SYSTEM— ULTRASTRUCTURAL REVIEW

Lecturer in Pathology, University Department of Pathology, Glasgow Western Infirmary

Lecturer in Anatomy, University of Glasgow

Regius Professor of Anatomy, University of Glasgow

BUTTERWORTHS

ENGLAND: BUTTERWORTH & CO. (PUBLISHERS) LTD.
LONDON: 88 Kingsway, WC2B 6AB

AUSTRALIA: BUTTERWORTH & CO. (AUSTRALIA) LTD.
SYDNEY: 586 Pacific Highway, 2067
MELBOURNE: 343 Little Collins Street, 3,000
BRISBANE: 240 Queen Street, 4,000

CANADA: BUTTERWORTH & CO. (CANADA) LTD.
TORONTO: 14 Curity Avenue, 374

NEW ZEALAND: BUTTERWORTH & CO. (NEW ZEALAND) LTD.
WELLINGTON: 26-28 Waring Taylor Street, 1
AUCKLAND: 35 High Street, 1

SOUTH AFRICA: BUTTERWORTH & CO. (SOUTH AFRICA) (PTY.) LTD.
DURBAN: 152-154 Gale Street

Suggested UDC Number: 611·3–018

ISBN 0 407 14850 7

Printed in Great Britain by Page Bros. (Norwich) Ltd.

THE DIGESTIVE SYSTEM— AN ULTRASTRUCTURAL ATLAS AND REVIEW

THE DIGESTIVE
AN
ATLAS AND

P. G. TONER

K. E. CARR

G. M. WYBURN

LONDON

Preface

This guide to the fine structure of the digestive system has two components, the text and the illustrations. The text describes the salient features of fine structure and directs the reader to a selection of recent literature. The micrographs have extended captions which draw attention not only to structures related to gastroenterology but also to points of general ultrastructural interest.

A work of this type necessarily reflects the authors' personal interests, imparting an element of bias to the presentation of the subject, but we hope that most specialists in gastroenterology will find in it something of value. We believe that it may also provide useful background knowledge for those with only limited access to 'live' electron microscopy, since it contains elements extending beyond the confines of gastroenterology to the more general field of cellular structure and function.

P.G.T.
K.E.C.
G.M.W.

Acknowledgements

Many of our colleagues have helped us by making available material utilized in the production of this book. These include Professor A. W. Kay and his staff in the University Department of Surgery, Western Infirmary, Mr. John Hutchison, Royal Infirmary and Dr. Anne Ferguson, formerly of the University Department of Medicine, Royal Infirmary and now of the University Department of Bacteriology and Immunology, Western Infirmary. Professor R. M. Kenedi kindly provided research facilities in the scanning electron microscope section of the Bio-Engineering research unit, University of Strathclyde. Mr. H. S. Johnston is responsible for a number of micrographs of human gastric biopsies and Dr. R. F. Macadam provided blocks of a carcinoid tumour and Dr. J. Shaw Dunn made other material available. Without their willing cooperation the book would have been incomplete.

It is a particular pleasure to acknowledge the technical assistance provided with a rare combination of helpfulness and efficiency by Mr. D. McSeveney and his staff, in the Electron Microscope Unit of the Department of Pathology. The heavy task of photographic work was undertaken by Mr. S. McCreanor and Miss Margaret Hughes, whose whole concern was to obtain the optimum result from any negative, however troublesome to print.

The work, the results of which are included in this book, was made possible by financial support received at various times from the Medical Research Council, Science Research Council, the Wellcome Foundation and the research fund of Glasgow University, for the purchase of equipment necessary for electron microscopy.

