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Immunohistochemical studies of endocrine cells in heterotopic pancreas

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Summary. Twenty-one specimens of heterotopic pancreas were investigated using the indirect immunoperoxidase method for insulin, somatostatin, glucagon, pancreatic polypeptide (PP) and gastrin. Ten specimens showed ducts, acini and islets, seven showed ducts and acini, and four showed a ductal component alone. Pyloric gland-like mucous glands were occasionally identified in association with the ductal component. In eight of ten lesions containing islets, the islets were round and had a clearly defined outline with many glucagon cells and either none or a modest number of PP cells (dorsal type). In the remaining two lesions, the islets showed varying sizes and irregular outline with many PP cells and a few or no glucagon cells (ventral type). In either type of islets, insulin and somatostatin were detected, but gastrin cells were absent. Some isolated endocrine cells were also present among the acinar and ductal components. Their occurrence in ducts was more frequent in lesions or areas mainly composed of the ductal compoment than in those with less prominent ductal tissue. In eight lesions a few gastrin cells were found in the ductal component which showed goblet cell metaplasia and pyloric gland metaplasia. An intimate relationship between goblet cell metaplasia and appearance of G cells is noteworthy.

Key words: Immunoperoxidase method – Heterotopic pancreas – Endocrine cell – Gastrin – Pyloric gland metaplasia

Introduction

Heterotopic pancreas is defined as pancreatic tissue occurring outside its usual location without an anatomical relation of either continuity or vascularization with the pancreas proper (Barbosa et al. 1946). Heterotopic pancreas is not rare, and has been reported to occur in 0.6% (Horgan 1921) to 13.7% (Feldman and Weinberg 1952) of autopsy series. Histologically,

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it consists of pancreatic acinar and ductal tissue, and islets are observed in one third of the lesions in routine haematoxylin and eosin-stained sections (Nickels and Lassonen 1970). There are only a very small number of reports about the nature of endocrine cells in heterotopic pancreas (Tomita and Kanabe 1983).

The aim of this article is to investigate the characterization of endocrine cells in the heterotopic pancreas, and to ascertain both the histogenesis of heterotopic pancreas and the cytogenesis of endocrine cells in the heterotopic tissue.

Materials and methods

Patients (Table 1). A total of 21 specimens of heterotopic pancreas were studied: 12 were found at autopsy, 8 were removed at surgery, and one was removed by endoscopic polypectomy at Tokai University Hospital, Isehara, Japan. The age of the patients ranged from 0 to 72 years (mean: 29.3). Fifteen of 21 patients were male, and the other were female. Eleven cases were found in the jejunum, followed by the duodenum (five cases), stomach (four cases) and ileum (two cases). The diameter of the heterotopic pancreases ranged from 0.2 to 3.0 cm. They were classified by Heinrich's classification (Heinrich 1909): 10 (48%) were classified as Type I, containing islet tissues as well as ducts and acini, 7 (33%) were classified as Type II consisting of ducts and acini without islet tissues, and 4 (19%) were classified as Type III consisting of ducts only. The specimens were fixed in 10–20% formalin, and prepared for paraffin-embedded consecutive sections at 4 μm thickness.

Table 1. Heterotopic pancreas examined

	Patient	Age	Sex	Size (cm)	Specimen	Heinrich's classification
Stomach	1	14	F	0.4 × 0.3	Polypectomy	I
	2	26	M	1.5 × 1.5	Autopsy	I
	3	48	M	1.4 × 1.2	Surgical	II
	4	52	F	1.1 × 1.1	Surgical	II
Duodenum	5	42	M	1.1 × 1.0	Surgical	I
	6	55	M	1.0 × 0.8	Autopsy	I
	7	61	M	0.7 × 0.5	Surgical	II
	8	69	M	0.8 × 0.7	Autopsy	II
	9	72	F	1.7 × 0.5	Autopsy	II
Jejunum	10	0	M	0.3 × 0.3	Autopsy	I
	11	0	M	1.0 × 1.0	Autopsy	I
	12	0	F	0.4 × 0.3	Autopsy	I
	13	41	M	0.6 × 0.6	Autopsy	II
	14	44	M	2.2 × 2.0	Surgical	I
	15	55	M	3.0 × 3.0	Autopsy	I
	16	68	M	1.0 × 1.0	Surgical	III
	17	69	F	1.1 × 1.0	Autopsy	III
	18	70	M	0.5 × 0.4	Autopsy	II
	19	70	M	0.8 × 0.7	Autopsy	III
Ileum	20	0	F	0.2 × 0.2	Surgical	III
	21	1	M	0.6 × 0.6	Surgical	I

