J. Anat. (1970), **107**, 3, pp. 491–504
With 13 *figures*
Printed in Great Britain

Secretory immunoglobulin in columnar epithelial cells of the large intestine

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(*Received* 28 *October* 1969)

INTRODUCTION

In an earlier study on the columnar epithelial cells of the large intestine of the monkey, attention was drawn to the several varieties of supranuclear inclusions or secretory granules found in many of the cells (Schofield, 1970). The histochemical reactions of the columnar cells, taken together with the fine structure of the secretory granules they contained, suggested that they elaborated a secretion which was protein in nature but differed from that of goblet cells. The suggestion was advanced that columnar cells with secretory granules are involved in the accumulation and discharge of immunoglobulins. Evidence derived from immunofluorescence studies for the local synthesis of immunoglobulins in the alimentary canal and associated extramural glands is mounting (South, Cooper, Hong, & Good, 1967; Gelzayd, Kraft & Kirsner, 1968; Tomasi & Bienenstock, 1968; Watson, 1969) but the ultrastructural basis for the results obtained in the intestines has received little direct attention. In the present paper the fine structure of columnar epithelial cells in the large intestine of man and sheep is examined and in man is correlated with the results of immunofluorescence studies on rectal mucosa. The hypothesis is advanced that secretory immunoglobulin is accumulated in the form of secretory granules by the columnar epithelial cells and that their secretions contribute to or form the glycoprotein surface coat or glycocalyx covering the luminal margin of the cells.

MATERIALS AND METHODS

Studies were carried out on the mucous membrane of the large intestine of man and sheep. Sheep tissues were taken from nine adult females and one wether under halothane anaesthesia. Human tissues were taken at 12 cm from the anal margin through a sigmoidoscope from four patients ranging in age from 58 to 69 years and attending an out-patients clinic for conditions found to be unrelated to disorders of the alimentary canal. In each case the biopsy area was judged normal both macroscopically and microscopically.

For ultrastructural study small blocks were fixed in 2% phosphate-buffered osmium tetroxide (Millonig, 1962) or in 4% phosphate-buffered glutaraldehyde and postfixed in osmium tetroxide (Sabatini, Bensch & Barrnett, 1963). Histochemical reactions for glycoproteins and glycolipids (Pearse, 1968) were carried out in tissue fixed in either 10% formalin or 4% glutaraldehyde, or in freeze-dried preparations fixed in formaldehyde vapour prepared in an atmosphere of 50% relative humidity.

Immunohistochemical investigations involved human rectal biopsies only. The tissues were rapidly frozen in isopentane cooled in liquid nitrogen and then maintained in dry ice. Cryostat sections 5 μm thick were air-dried at 4 – 10 °C and studied for immunofluorescence after staining. Rabbit antiserum directed against human IgA (Pentex) was used after dilution to 1 in 4 by the addition of phosphate-buffered saline (pH 7·3) to minimize non-specific staining. Goat antiserum directed against rabbit gammaglobulin was conjugated with fluorescein isothiocyanate and absorbed with human liver powder and bovine stomach homogenate. The specificity of the two antisera used was confirmed by microimmunodiffusion and immunoelectrophoresis. The indirect fluorescent antibody technique as described by Goldman (1968) was employed. Cryostat sections of gut were treated with rabbit antiserum directed against human IgA and subsequently with fluorescein-conjugated goat antiserum directed against rabbit γ-globulin. In control experiments, normal rabbit serum and normal goat serum conjugated with fluorescein were substituted for the specific antisera used. The preparations were examined immediately under a Leitz Ortholux microscope equipped with an HBO 200 W high-pressure mercury vapour lamp. The filters used comprised a UG1 exciter filter, a BG38 suppression filter and a K430 barrier filter.

RESULTS

Histochemical observations

In routine histological preparations of human and sheep tissues fixed in either aqueous formalin or aqueous glutaraldehyde a distinction could be readily made between goblet cells and columnar epithelial cells after Alcian blue or PAS staining. Goblet cells contained acid and alkaline mucoprotein and also PAS-positive material which was resistant to diastase digestion and was assumed to be glycoprotein. Columnar epithelial cells, or principal cells, showed no evidence of the presence of either glycoprotein or glycolipid. In freeze-dried preparations, however, staining of goblet cells with both PAS and Alcian blue was accentuated and many columnar cells in both the intestinal glands and, particularly in human tissues, the surface epithelium also gave a pronounced reaction with PAS. In the case of most of the surface columnar cells in both sheep and human intestine, the PAS reaction was found to be negative following incubation in the presence of diastase. A striking feature in freeze-dried preparations compared with tissues preserved in aqueous fixatives was the relatively large proportion of the mucous membrane represented by the interglandular part of the lamina propria; numerous plasma cells were readily distinguished in the lamina propria and after staining with PAS some few had a staining reaction similar to that of the columnar epithelial cells. Aggregates of intensely PAS-positive material were identified in other connective tissue cells of the lamina propria and these were found to be less numerous in sections which had been incubated in the presence of diastase.

