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# Studies on the G cells of the pyloric mucosa, the probable site of gastrin secretion

E. SOLCIA, G. VASSALLO, AND C. CAPELLA

*From the Institute of Pathological Anatomy, the University of Pavia, Italy*

Previous histological and histochemical studies on pancreatic tumours associated with the Zollinger-Ellison syndrome have shown that they are mainly composed of endocrine cells which, under proper conditions, display argyrophilia, toluidine blue metachromasia, and pseudoisocyanin fluorescence (Cavallero and Solcia, 1965; Potet, Martin, Thiery, Bader, Bonfils, and Lambling, 1966; Cavallero and Solcia, 1968). Since islet D cells of the normal human pancreas display the same staining properties (Solcia and Sampietro, 1965a; Epple, 1967; Fujita, 1968; Solcia, Vassallo, and Capella, 1968), it was postulated that these tumours originate from the neoplastic growth of D cells (Cavallero, Solcia, and Sampietro, 1967).

It has been well established that Zollinger-Ellison tumours produce and release a substance displaying all the chemical and physiological properties of gastrin, the polypeptide hormone identified in the antropyloric mucosa (Gregory, Tracy, French, and Sircus, 1960; Gregory and Tracy, 1964b; Gregory, Grossman, Tracy, and Bentley, 1967). Therefore, we considered the possibility that normal D cells may also secrete gastrin, and that endocrine cells staining exactly as the islet D cells or the cells of Zollinger-Ellison tumours may be present in the antropyloric mucosa and may be the site of gastrin secretion. In fact, in the antropyloric mucosa a type of endocrine cell has been found (the G cell), which was darkened by silver, stained metachromatically by toluidine blue, and showed white fluorescence with pseudoisocyanin (Solcia and Sampietro, 1965b; Solcia *et al*, 1968). The histochemical and ultrastructural features of this cell were in keeping with an internal secretion akin to a protein or peptide (Solcia, Vassallo, and Sampietro, 1967).

In the present paper the staining, ultrastructural, and distributive patterns of endocrine cells in the antropyloric mucosa of man and some mammals have been carefully reinvestigated with the aim of a better understanding of their morphological and functional significance. Comparative observations have been made on the pancreatic islets, with special reference to islet D cells, as well as on the endocrine cells of the fundic and duodenal mucosa.

## MATERIAL AND METHODS

Samples of the gastroduodenal mucosa and pancreas have been taken from anaesthetized guinea pigs, rabbits, dogs, cats, rats, mice, monkeys (*Macacus rhesus*), and man, and immediately processed for light and electron microscopy. For light microscopy the samples were fixed in Bouin, glutaraldehyde or a glutaraldehyde-picric acid-sodium acetate mixture, cut at 4 $\mu$ and stained by the following methods: Davenport's silver impregnation (Hellerström and Hellman, 1960), Bodian's silver impregnation (McManus and Mowry, 1960; Grimelius, 1964), toluidine blue or pseudoisocyanin following HCl-hydrolysis (Solcia *et al*, 1968), Masson-Hamperl's argentaffin reaction, diazonium reaction with Fast-black K and xanthydrol test for 5-hyroxytryptamine-producing enterochromaffin (Ec) cells (Solcia and Sampietro, 1967), the phosphotungstic acid-haematin (PTAHn) method for basic proteins (Terner, Gurland, and Gaer, 1964) with or without previous oxidation in acid permanganate, the xanthydrol test for islet A cells (Cavallero, Solcia, and Sampietro, 1968), the aldehyde fuchsin (Scott, 1952) and oxidation-pseudoisocyanin methods (Schiebler and Schiessler, 1959) for islet B cells.

For electron microscopy small pieces were fixed in 3% glutaraldehyde or in the Karnowsky mixture, refixed in osmium tetroxide and embedded in Epon 812 or in an Epon-Araldite mixture. Sections were double-stained with uranyl acetate and lead citrate.

## RESULTS

GASTRODUODENAL MUCOSA Several endocrine cells have been found to be scattered in the gastroduodenal mucosa of the different species under study. The general problems related to their identification have been fully considered elsewhere (Vassallo, Capella, and Solcia, 1969); in the present paper findings concerning antropyloric mucosa will be extensively described. Two cell types have been fully characterized: the argentaffin or enterochromaffin (Ec) cell and the G cell. Other endocrine cells, clearly differing from both Ec and G cells, have also been found.

*Ec cells* The Ec cells were selectively detected under light microscopy by their reactivity to the argentaffin, diazonium, and xanthydrol tests. Moreover, they were positive for the Bodian and

Davenport argyrophil methods,s tained black-blue to blue-violet by toluidine blue following HCl hydrolysis, sometimes showed some blue staining by the PTAHn method and displayed various degrees of fluorescence under ultraviolet light when stained by the HCl-pseudoisocyanin method. At electron microscopic examination Ec cells showed irregular, highly osmiophilic granules, a well developed Golgi complex, small mitochondria, scattered free ribosomes, and few iuxtanuclear arrays of endoplasmic reticulum. The size of the secretory granules was quite variable (from 150 to 400 mμ) according to the species examined and the tract of the gastroduodenal mucosa; in particular they were smaller in the gastric Ec cells than in duodenal ones (Fig. 7).

The distribution of Ec cells in the gastroduodenal mucosa was variable according to the species examined. In the pyloric mucosa they were quite numerous in the dog only, being relatively few in the other species.

