SCHAFER'S ESSENTIALS OF HISTOLOGY

DESCRIPTIVE AND PRACTICAL

For the use of Students

SIXTEENTH EDITION

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To the Memory of

SIR E. SHARPEY-SCHAFER, F.R.S.

who originally wrote
and subsequently edited
this book for close on
Fifty Years

PREFACE

To the Sixteenth Edition

Sixty-eight years have passed since this book first appeared from the pen of Sir Edward Sharpey-Schafer.

Certain changes have been made in revising this edition ; two new colour plates of blood cells replace the original plates and differ from it in character. Instead of a single cell of each type, several are depicted in order to make clear to the student their normal variations. Magnification and staining are the same—and hence comparable—in both plates. The contingent legend faces each plate ; this enables the student to compare the blood films under the microscope and the plates without reference to the main text.

Another innovation is the grouping together of the practical class instructions, which previously headed the chapters, in an Appendix at the end of the book. This we have done because we feel that what is essentially reading matter should be separated from the technical instructions only useful during practical classes in the laboratory.

A very brief historical introduction has been added and a number of inadequate photographs replaced for the sake of clarity by india ink drawings based on the originals. Correlation of histological structure with function is emphasised. Finally, both illustrations and text have gained by the better paper now available.

We are again much indebted to Dr. J. R. Baker and Dr. R. S. Creed for suggestions and advice.

It is a pleasure to thank Messrs. Spottiswoode, Ballantyne & Co. for their sustained solicitude in the production of this edition and we are particularly grateful to their readers for their comments, cross referencing and most careful checking of the proofs.

H. M. C.

R. H. D. S.

September, 1953.

POSTSCRIPT

It falls to me to record with great sorrow the death of Roland Short, my friend and co-editor.

His store of knowledge comprised not only medicine and a highly specialised competence in pathology, but also comparative anatomy. This wide knowledge, together with his fine scholarship and intellectual poise, proved invaluable assets in the work of the revision—work, may it be added, that was carried out in the greatest harmony.

De mortuis nil nisi bonum.

H. M. C.

November, 1953.

CONTENTS

THE ESSENTIALS OF HISTOLOGY

INTRODUCTORY.

Historical Note : Enumeration of Tissues : Cytology : The Structure and Functional Changes of Cells : Formation of the Tissues.

HISTORICAL NOTE.

THE name '*Histologie*' was due to the German anatomist A. K. F. Meyer, who so named the science in 1819; and later (1852), the English microscopist Quekett defined *Histology* as 'the science of the microscopic anatomy of animals and plants.' This definition holds good to-day if we correlate the then purely structural outlook with the present attachments of Histology to Physiology, Bio-chemistry and Physics.

The first major text-book on the subject was R. A. von Kölliker's 'Mikroskopische Anatomie' published in Germany in 1851. Its impact was such that an English translation was made in the following year. Both in range and accuracy Kölliker's text and figures of human tissues and organs are, even to-day, remarkable. There are, of course, many spaces to be filled in regarding the histological picture as we now know it, largely completed so far as tissue-structure is concerned but not, as yet, ready for final exhibition.

Now, why the lacunae in Kölliker's preliminary portrait? These notably include the finer details of the nervous system and the delineation of its tracts; the minute structure of the eye and ear; the classification and functional significance of the various types of blood-cells. We must remember that, a century ago, modern processes and instrumentation still required discovery and exploitation. The use of good fixatives to preserve tissues was almost unknown; only very simple microtomes were available and these seldom used; the ancillary methods of embedding had still to be devised, so also differential methods of staining to give colour-contrast to different types of cell, cell-inclusions and fibres (*e.g.*, muscle, collagen and elastic fibres).

At the time of publication of Kölliker's text-book the approach of the student was very different. The organs came 'fresh' from the dead house but usually without any form of preservation. They were examined either by dissociation with needles or after sectioning by hand with a barber's razor; staining was virtually unknown and the preparations were examined on the slide in water.

