

# ANIMAL MICROLOGY

*Practical Exercises in Zoölogical Micro-Technique*

*By*

MICHAEL F. GUYER

*Professor Emeritus of Zoölogy in the University of Wisconsin*

*With a Chapter on Drawing by*

ELIZABETH A. (SMITH) BEAN

*Former Assistant Professor in Zoölogy in the University of Wisconsin*

FIFTH REVISED EDITION

1953



THE UNIVERSITY OF CHICAGO PRESS

THE UNIVERSITY OF CHICAGO COMMITTEE  
ON PUBLICATIONS IN BIOLOGY AND MEDICINE

EMMET B. BAY • LOWELL T. COGGESHALL  
LESTER R. DRAGSTEDT • FRANKLIN C. McLEAN  
THOMAS PARK • WILLIAM H. TALIAFERRO

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO 37  
Cambridge University Press, London, N.W. 1, England  
The University of Toronto Press, Toronto 5, Canada

*Copyright 1906, 1917, 1930, 1936, and 1953 by The University of Chicago. All rights reserved. Published 1906. Second edition 1917. Third edition 1930. Fourth edition 1936. Fifth edition 1953. Second impression 1954. Composed and printed by THE UNIVERSITY OF CHICAGO PRESS, Chicago, Illinois, U.S.A.*

# ANIMAL MICROLOGY

## PREFACE TO THE FIRST EDITION

For the last ten years it has been a part of the writer's duties to give instruction in microscopic technic, and it has seemed to him that there is need for a series of practical exercises which will serve to guide the beginner through the maze of present-day methods, with the greatest economy of time, by drilling him in a few which are thoroughly fundamental and standard. The book is intended primarily for the beginner and gives more attention to the details of procedure than to discriminations between reagents or the review of special processes. The student is told what to do with his material, step by step, and why he does it; at what stages he is likely to encounter difficulties and how to avoid them; if his preparation is defective, what the probable cause is and the remedy. In short, the book attempts to familiarize the student with the little "tricks" of technic which are commonly left out of books on methods but which mean everything in securing good results.

A very brief, nontechnical account of the principles of the microscope is inserted (Appendix A) with the idea of giving the student just enough of the theoretical side of microscopy to enable him to get satisfactory results from his microscope. The microscope is so ably treated in the excellent works of Gage (*The Microscope*) and Carpenter (*The Microscope and Its Revelations*) that the writer feels himself absolved from any further responsibility in this matter.

The aim of the entire book is to be practical: to omit everything that is not essential; and, above all, to give definite statements about things. Appended to each chapter is a series of Memoranda which serve to supply additional information that is more or less pertinent without obscuring the main features of the method under consideration.

In Appendix B the formulas for a number of the most widely used reagents are given, with comments upon their uses and manipulation. Following this (Appendix C) is a concise table of a large number of tissues and organs with directions for properly preparing them for microscopic study.

Inasmuch as every experienced worker has his own "best" method for the preparation of almost any tissue, it is manifestly impossible to give all "best methods" in such a table. The writer believes, however, that the student will find the methods recommended all good ones which will yield satisfactory results.

In Appendix D some directions are given for collecting and preparing material for an elementary course in zoölogy.

It is hoped that the volume will prove of use: (1) as a class textbook; (2) as a guide to the independent individual worker (teacher, physician, college or medical student, or novice); (3) as a reference book for teachers, in the preparation of material for courses in elementary zoölogy, histology, or embryology.

In the matter of expressing his obligations the writer is at a loss to know just what to do. Many of the methods in microscopic technic have been handed down tradition-wise from one worker to another until their origin is unknown; they are the accumulated experiences of several generations of workers. Furthermore, many points have been absorbed, as it were, by the writer, from fellow-workers in the Universities of Chicago, Nebraska, and Cincinnati, respectively; consequently the obligation cannot be specifically expressed. Where the name of the originator of a method is known, due credit has been given. The books to which the author is most heavily indebted are the volumes of Gage and Carpenter, already mentioned, Lee's *Microtometist's Vade-mecum*, Whitman's *Methods in Microscopical Anatomy and Embryology*, Hardesty's *Neurological Technique*, Foster and Balfour's *Elements of Embryology*, Minot's *Laboratory Text-Book of Embryology*, Huber's translation of the Böhm-Davidoff *Text-Book of Histology*, Stöhr's *Text-Book of Histology*, Mallory and Wright's *Pathological Technique*, Bausch's *Manipulation of the Microscope*, and the *Journal of Applied Microscopy*. Grateful acknowledgment is also made to the various manufacturers of microscopic instruments and appliances for the loan of most of the cuts which have been used in this volume.

