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Pancreatic PP Cell Distribution and Hyperplasia

Immunocytochemical Morphology in the Normal Human Pancreas, in Chronic Pancreatitis and Pancreatic Carcinoma

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Summary. The endocrine pancreatic tissue from 13 patients with severe chronic pancreatitis, 5 patients with pancreatic duct carcinoma and 4 non-diseased pancreases was analysed by immunocytochemistry and morphometry. The controls revealed two distinct islet types with different regional distribution. The lower dorsal part of the pancreatic head contained islets with irregular outlines and a high number of PP cells (PP-cells 60.4 ± 4.1%; B-cells 29.4 ± 4.6%; A-cells 7.4 ± 1.5%; D-cells 2.8 ± 0.6%). The other parts of the pancreas contained compact islets with only a few PP cells (PP-cells 1.0 ± 0.4%; B-cells 69.3 ± 3.0%; A-cells 24.1 ± 2.1%; D-cells 5.8 ± 0.5%). In chronic pancreatitis the sclerotic tissue of the body and the tail region contained compact islets with altered cell inter-relationships when compared with controls. While the number of B-cells was diminished (48.5%), A and PP cells appeared to be increased in number (42.7 and 4.1%, respectively). Furthermore, ductulo-insular proliferations were conspicuous (nesidioblastosis) with budding-off of small endocrine cell clusters made up predominantly of A and PP cells. In 3 patients with pancreatic carcinoma increased numbers of PP cells and of A cells were found along the advancing edge of the carcinoma.

The data emphasize the necessity of taking into consideration regional PP cell distribution in each case in which an increase of PP cells is observed. True hyperplasia is found in chronic pancreatitis and, focally, in some cases with pancreatic carcinoma.

Key words: Endocrine pancreas – PP cells – Immunocytochemistry – Distribution – Normal pancreas – Chronic pancreatitis – Pancreatic carcinoma.

Introduction

Pancreatic Polypeptide (PP) is a 36 amino-acid straight chain polypeptide which is released from the pancreas after ingestion of food and which therefore may

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play a role in the complex process of digestion (Schwartz et al. 1976). PP producing cells have been localized in the islets and, also sporadically outside the islets of Langerhans, between acinar and duct cells (Larsson et al. 1975, 1976; Heitz et al. 1976; Orci et al. 1976; Baetens et al. 1977). So far, PP cells have not been observed in the extrapancreatic tissues of man.

Recently, it was shown that the distribution of PP cells is not homogenous throughout the pancreas. In the body and tail of the pancreas only a small number of PP cells can be seen. In contrast, this cell predominates in the lower areas of the pancreatic head (Orci et al. 1976; Larsson et al. 1976; Orci et al. 1978; Baetens et al. 1979; Rahier et al. 1979).

The aim of this study was to compare the distribution of PP cells and the other cell types known to occur in the normal endocrine pancreas with that found in chronic pancreatitis and pancreatic duct carcinoma.

Material and Methods

Collecting and processing of pancreatic tissue (Table 1): 4 entire pancreases were obtained at autopsy within 2 h after death from patients not suffering from diseases of the pancreas, gastrointestinal

Table 1. Clinical and morphological data

Case No.	Age/Sex	Pancreatic specimen	Histological diagnosis
Group I			
1	57/M	total pancreas	normal
2	51/M	total pancreas	normal
3	32/M	total pancreas	normal
4	35/M	total pancreas	normal
Group II			
5	37/M	tail	primary chronic pancreatitis
6	46/F	body-tail	primary chronic pancreatits
7	36/F	body-tail	primary chronic pancreatitis
8	38/M	tail	primary chronic pancreatitis
9	39/M	tail	primary chronic pancreatitis
10	40/F	tail	primary chronic pancreatitis
11	51/F	tail	secondary chronic pancreatitis
12	59/M	body-tail	secondary chronic pancreatitis
13	63/F	tail	secondary chronic pancreatitis
14	40/M	tail	secondary chronic pancreatitis
15	70/M	body-tail	secondary chronic pancreatitis
16	73/F	tail	secondary chronic pancreatitis
17	38/M	tail	secondary chronic pancreatitis
Group III			
18	50/M	head	pancreatic duct carcinoma
19	63/F	head	pancreatic duct carcinoma
20	61/M	head	pancreatic duct carcinoma
21	64/M	head	pancreatic duct carcinoma
22	54/M	head	pancreatic duct carcinoma

tract or liver (group I). Samples were taken from all areas according to the sampling procedure proposed by Orci et al. (1978). Surgical specimens were obtained from the body and the tail of the pancreas (group II) or were taken from the tumour and the surrounding pancreatic tissue (group III) from 6 patients with severe primary chronic pancreatitis and from 12 patients with secondary chronic pancreatitis due to duct obstruction by a carcinoma in the pancreatic head.

