their mutual interactions are reduced and their proliferative capacities stimulated, as in monolayer and suspension cultures, they usually lose many of the morphological characteristics and specific functions which they possessed in the intact organ. These cell cultures are used mainly for the study

of various aspects of cell proliferation and basic metabolism, and if one wishes to study the functions of differentiated tissues *in vitro*, it is necessary to employ organ culture techniques.

The use of these techniques enables the tissues to be maintained in a histologically and functionally differentiated condition where mitosis is restricted and cell migration largely eliminated. The term "organ culture" is for many purposes a misnomer because it is not possible at present to maintain all organs intact *in vitro* in an adequately functioning condition employing simple culture techniques.

As there is no circulation of medium in all but a few organ culture systems, at least one dimension of the cultured tissue should not exceed 1 to 2 mm in thickness in order to permit the diffusion of nutrients, oxygen, and metabolic waste products through the tissue. Therefore, when the organ is too large, a representative fragment or slice is cultured and a more accurately descriptive term for this is "organotypic culture," a term first employed by Maximow (1925). Loeb (1897) was probably one of the first to attempt to culture fragments of adult organs when he immersed pieces of rabbit liver and kidney in serum and did not detect histological evidence of tissue damage for at least 3 days. From 1926 on, the techniques were developed and used extensively by Fell and her associates. In recent years their use has spread throughout the world, undergoing many refinements and modifications to meet the requirements of individual investigators.

Most of these modifications have certain features in common imposed on them by the problems of diffusion and the reasons for which the technique is employed. The chief reason for using the technique is, or should be, that it is desired to investigate the properties, biosynthetic functions, sensitivities, and interactions of the cells in an organized tissue free of the complexities, often unknown or uncontrollable, which are present *in vivo*, where the tissue may be influenced by the rest of the animal through the medium of the circulating blood, the lymph, and the nervous system. A word of caution should perhaps be inserted here. The main advantage of using the organ culture technique in preference to the monolayer technique is that many of the specific functions and sensitivities of cells appear to be lost when they are separated from an organ and grown as individuals in monolayer or suspension culture. In recent years, however, considerable progress has been made in developing media and conditions in which certain monolayer cultured cells can retain the capacity to synthesize specific molecules characteristic of their differentiated state *in vivo* (Green and Todaro, 1967). If properly maintained, these cells do not "dedifferentiate" or change into cells which can only be described as fibroblasts, epithelial, or ameboid cells. If, therefore, it is possible to grow in monolayer culture the cells which