Kölliker's microscope would probably be condemned by the present-day medical student as only fit for salvage. The fine adjustment for accurate focus, the substage condenser for critical illumination and high aperture objectives (such as the apochromatic oil-immersion lens) not to mention the electron microscope still awaited discovery and the methods of industrial exploitation.

To sum up, the advances in a century of histology are primarily due to : (1) the development in methods of fixation and staining tissues for microscopic examination ; and (2) the development, optical and mechanical, of the microscope.

It is by the very inadequacy of Kölliker's methods that his achievement is crowned.

Animal Histology [1] is the science which treats of the minute structure of the tissues and organs of the animal body ; it is studied with the aid of the microscope, and is therefore also termed **Microscopic Anatomy.**

The study of histology is included in the medical curriculum because an adequate knowledge and understanding of it is necessary for the proper appreciation of both **Physiology** and **Anatomy.** It is also an essential preliminary to the study of the structure of diseased organs (**Pathology**). Practice in observing small but important differences of structure in various organs is excellent visual training for a medical student. Care and manual dexterity can be learnt in the handling of microscopes and in the preparation of specimens.

Every part or organ of the body consists of certain textures or tissues, which differ in their arrangement in different organs, but each of which exhibits characteristic structural features.

The chief methods of histological examination comprise :

1. **Dissociation** (or teasing).—The constituent cells of films of an organ or tissue are separated from each other by mechanical or chemical means. Dissociation with fine needles is the most common way of obtaining a sufficiently thin layer of cells or fibres for study with the microscope.

2. **The film or smear technique.**—This is used particularly in the examination of tissues of a semi-fluid or fluid nature, and is the standard method for blood and lymph. After making such a smear by mechanical means, it is customary to stain it. In this way differentiation between the various types of cell and cell-component is greatly aided.

3. **The sectional method.**—This involves the cutting of tissues into thin slices usually 5 to 50 μ in thickness.[2] It has the great advantage over methods 1 and 2 in that the relations of cells and tissues to one another are accurately preserved.

The following is a list of the principal tissues which compose the body :

Epithelial.

Connective : Areolar, Fibrous, Elastic, Reticular, Lymphoid, Adipose, Cartilage, Bone.

Blood and **Lymph.**

Muscular : Striated, Plain, Cardiac.

Nervous.

Some organs are formed of several of the above tissues, others contain only one or two.

[1] From ἱστός, a web or texture.

[2] The micron (or μ) is the standard unit of measurement in microscopy. $1\ \mu = \frac{1}{1000}$ of a millimetre $= \frac{1}{25000}$ of an inch.

It is convenient to include such fluids as the *blood* and *lymph* among the tissues, because they are studied in the same manner and contain cell-elements similar to those met with in some of the other tissues.

All the tissues are, prior to differentiation, masses of *cells* (embryonic cells). In some tissues elements become developed which take the form of *fibres*. Thus, the epithelial tissues are composed throughout life entirely of cells, only slightly modified in structure, and the nervous and muscular tissues consist of cells which are greatly modified to form the characteristic elements of those tissues. On the other hand, in the connective tissues an amorphous material becomes formed between the cells which is termed *ground-substance*. In this substance fibres make their appearance, sometimes, as in the fibrous connective tissues, in so large an amount as to occupy almost the whole of it, and greatly to preponderate over the cells. This ground-substance, possibly by virtue of its incorporating a certain amount of inorganic chlorides, has the property of becoming stained brown or black by silver nitrate and subsequent exposure to light, in which case the cells, which remain unstained, look like white spaces (cell-spaces) in the ground-substance (see fig. 87). It is possibly a gel permeated by the liquid tissue fluid. When an epithelial tissue or an epithelium-like arrangement of cells is similarly treated, the narrow interstices between the cells are also stained (see fig. 64), from which it is concluded that a similar substance exists in small amount between the cells of such a tissue. It has here been termed cement-substance, which may or may not be identical in nature with the ground-substance.

