J. Anat. (1970), **106**, 1, pp. 1–14
With 11 *figures*
Printed in Great Britain

Columnar cells with secretory granules in the large intestine of the macaque (*Cynamolgus irus*)

G. C. SCHOFIELD

Department of Anatomy, Monash University, Clayton, Victoria, Australia

(*Received* 31 *December* 1968)

INTRODUCTION

The categories of secretory cells present in the epithelium of the stomach and small intestine and their ultrastructure have been comprehensively reviewed by Ito (1967), Trier (1968) and Moe (1968). Undifferentiated cells which are assumed to represent a reserve of cells have also been described; in the gastric mucosa they are located mainly at the neck of the glands and possibly represent precursors of either the parietal or mucous neck cells (Johnson & Young, 1968); in the small intestine they are the most common cell type present and are confined to the intestinal glands (Trier, 1968).

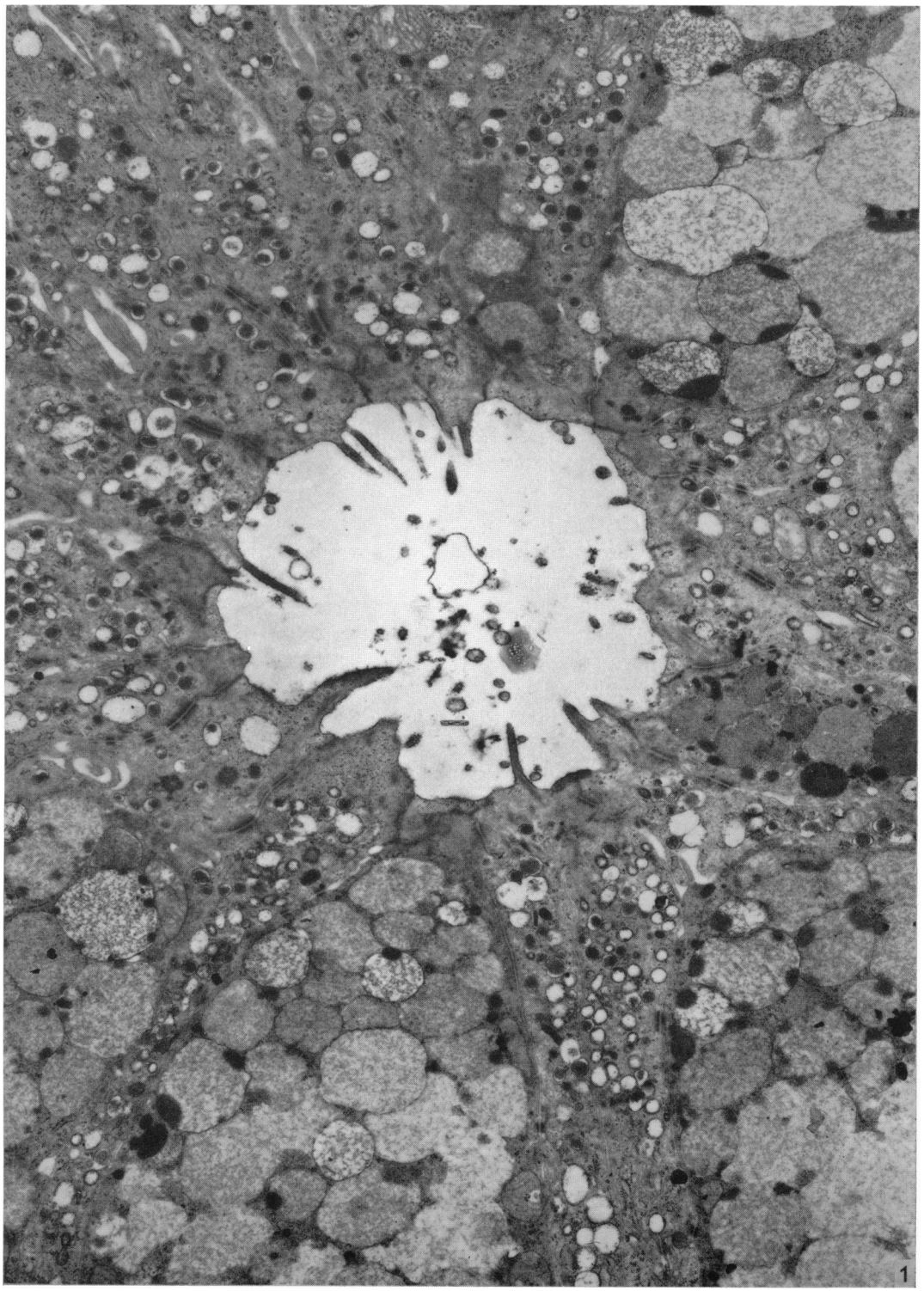
There are several accounts of the ultrastructure of the epithelium of the large intestine including studies on principal or columnar cells and goblet cells (Florey, 1960; Shearman & Muir, 1960; Hollmann, 1965; Freeman, 1966; Wetzel, Wetzel & Spicer, 1966), enterochromaffin cells (Schofield & Silva, 1968) and other cells classed as globule leukocytes, crystal-containing cells, undifferentiated cells and multivesicular cells (Silva, 1966; Carr & Whur, 1968; Lorenzsonn & Trier, 1968). The present study on the large intestine of the macaque (*Cynamolgus irus*) is concerned with the structure of columnar cells which present many of the features of the undifferentiated cells described in the stomach and small intestine of other species. They are also involved in secretory activities which are apparently unrelated to those of goblet cells and other epithelial cells in the large intestine.

MATERIAL AND METHODS

Pieces of the colon, rectum and anal canal were removed under ether anaesthesia from 11 adult macaque monkeys. Small blocks of tissue were fixed in 2% phosphate-buffered osmium tetroxide (Millonig, 1962) or in 4% phosphate-buffered glutaraldehyde and post-fixed in osmium tetroxide (Sabatini, Bensch & Barrnett, 1963). Histochemical reactions for acid phosphatase (Barka, 1963) were carried out in tissues fixed in 10% formalin or 4% glutaraldehyde.

Tissues fixed in a variety of solutions for light microscopy were also taken. They were either frozen-sectioned or dehydrated in graded alcohols, cleared in toluol and sectioned after wax impregnation. The preparations were subjected to the various histochemical procedures outlined in Table 1.

