A STUDENT'S HISTOLOGY

BY

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The study of living organisms appeals to those who appreciate variety of form, colour and function and, above all, the beauty of regular change associated with the interlocking of structure and kinetic function.

W. B. TURRILL.

Histology is interesting only when it gives a better insight into the function of the tissue or element under discussion . . . for however valuable the physiological observations may be, as long as their histological basis is disproportionately small or entirely missing, even the best founded physiological theories remain nebulous. Histology still remains the basis for all our physiological deductions and theories . . .

J. BOEKE.

In the following account the ordinary common distinction of structure and function will be used. The distinction rests upon abstractions but it is convenient for exposition. The notion of structure arises by considering the organism at an instant, abstracted from time. The abstraction is valuable because within the history of the organism there are relatively stable events which do not change much and these are called structure. In contrast there are unstable events and these are function. In the end the distinction is quantitative and rests upon the time scale we are using. What remains stable throughout an hour may not remain stable for ten years. . . . However, let us for a moment try to get behind the familiar abstraction of structure and function, which is at bottom merely a question of what changes slower or faster, and try to get beyond the classical concepts of matter.

A. D. RITCHIE.

TO

MY WIFE

PREFACE

THE printed page and the spoken word are the winds and tides that bring to the islands, which are our minds, the thoughts and ideas of other men's minds. It is however often difficult to be sure which page or whose word it was that initiated a line of thought that later became dominant in our minds. To make full and specific acknowledgement of my debts to others is not possible : there nevertheless remains a deep sense of indebtedness.

To some however my debt is clear.

I am grateful to Prof. R. C. Garry for his encouragement and for his permission to use to full advantage the collection of microscopic slides which have been built up over the years in the Histology Division of the Institute of Physiology. Some whose names are not known to me have left preparations which, after the passage of at least half a century, exhibit techniques that can still compare favourably with the best of to-day's methods. Other specimens were collected by Dr. A. McL. Watson or were his own work.

The recent additions to this collection and especially the specimens of the nervous system are very largely the work of Mr. F. W. Gairns. Without these this book might have been written but it could not have been illustrated from so many beautiful preparations. To Mr. Gairns I gladly acknowledge my indebtedness.

In the building up of this collection of specimens the Histology Division is grateful to the surgeons and pathologists who kindly take the time and trouble to send interesting material for both teaching and research purposes.

To Mr. R. Callander for his illustrations I am also deeply grateful. Nothing has been too much trouble to him in his efforts to give an accurate representation of the microscopic appearances. His artistic gift, his expert craftsmanship and his patience have made his contribution outstanding. It has been a pleasure to have his helpful co-operation.

To the following I am indebted for advice and for helpful criticism of drafts of parts of the manuscript: Dr. I. A. Boyd, Prof. D. F. Cappell, Dr. Mary C. Cumming, Dr. A. R. Currie, Prof. J. N. Davidson, Prof. J. B. Duguid, Dr. R. I. Shaw Dunn, Dr. S. C. Frazer, Prof. R. C. Garry, Dr. D. T. Gibson, Dr. A. J. Hale, Dr. A. Stewart Henderson, Prof. A. C. Lendrum, Dr. Ann B. McNaught, Dr. J. A. Milne, Dr. S. D. Morrison, Dr. H. N. Munro, Dr. J. Paul, Prof. G. Pontecorvo, Prof. T. Symington, Dr. A. P. M. Thompson, Dr. J. B. deV. Weir.

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To Mr. A. L. Goodall, Honorary Librarian of the Royal Faculty of Physicians and Surgeons of Glasgow, Prof. G. Moruzzi of the University of Pisa, and Mr. T. J. Shields of the British Medical Association Library, London, I extend my thanks for assistance and guidance on the biographical details in Appendix II.

To my daughter, Dr. Jean D. Garven, I am indebted for the preparation of the Index.

To those who have willingly lent microscopic preparations from which drawings were made, Dr. R. I. Shaw Dunn, Dr. A. D. Telford Govan, Dr. H. E. Hutchison and Prof. W. J. B. Riddell; to the members of the staff of the Institute of Physiology who have assisted in the proof reading; to the members of the technical staff, especially Mr. T. Gorrie for his constant help and good humour and Mr. D. Macallister for his photography; to Mr. Charles Macmillan and Mr. James Parker of Messrs. E. & S. Livingstone for their unfailing courtesy and patience; to Mr. Hislop for his interest and concern in the reproduction of the coloured figures: to all of these my grateful thanks are gladly given.

For the text as it now stands I alone am responsible. Any criticism which will improve it as a teaching medium I will welcome and appreciate.

