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Antro-Pyloric Gastrinoma Associated with Pancreatic Nesidioblastosis and Proliferation of Islets

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Summary. An adenoma in the antropyloric mucosa of a patient with recurrent duodenal ulcers was identified by immunofluorescence as a gastrinoma. Resected pancreatic tissue showed greatly increased numbers of islets and ducts. Insulin and glucagon cells were revealed by immunofluorescence not only in the islets but also in the duct epithelium. Signs suggesting neoformation of islets from the duct epithelium were observed.

In 1955 Zollinger and Ellison described a syndrome characterized by gastric hypersecretion and peptic ulceration, associated with a pancreatic islet cell tumour (for references see Polak *et al.*, 1972). The symptoms are known to be due to overproduction of gastrin by the tumour. In some patients close inspection of the pancreas failed to reveal a tumour, occasionally tumours were instead found in the duodenal wall (Guida *et al.*, 1966; Weichert *et al.*, 1967; 1971) or in the antropyloric region of the stomach (Polak *et al.*, 1972; Royston *et al.*, 1972). Sometimes, however, no tumour could be located; in some of these cases signs of tubulo-islet neoformation (nesidioblastosis) in the pancreas were observed (Bloodworth and Elliott, 1963). The present report describes a patient with recurrent duodenal ulcers, having an antro-pyloric tumour, showing gastrin-like immunoreactivity, associated with pancreatic nesidioblastosis and proliferation of islets.

Materials and Methods

Histological Stains. Specimens from tumour tissue including adjacent pyloric mucosa and from pancreas were fixed in 10% formalin, Bouin's fluid or a glutaraldehyde-picric acid-acetate mixture (GPA) (Solcia *et al.*, 1968). Paraffin sections were cut at 6 μ, deparaffinized in xylene and treated for immunohistochemistry (see below) or stained according to the following procedures: Hematoxylin-eosin, van Gieson, alkaline Congo red (Puchtler *et al.*, 1962), silver according to Grimelius (1968) or to Davenport as modified by Hellerström and Hellman (1960), argentaffin staining according to Masson-Hamperl (Singh, 1964), aldehyde fuchsin (Jennings, 1965), HCl-pseudoisocyanin (Solcia *et al.*, 1968), or lead-hematoxylin with or without previous HCl hydrolysis (Solcia *et al.*, 1969a). Fluorescence after treatment with HCl-pseudoisocyanin was examined in a fluorescence microscope equipped with an interference line filter transmitting light of the 546 nm wavelength and with a secondary filter having a transmittance above 580 nm.