The tissue samples were fixed in Bouin's solution. Serial paraffin embedded sections (3 µm) were stained with haematoxylin and eosin, periodic acid Schiff (PAS) and aldehyde fuchsin.

Immunocytochemistry. Insulin, glucagon, somatostatin and pancreatic polypeptide (PP) were visualized by using the indirect immunoperoxidase method (insulin) or by using the unlabelled antibody enzyme method (Sternberger 1979) as previously described (Klöppel et al. 1978).

Quantitative Analysis. Serial sections of two or three tissue blocks immunostained for A, B, D and PP cells were evaluated from each case. In the controls (group I) one tissue block contained the PP cell rich area of the pancreatic head, the other blocks were taken from other areas. A point counting system was used to determine the surface area occupied by the endocrine tissue per total parenchymal area. By means of a Leitz epidiascope the sections were projected on a transparent lattice with a net constant of 7.6 µm at a final enlargement of 380:1. Ten fields, containing 5 to 15 islets each, were evaluated. The sum of the areas occupied by each of the four investigated cell types was taken as the total endocrine area. Subsequently the relative area per total endocrine area occupied by each particular cell type was calculated.

The relative area of each cell type in the irregular islet type in the lower part of the pancreatic head and the compact type in the pancreatic body and tail were compared in controls (group I). In chronic pancreatitis (group II) only the islets in the sclerosed area were evaluated. In the cases with pancreatic carcinoma (group III) the endocrine cell area was not determined because of the focal changes in the distribution of the PP cells. Statistical evaluation was performed by the Wilcoxon's rank-sum test.

Results

Group I (Table 2)

The control pancreases contained two different islet types (Figs.1 and 2). In the lower part of the pancreatic head, particularly in its dorsal portion, but to some extent also in its anterior part, islets of varying size and irregular outline were found. They consisted of anastomosing cords and clusters of columnar cells, which were only occasionally sharply demarcated from the surrounding parenchyma by a narrow band of connective tissue. In the upper parts of the pancreatic head and in the other pancreatic regions the islets were of the well known compact type with a clearly defined outline as already described by Langerhans. PP cells were predominant in the irregular islets (Figs.3 and 4) in which they were mainly located at the periphery. In contrast, PP cells were rare in the compact islets. Moreover, single PP cells were found to be scattered both in the exocrine parenchyma and the ductular epithelium. The B-cell was the predominating cell type in the compact islets, followed by A, D and PP cells.

Group II (Table 3)

All specimens displayed a severe chronic pancreatitis with a 50 to 60% replacement of exocrine parenchyma by connective tissue. The sclerotic areas of the

Table 2. Relative area per total endocrine area (%) of insulin, glucagon, somatostatin and PP producing cells in the normal pancreas

Case No.	Insulin	Glucagon	Somatostatin	PP
1 head	32.7	7.4	2.5	57.4
tail	68.8	25.0	5.8	0.4
2 head	22.7	8.7	2.4	66.2
tail	71.3	22.7	5.2	0.8
3 head	30.7	5.3	3.6	60.4
tail	65.2	27.3	6.2	1.2
4 head	31.7	8.1	2.6	57.6
tail	71.1	21.2	5.7	1.4
Mean ± SD				
head	29.4 ± 4.6	7.4 ± 1.5	2.8 ± 0.6	60.4 ± 4.1
tail	69.3 ± 3.0	24.1 ± 2.1	5.8 ± 0.5	1.0 ± 0.4

Table 3. Relative area per total endocrine area (%) of insulin, glucagon, somatostatin and PP producing cells in the pancreatic body or tail in chronic pancreatitis (group II)

Case No.	Insulin	Glucagon	Somatostatin	PP
5	27.6	64.7	6.2	1.4
6	52.8	38.0	5.3	3.9
7	39.3	48.9	8.1	3.7
8	31.6	61.1	5.3	1.9
9	48.2	46.6	3.1	2.1
10	56.6	34.9	4.9	3.6
11	63.2	30.9	4.4	1.5
12	40.2	45.4	3.0	11.4
13	57.9	35.5	4.4	2.2
14	49.6	42.3	5.1	3.0
15	51.9	34.1	7.8	12.9
16	57.5	37.0	3.7	1.8
17	53.7	36.0	5.9	4.4
Mean ± SD	48.5 ± 10.7[a]	42.7 ± 10.4[a]	5.2 ± 1.6	4.1 ± 3.6[a]

[a] Statistical significance ($P < 0.001$) by comparison to compact islets of normal controls

