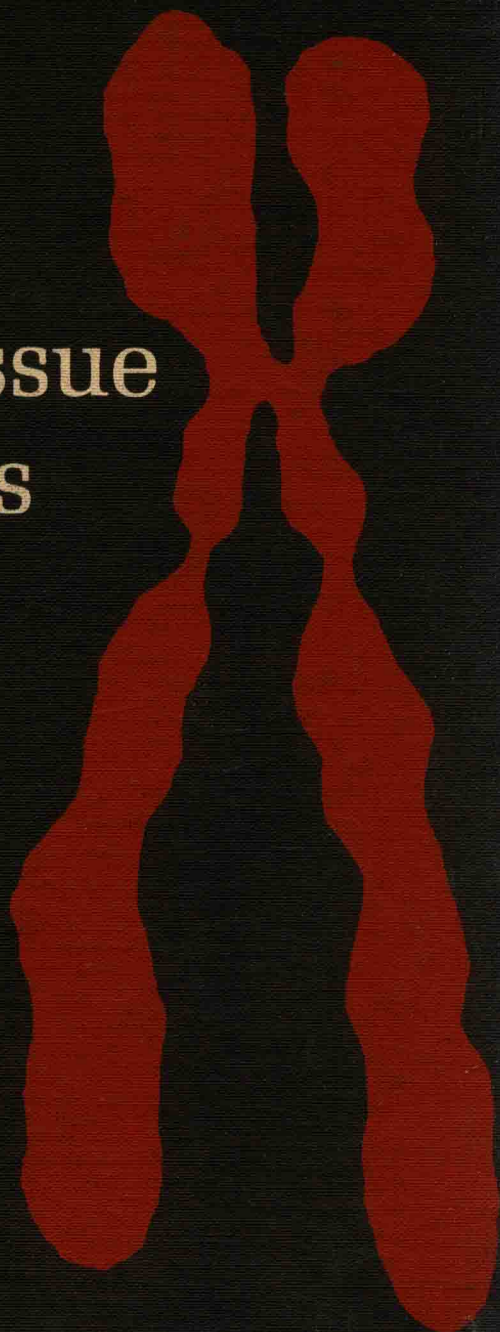


Gretchen L. Humason

Animal Tissue Techniques



Third Edition

Animal Tissue Techniques

Gretchen L. Humason

OAK RIDGE ASSOCIATED UNIVERSITIES



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Animal Tissue Techniques

A Series of Books in Biology

EDITORS Donald Kennedy
Roderic B. Park

Preface

This book of basic and standard histological procedures (and some specialized techniques) was designed to meet the diverse needs of premedical students, medical technicians, zoology majors, and research assistants. Most histological reactions follow a logical and specific sequence, and I have attempted to include simplified discussions of the basic methods that are applicable both to normal and to pathological conditions in zoology and medicine.

It is not intended that this text should be a complete reference book on histology; the experienced worker knows of numerous such tomes, as well as journals that specialize in histology and related topics. However, special methods of wide usage and exceptional merit are included, particularly those that are not overly complicated or unpredictable. It is hoped that the technician, once familiar with the material covered here, will watch the literature for modifications and improvements of standard techniques; in this way, with this book as a foundation, his work can be kept up to date and, perhaps, simplified.

Methods for fixation are fairly well established, with only occasional variations. The section on fixation presented herein is as modern as I can make it, and it includes a brief description of the chemicals employed. Old

staining techniques continue to be perfected and new ones developed; I have tried to include the best of these and, for the sake of the student, to adapt them to the standard three-hour laboratory period and to the kinds of equipment most widely available. Some special methods that are more time-consuming have been included for special projects and research. They have been simplified, wherever possible, to serve as introductory techniques for the student who plans to proceed to more complicated techniques later.