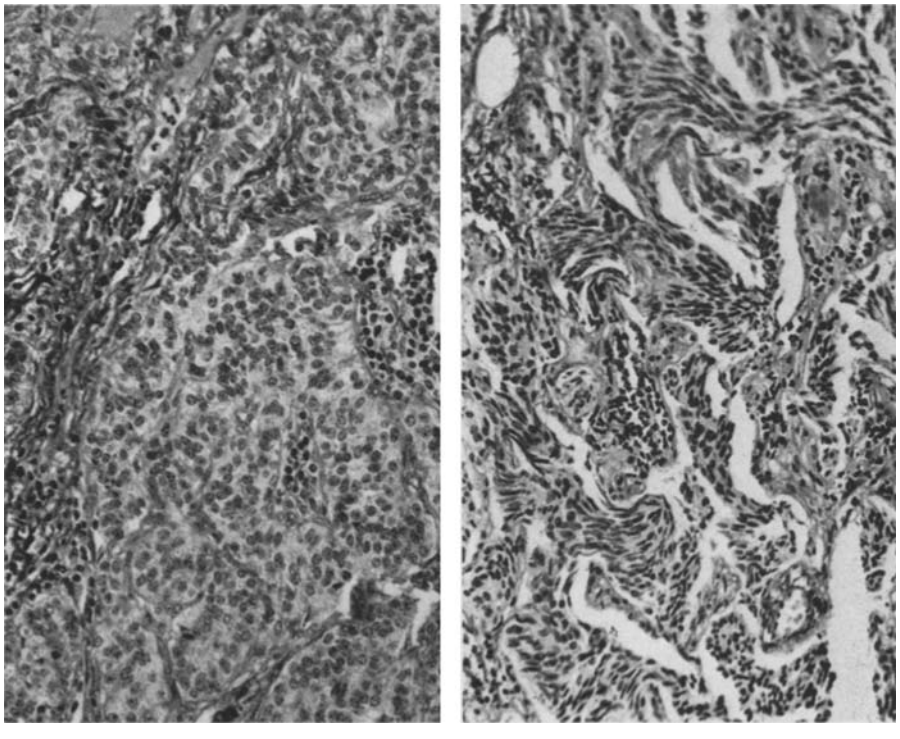
Immunohistochemistry. Sections to be used for the demonstration of gastrin were cut from specimens fixed in Bouin or formalin, for glucagon and insulin from formalin-fixed specimens—the sections to be used for insulin immunofluorescence were refixed in Bouin for 2 hours. Deparaffinized sections were hydrated through graded ethanol solutions and rinsed in 0.01 M phosphate buffer, pH 7.2, which was 0.9% with respect to saline. Antisera directed against gastrin or glucagon were obtained from rabbits. The gastrin antiserum (2609) and the glucagon antiserum (K 4302, VII) have been characterized in detail elsewhere (Holst and Aasted, 1973; Rehfeld *et al.*, 1972). Antiserum directed against insulin was obtained from guinea-pigs immunized with porcine crystalline insulin (Novo). A drop of antiserum (diluted 1:20 for antigastrin and anti-insulin, undiluted for anti-glucagon) was applied onto the sections which were then incubated for 30 minutes at room temperature. The sections were washed thoroughly in several changes of buffered saline whereafter antiserum from goat directed against rabbit IgG globulin (for glucagon and gastrin immunofluorescence) or guinea-pig IgG globulin (for insulin immunofluorescence) and labelled with fluorescein isothiocyanate (FITC (Miles) was applied for another 30 minutes in dilution 1:20. Finally, the sections were washed in buffered saline, mounted in buffered glycerine (pH 7.2) and examined in a fluorescence microscope (primary filter Schott BG 12, secondary filter OG 4). Controls were as follows: a) the first layer was omitted; b) the second layer was omitted; c) both layers were omitted; d) normal rabbit serum was applied as first layer; e) the antisera against gastrin, glucagon and insulin were permitted to react with gastrin (10 μg/ml), glucagon (10 μg/ml) and insulin (50 μg/ml) respectively before applying them as first layers.

Case Report

Male, born in 1907. Several attacks of urolithiasis in 1949 and 1971. Since 1962, episodes of epigastric pain, abdominal distension and on several occasions also hematemesis. Recurrent duodenal ulcers had been diagnosed on radiological examination. In July 1972 the patient was readmitted to the hospital because of hematemesis. X-ray showed an ulcer in the duodeno-pyloric region. Laparotomy revealed a grossly scarred pylorus with a fresh ulcer. Selective vagotomy with pyloroplasty was planned but at incision of the pylorus a small tumour was observed in the wall. Upon palpation of the pancreas no tumour was found. Resection of the stomach *ad modum* Billroth II was then added to the vagotomy. Postoperative recovery was complicated by pulmonary atelectasis with fever. One year after, the patient is without abdominal discomfort.

Pathology

Gross examination of the resected specimen revealed scarring of the pyloric region, with an active ulcer measuring 1 cm in diameter. In addition, there was a round tumour, approximately 1 by 1 cm, situated in the submucosa of the inferior aspect of the pylorus. It showed homogenous, yellow-brown cut surfaces and a well demarcated, polycyclic outer contour. In most parts, the histological picture resembled that of a pancreatic insuloma, consisting of clusters and columnar strands of epithelial cells with regular, rather pale nuclei and weakly eosinophilic cytoplasmic granules (Fig. 1 a). In some areas, the tumour tissue was highly vascularized, forming blood-containing vascular channels, occasionally lined by tumour cells. Other parts mimicked a spindle cell tumour, consisting of elongated cells arranged in streaks and whorles (Fig. 1 b). The tumour did not contain any amyloid, as defined by the alkaline Congo red stain. Mitotic figures were rarely



a b

Fig. 1. Antro-pyloric tumour, van Gieson stain. a Predominating tumour growth pattern: Columnar strands of regular cells (× 220). b A small area had the appearance of a spindle cell tumour with elongated cells arranged in streaks and whorles (× 180)

seen. There was no infiltration to adjacent muscularis propria. The overlying mucosa with the superficial part of the tumour was ulcerated.