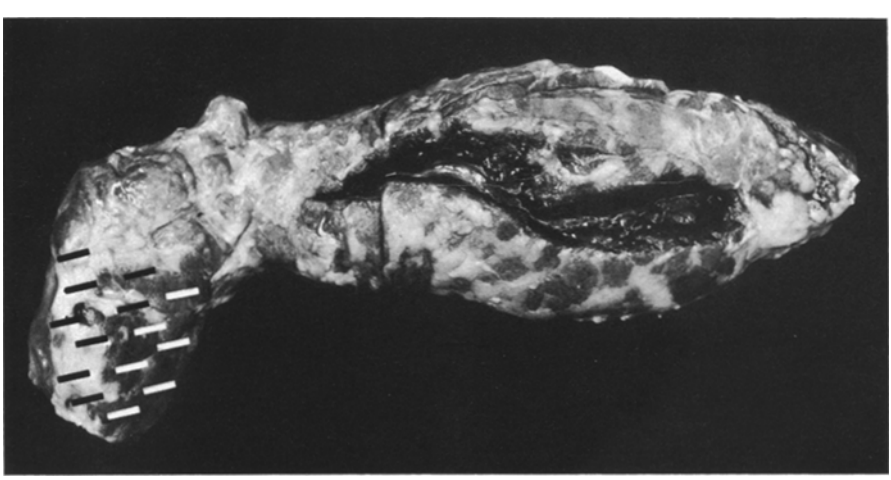

Fig. 1. Topography of PP cell rich area (*hatched area*) in the pancreatic head

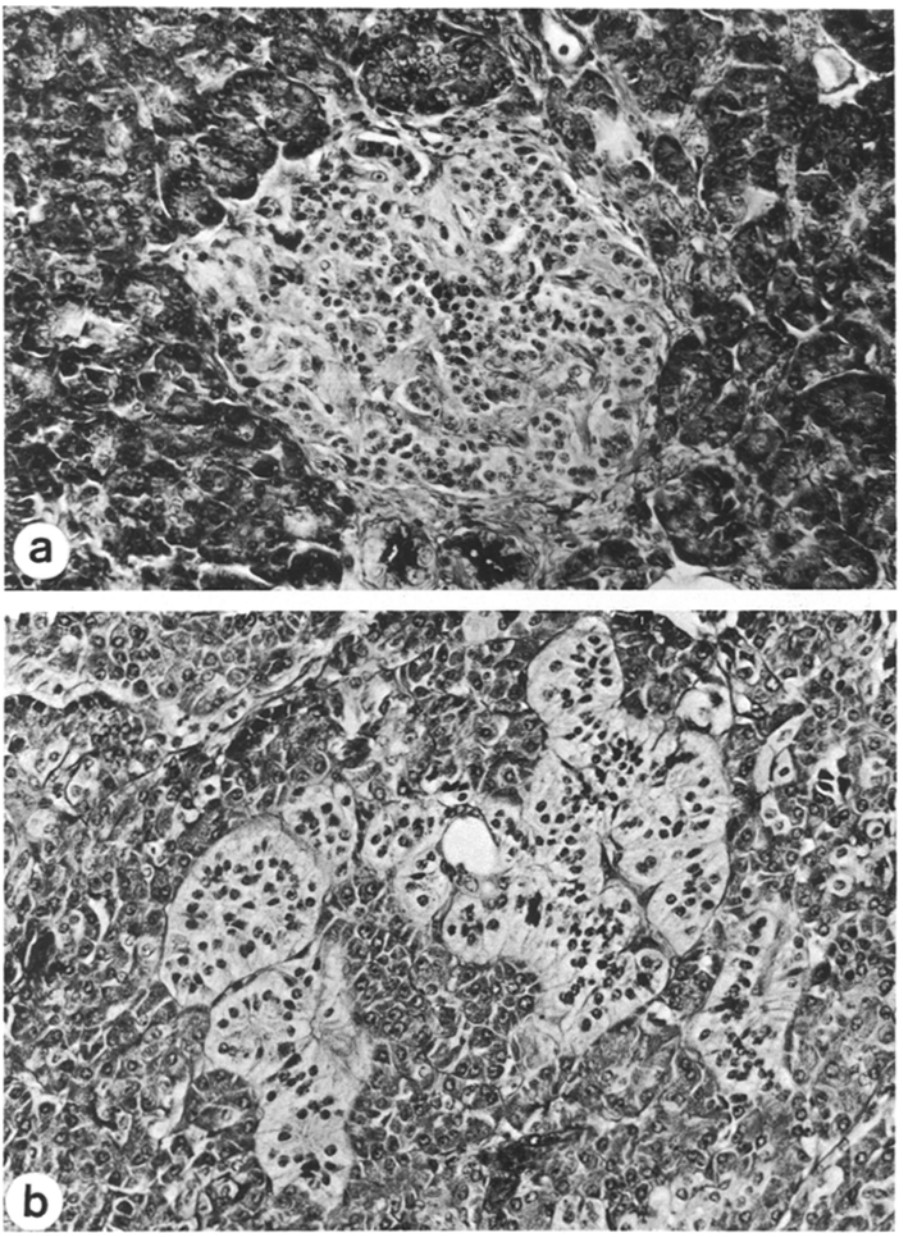

Fig. 2a and b. Islet patterns in the normal pancreas. **a** Compact islet of the pancreatic body and tail. **b** Islet with irregular outline in the lower part of the pancreatic head, characterized by anastomosing cords and clusters of columnar cells. PAS ×250