Some instructors may not agree with the way in which I have organized the text, but to me it is a logical one. Thus, fixation is treated first, because it is usually the first process in tissue preparation; this is followed by embedding in some kind of medium, sectioning on a microtome, mounting sections on slides, and, finally, staining them with the help of a microscope. A logical arrangement of staining methods is hard to come by, so I have followed my own inclinations: some sections are organized by related tissues, others by related methods. The latter was considered desirable for such processes as silver impregnation, metachromasia, and the use of Schiff reagent. The final chapters include such specialized techniques as histochemistry, chromosome preparation, autoradiography, and invertebrate mounts. Wherever possible, I have referred to my own experience with these methods to help students succeed with their first efforts, and I have included modifications that might appeal to other adventurous technicians.

This book is in four parts. Part I covers those basic procedures and general considerations with which every tissue technician should be familiar. Part II provides detailed information about specific staining methods for most tissues. An instructor might choose a few favorite methods from this section to round out a course, while the professional technician will find here most of the specific methods required on the job. Part III deals with special procedures, those that are special in the sense that they are not common in most laboratories, although they may be very important in some. Although the discussion of some of these procedures is brief, references have been cited extensively for the benefit of those who might wish to refer to more thorough discussions. Part IV is devoted largely to laboratory aids and the preparation of solutions—useful information in any laboratory.

In the third edition, *Animal Tissue Techniques* has been extensively revised and updated. Many of the changes have been to improve its usefulness for graduate and undergraduate teaching. The typography has been altered and the design improved with an eye to making the book more readable and, hence, more useful to students and technicians alike.

Too, the list of references has been carefully emended to cover recent important publications in the field.

To have included everything necessary to satisfy everyone and still to have kept the price of the book within the means of the average student would have been impossible. Some topics, necessarily, have been treated only in passing. The electron microscope, for example, is much too specialized for students in beginning technique classes, and an entire book could be devoted to instructing students in its operation alone. The topic of photomicrography is equally complex. Methods for preparing plastic whole mounts have not been included; excellent leaflets on the subject are published by the companies that supply the materials necessary for their preparation. Good color photographs are helpful, but they are also, unfortunately, expensive—even a few of them can add appreciably to the cost of a book. In my teaching, I have used a demonstration set of slides to help my students recognize proper staining. The set started with a few of my own slides, and it was gradually enlarged by additions from the students in my classes. The students were happy to contribute examples of their best work, and the collection eventually increased to several hundred excellent slides. Other instructors might consider building a study collection of slides in the same way.

I have derived invaluable personal satisfaction from my association with students. I am grateful to them for helping me to develop my tolerance and patience—two qualities that are essential in my profession. I am grateful to them, too, for what they have helped me to learn, for there is no surer way to master a subject than to teach it to others. One former student in particular should receive credit for her encouragement and for prodding me toward writing this book—Marlies Natzler of the University of California at Los Angeles.

Grateful acknowledgements are also due to Marvin Linke, Jeanne Simons, and Leta Burleson, the three artists who contributed to the three editions of this book; to Julie Langham, for help with photography; to Nellie M. Bilstad, for valuable suggestions; to the Cytogenetics Division of Oak Ridge Associated Universities, for information about late developments in chromosome preparation; to the Zoology Department of the University of California at Los Angeles for the lessons I learned there as a student, a departmental technician, and a lecturer; and to Dr. C. C. Lushbaugh, for his continued encouragement.

October 1971

Gretchen L. Humason

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PART **I**

**BASIC
PROCEDURES**

CHAPTER 1

Fixation

As soon as a tissue ceases to be alive, its cells start to change. Multiplying bacteria begin to destroy them, and the process of autolysis (self-digestion) by contained enzymes begins to dissolve them. The activity of these enzymes is reversed from that in live cells; instead of synthesizing amino acids into proteins, they begin to split proteins into amino acids. These amino acids diffuse out of the cells; as a result cell proteins are no longer coagulable by chemical reagents. These cell changes are called postmortem conditions and must be prevented if tissue is to be examined in the laboratory.

The prevention of postmortem conditions is the primary objective of tissue preparation, but it is also necessary to treat tissue to differentiate the solid phase of the protoplasm from the aqueous phase, to change cell parts into materials that will remain insoluble during subsequent treatment, and to protect cells from distortion and shrinkage when subjected to such fluids as alcohol and hot paraffin. Other important objectives of tissue preparation are to improve the staining potential of tissue parts and to alter their refractive indices for better visibility.