M. F. G.

## PREFACE TO THE SECOND EDITION

The favorable reception accorded the first edition of *Animal Micrology* has encouraged the author to believe that a second edition, incorporating some of the many new methods which have appeared during the last ten years, would be equally welcome. The general plan of the book has not been altered (see Preface to the First Edition, on a preceding page), although changes have been made on nearly every page, many sections have been entirely rewritten, and two new chapters, one on "Cytological Methods," the other on "Drawing," have been added. The chapter on drawing was prepared by Dr. Elizabeth A. Smith.

In spite of a determined effort to limit the book to its former size, it has expanded by over fifty pages. For every method dropped, there seemed to be a host of good new ones demanding recognition. These in the main, however, have been left to the encyclopedia and the various technical books and journals listed at the end of the volume. As in the first edition, the policy has been, not to attempt to give all "best" methods, but rather to select representative good ones which have proved their worth by satisfactory tests in American laboratories.

Whatever merit the new edition may prove to have over that of the earlier one is due in no small measure to the many helpful suggestions of my colleagues in other colleges and universities. I am particularly indebted in this respect to Professors C. E. McClung, R. R. Bensley, H. McE. Knower, F. L. Landacre, F. C. Waite, B. M. Allen, George R. La Rue, Edward L. Rice, F. D. Barker, R. M. Strong, and H. L. Wierman, and to Doctors Elizabeth A. Smith and C. H. Heuser.

M. F. G.

## PREFACE TO THE THIRD EDITION

The continued use of this book in both American and foreign laboratories encourages the author to believe that it still satisfactorily meets the needs of beginners in microscopic technic. That they may have the benefit of the more important advances of recent years, this third edition has been prepared. As in former editions, the effort has been to make it, not an encyclopedia of technic or a catalogue of all possible procedures, but a representative series of reliable standard methods.

For helpful suggestions in connection with the present edition, I am particularly indebted to Harold W. Beams, Joseph B. Goldsmith, Christopher J. Hamre, Frederick L. Hisaw, Steven J. Martin, Harvey M. Smith, Opal M. Wolf, and Chao-Fa Wu.

M. F. G.



## PREFACE TO THE FOURTH EDITION

If the author may judge from the continued use of this book in many laboratories for some thirty years, together with comments he has had on it from time to time, it has become a sort of stand-by for beginners in animal microscopy, for many physicians who wish to do microscopic investigation, and for teachers who are their own technicians. He feels obligated, therefore, to pass on to such users any knowledge of outstanding advances in technic that comes his way which may save their time materially or make for greater precision. Because of its time-saving advantages and also its value with tissues which become unduly hard, brittle, or friable under ordinary treatment, the introduction of the dioxan method (chap. vii) would alone warrant a new edition. Minor changes, mainly additions, have been made on many pages, however; and such new special technics as have proved to be exceptionally valuable in the author's own laboratory or in those of his immediate associates have been included.

Grateful acknowledgment is made to Harland W. Mossman, Pearl E. Claus, Nellie M. Bilstad, Ernst J. Dornfeld, Melvin Doner, James O. Foley, and Merlin L. Hayes for helpful suggestions.

M. F. G.

## PREFACE TO THE FIFTH EDITION

Although this book was first published over forty-five years ago, its steadily increasing use in various laboratories as a textbook and for reference makes it imperative that it be revised from time to time, with the addition of new technics and the replacement of various older ones. It is left to earlier editions to provide such of the latter as may still occasionally be desired.

While interest is at present probably keener in the chemical constitution of living matter than in its histological makeup, still a knowledge of the structural background of what we are analyzing chemically is indispensable. The fact remains that in plants and animals the chemical constituents are very definitely combined and arranged into characteristic larger units, and it is these that the histologist is viewing and naming.

Inasmuch as Appendix C on the preparation of individual tissues and organs has come to be widely used for reference in many laboratories, the author has had it carefully reviewed by Dr. Pearl E. Claus (Mrs. Eugene Whitehead), who gives both introductory and advanced cytological courses in technic in the zoölogical department of the University of Wisconsin. He also is greatly indebted to various others of his associates for their suggestions and assistance.

