CHURCHILL LIVINGSTONE

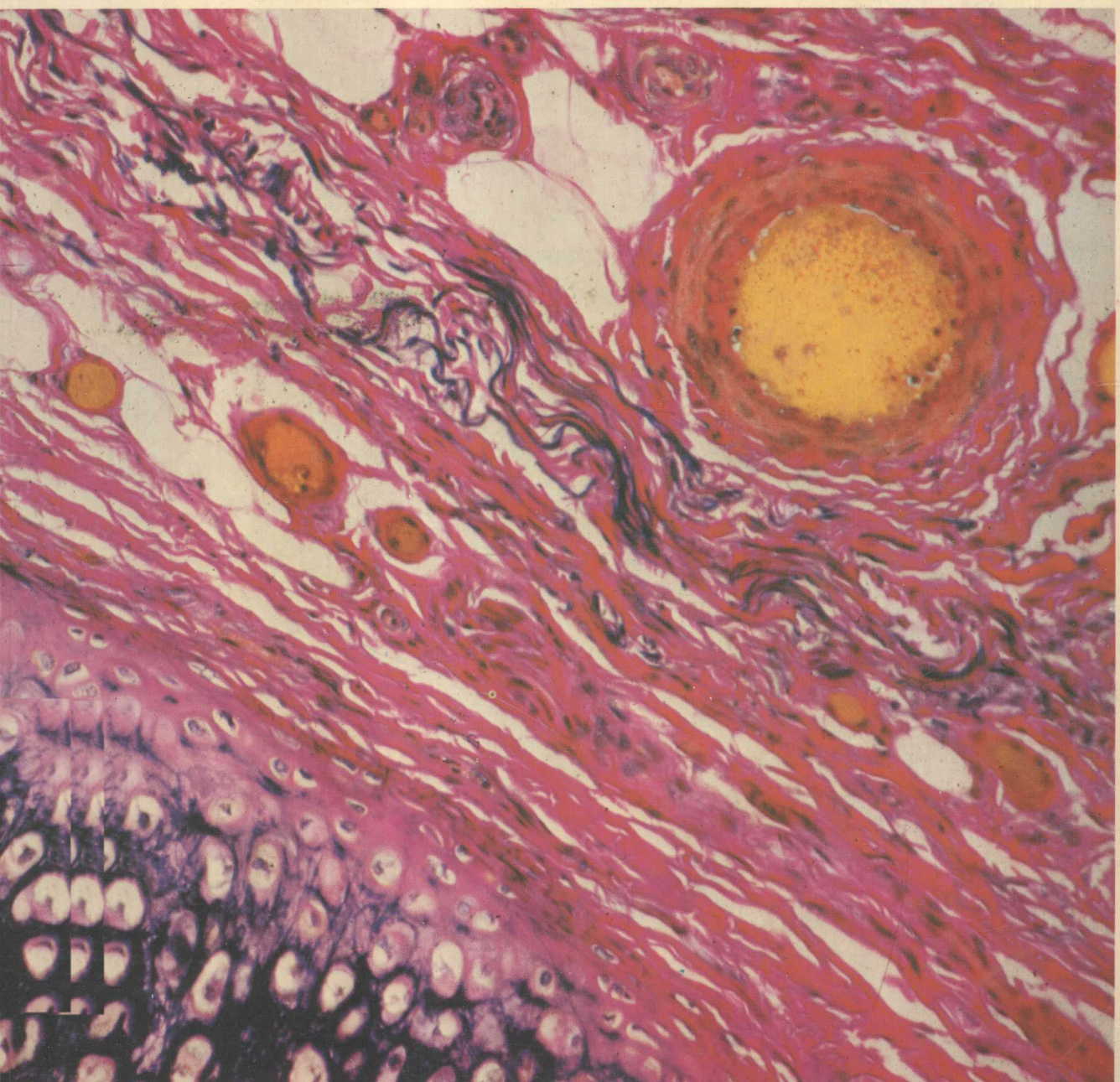
P. R. Wheater
H. G. Burkitt
V. G. Daniels

Illustrated by P. J. Deakin

FUNCTIONAL HISTOLOGY

A TEXT AND COLOUR ATLAS

Foreword by R. Warwick



Functional Histology

A TEXT AND COLOUR ATLAS

Paul R. Wheater
BA Hons (York), B Med Sci Hons (Nott), BM BS (Nott)
The Queen's Medical Centre, University of Nottingham

H. George Burkitt
BDSc Hons (Queensland), M Med Sci (Nott) FRACDS
The Queen's Medical Centre, University of Nottingham

Victor G. Daniels
BSc Hons (Lon), MB BChir (Cam), PhD (Sheff)
Lecturer in medical education, University of Dundee

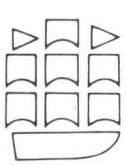
Illustrated by

Philip J. Deakin
BSc Hons (Sheff), MB ChB (Sheff)
The Medical School, University of Sheffield

Prepared in the Department of Pathology, University of Nottingham

Foreword by

Roger Warwick, BSc, PhD, MD
Professor of Anatomy
Guy's Hospital Medical School
University of London



CHURCHILL LIVINGSTONE
EDINBURGH LONDON AND NEW YORK 1979

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by Churchill Livingstone Inc., 1560 Broadway, New York, N.Y. 10036, and by associated companies, branches and representatives throughout the world

First published 1979
Reprinted 1980
Reprinted 1981
Reprinted 1982 (twice)
First ELBS Edition published 1982
First edition reprinted 1984 (twice)

ISBN 0 443 01658 5 (cased)
ISBN 0 443 01657 7 (limp)

British Library Cataloguing in Publication Data
Wheater, Paul R
Functional histology.
1. Histology
I. Title II. Burkitt, H George
III. Daniels, Victor G
599'.08'24 QM551 79–40314

Printed in Great Britain by Jarrold & Sons Ltd, Norwich

Functional Histology

Foreword

A new book, added to the current spate of publications, must offer some special qualities to commend it amongst its rivals—perhaps a newness of approach to its subject, perhaps an unusual excellence in content. This volume satisfies both criteria.