The cells of a tissue are not always separate from one another, but are in some cases connected by bridges of the cell-substance, which pass across the intercellular spaces. This is especially the case with the cells of the higher plants, but it has also been found to occur in many animal tissues; *e.g.*, in some varieties of epithelium (see figs. 60, 61). Occasionally the connexion of the cells of a tissue is even closer, and lines of separation between them are faint or absent. The term *syncytium* is given to any such united mass of cells.

CYTOLOGY: THE STRUCTURE AND FUNCTIONAL CHANGES OF CELLS.

Cytology deals with the structure and function of cells as opposed to that of organised tissues which forms the subject matter of histology. Although it is impossible to draw a sharp line between the two subjects, in practice it is convenient to refer to them as distinct.

A cell (fig. 1), is a minute portion of living substance (*protoplasm*) which is enclosed by a *cell-membrane* and always contains a specially differentiated part which is known as the nucleus.

Despite great technical difficulties, information about the physical state and chemical nature of the cell constituents has been obtained by a variety of ingenious methods. This knowledge, together with changes observed in different functional states, is justifiably becoming of ever-increasing importance in the study of Physiology and Bio-chemistry. Changes in the size

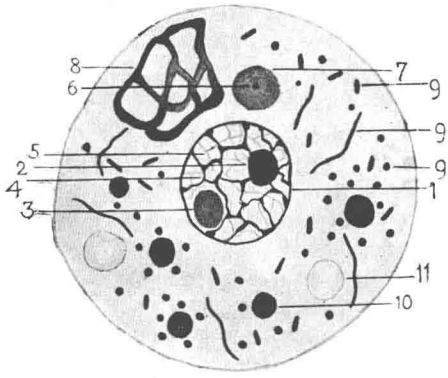

FIG. 1.—DIAGRAM OF CELL.
(H. M. Carleton.) Highly magnified.

1, nucleus; 2, karyosome; 3, nucleolus (plasmosome); 4, chromatin meshwork of nucleus; 5, 'linin' meshwork; 6, centriole; 7, centrosome; 8, Golgi apparatus; 9, 9, 9, mitochondria (granular, rod-shaped, thread-like); 10, metaplasmic inclusions; 11, vacuoles.

Some of the structures shown in this diagram are visible only in fixed and stained specimens. Others, such as 11, are best observed in vitally stained material.

and shape of cells and alterations in the various inclusions (particularly the Golgi apparatus) can form a reliable index of cellular activity.

CYTOPLASM.

This term is usually applied to the whole substance of a cell with the exception of the nucleus. It is composed of an apparently undifferentiated part which forms the main mass of most cells and several differentiated components such as the cell-membrane, mitochondria, Golgi apparatus, etc.

In general the ground cytoplasm of the living cells appears homogeneous by the ordinary methods of direct illumination. But examination under the oblique light rays of the dark ground condenser reveals colloidal particles, sometimes in obvious Brownian motion. Various appearances of structure —granular, reticular, fibrillar—may be exhibited after fixation, but these, in many cases, have been determined by the action of the fixative which has been employed, and do not necessarily represent the true state of the living cell. Water, protein and fat are present. The fat may form the wall of intracellular compartments, within which are protein and water. In some cells it is possible to cause reversible coagulation of the protein.

Inorganic salts are present and can be demonstrated by the technique of micro-incineration. To do this a section is carefully heated to drive off the organic substances and leave the inorganic. These are then localised in a pattern corresponding to that of the cells of the section. To some extent the type of salt may be identified. By this and other methods it has been found that the salts of the cell sap differ markedly from those of tissue fluid. Potassium salts are particularly high in concentration. Sodium and chloride ions are in much smaller concentration than in tissue fluid.

It has also proved possible to estimate the viscosity of cytoplasm by studying the rate of movement of particles through cells either under centrifugal force or, in the case of iron particles, in a magnetic field. The results obtained are variable and seem to indicate that it is high and subject to fluctuations.

Micro-dissection.—The dissection of living cells is effected by microscopically fine quartz or glass needles, mounted in a special apparatus for manipulating them mechanically. By using hollow needles, fluids can be introduced into the cell through the surface film. By this means it has been possible, with the aid of indicator dyes, to determine the pH of the cytoplasm in living cells.