H. S. D. GARVEN.

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TO THE STUDENT

1. The Latin and Greek roots at the beginnings of sections and paragraphs are given to enable the student to understand the meaning of the English scientific and medical terms and therefore more readily remember them. It is interesting to note how frequently the adjective is derived from the Latin and the name of the diseased state from the Greek, *e.g.* renal and nephrosis, pulmonary and pneumonia, mammary and mastitis, etc.

2. The descriptions given in the text are for human cells, tissues and organs unless definite reference is made to other species.

3. The drawings and photomicrographs are also from human material, with a few exceptions which are specifically labelled. The diagrams are not all from human material: they show mainly the more generalized vertebrate or mammalian form.

4. The drawings and photomicrographs indicate, either in the figure or in the legend, the degree of magnification. To assist comparison all the magnifications lie within a definite series (×2·5, ×5, ×10, ×20, ×40, ×80, ×150, ×400, ×800, ×1600).

5. As far as possible the text and the illustrations should be used in conjunction with the actual examination of microscopic specimens.

6. References to original work have not been included in the text: they have indeed been deliberately omitted. To assist the senior student there is provided in Appendix I a short list of books, monographs and papers which may be consulted for further reading.

7. In Appendix II a list of histologists and other workers is given. Many of the names in the list have become part and parcel of the language of histology. The short biographical notes will enable the student to see the international character of scientific knowledge. The list is a very inadequate acknowledgement of our debt to workers of many nationalities.

8. In the biological world textbook description must steer a course between the Scylla of elaborate detail that obscures the general pattern and the Charybdis of oversimplification that results in frank stultification. How far this book succeeds will be a matter of opinion. That the author is more concerned to give to his student reader a point of view than a detailed compendium of facts is, he hopes, clear.

LIST OF CONTRACTIONS

Adrenocorticotrophic hormone	ACTH
Ångstrom unit	Å
Arterio-venous anastomosis	A.V.A.
Atomic weight	at. wt.
Atrio-ventricular	A.V.
Autonomic interstitial cell	A.I.C.
Blood pressure	B.P.
Blood vessel(s)	B.V.
Central nervous system	C.N.S.
Cerebro-spinal fluid	c.s.f.
Degree (Centigrade)	°C.
Deoxyribonucleic acid	DNA
3.4-Dihydroxyphenylalanine	DOPA
Eosin	E.
Figure, Figures	Fig. Figs.
Follicle stimulating hormone	FSH
Gram	g
Gravity	*g*
Greek	Gr.
Growth hormone	GH
Haemalum or Haematoxylin	H.
Haemalum and Eosin	H. & E.
Haemalum, Eosin, Phloxine, Tartrazine	H.E.P.T.
Negative logarithm of Hydrogen ion concentration	pH
Iron haematoxylin	I.H.
Interstitial cell stimulating hormone	ICSH
Latin	L.
Longitudinal section	L.S.
Luteinizing hormone	LH
Lymphatic vessel(s)	L.V.
Mean corpuscular diameter	M.C.D.
Mean corpuscular haemoglobin	M.C.H.
Mean corpuscular haemoglobin concentration	M.C.H.C.
Mean corpuscular volume	M.C.V.
Methylene Blue	M.B.
Methylene Blue and Eosin	M.B.& E.
Oblique section	O.S.
Orange G.	O.G.
Page, pages	p, pp
Periodic acid Schiff reaction	P.A.S.
Peripheral nervous system	P.N.S.
Red blood corpuscle(s)	R.B.C.
Resorcin fuchsin	R.F.
Reticulo-endothelial	R.E.
Ribonucleic acid	RNA
Specific gravity	sp. gr.
Somatotrophic (Growth) hormone	STH
Thyroid-stimulating hormone	TSH
Transverse section	T.S.
Ultra-violet (light)	U.V.
van Gieson	V.G.
White blood corpuscle(s) (leucocytes)	W.B.C.

PREFIXES USED TO SIGNIFY MULTIPLES OF TEN

Tera-	T	$\times 10^{12}$	
Giga-	G	$\times 10^{9}$	
Mega-	M	$\times 10^{6}$	
kilo-	k	$\times 10^{3}$	
centi-	c	$\times 10^{-2}$	
milli-	m	$\times 10^{-3}$	
micro-	μ	$\times 10^{-6}$	
nano-	n	$\times 10^{-9}$	
pico-	p ($\mu\mu$)	$\times 10^{-12}$	

Examples

metre	m
centimetre	cm
millimetre	mm
micron	μ
millimicron	mμ
gram	g
kilogram	kg
milligram	mg
picogram	pg
litre	l
millilitre	ml
a million	M
square centimetre	cm^2
cubic millimetre	mm^3

CONTENTS

CHAPTER 1

THE CELL AND GENERAL CONSIDERATIONS

1. The study of histology. 2. The cell. 3. Methods of examination of cells. 4. Birefringence or anisotropy in biological microscopic investigations. 5. Practical note on staining methods. 6. Routine for examination of a permanently mounted specimen. 7. Sizes and magnifications. 8. Structure and function of different parts of the cell. 9. Modifications of the cell membrane. 10. Cells of the vertebrate body. 11. Cell division. 12. Surfaces. 13. Organization of the vertebrate body. 14. Microscopic appearances and mental orientation.