Ultrastructural observations

In the sheep

In both osmium- and glutaraldehyde-fixed tissues the predominant epithelial cells of the mucous membrane comprised goblet cells and columnar cells (Fig. 1). Mucino-

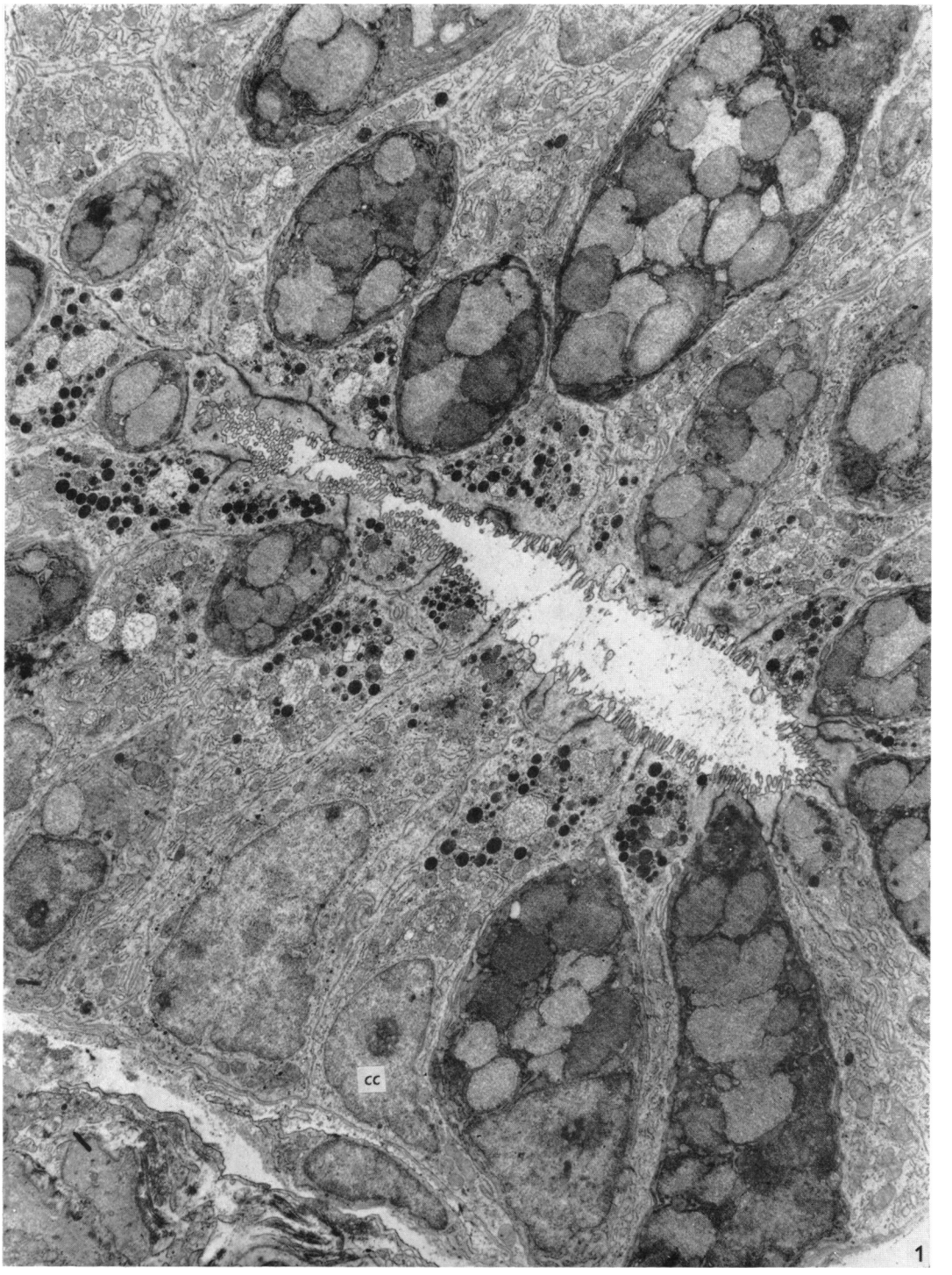


Fig. 1. Sheep colon. An intestinal gland containing goblet cells and columnar cells with secretory granules ranging from dense granules to lightly stained vesicles. One of the columnar cells (*CC*) is shown at higher magnification in Fig. 7. × 5000.