Proceeding in this way, R. Chambers found the pH of the living nucleus in various cells of *Rana* and *Necturus* to be about 7·5, whether normal or injured, but the pH of the cytoplasm to be distinctly more acid, being about 6·9 in the normal state, and about 5·3 when injured and cytolysing. J. and D. M. Needham found the pH of the cytoplasm of *Amœba proteus* to be about 7·5: Pollack puts it lower (6·6 to 7·2), but the variety of Amœba may have been different. The cytoplasm has considerable buffering power and resists the action of acids if not in excess. The oxidation-reduction intensity (rH) has been similarly determined.

Remarkable results have been obtained by injecting into the interior of the cytoplasm substances which are toxic when added to the environment. Thus Pollack, working with Chambers, found that a solution of picric acid or fairly strong alcohol can be introduced into the cytoplasm without producing any deleterious effect, and Brinley makes the same statement for hydrocyanic acid and cyanides.

The injection of salts of the monovalent electrolytes, Na and K, increases the fluidity of the cytoplasm, whilst the salts of Ca and Mg produce coagulation. The electrolytes appear to maintain a balanced condition of the colloids in living protoplasm, being so proportioned that the coagulating action of one kind is offset by the dispersive action of the other kind.

If the surface pellicle of a cell is torn the fluid protoplasm may exude, but the exudation immediately forms a new membrane around itself; this, however, occurs only in the presence of calcium salts.

By the method of micro-dissection it has also been determined that the astral configurations (division, spindle, etc.) seen during cell-division are gelated portions of the cytoplasm. The mitochondria and the chromosomes also appear to be gels: they can be stretched by the micro-needles and on release regain their original length.

1. **Cell-membrane.**—A fine pellicle covers the exterior of the cytoplasm of all living cells. It acts mechanically as a retaining membrane and to prevent damage from the exterior. But it also has an important function in regulating the inflow and outflow of water and dissolved substances. It probably consists of a bimolecular film of lipoprotein molecules supported by a condensation (gel) of protein of the cytoplasm—ectoplasm.

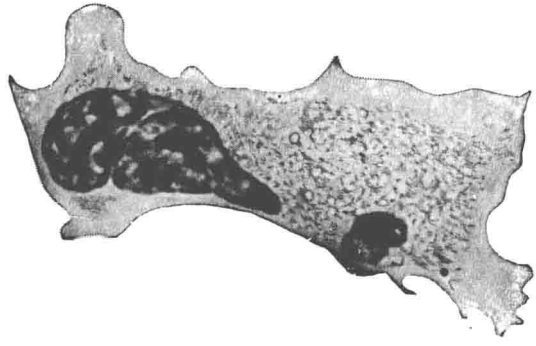

FIG. 2.—PHOTOGRAPH OF LEUCOCYTE OF TRITON, FIXED WHILST IN AMŒBOID CONDITION BY JET OF STEAM DIRECTED ON TO COVER-GLASS, AND SUBSEQUENTLY STAINED WITH HÆMATOXYLIN. (E. Sharpey-Schafer.) × 1360. Untouched photograph.

The protoplasm shows an internal granular endoplasm and a clear ectoplasm.

Micro-dissection has shown that the cell membrane can be considerably stretched without rupturing it. The gap caused by further stretching can easily be bridged once more by a newly formed membrane provided that the injury is not too great.

The cell membrane is not freely permeable to all dissolved substances. Water can usually pass it, but inorganic solutes, particularly cations, pass slowly or not at all except when the membrane is modified during activity. Organic substances, particularly if fat soluble, pass easily unless their molecular weight is high. It is held (Höber) that a partial explanation of cell permeability can be obtained by assuming that it is lipoprotein in nature; fat-soluble substances pass by dissolving in the lipid; other substances pass by entering pores of varying sizes.