1. THE STUDY OF HISTOLOGY

THE Greek word *histos* means a web ; this has been adopted as the term for a tissue in the biological sense. Histology therefore means the study of tissues. To-day histology is used in the wide sense, the study of the fine structure or minute anatomy of the normal body, as it is revealed by various techniques in magnifications of very varying degree. It includes cytology (Greek *cytos*, a cell), the study of cells, *i.e.* the anatomical units of the body, as well as the study of the tissues and of the organs. It includes also histo-chemistry, or the microscopic study of the chemical components of the cells and tissues.

The student of histology is therefore engaged in storing in his mind a series of mental pictures, visual memories of the normal minute structure of the different parts of the body, which he can recall at will. This volume is a guide to that end, but it is not a substitute for hours of careful examination of actual microscopic specimens. As an aid to the systematic examination and the imprinting of the picture on the memory, no method can equal the making of accurate drawings.

Histology has a value to the student as a scientific discipline. In his histological study he can, if he so wishes, train his powers of observation. In the development of a microscopic ' awareness ' he can learn the value of orderliness in method : he may develop the ability to see ' the wood ' as well as ' the trees '.

If during the course of his microscopic observation, the student also tries to link the visual memories of structure with the function which the structure fulfils, the mental associations so formed will lighten the burden of his physiological studies.

For the medical student the study of the normal cells, tissues and organs is the basis on which later he will superimpose the abnormal or pathological. Without an adequate basis of the normal how can he determine when any particular appearance has overstepped the normal limits and become pathological?