*G cells* The G cells stained red to violet by the HCl-toluidine blue method and invariably showed a white fluorescence by the HCl-pseudo-isocyanin method (Fig. 1). By coupling the HCl-toluidine blue method to the diazonium reaction, it was easy to distinguish in the same section black-stained Ec cells from red-stained G cells. Following the diazonium-HCl-pseudoisocyanin technique, G cells displayed fluorescence under ultraviolet light whereas Ec cells completely lacked such reaction. G cells of the guinea pig, cat, and man were also positive with the Davenport argyrophil method, but were negative with the argentaffin reaction. They were also unreactive with the PTAHn, aldehyde fuchsin, and oxidation-pseudoisocyanin methods.

Under electron microscopic examination this cell type exhibited various amounts of secretory granules mainly grouped at the infranuclear portion of the cell. These granules were enveloped by a thin membrane which was usually rounded. The internal core appeared in some instances small (150 to 200 mμ), relatively dense and homogeneous, in some others larger (250 to 300 mμ), with a low electron opacity and evidently granular; all the intermediate stages were present. The first pattern was more frequently found in the guinea pig, the second one in the dog, rat, and rabbit, intermediate features in man, cat, and monkey. But, these changes in the granules occurred also in the same animal, both in different cells and in the same cell (Figs. 3, 5, 6, and 11). Different functional stages of cells and granules might explain such variability of morphological patterns.

The ergastoplasm of G cells was well developed, particularly in those storing few granules; a prominent Golgi apparatus was generally located in a supranuclear site; mitochondria, lysosomes, and small vesicles were scattered in the cytoplasm. A thin cytoplasmic process was usually seen to reach the luminal surface of the pyloric glands in a narrowed area; here, it was frequently covered, at least in such species as guinea pig, rabbit, and man, by a tuft of microvilli. As a rule the G cell, which at its base was directly applied on the basal membrane of the glands, was closely connected to the adjacent exocrine cells by desmosomes and terminal bars in its apical portion, while such junctions did not occur in the rest of the lateral surface, where extracellular spaces and canaliculi were observed (Figs. 4 and 6).

Large numbers of G cells were found in the antropyloric mucosa of all species examined; as a rule, they were more numerous in the pylorus, and progressively decreased from this site to the mucosa adjacent to the corpus. Similar cells were found in the fundic and duodenal mucosa of some species, particularly in the neighbourhood of the pyloric mucosa. Their concentration was very low in comparison with that of pyloric G cells, to which it is not clear whether they should be related.

In species having a thin mucosa, as for instance in the mouse, guinea pig, and rabbit, G cells were found in the deeper half of the pyloric glands. In the dog and cat, they were grouped mainly in the middle third of the mucosa, scattered between the deeper mucous neck cells and the more superficial mucoid cells, although, particularly in the dog, they occurred in the deeper third also. A similar distribution was observed in man, although as the zone of mucoid cells is higher in man, the human G cells were slightly more superficial than in the dog. As a rule G cells were very scarce in the epithelium lining the gastric surface and gastric pits.

*Other endocrine cells* These cells, clearly differing from both Ec and G cells, showed morphological features different from one species to another and from one portion of the gastrointestinal mucosa to another. Thus, it appears doubtful how many cell types they represent and what is the relationship between the cells of different species. (A detailed description of such cells has been given elsewhere by Vassallo *et al*, 1969.) They were generally negative for diazonium and argentaffin reactions; partly they stained red-violet to blue-violet by HCl-toluidine blue and showed fluorescence by HCl-pseudoisocyanin and partly gave argyrophilia by Bodian's or Davenport's methods; some of them stained blue by the PTAHn method.

Electron microscopically various kinds of cells have been observed. Some cells, which have been previously described by Orci, Pictet, Forssmann, Renold, Rouiller (1968), showed round, dense granules resembling those of A cells in the pancreatic

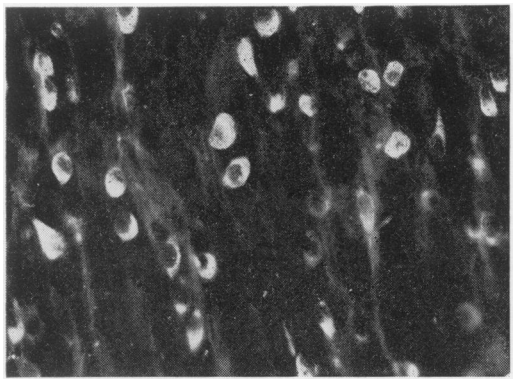

FIG. 1.

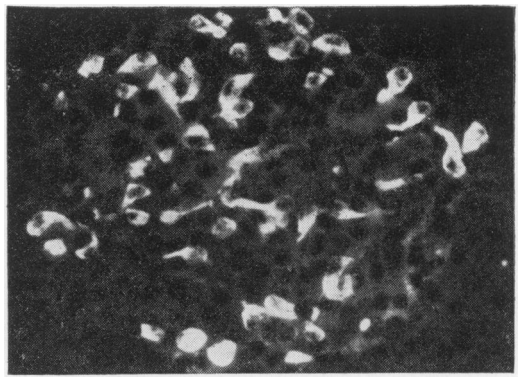

FIG. 2.