Firstly, it is deliberately planned to assist the reader not only as an armchair textbook but equally as a book for work in the laboratory. The main text, succinct and not over-burdened with details, deals primarily with principles, generalisations, and functional considerations, but is nevertheless closely linked to appropriate illustrations. The captions to these, being chiefly concerned with structural details, directly adjoin the corresponding illustrations.

Secondly, the illustrations are indeed of unusual excellence. The diagrams are clear, concise and well conceived and are a credit to the illustrator and, of course, to the advice of the three other members of the authorship. These three authors are almost entirely responsible for all the photographs (except for those taken by electron microscope) and also for the preparation of materials for them. As a result of these efforts they have assembled, specifically for this book, a magnificent display of mammalian cells and tissues (largely from human sources), which in clarity and accuracy, in scope and quality, surpasses any similar series of illustrations in books of such moderate size.

The volume is most interesting and attractive in conception and format. It has freshness and yet authority, which doubtless are derived from the comparative youthfulness of its originators and from their collectively wide and varied experience of teaching and research in biology and medicine. Evidently also, they have not forgotten the problems of student days, and have therefore produced a book which, without obscurity or condescension, both instructs and stimulates. Its appeal should be wide in biological, medical and ancillary fields, and I wish its authors much success.

London, 1979 Roger Warwick

Preface

Histology has bored generations of students. This is almost certainly because it has been regarded as the study of structure in isolation from function; yet few would dispute that structure and function are intimately related. Thus, the aim of this book is to present histology in relation to the principles of physiology, biochemistry and molecular biology.

Within the limits imposed by any book format, we have attempted to create the environment of the lecture room and microscope laboratory by basing the discussion of histology upon appropriate micrographs and diagrams. Consequently, colour photography has been used since it reproduces the actual images seen in light microscopy and allows a variety of common staining methods to be employed in highlighting different aspects of tissue structure. In addition, some less common techniques such as immunohistochemistry have been introduced where such methods best illustrate a particular point.

Since electron microscopy is a relatively new technique, a myth has arisen amongst many students that light and electron microscopy are poles apart. We have tried to show that electron microscopy is merely an extension of light microscopy. In order to demonstrate this continuity, we have included resin-embedded thin sections photographed around the limit of resolution of the light microscope; this technique is being applied increasingly in routine histological and histopathological practice. Where such less conventional techniques have been adopted, their rationale has been outlined at the appropriate place rather than in a formal chapter devoted to techniques.

The content and pictorial design of the book have been chosen to make it easy to use both as a textbook and as a laboratory guide. Wherever possible, the subject matter has been condensed into units of illustration plus relevant text; each unit is designed to have a degree of autonomy whilst at the same time remaining integrated into the subject as a whole. Short sections of non-illustrated text have been used by way of introduction, to outline general principles and to consider the subject matter in broader perspective.

Human tissues were mainly selected in order to maintain consistency, but when suitable human specimens were not available, primate tissues were generally substituted. Since this book stresses the understanding of principles rather than extensive detail, some tissues have been omitted deliberately, for example the regional variations of the central nervous system and the vestibulo-auditory apparatus.

This book should adequately encompass the requirements of undergraduate courses in medicine, dentistry, veterinary science, pharmacy, mammalian biology and allied fields. Further, it offers a pictorial reference for use in histology and histopathology laboratories. Finally, we envisage that the book will also find application as a teaching manual in schools and colleges of further education.

Nottingham, 1979

Paul R. Wheater
H. George Burkitt
Victor G. Daniels

Acknowledgements

With few exceptions, each of the illustrations was specially prepared for the book. Whilst accepting full responsibility for the entire contents, the authors are indebted to many individuals who have made invaluable contributions in their specialised fields.

Most of the tissue preparation and photomicrography was performed within the Departments of Pathology and Human Morphology of the Queen's Medical Centre, University of Nottingham. The authors are thus extremely grateful for the generous co-operation of Professors I. M. P. Dawson and R. E. Coupland. Special thanks are due to Mrs Janet Palmer of the Department of Pathology, who gave tireless assistance in the preparation of many of the tissues for light microscopy which were used in this book and many more preparations, for which space was not available. Similarly, our thanks are conveyed to Mr Paul Beck of the Department of Human Morphology for producing a large number of valuable specimens. Many of the electron micrographs were made available by Mr John Kugler and Mrs Annette Tomlinson, also of the Department of Human Morphology; to both we are deeply indebted.

Other people freely made available their resources: Mr Peter Crosby of the Department of Biology, University of York provided all the scanning electron micrographs, and his colleague Mr Brian Norman provided several light microscopic sections; Dr Robert Lang, also of York University, provided the freeze-etched preparation used in Figure 1.8; Mr Donald Canwell of the Physiological Laboratory, University of Cambridge contributed several sections from his personal collection; Mr Nigel Cooper of the Department of Zoology, University of Cambridge, provided the electron micrographs for Figure 13.18; Dr Graham Robinson and Mr Stan Terras of the Department of Pathology, University of Nottingham each provided several electron micrographs, and they and their colleague, Miss Linda Burns, provided all the thin resin sections used for light microscopy; Dr David Tomlinson and Dr Terry Bennett of the Department of Physiology, University of Nottingham contributed Figures 7.14 and 7.17 respectively; Dr Pat Cooke of the Department of Genetics, City Hospital, Nottingham lent the chromosome preparation used in Figure 1.19; Dr David Ansell of the Department of Pathology, City Hospital, Nottingham, Dr Hugh Rice and Dr Peter James of the Department of Pathology, Nottingham General Hospital, and Dr Pauline Cooper of the Department of Pathology, Addenbrooke's Hospital, Cambridge made available various tissue specimens and slides. Mr Peter Squires and Mr Hugh Pulsford of Huntingdon Research Centre, Cambridgeshire were a great source of help in providing the primate tissues used when suitable human tissues were unavailable. To all of these kind and co-operative people we express our sincere thanks.

