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Studies on the structure and distribution of immunoglobulin A-containing cells in the gut of the pig

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INTRODUCTION

In previous studies on the large intestine of man, sheep and monkeys, secretory granules which appeared to contribute to the glycoprotein enteric surface coat were identified in columnar epithelial cells; in human rectum, the epithelial cells with secretory granules were also shown to contain aggregates of immunoglobulin of the class IgA and it was suggested that one determinant of immune competence in the intestines is IgA liberated at the luminal margin from secretory granules in the epithelial cells (Schofield, 1970; Schofield & Atkins, 1970).

Recently, immunoglobulin comprising IgA and IgM classes has been shown to be present in both crypt epithelium and plasma cells of the small intestine of the pig (Porter & Allen, 1970; Allen & Porter, 1970). In the present investigation, ultrastructural and histochemical features of immunoglobulin-containing cells in the pig are examined in selected parts of the stomach and intestines.

MATERIALS AND METHODS

Studies were carried out on gastro-intestinal tissues obtained from ten healthy adult pigs within minutes after death. Specimens were taken from the pyloric antrum, proximal part of the duodenum, terminal ileum, caecum, and terminal part of the colon.

For ultrastructural studies small blocks were fixed in 4 % phosphate-buffered glutaraldehyde (Sabatini, Bensch & Barrnett, 1963), and postfixed in 2·5 % osmium tetroxide in potassium dichromate (Dalton, 1955). Histochemical reactions for glycoprotein and glycolipids were carried out on freeze-dried and formaldehyde-fixed tissues using techniques described previously (Schofield & Atkins, 1970).

Immunohistochemical studies were performed using rabbit antisera directed against an immunoglobulin-containing fraction of colostrum obtained from pigs during the period ranging from 2 days before to 3 days after farrowing. Essentially, the method of Porter (1969) was used to isolate pig IgA from colostrum; gel-filtration chromatography on a Sephadex G-200 column using 0·1 M phosphate buffer (pH 7·0) was carried out, followed by anion exchange chromatography on a diethylaminoethyl-cellulose (DEAE-cellulose) column using 0·02 M phosphate buffer (pH 7·6) containing 0·125 M NaCl. One colostral fraction appeared homogeneous in its electrophoretic migration and was identified as an immunoglobulin by agar-gel

electrophoresis and by immunoelectrophoresis using rabbit antiserum directed against γ-globulins derived from pooled pig serum; thus, a line of identity between pig serum globulins and colostral fraction was obtained. Cross-reaction also occurred between the colostral fraction and antisera specific for human serum IgA (Hoechst). Following immunization of rabbits with the immunoglobulin-containing colostral fraction, the appearance of specific antibody in the rabbit sera was established by micro-immunodiffusion. On immuno-electrophoresis the colostral fraction formed a pattern characteristic of IgA (Vaerman & Heremans, 1970); immunodiffusion studies also showed the presence of minimal activity against another component of the colostral fraction, for a second faint precipitin line was seen which persisted following absorption of the antisera used with pig serum globulins.

IgG from rabbit pre-immune and immune sera was isolated using DEAE-cellulose (Reif, 1969). Immunological cross-reactivity due to antigenic determinants shared with other pig immunoglobulins was removed by absorption with IgM prepared by ultrafiltration of serum using a Diaflo XM-300 membrane. IgG from rabbit sera was conjugated with fluorescein isothiocyanate following the techniques outlined by Nairn (1969). The conjugates were then purified by gel-filtration chromatography using a Sephadex G-25 column in phosphate-buffered saline (pH 7·1); absorption of the conjugates with lyophilized rat liver powder and mouse kidney homogenate was also carried out in order to reduce non-specific staining.

Tissues for immunohistochemical study were rapidly frozen in isopentane cooled in liquid nitrogen. Cryostat sections 5 μm thick were fixed immediately in either methanol or 95 % ethanol at room temperature for 30 min (Allen & Porter, 1970). After immunofluorescence staining 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 UG 1 exciter filter, a BG 38 suppression filter, and a K 430 barrier filter. The specificity of immunofluorescence staining achieved was confirmed by the use of a number of procedures in control tissues: thus staining with conjugated specific immune serum was not seen in pig tissues subjected to prior treatment with unconjugated specific immune serum but was present in preparations subjected to prior treatment with pre-immune serum. Again, pig tissues were not stained when conjugated pre-immune serum was used. Finally, sections of chicken and rat intestinal and lymphoid tissues, processed as for pig tissues, failed to stain with conjugated immune serum.

