HANDBOOK OF HISTOPATHOLOGICAL TECHNIQUE

(INCLUDING MUSEUM TECHNIQUE)

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FOREWORD

THERE HAS always been a tradition of exemplary technique in British pathological histology. It can well bear comparison today with any in the world. Much of this satisfactory state of affairs is due to the technologists, who had themselves initiated or handed down standards of virtuosity which, however, too often remain unrecorded, and who diffuse their influence over a narrow field.

Mr. Charles Culling is well known as a teacher and as an exponent of his speciality, and has trained many who are themselves now experts. His experience includes a period of authority as Serjeant-Major in the Central Military Pathological Laboratory of India, Poona, although most of his work has been in the laboratories at Westminster Hospital School of Medicine.

In this book he has set out the methods which he himself employs, with all the detail which makes the difference between success and failure. He has also given an account of the methods in use in our museum which, it is hoped, may prove of value to all whose privilege it is to prepare for posterity the dissections and illustrating material of today.

It is a great pleasure to me personally to introduce the work of one whose standards have been so uniformly high, and I do so in the confidence that this book will help many to follow in his footsteps.

R. J. V. PULVERTAFT

PREFACE

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

July, 1957

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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.

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.

INTRODUCTORY

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.

CHAPTER 1

THE CELL

THE CELL (Fig. 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.

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. When a cell divides the chromatin is aggregated to form the chromosomes, the number of which is characteristic of the species. In human cells there are 48 chromosomes. These chromosomes carry hereditary characteristics and on division split along their length, one-half of each going to each new or daughter cell.

Nucleoli

The nucleus generally contains one or more refractile particles which are known as nucleoli. These nucleoli are divided by some workers into two groups, plasmosomes and karyosomes.

Plasmosomes.—Plasmosomes may be present in the nucleus and generally are easily differentiated from karyosomes by their regular spherical shape. They are said to be composed

of protein other than nucleoprotein. Their function is not known.

Karyosomes.—There may be more than one in a nucleus and when present they are less regular in outline than the plasmo-

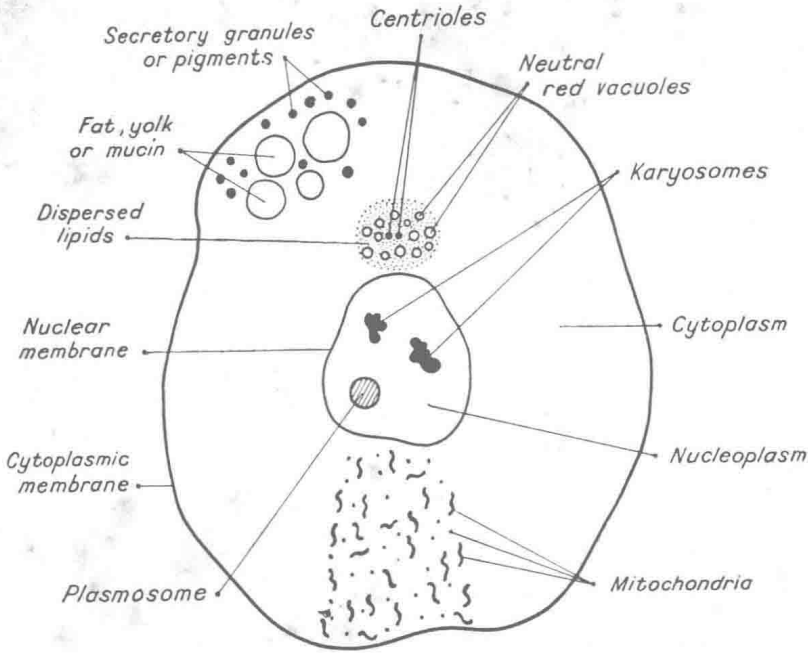

FIG. 1.—Diagram of a living cell showing its component parts and possible inclusions. The Golgi apparatus would be present in place of the neutral red vacuoles, if the cell were fixed. The inclusion of fat, yolk, mucin, secretory granules, and pigments is purely diagrammatic; it is unlikely that more than one type would be present. Should such inclusions be present they are normally distributed throughout the cytoplasm.

somes. They are composed of chromatin (nucleoprotein) and are thought to be reservoirs called upon during cell division (mitosis).

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.

THE CYTOPLASM

Cytoplasmic membrane

The cell is bounded by a semi-permeable membrane thought to be composed of protein and lipid particles. This permits the exchange of food and secretory products largely under the influence of osmotic pressure. Using a micro-manipulation apparatus fine pipettes can be introduced through this membrane into the cytoplasm without, in themselves, causing death of the cell.

Cytoplasm (*ground substance*)

The cytoplasm is a homogeneous, watery solution, basic in reaction, of protein, various salts and sugars.