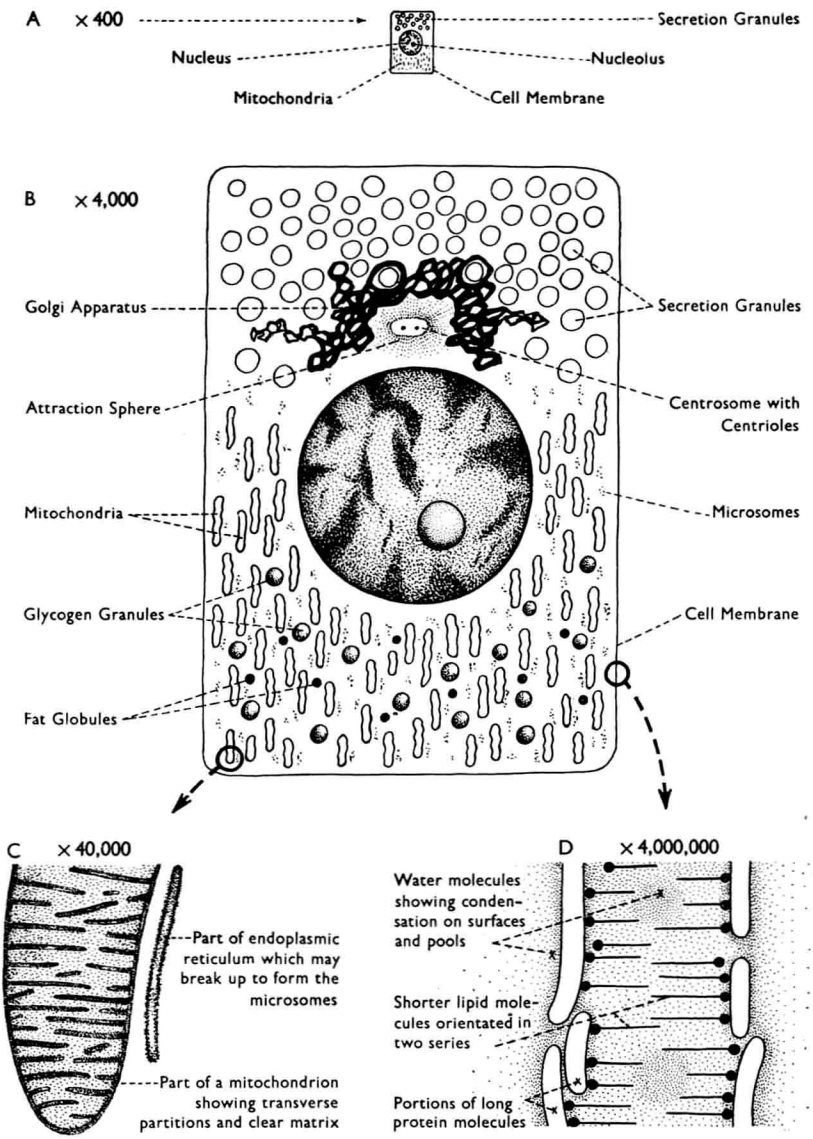

FIG. 1, 1

A generalized animal cell of secretory type and its parts at four different magnifications: A ×400, B ×4,000, C ×40,000, after Palade, and D ×4,000,000.

2. THE CELL

L. *cellula* Gr. *cytos*

The cell is the fundamental structural (morphological) and functional (physiological) unit of all living organisms, whether plants or animals. It is an organized unit, which in the simplest terms can be said to consist of a minute speck of protoplasm, a quantity of living cell substance—the cytoplasm—surrounding a kernel, the nucleus (Fig. **1,** 1A). This involves a surface of separation between the cell and its environment—the cell membrane, and also a surface between cytoplasm and nucleus—the nuclear membrane. In recent experimental procedures it has become clear that there can be very considerable interference with the organization of the cell without the occurrence of the death of the cell, for the cell has remarkable ability to reorganize itself and return to its original organized state. If, however, the interference is greater, the cell fails to return to its former state and dies. Within this organization a constant whirl of activity goes on: it is no static state. Death is the permanent stoppage of these cycles and phases of activity. Equally remarkable is the cell's ability to preserve, within this scene of intense physical and chemical change, its own essential individuality, identity and specificity.

Historical.—Hooke, a contemporary of Newton, in 1665 first used the term cell to describe the small hollow spaces which he found present in the bark of trees. Here the living portion of the cell had disappeared and he really was describing the space enclosed by the still intact cellulose wall. Malpighi (1625-91) who did such remarkable work with lenses of low magnifications, described vesicles and utricles in animal tissues. But it was only in 1831 with Brown, of Brownian movement fame, that the modern conception of a quantity of 'cellular juice' enclosing a nucleus was first enunciated. With Schleiden, in the botanical world, in 1838, and Schwann, in the zoological world, in 1839, the cell theory began to crystallize in men's thoughts. This theory put forward for the first time the idea that all organisms are essentially composed of these small units or cells. Some organisms remain as one cell throughout their life-span: others are aggregates of many cells, in the human body, some thousand million million cells or more. The term protoplasm was first used by Max Schultz in 1861. Kölliker in 1884 first made it clear that all organisms begin as a single cell, the fertilized ovum or zygote, itself the union of male and female specialized cells or gametes.

Beginning then with the fundamental unit, the cell, it was soon clear that numbers of similar cells existed together in masses or 'webs' and for this accumulation of like elements and their products the word tissue was used. The tissues are arranged in special patterns to form the various 'organs' of the body. Groups of organs are

closely linked to one another in the individual 'systems' of the body. In the normal working of the body which we call health the various systems act together in harmony to bring unity and individuality.

In the strict sense of the term, the cell is a living unit completely isolated from its fellows structurally by its membrane. In certain parts of the body the units are not in fact isolated completely but are joined together by their outreaching processes to form a network of cells: such an arrangement is known as a syncytium (Greek *syn*—together; *cytos*—a cell). The degree of independence of the units in such a syncytium may in part be determined by the diameter of the fine connecting links and also by the specialized or unspecialized nature of these connecting processes. In other instances even the appearance of isolation is lost and the structure is one of a large mass of cytoplasm in which lie numerous nuclei: such large elements are known as syncytial or plasmodial masses. In some instances the line of division between such masses and multinucleate giant cells is hard to draw. In these syncytial masses it may be that a true fusion of previously isolated units has taken place; in others it may well be that the nucleus divides, while the cytoplasmic mass increases in size but does not divide.

3. METHODS OF EXAMINATION OF CELLS

A widening range of methods has in recent years enlarged and extended the original simple conception of the cell, as membrane, cytoplasm and nucleus. A very large accumulation of factual information is now available to us as a result of this great variety of procedures. These may be divided into methods of examining (1) the living cells and (2) dead cells.