Mr Bill Brackenbury of the Department of Pathology, University of Nottingham very skilfully performed all the macrophotography. All the remaining colour photomicrography was performed by one of the authors (P.R.W.). The onerous task of typing the manuscript was carried out with skill and great patience by Mrs Christine Stevens.

The authors express their warmest thanks to Dr Alan Stevens of the Department of Pathology, University of Nottingham who performed the role of scientific editor with seemingly limitless dedication, insight and enthusiasm.

Finally, we would like to express our thanks to the staffs of Churchill Livingstone and Jarrold & Sons Ltd for their unstinting assistance.

P.R.W.
H.G.B.
V.G.D.

Contents

1. The cell

Introduction

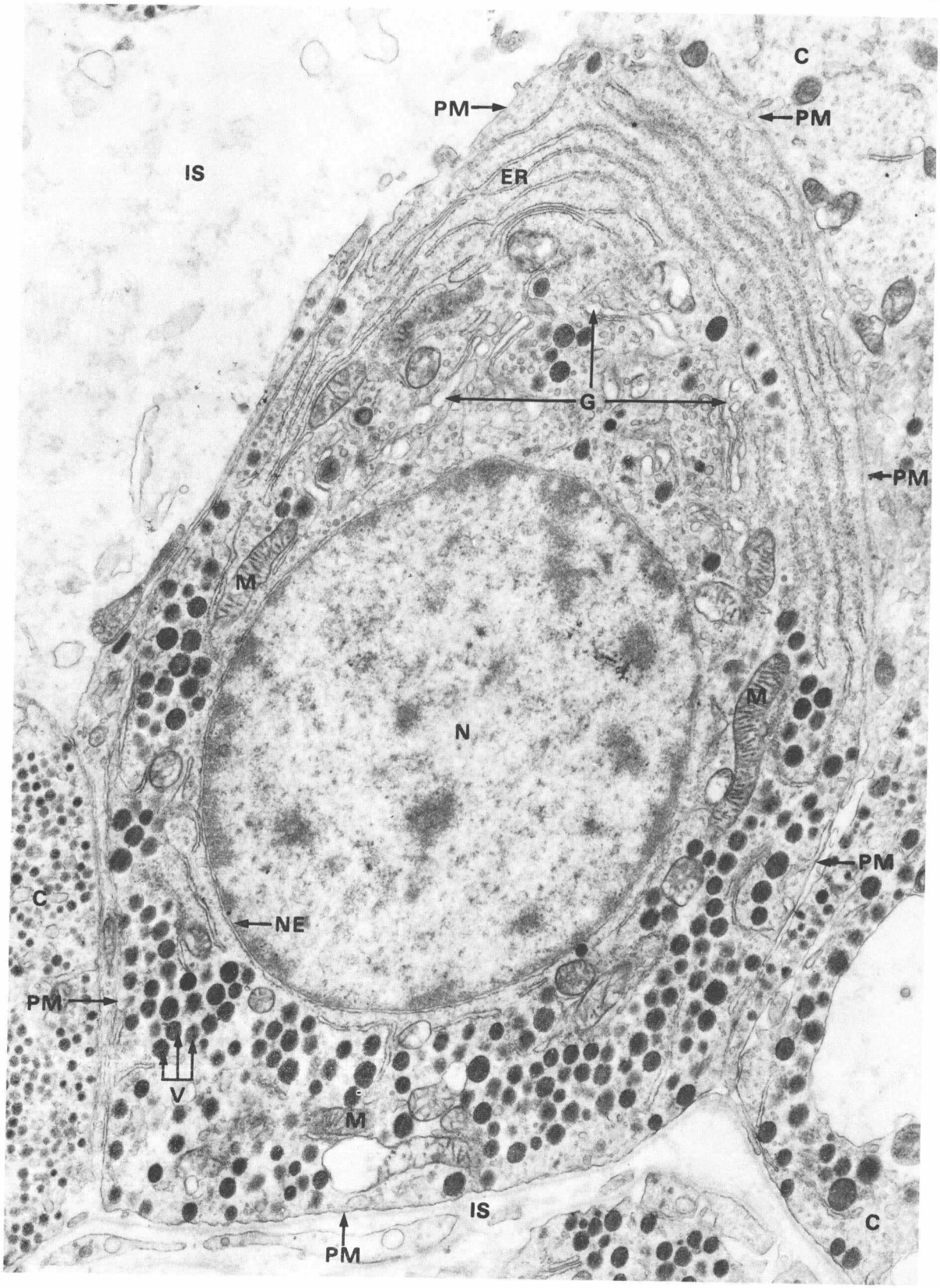
The cell, the functional unit of all tissues, has the capacity to perform individually all the essential life functions. Within the various tissues of the body, the constituent cells exhibit a wide range of specialisations which are, nevertheless, merely amplifications of one or more of the fundamental cellular processes. Reflecting their particular functional specialisations, mammalian cells have an extraordinary range of morphological forms yet all cells conform to a basic model of cell structure.