FIGS. 1 and 2. *Non-Ec endocrine cells (mainly G cells) of the guinea pig pyloric mucosa (Fig. 1) and D cells of a guinea pig pancreatic islet (Fig. 2) showing white fluorescence under ultraviolet light. Diazonium-HCl-pseudoisocyanin method.* × *350* and × *300.*

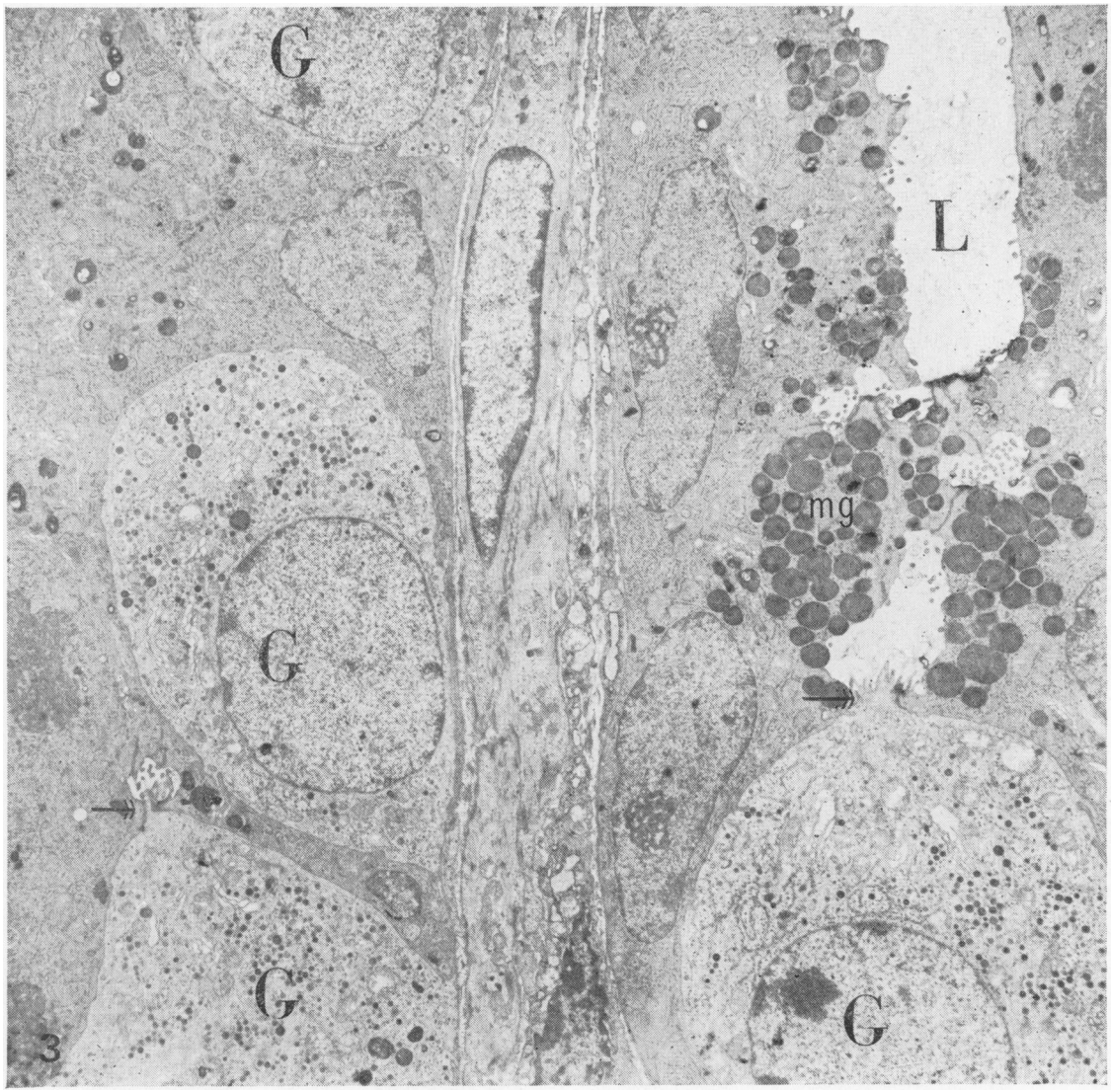


FIG. 3. *Four G cells in the guinea pig pyloric mucosa; two of them reach the glandular lumen (L) at arrows. mg = mucous granules.* × *5,000.*

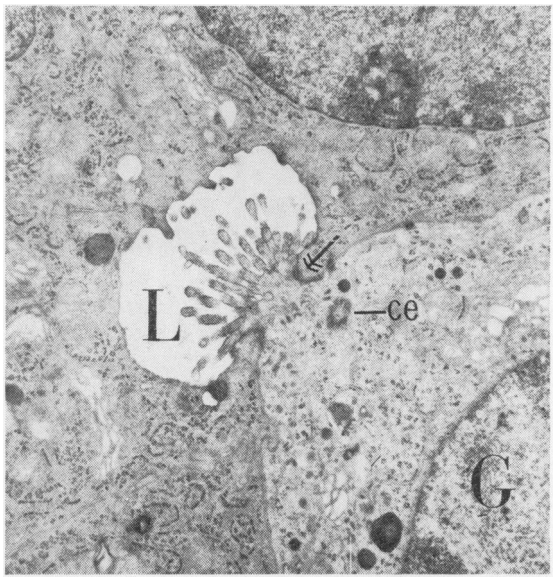


FIG. 4. *Guinea pig G cell showing a tuft of microvilli spreading in the lumen of a pyloric gland. ce = centriole; junctional complex at arrow. × 9,000.*

FIG. 5. *G cell of the dog pyloric mucosa. × 15,000.*

FIG. 6. *Human G cell with basally situated granules lying on the basal lamina of a pyloric gland. Cp = capillary vessel. × 11,000.*

FIG. 7. *Enterochromaffin (Ec) cell with dense irregular granules in the human antral mucosa. Ox = oxyntic cell. × 11,000.*

FIG. 4.