We are grateful to the editors of the journals *Gut*, *Journal of Pathology and Bacteriology*, *Journal of Ultrastructure Research* and *Journal of Anatomy* and to the publishers E. and S. Livingstone, for permission to use micrographs which originally appeared in their publications. Acknowledgement is hereby made to the following sources:

Figures 2.4b and *2.6b:* from Toner, P. G., Carr, K. E., Ferguson, A. and Mackay, C. (1970). 'Scanning and Transmission Electron Microscopic Studies of Human and Intestinal Mucosa.' *Gut* **11**, 471–481

Acknowledgements

Figures 2.2, 2.3b, 2,4a and *2.5*: from Toner, P. G. and Carr, K. E. (1969). 'The Use of Scanning Electron Microscopy in the Study of the Intestinal Villi.' *J. Path. Bact.* **97**, 611–617

Figures 3.30, 3.31a, 3.33, 3.34, 3.36a, 3.37a, 3.38, 3.39, 3.40, 3.43 and *3.44*: from Toner, P. G. and Ferguson, A. (1971). 'Intraepithelial Cells in the Human Intestinal Mucosa.' *J. Ultrastruct. Res.* **34**, 329–344

Figure 3.46a: from Carr, K. E. (1967). 'Fine Structure of Crystalline Inclusions in the Globule Leukocyte of the Mouse Intestine.' *J. Anat.* (*Lond.*) **101**, 793–803

Figures 1.14, 1.17a, 1.22, 1.25b, 2.10, 2.12b, 2.13, 2.15b, 2.24, 4.9, 4.13, 4.16, 4.18a, 4.24a, 4.25, 4.32 and *5.11*: from Toner, P. G. and Carr, K. E. (1968). *Cell Structure: An Introduction to Biological Electron Microscopy*. London and Edinburgh; E. and S. Livingstone

Contents

CHAPTER 1

Oesophagus and Stomach

Ultrastructural studies of the gastrointestinal tract have shown a tendency to concentrate on areas of current physiological interest, such as the stomach with its secretory function and the small intestine with its absorptive capacity. As a result the epithelium of the oesophagus has been neglected to some extent by the electron microscopist despite fine structural features worthy of consideration. It is true that the human oesophagus does little more towards digestion than conduct food from the mouth and pharynx to the stomach. The mucosa, however, must be resistant to physical trauma including heat, cold, abrasion and chemical injury from the components of food and drink, and must remain largely impermeable to the fluid environment to which it is exposed. In many species the stratified squamous oesophageal epithelium meets these requirements by keratinization, a specialization of an essentially protective nature. Keratinization is related to the character of the diet. In those species with a coarse diet consisting largely of vegetable material, the laboratory rodents for example, the oesophagus becomes thickened and keratinized after birth, while a thinner and less obviously keratinized layer is found in animals with a softer diet. In man the cells of the superficial layer of the oesophagus retain their nuclei and there is no evidence of keratinization. The rat or mouse oesophagus, which illustrates parts of this section, undergoes full keratinization, the successive layers of cells representing different stages in this continuous process of maturation, which can be seen as a protective adaptation to prevailing conditions. The buccal mucosa, which largely shares the oesophageal environment, displays fine structural features of a similar type (Listgarten, 1964; Hashimoto, Di Bella and Shklar, 1966; Parakkal, 1967; Susi, 1969).

The cells of the basal layers of the epithelium (*Figure 1.1*) are small, rounded and relatively compact, with a large nucleus which fills much of the cell and is often indented. Occasional mitotic figures (*Figure 1.2*) are encountered in the basal layer, the chromosomes appearing as usual as irregular chromatin masses without a limiting membrane. The cell base rests on a continuous and apparently homogeneous basal lamina, about 50 nm in thickness, which closely follows the contours of the cell membrane, but is separated from it by a narrow pale interspace. The basal lamina has no clearly defined substructure other than a suggestion of a filamentous background which may represent a precipitation artefact induced by fixation. In some situations, such as the human gingival epithelium, delicate cytoplasmic pegs extend downwards from the cell, forming a serrated basal profile which

is followed closely by the basal lamina. This pattern might contribute to the mechanical strength of the mucosa in a site particularly liable to trauma.

The lateral and the distal surfaces of the cells in the deep layers of the oesophageal epithelium come into contact with the corresponding faces of adjacent cells and are linked to them by frequent adhesion specializations. The apposed cell membranes have elaborate interdigitating folds and projections which may serve as a mechanical linkage between cells. There is the normal interspace of between 10 and 20 nm separating the cell surfaces at most points with areas of wider separation perhaps attributable in part to fixation contraction (*Figure 1.3*). At high resolution the cell membrane shows the recognized trilaminar fine structure common to most biological membranes. Numerous desmosomes link adjacent cells, their number, size and prominence emphasizing the importance of cellular adhesion in determining the robust mechanical nature of this epithelium (*Figures 1.3*, *1.4a* and *b*, *1.5b*).