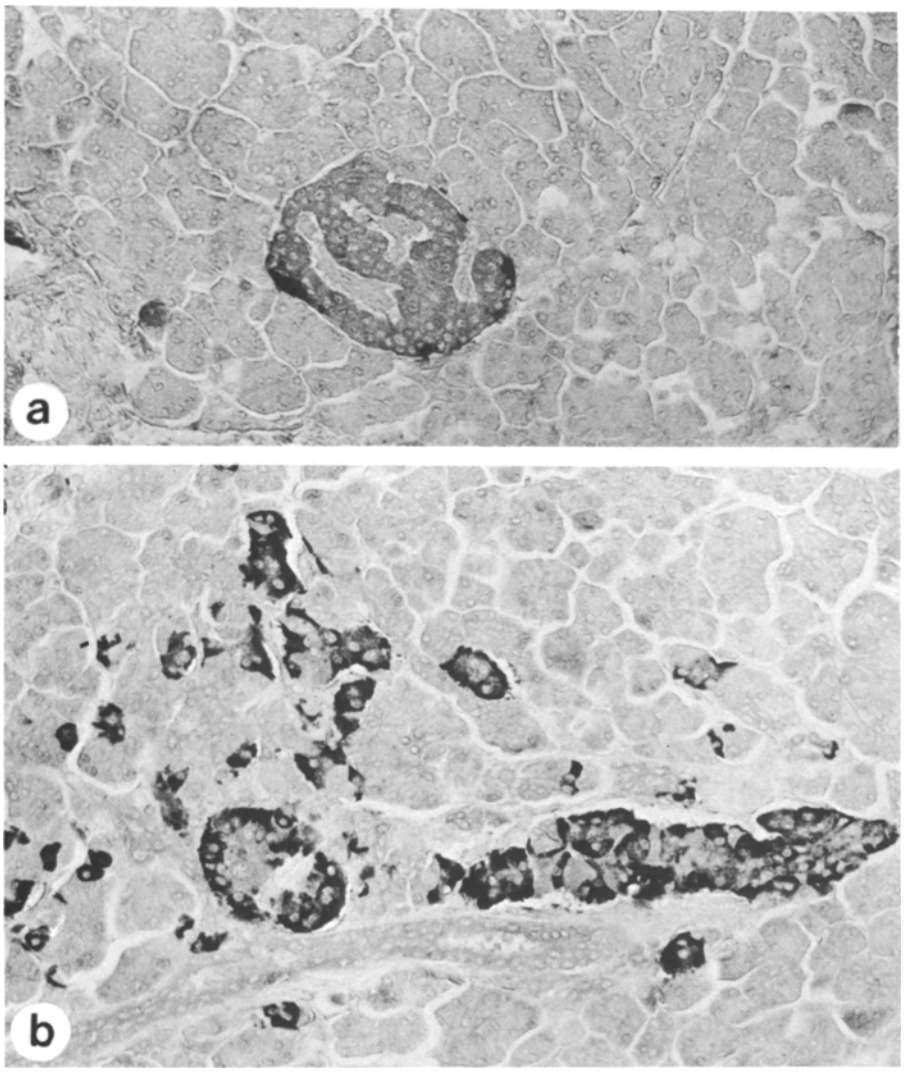

Fig. 3a and b. Normal pancreas. PP cell distribution in a compact islet (**a**) and an islet with irregular outline (**b**). Immunocytochemical staining for PP. ×250

pancreatic body or tail region contained compact islets of varying size exclusively. Furthermore, small ductulo-insular proliferations were seen to occur in their vicinity. The immunocytochemical findings in the compact islets within the sclerotic areas differed from those in the controls. The relative endocrine area occupied by A cells and PP cells was increased (Fig. 5). As in the controls A and PP cells were located mainly at the islet periphery. While the B cell area was considerably diminished, that of D cells remained constant. The nesidioblastic buds frequently were made up of PP and A cells.

