HISTOLOGICAL TECHNIQUE

CARLETON AND DRURY

THIRD EDITION

OXFORD

HISTOLOGICAL TECHNIQUE

FOR NORMAL AND PATHOLOGICAL TISSUES AND THE IDENTIFICATION OF PARASITES

BY

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SYNOPSIS OF RECOMMENDED METHODS FOR GENERAL HISTOLOGY

GENERAL

Avoid autolysis. Never let tissues dry. Do not distort by handling or crushing. Tissues removed by diathermy may show the 'streaming' artifact. Contraction of some tissues during processing (nerves, muscle, skin) may be diminished by pinning them (or allowing them to adhere) to a piece of thin card. Obtain thin (3 mm.) slices of tissue for immediate fixation.

FIXATION

Mercuric chloride-formaldehyde (p. 23) for 12–24 hours is useful for general purposes. Mercury precipitates must be dissolved out by treatment of the unembedded tissue with iodine-alcohol (p. 21) or by passing the unstained sections on the slide through the iodine-thiosulphate sequence (p. 96).

Susa (p. 20) gives excellent rapid fixation, and time is gained by the transfer to 96 per cent. alcohol.

Formaldehyde (p. 23) is useful if museum specimens are required in addition to sections. Also for prolonged storage of specimens. Original colours can be restored. Most staining procedures can be performed after fixation in **10 per cent. formol/saline** (p. 256), especially if sections are **post-chromated** on the slide by the chromic acid-bisulphite sequence (p. 107). **Formol-acetic** (p. 256) is a more rapid and precise fixative which is useful for surgical biopsies.

Bouin's fluid (p. 21) and **Zenker's fluid** (p. 22) are both excellent for the aniline blue methods of the Mallory type (p. 107). Both fixatives must be washed out of the tissues before final dehydration.

Carnoy's fluid (p. 25) is very useful for the rapid fixation of urgent biopsy specimens.

DEHYDRATION AND EMBEDDING

Dehydrate in graded alcohols (or dioxane, p. 41).

For **routine specimens**, embed in paraffin, after clearing in xylol, benzene, or chloroform.

For **delicate objects**, the cedar-wood oil method (p. 35) is recommended.

For **large objects** and certain central nervous system techniques, embed in celloidin (p. 47).

Slices of **whole organs** can be embedded in gelatine, cut as frozen sections on a large base sledge microtome and mounted on paper (p. 267).

SECTION CUTTING

Cambridge rocking microtome (p. 51) for serial sections of small blocks.

A **rotary microtome** (p. 52) is better for larger tissues.

For separate sections from large blocks or hard tissues the **base sledge microtome** (p. 53) is recommended.

Fix the sections on the slide with the gylcerol-albumen mixture (p. 62), starch paste (p. 62), or cover with a thin film of 0·5 per cent. celloidin in alcohol-ether.

For rapid sections, fat stains, and certain central nervous system techniques, cut frozen sections with the **freezing microtome** (p. 73).

MOUNTING MEDIA

Synthetic mounting media such as **DePeX** (p. 90) dry quickly and are colourless. Fading of stained sections is slight. For aqueous mounting use **glycerol jelly** (p. 91). Store all stained sections in the dark.

STAINING METHODS

(*See back endpaper*)

HISTOLOGICAL TECHNIQUE

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PATHOLOGICAL TISSUES AND THE
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H. M. CARLETON

1896–1956

TO THE MEMORY OF

SIR CHARLES SHERRINGTON

O.M., F.R.S., M.D.