RESULTS

Histochemical observations

In both freeze-dried tissues and tissues fixed in aqueous formaldehyde PAS-positive aggregates identified as glycoprotein were seen in the glandular epithelium of the pyloric antrum, in the epithelium of Brunner's glands and in epithelial cells of the glands of the small and large intestines. The majority of epithelial cells in both the pyloric antrum and Brunner's glands were indistinguishable in respect of intensity and content of PAS-positive material, and all but a few cells seen at the fundus of some pyloric glands were packed with granules. In the intestines a pronounced staining reaction was seen in goblet cells, and PAS-positive granules were also seen

in the supranuclear region of columnar cells of intestinal glands. In columnar cells, the granules were best seen in freeze-dried preparations, but were also present in wax-embedded tissues after incubation in the presence of diastase, and were similar in distribution to electron-dense granules seen in ultrastructural preparations of pig intestinal epithelium.

Numerous cells identified as plasma cells were seen in the lamina propria of the intestines and in some the cytoplasm was markedly PAS-positive. Comparatively few plasma cells were seen in the pyloric antral mucosa and in the connective tissues investing secretory units of Brunner's glands.

Immunohistochemical observations

Intestinal mucous membrane

In all areas of intestine studied, intense immunofluorescence staining was seen, both in cells identified as plasma cells in the lamina propria and in columnar epithelial cells of intestinal glands, when fluorescein-labelled rabbit antiserum directed against pig colostral IgA was used (Figs. 1–3). The distribution of plasma cells in the lamina propria was generally similar throughout the intestines, except in the ileum, where they showed a striking increase in numbers in relation to Peyer's patches (Fig. 3); here they were also present in smaller numbers in the peripheral part of the submucosal lymphoid nodules. Most plasma cells in the intestines showed homogenous cytoplasmic staining but some contained discrete fluorescent granules.

Most columnar epithelial cells of the intestinal glands showed immunofluorescence staining which was usually more pronounced in the apical region but in some cells was uniform throughout the cytoplasm. In general, intensity of staining in columnar epithelial cells was greater in the colon than in the duodenum, and greater in the duodenum than in the ileum. In all areas examined, staining in surface cells was less obvious than in columnar cells of the intestinal glands. Goblet cells throughout the intestines were conspicuously devoid of immunofluorescence staining. Control staining of serial sections also failed to show fluorescence in either epithelial cells or cells of the lamina propria.

Pyloric glands and Brunner's glands

With rabbit antiserum directed against pig colostral IgA, immunofluorescence staining was seen in only a few plasma cells in interglandular tissues in the pyloric antrum and at the periphery of lobules of Brunner's glands. Glandular epithelium in both areas and surface epithelial cells in the pyloric antrum remained unstained.

Ultrastructural observations

Intestinal mucous membrane

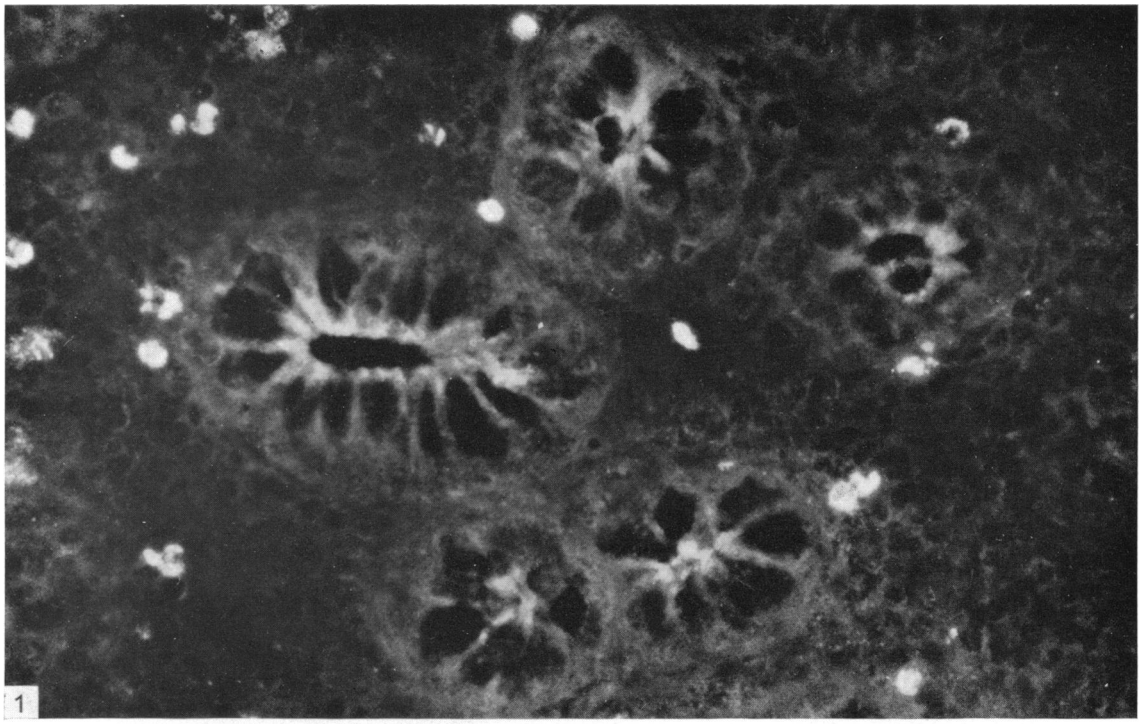
In the colon, the predominant epithelial cells comprised goblet cells and columnar cells. Goblet cells were not appreciably different in form from those in the large intestine of man, monkey, and sheep; mucinogen granules aggregated in the supranuclear region showed considerable variation in size, shape and electron density, and in some preparations the cytoplasm bounding the mucinogen mass was electron-dense and especially prominent.