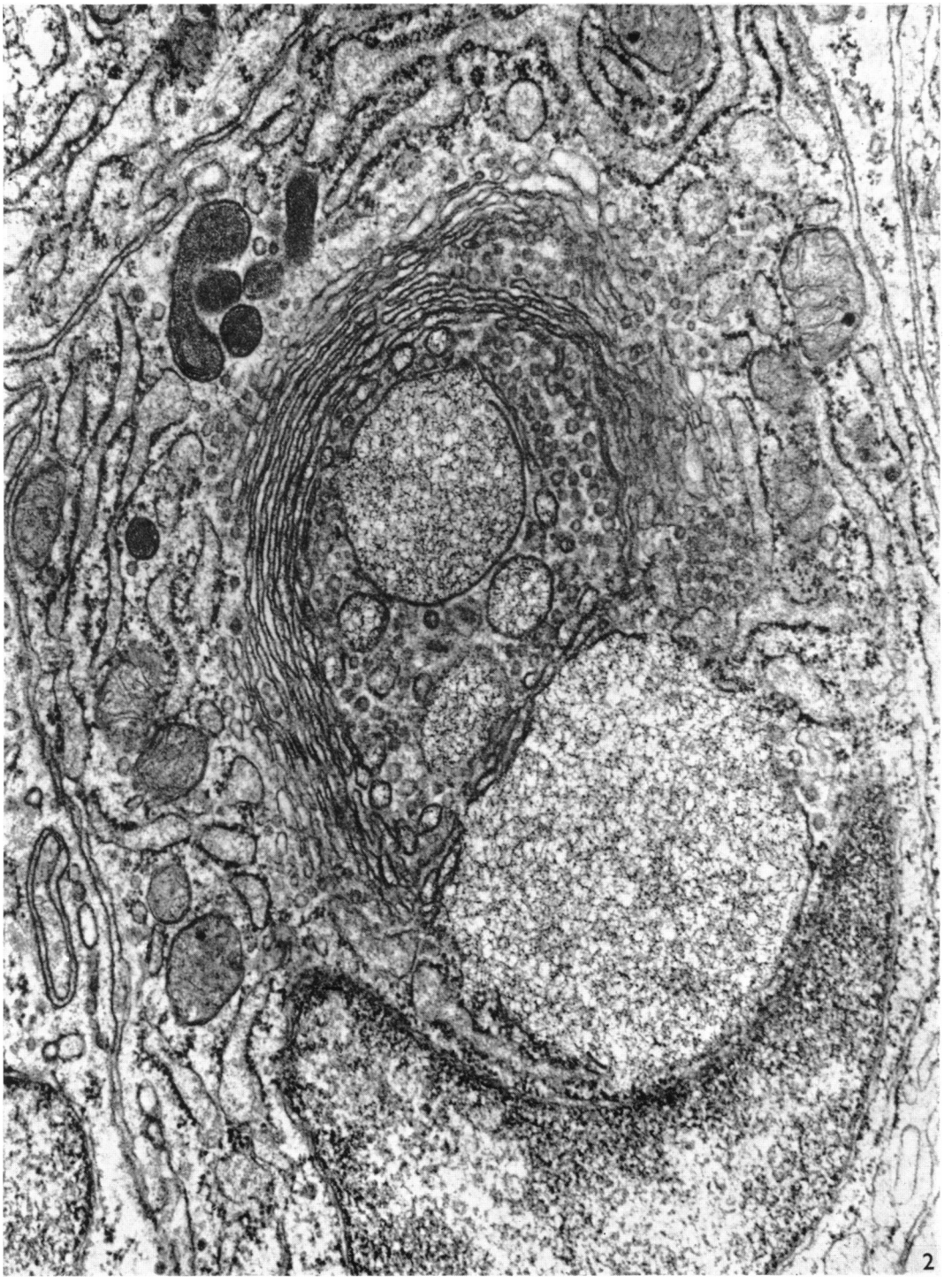


Fig. 2. Sheep colon, intestinal gland. An epithelial cell containing several lightly stained secretory granules in the Golgi region. Other more densely stained structures, possibly early secretory granules, are also seen outside the upper left margin of the Golgi zone. Compare this cell with the columnar cell illustrated in Fig. 7. × 38000.

gen granules aggregated in the supranuclear region of the goblet cells showed considerable variation in shape and electron density and, in cells bulging into the lumen, they occupied the entire apex except where rudimentary microvilli were present. Individual mucinogen granules were usually regular in texture, although some showed a lightly stained peripheral margin, and were either continuous with adjacent granules or separated from them by narrow strips of darkly stained cytoplasm containing ribosomes.

Almost all columnar cells in the intestinal glands contained membrane-bounded inclusions in the supranuclear region. Two main types were seen, dense spherical granules up to 0·5 μm in diameter and larger spherical or irregularly shaped vesicles with electron-lucent contents (Figs. 1, 4, 7). A gradation between dense granules and vesicles was seen in most cells with intermediate forms, represented by a darkly stained fibrillar core surrounded by an electron-lucent halo, predominating in some. Vesicles in some cells were similar in texture and electron density to the more lightly stained of the mucinogen granules in goblet cells, but they were usually smaller in size. Coalescence of spherical vesicles occurred near the microvillous border leading to the formation of larger irregularly shaped vesicles which frequently enveloped the smaller dense granules also found in this region (Fig. 4). The vesicles contained a filamentous network lying within an apparently structureless matrix and often apparently continuous through localized deficiences in the bounding membrane with the surrounding ribosome-rich cytoplasm. Some of the enlarged inclusions bulged into the lumen of the intestinal glands and were separated from it by plasma membrane only (Fig. 4); such inclusions, unlike aggregates of mucinogen granules in goblet cells, did not occupy fully the apex of the cell. Many columnar cells with numerous inclusions presented a scalloped free margin and appeared to be in the process of discharging the contents of an apical vesicle into the adjacent lumen. The contents of both discrete vesicles and those discharging into the lumen of the glands showed a striking resemblance to the surface coat found at the luminal margin of the glandular epithelium.

Wedge-shaped epithelial cells without obvious secretory granules were also seen occasionally at the fundus of the intestinal glands (Fig. 3). Their narrow apices were surmounted by relatively few microvilli. Their cytoplasm was lightly stained and contained granular endoplasmic reticulum in the perinuclear region and agranular reticulum nearer the apex; mitochondria, ribosomes and Golgi apparatus were unexceptional in their form and distribution. Such cells appeared to be the least differentiated of the epithelial components seen in the large intestine and cells of similar appearance were carefully surveyed for evidence of the earliest development of secretory granules within them. Some cells contained a few inclusions which were confined to the Golgi region and resembled mucinogen granules (Fig. 2); others also contained mucinogen-like inclusions in the Golgi region but these were accompanied by one or more dense granules similar to those found in many of the actively secreting columnar epithelial cells. It seems likely, therefore, that even if goblet cells and columnar cells with secretory granules are distinct cell types, the respective stem cells from which they arise are indistinguishable from each other.