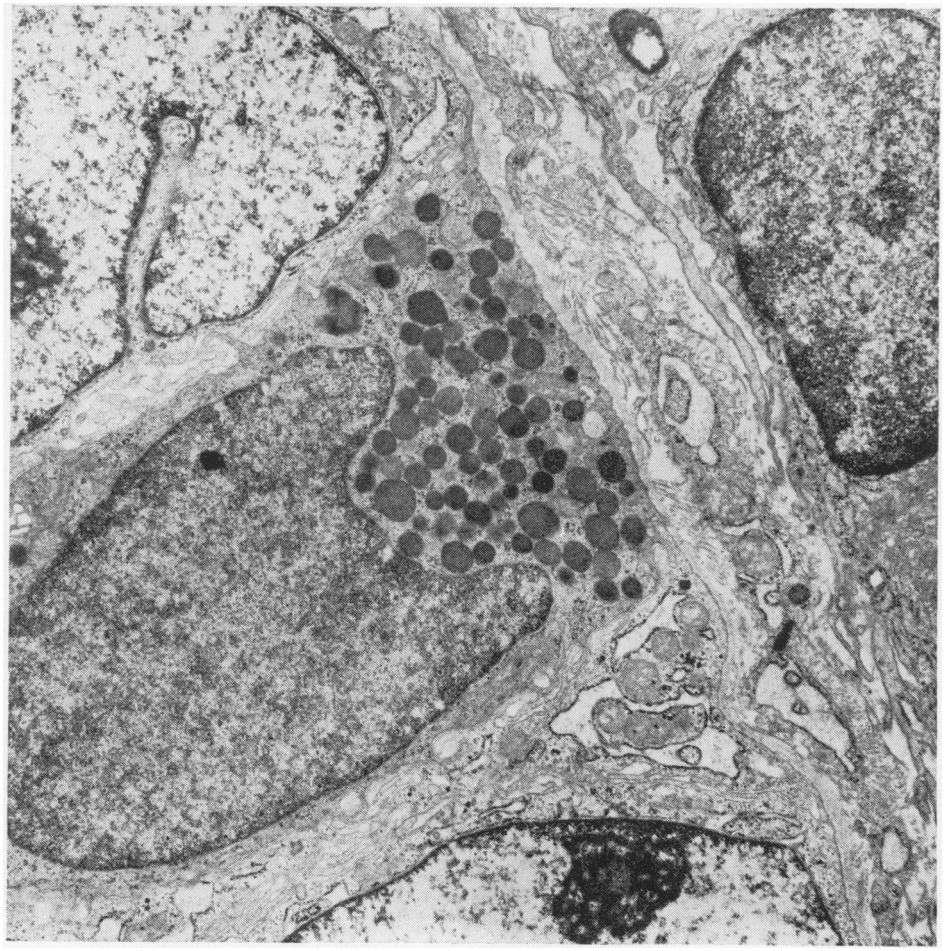

FIG. 6.

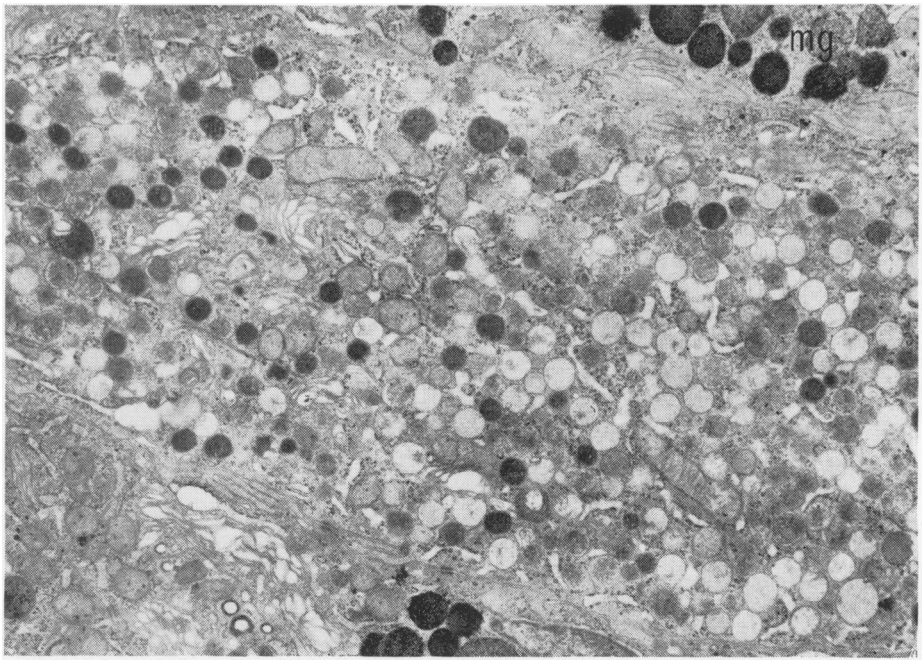


FIG. 5.

