Handbook of Histopathological and Histochemical Techniques

Third Edition

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(including museum techniques)

THIRD EDITION

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With a Foreword by

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Foreword

Despite the number of excellent texts on histological techniques and histochemistry now available, there is an unfortunate dearth of definitive works to guide the histopathologist or technologist in a practical fashion through the pitfalls of histological technique and at the same time provide in a clear, succinct manner the relevant supporting data and attendant bibliographical review. This new edition of *Handbook of Histopathological and Histochemical Techniques* does, I believe, fill this need.

During post-graduate study in England and the United States of America, I became aware of the meticulous and superb preparations that can be expected from first-class histological technologists. Charles Culling is an expert in his field and, utilizing his many years of experience in teaching and in applying these techniques, many of which he has in fact devised or modified, he has brought to a clear focus those methods which have proved most useful and practical.

He has added a wealth of new material to this edition, including an exhaustive survey of carbohydrate and protein histochemistry. The excellent chapters on fluorescent and immunofluorescent methods, the technical aspects of electron microscopy, and those dealing with fixation, lipids and enzymes, have all been revised and up-dated.

This work is, in fact, a complete book of methodology correlated with a discussion of the relevant theory for each method outlined.

When using this book one experiences the feeling that an expert is looking over your shoulder ready to offer advice. I believe it will be invaluable to all those with an interest in histology and histochemistry, both as a reference text and at the bench. It should prove of particular assistance to selected undergraduate students in the biological and health sciences, pathologists–both in the hospital setting and in experimental research–technologists and workers in various research fields.

W. L. DUNN

Preface to the Third Edition

Since the second edition, there has been a phenomenal expansion in the field of histochemistry and perhaps, even more particularly, in the literature pertaining to it. In our laboratory, over these nine years, we have endeavoured to test most of the new methods that have been described and to determine their specificity, usefulness and reliability of performance as routine methods. We have probably missed many of those published and perhaps been unfair to some we tried, but an effort has been made to incorporate in this new edition those methods that have been deemed useful and reasonably reliable. All books share the bias of their author and although I have endeavoured to reduce this to a minimum, the chapters on fixation and carbohydrates will, by their bibliographies, attest to mine. I have, for the first time, included a chapter on proteins which I hope embodies all the currently useful methods for their identification and/or demonstration. All the other chapters have been updated as far as possible; for example, the cell, enzymes, chromosome techniques, lipids, pigments, and so on. The chapters on museum technique, while unchanged, have been left in as one of the few textbook sources of information on the subject.

My continued association with students has helped tremendously with the preparation of the material and my association with post-graduate students in histochemistry, particularly those from the allied sciences, has broadened my concepts and will, I hope, make this volume more useful to them.

Due to the pressure of time and space, there are almost certainly errors of commission and omission and for these I apologize.

C. F. A. CULLING

Preface to the First Edition

When invited by the publishers to write this book, I was pleased to accept in view of the fact that having studied and practised this subject for over twenty years I felt there was a need for a textbook covering a wider field. Teaching and examining candidates for the Institute of Medical Laboratory Technology final examination in histopathological technique has emphasized this point, and since it embraces every aspect of the subject I have kept the Institute of Medical Laboratory Technology examination in mind while writing. I hope that this book will also be of use to those wishing to learn or practise histopathology or histology–such as students of biology, physiology or medicine. It should prove of value not only as a textbook from which to learn the subject, but also as an up-to-date reference book.

If the contents appear to be unbalanced in some respects, for example, the greater attention given to the anatomy of the central nervous system, and the composition and classification of the lipids and connective tissue, it is because my experience in teaching leads me to believe that a great deal of difficulty in learning and practising techniques is due to a lack of basic knowledge, particularly in these subjects. For similar reasons I make no apology for the amount of space given to microscopy.

Histochemical methods are playing an increasingly larger part in the histopathology laboratory and, although most of the traditional methods have been included, new methods are also given if they have proved reliable.

The term 'histochemistry' has come into prominence in recent years as the study of the chemistry of tissue components by histological methods, and it is probable that the impetus given in the post-war years

to this type of method, with its greater accuracy and control, is responsible for the impression that it is of recent origin. In fact Raspail, in 1830, wrote an essay (*Essai de Chimie Microscopique applique a la Physiologie*) which is generally accepted as the beginning of recorded histochemistry.

The point at which histopathology ends and histochemistry begins is impossible to determine and, although often regarded as an entirely separate subject, histochemistry is, in fact, the basis of many so-called histopathological methods.

I have been fortunate in being able to call on many colleagues at the Westminster Hospital Medical School for advice and helpful criticism; to them I offer my sincere thanks.