FORMERLY WAYNFLETE PROFESSOR OF PHYSIOLOGY
IN THE UNIVERSITY OF OXFORD

FOREWORD

H. M. CARLETON

1896–1956

HARRY CARLETON was an incomparable histologist who preserved the traditions of the great pioneers of the nineteenth century in a rapidly changing world. A pupil of Gustav Mann, whom he succeeded at Oxford, and of Champy, he inherited their common sense and uncanny intuition, and welded these qualities with his innate artistry and broad outlook into something that was unique and inspiring. His unrivalled practical knowledge of technique was freely drawn upon by J. S. Haldane, Sherrington, Florey, and a host of others. During the Second World War he gave valuable service in pathology as a member of Barcroft's team for chemical defence at Porton. His wit and gentle cynicism made him a delightful companion and he weathered many a trying emergency through his sincerity and good humour. As Editor of Shäfer's *Essentials of Histology* for some years, and author of *Histological Technique*, the revision of which he had just completed before he died, Carleton achieved an international reputation for reliability and shrewdness with the everyday obstacles of the practical histologist. His scientific papers, though not numerous, went straight to the heart of the matter with which he was concerned and reflected the care that he gave to every question.

Those of us who enjoyed his friendship will miss this generous, warm-hearted man; there must be countless others to whom remembrance of Harry Carleton will long remain a fragrant memory.

G. R. CAMERON

INTRODUCTION

THE aim of this new edition is to give, as before in compact form, the chief methods employed in the microscopical examination of human and other mammalian organs.

Some of the old matter has been deleted. Thus the introductory chapter (The Cell as a Physico-chemical Entity) is suppressed. On the other hand the chapter on methods of preparation and examination has been considerably extended. The colloidal nature of living protoplasm is emphasized again in regard to the effects produced on its structure by fixatives.

The worker in biology or medicine must be an Aphrodite, quick to transform the cold ivory of Pygmalion's technical result into the glow of the living cell. To further this the question of artifacts in fixed and stained preparations is also stressed.

To include *all* the methods of normal and morbid histology in a manual of moderate size is manifestly impossible. The following plan has therefore been adopted in these pages:

First, to describe in detail the fundamental processes of Fixation, Embedding, Section-cutting, and Staining. With regard to fixation and staining, a number of 'Type Methods' of wide application have been chosen. Once the principles and practice of these have been mastered by the beginner, the application of the special methods should be easy.

Secondly, to provide workers with accessory and special methods. The methods most suitable for the various organs and tissues are dealt with here. They are necessarily a selection, and by no means represent the totality of the technical approaches known. It has thus been necessary to adapt the length of the sections dealing with the methods for the organs and tissues to the importance of the subject. That is why the technique for the microscopic study of Tendon is described in a few paragraphs, while that for the Nervous System entails many pages.

Thirdly, to set out the methods for the identification of *morbid* changes, or parasites of various sorts, in tissues. Whenever such changes, or organisms, have important clinical bearings the technique most suitable for their identification is included. Methods for examining body fluids and dejecta for parasites are also given.

A word regarding the 'Notes' which are generally appended to the methods. A successful technique depends largely on one's knowledge of various minor 'tips'. Anyone who has worked with an experienced laboratory technician realizes that the degree of success in any manipulative science is largely dependent on the knowledge of small hidden difficulties and the means for their avoidance. (I am glad to record that such collaboration has often been my privilege.) In order to make this book as practical as possible, such pitfalls are freely indicated—even at the risk of seeming superfluous.

In this, as in previous editions, the vast majority of the methods have been personally tested.

A short and general list of references is appended. It refers the reader to standard works, or important monographs, in which full bibliographies may usually be found. This manual would be unduly lengthened by anything of the nature of a full bibliography. Nevertheless the references provided will enable anybody to gain access to the literature.

In this task of revision I have had the generous help of Dr. R. A. B. Drury who, until recently, was Lecturer in Morbid Anatomy in University College Hospital Medical School. He is entirely responsible for the chapters on Methods for Histo-pathological Processes, Bacteriological Methods, and Methods for Spirochætes. Many of the new methods described here are actually in use, or have been tested in the Department of Morbid Anatomy at University College Hospital Medical School, and I am grateful to Professor G. R. Cameron for the help and encouragement afforded Dr. Drury.

My acknowledgements are many: Sir Charles Sherrington died in 1952. All that I can do is to continue, in affectionate and admiring memory, the dedication of previous editions.