M. F. G.

## CONTENTS

INTRODUCTORY . . . . .	1
Apparatus and Supplies Required, 1; General Rules, 4.	
I. PREPARATION OF REAGENTS . . . . .	6
Practical Exercises, 6; Memoranda, 10-12.	
II. GENERAL STATEMENT OF METHODS . . . . .	13
Killing, Fixing, and Hardening, 13; Washing, 15; Dehydrating, 16; Preserving, 16; Staining, 17; Clearing, 19; Mounting, 20; Imbedding, 20; Affixing Sections, 21; Decolorizing, 22; Bleaching, 22; Corrosion, 22; Decalcification and Desilicidation, 23; Injection Methods, 23; Isolation of Histological Elements, 23; Normal or Indifferent Fluids for Examining Fresh Tissues, 23; General Scheme for Mounting Whole Objects ( <i>in toto</i> Preparations) or Sections, 24.	
III. KILLING AND FIXING . . . . .	25
Cautions, 25; Fixing with Zenker's Fluid, 26; Fixing with Bouin's Fluid, 26; Formalin as a Fixing Reagent, 27; Memoranda, 27-31.	
IV. SIMPLE SECTION METHODS . . . . .	32
Freehand Section Cutting, 32; Memoranda, 33, 34.	
V. THE PARAFFIN METHOD: INFILTRATION AND SECTIONING . . . . .	35
The Method, 35; Memoranda, 41-44; Difficulties Likely To Be Encountered in Sectioning in Paraffin, and the Probable Remedy, 44.	
VI. THE PARAFFIN METHOD: STAINING AND MOUNTING . . . . .	47
Staining with Hematoxylin, 47; Double Staining in Hematoxylin and Eosin, 49; Double Staining in Cochineal and Lyons Blue, 49; Staining with Heidenhain's Iron-Hematoxylin, 49; Iron-Hematoxylin with Other Stains, 51; Staining in Bulk before Sectioning, 51; Paraffin Method for Delicate Objects, 52; Euparal as a Mounting and Preservation Medium, 53; Memoranda, 53-58.	
VII. THE DIOXAN METHOD . . . . .	59
Why Valuable, 59; The Method, 59; With the Freezing Method, 61; Memoranda, 60-62.	
VIII. THE CELLOIDIN METHOD . . . . .	63
The Method, 63; Staining Celloidin Sections in Hematoxylin and Eosin, 66; Memoranda, 66-70.	
IX. THE FREEZING METHOD . . . . .	71
The Method, 72; Chemosurgical use, 75; Memoranda, 73-75.	

X. METALLIC SUBSTANCES FOR COLOR DIFFERENTIATION . . . . .	76
A Golgi Method for Nerve Cells and Their Ramifications, 76; Memoranda on Golgi Methods, 77, 78; Other Silver Nitrate Methods, 78; Protargol, 80; Memoranda on Silver Methods, 79-81 Gold Chloride Method for Nerve Endings, 81.	
XI. ISOLATION OF HISTOLOGICAL ELEMENTS: MINUTE DISSECTIONS . . . . .	83
Dissociation by Means of Formaldehyde, 83; Isolation of Muscle Fibers by Maceration and Teasing, 83; Maceration by Means of Hertwig's Fluid, 84; Mall's Differential Method for Reticulum, 84; Dublin's Method for Reticular Connective Tissue, 85; Maceration Technics Recommended for Various Tissues, 85; Minute Dissection and Mounting of Various Parts of Insects, 86; Illuminating Living Structures, 88; Memoranda, 87, 88.	
XII. TOOTH, BONE, AND OTHER HARD OBJECTS . . . . .	89
Sectioning Decalcified Tooth, 89; Sectioning Decalcified Bone, 89; Sectioning Bone by Grinding, 89; Memoranda, 90, 91.	
XIII. INJECTION OF BLOOD AND LYMPH VESSELS . . . . .	92
Red Injection Mass, 92; Blue Mass, 92; Yellow Mass, 92; Rubber Injection, 93; Injecting with a Syringe, 93; Micro-injection of Embryonic Vessels, 96; Corrosion Methods, 99; Memoranda, 95-100.	
XIV. OBJECTS OF GENERAL INTEREST: CELL-MAKING, FLUID MOUNTS, <i>in toto</i> PREPARATIONS, ETC. . . . .	101
Turning Cells, 101; Mounting in Glycerin (Water Mites, Transparent Larvae), 102; Killing and Mounting Hydra, 102; Mounting in Glycerol (Small Crustacea, etc.), 103; Mounting in Balsam (Flat Worms, Mosquito, Gnat, Aphid), 104; Opaque Mounts (Beetles, Wings of Moths and Butterflies, Head of Fly, Foreleg of <i>Dytiscus</i> ), 105; Dry Mounts, 106; Spalteholz Method of Clearing Total Specimens, 110; Memoranda (Including Directions for Mounting Other Forms), 106-13.	
XV. BLOOD . . . . .	114
Examination of Fresh Blood, 114; Effects of Reagents, 114; To Demonstrate Blood Platelets, 114; Stained Preparation of Fibrin, 115; Crystals of Blood, 115; Cover-Glass Preparations (Dry), 115; Rapid Method, 116; Enumeration of Blood Corpuscles, 116; Observation of the Blood Current, 118; Inflammation, 119; Blood Species, 121; Memoranda, 119-21.	
XVI. BACTERIA . . . . .	122
Bacterial Examination, 122; Cover-Glass Preparations from Fluid Media, 122; Staining and Mounting, 123; Bacteria in Tissues, 124; Methylene Blue Stain for Bacteria in Tissues, 124; Gram-Weigert	