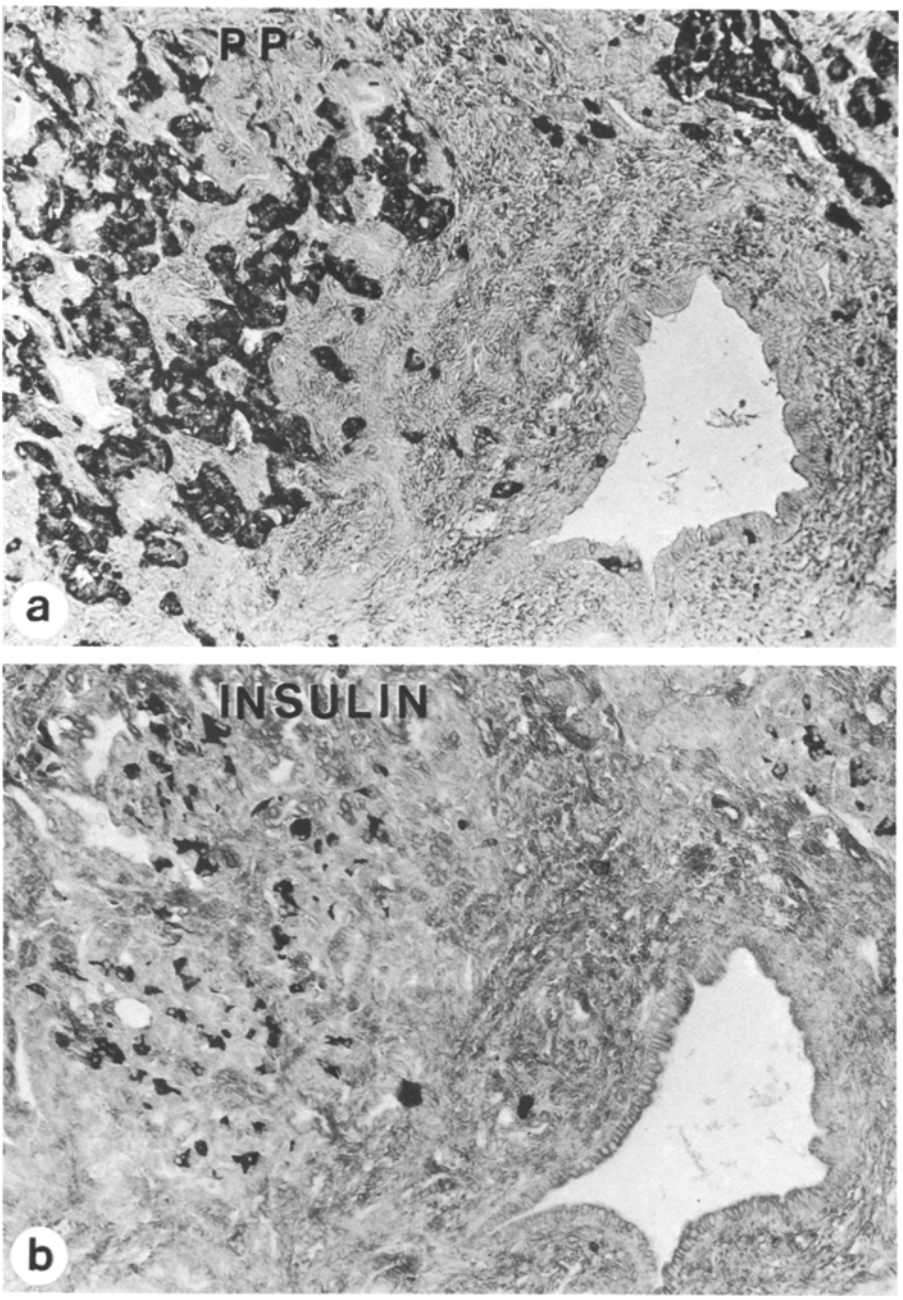

Fig. 4a and b. Normal pancreas. Immunocytochemical staining for PP (**a**) and insulin (**b**) in consecutive sections of an islet with irregular outline. ×140