Preparations for electron microscopy were dehydrated in graded acetone solutions and embedded in Araldite. Sections were stained with uranyl acetate (Watson, 1958)


1

and lead citrate (Reynolds, 1963), and were examined in a Siemens Elmiskop IA at 80 kv. Thick sections of Araldite-embedded material were also examined with the light microscope after staining procedures described for paraffin-embedded specimens.

OBSERVATIONS

Cells of the epithelium

Distinct cell types were identified in the epithelium of the colon, rectum and glandular part of the anal canal and classified on the basis of their form, position and histochemical reactions. Goblet cells and columnar cells extended throughout the thickness of the epithelium but enterochromaffin cells, and occasionally also mast cells, were found mainly in the basal part of the epithelium. Other cells, including lymphocytes and eosinophil and neutrophil leukocytes, were also identified in the epithelium. The fine structure of the cell types referred to resembled that described in other species except for the columnar cells, which were surprisingly varied in appearance; almost all of the columnar cells in the intestinal glands contained granular or vesicular inclusions (Fig. 1). The histochemical reactions and fine structure of the columnar cells were studied in more detail.

Histochemical reactions of columnar cells

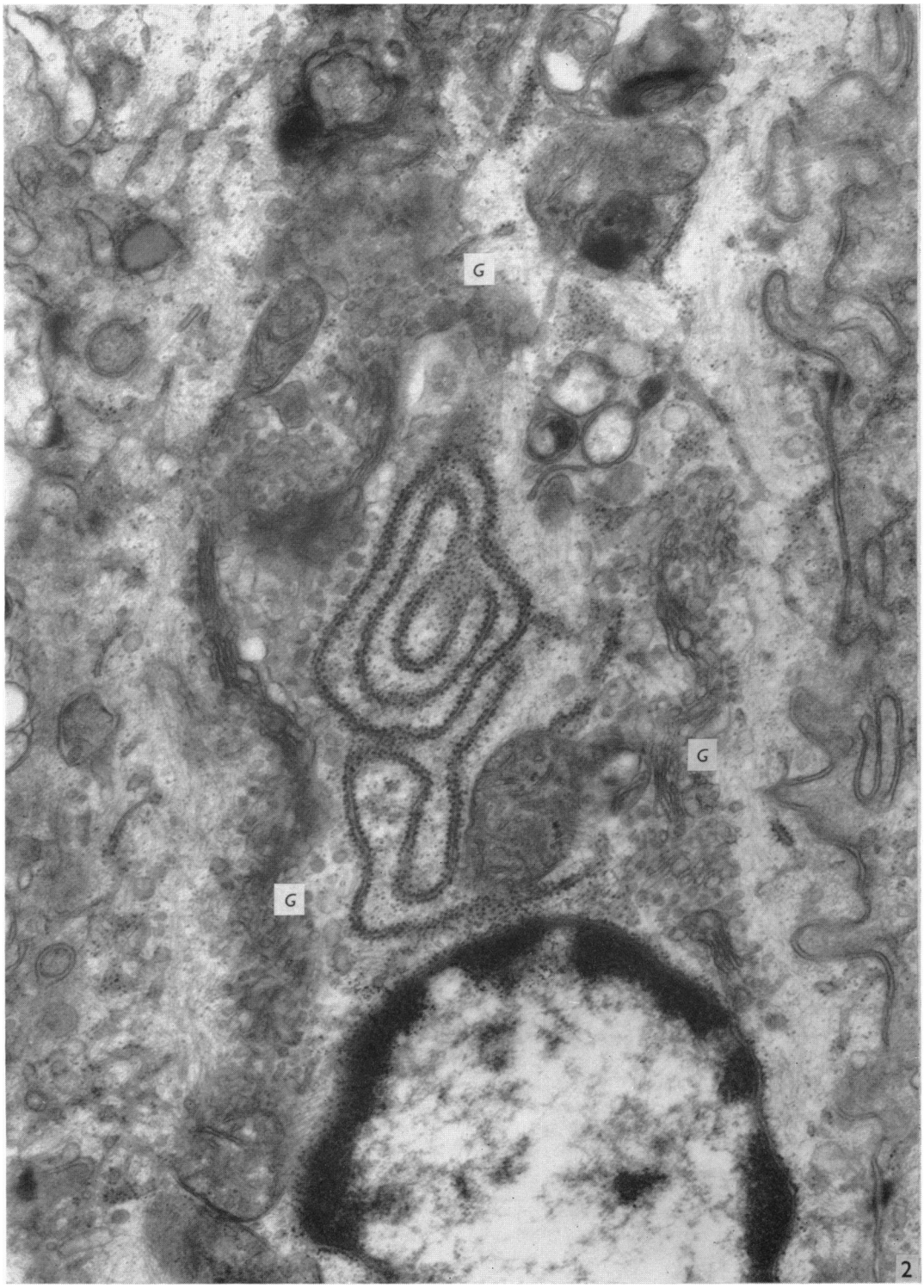
In paraffin or frozen sections the columnar cells were aggregated most prominently at the fundus of the intestinal glands and at the lumenal surface of the large intestine. In thinner (1 μm) sections of Araldite-embedded specimens, the columnar cells also occupied the body and neck of the intestinal glands, where they formed slender profiles between the more prominent goblet cells; the cytoplasm was apparently homogeneous in most of the columnar cells but some contained fine, clear supranuclear vacuoles in toluidine blue-stained preparations. The various histochemical procedures used and the reactions of the cytoplasm of columnar and other epithelial cells in cryostat or paraffin-embedded preparations of the large intestine are included in Table 1. These studies suggested that the columnar cells contained protein but not carbohydrates, lipids or fluorescent amines in amounts detectable with routine histochemical procedures.

Fine structure of columnar cells

The columnar cells were cylindrical in shape; they extended throughout the thickness of the epithelium and presented microvilli which varied appreciably in number and appearance in different areas. The columnar cells were the predominant cell type at the surface of the mucous membrane and at the fundus of the intestinal glands and elsewhere in the intestinal glands were at least as numerous as goblet cells.

Adjacent columnar cells were linked by junctional complexes (maculae and zonulae adherentes and zonulae occludentes), some apparently continuous with fibrillar elements within the cytoplasm (Figs. 4, 6). Interdigitations which sometimes were

Fig. 1. An electron micrograph of the cells bordering the lumen of the fundus of an intestinal gland of the colon. Portions of six goblet cells, none of which here reach the lumen, can be seen. The remaining columnar cells contain inclusions ranging from dense granules to vesicles with lightly stained contents; the few microvilli present are elongated and some are broadly based. $\times$ 11 500.


