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*Histological and
Histochemical Technics*

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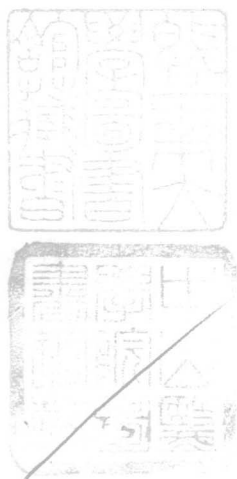
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HAROLD A. DAVENPORT, M.D.

*Professor of Anatomy, Northwestern University Medical School,
Chicago, Illinois*

*Histological and
Histochemical Technics*



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Preface

Accepting the risk that the preface may be an unread portion of a book, and keeping in mind that it offers a good opportunity for an author to be facetious or trite, or both, I begin by congratulating the reader who starts with the preface.

The text which follows presents moderately condensed reading matter for the beginner by giving a general survey of theory and practice in chapters 1-9, details of fixing fluids, stains and staining technics in chapters 10-13 and an elementary survey of histochemistry in chapters 14-20. In presenting theory, I have assumed that the reader has some knowledge of biology, physics and chemistry—at least such knowledge as would be obtained by taking these courses in high school. Emphasis is placed on the close relationship between biological science and technical methods, with physical and chemical phenomena used as explanatory media. I have attempted to give the reasons for doing things either as they are done, or as they might be done better. This form of presentation should give the student a background for a critical attitude toward the procedures used. An understanding of the purpose of each step in a given procedure will be more conducive to success than the mere following of a routine.

In presenting methods, arrangement has been chiefly on the basis of the nature of the method itself, rather than on the type of specimen or tissue component for which it might have been intended originally. This form of presentation should serve to emphasize similarities and differences among methods. It provides a practical means of limiting the number of technics needed for discussion, since representative technics

can be used as examples of related ones. Larger texts and original literature should be consulted if more complete coverage of special procedures is desired, because an essential objective is the limitation of subject matter in this textbook to a coverage suited to undergraduate courses.

Comments on procedure have been based partly on first-hand knowledge, partly on readings from books and periodicals and partly on just plain hearsay. Giving good advice on how to perform a technic efficiently may require a strange mixture of known facts, half-truths and utter empiricism. Since many, if not most, methods have been developed empirically, and since much time is required to analyze a method by controlled experiments, it is not so strange that they often contain superfluous and illogical steps. The complexity and background of histological technic is such that there seems to have been a tendency to venerate the complex instead of seeking the simple, but as the physico-chemical bases of methods become better established, illogical and superfluous steps tend to disappear. Meanwhile, it is fortunate that most of such steps are harmless. In the discussions of various procedures, I have attempted to present the subject matter in a way that will help to dispel some of the mystery which may have plagued the student or technician who is overawed by the seemingly erudite nature of recipes.

The reader should consider that, during its development, histological technic as such has seldom been the subject of research, but instead has served as a tool for research in histology and cytology. For this reason, information that might be obtained by subjecting a method to critical study is not always available. Present trends in research favor histochemistry as a means to study cellular composition and function and, as a result, a more comprehensive knowledge of technic has become necessary. Whereas in anatomical studies a stain that reveals certain cellular or other morphological features is acceptable regardless of how obtained, in histochemical studies the means by which a stain is achieved are generally the useful criteria.

Lest the beginner be tempted to accept the lore of the past on faith alone, I suggest applying the following questions to each individual step of a technic: (1) What does it accomplish? (2) Is the reagent used specific for its purpose, or could others be used equally effectively? (3) What might be the effect of varying time, temperature, concentration or other feature of the process? (4) Is the step essential; and, if so, what would be the effect of its omission? Questions of this sort should be helpful in learning the logical sequence of a procedure, and in discriminating between the critical and the uncritical steps. When answers to these questions are known, the processing of tissues becomes a logical sequence of events that meets the requirements of both science and utility.

Although there is much in common between the ordinary histological methods and histochemical procedures, histochemistry has been presented in the last seven chapters as a separate section. I have made this arrange-

ment of subject matter to facilitate the presentation of aims and requirements of histochemistry, and to classify chemical attributes of tissue components. The treatment of the subject is admittedly cursory but should be sufficient for an introduction to this relatively new and very active field of histological and cytological study.

HAROLD A. DAVENPORT

Northwestern University Medical School

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H. A. D.

Introduction

Two main types of abbreviations are common in scientific writing: a shortening of a word to one or more of its letters, and the creation of a symbol for one or more words by initial letters. An example of the first kind is familiar in *aq.* for aqueous, *conc.* for concentrated and *soln.* for solution. Examples of the second kind are *LVN* for low viscosity nitrocellulose, and *CRAF* for a fixing fluid that is a mixture of chromic acid, acetic acid and formalin. Older usage required a period after all abbreviations, but recent trends have been toward the omission of periods; thus *mm*, *cm*, *ml*, *ft*, *lb*, *sec*, *min*, *hr* appear without a period. Some abbreviations, such as *C. P.* (chemically pure) and *U. S. P.* (United States Pharmacopoeia), are keeping their periods for the time being. Also there is a hesitancy to drop the periods from such abbreviations as *aq.*, *soln.*, *conc.*, *et al.*, *i.e.*, *e.g.* and others that have been in common use for a long time and are not included in the group that relate to time, temperature, weights and measures. Possibly all periods after abbreviations will disappear eventually.

In this book, the attempt has been made to conform with the advanced, present usage of the style of abbreviations. The reader will need to know the ones in common use, particularly those for time, weights and measures. A list of the needed ones follows.

<i>Time</i>		<i>Weight</i>		<i>Distance</i>	
sec	second	μg	microgram	$\text{m}\mu$	millimicron
min	minute	mg	milligram	μ	micron
hr	hour	kg	kilogram	mm	millimeter
day	(not abbreviated)				
wk	week				
mo	month				
yr	year	cm^3	cubic centimeter (not used for fluid volumes)		
		ml	milliliter (used for fluids)		
		lit	liter (often spelled out)		

Volume

Note that the singular form only of the abbreviation is used for both singular and plural; min means either minute or minutes, gm means gram or grams.

Although the Greek mu (μ) is used to mean 0.001 millimeter, it may be used also as a prefix to mean one millionth of some other quantity; for examples, μsec means 0.000,001 second and μg means 0.000,001 gram or 0.001 mg. The letter m as a prefix means one thousandth; for examples, $\text{m}\mu$ (millimu) 0.001 micron or 0.000,001 mm. The Angström unit (abbreviated Å), used in connection with histology chiefly for the wave lengths of light, is 0.1 $\text{m}\mu$, hence 5000Å equals 500 $\text{m}\mu$. The grain of apothecaries' weights is abbreviated gr, and care should be taken that this symbol is not used for gm when gram is meant.

The much used cc, meaning 0.001 liter, is an obsolescent term, but it dies hard. The distinction between cc and ml so far as histological technic is concerned is purely academic.

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