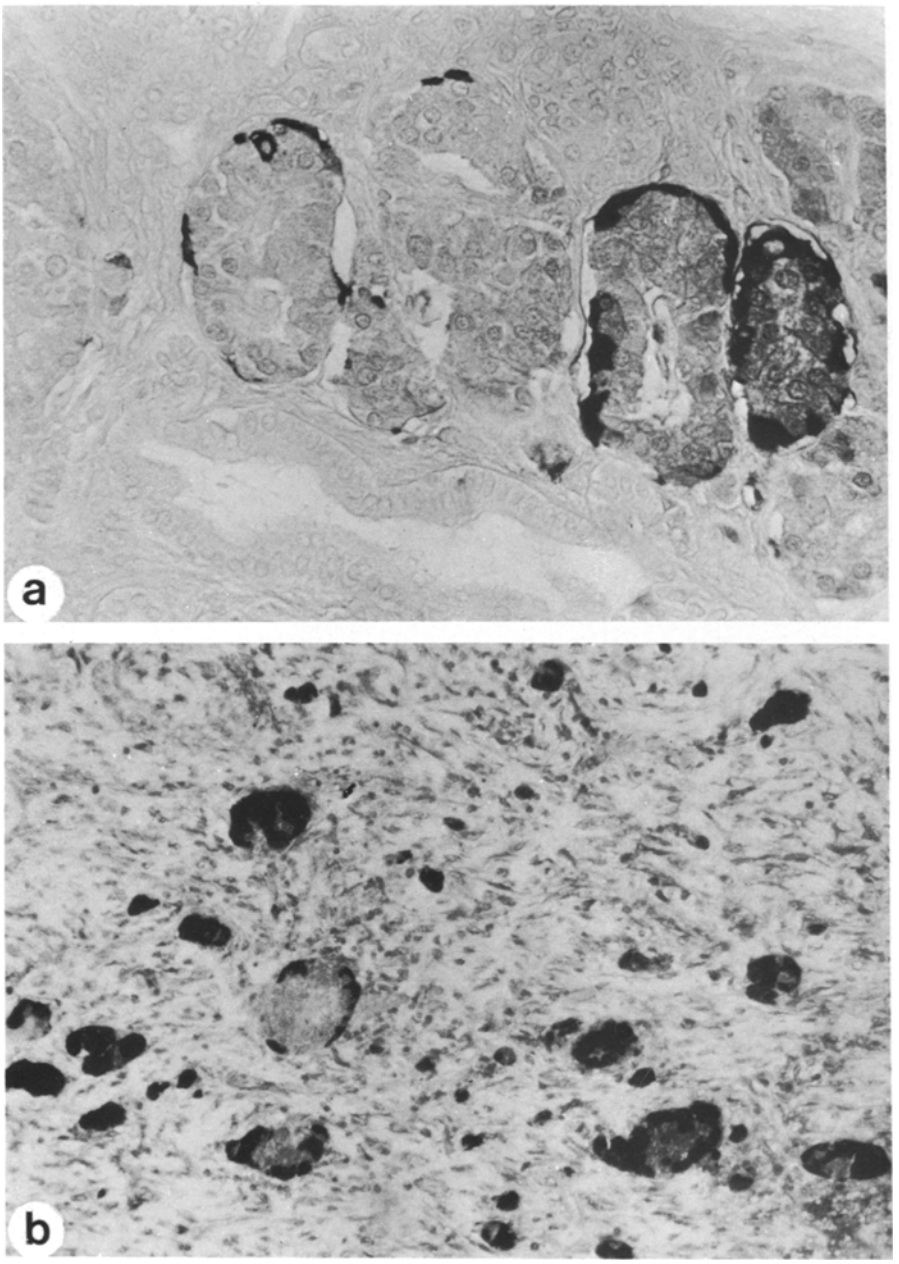

Fig. 5a and b. Immunocytochemical staining for PP cells in chronic pancreatitis (tail). **a** Groups of islets with increased number of PP cells (compare with Fig. 3a). **b** Sclerotic tissue containing numerous small clusters of PP cells. $\times 140$