G
G
G
2

quite complex occurred between the basal aspects of adjacent cells and less frequently nearer their free border. The nuclei were basally placed and generally ovoid in shape, and the nuclear envelope was frequently infolded with ribosomes on its outer surface. Granular endoplasmic reticulum was prominent in some cells, especially those containing dense spherical inclusions, but was generally less abundant than in goblet cells; agranular reticulum was found mainly in complex and extensive Golgi areas (Fig. 2). Free ribosomes and mitochondria were distributed throughout the cytoplasm and were also often aggregated in the apical part of those cells which contained dense spherical inclusions in the submicrovillous zone.

Table 1. *Reactions of epithelial cells in the monkey colon*

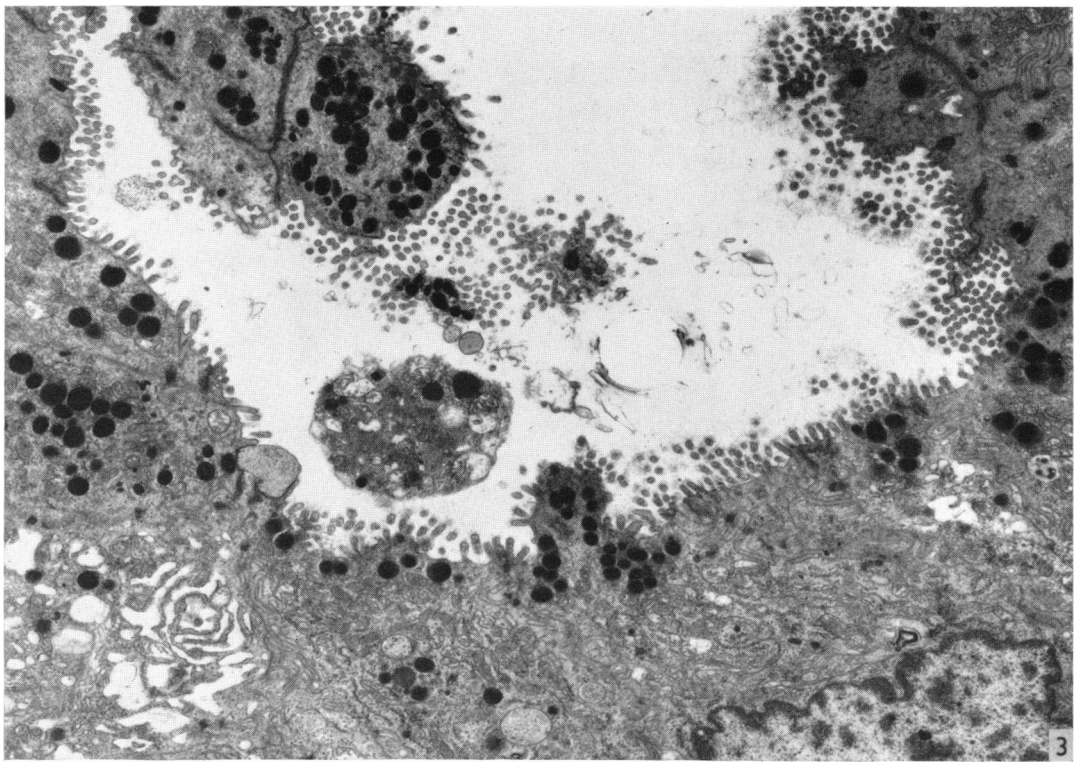
Methods	GC	CG	CS	EC
Periodic acid-Schiff (P)	+++	0	0	.
Mucihaematin (C)	+++	0	0	.
Mucicarmine (C)	+++	0	0	.
Alcian blue, pH 2 (P)	+++	0	0	.
Bismark brown (T)	+++	0	0	.
Potassium permanganate/aldehyde fuchsin (T)	+++	+	+	.
Acid solochrome cyanine (P)	0	++	+++	.
Sakaguchi (G)	0	+	+	.
Coupled tetrazonium (G)	+	+++	+++	.
DNFB/H-acid (T)	+	+++	+++	.
Performic acid-Schiff (P)	0	+	+	.
Ninhydrin-Schiff (T)	0	+	+	.
Ferric ferricyanide (P)	+	0	0	.
Activated protargol (S)	0	0	0	+++
Hexamine silver (S)	0	0	0	+++
Fluorescence (S)	0	0	0	+++
Sudan black (P)	0	0	0	0

Methods used were according to Culling, 1957 (C); Gurr, 1960 (G); Pearse, 1961 (P); Schofield *et al.* (1967) (S); and Thompson, 1966 (T). The reactions of the cytoplasm of goblet cells (GC), columnar cells of the glandular (CG) and surface epithelium (CS), and enterochromaffin cells (EC) are classed as negative (0), weak (+), moderate (++) and strong (+++).

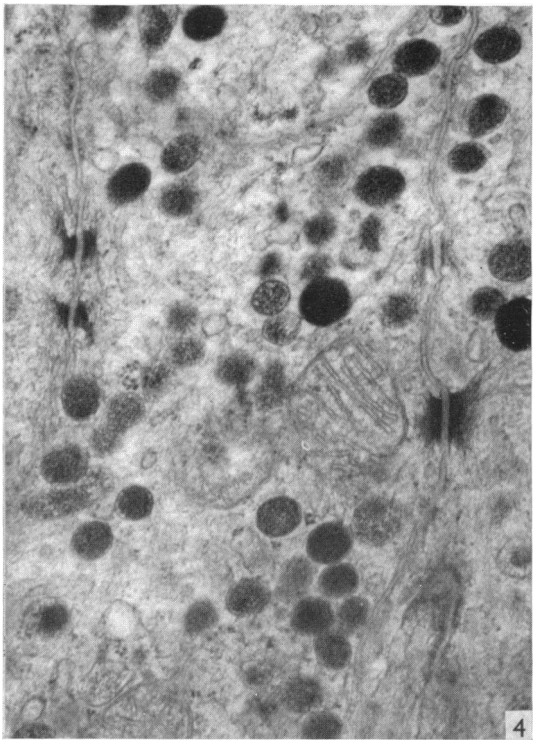
Supranuclear inclusions, which ranged from dense spherical granules (Figs. 1, 4, 6) to irregularly shaped vesicles (Figs. 1, 5, 6), were the most prominent feature of the columnar cells in the intestinal glands and, in some few cells, vesicles were seen apparently discharging contents into the lumen of the glands. Dense granules were also seen in the superficial cells of the lining of the glandular part of the anal canal (Fig. 3) but not in the surface epithelium lining the lumen of more proximal parts of the large intestine.