Mucin histochemistry. For the identification and characterization of metaplastic changes, Alcian blue (pH 2.5)-periodic acid-Schiff (AB-PAS) staining and paradoxical Concanavalin A staining were used in addition to haematoxylin and eosin (H&E). The paradoxical Concanavalin A staining, class III, is highly specific for mucins of pyloric glands, mucous neck cells, Brunner's glands and metaplastic (pseudo)pyloric glands (Katsuyama and Spicer 1978; Tsutsumi et al. 1984a). Sections from normal and metaplastic gastric antrum were used as positive staining controls.

Antisera. The following antisera with known immunological specificities (Yanaihara 1980; Tsutsumi 1984; Tsutsumi et al. 1983; 1984a and b) were used as primary antibodies for the immunohistochemistry. These included guinea pig antihuman little gastrin serum (GP-1304, a geneous gift from Dr. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka, Japan), guinea pig antiporcine insulin serum (NY-1, Yanaihara), rabbit antisomatostatin serum (DAKO, USA), rabbit antiglucagon *C*-terminal 19–29 serum (GC-5, Japan Immunoresearch Laboratories, Takasaki, Japan), rabbit antihuman pancreatic polypeptide (PP) serum (615-1054-B-248-19, kindly supplied by Dr. R.E. Chance, Lilly Laboratories, Indianapolis, USA) and rabbit antiporcine gastrin-releasing peptide (GRP) serum (R-6902, Yanaihara) which recognizes both the *N*- and *C*-terminal portions of porcine GRP. These antisera were used at a dilution of 1:1,000 except for anti PP serum which was diluted at 1:10,000. As reported previously (Tsutsumi et al. 1984b), antiporcine GRP serum R-6902 specifically stains almost all of the pancreatic ductal cells including centroacinar cells, whereas it does not stain acinar and islet cells. Some substance(s) cross-reacting to the *N*-terminal fragment of porcine GRP is considered to be present in the cytosol of the pancreatobiliary ductal cells (Tsutsumi et al. 1984b).

For secondary antibodies, horseradish peroxidase (HRP)-labeled antirabbit IgG goat IgG Fab fragments (prepared in our laboratory), HRP-labeled antiguinea pig IgG rabbit IgG (purchased from Miles Laboratories, USA) were used. Negative controls included nonimmune sera from a rabbit and guinea pig at a 1:500 dilution.

Immunohistochemistry. Immunohistochemical studies were carried out by the indirect immunoperoxidase method using antisera listed above. Incubation with the antibodies and rinsing with 0.01 M phospate-buffered saline, pH 7.2 in each step were performed at room temperature for 30 min. Endogenous peroxidase was inactivated by dipping deparaffinized sections in 0.5% periodic acid at room temperature for 10 min prior to the immunostaining. The working solution for the HRP reaction contained 30 mg% 3,3′ diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Osaka, Japan) and 10 mM hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6. Nuclear couterstaining was performed with 1% methyl green solution, buffered by 0.1 M veronal acetate at pH 4.0.

To confirm the specificities of each immunostaining, the primary antisera were preincubated with an excess amount (5 μg/ml) of the corresponding synthetic or purified peptides at 37° C for 60 min (preabsorption tests) as reported previously (Tsutsumi et al. 1983; Tsutsumi 1984). Normal pancreatic tissue (head and tail) obtained at autopsy and normal antral mucosa from surgical material were used for positive staining controls.

Result

Positive control specimens of the pancreas and stomach were clearly stained for the corresponding substances. Negative control sections incubated with normal rabbit or guinea pig serum failed to stain any tissue components. Preabsorption experiments using the peptide antigens further confirmed specificities of the immunostaining.