I am especially grateful to Professor R. J. V. Pulvertaft for his constant encouragement and criticism of the script; Dr. E. Ball for his invaluable advice and correction of manuscript and proofs; Dr. J. D. Billimoria and Mr. J. F. Wilson for technical advice; Professor D. S. Russell, Director of the Institute of Pathology, the London Hospital, for the use of material; and Mr. H. J. Oliver and Mr. V. S. Trenwith, of the London Hospital, for advice and criticism of proofs; Mr. J. R. Stokes, who took many of the colour photographs; and Dr. P. Hansell and Mr. L. Hill, Department of Medical Photography and Illustration, for assistance in preparing photographic material.

I record my thanks to those commercial firms who have kindly supplied blocks or photographs for inclusion in the book; Miss A. M. R. Collard, for secretarial assistance; and to my publishers, who have been most co-operative and helpful at all times.

C. F. A. CULLING

Acknowledgements

If I have learned anything over the years, since writing the first edition of this work, it is that the quality and breadth of one's thinking is remarkably dependent upon one's colleagues, both past and present. I have been extremely fortunate in this regard and I thank them all most sincerely. Most particularly, I would thank Mr. J. F. Wilson at the Westminster School of Medicine, London, who first taught me how to cut and stain a section and who is perhaps more responsible than any other for any success I have achieved in this field—for me he will remain the doyen of histopathological technique.

I am especially grateful to Doctor W. L. Dunn, Professor and Head of the Department of Pathology at the University of British Columbia, and to Doctor H. E. Taylor (formerly Head of the Department who is now Director of Personnel Support for the Medical Research Council of Canada), who have both given unstintingly of their time to encourage, discuss and criticize material for me.

I would also particularly thank Doctor P. E. Reid, my research associate, whose knowledge and time I have drawn upon in large measure; he has shared my concerns, generally filled the role of critic, abstractor and enthusiast when the volume of literature seemed unending.

Doctor Philip S. Vassar has continued to give freely of his time, knowledge and up-to-date and encompassing files of published papers, all of which have been invaluable.

Doctor W. H. Chase, who wrote the chapter on the electron microscope in the last edition, has yet again generously assisted me with this task, for which I thank him most sincerely. My colleagues at the University of British Columbia, too numerous to mention, I thank no less sincerely.

ACKNOWLEDGEMENTS

To my technical assistants, particularly Mrs. Barbara Barkoczy, Mrs. Alison Russell, Mrs. Linda Trueman and Miss Maureen Day, I express my gratitude for their patience and perseverance with many of the more difficult techniques they worked on.

These acknowledgements would not be complete without mention of my wife, Lois, whose patience has been unending and support tireless.

Many of the new methods published or modified arose from research supported by grants to Doctor H. E. Taylor, Doctor W. L. Dunn or to myself from the Medical Research Council of Canada.

I would like to record my thanks to Mrs. Audrey Spencer for secretarial assistance, to the staff of the Woodward Library, University of British Columbia, who were of great assistance in literature surveys, and to my publishers who have been most understanding and co-operative at all times and in all ways.

Vancouver, B. C. C. F. A. CULLING

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Part I – Introductory

Histopathological technique is that branch of biology concerned with the demonstration of minute tissue structures in disease. Since the differences between diseased and normal tissues are often slight, it follows that the majority of the methods involved may be used for both.

Before such structures can be demonstrated the tissue must be prepared in such a manner that it is sufficiently thin (one to two cells thick) to be examined microscopically, and that the many and complex structures which go to make up tissue may be differentiated. This differentiation is usually achieved by selective colouring, and, since it is impossible to demonstrate all these structures in one preparation, methods are employed which stain one or more in each section or slice of tissue.

The term 'histochemistry' has come into prominence in recent years as the study of the chemistry of tissue components by histological methods, and it is probable that the impetus given in the post-war years to this type of method, with its greater accuracy and control, is responsible for the impression that it is of recent origin. In fact, Raspail, in 1830, wrote an essay, *Essai de Chimie Microscopique Applique a la Physiologie*, which is generally accepted as the beginning of recorded histochemistry.

Mann (1902) said 'the object of all staining is to recognize microchemically the existence and distribution of substances which we have been made aware of macrochemically. It is not sufficient to content ourselves with using acid and basic dyes and speculating on the acid or basic nature of the tissues, or to apply colour radicles with oxidizing or reducing properties. We should find staining reactions which will indicate the presence of certain elements such as iron, phosphorus,

carbohydrates, nucleus or protamines, and so on.' Mann's words are as cogent today as they were 55 years ago when they were written.

There are special methods of preserving and preparing the tissue in mass, known as fixation, which precede the special staining methods employed. This process of fixation is used even when tissue or body fluid is smeared on glass slides.