Columnar cells with few inclusions. These cells contained inclusions, which were confined in the main to the submicrovillous zone, of two main varieties. The first comprised spherical dense granules up to 0·5 μm in diameter resembling those illustrated in Fig. 3; they were not obviously membrane-bounded, possibly due to

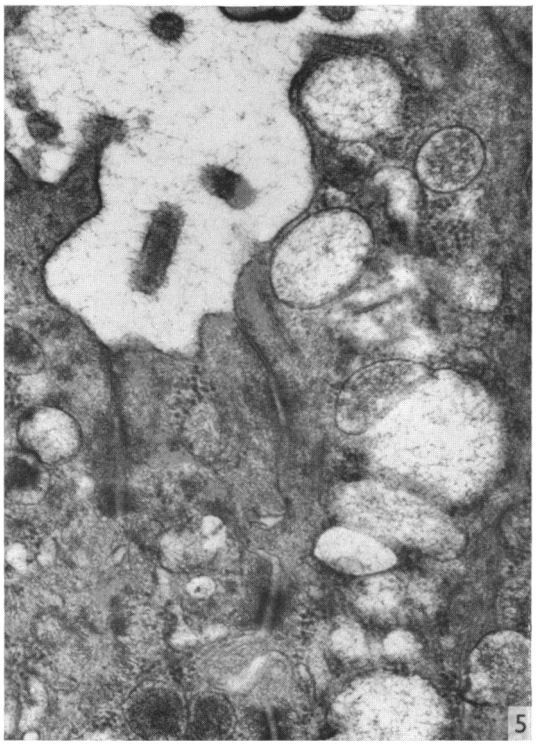
Fig. 2. The supranuclear region of a columnar cell in an intestinal gland of the rectum. An extensive horseshoe-shaped Golgi complex (*G*) can be seen embracing granular endoplasmic reticulum and a mitochondrion. × 34500.



3



4



5

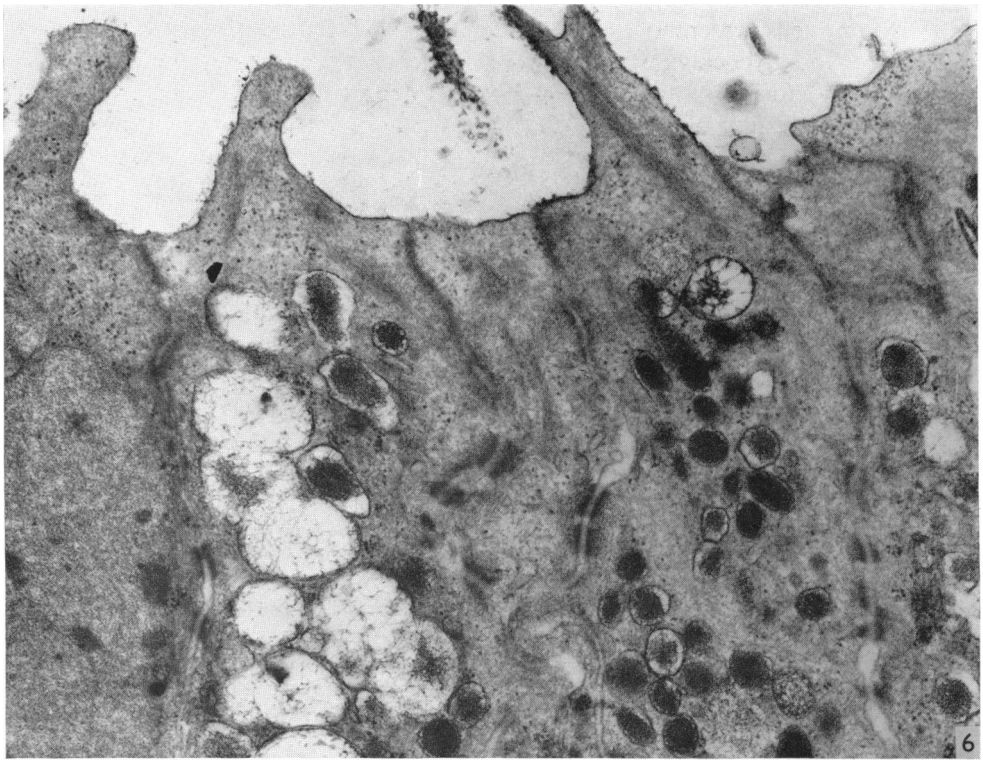
the density of the granule contents, and the cytoplasm surrounding them often contained conspicuous aggregates of mitochondria, ribosomes and both smooth and rough endoplasmic reticulum. The microvilli of cells with dense granules were usually relatively short, regular in outline and covered by a distinct external coat or glycocalyx; clumps of irregularly shaped and irregularly oriented microvilli arising from a common stem were also seen (Fig. 10). The second main variety of inclusion comprised small spherical vesicles some of which contained ill-defined granular contents (Fig. 7). There were few mitochondria or ribosomes and little endoplasmic reticulum in the surrounding cytoplasm which was apparently structureless apart from strands of filaments continuous with junctional complexes, with the terminal web and with the cores of the microvilli. The microvilli in many of these cells were glycocalyx-covered and elongated with a core of parallel filaments, or possibly microtubules, extending as bands of variable length into the submicrovillous zone; bands from adjacent microvilli joined a terminal web which was sinuous, sometimes laminated, and often deficient.