In some areas of colon almost all columnar epithelial cells in the intestinal glands contained numerous small spherical electron-dense inclusions up to 1 μm in diameter and predominantly supranuclear in position (Figs. 4, 6); at high resolution it was possible to distinguish a membrane bounding some of the granules. Electron-lucent vesicles, spherical or irregular in shape and some containing a more darkly stained filamentous core, were also seen in the supranuclear region of many of the cells; some vesicles at the apical margin of the cells were in the process of discharging contents similar in texture and staining reaction to the surface coat enveloping neighbouring microvilli. Such cells also contained inclusions which were intermediate, in form and staining reaction, between dense granules and discharging vesicles, and appeared to represent stages in the transformation of granules into secretory products. As in the case of human, monkey and sheep colon, the use of the term *secretory granule* for the range of inclusions present in columnar epithelial cells appears appropriate. The Golgi region of some columnar epithelial cells contained electron-dense and irregularly shaped inclusions which were identified as secretory granules in the process of being formed (Fig. 7). Some few columnar cells also contained infranuclear granules resembling those aggregated more profusely in the supranuclear region.

In other areas of the colon columnar epithelial cells of intestinal glands generally contained fewer supranuclear secretory granules but even in these areas most cells contained at least one such granule. Columnar cells in surface epithelium contained relatively large numbers of mitochondria in the infranuclear region, but secretory granules were generally absent. Plasma cells in the lamina propria of the colon were readily identified by their nuclear form and by their reticulum-bounded cisternae containing filamentous electron-lucent material. In a number of plasma cells membrane-bounded granules, resembling the secretory granules found in columnar epithelial cells, were found between cisternae and also in the Golgi region (Fig. 5).

Columnar epithelial cells with supranuclear secretory granules resembling those found in the colon were also seen in intestinal glands of the ileum and duodenum. In the ileum, the cells were fewer in number and contained fewer granules than in most areas of the colon. In the duodenum, cells with supranuclear secretory granules were especially prominent at the fundus of intestinal glands; the granules were generally less densely stained than those in the colon and in many preparations secretory granules which were largely intact were also seen in the lumen of intestinal glands (Fig. 8).

Figs. 1, 2, 3. Fluorescein-conjugated antiserum directed against pig colostral IgA has been applied to methanol-fixed sections of pig intestines. Fluorescence in epithelium is located mainly in the apical cytoplasm of columnar cells, being particularly prominent in glands of the colon (Figs. 1, 2), and is absent from the mucinogen mass of goblet cells. Plasma cells throughout the lamina propria are also stained, and are especially numerous in relation to lymphoid follicles of Peyer's patches (Fig. 3). Groups of autofluorescent cells can also be seen in the submucosal lymphoid nodule and in its mucosal extension. Fig. 1, $\times 500$; Fig. 2, $\times 180$; Fig. 3, $\times 72$.



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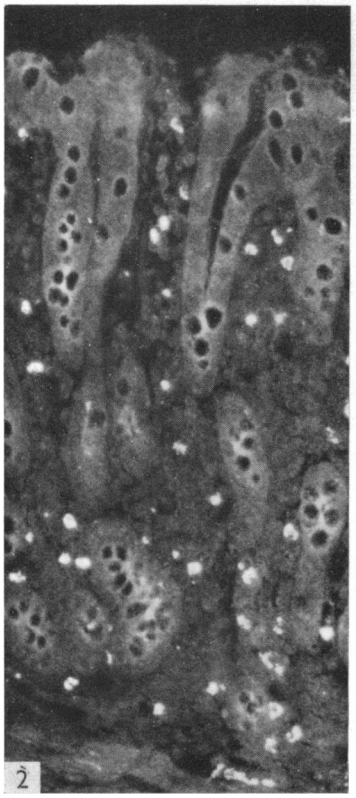