The resected specimen also contained pancreatic tissue situated in the omentum outside the pylorus. This specimen showed a marked proliferation of ducts and islet tissue (Fig. 2a). Small clusters of light cells, closely resembling islet cells were seen intermingled with duct epithelium and in apparent interposition between the basement membrane and the columnar duct cells (Fig. 2c). Transitions between ductal and islet type organization of these cells were frequent, suggesting a gradual development of islets from cells (nesidioblasts) within the duct epithelium (Fig. 2b and c). The ducts and islets were enclosed in a rich connective tissue stroma. In some parts, the ducts and islets were very numerous and some islets were enlarged with an apparent reduction of exocrine parenchyma. The picture resembled that seen after occlusion of the pancreatic duct with a relative increase in number of islets. The larger islets often contained eosinophilic, hyalin-like deposits intermingled with the epithelial cells. These deposits could be stained with alkaline Congo red and displayed a distinct yellow and apple-green birefringence, characteristic of amyloid, when examined in polarized light.

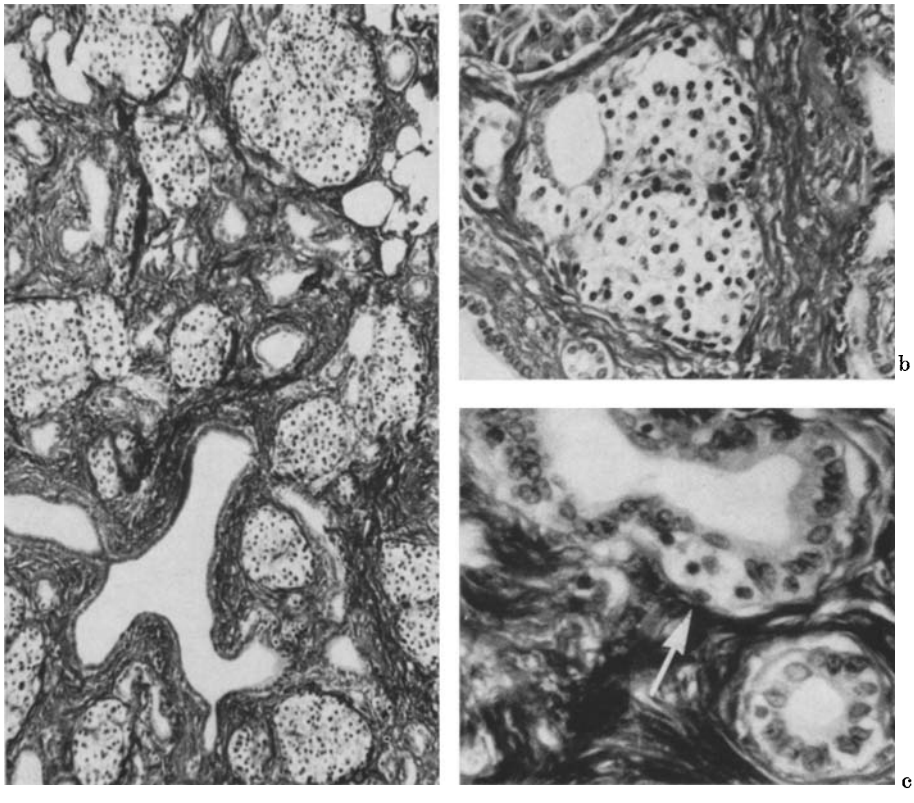
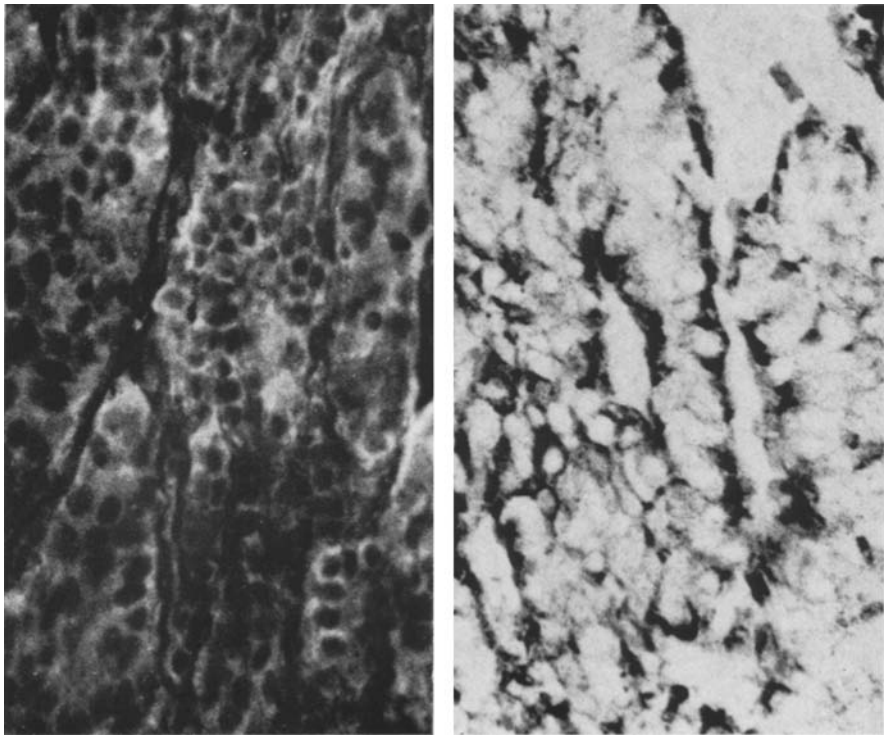