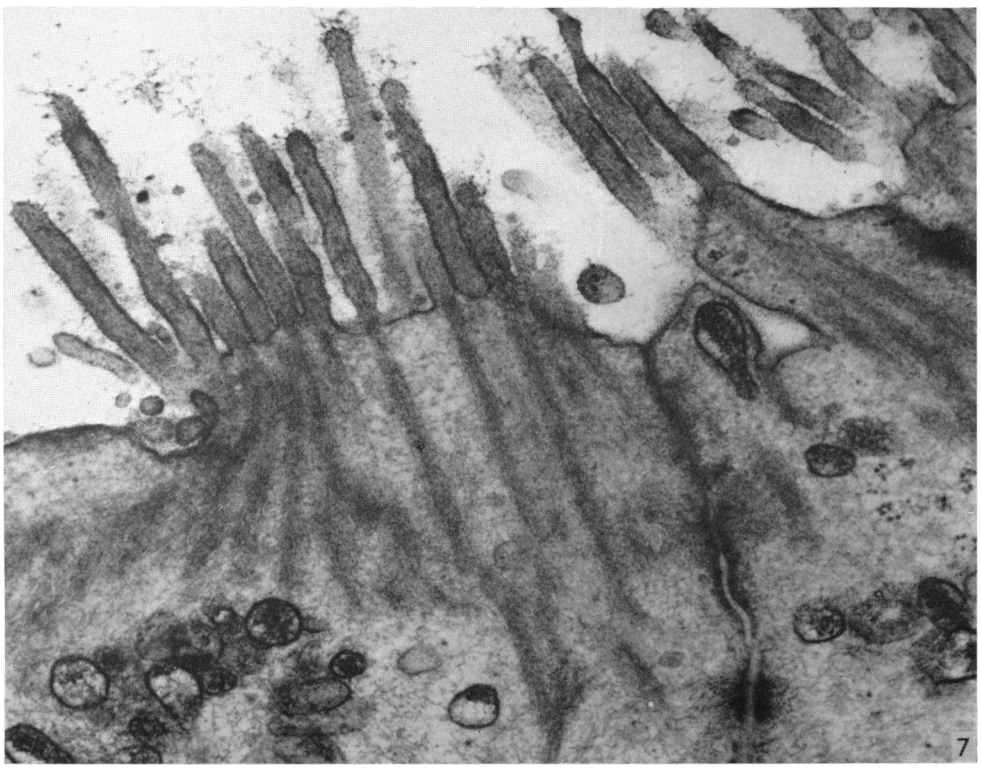
Columnar cells with numerous inclusions. Most columnar cells of the intestinal glands contained many supranuclear inclusions which ranged from the dense spherical ones already described to larger lightly stained vesicles. In most cells one or other variety predominated but intermediate forms were also present (Fig. 6). The variation in the structure of the inclusions was possibly attributable to different phases of development rather than differences in type. Columnar cells with predominantly dense inclusions also contained other, usually larger, inclusions with a more lightly stained granular core separated from a bounding membrane by an electron-lucid or faintly granular halo (Fig. 6). Some of the lightly cored inclusions were of considerable size, due apparently to coalescence of adjacent small inclusions (Fig. 8). Mitochondria were not prominent in the submicrovillous zone of these cells but there were numerous ribosomes in the cytoplasm surrounding the inclusions. In other columnar cells larger lightly stained granular inclusions predominated. They showed an expansion of the electron-lucent area internal to the bounding membrane and, here, delicate strands extended as a reticulum from the central core to the peripheral membrane (Fig. 9). In many columnar cells the greater part of the apex had a vacuolated appearance with vesicles extending towards the free border (Figs. 5, 6) and, in some few cases, apparently discharging contents whose texture was not unlike that of the glycocalyx covering the free surface of the columnar epithelial cells. In some respects, the content of the vesicles also resembled that of mucous granules in goblet cells, irrespective of the fixative used, but they were less densely stained and clearly originated from inclusions found almost exclusively in the apical part of the columnar cells rather than in the Golgi region. The slender outline of the columnar cells was not influenced by differences in the number and form of their inclusions.

Fig. 3. Surface epithelial cells bounding the lumen of the glandular part of the anal canal. Dense spherical inclusions can be seen in the apical part of the cells. The microvilli are small and relatively regular. × 5500.

Fig. 4. The supranuclear region of columnar cells in a rectal gland showing desmosomes and dense inclusions. × 32000.

Fig. 5. Columnar cells bounding the lumen of an intestinal gland of the anal canal. Vesicles with lightly stained contents can be seen adjacent to the surface plasma membrane. × 39000.


6


7

Microvilli in cells with numerous vesicles were few and irregularly shaped (Figs. 1, 5, 6) and some cells presented a single, thick, elongated microvillus in which both the tubular core and glycocalyx were indistinct. Other large microvilli appeared to have been formed by fusion of adjacent microvilli, for their broad base occupied a large part of the free surface of the parent cell as seen in section.

Acid phosphatase techniques showed the presence of numerous lysosomes in cells of the lamina propria but surprisingly few of the inclusions in columnar cells of the intestinal glands showed a positive reaction.

Columnar cells lining the lumen of the colon and rectum, unlike those of the intestinal glands and the superficial cells of the glandular part of the anal canal, were a relatively homogeneous group and did not contain numerous granular inclusions or large vesicles (Fig. 11). However, minute vesicles were seen in the submicrovillous zone and also in the intermicrovillous spaces; their significance was not clear. The microvilli were more regular in diameter and height than in cells of the intestinal glands. Mitochondria were numerous in the supranuclear region and granular and agranular endoplasmic reticulum was distributed regularly and in small amounts throughout the cells.