In the human rectum

Goblet cells in the human rectum were not appreciably different in form from those in sheep and their mucinogen granules conferred a variegated appearance on the supranuclear region with more lightly stained granules predominating in some cells (Fig. 5).

Secretory granules in columnar cells of the rectum were less conspicuous in man than in sheep. Some of the columnar cells of the intestinal glands contained both dense granules and lightly stained vesicles in the supranuclear region (Fig. 8). In most columnar cells with inclusions the secretory granules were lightly stained with or without a more darkly stained filamentous core (Figs. 6, 9), and showed a range of variation in size, shape, and contents similar to that of the electron-lucent vesicles in sheep. In vesicles adjacent to or discharging into the lumen, the filamentous contents showed a striking resemblance to the surface coat enveloping neighbouring microvilli (Figs. 6, 9, 10). Goblet cell secretion in the lumen of intestinal glands often had a flocculent appearance and was appreciably different from that of the columnar cells (Figs. 8, 10). The apical cytoplasm of the columnar cells contained numerous free ribosomes but comparatively little endoplasmic reticulum. Mitochondria were also numerous in the apical region, including the area immediately subjacent to the microvillous border, and here were often apparently in contact with the secretory granules (Fig. 9).

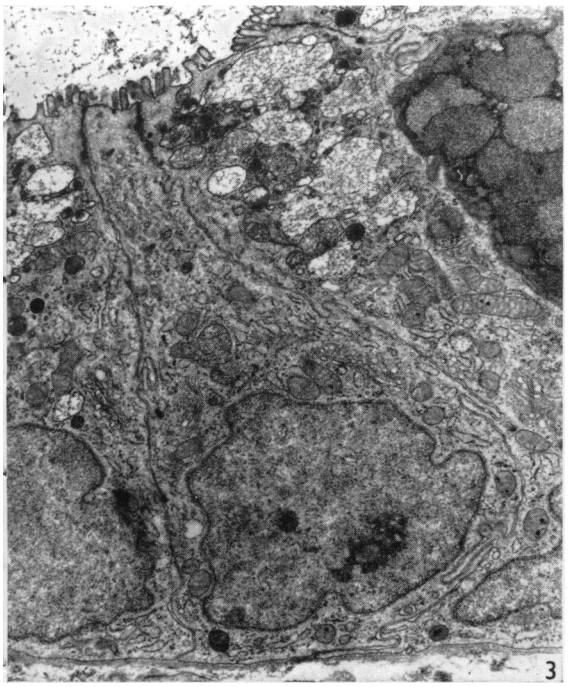
A striking feature of the columnar cells of the surface epithelium was the profusion of small dark irregularly shaped mitochondria in the infranuclear region and in some areas profiles of adjacent cells contained over a hundred mitochondria each. Glycogen particles occurred in the infranuclear region also but were more conspicuous in the supranuclear region where they were frequently aggregated in prominent masses sufficiently large to be resolved by the light microscope. Granular and electron-lucent inclusions similar to those found in columnar cells of the intestinal glands were seen in about one in three of the cells of the surface epithelium; they were thus fewer in number and correspondingly less conspicuous than in cells of the glandular epithelium. Microvesicles attached to or lying between the microvilli were a constant feature of surface epithelial cells in which the palisade arrangement of microvilli was more regular than in the glandular epithelium.

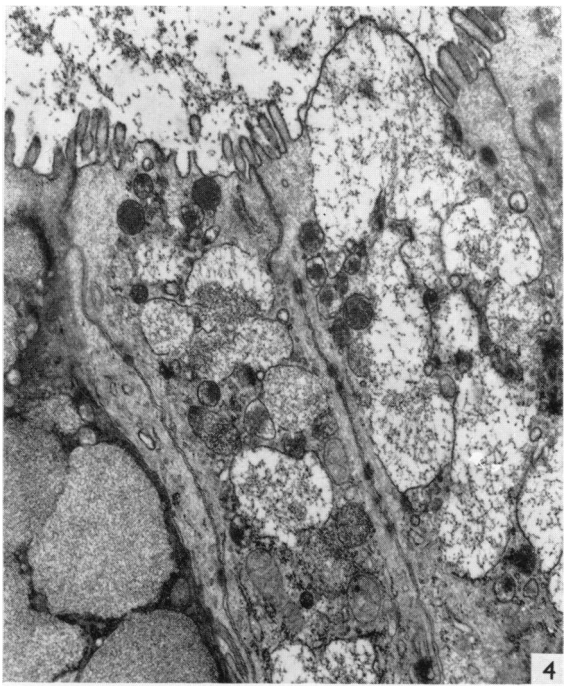
Fig. 3. Sheep colon. Fundus of intestinal gland. A relatively undifferentiated epithelial cell flanked by columnar cells both of which contain a variety of secretory granules. × 7000.