Method for Bacteria in Tissues, 124; Hanging-Drop Preparations, 125; Memoranda, 126-29.

XVII. SOME EMBRYOLOGICAL METHODS: SECTIONS AND *in toto* MOUNTS OF FROG AND CHICK; OTHER FORMS . . . . . 130

The Frog, 130; Section Method, 130; Whole Mounts, 131; Memoranda on Amphibian Material, 132-34; The Chick, 134; General Memoranda on Embryological Methods and Materials, including *in vitro* Cultures, 136-49; Ethyl Metacrylate Mounting, 149; Mesenchymal Derivatives, 149

XVIII. SOME CYTOLOGICAL METHODS . . . . . 150

General Remarks, 150; Mitosis, 151; Testis of Crayfish, Sections, 151; Smears, 152; Blastodisk of Whitefish, 152; Testis of Necturus, 152; Somatic Cells of *Ambystoma*, 153; Living Cells, 153; Mitochondria, 154; Golgi Apparatus, 158; Staining of Living or Fresh Tissues, 160; Quartz Rod Illumination, 160; Tests for Certain Cellular Structures, 161; Special Methods, 166; Allen's B-15 and B-20 Methods, 166; Hance's Cold Method, 167; Gomori's Method, 167; Feulgin's Technic, 169; Schiff's Reagent, 169; Photographing Cellular Structures, 170; Accessory Chromosomes, 171; Aceto-carminc Preparations, 171; Protoplasmic Currents, 172; Celloidin instead of Paraffin, 172; Urea in Fixing Fluids, 172; Drop-Method of Changing Fluids, 172; Dehydration by Dialysis, 173; Tissues of Young Adults Desirable, 173; Dissection of Living Cells, 173; Estimation of Carbon Dioxide, 173; Euparal, 174; Hydrogen-Ion Indicators, 176; Memoranda, 171-76.

XIX. RECONSTRUCTION OF OBJECTS FROM SECTIONS . . . . . 177

Reconstruction in Wax, 177; Geometrical Reconstruction, 178; Blotting-Paper Method, 179; Rolling Drawings into the Wax, 179; Photography in Reconstruction Work, 179; Cutting Out Wax Plates, 180; Memoranda, 178, 180.

XX. DRAWING . . . . . 181

Materials for Class Work, 181; Methods of Representation, 182; Outline, 182; Depth, 182; Ink Drawings, 182; Pencil Drawings, 183; Wash Drawings, 184; Size and Arrangement of Drawings, 184; Labeling, 185; Modes of Representation for Special Courses, 185; Embryology, 186; Histology, 186; Cytology, 187; Drawings for Publication, 187; Materials for Manuscript Drawings, 187; Camera Lucida, 188; Reduction, 188; Line Process, 189; Halftone, 189; Wash and Combination Drawings for Reproduction, 190; Lithography, 191; Arrangement for Reductions, 191; Lettering, 191.