DISCUSSION

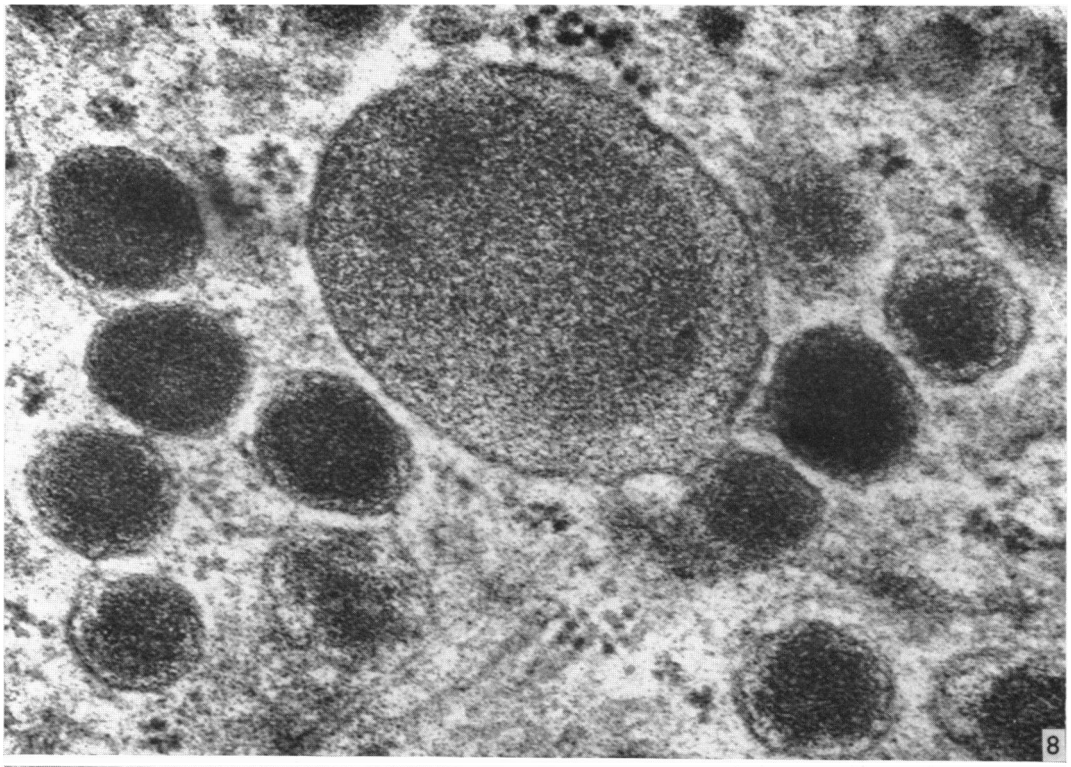
It is probable that more than one cell type is included under the heading of columnar cell in the colon of the Cynamolgus macaque. Certainly there were conspicuous differences, apparent only at high resolution, between columnar cells in the intestinal glands and at the surface of the mucous membrane. Columnar cells of the intestinal glands also differed appreciably in different areas but almost all contained supranuclear inclusions which were mainly in the submicrovillous zone. It is possible to interpret the wide range of variation in the form of the inclusions, occurring even in individual cells, as indicating a progressive transformation of dense granules into vesicles with lightly stained contents. Discharge of the contents of the vesicles into the lumen of the intestinal glands, possibly by either apocrine or merocrine secretion, thus appeared to represent the terminal event of a secretory cycle in which almost all of the columnar cells of the intestinal glands are involved. It seems reasonable, therefore, to use the term secretory granule in reference to the contents of the columnar cells. Microvilli of the secretory columnar cells of the intestinal glands also varied in form. In cells with a few dense inclusions, the palisade of microvilli was less regular than that of columnar cells lining the lumen of the intestine; some microvilli shared a common stem containing multiple bands of filaments or microtubules which extended towards the terminal web in the apical part of the cell. The surface coat or glycocalyx (Bennett, 1963) on microvilli of these cells was conspicuous. In cells with numerous secretory granules the microvilli were unusually large and broadly based and were not coated with a prominent glycocalyx.

In many respects the secretory columnar cells in the large intestine of the monkey

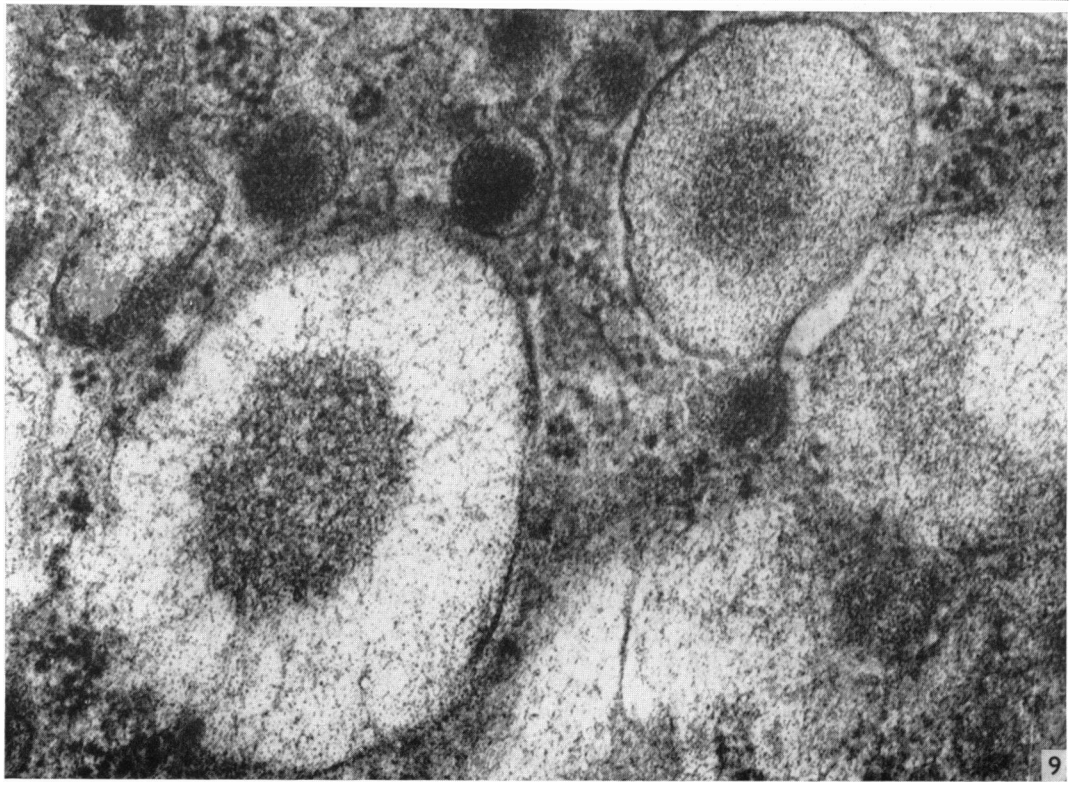
---

Fig. 6. Columnar cells in an intestinal gland of the colon. Neighbouring cells contain different varieties of inclusions and both are surmounted by broadly based microvilli. × 26500.

Fig. 7. Columnar cells with a few inclusions in the submicrovillous zone. The microvilli are elongated and connected to the terminal web by bands of microtubules. × 43000.