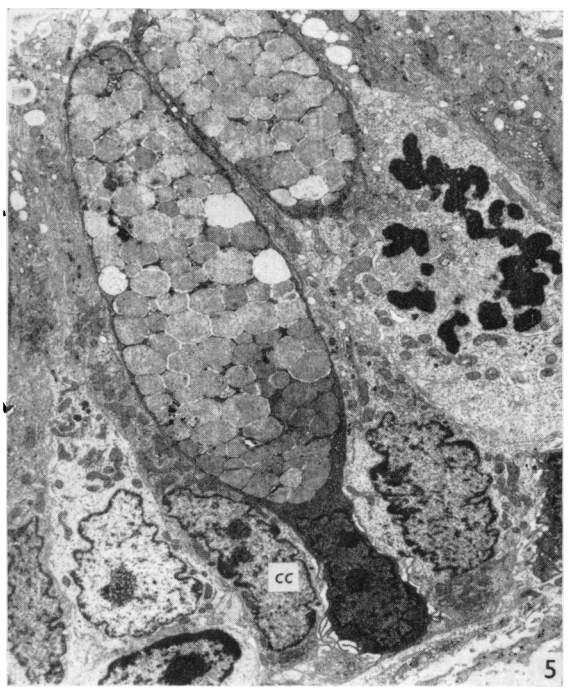
Fig. 4. Sheep colon, intestinal gland, showing the apical region of two columnar cells. In the cell on the right there is coalescence of secretory granules forming an electron-lucent mass which is bulging into the adjacent lumen. × 14 000.

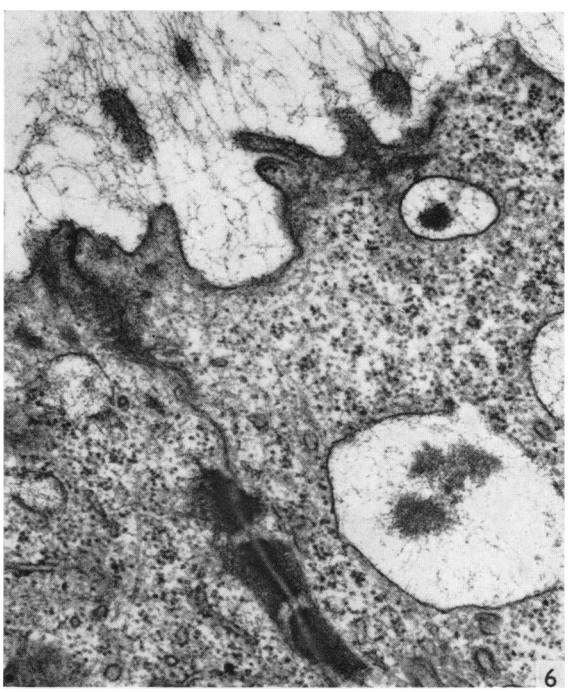
Fig. 5. Human rectum, intestinal gland. Portions of two goblet cells and a cell in mitosis can be seen. One of the columnar cells (*CC*) contains electron-lucent secretory granules at its luminal margin. Similar granules can be seen in other cells. × 2600.

Fig. 6. Human rectum. Vesicular type secretory granules at the apical region of a columnar cell of an intestinal gland. One of the vesicles is discharging contents into the lumen. × 31 000.



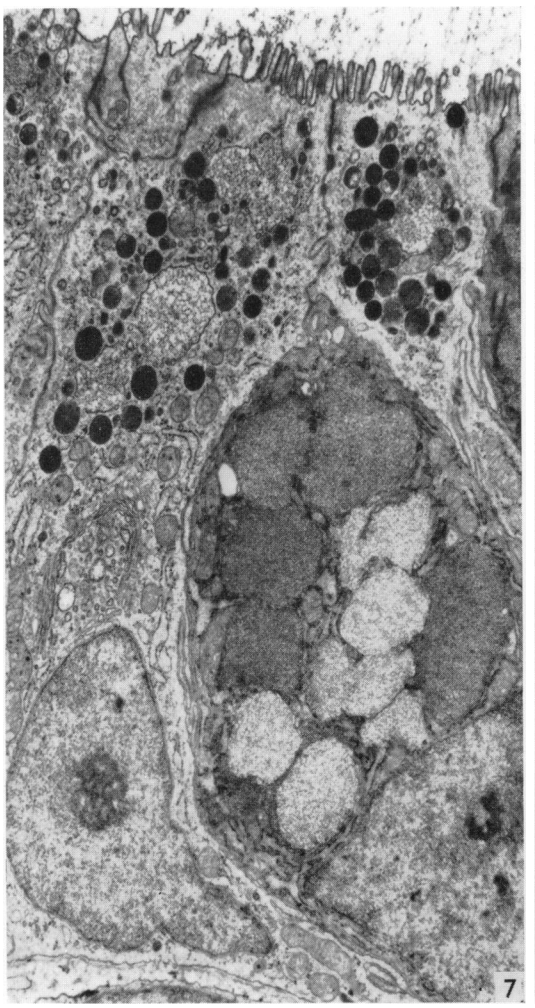






Immunohistochemical observations

Intense immunofluorescence staining was seen in most columnar epithelial cells of the intestinal glands in each specimen studied (Figs. 11, 12, 13). The intensity of the staining reaction was usually more pronounced in the apical region but in some cells was uniform throughout the cytoplasm. Linear staining was seen at the lateral and luminal margins of the columnar cells and was also present but less intense in the