In certain types of cell the cytoplasmic structure may be differentiated and such differentiation appears relatively fixed, like the striations seen in voluntary muscle fibres. The cytoplasm is a fluid of low viscosity, but towards the surface it is usually more of a gel in consistency. It should be remembered, however, that it is capable of rapidly reversible sol–gel transformation. It has an osmotic pressure equal to about 0·9 per cent sodium chloride.

Neutral red vacuoles and Golgi apparatus

Neutral red vacuoles are minute spherical structures rendered visible in living cells by neutral red. Such bodies are seen only in the living cell and it is thought by many workers that, following the fixation of a cell, the neutral red vacuoles and the area of dispersed lipid which surrounds them form an artefact known as the Golgi apparatus, which can be demonstrated by silver or osmium tetroxide techniques.

Mitochondria

Mitochondria are minute bodies, usually several hundred in number. They may be either filamentous or granular and are generally scattered throughout the cytoplasm, but occasionally congregated near the nucleus. In the living cell they are motile. They are lipo-protein in nature but little is known of their function. They are quickly affected by temperature and autolysis and are among the first structures to disappear on death

of the cell; for this reason early fixation is necessary for their demonstration.

Centriole (*cytocentrum, centrosphere*)

Near the nucleus of most living cells can be seen a condensation of protoplasm which contains one or two small, refractile bodies known as centrioles. During mitosis the two centrioles move to opposite poles of the cell and support a skein of fine protoplasmic rays, known as the achromatic spindle, which is crystallized protoplasm and may be demonstrated with a polarizing microscope. Along this spindle the chromosomes, after division, arrange themselves. The centriole is said to be absent from nerve cells, which, being highly differentiated, have lost the power of division.

Cytoplasmic inclusions

These are inert, non-protoplasmic substances found within the cytoplasm, and may be products of the cell's own activity, or may be taken up from the surrounding medium.

The more important inclusions are:

(*a*) *Fat*, which occurs as globules within the cytoplasm, often forcing the nucleus and other intra-cellular materials to one side.

(*b*) *Yolk*, which is similar to fat but usually more yellow in colour. It is differentiated by dissolving out the fat, the yolk having a protein base which is subsequently demonstrable.

(*c*) *Glycogen*, which occurs in a watery solution of colloidal nature. After fixation it is seen as fine granules or an amorphous mass, dependent on the type of fixation technique employed.

(*d*) *Mucin*, which is first demonstrable in mucin-producing cells as minute granules known as mucigen. These become droplets of mucin which coalesce, producing the typical distension of the 'goblet cell.'

(*e*) *Secretory granules and pigments.*—Remnants of ingested material, such as cell membranes and bacteria, are seen occasionally in the cytoplasm of some cells; for example, histiocytes.

CELL DIVISION (MITOSIS)

With the exception of certain specialized cells (for example, nerve cells), cells multiply by division. The normal mechanism of division is a complicated one known as *mitosis*, but certain cells (for example, cartilage cells) undergo simple division (*amitotic* division) like bacteria.

The process of mitosis is divided into four stages (Fig. 2):

(i) Prophase.
(ii) Metaphase.
(iii) Anaphase.
(iv) Telophase.

Prophase

The chromosomes become visible by concentration of the chromatin; they at first form a long continuous skein which later divides into separate chromosomes. At the same time

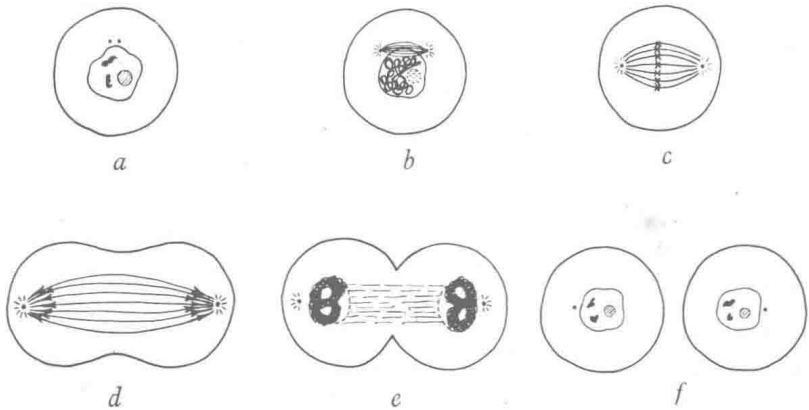

FIG. 2.—Diagram showing the four phases of division of a cell. (*a*) Normal cell having a resting nucleus, with one plasmosome and two karyosomes. There are two centrioles. (*b*) Prophase: formation of the achromatic spindle has commenced, and the chromatin has condensed into a continuous skein. (*c*) Metaphase: the chromosomes have arranged themselves in the centre of the cell, and each has split into two. (*d*) Anaphase: the chromosomes have moved along the achromatic spindle to opposite poles of the cell, and the cytoplasmic membrane has become constricted. (*e*) Telophase: the chromosomes have begun to coalesce, and the cytoplasmic membrane has become more constricted. (*f*) Telophase: the cytoplasmic membrane has divided, and a nuclear membrane has formed in each of the two daughter cells.

the two centrioles move to opposite poles of the cell (if there is only one it first divides), and the nuclear membrane begins to disappear. Between the centrioles fine fibres appear, and it is on these fibres (the achromatic spindle) that the chromosomes arrange themselves, and appear to move after division (Fig. 2*b*).

