

# TISSUE CULTURE

*Methods and Applications*

*Edited by*

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*Biomedical Division*

*The Samuel Roberts Noble Foundation, Inc.*

*Ardmore, Oklahoma*



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## *Preface*

Tissue culture research has been expanding explosively during the past decade, and the initial concept of this book was simply to collate some of the newer tissue culture methods, particularly those involving some degree of automation. This plan was altered in favor of a treatise that not only brings tissue culture methodology up-to-date but also includes representative protocols for the application of these techniques. Accordingly, over a hundred authors have pooled their efforts to produce this volume. In so doing, the mutual hope is that it will serve as a reference portfolio to help both the novice and veteran researcher "get on with the job" in an inspired, efficient, and productive manner.

Since there are several excellent books available that describe many of the basic techniques of tissue culture, some of the classic procedures, e.g., hanging-drop cultures, are not included. Methodology in tissue culture has progressed dramatically, however, and much of this gain has not been summarized in book form. In fact, over eighty percent of the procedures included are derived from research conducted in the last eight years. Moreover, tissue culture application has mushroomed so in recent years that it has become a part of diverse biological inquiries. Thus, tissue culture methods per se and applications are scattered profusely throughout the contemporary scientific literature. This book attempts to present an overview of up-to-date procedures for working with cells in culture and for using them in a wide variety of scientific disciplines.

The reader will find a series of protocols useful for dissociating tissues (Section I) followed (Section II) by exemplary procedures for establishing primary cultures of cells from vertebrate, invertebrate, and plant sources. Once cultures are established, it is important to harvest them (Section III), reproduce them (Section IV), or clone them (Section V) efficiently and properly. In some instances it is most desirable to automate systems, regulate culture environments, or work with massive quantities of cells (Section VI), and for certain purposes not be limited necessarily to culture supports only of glass or plastic (Section VII).

Standardized procedures for evaluating the progress and behavior of cells in culture are presented in Section VIII, including enumerations of cells and nuclei and analyses of cell cycle events. Some of the more recent "tricks of the trade" in microscopy applied to tissue culture are described in Section IX, including aspects of light, electron, and fluorescence microscopy.

The next four sections (X-XIII) are intended to indicate the tremendous latitude that tissue culture has in science. More importantly, they may provide

inspiration for further work. Although a dozen or more subjects might have been chosen for illustration, these sections have been confined to descriptions of procedures for cell hybridization, virus propagation and assay, production of hormones and intercellular substances, and the use of tissue culture in the diagnosis and understanding of disease.

The last portion of this book (Section XIV) deals with quality control measures in the tissue culture laboratory, a first consideration for anyone employing cells in culture. This aspect of tissue culture has been surveyed often in the literature and in the relatively few existing books on the subject. However, just as in all other phases of tissue culture methodology and application, the techniques of sterility testing, cell species (and intraspecies) identifications, standardizations of media preparation, etc., have much improved during the past decade. Thus in some cases, and especially for those relatively unfamiliar with working with cell cultures, this last section may be read first.

Most tissue culture work to date has dealt with mammalian, avian, and plant material. Especially in recent years, though, there has been considerable pioneering work with amphibian and fish tissues and with cells from invertebrate species. The latter are of interest, to cite just one reason, because insects are vectors for a number of human diseases. Also in recent years some very important observations have been made on characteristics of cells in culture. These include cells which continue to show specialized functions or express genetic abnormalities *in vitro*. All of these and other recent and exciting developments in methodology and application are represented by one or more well-documented procedures.

There are thousands of people in the world of science working with cells in culture who have only a peripheral interest in methodology *per se*. This is because their primary research expertise lies in another discipline; for them, the use of cells in culture is but one possible exploitable technology. These investigators may find this book in the same vein as the young high school girl in biology class who was assigned a book about penguins for outside reading. When asked about the book by her instructor, she replied in effect that it was interesting but that it told her more about penguins than she really wanted to know. On the other hand, there are thousands of students of cells in culture for whom it is impossible, of course, to include in one volume everything they always wanted to know about tissue culture. Our hope is that something in this book will be of value to all using tissue culture.

We express our deep appreciation for the enthusiastic cooperation of the contributors, many of whom felt as we did that a compilation on tissue culture of this nature would be valuable. Thanks are due also to the staff of Academic Press for their encouragement and cooperation. In addition, we are much indebted to other people who have been directly connected with this endeavor. Assistance in editing, retyping manuscripts, and indexing was supervised by Gwen Taft, who had very capable help from Jane Lawrence, Marcia Remondino, and Linda Platt. Necessary printed informational material for the large number of contributors and assistance in photography were provided by Glenn Elmore and Ivan Lawson. Finally, we express our appreciation to the Board of Trustees of The Samuel Roberts Noble Foundation, Inc., who authorized a subvention which helped

reduce the cost of the book in the hope that by so doing the information generously provided by the many contributors might be more readily available to students and life science researchers alike throughout the world.

If the reader finds this volume a useful guide and reference work, or, more importantly, derives stimulation to conduct *in vitro* studies of vertebrate, invertebrate, or plant cells more meaningful than those described to date, then the contributions of all will have been worthwhile.

PAUL F. KRUSE, JR.  
M. K. PATTERSON, JR.

reduced the cost of the task in the hope that by reducing the information given, only provided by the many contributors might be more readily available to scientists and life science researchers alike throughout the world.

At the same time, the volume of useful work and information work in the field of biology is increasing so rapidly that the number of contributors is increasing at a rate which is not commensurate with the amount of work that can be done. It is therefore necessary to select those contributors who are most likely to be of use to the community of life scientists.

Paul F. Patterson, Jr.  
Editor, *Journal of Biological Chemistry*



*Dr. Paul F. Kruse, Jr.*

(1921-1973)

On January 31, 1973, Dr. Paul F. Kruse, Jr., died after a brief illness. Because of his dedication and contributions to tissue culture and to this book, the coeditor and contributors dedicate this volume to his memory.

Dr. Kruse was Director of the Biomedical Division and Vice President of The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma. He was Treasurer of the Tissue Culture Association (1966-1971) during the period of construction of The W. Alton Jones Cell Science Center. He served on the Executive Committee, was Chairman of the Publicity and Endowment Committee, and was a visiting staff lecturer at the Center. He was a member of the Association's committees on NASA programs and International Science Fairs, he was on the Advisory and Peer Review Committee of the American Type Culture Collection, and was a member of its Board of Trustees. He was a member of numerous site review teams and a consultant on governmental, university, and industrial programs.

In spite of the hours demanded of him for administrative duties, Dr. Kruse found time for research. The perfusion culture system he developed was the prototype of the systems commercially available today. He applied the method to the study of metabolism and growth of cells under "more physiological conditions."

Dr. Kruse insisted that this book be edited in such a manner that it would be useful to investigators at all levels. His interest in tissue culture was exemplified by his vigorous pursuit of a subvention so the cost of this book would not be prohibitive for anyone. All editorial royalties are toward this end.

In all things, Paul Kruse truly expressed his beliefs in both words and actions.

M. K. PATTERSON, JR.

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