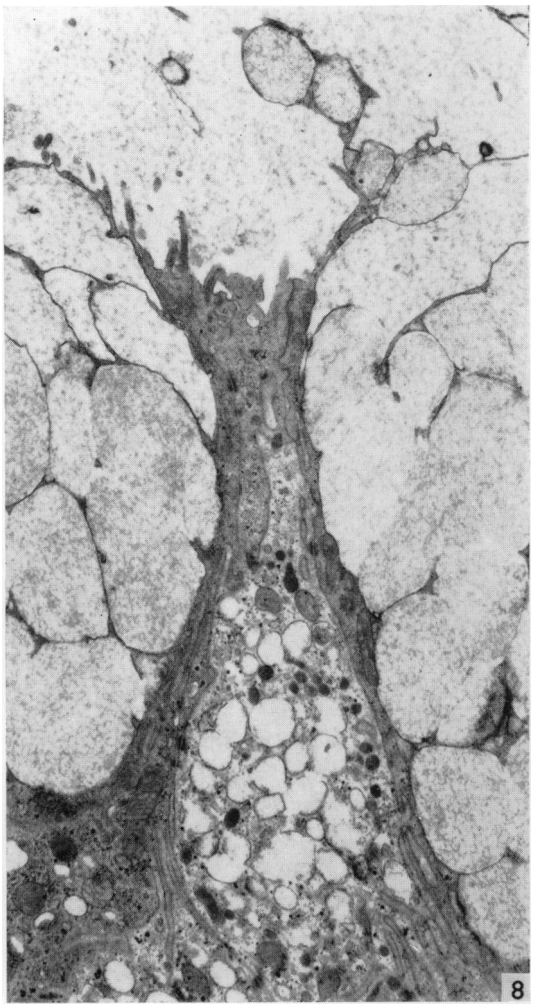


Fig. 7. Sheep colon. One of the areas illustrated in Fig. 1 is shown here at higher magnification. A variety of secretory granules can be seen in the columnar cells and one of the cells contains small electron-lucent inclusions in the Golgi region. × 10 000.

Fig. 8. Human rectum, intestinal gland. A columnar cell flanked by goblet cells and containing both electron dense and electron-lucent inclusions. The goblet cell on the right is apparently discharging mucinogen granules. Other granules in both goblet cells have a flocculent appearance similar to that of the material seen within the lumen. × 8000.