APPENDIX A. THE MICROSCOPE AND ITS OPTICAL PRINCIPLES . . . . . 195

Optical Principles, 195; Lenses, 196; Images, 197; The Simple Microscope, 198; The Compound Microscope, 198; Defects in the

Image, 200; Nomenclature or Rating of Objectives and Oculars, 203; Some Common Microscopic Terms and Appliances (Alphabetically Arranged), 205; Manipulation of the Compound Microscope, 224.	
APPENDIX B. SOME STANDARD REAGENTS AND THEIR USES . . . .	228
Fixing and Hardening Agents, 228; Stains, 240; Herxheimer's Solution, 261; Normal or Indifferent Fluids, 263; Dissociating Fluids, 264; Decalcifying Fluids, 265.	
APPENDIX C. TABLE OF TISSUES AND ORGANS, WITH METHODS OF PREPARATION . . . . .	267
APPENDIX D. PREPARATION OF MICROSCOPIC MATERIAL FOR A GENERAL COURSE IN ZOÖLOGY . . . . .	283
APPENDIX E. TABLE OF EQUIVALENT WEIGHTS AND MEASURES . . .	307
APPENDIX F. REFERENCES . . . . .	309
INDEX . . . . .	311

## INTRODUCTORY

### APPARATUS AND SUPPLIES REQUIRED

The student should provide himself with the following supplies:

One half-gross box best-grade glass slides, standard size ( $25 \times 75$  mm.).

One-half ounce, 18 mm. or  $\frac{3}{4}$  inch, round cover glasses, medium thickness, (0.18 mm.)

Thirty  $25 \times 50$  mm. cover glasses, medium thickness.

Two or three Pillsbury slide boxes (Fig. 1).

One box of labels for slides.

Three to six camel's-hair brushes (Fig. 2).

Six pipettes (Fig. 3).

One package Valet razor blades.

One set of dissecting instruments as follows:

One large scalpel or cartilage knife (Fig. 4).

One small scalpel (Fig. 5).

Two needles (Fig. 6).

One fine straight scissors (Fig. 7).

One fine straight dissecting forceps, file-cut points (Fig. 8).

One blowpipe (Fig. 9).

One section lifter (Fig. 10).

To which may well be added:

One heavy scissors (Fig. 11).

One curved scissors (Fig. 12).

One heavy forceps (Fig. 13).

One fine forceps, curved tips (Fig. 14).

One horn spoon.

One desk memorandum calendar.

Blank cards (about  $75 \times 100$  mm.) for keeping records of experiments. The kind of card used for library card catalogue will do.

One section razor (Fig. 15), or one microtome blade.

A piece of moderately heavy copper wire with one end hammered out to a width of 7-10 mm.

A sheet of aluminum foil.

Towels.

A glass-marking pencil (wax) or writing diamond will be found useful.

Coarse carborundum "engraver's pencil points," which may be purchased for 75 cents a dozen, are very satisfactory for marking glass, according to Professor C. E. McClung.

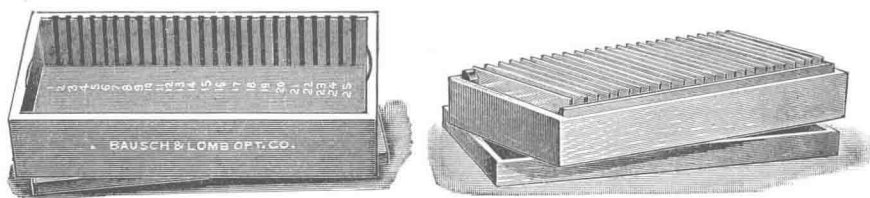


FIG. 1



FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIG. 7



FIG. 8



FIG. 9



FIG. 10



FIG. 11





FIG. 15

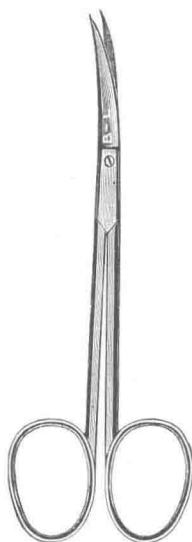


FIG. 12



FIG. 13



FIG. 14



FIG. 16



FIG. 17



FIG. 18



FIG. 19



FIG. 20

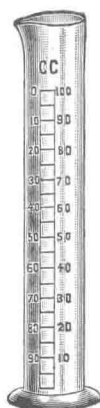


FIG. 21



FIG. 22



FIG. 23