THE EXAMINATION OF THE LIVING CELL

Methods of Preparation

This presents a number of problems. The cell must be kept alive during the actual examination and precautions taken so that conditions are not set up which adversely affect the living cell. In such unnatural conditions the cells may undergo abnormal changes which if allowed to continue may cause the death of the cell. Many of these methods are therefore available to us only for a certain relatively short interval of time, after which abnormal activities supervene. All demand a degree of technical ability. Living cells may be taken from the body and examined under suitable conditions, *e.g.* the white blood cells on a suitably warm stage of a microscope. For solid tissue this method has grave limitations.

Much has been learnt from the method of tissue culture (Harrison; Strangeways). In this method the cells of the tissues are cultured

in test tubes as are bacteria. In such conditions the cells are freed from their normal restrictive environment and proliferate freely for a time: a few of these cells are then removed to a fresh tube (sub-culture).

Cells of the tissues of the body can be grown *in vitro* if the environmental factors and the constitution of the medium fulfil certain conditions. The temperature must lie within narrow limits near the normal body temperature. Oxygen must be freely available. The medium must have a suitable osmotic pressure and a balanced ratio of anions and kations, with a pH lying within a limited range: sodium, potassium, calcium and other ions must be present in ratios approximating to those in the body fluids. Glucose, as a source of energy, and combined organic nitrogen in the form of amino-acids are also necessary. If the culture is to continue to grow, growth-promoting factors, such as are present in embryonic tissue juice, must be present. Growth normally begins in cells resting on a surface.

These conditions are instructive as similar conditions must presumably prevail in the normal body when growth or replacement of cells is taking place.

Cells removed from such cultures are easily examined on a slide. In this way heart muscle cells have been found to contract rhythmically and growing nerve fibres to form myelin.

The examination of living cells outside the body may use

(1) *Ordinary daylight.*—(*a*) Light rays are reflected and absorbed by the cells and their parts to give a black and white picture. If only certain rays within the spectrum are involved, the picture obtained is coloured.

(*b*) To assist in the differentiation of the parts, dyes which are harmless to the cells, or are relatively so, are used and deductions can be made from the resulting coloured picture, as for example when the solubility of the dye in different media is known.

(*c*) Light in passing through the cell may be retarded in its velocity, *i.e.* refraction may take place. This may only reveal itself in the ordinary microscope at the edges of small bodies; the degree of retardation (or 'out-of-stepness') with the other rays may, in phase contrast microscopes, be revealed as intensity changes. A further extension of this technique is developed in the interferometric microscope.

(*d*) Dark-ground illumination may be used. This is a refinement in the use of the Faraday-Tyndal phenomenon. In the same way as a beam of bright light entering a darkened room reveals the presence of dust particles floating in the air, so in a beam of light directed across the microscope stage, some of its component rays may be

deflected into the objective lens and seen as a bright point of light on a dark ground. Granules and surfaces can in this way be visualized in the otherwise black field.

(2) *Polarized light.*—In this light of monoplanatic vibration, the plane of vibration may be altered on passing through certain substances: small objects with this property may therefore be distinguished. In some instances the beam may be split up into light vibrating in two different planes. Materials which have this beam-splitting property are said to be birefringent, or possessed of birefringence. Information on the physical structure of elements can therefore be obtained (*see* p 8).

(3) *Ultra-Violet (U.V.) light.*—This light is of shorter wavelength and can therefore give better definition and greater resolution; it also shows a different absorption picture. This method is an expensive one as quartz lenses are necessary for all the optical parts.

The examination of living cells within the body

(1) The depth of penetration of the rays of ordinary light into the tissues of the body is limited but in certain sites, *e.g.* the cuticle over the lunule of the nail and the cornea, interesting observations have been made.

(2) The exposure of internal organs can be so arranged that there is a minimal disturbance of the normal environmental temperature, humidity, etc. Here the technique of the internally reflecting quartz rod may be used to illuminate the exposed surface, *e.g.* Knisely on the spleen.

(3) The insertion of transparent chambers into the body, as in the rabbit's ear, permits the examination of the living growing tissues at relatively high magnifications.

Additional Methods.—A number of other new techniques has also contributed to our knowledge:

(1) The micro-manipulation techniques of Chambers and de Fonbrune. With these delicate instruments the art of micro-surgery has developed. In this it is now possible to cut individual cells in pieces, to tear open microscopic membranes, to inject fluids into the cell or pipette off from the cell certain parts, to remove surgically certain parts and to do transposition and grafting experiments.

(2) The ultra-centrifuge. With the high speeds developed in this instrument cells may be subjected to gravitational forces equal to 1,000 *g* and even 100,000 *g*. This results in a stratification of the cell contents, with the lightest elements at one end and the heaviest at the other. The individual layers may then be stained or separated from one another and examined.