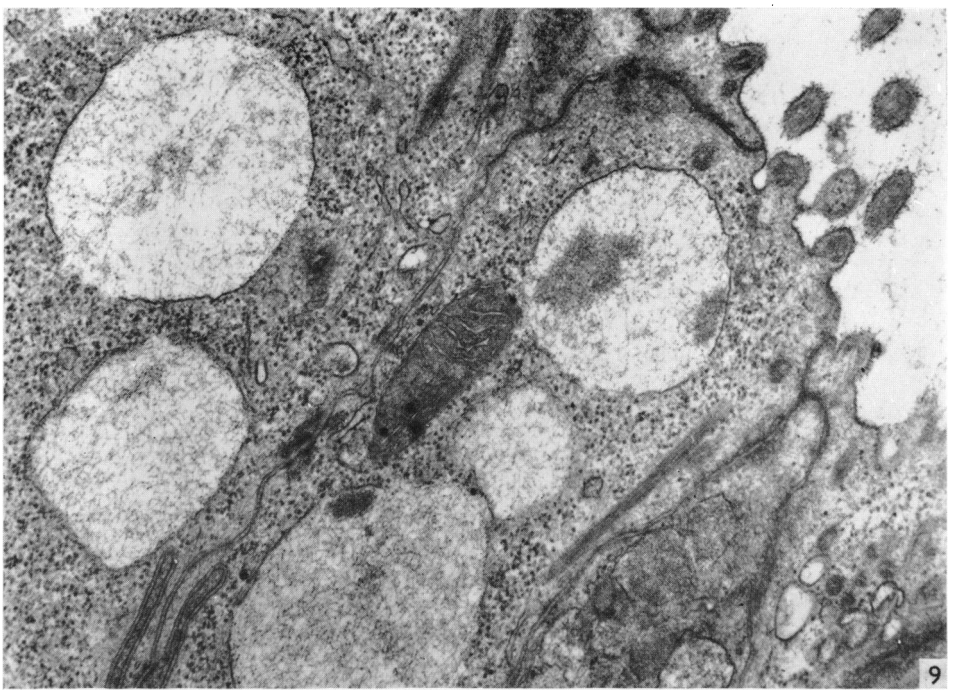
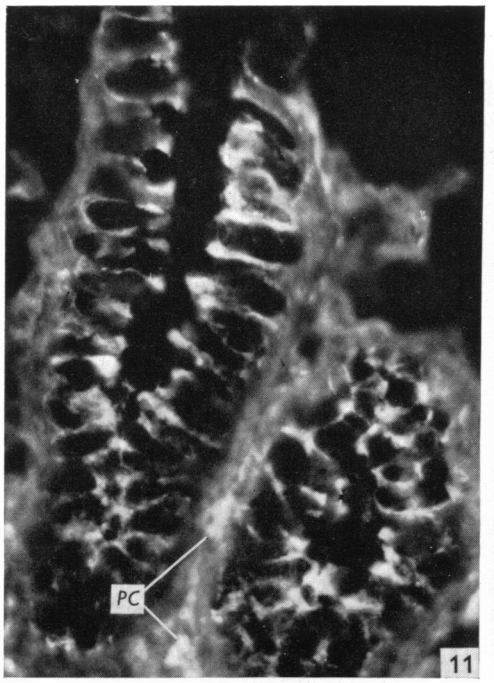
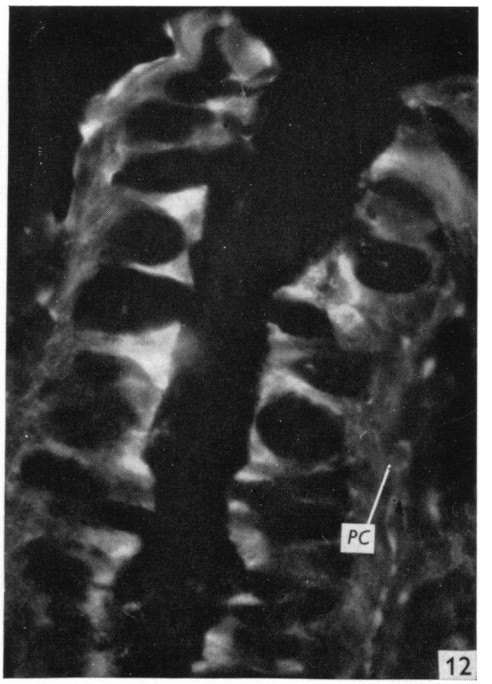
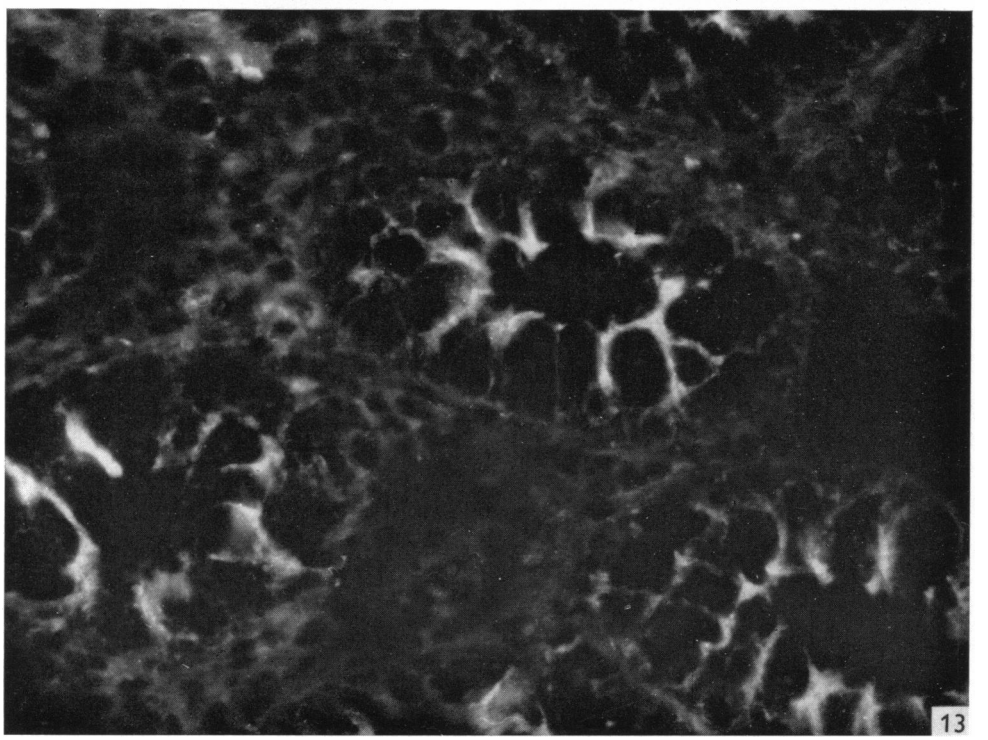


Fig. 9. Human rectum, intestinal gland. Electron-lucent secretory granules can be seen in the apical region of columnar cells. Note the filamentous appearance of the contents of the granules. In several inclusions, denser areas possibly representing remnants of electron dense granules can also be seen. × 29 000.

Fig. 10. From the same specimen as Fig. 9 showing two secretory granules discharging contents. Note the similarity between the enteric surface coat and the contents of the secretory granules. × 39 000.







Figs. 11–13. In these preparations fluorescein-labelled antiserum directed against human IgA has been applied to sections of human rectal mucous membrane. Fluorescence in the epithelium is seen in columnar cells but not in goblet cells; in most of the columnar cells fluorescence is more pronounced in the apical region. Staining is also seen in plasma cells (*PC*) in the lamina propria. Fig. 11, ×240; Fig. 12, ×375; Fig. 13, ×530.

region of the basement membrane of the epithelium. A network of weakly fluorescent material was seen in the lumen of some of the intestinal glands.

The majority of goblet cells identified by their shape and in some preparations also by their subsequent reaction with Alcian blue were conspicuously devoid of immunofluorescent staining but some few cells appeared to contain weakly immunofluorescent areas which possibly represented individual mucinogen granules. Immunofluorescent cells seen in the lamina propria were identified as plasma cells; they were equally common in the interglandular and subglandular parts of the lamina propria.

Control staining of serial sections failed to show fluorescence in cells of surface or glandular epithelium or in cells of the lamina propria.