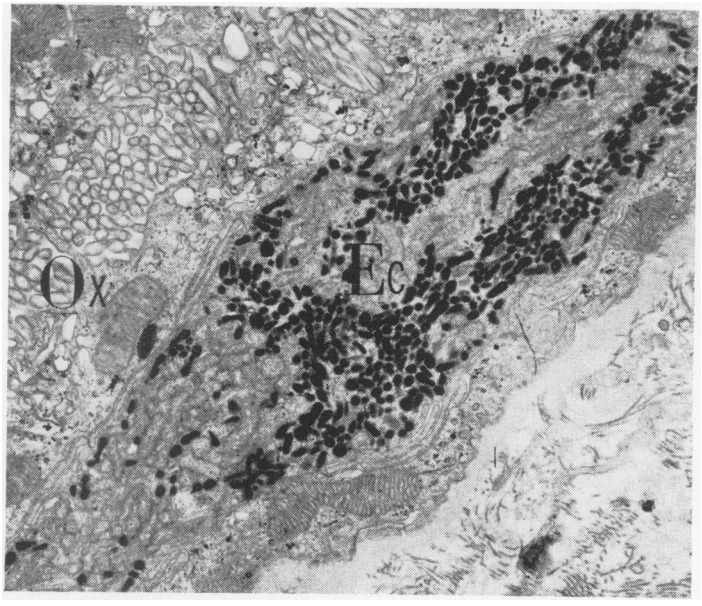


FIG. 7.

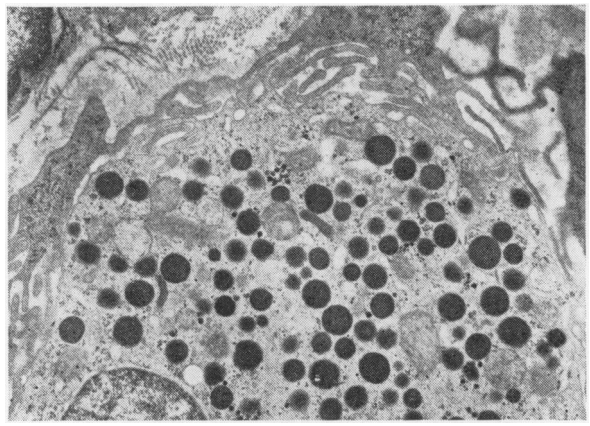

FIG. 8. *Endocrine cell with large dense granules in the human gastric mucosa.* × *11,000.*

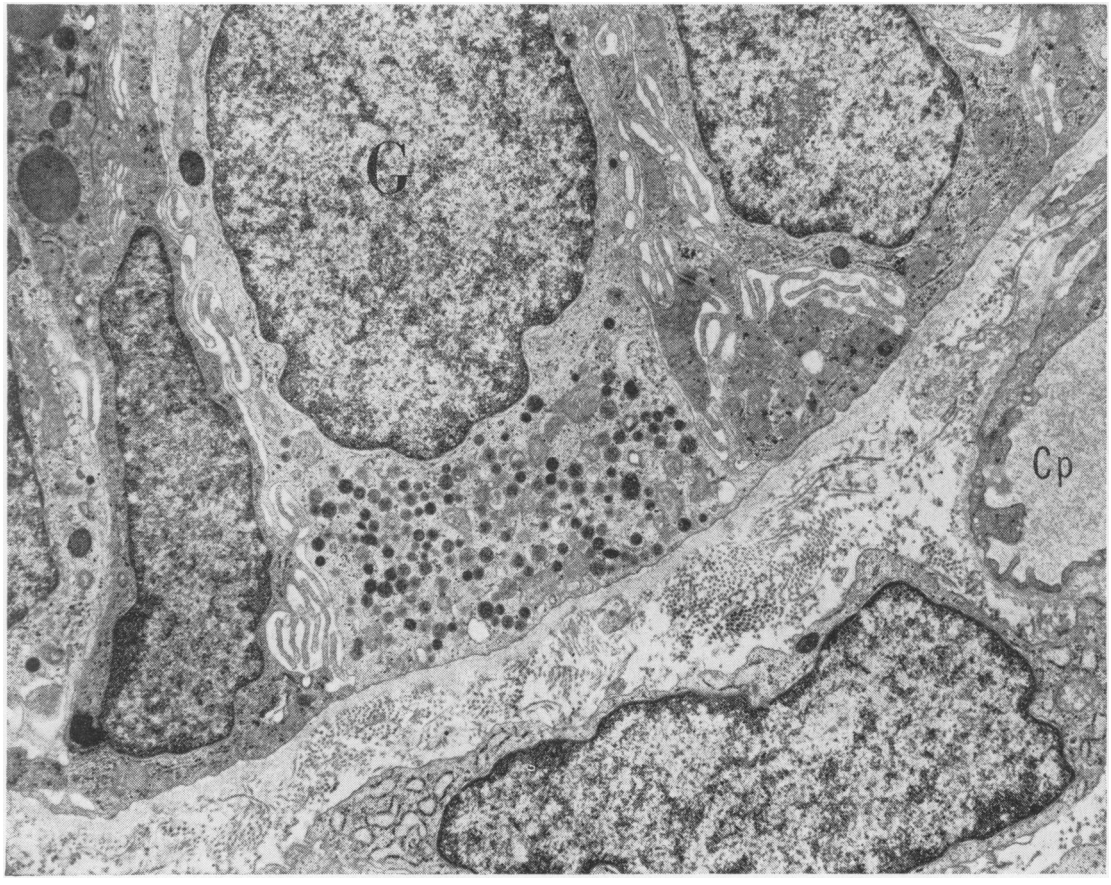


FIG. 9. *Cell with basally situated large granules of relatively low density lying on the basal lamina of a human pyloric gland.* × *11,000.*