Figure 1.1. Rat buccal epithelium, rat tongue, magnification: 3,600. This micrograph shows an oblique plane of section through the deeper layers of the buccal epithelium. The more basal cells, with a high nucleocytoplasmic ratio, lie at the upper and lower margins of the field, the middle zone being occupied by slightly more superficial cells. The compact, slightly crenated nuclei contain quite prominent nucleoli. The cells are separated by a distinct intercellular space which is crossed by tongues of cytoplasm which interdigitate with one another. In many cases these processes are linked by large prominent desmosomes, their attached filaments staining densely in this preparation. The desmosomes, the filaments, the processes and the intercellular space contribute together to the familiar light microscopical picture of the 'prickles' or 'intercellular bridges' of squamous epithelial cells. In the lower left hand corner of the field a small oval cell (L) lies between the epithelial cells; this is probably an intra-epithelial lymphocyte (*see* page 166)

Figure 1.2. Rat buccal epithelium, rat tongue, magnification: 15,000. In this view of the basal layer of the epithelium the nuclei (N) of two basal cells are in contrast with the mitotic figure which occupies the centre of the field. The chromosomes (C) appear in section as irregular and rather poorly delimited dense granular masses. A few of the microtubules which make up the mitotic spindle can just be distinguished, but the centrioles lie outside the plane of section, which probably passes through only one of the chromosome masses. The cell is still linked to its neighbours by desmosomes and the tonofibrils are scattered around the periphery, along with the mitochondria, many of which show some swelling artefacts (M). Several groups of characteristic membranes are found in the cytoplasm (arrow). These may give rise to the nuclear envelope which must re-form at telophase. A basal lamina (B) underlies the epithelium, separating the cells from the collagenous connective tissue below

Figure 1.3. Rat buccal epithelium, rat tongue, magnification: 12,000. These cells are situated just above the most basal layer of the epithelium. Their cytoplasm is a little more bulky than that of the basal cells. The intercellular spaces (S) are prominent. They are crossed by cytoplasmic processes, some of which are joined by desmosomes, details of which are not clearly seen at this magnification. The position of each desmosome is, however, marked by the presence of bundles of densely stained cytoplasmic filaments (F) which appear to run from cell to cell, but which are actually inserted into the cytoplasmic sides of the areas of membrane specialization which constitute the desmosome. These filaments apparently connect with the network of tonofibrils which form coarse bundles elsewhere in the cytoplasm. The background of the cytoplasm contains numerous groups of ribosomes and a moderate number of mitochondria (M)