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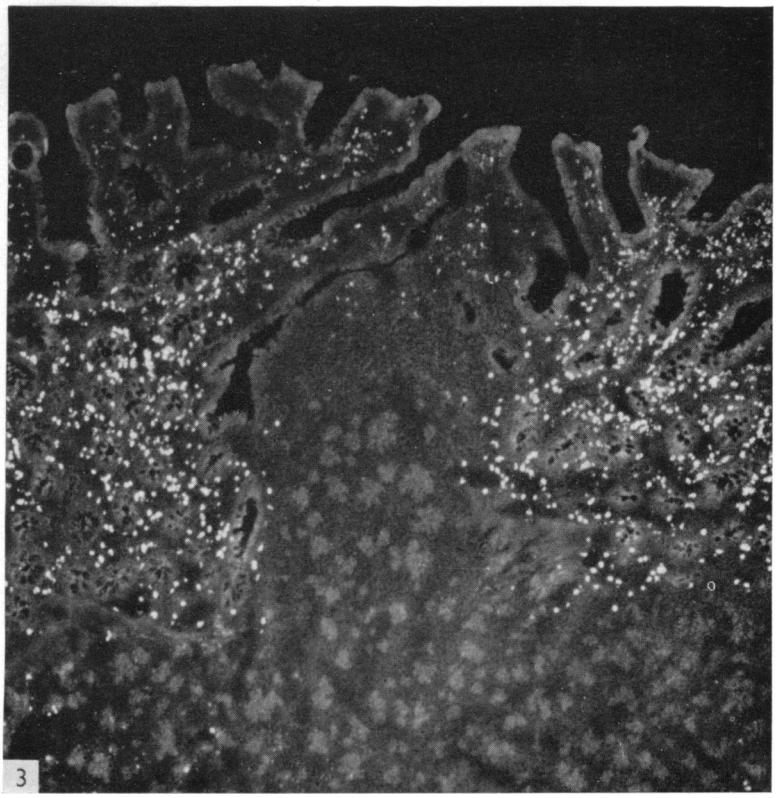

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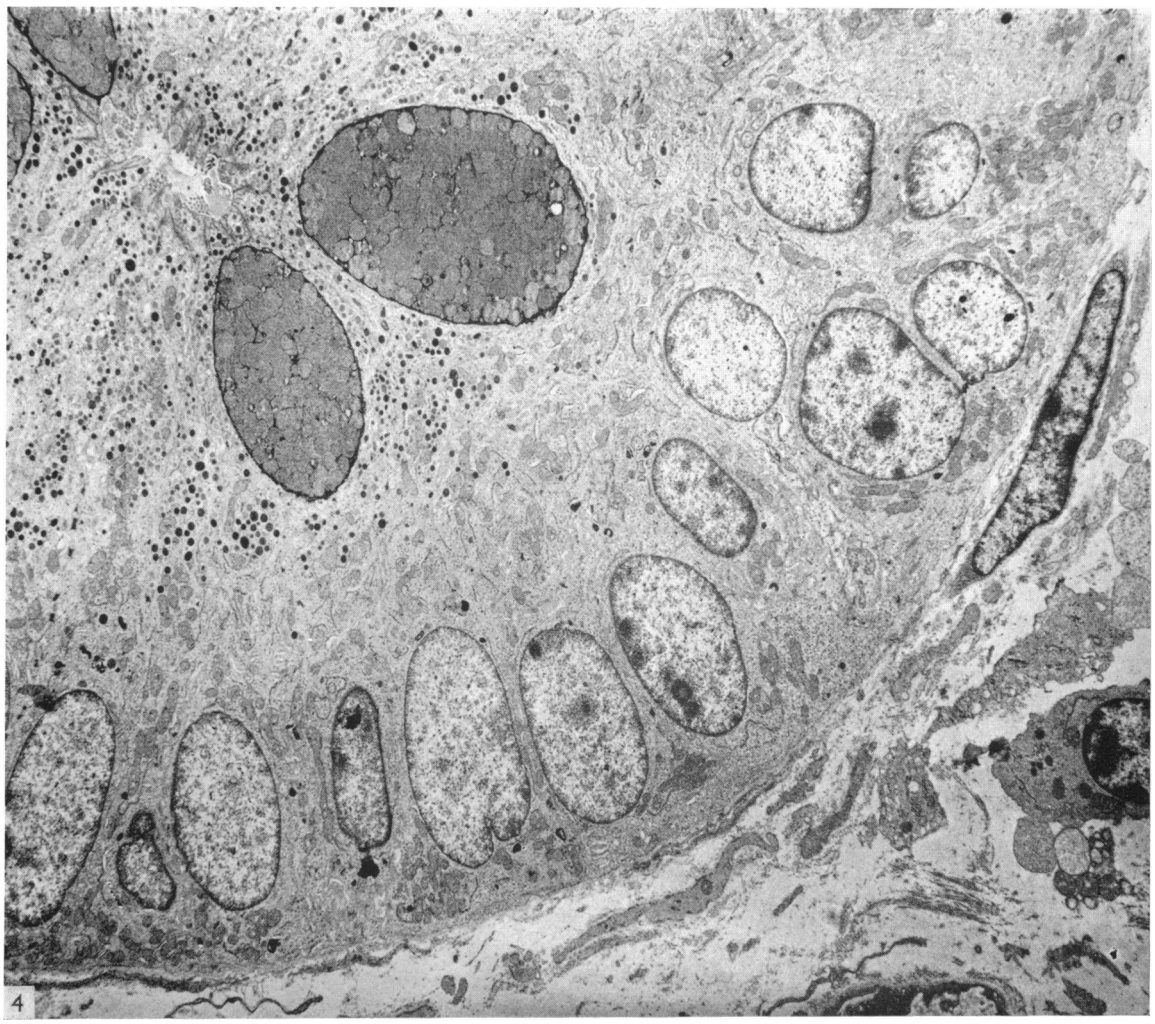