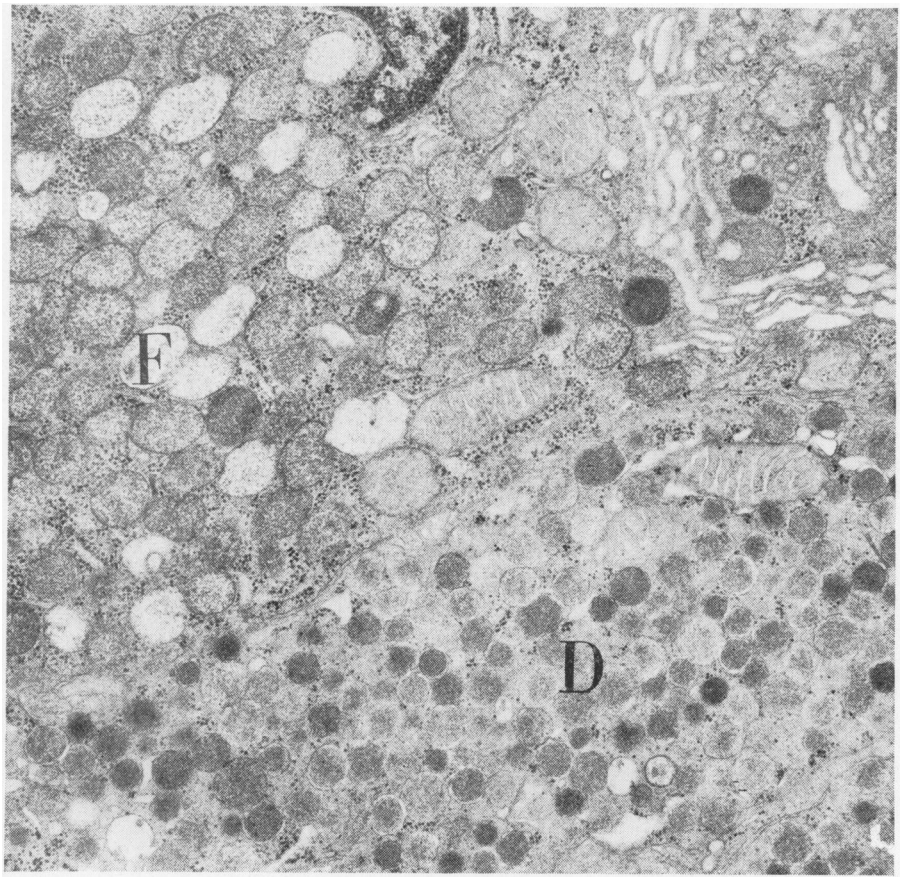


FIG. 10. *F and D cells of a pancreatic islet in the dog uncinate process. × 25,000.*

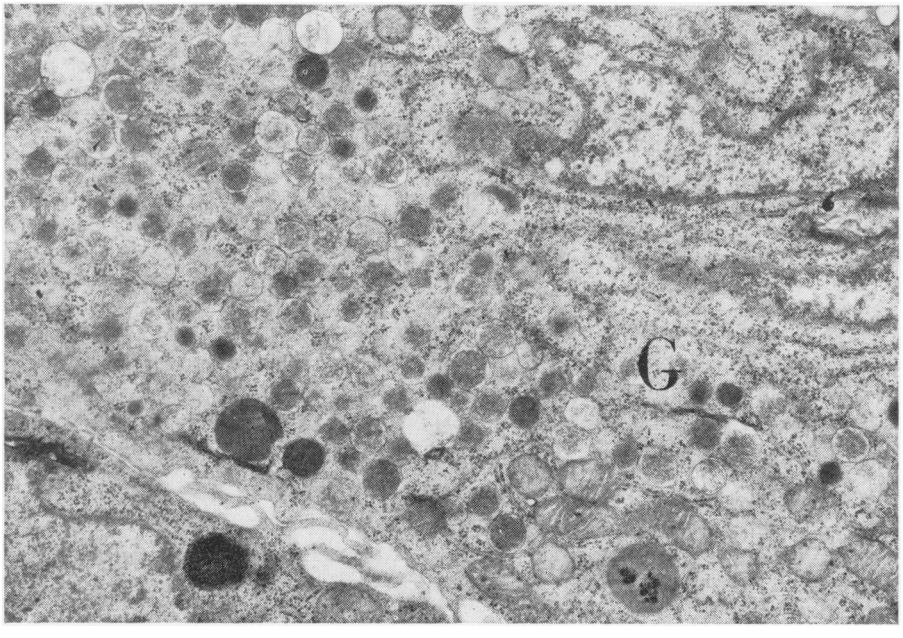


FIG. 11. *G cell of the dog pyloric mucosa: compare its granules with those of islet cells in Figure 10. × 25,000.*