Even with primitive light microscopy, it was evident that cells were divided into at least two components, the *nucleus* and the *cytoplasm*, and as microscopical techniques advanced it became increasingly obvious that both the cytoplasm and the nucleus contained a number of subcellular elements which were called *organelles*. The advent of electron microscopy (EM) permitted description of the ultrastructure of these and many more organelles beyond the limit of resolution of the light microscope; the light microscope cannot resolve structures smaller than 0·5 μm (500nm). Much of present knowledge about cell structure is based upon electron microscopy, but most cellular functions take place at the biochemical level which is even beyond the resolving capacity of the electron microscope; currently, structures smaller than about 1·0nm (10Å) are not generally resolvable. Microscopy is only one of many techniques which have been used to further the understanding of cell function and structure.

Fig. 1.1 The cell *(illustration opposite) (EM × 15000)*

The basic organisational features common to all cells are illustrated in this electron micrograph of a hormone-secreting cell from the pituitary gland. All cells are bounded by an external limiting membrane called the *plasma membrane* or *plasmalemma* **PM** which serves as a dynamic interface between the internal environment of the cell and its various external environments. In this particular example, the cell interacts with two types of external environment: adjacent cells **C** and intercellular spaces **IS**.

The nucleus **N** is the largest organelle and its substance, often referred to as the *nucleoplasm*, is bounded by a membrane system called the *nuclear envelope* **NE**. The cytoplasm contains a variety of organelles most of which are also bounded by membranes. A diffuse system of membrane-bound tubules, saccules and flattened cisterns, collectively known as the *endoplasmic reticulum* **ER**, pervades the cytoplasm. A more distended system of membrane-bound saccules, the *Golgi apparatus* **G**, is usually found close to the nucleus. Scattered free in the cytoplasm are a number of relatively large, elongated organelles called *mitochondria* **M** which have a smooth outer membrane and a convoluted inner membrane system. In addition to these major organelles, the cell contains a variety of other membrane-bound structures, an example of which are the numerous, electron-dense *secretory vacuoles* **V** seen in this micrograph. Thus the cell is divided into a number of membrane-bound compartments each of which has its own particular biochemical environment. The organelles are suspended in a fluid medium called the *cytosol* which itself constitutes a discrete biochemical environment.



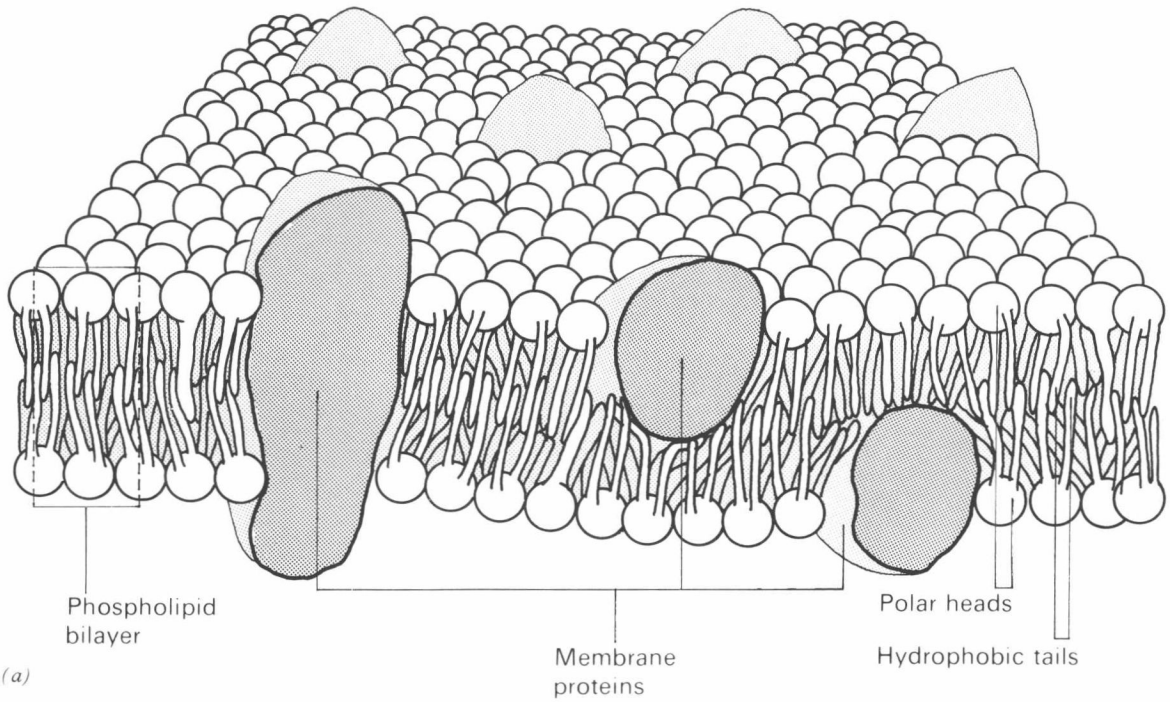


Fig. 1.2 Membrane structure

(a) Schematic diagram (b) EM × 167 400

Despite intensive investigation, the structure of cell membranes is still not known with certainty; however, a theoretical model has been progressively developed which satisfactorily incorporates much of the currently available biochemical and histological evidence.

Towards the end of the last century, it was observed that lipids rapidly gain entry into cells, and it was postulated that the 'cell boundary' was composed of lipid. In the 1920s it was found that, by measuring the minimum area that could be occupied by a monolayer of lipids extracted from a defined number of red blood cells, there was enough lipid present in the monolayer to cover each cell twice. From this it was concluded that the cells were bounded by a double layer of lipid. Later, it was proposed that cell membranes are symmetrical structures consisting of a bilayer of phospholipid molecules sandwiched between two layers of protein. This model, however, failed to explain the selective permeability of most cell membranes to molecules which are not lipid soluble such as glucose, sodium ions and potassium ions. These difficulties were theoretically overcome by postulating the existence of 'pores' composed of protein, through which hydrophilic molecules could readily be transported by passive or active mechanisms. As a result of electron microscopic studies in the late 1950s, the concept of the 'unit membrane' was devised, in which it was envisaged that all cell membranes have the same structure, since they all appeared to have the same trilaminate ultrastructure.