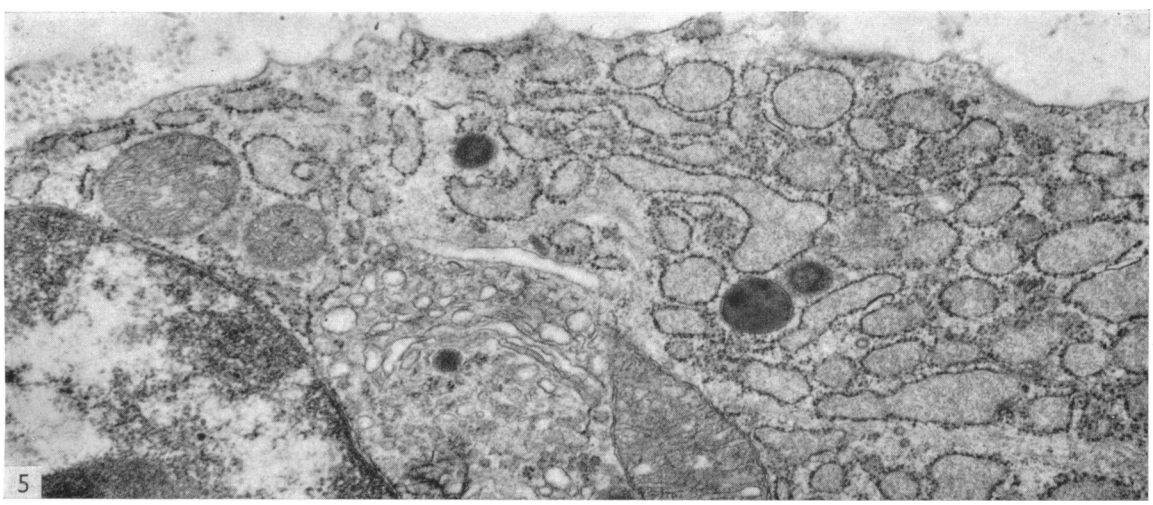
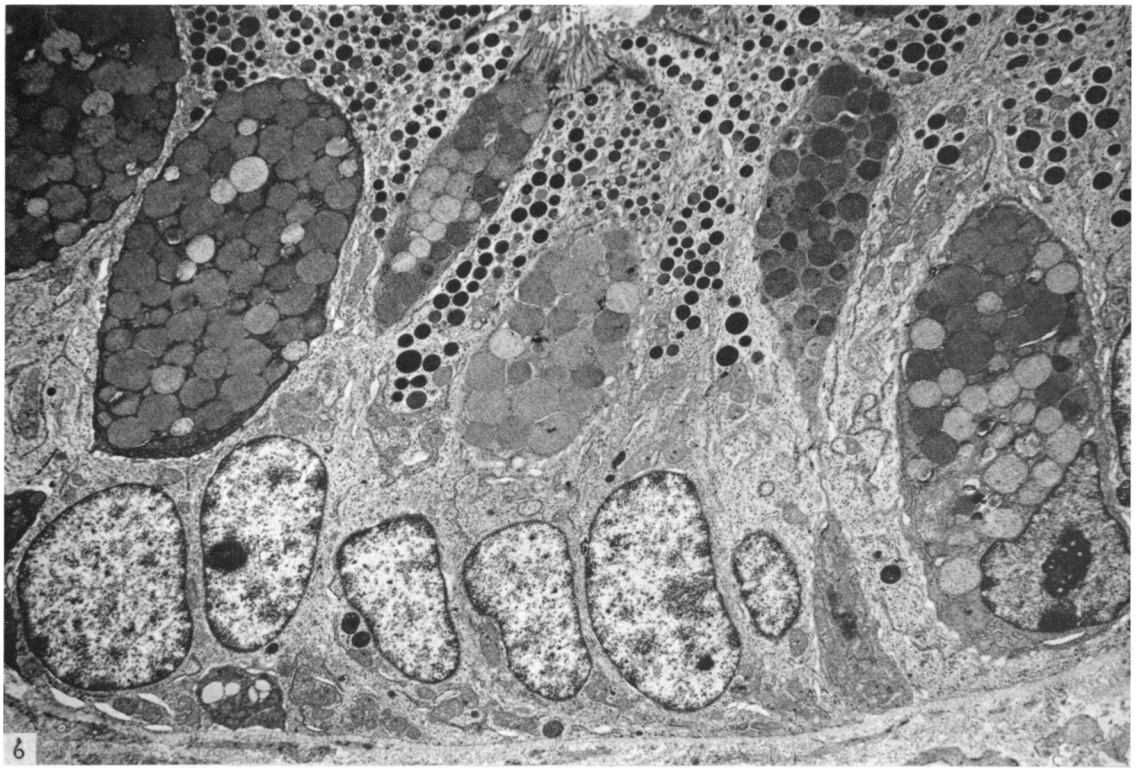


Fig. 4. Pig colon. Section of an intestinal gland lined by goblet cells and by columnar cells containing electron-dense granules. ×2800.