Endocrine cells in islets of heterotopic pancreas

In all 10 cases that contained islets tissues in H&E sections, four types of endocrine cells including insulin cells (B cells), somatostatin cells (D cells), glucagon cells (A cells) and pancreatic polypeptide cells (PP cells)

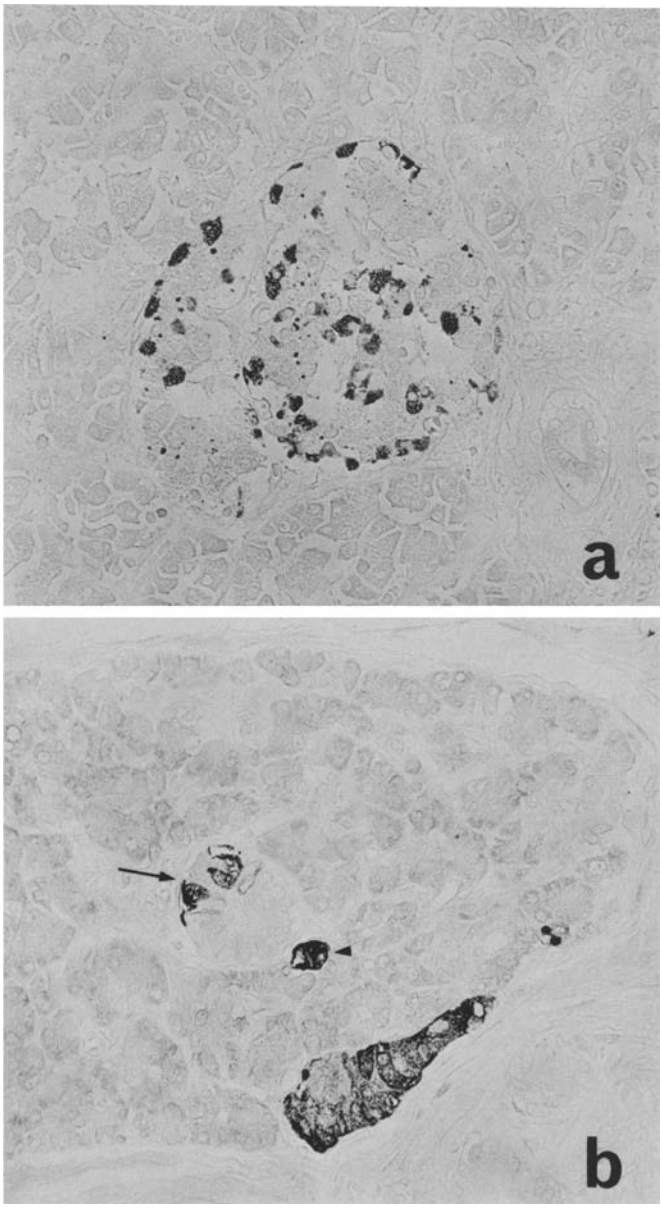

Fig. 1 a, b. Two different types of islet tissue in heterotopic pancreas: dorsal type (**a**, case 2) and ventral type (**b**, case 15). Indirect immunoperoxidase staining for glucagon (**a**) and pancreatic polypeptide (**b**). × 300. Two different types of islet tissue are recognized in heterotopic pancreas. In one type, the dorsal type (**a**), the islet shows a round shape with a clearly defined outline, and is glucagon-rich. The number of pancreatic polypeptide-positive cells ranged from zero to a modest number. In another type, the ventral type (**b**), the islet, showing varying sizes and irregular outlines is glucagon-poor and pancreatic polypeptide-rich. A small duct (*arrow*) and acinar area (*arrowhead*) contain isolated PP cells.