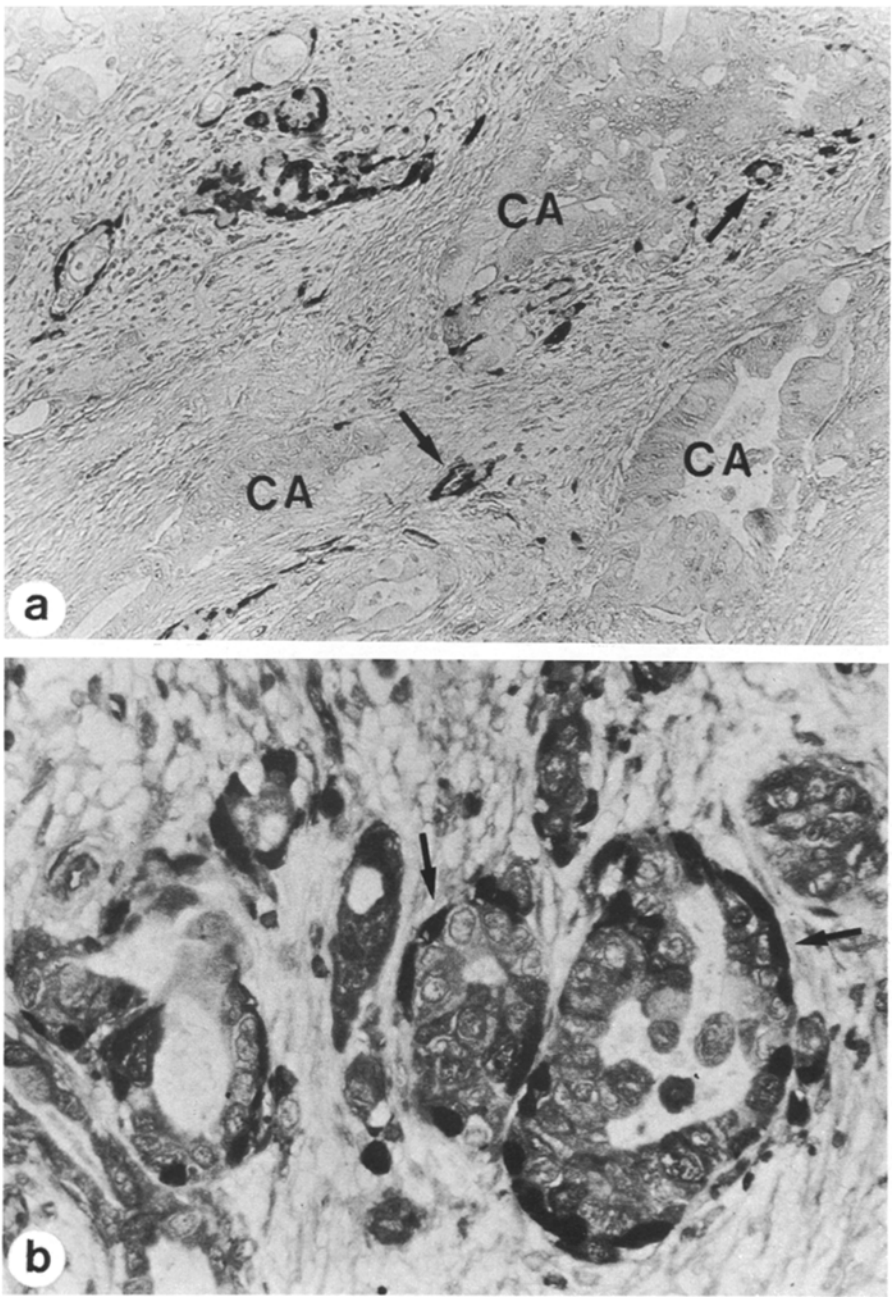

Fig. 6a and b. Immunocytochemical staining for PP in pancreatic carcinoma. **a** Groups and single PP cells (*arrows*) scattered between carcinomatous ducts (*CA*). ×140. **b** PP cells closely associated with atypical duct cells (*arrows*). ×640