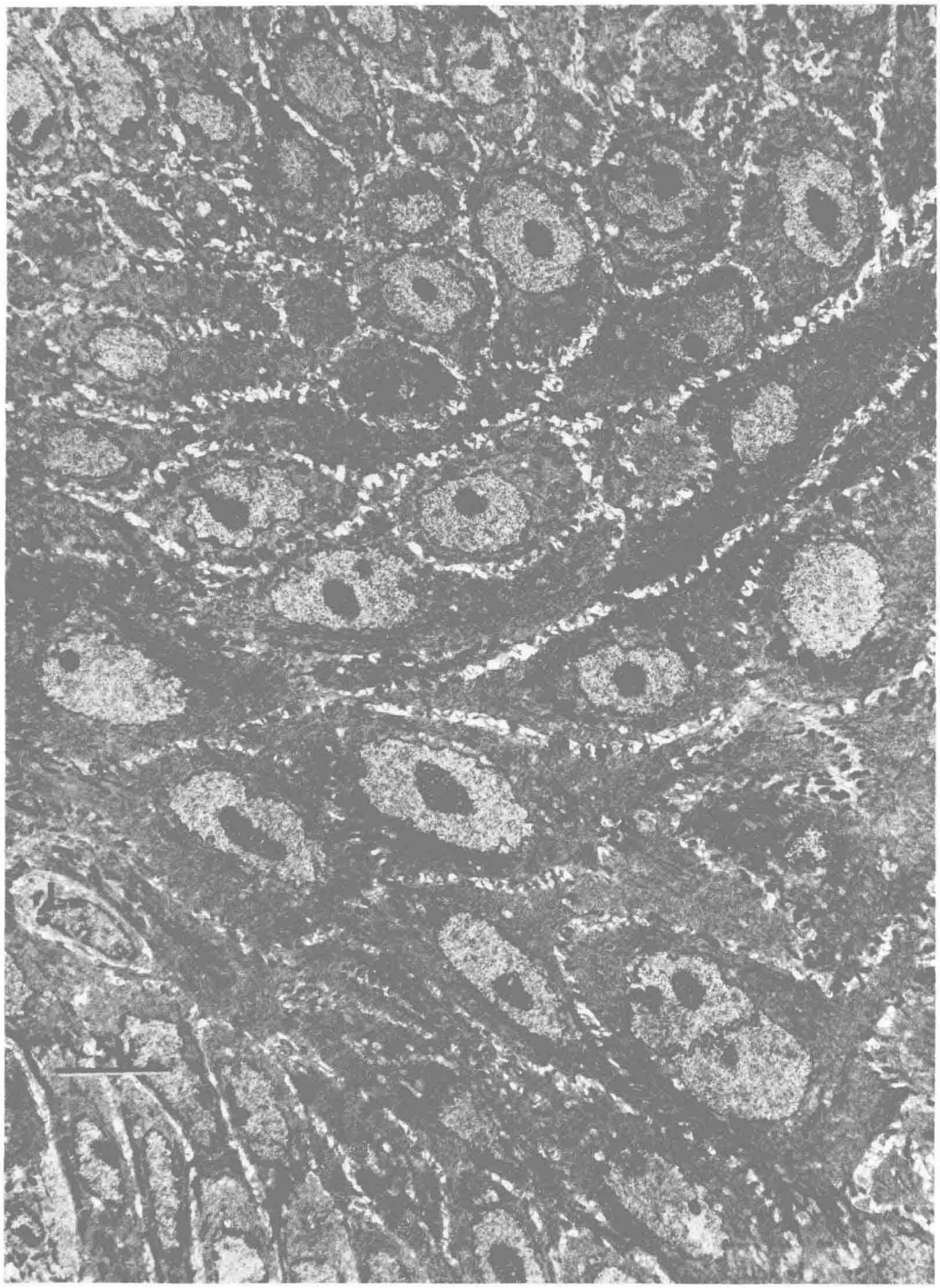

Figure 1.1.