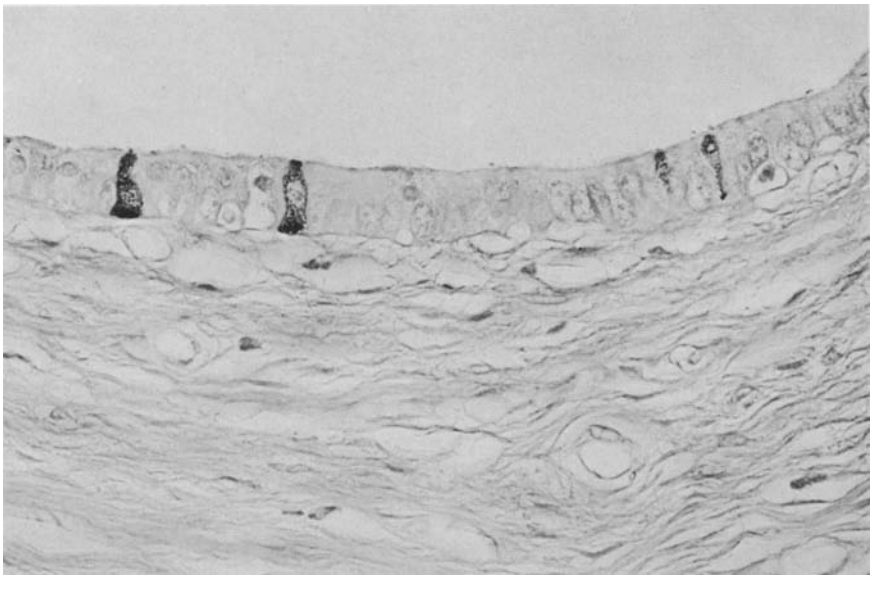

Fig. 2. Endocrine cells in the ductal component of heterotopic pancreas, case 3. Indirect immunoperoxidase staining for somatostatin. × 300. All four kinds of the endocrine cells seen in islet tissue are scattered among the ductular epithelium as an isolated open type cell. Somatostatin and pancreatic polypeptide are most commonly found in the ductal endocrine cells

were found in the islet tissues. B cells and D cells were distributed in the same pattern as in normal pancreas. As for the distribution of A cells and PP cells, two different types of islet tissues were recognized in the heterotopic pancreas. In one type (8 cases), the islets showing a round shape with a clearly defined outline were rich in A cells with either, none or a modest number of PP cells (Fig. 1a). In another type (2 cases), the islets showed varying sizes and irregular outlines, and were glucagon-poor and PP-rich (Fig. 1b). These two lesions were located at the jejunum and ileum (cases 15 and 21). In normal pancreas, the former was indentified as dorsal type while the latter was called the ventral type (Orch and Perrelet 1981). Gastrin cells (G cells) were absent from the islets.

Endocrine cells in acinar and ductal tissues of heterotopic pancreas

All four kinds of isolated endocrine cells were also frequently scattered both in the acinar area and in the ductal epithelium, which specifically stained positive with antiporcine GRP serum R-6902. All of the lesions examined showed the presence of varying numbers of endocrine cells in the acinar and/or ductal components. With the immunostaining, abortive islet-like small clusters of endocrine cells were commonly and clearly shown in acinar areas of the Type II lesions. Generally, the number of the intraductal endocrine cells was more than those seen in the normal pancreas. Their occurrence in ducts was more frequent in the lesions or areas mainly composed of the ductal component than in those with less prominent ductal

