Colour Atlas of Histology

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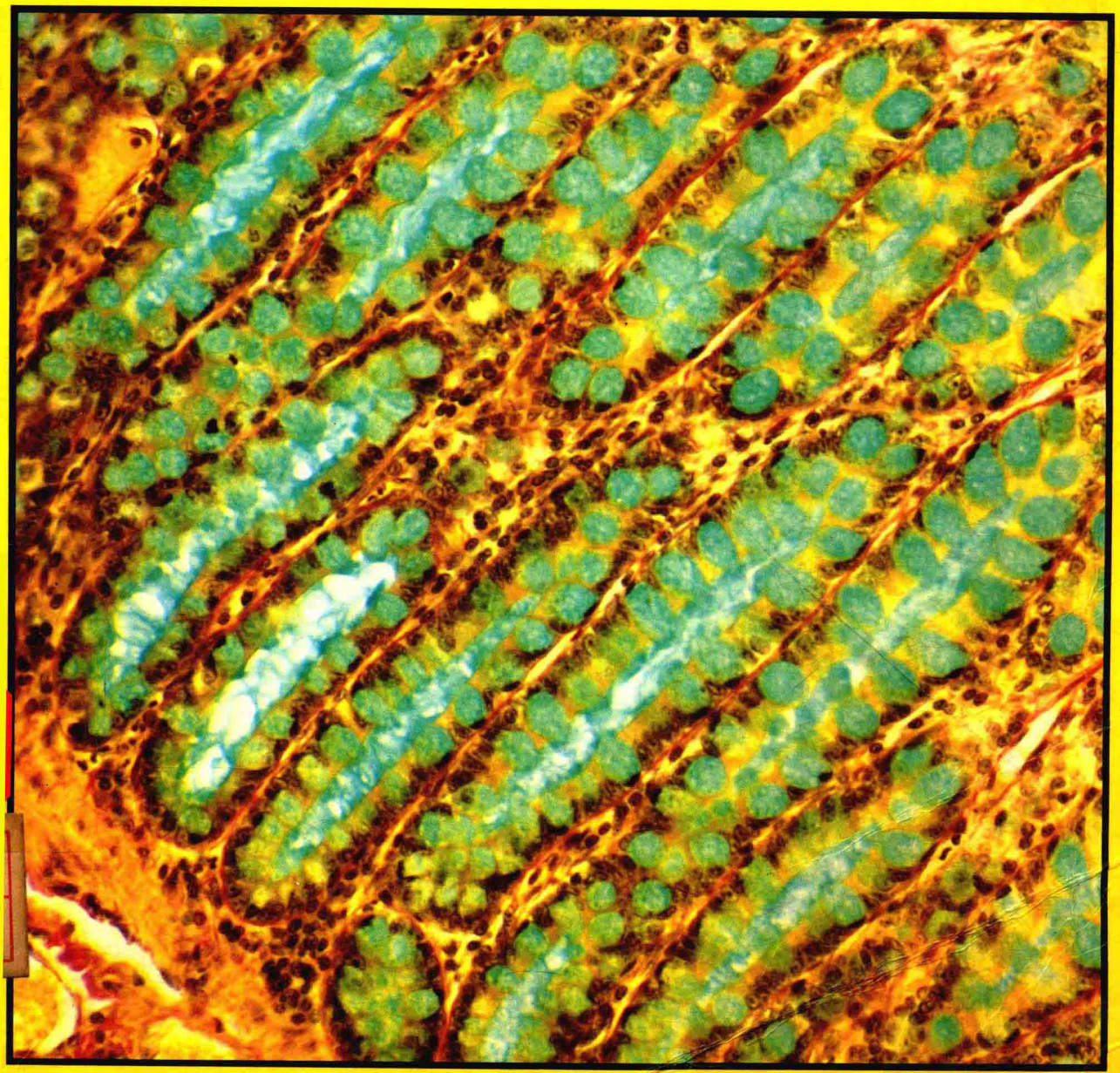

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Cover photomicrograph: Glands of the large intestine
(Alcian blue/Van Gieson stain)

Preface

The motivation for producing this book was derived from the enthusiasm of various teacher colleagues who have read an earlier publication *Functional Histology; a text and colour atlas*, Churchill Livingstone, Edinburgh (1979) of which two of us (P.R.W. and H.G.B.) were the principal authors. *Functional Histology* was aimed at medical and other university students specialising in biology; however, it has also found increasing use as a teaching manual by secondary school teachers. In this respect, *Functional Histology* is unnecessarily detailed and comprehensive for use by school students themselves and this book has, therefore, been prepared especially with their needs in mind.