The current concepts of membrane structure are shown diagrammatically above. In this model, cell membranes are considered to consist of a bilayer of phospholipid molecules; the hydrophilic (lipid-insoluble) portions of the

phospholipid molecules of each layer are aggregated at the surface with their hydrophobic 'tails' projecting into the centre of the membrane where they interact with the hydrophobic 'tails' of the opposed phospholipid layer. The weak intermolecular forces which hold the bilayer together allow individual molecules of phospholipid to move relatively freely within each layer. Cell membranes are therefore highly fluid in nature, yet have the ordered structure of a crystal. Cholesterol molecules are incorporated in the hydrophobic regions of the membrane and modify the fluidity of the membrane. In this model, proteins are scattered in the phospholipid bilayer, some of them extending through the entire thickness of the membrane to be exposed to each surface; it is proposed that these molecules function as 'pores' through which hydrophilic molecules are transported either passively or actively. These proteins, and others which do not span the whole width of the membrane, are also freely mobile within the plane of the phospholipid bilayer. This model is known as the *'fluid mosaic model'* of membrane structure.

On the external surface of the plasma membranes of animal cells, many of the membrane proteins and some of the membrane lipids are conjugated with short chains of polysaccharide; these glycoproteins and glycolipids project from the surface of the bilayer forming an outer coating which may be analogous to the cell walls of plants, bacteria and fungi. This polysaccharide layer has been termed the *glycocalyx* and appears to vary in thickness in different cell types; whether an analogous layer exists on all membranes or only at the external surface is unknown. The function of the glycocalyx is obscure, but there is evidence that it may be involved in cell recognition phenomena, in the formation of intercellular adhesions, and in the adsorption of molecules to the cell surface. Alternatively, the glycocalyx may simply provide mechanical and chemical protection for the plasma membrane.

The electron micrograph in (b) provides a high magnification view of a plasma membrane; this example illustrates the minute surface projections of a lining cell from the small intestine. All membranes have a characteristic trilaminate appearance comprising two electron-dense layers separated by an electron-lucent layer. The outer dense layers are thought to correspond to the hydrophilic 'heads' of phospholipid molecules whilst the electron-lucent layer is thought to represent the intermediate hydrophobic layer mainly consisting of fatty acid side chains. On the external surface of the plasma membrane an outer fibrillar coat, called the *'fuzzy coat'*, represents the glycocalyx **G**. This is an unusually prominent feature of small intestinal lining cells.

Transport across plasma membranes

Plasma membranes mediate the continuous exchange of metabolites between the internal and external environments of the cell in four principal ways. These mechanisms enable the cell to control the quality of its internal environment with a high degree of specificity.

(i) Passive diffusion: this type of transport is entirely dependent on the presence of a concentration gradient across the plasma membrane. Lipids and lipid-soluble metabolites such as ethanol pass freely through plasma membranes; plasma membranes also offer little barrier to the diffusion of gases such as oxygen and carbon dioxide. The plasma membrane is, in general, impermeable to hydrophilic molecules; nevertheless some small molecules including water and urea, and inorganic ions such as bicarbonate, are able to pass down osmotic or electrochemical gradients through the membrane via hydrophilic regions, the nature of which remains obscure.

(ii) Facilitated diffusion: this type of transport is also concentration-dependent and involves the transport of larger hydrophilic metabolites such as glucose and amino-acids. The process is strictly passive but requires the presence of so-called 'carriers' to which the metabolites bind specifically but reversibly in a manner analogous to the binding of substrate with enzyme.

(iii) Active transport: this mode of transport is not only independent of concentration gradients but also often operates against extreme concentration gradients. The classical example of this form of transport is the continuous transport of sodium out of the cell by the so-called 'sodium pump'; this process requires the expenditure of energy provided in the form of ATP. It is postulated that this form of transport occurs through 'dynamic pores' consisting of proteins or protein systems which span the plasma membrane. Both active and passive transport processes are enhanced by increasing the area of the plasma membrane by folds or projections of the cell surface as exemplified by the absorptive cells lining the small intestine (see Fig. 1.2).

(iv) Bulk transport: bulk transport involves engulfment of large molecules or small particles by cytoplasmic extensions, thus forming membrane-bound vacuoles within the cytoplasm. When this process involves the creation of small vacuoles it is known as *pinocytosis*, and when large vacuoles are formed it is called *phagocytosis*. The term *endocytosis*, encompassing both processes, is probably a more appropriate term for bulk transport into the cell. Endocytotic vesicles either discharge their contents directly into the cytoplasm or fuse with membrane-bound organelles called *lysosomes*; lysosomes contain more than twelve different enzymes which are capable of degrading carbohydrates, lipids, proteins, nucleic acids and other organic molecules. Lysosomal enzymes digest

engulfed material which is then made available for metabolic processes. In many secretory processes, bulk transport also occurs in the opposite direction when it is termed *exocytosis*.

Histologically, the passive and active processes of transport can only be observed indirectly; for example, cells suspended in hypotonic solutions swell due to passive uptake of water whereas cells placed in hypertonic solutions tend to shrink due to outflow of water. Radio-isotope labelling techniques can be used to follow active transport processes. Bulk transport, however, is readily observable by microscopy.