Fig. 5. Pig colon. Plasma cell of the lamina propria containing several membrane-bounded and electron-dense inclusions located between cisternae and in the Golgi zone. ×26600.



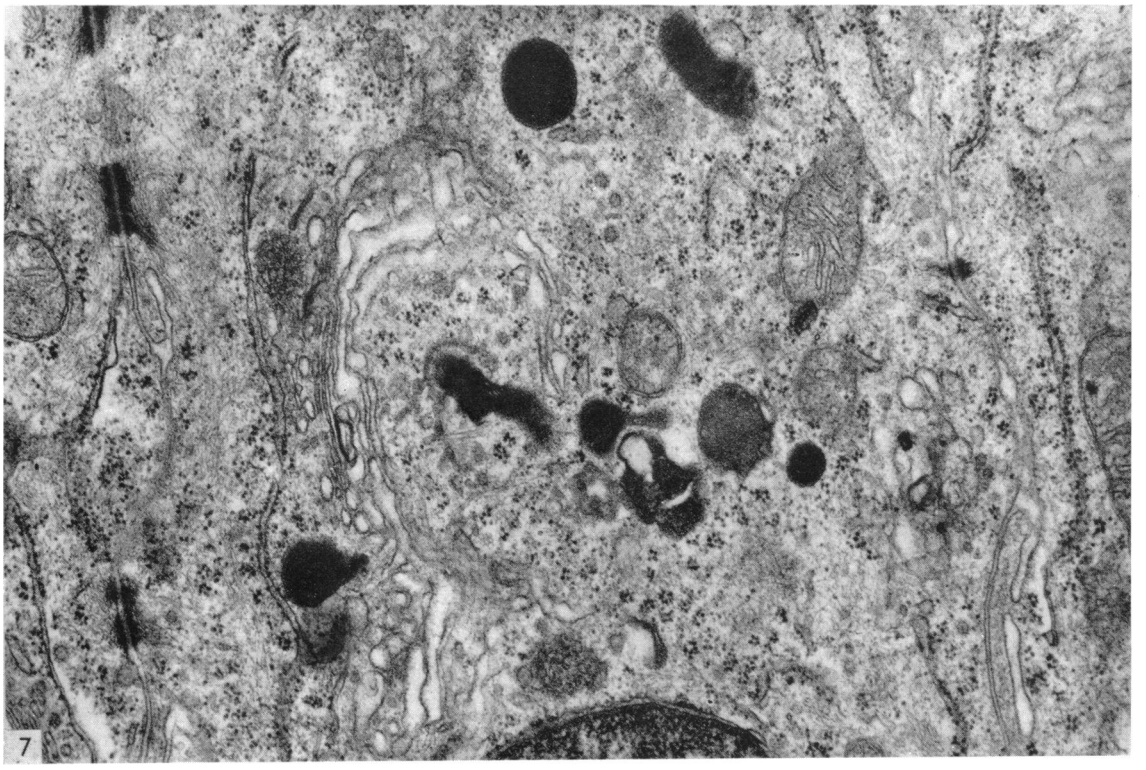
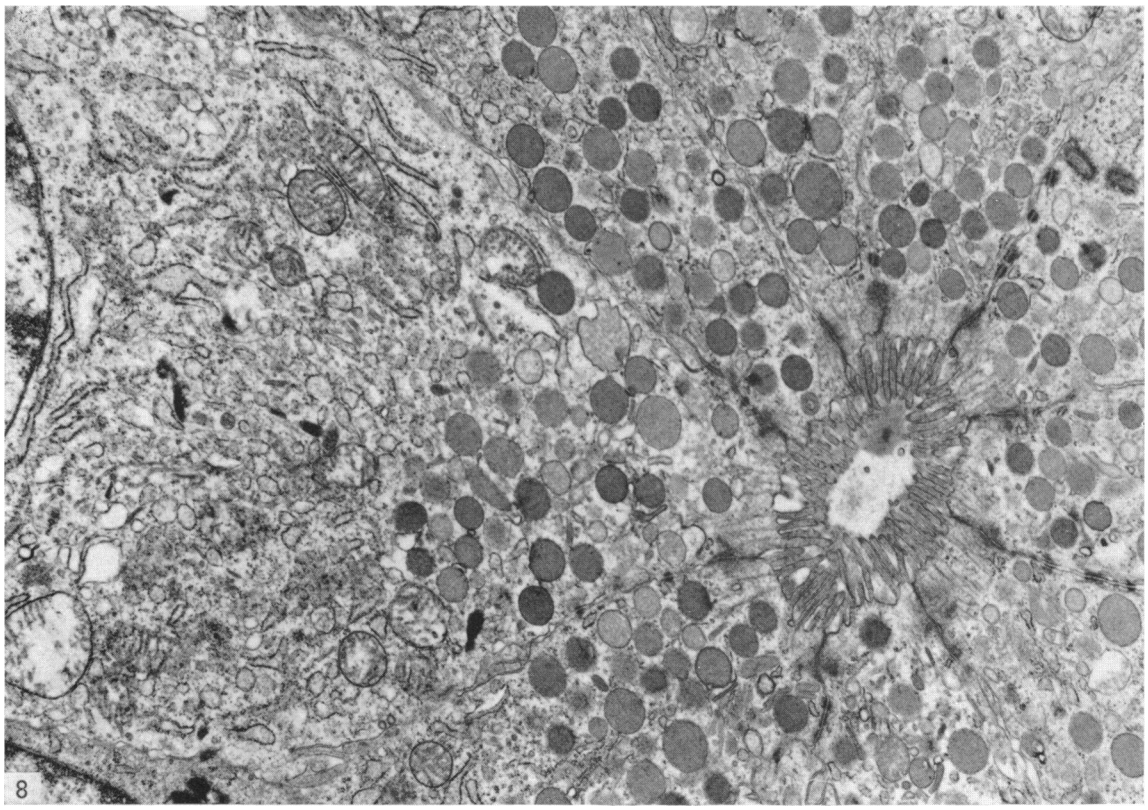