This book is divided into three sections. The first section deals with the structure and function of the cell; in addition to the micrographs, mainly electron micrographs, the text and expanded captions cover all the major aspects of mammalian cytology required by A-level syllabuses. The second section introduces the basic tissue types, each chapter or new subsection commencing with a short introductory text summarising the main principles. The tissue types are then illustrated by appropriate colour micrographs, two and three dimensional diagrams and electron micrographs. The third section embraces the histology of the various organ systems, adopting a similar approach to that of the second section.

Wherever possible we have tried to avoid unnecessary jargon in the interests of simplicity. Throughout this work we have included ultrastructural details wherever it seemed to further understanding of the tissue structure and function. We make no apologies for covering some topics not yet established in school syllabuses, for example the immune system, in anticipation of their increasing importance in the future.

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Notes on staining techniques

1. Haematoxylin and eosin (H & E)

This is the most commonly used technique in animal histology and routine pathology. The basic dye haematoxylin stains acidic structures a purplish blue. Nuclei and rough endoplasmic reticulum, for example, both have a strong affinity for this dye owing to the high content of DNA and RNA respectively. In contrast, eosin is an acidic dye which stains basic structures red or pink. Most cytoplasmic proteins are basic and hence cytoplasm generally stains pink or pinkish red. In general, when the H & E staining technique is applied to animal cells, nuclei stain blue and cytoplasm stains pink or red.

2. Periodic acid–Schiff reaction (PAS)

Staining techniques which specifically stain components of cells and tissues are called histochemical staining techniques. Such techniques are invaluable for the understanding of cell and tissue structure and function, and for making a diagnosis on diseased tissues. The PAS reaction stains glycogen a deep red colour, traditionally described as magenta. The mucin produced by goblet cells of the gastro-intestinal and respiratory tracts stains magenta with this technique (and is therefore termed PAS positive). Basement membranes and the brush borders of kidney tubules and the small and large intestines are also PAS positive, as is cartilage and to some extent collagen.

3. Masson's trichrome

This technique is a so-called connective tissue technique since it is used to demonstrate connective tissue elements – principally collagen. As its name implies, the staining technique produces three colours; nuclei and other basophilic structures are stained blue, collagen is stained green or blue depending on which variant of the technique is used, and cytoplasm, muscle, erythrocytes and keratin are stained bright red.

4. Alcian blue

Alcian blue is a mucin stain which may be used in conjunction with other staining methods such as H & E or van Gieson (see below). Certain types of mucin, but not all, are stained blue by the Alcian blue method, as is cartilage. When the technique is combined with van Gieson, the Alcian blue colour becomes green.

5. Van Gieson

This is another connective tissue method in which collagen is stained red, nuclei blue and red cells and cytoplasm yellow. When used in combination with an elastic stain, elastin is stained blue/black in addition to the results described above. This staining technique is particularly useful for blood vessels and skin.

6. Reticulin stain

This method demonstrates the reticulin fibres of connective tissue which are stained blue/black by this technique. Nuclei may be counterstained blue with haematoxylin or red with the dye, neutral red.

7. Azan

This technique is traditionally classed as a connective tissue method but is excellent for demonstrating fine cytological detail, especially in epithelium. Nuclei are stained bright red; collagen, basement membrane and mucin are stained blue; muscle and red blood cells are stained orange to red.

8. Giemsa

This technique is a standard method for staining blood cells and other smears of cells. Nuclei are stained dark blue to violet, background cytoplasm pale blue and red cells pale pink.

9. Toluidine blue

This is a basic stain which stains acidic components various shades of blue. It is commonly employed on thin, resin-embedded specimens. Some tissue components are able to turn the blue dye red – a phenomenon known as metachromasia.