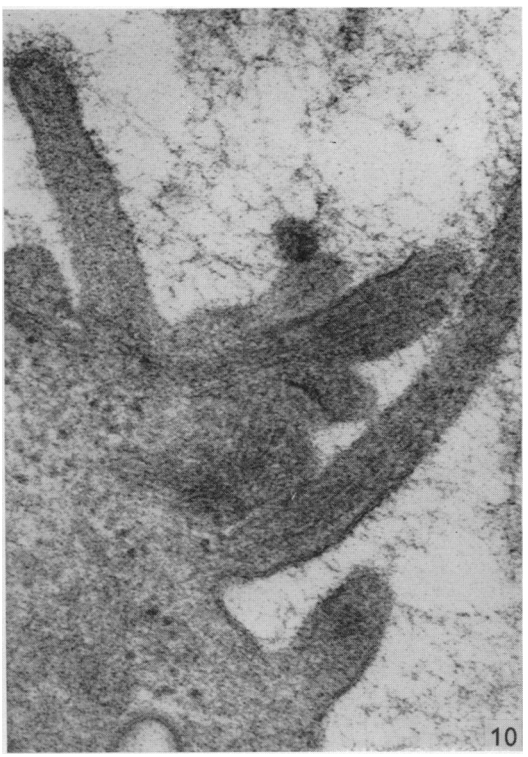


8



9

resembled the undifferentiated epithelial cells of the stomach and intestines described by Wetzel *et al.* (1966), Johnson & Young (1968) and Trier (1968) and the vacuolated cells described by Hollmann (1965); some of their inclusions also resembled those found in mucous neck cells of the stomach (Ito, 1967) or even in Paneth cells (Moe, 1968). Little conclusive evidence was obtained from histochemical studies as to the nature of the secretion of the columnar cells of the colon but clearly it differs from mucus produced by goblet cells and does not appear to correspond to the acid mucosubstances identified by Wetzel *et al.* in non-goblet cells of the mouse colon.



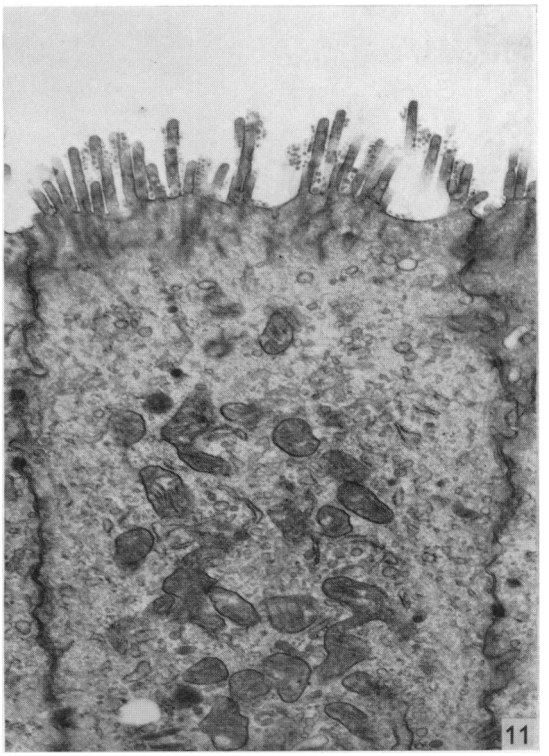


Fig. 10. A clump of microvilli arising from a columnar cell which contained few inclusions. Rectal gland. The surface coat is prominent. × 78000.

Fig. 11. A columnar cell of the surface epithelium of the colon showing mitochondria and agranular endoplasmic reticulum. No secretory granules are present and the microvilli are more regular than those of most cells found in the intestinal glands. × 11000.

Fig. 8. Membrane-bounded inclusions in the supranuclear region of a columnar cell in an intestinal gland of the anal canal. Several small inclusions can be seen and one is apparently continuous with a larger inclusion in which the central region is also more densely stained. Glutaraldehyde fixation. × 101000.

Fig. 9. From the same region as Fig. 8 showing still larger inclusions. The electron-lucent area seen in secretory granules in the previous figure is expanded and traversed by strands linking the granular core with the bounding membrane. Glutaraldehyde fixation. Compare with Fig. 6. × 87500.

Histochemical evidence at a light microscopic level suggests that it is protein and also that it comprises a considerable proportion of the secretion of the large intestine of the monkey, for the secretory columnar cells were about as numerous as the goblet cells although occupying less volume in the epithelium.

If the columnar cells with numerous inclusions are to be regarded as undifferentiated cells, an interpretation which is not entirely satisfactory in view of their apparent role in the elaboration and discharge of secretion, the question follows as to what other cell types arise from them. The dark slender goblet cells with extensive accumulations of granular endoplasmic reticulum and only a few mucous granules in the Golgi region were the least differentiated goblet cells identified but even these did not show any marked resemblance to the columnar cells in which the cytoplasmic matrix was invariably less densely stained with secretory granules generally subapical in position. Enterochromaffin cells were much less numerous than the columnar cells and their distinctive granules were mainly infranuclear. If the granular columnar cells of the intestinal glands replace those on the surface, their relocation must be associated with either a loss of secretory activity, more in keeping with a process of dedifferentiation than differentiation, or a change in its rate or extent, possibly associated with the development of absorptive functions.

The possibility that the granular appearance of the columnar cells was due to the presence of pathogens in the intestinal flora required careful consideration, for Cynamolgus colonies are notoriously prone to intestinal infections with micro-organisms ranging from *Shigella flexneri* to enteroviruses. However, each area of large intestine studied was not appreciably different in light microscopic appearance from that of normal human adults, in spite of the unusual form of the microvilli seen in many of the cells at high resolution. The presence of secretory granules in the columnar cells of the large intestine may therefore be regarded as normal for the colony even if representing an exaggerated physiological response of the cells to intestinal organisms present. This view is reinforced by preliminary studies on other species, including mice and sheep, in which columnar cells with similar secretory granules have also been identified in the large intestine.

Interpretations of the significance of the secretory granules of the columnar cells are likely to be influenced by a knowledge of their biochemical structure. If the protein nature of the inclusions is confirmed, the possibility arises that they comprise aggregates of immunoglobulins. The general problem of the categories of immunoglobulins present in tissues of the alimentary system and the sites at which they are elaborated have been examined by a number of investigators (Crabbé & Heremans, 1966; Rossen *et al.* 1968). A recent suggestion is that one of the immunoglobulins (IgA) present in the secretory epithelium of salivary glands is formed initially in adjacent plasma cells, then transferred to the epithelial cells, where it is united noncovalently with 'transport piece', a beta-globulin produced separately at this site, before the two components are secreted on the mucous surface (South, Warwick, Wollheim & Good, 1967). Gelzayd, Kraft & Kirsner (1968) also reported the presence of immunoglobulins in the apical part of epithelial cells of human rectal glands and referred to a unique type of local immunoglobulin system, the biological importance of which they were uncertain. The secretory columnar cells described in the present study correspond in position at least to the globulin-containing cells described by

Gelzayd *et al.*; their structure is consistent with a capacity for protein synthesis and the possibility that they are involved in the accumulation and discharge of immunoglobulins merits further investigation.