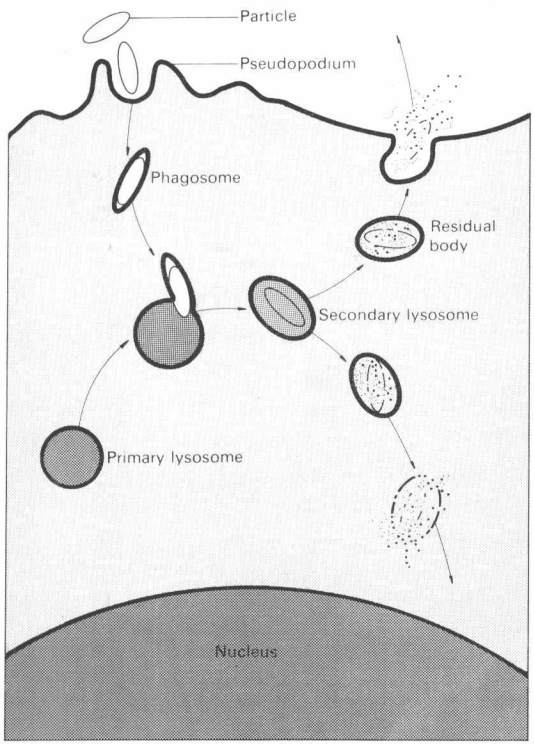
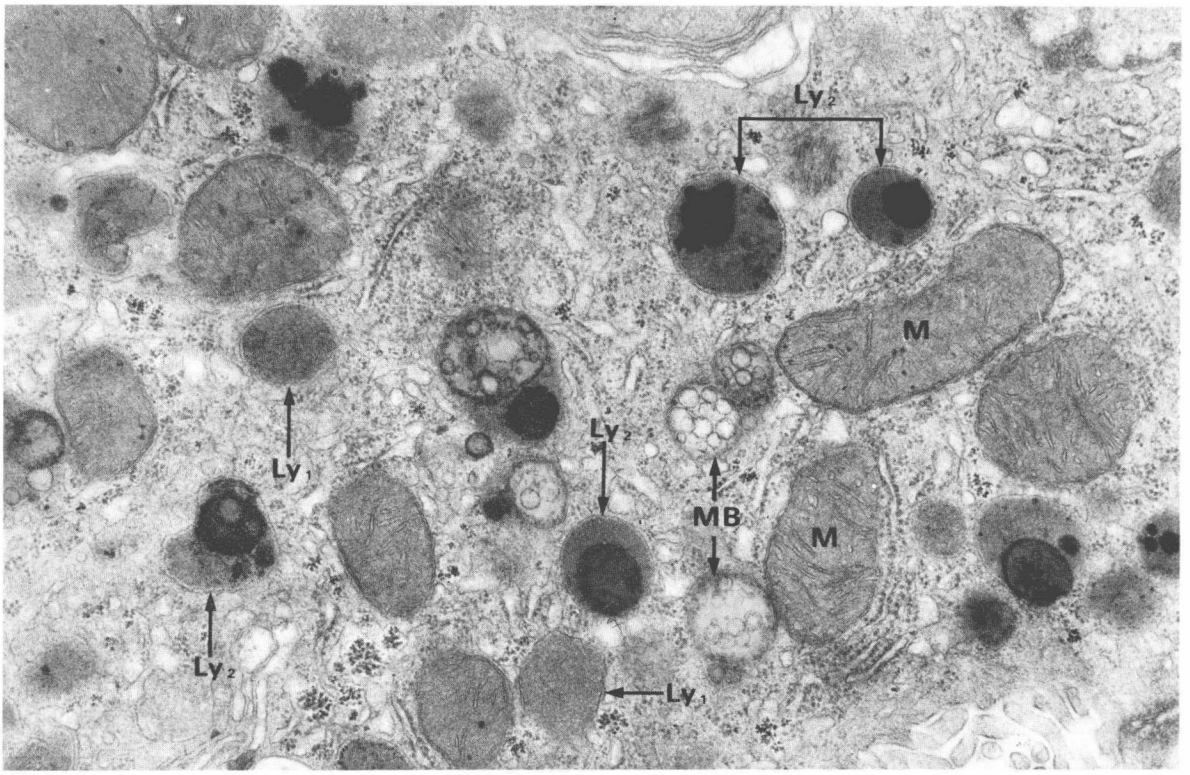


Fig. 1.3 Endocytosis

This diagram summarises the main steps in endocytosis of particulate matter. The first stage of phagocytosis involves recognition of a particle; this then becomes surrounded by cytoplasmic extensions called *pseudopodia*. When the particle is completely surrounded, the plasma membrane fuses and the membrane surrounding the engulfed particle forms a vesicle, known as a *phagosome* or *endocytotic vesicle*, which detaches from the plasma membrane to float freely within the cytoplasm. The phagosome is then in some way recognised by one or more *primary lysosomes* which fuse with the phagosome to form a *secondary lysosome*. This exposes the engulfed material to a battery of lysosomal enzymes. When digestion is complete, the lysosomal membrane may rupture, discharging its contents into the cytoplasm. Undigested material may remain within membrane-bound vesicles called *residual bodies*, the contents of which may be discharged at the cell surface by exocytosis; alternatively residual bodies may accumulate in the cytoplasm.

Lysosomes are also involved in the degradation of cellular organelles, many of which have only a finite lifespan and are therefore replaced continuously; this lysosomal function is termed *autophagy*. Most autophagocytic degradation products are reutilised by the cell, but some indigestible products accumulate and become indistinguishable from the residual bodies of endocytosis. With advancing age, residual bodies accumulate in the cells of some tissues and appear as brown so-called *lipofuscin granules*.