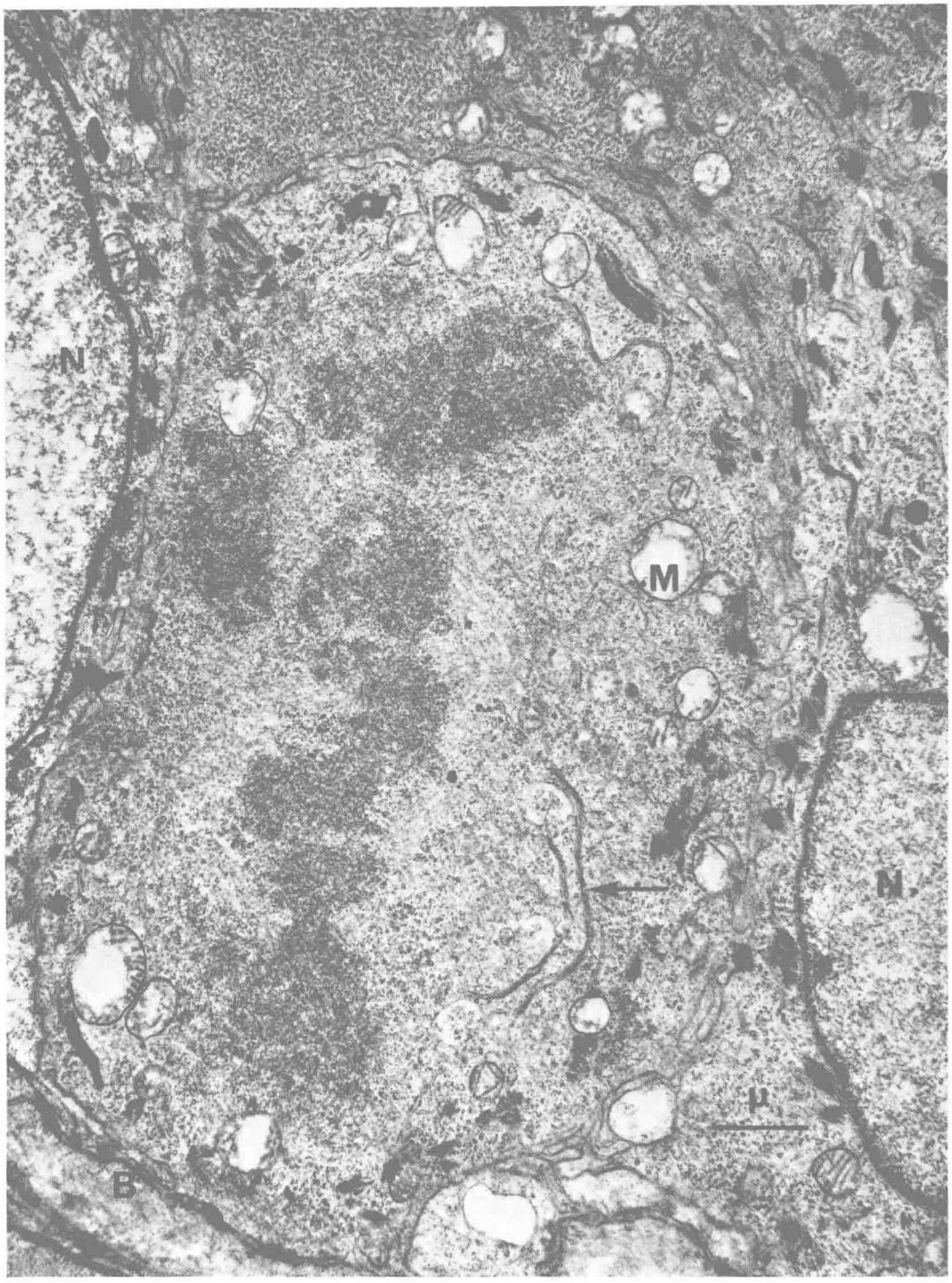

Figure 1.2.