Fig. 2. Pancreatic specimen, van Gieson stain. a Low magnification to show proliferation of islets and ducts in a rich connective tissue stroma (× 90). b Islet showing remnant of ductal-type arrangement (× 180). c A small cluster of light cells (arrow) resembling islets cells are interposed between the basement membrane and the duct cells proper (× 450)

Histochemistry and Histology

Tumour. Immunohistochemical staining of sections from tissue specimens fixed in Bouin or formalin gave a strong reaction with anti-gastrin serum in the majority of the tumour cells (Fig. 3a) whereas staining with anti-glucagon or anti-insulin sera gave negative results. The intensity of the immunofluorescence varied in different parts of the tumour. Controls were negative. A polarity in the cells was sometimes noticeable in that the fluorescence was concentrated in the part adjacent to the basement membrane. The cells were argyrophil with the Grimelius but not with the Hellerström-Hellman technique (Fig. 3b) and they were non-argentaffin. Further, the cells stained with HCl-lead-hematoxylin and HCl-pseudoisocyanin.

Antro-Pyloric Mucosa. Immunofluorescent staining for gastrin revealed a fairly large number of gastrin cells predominating in the middle third of the glands (Fig. 4). When restained the gastrin cells were found to be argyrophil with the Grimelius but not with the Hellerström-Hellman technique and they were non-



a b

Fig. 3. a Immunofluorescence of gastrin in cells of the antro-pyloric tumour (× 540). b Argyrophil staining (Grimelius technique) of tumour cells (× 540)

argentaffin. Thus, their staining properties agreed with those of the tumour cells and with those described by Vassallo *et al.* (1972) for the antral gastrin cell.

Pancreas. The islets were extremely numerous and prominent (Fig. 2a). The immunohistochemical technique showed a large number of insulin cells within the islets (Fig. 5a). In addition, scattered or clustered insulin cells were frequently found intermingled with or beneath the duct epithelium (Fig. 6). The insulin cells in the islets as well as those in the ducts were strongly aldehyde fuchsin-positive (Fig. 6b) and non-argyrophil. Immunofluorescent staining for glucagon demonstrated numerous cells in the islets and scattered cells in the duct epithelium (Fig. 5b). Controls were negative. The glucagon cells were argyrophil (Grimelius technique) and did not stain with aldehyde fuchsin. A characteristic feature was that certain islets seemed to contain almost exclusively insulin cells whereas others contained mainly glucagon cells. Attempts to demonstrate pancreatic gastrin in the formalin-fixed paraffin sections failed. Silver staining according to Hellerström-Hellman showed A_1-cells to be present in a moderate number in the islets as well as in the duct epithelium (Fig. 7). These cells are the proposed

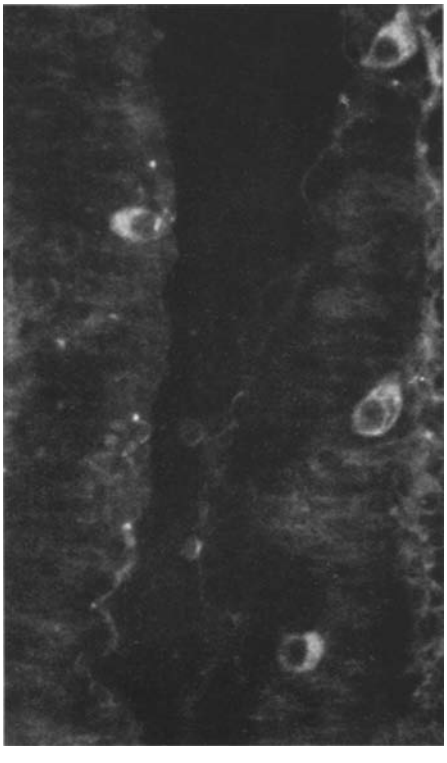


Fig. 4. Immunofluorescent gastrin cells in non-afflicted antro-pyloric mucosa. (× 470)