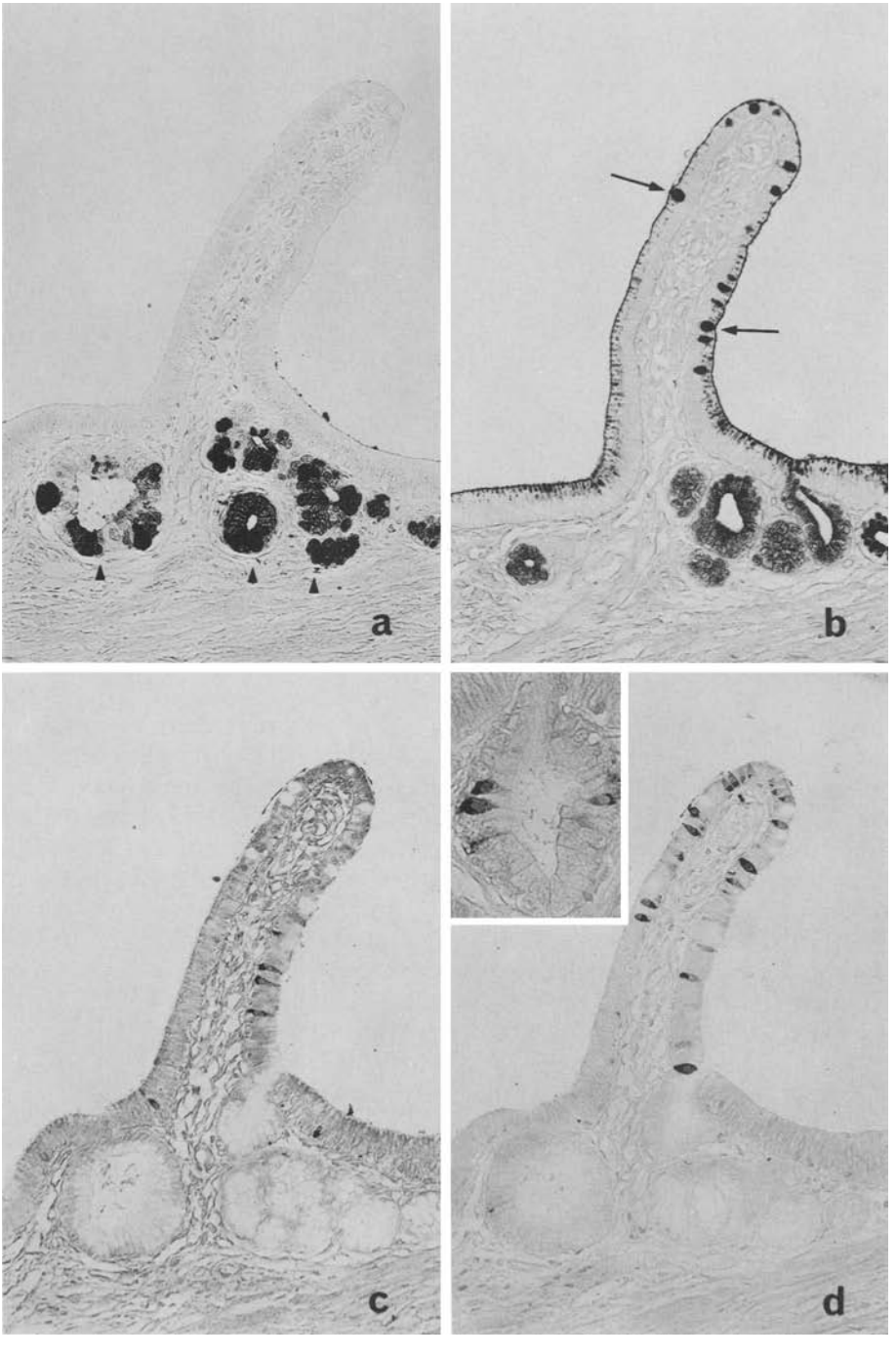
a
b
c
d

Table 2. Pyloric gland metaplasia (PGM), goblet cell metaplasia (GCM) and G cells in heterotopic pancreas

Heinrich's classification	I ($n=10$)	II ($n=7$)	III ($n=4$)
PGM	3/10 (30%)	4/7 (57%)	3/4 (75%)
GCM	2/10 (20%)	3/7 (43%)	3/4 (75%)
G cell	2/10 (20%)	3/7 (43%)	3/4 (75%)

tissue. The hormones most commonly found in the ductal endocrine cells were PP and somatostatin (Figs. 1b and 2).

However, parts of the ductal tissue were accompanied by small mucous glands of the pyloric type (pyloric gland metaplasia), which were strongly stained with paradoxical Concanavalin A staining (Fig. 3a). The metaplastic pyloric glands were identified in 10 of 21 lesions, and they were found in the following order: Type III > Type II > Type I (Table 2). With AB-PAS staining, metaplastic pyloric glands were PAS-positive and AB-negative as were normal pyloric glands. At times, the duct-lining layer in the area with pyloric gland metaplasia contained a few goblet cells which were strongly alcianophilic and also PAS-reactive (goblet cell metaplasia) (Fig. 3b). Ducts showing goblet cell metaplasia were almost always accompanied by pyloric gland metaplasia, while ducts with metaplastic pyloric glands but without goblet cells were often present. In the metaplastic ductal tissue especially with goblet cell metaplasia, D cells (Fig. 3c) and G cells (Fig. 3d) were not infrequently observed in the duct-lining layer, while B cells, A cells and PP cells were seldom found. The G cells and D cells were also distributed in the metaplastic pyloric glands (Fig. 3d, inset). An intimate relationship between goblet cell metaplasia and appearance of G cells should be noted. G cells were totally absent from the acinar areas and nonmetaplastic ducts.

Discussion

Several theories have been presented to explain the origin of heterotopic pancreas. However, the exact mechanism has not yet been elucidated. Three theories have been proposed. Warthin (1904) proposed that heterotopic pancreas is formed by the lateral budding of the primitive pancreatic ducts