To Professer A. C. Hardy, F.R.S., I record my most grateful thanks for accommodating me in his department.

Dr. John R. Baker, in charge of the Cytology laboratory of this department, has again given much valuable advice, and I am further indebted to him for giving me every facility for testing methods. To Mrs. Barbara Luke, his technical assistant, I record my grateful acknowledgements. I am indebted to Mr. Savile Bradbury for much excellent counsel in regard to Histo-chemical methods and chemical nomenclature, and Mr. William Galbraith has given me information —incorporated and acknowledged in the text—regarding the microscope.

Ten of the illustrations are new, and of these, eight, namely figures 3, 4, 5, 6, 7, 11, 13, and 15, were specially drawn for me by Mr. J. T. Y. Chou to whom I am duly grateful. They replace photographs used in the earlier editions and give a very much clearer representation.

Messrs. Percy Trotman and Leonard Small of this department have in many ways facilitated my work. To the staff of the Radcliffe Science Library I am most grateful for their unstinted help in locating matter for this new edition.

H. M. C.

Department of Zoology and
Comparative Anatomy, Oxford
November 1956

TABLE FOR THE DILUTION OF 96 PER CENT. ALCOHOL

[Alcohol in Great Britain is currently supplied (since World War II) in a concentration of 94 to 95 per cent. But for all general purposes these figures are sufficiently accurate.]

Grade Required	*Volumes of Rectified Spirit*	*Volumes of Water*
90 per cent.	83·5	16·5
80 ,,	93·5	6·5
70 ,,	72·9	27·1
60 ,,	62·5	37·5
50 ,,	52·1	47·9
40 ,,	41·6	58·4
30 ,,	31·2	68·8

TABLE FOR THE DILUTION OF COMMERCIAL (40 PER CENT.) FORMALDEHYDE SOLUTION

Grade of Formaldehyde Required	*Volumes of 40 per cent. Formaldehyde*	*Volumes of Water or Saline Solution*
3 per cent.	7·5	92·5
5 ,,	12·5	87·5
8 ,,	20·0	80·0
10 ,,	25·0	75·0
15 ,,	37·5	62·5
20 ,,	50·0	50·0

TABLE FOR THE PREPARATION OF VARIOUS GRADES OF ALCOHOL FROM 90 PER CENT. DOWNWARDS (AFTER GAY-LUSSAC)

NOTE.— The intersection of the horizontal row (= grade required) and the vertical row (= original grade) indicates the number of volumes of water required for every 100 *volumes of the original grade of alcohol*. Thus, to prepare 60 per cent. alcohol from 85 per cent. alcohol, 100 volumes of the *latter* should be diluted with 44·48 volumes of water.

Weaker Grade Required	*Original Grade*								
	90 per cent.	*85 per cent.*	*80 per cent.*	*75 per cent.*	*70 per cent.*	*65 per cent.*	*60 per cent.*	*55 per cent.*	*50 per cent.*
85 per cent.	6·56	..	..	..	..	..	..	..	..
80 „	13·79	6·83	..	..	..	..	..	..	..
75 „	21·89	14·48	7·20	..	..	..	..	..	..
70 „	31·05	23·14	15·35	7·64	..	..	..	..	..
65 „	41·53	33·03	24·66	16·37	8·15	..	..	..	..
60 „	53·65	44·48	35·44	26·47	17·58	8·76	..	..	..
55 „	67·87	57·90	48·07	38·32	28·63	19·02	9·47	..	..
50 „	84·71	73·90	63·04	52·43	41·73	31·25	20·47	10·35	..
45 „	105·34	93·30	81·38	69·54	57·78	46·09	34·46	22·90	11·41
40 „	130·80	117·34	104·01	90·76	77·58	64·48	51·43	38·46	25·55
35 „	163·28	148·01	132·88	117·82	102·84	87·93	73·08	58·31	43·59
30 „	206·22	188·57	171·05	153·61	136·04	118·94	101·71	84·54	67·45

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