(a)

(b)

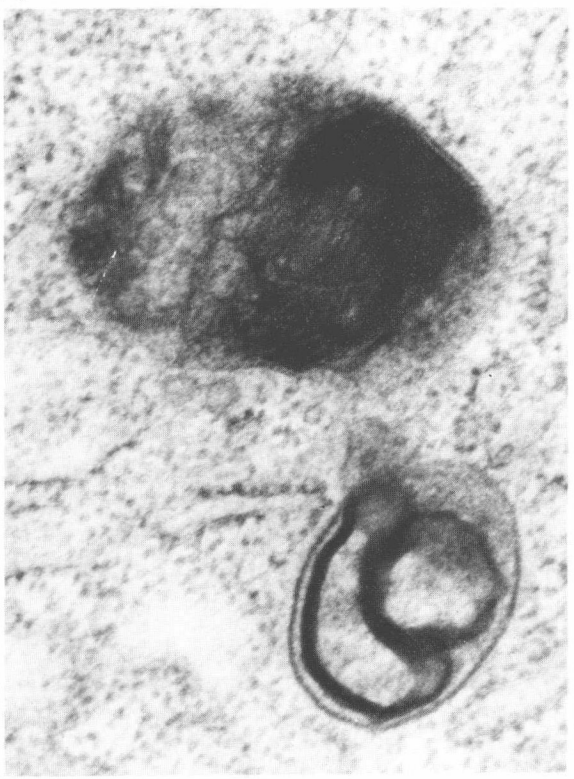


Fig. 1.4 Lysosomes

(EM (a) ×27000 (b) ×95000)

These micrographs show the typical appearance of lysosomes and residual bodies. Micrograph (a) shows part of the cytoplasm of a liver cell. Primary lysosomes **Ly_1** vary greatly in size and appearance but they are recognised as membrane-bound organelles containing a granular, amorphous material. Secondary lysosomes **Ly_2** are even more variable in appearance but are recognisable by their diverse particulate content some of which is extremely electron-dense. The distinction between residual bodies and secondary lysosomes is often difficult, but one distinctive type of residual body, the so-called *multivesicular body* **MB**, is seen in this micrograph. Multivesicular bodies are membrane-bound vesicles containing a number of smaller vesicles which are thought to represent the debris of cell membrane degradation. Note the size of lysosomes relative to mitochondria **M**.

In micrograph (b), two residual bodies, also from a liver cell, are shown at high magnification.

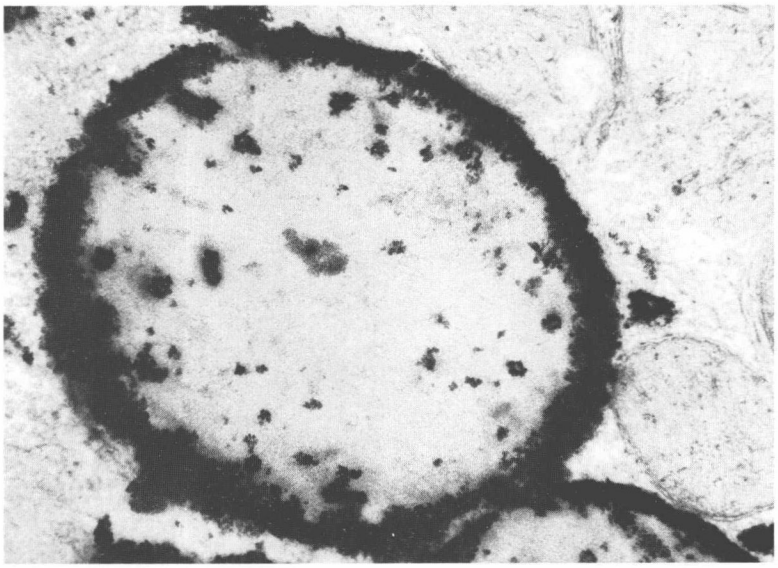


Fig. 1.5 Lysosomes

(EM histochemical method for acid phosphatase × 45000)

Histochemical methods can be used to demonstrate sites of enzyme activity within cells. Such a method has been used in this preparation to demonstrate the presence of *acid phosphatase* within lysosomes; the enzyme activity is represented by a very dense deposit within lysosomes. Acid phosphatase is one of several hydrolytic enzymes characteristic of lysosomes which can be used as histochemical markers for these organelles.

Protein synthesis

Proteins are not only a major structural component of cells but, in the form of enzymes, mediate every metabolic process within the cell. Thus the nature and quantity of proteins present within any individual cell determines the activity of that cell. Both the structural proteins and enzymes of the cell are subject to wear and tear and are replaced continuously. Many cells also synthesise proteins for export; such proteins include glandular secretions and extracellular structural components of tissues. Protein synthesis is, therefore, an essential and continuous activity of all cells and the major function of some cells.

The principal organelles involved in protein synthesis are the *nucleus* and *ribosomes*. The nucleus of every cell contains within its complement of DNA a template for each protein that can be made by that individual as a whole. However, most cells only synthesise a certain defined range of proteins which are characteristic of the particular cell type and therefore only part of the DNA template is utilised. The process of protein synthesis involves *transcription* of the DNA code for a particular protein by synthesis of the specific, complementary messenger RNA (mRNA) molecule. The mRNA molecule then enters the cytoplasm to associate with ribosomes upon which protein synthesis occurs; the amino-acid sequence of the resulting protein is determined by *translation* of the mRNA code.