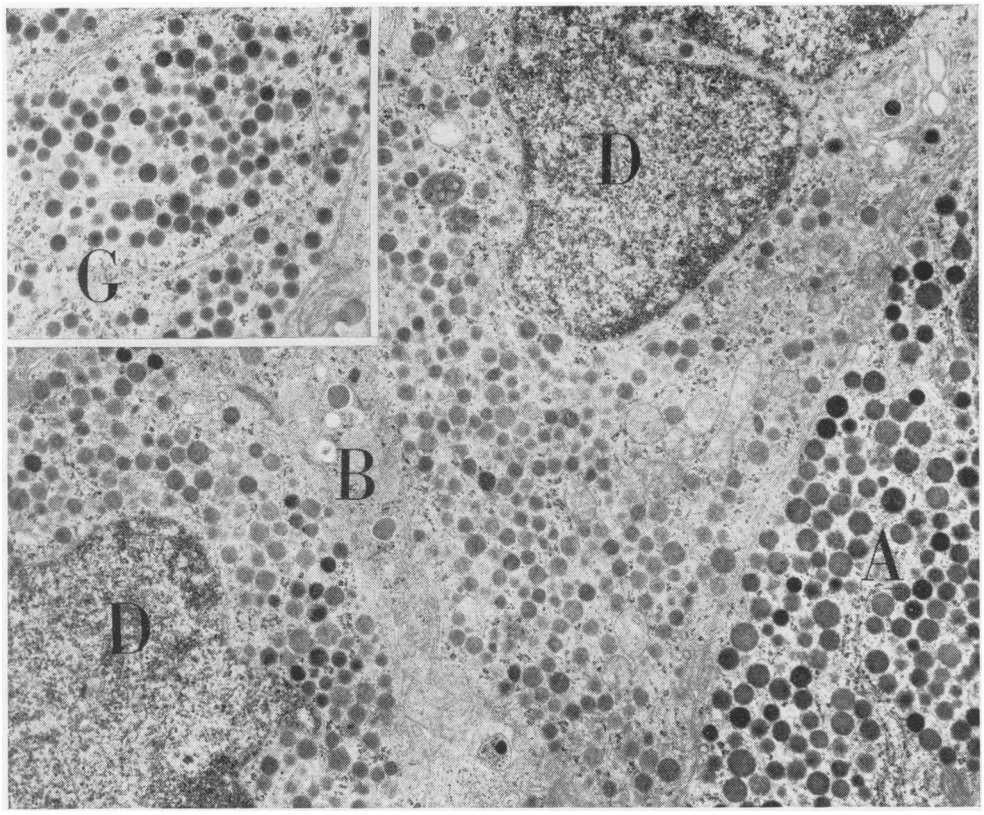


FIG. 12. *Guinea pig pancreatic islet showing an A cell, a B cell and two D cells.* × *13,000. Compare the secretory granules of D cells with those of a guinea pig G cell (insert* × *13,000.)*

islet; they were numerous in the fundic mucosa (particularly of the rat, dog, and mouse) and duodenal mucosa (cat, dog, man) but were scarce in the pyloric mucosa. Cells otherwise similar but with a more irregular profile have been found to be numerous in the rabbit and monkey stomach as well as in the monkey duodenum; these correspond to PTAH-positive cells shown by light microscopy. Cells with large, variously dense, irregular granules were numerous in the stomach of man, dog, and cat; their endocrine nature was suggested by the basal site of the granules, which were never seen near the gland lumen (Figs. 8 and 9).

Some cells showed small, round granules with a thin wall separating the external membrane from a core of moderate electron opacity; other cells stored granules with an irregular dense core eccentrically placed in clear vesicles. These cells, which were more evident in the stomach of the cat, man, rabbit, rat, guinea pig, and monkey, were also found by Forssman, Orci, and Rouiller (1968), who noted their similarity to catecholamine-storing cells.

PANCREATIC ISLETS As a whole, our results on islet A and B cells were comparable to those reported in previous papers (Solcia and Sampietro, 1965a; Caramia, Munger, and Lacy, 1965; Sato, Herman, and Fitzgerald, 1966; Cavallero and Solcia, 1968). Particular attention has been paid to the remaining islet cells.

*D cells* D cells stained red to violet with HCl-toluidine blue and showed intense white fluorescence after HCl-pseudoisocyanin (Fig. 2); they were silver-impregnated by both Davenport's and Bodian's methods. D cells failed to show blue staining after PTAHn and blue-gray staining after xanthydrol, thus clearly differing from A cells; they failed also to react to the methods for B cells and Ec cells. By electron microscopy, D cells were identified by their small (150 to 250 m$\mu$) round granules of low electron opacity; ergastoplasma, Golgi, and mitochondria were well represented. As regards both staining and ultrastructural features the secretory granules of D cells in the guinea pig and dog but not in the cat and rabbit were similar to those of

some G cells, particularly when G cells have smaller and more regular granules (Figs. 10 to 12). The D cells, which generally accounted for about 5 to 10% of all islet cells, showed different distributive patterns according to the species examined, being found at the islet periphery in the rabbit, rat, and mouse, and scattered on the whole islet in the other species; some few cells were also found to be scattered in the exocrine parenchyma.