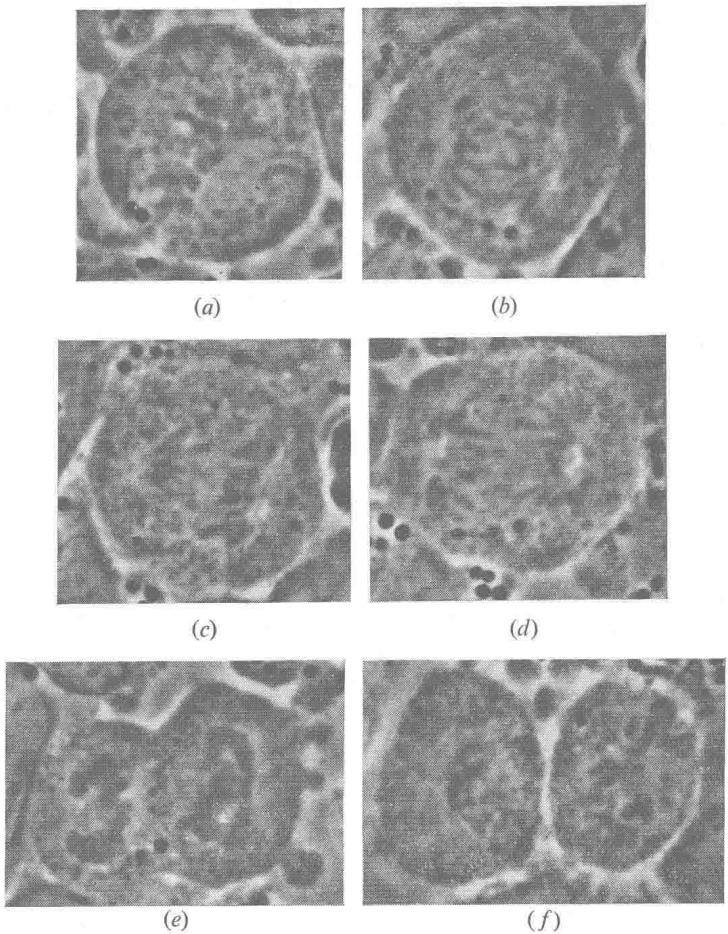

FIG. 3.—Phase-contrast microphotographs of bone-marrow cell showing mitosis. (*a*) Metaphase: chromosomes are seen in the central region of the nucleus, and are in the process of dividing. (*b*), (*c*) and (*d*) Anaphase: the chromosomes are moving along the achromatic spindle to opposite poles of the nucleus. (*e*) Telophase: the chromosomes coalesce, and the cytoplasm becomes constricted. This stage is a very rapid one, and is accompanied by violent bubbling movements at the periphery of the cytoplasm. This latter action is seen quite clearly on the right of this photograph. (*f*) Telophase: the nucleus is formed, and the constriction of the cytoplasmic membrane is continued to form two daughter cells.

Note.—The stage of prophase is not shown in this series owing to the impossibility of deciding which particular cells will mitose. Consequently a cell is usually in anaphase when first noticed.

Metaphase

The centrioles are at opposite poles; the chromosomes are shorter and thicker and are arranged in the central region of the spindle. Each chromosome now splits into two (Figs. 2*c* and 3*a*).

Anaphase

At this stage the chromosomes move along the spindle to opposite poles (Figs. 2*d* and 3*b*, *c* and *d*).

Telophase

In this final stage, the sets of chromosomes having reached opposite poles, nuclear membranes are formed around each of the daughter nuclei, the chromosomes gradually expanding and dissolving. The cytoplasmic membrane itself divides, having become constricted during telophase and finally is only connected by a fine thread which ruptures as the cells move apart (Figs. 2*e* and *f* and 3*e* and *f*).

COLLOIDAL CONCEPTION OF TISSUE

In order to correlate the mobility of cells in certain conditions with the apparent immobility of tissues, it is necessary to have some knowledge of colloidal theory.

The cytoplasm of most cells allows free movement of granules within it, showing that it has a low viscosity, yet it maintains its shape by an outer area which is more of a gel. Both these areas are capable of reversal and the cell may, in certain circumstances, become almost liquid, or if injured or dead may revert wholly to a stiff gel. Damage to the cytoplasmic membrane will normally result in gel formation under the damaged area until repair has taken place.

This reversion from sol to gel and *vice versa* is possible because of the colloidal nature of protoplasm.

COLLOIDS

When a powdered solid is put into a fluid, it may remain suspended in that fluid and be easily removable by filtration through paper; or it may disperse as single molecules which