Ribosomes are minute cytoplasmic organelles, each composed of two subunits of unequal size. Each subunit consists of a strand of RNA (ribosomal RNA) with associated ribosomal proteins; the ribosomal RNA strand and associated proteins are folded to form a condensed, globular structure. Ribosomes are highly active structures with specific receptor proteins which align mRNA strands so that transfer RNA (tRNA) molecules carrying the appropriate amino-acids may be brought into position prior to the addition of their amino-acids to the growing polypeptide chain. Other ribosomal proteins are involved in catalysing peptide bond formation between amino-acids. Individual ribosomes are too small to be clearly resolved by electron microscopy although they are visible as small electron-dense masses at high magnification; nevertheless, the detail of ribosome structure and function are well established at the molecular level. Ribosomes are found free in the cytoplasm either singly or as small aggregations called *polyribosomes* or *polysomes*; ribosomes are also attached to the surface of the extensive intra-cytoplasmic membrane system known as the endoplasmic reticulum (see Fig. 1.9).

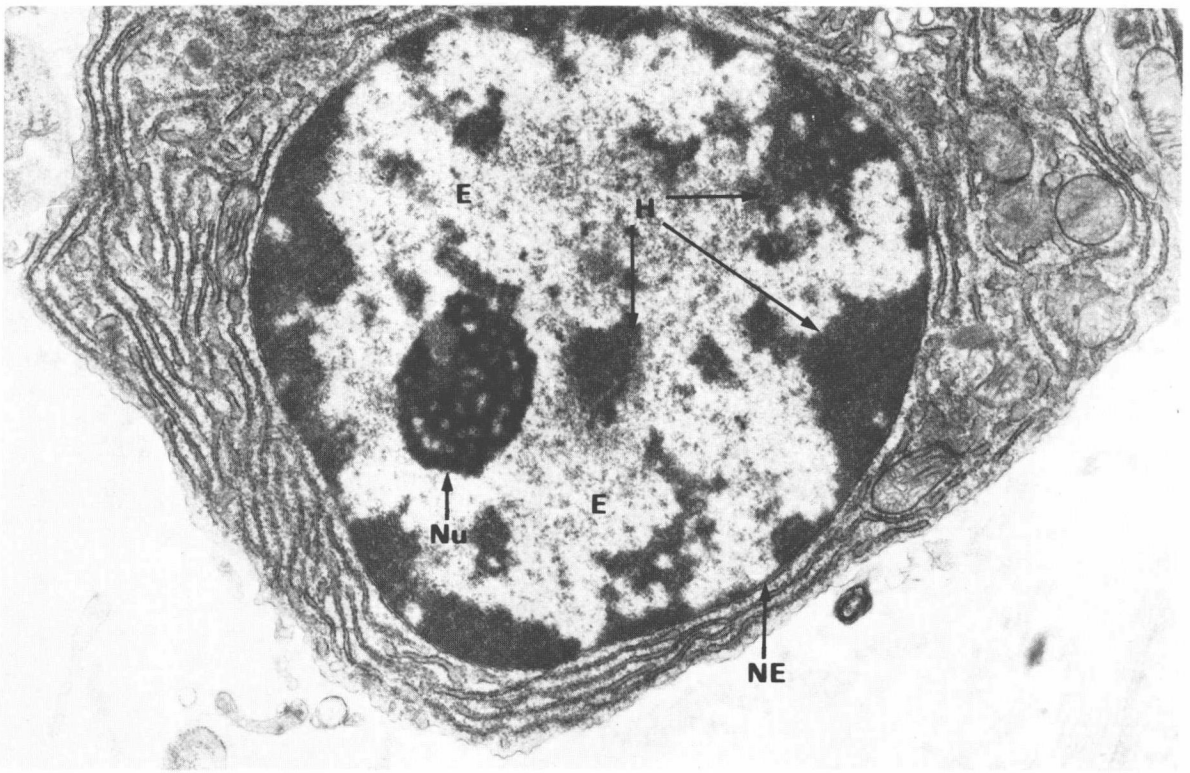


Fig. 1.6 Nucleus

(EM × 15400)

This micrograph illustrates the typical nucleus of a highly active, protein-secreting cell. The nuclear envelope **NE**, separating the nuclear contents from the cytoplasm, is barely visible at this magnification. The nucleus not only contains DNA, which comprises less than twenty per cent of its mass, but also contains a large quantity of protein called *nucleoprotein*, and some RNA. Most of the nucleoprotein is intimately associated with DNA; the remainder consists of enzymes responsible for RNA and DNA synthesis. The nuclear RNA represents newly synthesised messenger, transfer and ribosomal RNA which has not yet passed into the cytoplasm.

Except during cell division, the chromosomes, each comprising a discrete length of the DNA complement, exist as tangled strands which extend throughout the nucleus and cannot be visualised individually by direct electron microscopy. Nuclei appear as heterogeneous structures with electron-dense and electron-lucent areas. The dense areas, called *heterochromatin*, represent that portion of the DNA complement and its associated nucleoprotein which is not active in protein synthesis. Heterochromatin **H** tends to be clumped around the periphery of the nucleus but also forms irregular clumps throughout the nucleus. In females, the quiescent X-chromosome (equivalent to the Y-chromosome of the male) forms a small discrete mass known as a *Barr body*; Barr bodies are seen at the edge of the nucleus in a small proportion of female cells when cut in a favourable plane of section. The electron-lucent nuclear material, called *euchromatin* **E**, represents that part of the DNA which is active in protein synthesis. Collectively, heterochromatin and euchromatin are known as *chromatin*, a name derived from the strongly coloured appearance of nuclei when stained for light microscopy.

Many nuclei, especially those of cells highly active in protein synthesis, contain one or more extremely dense structures called *nucleoli* **Nu** which are the sites of ribosomal RNA synthesis. Each cell type has a characteristic nucleolar morphology. In general, the degree of activity of any cell may be judged by the ultrastructural appearance of its nucleus. Relatively inactive cells have small nuclei in which the chromatin is predominantly in the condensed form (heterochromatin) and in which the nucleolus is small or absent.