Fig. 6. Pig colon. Fundus of an intestinal gland. Numerous secretory granules can be seen in the cytoplasm of the columnar epithelial cells. × 3000.

Fig. 7. Pig colon. Columnar cell lining an intestinal gland. The membrane-bounded electron-dense inclusions in the Golgi region are identified as early secretory granules. × 28000.



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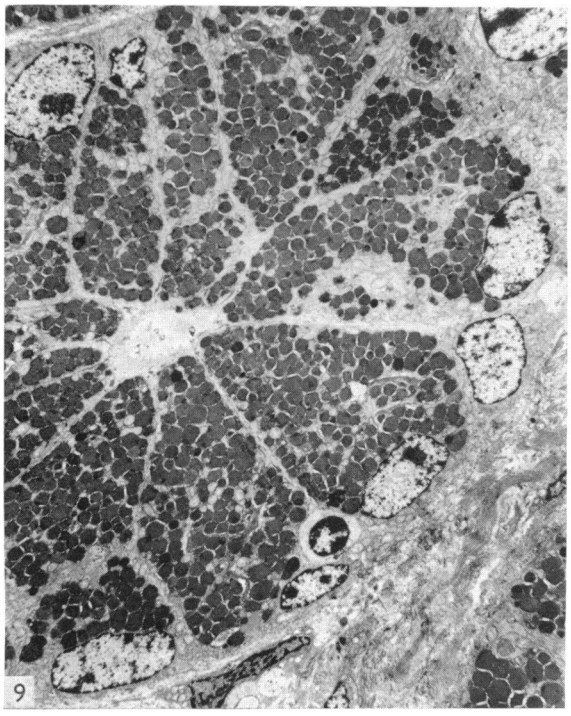

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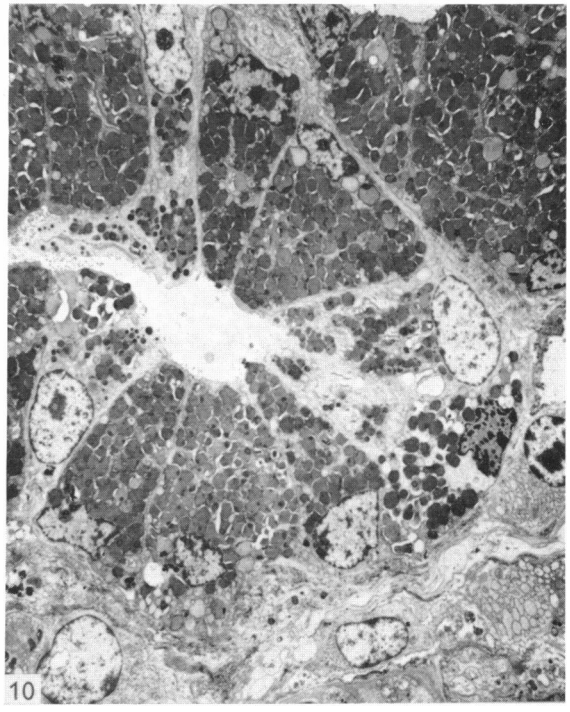

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Pyloric glands and Brunner's glands

The majority of glandular epithelial cells in both areas were packed with supranuclear secretory granules. Individual granules were appreciably larger than those in intestinal epithelium; they had a diameter of up to 2·5 μm and were relatively homogenous in appearance (Figs. 9, 10).