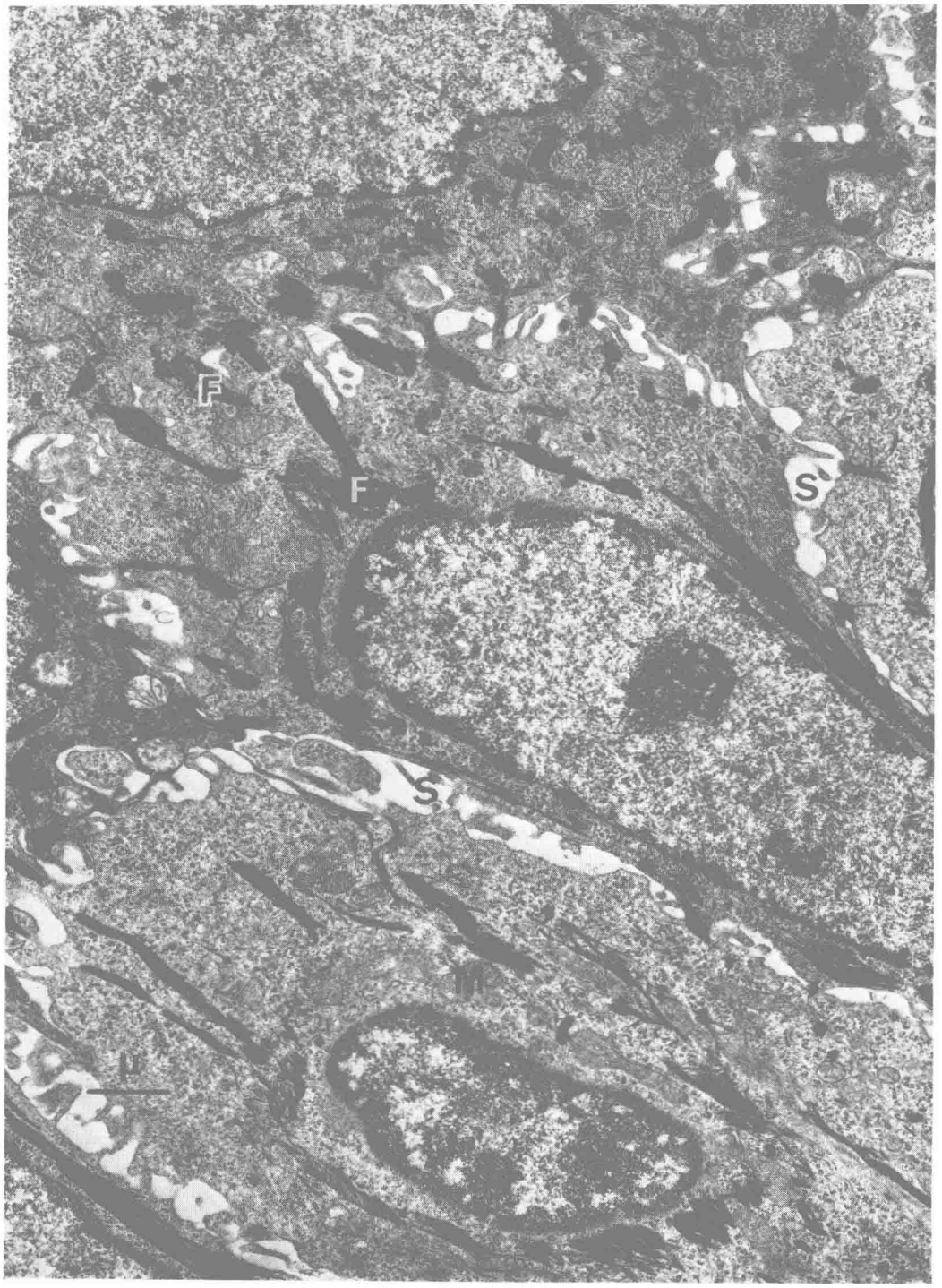

Figure 1.3.

These plaque-like adhesion points are highly specialized, with a central linear aggregate of intercellular dense material flanked by matching membrane specializations of the cells involved (*Figure 1.4b*). The thin dense central linear component of the desmosome, the intercellular contact layer, bisects the intercellular space and may represent a localized condensation of the external coat or glycocalyx, normally unstained in conventional preparations. This layer may act as intercellular 'cement' material essential to the mechanical properties of the desmosome. In the two identical flanking zones the trilaminar cell membrane structure is reinforced by cytoplasmic aggregates of dense material. At high resolution both dense laminae are slightly thickened, giving the sectioned desmosome a characteristic laminated appearance. A linear thickening applied to the inner aspect of the membrane serves as an attachment for the numerous cytoplasmic filaments which converge on the desmosome from different directions (*Figures 1.3, 1.4, 1.5b*).

At the desmosome the normally undulating contours of the contact surfaces are straight, suggesting that the presence of these specializations endows the cell membrane with an unaccustomed rigidity. The cytoplasmic filaments which are inserted into the desmosome form, in aggregate, dense cytoplasmic bundles (*Figures 1.1, 1.2, 1.3*), which represent the tonofibrils recognized by light microscopy. Their sub-units, the tonofilaments, may represent an essentially continuous interconnecting network extending widely through the cytoplasm (*Figures 1.4, 1.6a*) and forming in effect a system of 'guy ropes' anchoring the cells to their adhesion points. These desmosomes with their attached filaments are the 'intercellular bridges' of light microscopy, the ultrastructural basis for which can well be seen in low magnification micrographs (*Figure 1.1*). There is, however, no cytoplasmic continuity at the desmosome, nor do tonofilaments cross the cell membrane or the intercellular gap at these points. The 'bridges', in other words, are adhesion specializations which do not involve any form of cytoplasmic continuity. It must be remembered that the typical laminated pattern of the desmosome is only seen when the membranes lie vertical to the plane of section, an obliquely sectioned desmosome presenting a blurred and confusing image recognizable mainly by the persisting pattern of converging filaments on either side (*Figures 1.4a, 1.5b*). The disc-like nature of the desmosome becomes obvious in this plane of section. The basal aspect of the basal cell, in contact with the basal lamina, has no true desmosomes, since these are essentially paired structures. There are, however, half desmosomes with inserted tonofilaments of the normal type