In accordance with the observations of Munger, Caramia, and Lacy (1965), in the uncinate process of the dog pancreas a peculiar type of cell was found, the F cells, which were previously described by Bencosme and Liepa (1955) as 'X' cells (Fig. 10). They stained violet-red with HCl-toluidine blue, showed fluorescence with HCl-pseudoisocyanin, and displayed argyrophilia when Davenport's or Bodian's method was used. Under electron microscopy, they were filled with large granules (200 to 400 m$\mu$) of low electron opacity and irregular shape. The F cells were found not only in the islets but also in the wall of ducts and acini of the dog pancreas. Cells displaying ultrastructural features different from those of A, B, and D cells were occasionally found also in the cat, guinea pig, and monkey pancreas.

## DISCUSSION

In recent years, growing evidence has been accumulated from biochemical and functional studies in support of the view that gastrin really represents a definite hormone arising from the pyloric mucosa (Gregory and Tracy, 1964a; Gregory, Tracy, and Grossman, 1966; Elwin and Uvnäs, 1966). Thus, it seems probable that, as happens for many hormones so far identified, this factor is secreted in a specific endocrine cell. The present investigations, in accordance with previous studies (Solcia and Sampietro, 1965b; Solcia *et al*, 1967; Orci *et al*, 1968; Carvalheira, Welsch, and Pearse, 1968; Vassallo, Solcia, and Capella, 1968; Forssmann *et al*, 1968) clearly show that large numbers of cells displaying the features of endocrine cells are present in the pyloric mucosa of mammals. Among these, a cell type has been identified, the G cell, which occurs in large numbers in the pyloric mucosa of all species examined but infrequently in the other parts of the gastroduodenal mucosa. It displays the features of cells secreting protein hormones, *ie*, specific secretory granules of protein nature and well developed ergastoplasma and Golgi complex.

In our opinion the above findings support the hypothesis that G cells secrete gastrin, the only peptide or protein hormone so far extracted, mostly although not exclusively, from the pyloric mucosa. The staining properties of granules stored in the G cells, which suggest the presence of an acid protein with many side-chain carboxyl groups (Solcia and Sampietro, 1965b, c), appear to be in keeping with the known chemical properties of gastrin (Gregory, Hardy, Jones, Kenner, and Sheppard, 1964; Tauber and Madison, 1965). Further support for the gastrin hypothesis comes from the recent paper of Bromé, Fyrö, and Olbe (1968) dealing with the localization of gastrin in the dog and cat pyloric mucosa. The hormone was found in the basal two-thirds of the glands, with maximal concentration in the middle third. Such a distribution exactly reproduces the above reported distributive patterns of G cells in the same species.

It should be noted that, if G cells secrete gastrin, their special relationship with the gastric lumen might be of some importance for the reception of the chemical stimuli acting on gastrin secretion. Nerve endings, whose existence in the gastric mucosa has been postulated by physiologists, in our electron microscopic observations were found to be present only below the basal membrane of the glands; they lacked any direct contact with the lumen or any specialized connexion with the epithelial cells. Unpublished observations on the guinea pig and rat gastric mucosa enable us to confirm that, as first noted by Carvalheira *et al* (1968), G cells display a high cholinesterase activity; endocrine non-G cells of the rat fundic mucosa are also reactive.

Recently, we observed an increase of argyrophil-metachromatic cells in the pyloric mucosa of a few patients affected by duodenal ulcer and hypersecretion, as well as a diffuse hyperplasia of such cells in a case of postanastomotic ulcer as a sequel to a gastroenteroanastomosis (Solcia, Vassallo, and Sampietro, 1968). These findings suggest seeking an opportunity of performing an extensive light and electron microscopic study of G cells in states of altered gastric secretion.

The above reported findings show that metachromatic basophilia and argyrophilia cannot be considered peculiar properties of islet D cells and pyloric G cells. Islet F cells and part of the endocrine non-G cells in the gastroduodenal mucosa are also reactive for these methods. Thus, argyrophil-metachromatic non-D cells, although not yet detected in human islets, might also be considered, besides D cells, as the possible origin of the argyrophil-metachromatic cells found in Zollinger-Ellison tumours. Moreover, it remains uncertain whether the comparable staining properties and ultrastructural similarities that we found between islet D cells and some gastrointestinal cells have any significance from the functional point of view. On cytological and cytochemical grounds we were unable to identify unequivocally, as did Forssmann *et al* (1968), D cells of the gastrointestinal mucosa.

As regards the function of the gastrointestinal endocrine cells other than Ec and G cells, nothing is presently known, apart from the enteroglucagon hypothesis suggested by Orci *et al* (1968) for the A-like cells, and the catecholamines hypothesis suggested by Forssmann *et al* (1968) for the small granules cells. It is possible that at least some of the intestinal cells with round dense granules produce secretin, if the close structural resemblance between this intestinal hormone and glucagon (Mutt and Jorpes, 1967) is taken into account.

## SUMMARY

A type of endocrine cell, the G cell, has been detected in the antropyloric mucosa of man and several mammals, which displays all the features of cells secreting protein hormones, *ie*, specific secretory granules of a protein nature, well developed ergastoplasma, and a prominent Golgi complex. The distribution of the G cells in different parts of the gastrointestinal mucosa as well as in different zones of the pyloric glands corresponds strictly to the distribution of the hormone gastrin, suggesting that such a protein hormone may be secreted by G cells. At least in part, the G cells show staining and ultrastructural similarities with the D cells of the pancreatic islets, but clearly differ from enterochromaffin cells and other endocrine cells of the gastrointestinal mucosa.

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