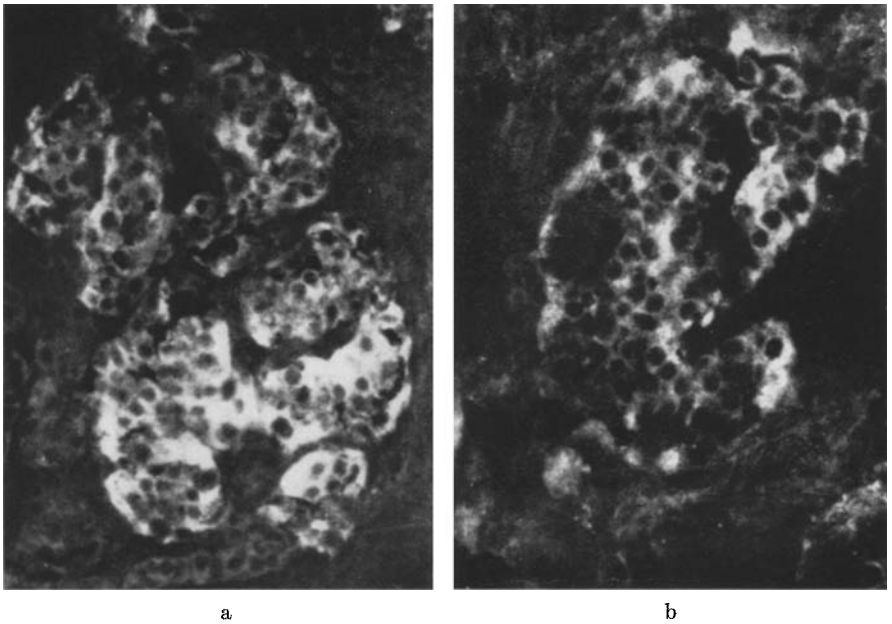


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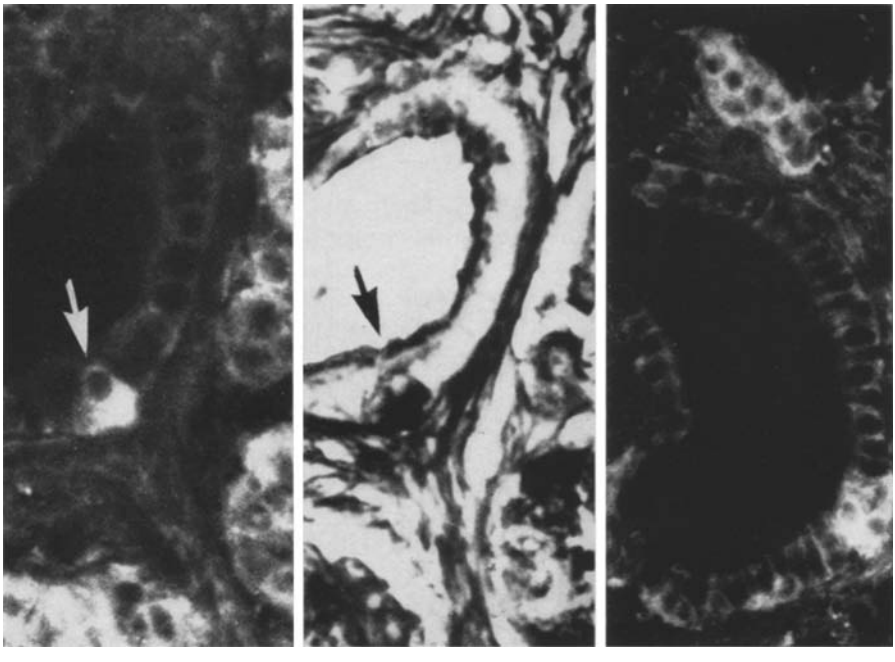
Fig. 5. a Immunofluorescence of insulin in islet dominated by insulin cells (× 280). b Immunofluorescence of glucagon in islet dominated by glucagon cells (× 300)



a b c

Fig. 6. a Pancreatic duct adjacent to islets of insulin cells. Immunofluorescence of insulin also in single cell of the duct epithelium (arrow) (× 580). b Same section restained with aldehyde fuchsin showing insulin cell in the duct epithelium (arrow) (× 580). c Large duct with several accumulations of insulin cells within the duct epithelium (× 340). This figure (and Fig. 2b and c) possibly illustrates the various stages of islet development