---

Figure 1.4a. Rat buccal epithelium, desmosomes. Rat tongue, magnification: 40,000. The plane of section in this case has cut across an area of complex interdigitation of cell membranes, with numerous desmosomes. The filaments associated with the desmosomes are in many places cross-sectioned, appearing as dense dots (arrows). The adjacent cytoplasm shows bundles of filaments in longitudinal section with numerous scattered ribosomes (R). A number of small, ill-defined granules lie in the peripheral cytoplasm (G). These are the membrane-coating granules, mostly cut in cross-section in this case

Figure 1.4b. Desmosomes, rat oesophagus, magnification: 65,000. Higher magnification micrograph showing several desmosomes between adjacent epithelial cells. Their laminated structure is clearly seen except at one point where the desmosome turns obliquely to the plane of section (D). For the same reason the boundary between cells is obscure at various points (arrows). In addition to the numerous cytoplasmic filaments (F), some cut in cross-section, there are several membrane-coating granules, their laminated pattern being just distinguished (G)

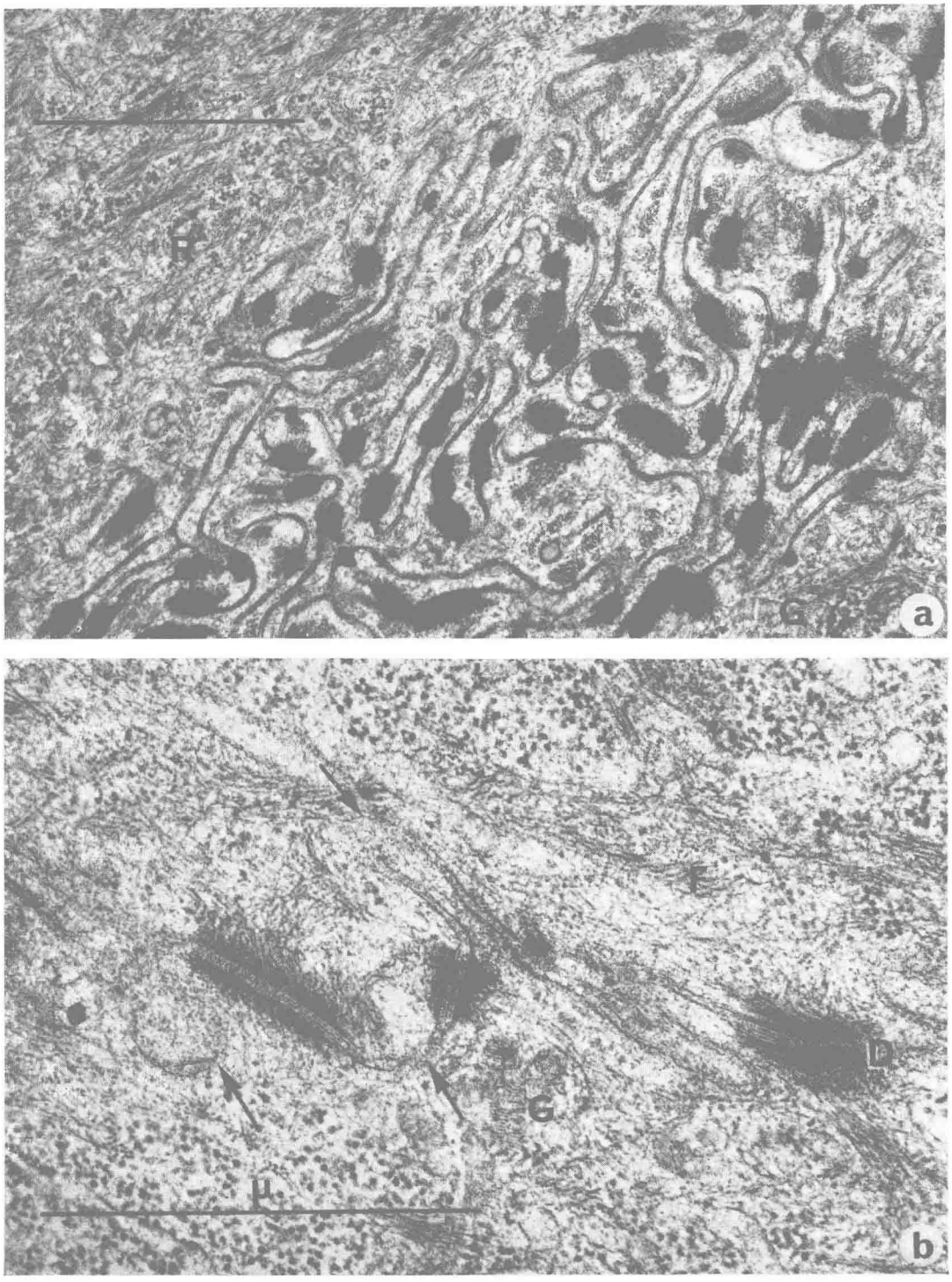

Figure 1.4.

and membrane specializations equivalent to one cell's contribution to the desmosome structure (*Figure 1.5a*). External to the cytoplasmic specializations of the half desmosome, there is usually a small plaque of extracellular material representing the 'cement' layer of the conventional desmosome.

The cytoplasm of the basal cell is relatively unspecialized (*Figure 1.2*). Ribosomes are present in moderate numbers, mostly lying free in the cytoplasm rather than attached to membranes of the endoplasmic reticulum. Membrane systems are in general sparse; the typical Golgi apparatus is of small size and the endoplasmic reticulum is represented by scattered cisternae and vesicles. Mitochondria, round or oval in profile and poorly organized, are relatively few in number. At various points in the cell there are densely staining bundles of cytoplasmic filaments similar to those attached to the desmosomes (*Figures 1.2, 1.3*). While their three dimensional relationships are to some extent uncertain, it seems likely that these filament bundles represent an interconnected network, the prominence of which may be related to the mechanical strength of the epithelium. The tonofilaments take up a peripheral distribution in mitosis (*Figure 1.2*). The lack of evidence of cytoplasmic differentiation and the occasional mitosis in the basal layer are characteristic of cells in a germinal layer, the source of replacements for shed cells in an epithelium.