DISCUSSION

It is possible to distinguish quite readily between goblet and columnar cells in the large intestine of man and sheep on the basis of differences in their form, in their response to Alcian blue staining and, in the case of human cells, in their immunohistochemical reactions to fluorescein-labelled antibody directed against serum IgA. However, the two cell types do present some features in common. Both are clearly secretory cells and, in freeze-dried tissues, both respond to PAS staining, although the reaction is less pronounced in columnar cells. The PAS positive material in columnar cells does not appear to be glycolipid for it does not react to Sudan black. Ultrastructurally, the apparent precursor cells of columnar and goblet cells are indistinguishable and, furthermore, the Golgi region of many columnar cells with dense apical granules contains newly formed inclusions resembling mucinogen granules found in mature goblet cells. The possibility exists, therefore, that columnar cells and goblet cells are not necessarily committed to one particular role and to the manufacture of a particular type of secretory product throughout their life-span. Further studies using the techniques employed by Lev & Gerard (1967), Bertolini (1968), Bradbury & Stoward (1967, 1968) and Merzel & Leblond (1969) for demonstrating the early appearance of various types of epithelial mucins and the cells containing them will assist in establishing whether or not goblet cells and the columnar cells of the present study are cells of separate lineage as differences in their secretion and secretory mechanisms would suggest.

The ready availability of antisera directed specifically against human serum IgA led to the selection of human gut for immunohistochemical studies, and the observation that columnar and plasma cells in the human rectum contain IgA, whereas goblet cells do not, confirms findings by previous workers (Gelzayd *et al.* 1968). In tissues stained with PAS the glycoprotein aggregates in columnar cells of the large intestine of man, and also sheep and macaques, thus appear to correspond to those found in PAS-positive plasma cells, possibly those cells which in ultrastructural preparations are seen to contain distended cisternae. It is not possible at present to be certain that the immunoglobulin material found in the columnar cells corresponds to the secretory granules they contain. However, preliminary studies on monkey colon using ferritin-labelled antiserum directed against monkey γ-globulin points to the presence of globulin in some of the vesicles in columnar cells; homogenates of monkey and sheep colonic mucous membrane prepared by differential centrifugation

and examined by gel diffusion and electrophoresis also show the presence of immunoglobulins in various fractions, including a low-speed particulate fraction containing inclusions characteristic of columnar cells (Schofield, 1969).

Whatever the nature of the secretion elaborated by the columnar cells of large intestine, its morphological resemblance to the surface coat enveloping microvilli commands attention. Pittman & Pittman (1966) also described secretory vesicles in columnar cells of the human colon and, as in the present study, they found that the secretory vesicles contained filamentous meshworks resembling those in the surface coat. Some of the earlier studies on the enteric surface coat, also known as the extraneous coat or glycocalyx, suggest that it is an integral part of the plasmalemma and that it is intrinsic to the epithelial cells with which it is associated (Rifaat, Iseri & Gottlieb, 1965; Ito, 1965). Should subsequent studies on the gut involving the use of ferritin-labelled antiserum directed against IgA show localization within both secretory granules of the columnar cells and the surface coat at their luminal borders, the view that the cells contribute an immunoglobulin secretion to the enteric surface coat, perhaps as part of an intestinal defence mechanism, will have been strengthened.

A number of hypotheses exist as to the manner in which IgA of plasma cell origin and secretory piece are combined to form secretory immunoglobulin (Tomasi & Bienenstock, 1968; Tourville, Adler, Bienenstock & Tomasi, 1969). The schemata suggested by Heremans & Crabbé (1967) and South, Warwick, Wollheim & Good (1967) envisage a predominantly noncovalent linkage between the two components, either within the epithelial cells lining the alimentary canal and extramural glands or within the lumen. However, Tomasi & Czerwinski (1968) found disulphide bonding in most secretory immunoglobulin aggregates. South, Cooper, Hong & Good (1967) cite evidence that secretory piece is located specifically in duct epithelial cells of salivary glands and refer to the need for complementarity of molecules of IgA and secretory piece before union leading to the formation of secretory immunoglobulin can be achieved. Tourville *et al.* (1969) also located secretory piece on the luminal side of the basement membrane in human rectal epithelium but mainly in goblet cells and intercellular spaces. It would be of interest to determine the relative proportions of IgA and secretory piece in intestinal epithelial cells and whether or not their combination, if it occurs within the cells, is dependent on molecular aggregates which have a form susceptible to ultrastructural resolution. It is not impossible that the dense granules of columnar epithelial cells represent regions where synthesizing and bonding activities, involving IgA of plasma cell origin and secretory piece of epithelial origin, occur under the influence of high energy molecular assemblies. Isolation and analysis of the granules would seem to be an appropriate precedure to follow in subsequent studies, for whatever their nature they are likely to represent sites of intense activity in the secretory columnar cells of the intestine.

SUMMARY

The epithelium lining the large intestine has been studied in man and sheep using histochemical, immunohistochemical, and ultrastructural methods of investigation. Both goblet cells and columnar epithelial cells are involved in the accumulation and discharge of secretory products which appear to be glycoprotein in nature. In the

human intestine, columnar cells, unlike goblet cells, contain immunoglobulin (IgA) aggregated most prominently in the supranuclear region. Differences in the immunohistochemical response of the two types of epithelial cell may be related to differences in the ultrastructural appearance of their secretions, for material in mucinogen granules of goblet cells frequently has a flocculent appearance, whereas the contents of mature secretory granules of columnar cells resemble closely the enteric surface coat enveloping microvilli. Suggested roles for columnar epithelial cells of the large intestine are an involvement in the elaboration of an immunoglobulin-containing surface coat and a contribution to intestinal defence mechanisms.

The authors are indebted to Mr A. R. Kreibich, Mr A. R. Davis 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.

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