The membrane is undoubtedly modified in electrical polarisation and in permeability during activity such as nerve conduction or muscle contraction. Pseudopodia formation may be accompanied by, or may be caused by, changes in the cell-membrane. In a phagocytic cell such as the macrophage some substances are taken in by enclosure of a droplet of water by the pseudopodia. The contents of the vacuole are absorbed and the water passes out through the cell-membrane. This process, termed Pinocytosis by Lewis, is best appreciated by seeing his film on the subject. This process may occur in other types of cell. Finally, it is likely that many reactions of the cell occur by adsorption of the substrates and enzymes at the cell surface.

2. **Centriole.**—All cells of the higher animals which are still capable of mitotic division contain this body. Highly specialised cells, which have lost the capability of reproduction, such as nerve-cells, are without it. In rounded or polyhedral cells the centriole lies close to the nucleus; it is often double. In elongated cells (*e.g.*, in columnar epithelium) it usually lies between the nucleus and the free end of the cell. It is very resistant to reagents. It is deeply stained by iron-hæmatoxylin. When a cell is about to divide, its centriole divides first, and the two centrioles thus produced gradually separate from one another and pass to opposite poles of the cell. From each of the two centrioles a number of what appear to be fine fibres diverge towards the equator of the dividing nucleus and, joining with those from the opposite centriole, constitute what is known as the *achromatic spindle* (p. 22), to which the divided chromosomes of the nucleus become attached, and along which they appear to be guided towards the centrioles to constitute the daughter nuclei.

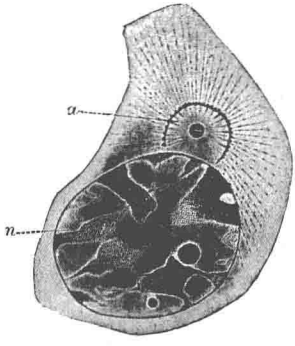

FIG. 3.—A CELL (WHITE BLOOD-CORPUSCLE) SHOWING ITS CENTRIOLE AND CENTROSOME. (M. Heidenhain.)

In this, as in most cases, the centrosome, *a*, lies near the nucleus, *n*.

In some cells the centrioles are multiple; this is frequently the case with leucocytes, and always with giant-cells found in bone-marrow and elsewhere. The cytoplasm immediately surrounding the centriole is often different in appearance

from the rest, and forms a small sphere known as the *centrosome*. This is sometimes itself enveloped by a feltwork of irregular filamentous particles which form

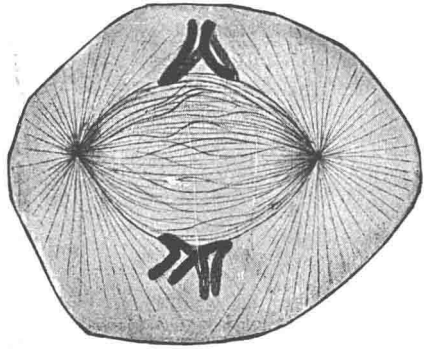

FIG. 4.—SPERMATOCYTE OF SALAMANDER, SHOWING ACHROMATIC FIBRES OF SPINDLE AND OTHER FIBRES RADIATING FROM CENTRIOLES. (Flemming.)

Four chromosomes are represented at the equator of the spindle.

a capsular covering to it (M. Heidenhain, Champy and Gley). No centriole has been found in the cells of the higher plants, although centrosomes and archoplasmic fibres are well marked in them, especially during cell-division.