10. Silver and gold methods

These methods were extremely popular at the end of the nineteenth century and are occasionally used today to demonstrate such fine structures as cell processes (as in neurones), motor end-plates and intercellular junctions. Depending on the method used, the end product is either black, brown or golden.

11. Chrome alum haematoxylin

This method is rarely used and is similar to the H & E method in principle, in that nuclei are stained blue and cytoplasm is stained red. Empirically this method demonstrates the alpha cells of the pancreas as pink cells and the beta cells as blue.

12. Isamine blue/eosin

This method is also similar to the H & E method but the blue component is rather more intense.

13. Nissl and methylene blue methods

These techniques use a basic dye to stain the rough endoplasmic reticulum found in neurones: when this is seen as clumps it is called Nissl substance.

14. Sudan black and osmium

These dyes stain lipid-containing structures, such as myelin, a brownish-black colour.

Part A Cell structure, function and replication

Cell structure and function

Cell cycle and replication

1. Cell structure and function

Introduction

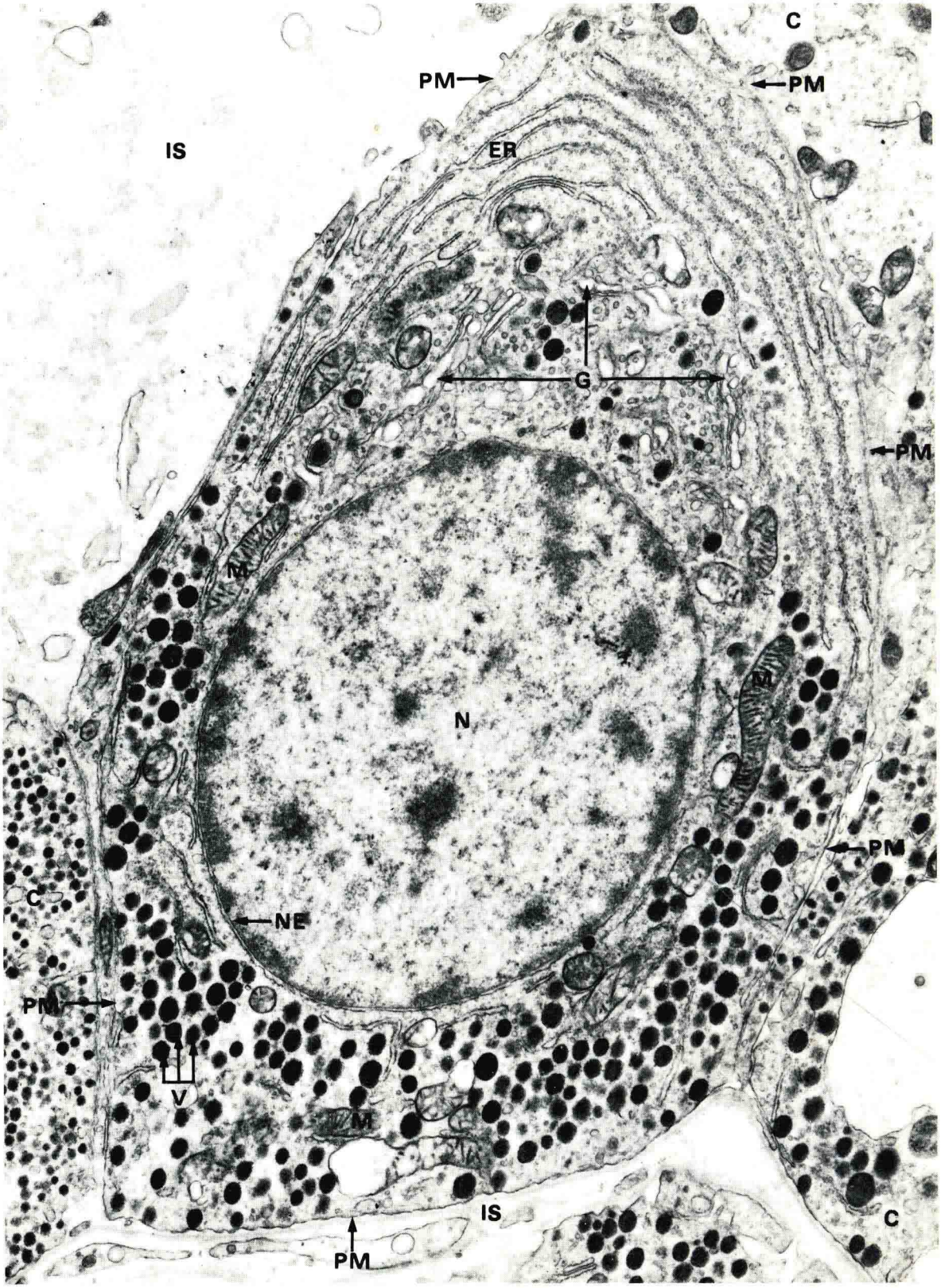
The cell, the functional unit of all tissues, has the capacity to perform individually all the essential life functions. Within the various tissues of the body, the constituent cells exhibit a wide range of specialisations which are, nevertheless, merely amplifications of one or more of the fundamental 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.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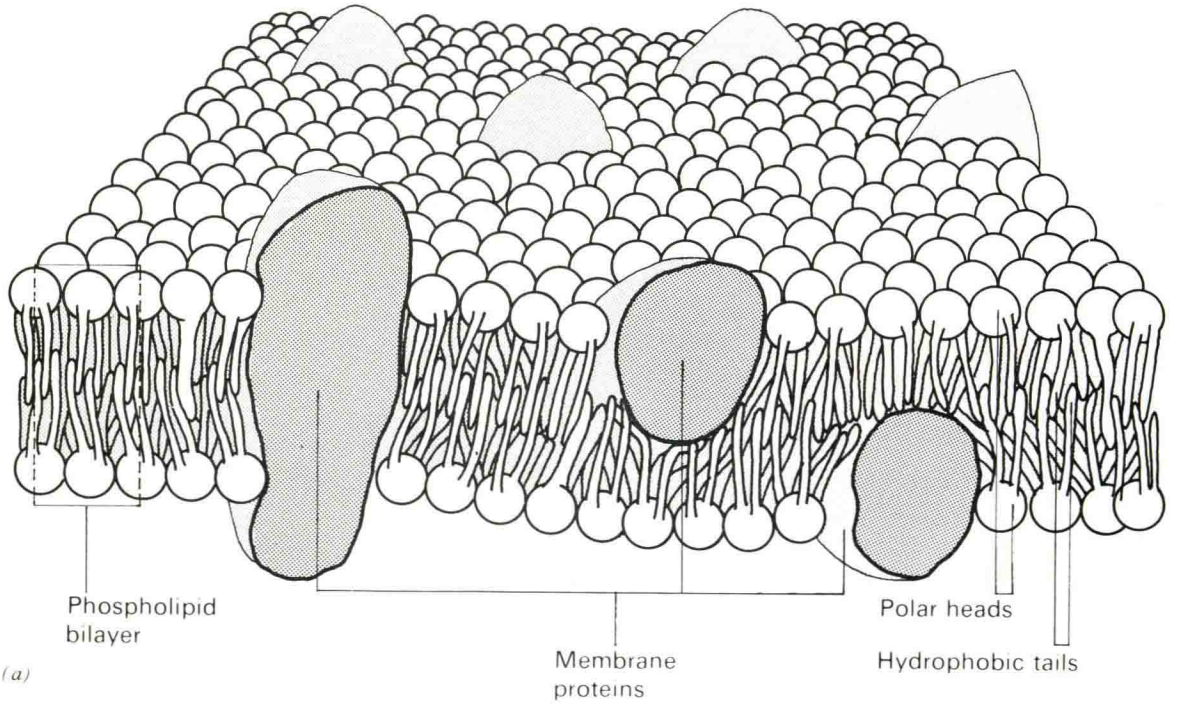
Phospholipid bilayer
Membrane proteins
Polar heads
Hydrophobic tails

(a)


G

(b)

Fig. 1.2 Membrane structure

(a) Schematic diagram (b) EM $\times 330000$

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 (opposite). 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.

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