Fig. 3a–d. Close relationship between metaplastic changes of the ductal component and the occurrence of somatostatin and gastrin immunoreactive cells. Case 3, adjacent sections. **a** paradoxical Concanavalin A staining, ×150; **b** Alcian blue (pH 2.5)-PAS staining, ×150; **c** indirect immunoperoxidase staining for somatostatin, ×150; **d** indirect immunoperoxidase staining for gastrin, ×150 (inset ×300). This part of the ductal tissue in heterotopic pancreas shows pyloric gland metaplasia (*arrowhead*), which is specifically identified by paradoxical Concanavalin A staining **a**, and goblet cell metaplasia (*arrows*), which is clearly seen by AB-PAS staining **b**. Isolated open type cells containing somatostatin **c** and gastrin **d** are distributed in the same metaplastic duct. Occurrence of gastrin cells in metaplastic pyloric glands is shown in the inset of **d**

as they penetrate the intestinal wall, the mass of pancreatic tissue thus formed being snared off and carried by the longitudinal growth of the intestine either upward or downward. Horgan (1921) suggested that small buds from the branching ends of either the anterior or posterior pancreatic anlage which has not yet coalesced become attached to the gut wall and remain separately grafted in a new location in the gut wall. Lordy (1930) explained the occurrence of heterotopic pancreas by stating that it results from the persistence or imcomplete regression of the left ventral anlage which is normally destined to atrophy.

It has been demonstrated that the morphology and hormonal contents of islets show a diversity in different portions of the pancreas (Baetens et al. 1979; Bommer et al. 1980). The lower dorsal part of the pancreatic head including the uncinate process contains islets with irregular outlines, a high number of PP cells and a few A cells. The other part of the pancreas possesses round islets with many A cells and only a few PP cells. The former has been called the duodenal or ventral type, while the latter has been called the dorsal type (Orci and Perrelet 1981).

Recently, Tomita and Kanabe (1983) studied 17 cases of heterotopic pancreas with the immunohistochemical technique for detecting insulin and PP immunoreactive islets. They concluded, using PP cells as a marker for the ventral anlage, that heterotopic pancreases appear to derive from either PP-rich ventral or PP-poor dorsal anlage. In the present study, heterotopic pancreases containing apparent islet tissue could again be classified into two types: the ventral type for two lesions and the dorsal for eight lesions. Thus our results support their findings and the theory of Lordy (1930) seems the least likely to be true.

It is worthy of note that all four kinds of endocrine cells seen in the islet tissue were also distributed in the ductal system. The more prominent the ductal component is in the heterotopic tissue, the more frequent the occurrence of the ductal endocrine cells. A similar phenomenon has been found in the atrophic pancreas with chronic inflammation and fibrosis (Bommer et al. 1980; Klöppel et al. 1983), where plentiful endocrine cells are distributed in the remaining duct system associated with features of budding from the duct (nesidioblastosis) (Bartow et al. 1981; Gould et al. 1983). "Pseudoneoplastic" neoformation of endocrine cells from the duct is not rare in chronic pancreatitis (Bartow et al. 1981). Thus, the cellular origin of endocrine component in heterotopic pancreas is likely to be ductal and endodermal, as it is in normal pancreas (Andrew 1984).

In addition, we described here two kinds of metaplastic lesions of the duct cells in heterotopic pancreas; pyloric gland metaplasia and goblet cell metaplasia. Metaplastic (pseudo)pyloric glands are specifically visible with paradoxical Concanavalin A staining, class III (Tsutsumi et al. 1984a), while metaplastic goblet cells are clearly identified by AB-PAS staining as in normal intestinal goblet cells. These metaplastic changes have been described commonly in aged pancreases and considered to be a kind of aging process (Feldman 1955, Katsuyama et al. 1980). Interestingly enough, a few G cells were frequently found in the ducts with such metaplastic changes. D cells

were also present in the metaplastic area, whereas B cells, A cells and PP cells were scarcely noted. The occurrence of G cells especially showed a close relationship to goblet cell metaplasia, in which metaplastic pyloric glands were commonly seen. Tsutsumi et al. (1984a) recently reported the similar neoformation of G cells in metaplastic lesions of the gallbladder. In the gallbladder, goblet cell metaplasia was intimately related to the occurrence of endocrine cells including G cells, D cells and argentaffin cells, while mucosa without goblet cell metaplasia was devoid of such endocrine cells (Tsutsumi et al. 1984a). One of the authors has also confirmed the appearence of G cells and argentaffin cells in metaplastic ducts in the aged human pancreas (Tsutsumi; unpublished observation). Whereas the functional significance of gastrin in the metaplastic lesions in heterotopic pancreas remains to be elucidated, the fact that G cells may appear in metaplastic pancreatic ducts should be evaluated in view of the ontegeny of G cells in the fetal and neonatal pancreas (Larsson 1977) and the occurrence of G cell neoplasms from the adult pancreas.

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