As the cells of the epithelium migrate, in the normal course of renewal, towards the lumen of the oesophagus where they are finally shed, their maturation is accompanied by progressive changes in fine structure. The cells of the middle layers are larger but more flattened and their nuclei are elongated. Cytoplasmic volume increases out of proportion to nuclear size. Contact relationships are relatively unchanged and numerous desmosomes are found, as well as quite complex interlocking cytoplasmic pegs (*Figures 1.4a, 1.5b, 1.6b*). The Golgi apparatus is larger than in the basal cells and seems to be taking part in the elaboration of small cytoplasmic granules

---

Figure 1.5a. Oesophageal epithelium, rat oesophagus, magnification: 47,000. This is the basal aspect of the oesophageal epithelium, the cells being separated from the underlying connective tissue by the basal lamina (B). Parts of two basal cells lie in close contact, with a desmosome (D) linking their surfaces. Along the basal surface half desmosome specializations (X) are clearly seen, while at one point (arrow) a micropinocytotic vesicle can be made out. The cytoplasm contains ribosomes and bundles of fibrils (F) cut both in longitudinal and in transverse section. A few mitochondria are present and the small segment of the nucleus of one cell has a well-defined nuclear pore (P) interrupting the nuclear envelope. Note the dense cytoplasmic aggregate on the cytoplasmic side of desmosome and hemidesmosome specializations and the external lamination which characterizes the latter

Figure 1.5b. Rat buccal epithelium, rat tongue, magnification: 32,000. This field contains parts of several cells from the granular layer. The cytoplasmic filaments are rather more scattered than in the deeper layers illustrated in the previous pictures and the cytoplasm is distinguished by the presence of large dense keratohyalin granules (K), which may also on occasion lie within the nucleus. No internal structure can be made out in these granules. The cytoplasm contains numerous ribosomes. The details of the desmosomes are only clearly made out when the plane of section is vertical to the plane of the membranes. One desmosome (D 1) shows the laminated structure quite clearly, but most of the others (D 2) are sectioned so obliquely that no internal structure can be seen. The filaments inserted into the desmosome appear in the latter case as a diffuse dense plaque. At various points between the desmosomes the obliquity of the membranes of adjacent cells leads to a false impression of cytoplasmic continuity (arrows). The cells are, however, entirely separate