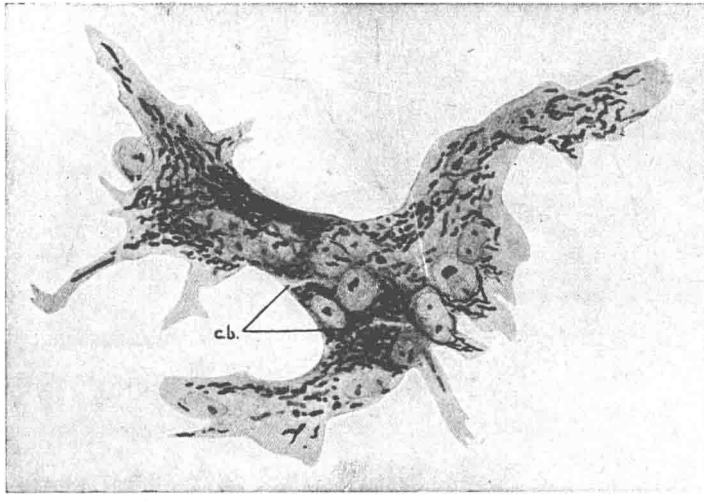

FIG. 5.—A GIANT-CELL (FROM BONE OF EMBRYO CHICK OF 10½ DAYS) STAINED WITH IRON-HÆMATOXYLIN TO EXHIBIT THE MITOCHONDRIA. (Honor B. Fell.)

The cell has been produced by the coalescence of smaller cells: cell-boundaries are seen at *cb*.

3. **Mitochondria.**[1]—These are either punctate, rod-like, or filamentous bodies (fig. 5); they appear to exist in all cells, plant and animal. They can be observed in the living cell when viewed with dark ground illumination

[1] From μίτος, a thread, and χόνδρος, a grain.

(fig. 6); they then often appear to be in constant movement. Filamentous mitochondria have been seen to segment and reunite. In round or polyhedral cells the mitochondria are generally evenly distributed (fig. 5), but in elongated cells, such as the columnar cells of the intestine, they form two groups, one at each end of the cell. It has been found possible to separate mitochondria from other cell components by crushing cells and submitting a suspension of the debris to differential centrifuging. The analysis of the preparations reveals that mitochondria are composed of lipoprotein. The fatty substances include neutral fat, phospholipids and cholesterol. Several components of the oxidative systems of the cell can be demonstrated, including cytochrome, cytochrome oxidase, succinodehydrogenase. Glutathione has

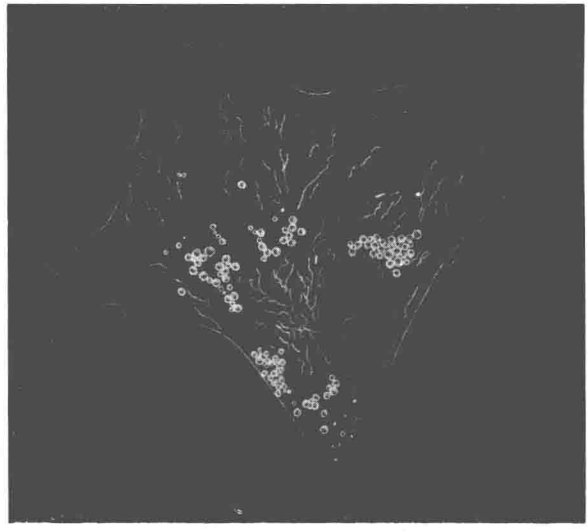

FIG. 6.—A LIVING CELL FROM A CULTURE OF EMBRYONIC CHICK TISSUE, SHOWING NUCLEUS WITH NUCLEOLI, THREAD-LIKE MITOCHONDRIA AND FAT-GLOBULES AS SEEN BY DARK GROUND ILLUMINATION. (Drawn by Honor B. Fell.) Magnified about 1000 diameters.

also been identified by histochemical tests. Phosphatase is present. It seems likely that, although not directly participating in the anabolic activity of the cell, their presence is necessary to provide the requisite energy from oxidative processes.

Histologically, mitochondria can be demonstrated in the living cell by staining with Janus green. In the fixed cell, acid fuchsin and Heidenhain's hæmatoxylin may be used.

4. **Microsomes.**—Recently, great interest has been aroused by the discovery of these submicroscopic particles in the cytoplasm (Claude). They probably occur in all cells and may constitute up to 25 per cent. of the dry weight of the cell. They cannot be seen with an ordinary microscope because they are too small. Pure preparations of them have been made by crushing cells and submitting the debris to differential centrifuging. It is found that