When blocks of tissue are to be examined they must, after fixation, be cut into thin slices or sections. In order that such sections may be cut and manipulated they are normally impregnated and embedded in a firm medium, usually paraffin wax. The various methods of examination of tissue cells and structures are summarized in Chapter 2.

Chapter 1 describes the structure and contents of the cell, since it is considered that to attempt to practise histopathological technique without a knowledge of the cell is analogous to trying to drive a car without any knowledge of its controls.

Chapter 1

The Cell

The body is composed of tissues. Each tissue is composed of units of living matter (cells) and non-living fibres. The cells have certain common characteristics which are dealt with later in this chapter, but they are of various types, each of a specialized nature, differing from others in shape, size and function. The type of arrangement of cells and fibres enables the various tissues to be recognized.

As seen in the normal histological preparation, the fixed cell can do no more than bear a resemblance to the living, and the method of processing and staining will determine how near that resemblance will be. Long study of histological preparations leaves the observer unprepared for the fascinating picture of living cell cultures revealed by phase-contrast microscopy. By this technique the cells can be seen as living entities, sometimes actively moving in the preparation (for example, polymorphonuclears, lymphocytes, histiocytes), but always showing activity within. Many aspects of living cells can be seen by modern microscopy, which enables them to be studied in some detail, but even this detail is limited and by no means all the components can be seen and recognized.

A knowledge of normal histology is of immense help in practising histological technique, and those who hope to master the subject are advised to study it. If techniques are to be understood and controlled a knowledge of general cell structure is essential, and for that reason will be dealt with in some detail.

The cell *(Figure 1.1)* is to living tissue what the molecule is to chemistry; that is to say, it is the unit of which larger masses are built, and which cannot be further divided without losing its identity. The cell is composed of a *nucleus* surrounded by *cytoplasm*, each of these being enclosed by a membrane.