DISCUSSION

Three distinct groups of epithelial inclusions, each containing glycoprotein demonstrable with the PAS technique, were identified in the tissues examined in the present study. Epithelial cells of pyloric glands and Brunner's glands, intestinal goblet cells, and columnar epithelial cells of intestinal glands, respectively, all contain secretory granules with a distinctive and easily recognizable form in both light- and electron-microscopic preparations. Secretory granules in goblet cells and in the epithelium of pyloric glands and Brunner's glands are relatively large and form a major cytoplasmic constituent; unlike secretory granules in columnar epithelial cells of intestinal glands, they are not stained by fluorescein-labelled rabbit antiserum directed against pig colostral IgA and, whatever their role as secretions or components of secretions, it is clear that they are not involved in the elaboration of the IgA component of secretory immunoglobulin. This view is strengthened by the observation that relatively few plasma cells were seen in the mucosa of the pyloric antrum or in Brunner's glands.

The immunohistochemical techniques used in the present study have not enabled 'secretory piece', known to be a component of secretory immunoglobulin and believed by Tourville, Adler, Bienenstock & Tomasi (1969) to be present in goblet cells, to be identified in the tissue preparations studied. Some minor factor, additional to IgA and possibly representing 'secretory piece', was detected in the immunoglobulin-containing colostral fraction used to prepare an antiserum for immunofluorescent staining, but whatever its nature its presence did not lead to staining of the aggregates of mucus present in goblet cells.

In respect of their form and staining reactions, including immunohistochemical reactions, the columnar epithelial cells of the pig colon resemble closely those found in the human rectum (Schofield & Atkins, 1970). The cells thus appear to represent sites at which IgA, presumably of plasma cell origin, is accumulated prior to its discharge into the lumen of intestinal glands. As in the case of the human intestine, the contents of discharging secretory granules and the glycoprotein enteric surface coat enveloping microvilli have a similar appearance and the possibility thus exists that IgA has an important role as a component of the enteric surface coat in intestinal defence mechanisms. In the present study it was possible to examine under

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Fig. 8. Pig duodenum. Fundus of an intestinal gland of the mucous membrane. Secretory vesicles and granules varying in electron density are abundant in the apical cytoplasm. × 8000.

Figs. 9, 10. Pig pyloric antrum (Fig. 9) and Brunner's gland (Fig. 10). Glandular epithelial cells in both areas contain numerous large secretory granules. Very few plasma cells are seen in the adjacent connective tissue. Fig. 9, × 1100; Fig. 10, × 1100.

appropriate conditions the distribution of IgA aggregates at a number of levels in the gastrointestinal tract. It is believed that variations in the amount of a substance present in tissues and reacting with specific fluorescein-labelled antiserum are not necessarily represented by comparable variations in intensity of immunofluorescence staining (Nairn, 1968). It follows that estimates of the relative amounts of IgA at different levels of gut based on relative intensity of the staining reactions obtained may not be entirely valid. Nevertheless, if one ultrastructural correlate of the immunofluorescence staining achieved in pig intestinal epithelium is the presence of intraepithelial secretory granules, a possibility which requires further study using cell fractionation techniques, it would appear from both immunohistochemical and ultrastructural observations that IgA aggregates in intestinal epithelium occur in greater amounts in colon and duodenum than in the ileum. The significance and the morphological consequences of the observation that IgA-containing plasma cells are more numerous in the region of Peyer's patches than elsewhere in the gut have yet to be established. It is possible, for example, that Peyer's patches and possibly other lymphoid aggregates in the gut are sites at which secretory immunoglobulin is liberated in greater amounts than elsewhere.

It has not been possible to identify ultrastructurally those plasma cells which were shown to contain IgA. The dense granules found within some of the plasma cells in the lamina propria are strikingly similar in form to those found in columnar epithelial cells and like them appear to be formed in the Golgi region. Allen & Porter (1970) have shown that both IgA and IgM are present in the mucous membrane of the small intestine of the pig and further studies using labelled antibody may enable the categories of cells containing each class of immunoglobulin to be identified.

SUMMARY

Selected areas of the gastro-intestinal tract of the pig have been studied using histochemical, immunohistochemical and ultrastructural techniques. Intra-epithelial glycoprotein aggregates were identified in glands of the pyloric antrum, in Brunner's glands, and in intestinal mucous membrane where some plasma cells were also PAS-positive. Intra-epithelial IgA was found only in columnar cells of intestinal mucous membrane and was most pronounced in colon and duodenum, regions where the columnar cells contain numerous granular and vesicular inclusions which may represent aggregates of secretory immunoglobulin. IgA was also seen in plasma cells of intestinal mucous membrane some of which contained granules resembling those seen in columnar epithelium. IgA-containing plasma cells were more numerous in the region of Peyer's patches than elsewhere in the gut.

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