SUMMARY

The histochemical reactions and fine structure of columnar epithelial cells of the large intestine have been studied in the macaque (*Cynamolgus irus*). Several varieties of supranuclear inclusions were found in the columnar cells of the intestinal glands and in the surface cells of the glandular part of the anal canal; these ranged from dense spherical granules to irregularly shaped vesicles, some of which were seen discharging their contents into the adjacent lumen. It is suggested that an appropriate term of reference for the inclusions identified in the columnar cells is secretory granule.The microvilli of the secretory columnar cells of the intestinal glands showed an unusual range of variation in form, due possibly to variations in cell activity. Surface columnar cells other than those in the anal canal did not contain collections of secretory granules and their microvilli were more regular in shape.

The fine structure and histochemical reactions of the secretory columnar cells of the intestinal glands indicate that their secretion differs from that of goblet cells and evidence available so far suggests that it is protein in nature. The possibility that the columnar cells are involved in the accumulation and discharge of immunoglobulins is discussed.

The author is indebted to Dr W. R. Lane, Dr N. B. Walden and the Commonwealth Serum Laboratories, Melbourne, for the supply of macaques, to Mr A. R. Kreibich and Mr M. M. Richards for technical assistance, and to Mr J. S. Simmons, F.R.P.S., for the photographic enlargements.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

REFERENCES

BARKA, T. (1963). Electron histochemical localization of acid phosphatase activity in the small intestine of mouse. *J. Histochem. Cytochem.* **12**, 229–238.

BENNETT, H. S. (1963). Morphological aspects of extracellular polysaccharides. *J. Histochem. Cytochem.* **11**, 14–23.

CARR, K. E. & WHUR, P. (1968). Ultrastructure of globule leucocyte inclusions in the rat and mouse. *Z. Zellforsch. mikrosk. Anat.* **86**, 153–162.

CRABBÉ, P. A. & HEREMANS, J. F. (1966). The distribution of immunoglobulin-containing cells along the human gastrointestinal tract. *Gastroenterology* **51**, 305–316.

CULLING, C. F. A. (1957). *Handbook of Histopathological Technique*. London: Butterworth & Co.

FLOREY, H. W. (1960). Electron microscopic observations on goblet cells of the rat's colon. *Quart. Jl Exp. Physiol.* **45**, 329–336.

FREEMAN, J. A. (1966). Goblet cell fine structure. *Anat. Rec.* **154**, 121–147.

GELZAYD, E. A., KRAFT, S. C. & KIRSNER, J. B. (1968). Distribution of immunoglobulins in human rectal mucosa. *Gastroenterology* **54**, 334–340.

GURR, E. (1960). *Methods of Analytical Histology and Histo-chemistry*. Baltimore: Williams and Wilkins.

HOLLMANN, K. H. (1965). Über den Feinbau des Rectumepithels. *Z. Zellforsch. mikrosk. Anat.* **68**, 502–542.

ITO, S. (1967). Anatomic structure of the gastric mucosa. In *Handbook of Physiology: Alimentary Canal, Vol.* 2. Washington, D.C.: American Physiological Society, pp. 705–741.

JOHNSON, F. R. & YOUNG, B. A. (1968). Undifferentiated cells in gastric mucosa. *J. Anat.* **102**, 541–551.

LORENZSONN, V. & TRIER, J. S. (1968). The fine structure of human rectal mucosa. *Gastroenterology* **55**, 88–101.

MILLONIG, G. (1962). Further observations on a phosphate buffer for osmium solutions. In *Electron Microscopy*, Vol. 2. New York and London: Academic Press, p. P-8.

MOE, H. (1968). The goblet cells, Paneth cells, and basal granular cells of the epithelium of the intestine. *Int. Rev. gen. exp. Zool.* **3**, 241–287.

PEARSE, A. G. E. (1961). *Histochemistry. Theoretical and Applied.* London: Churchill.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

ROSSEN, R. D., COUNCILMAN, M., HSU, K. C., BUTLER, W. T. & ROSE, H. M. (1968). Localization of IIS external secretory IgA by immunofluorescence in tissues lining the oral and respiratory passages in man. *J. Immunol.* **100**, 706–717.

SABATINI, D. D., BENSCH, K. & BARRNETT, R. J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**, 19–58.

SCHOFIELD, G. C. & SILVA, D. G. (1968). The fine structure of enterochromaffin cells in the mouse colon. *J. Anat.* **103**, 1–13.

SCHOFIELD, G. C., HO, A. K. S. & SOUTHWELL, J. M. (1967). Enterochromaffin cells and 5-hydroxytryptamine content of the colon of mice. *J. Anat.* **101**, 711–721.

SHEARMAN, D. J. C. & MUIR, A. R. (1960). Observations on the secretory cycle of goblet cells. *Quart. Jl exp. Physiol.* **45**, 337–342.

SILVA, D. G. (1966). The fine structure of multivesicular cells with large microvilli in the epithelium of the mouse colon. *J. Ultrastruct. Res.* **16**, 693–705.

SOUTH, M. A., WARWICK, W. J., WOLLHEIM, F. A. & GOOD, R. A. (1967). The IgA system. III. IgA levels in the serum and saliva of pediatric patients—evidence for a local immunological system. *J. Pediat.* **71**, 645–653.

THOMPSON, S. W. (1966). *Selected Histochemical and Histopathological Methods.* Springfield: Charles C. Thomas.

TRIER, J. S. (1968). Morphology of the epithelium of the small intestine. In *Handbook of Physiology: Alimentary Canal*, Vol. 3. Washington, D.C.: American Physiological Society, pp. 1125–1175.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *J. biophys. biochem. Cytol.* **4**, 475–478.

WETZEL, M. G., WETZEL, B. K. & SPICER, S. S. (1966). Ultrastructural localization of acid mucosubstances in the mouse colon with iron-containing stains. *J. Cell Biol.* **30**, 299–315.