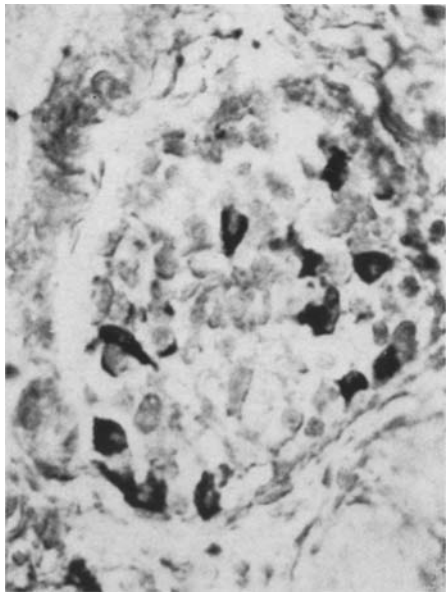


Fig. 7. Silver staining according to Hellerström-Hellman, showing A_1-cells in pancreatic islet (× 400)

storage site of pancreatic gastrin (Lomsky, Langr and Vortel, 1969; Greider and McGuigan, 1971; Polak *et al.*, 1972).

Discussion

Because of the clinical features (recurrent duodenal ulcers), the staining properties which were similar to those of the antro-pyloric gastric cell, and the presence of gastrin-like immunoreactivity, the antro-pyloric tumour was classified as a gastrinoma. There has been some controversy regarding the argyrophilia of the antral gastrin cell (*cf.* McGuigan and Greider, 1971a, b; Mitschke, 1971; Solcia, Capella and Vassallo, 1971; McGuigan, Greider and Grawe, 1972). By now it appears well established that this cell is argyrophil with the Grimelius technique (Solcia, Vassallo and Capella, 1969b; Pearse and Bussolati, 1972; Mitschke and Becker, 1973; *cf.* Solcia, 1972). Gastrin is believed to be stored also in the A_1-cells of the pancreatic islets (Lomsky *et al.*, 1969; Greider and McGuigan, 1971; Polak *et al.*, 1972). These cells stain differently from the gastrin cells of the antro-pyloric mucosa in that they are argyrophil with the Hellerström-Hellman (1960) technique but non-argyrophil with the Grimelius (1968) technique. The tumour cells were strongly argyrophil with the latter method. The staining properties and the localization of the tumour suggest that the tumour has originated from the gastrin cells of the antro-pyloric mucosa rather than from ectopic pancreatic tissue. However, it should be noted that pancreatic gastrinomas sometimes exhibit staining characteristics similar to those of the antral gastrin cell (for a discussion see Creutzfeld *et al.*, 1969, 1971; Vassallo *et al.*, 1972). One similar case of gastrin-storing tumour in the pyloric antrum was recently described by Royston *et al.* (1972). These tumour cells could be stained with lead-hematoxylin and silver (Bodian) but not with argentaffin techniques.

Hyperplastic ducts with buds of endocrine cells showing gastrin immunofluorescence was described by Solcia *et al.* (1970) in connection with a *pancreatic* gastrinoma. The present *antro-pyloric* gastrinoma was also associated with marked proliferation of endocrine cells from the pancreatic duct epithelium. However, these buds never showed gastrin immunofluorescence and only a small number of A_1-cells could be demonstrated with the Hellerström-Hellman technique. Instead, the buds and islets consisted almost exclusively of insulin and glucagon cells, often with one cell type predominating in individual islets. Conceivably, this reflects a bipolar organization with insulin cells and glucagon cells separated. This is characteristic also of developing islets in the human foetal pancreas (Robb, 1961). The results indicate that mature endocrine cells occur in the duct epithelium; later on such cells may give rise to islets. It is possible that the characteristic proliferation pattern of the endocrine cells (see Figs. 2 and 6) illustrates various stages in islet development ("tubulo-islet neoformation") (Bloodworth and Elliott, 1963; Yang and Hunter, 1959).

The combination of gastrinoma and pancreatic nesidioblastosis with proliferation of islets may be part of a more generalized endocrine disorder (multiple endocrine adenomatosis) (Ballard *et al.*, 1964; Vance *et al.*, 1972). An alternative speculation is that the islet proliferation is secondary to trophic influence from the gastrinoma.

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