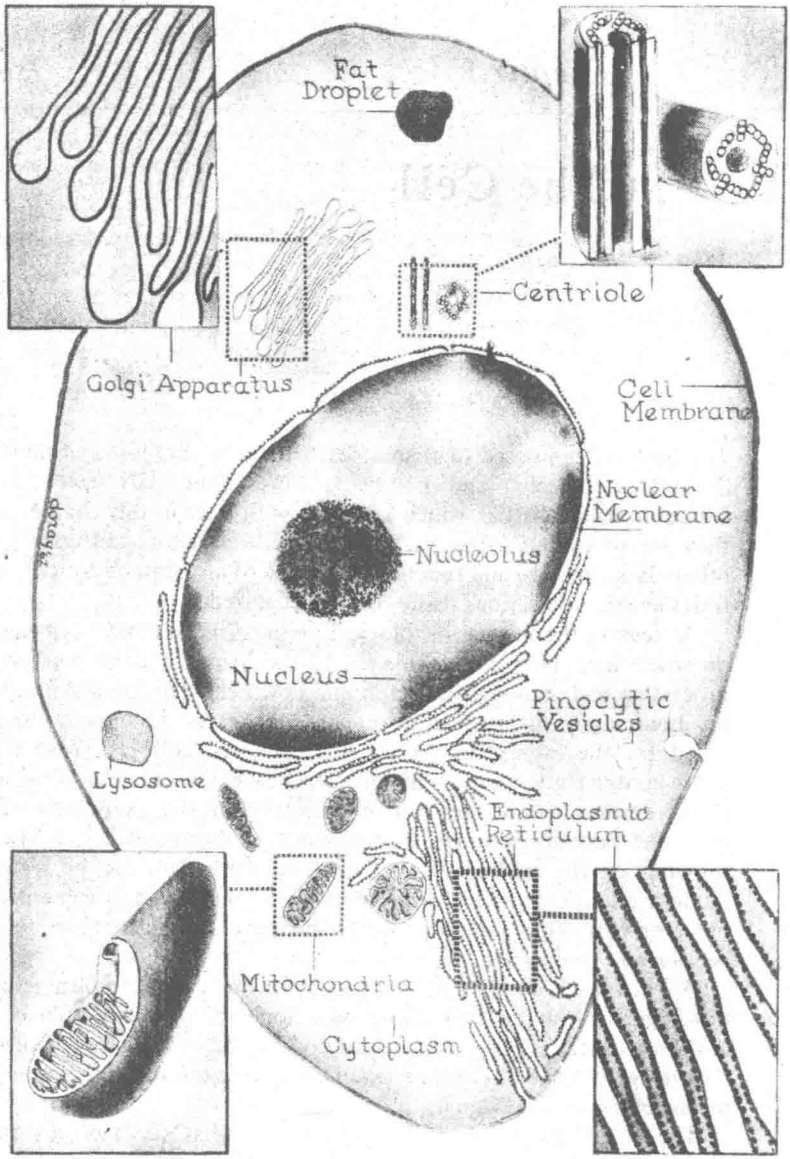

Figure 1.1 – Modern diagram of a normal cell

With modern methods of experimental cytology and cytochemistry allied to electron microscopy our knowledge of the cell in recent years has increased enormously. The modern diagram of a cell *(Figure 1.1)* based on electron micrographs and cytochemical research is vastly different from that of even a few years ago. It should however be remembered that the conventional diagram *(Figure 1.2)* is the image as seen by the light microscope and as such may still serve some purpose.

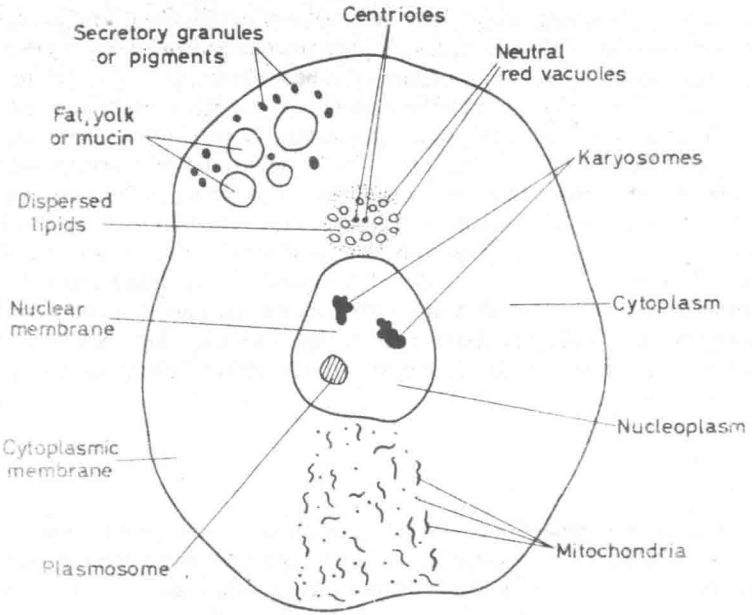

Figure 1.2 – Diagram of a living cell showing its component parts and possible inclusions. The Golgi apparatus would be present in place of the neutral red vacuoles, if the cell were fixed. The inclusion of fat, yolk, mucin, secretory granules, and pigments is purely diagrammatic; it is unlikely that more than one type would be present. Should such inclusions be present they are normally distributed throughout the cytoplasm

THE NUCLEUS

Nucleoplasm

The ground substance of the nucleus is a colloidal solution of proteins with various salts. The application of most fixatives causes the proteins

to be precipitated as a fine mesh with aggregates of protein at the intersections. Most of the protein is bound to nucleic acid (deoxyribonucleic acid–DNA) forming *nucleoprotein* which, because of its acid reaction, stains intensely with basic dyes. The darkly staining part of the nucleus is known as chromatin; the pale part is known as achromatin, and under certain circumstances as parachromatin.

The structure and function of the nucleic acids (DNA and RNA) is dealt with elsewhere (page 245), and will not be discussed here except to say they are the most important single cellular constituents. DNA, in addition to being self-replicating, has been shown to be the architect of the cell directing, through its intermediary the RNA, every function.

In human cells there are 46 chromosomes. These chromosomes carry in their DNA hereditary characteristics thought to be dependent on the code of nitrogenous bases, and on division split along their length, one half of each going to each new or daughter cell. In the resting cell, although they cannot at present be differentiated as separate structures, the chromosomes are believed to lie uncoiled in a delicate lace-like structure in which the DNA and other components are able to interact with the surrounding medium. It is in this form that the DNA is most active and when it is tightly coiled into the classical chromosome the DNA is inert.

Nucleoli

The nucleus generally contains one or more refractile particles which are known as nucleoli. These are easily recognizable in a resting nucleus by their regular spherical shape. Under the electron microscope they are seen to be composed of large numbers of small granules which are similar in appearance to the ribosomes of the cytoplasm. They are rich in RNA and are thought to be active in the synthesis of protein.

Nuclear Membrane

The nuclear membrane which surrounds the nucleus is not thought to be permeable in the normal sense of the word. Rupture of this membrane is a sign of imminent death of the cell.

It will be seen in *Figures 1.1* and *1.3e* that the nuclear membrane has now been shown to be a double membrane in which there appears to be annuli or holes (nuclear pores) in the outer layer which are open to the cytoplasm. Some workers believe that it is through these annuli that contact with the cytoplasmic or endoplasmic reticulum is made.