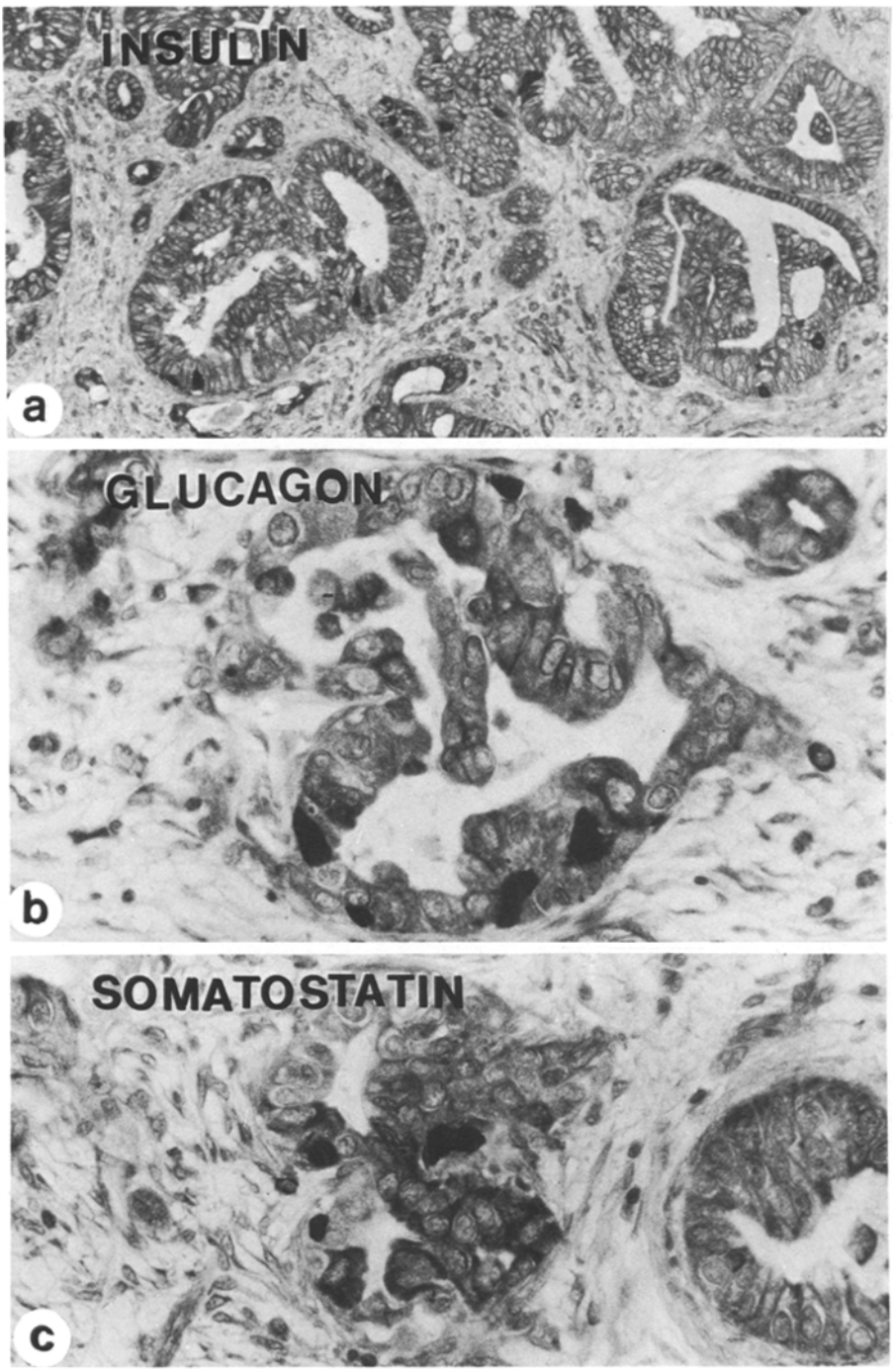

Fig. 7a–c. Immunocytochemical staining of different endocrine cells associated with a pancreatic duct carcinoma. **a** ×140, **b** and **c** ×350

Group III

All patients suffered from a ductal adenocarcinoma of the pancreatic head. At the advancing edge of the tumours some carcinomatous ducts were surrounded by small endocrine cell clusters, and sometimes endocrine cells were found in close contact with the atypical duct epithelium. At immunocytochemistry these cells contained mainly PP (Fig. 6) and glucagon, and to a lesser degree insulin and somatostatin (Fig. 7). These endocrine cell accumulations, occurring focally in 3 patients, could easily be distinguished from the PP cell rich islets in the pancreatic head, which were also often seen in the cases with pancreatic head carcinomas.

Discussion

Our results confirm recent observations of a heterogenous PP cell distribution in the normal human pancreas (Orci et al. 1978; Paulin and Dubois 1978; Gersell et al. 1979; Rahier et al. 1979), which has also been recognized in rats and dogs (Baetens et al. 1979; Gersell et al. 1979). Islets of the tail, body and upper parts of the head of the pancreas contain only a small number of PP cells which are mainly localized at their periphery. These islets, representing the well known islet type in the pancreas (Langerhans, 1869), are characterized by its compact oval shape and a marked predominance of B cells. By evaluating the relative endocrine cell area occupied by each cell type B cells were found to make up 70% of the area in compact islets, A cells 25%, D cells 6% and PP cells 1%. These numbers correspond quite well with earlier data based on a cell counting method (Klöppel et al. 1978). The opposite result was observed in the islets of irregular size and shape which occur in the lower dorsal, but sometimes also in the lower anterior portion of the pancreatic head. Here the PP cell is the dominating endocrine cell type. In these islets with irregular outlines (Mc Callum 1907; Cecil 1911) the PP-cells constitute 60% of the total endocrine area while B-cells make up 30%, A cells 7.4%, and D cells 2.8%. This proportional distribution remains fairly constant in the islets. The total area occupied by PP cell rich islets, however, and thus the total PP cell mass seems to vary greatly from one case to another. This also appears from other studies. In the newborn pancreas the area occupied by PP cell rich lobules was found to constitute from 13.2 to 16.7% of the volume of the entire gland (Rahier et al. 1979) while in the adult pancreas it makes up from 6% to 24% (Orci et al. 1978).

It is not yet known whether the PP cell predominance in the area of the pancreatic head, probably deriving from the ventral pancreas anlage (Baetens et al. 1979), is of any functional significance. Investigations of the PP cell system in primary or secondary chronic pancreatitis, in which both the digestion as well as the endocrine function of the pancreas are impaired, might provide an answer to this question. In this disease the histological and cytological architecture of the islets in the sclerotic tissue is markedly changed (Klöppel et al. 1978). For reasons unknown, the number of B cells is reduced, while that of A and PP cells is unequivocally increased. This increase is particularly true

for PP cells, since all the pancreatic specimens evaluated where obtained from the body and the tail of the pancreas, where in normal specimens no PP cell rich islet areas occur. These data, determined by point counting morphometry, confirm earlier results (Klöppel et al. 1978), although the increase in PP cells is now demonstrated more clearly. The increase in PP and A cells appears to be a relative hyperplasia due to the reduction of the number of B cells. However, the finding of a ductulo-insular proliferation (nesidioblastosis) in which mainly A and PP cells are involved, suggests that the increase in PP and A cells may not be merely secondary to the reduction of B-cells.

It is well known that B cell responsiveness to glucose is impaired in chronic pancreatitis (Bank et al. 1975), while the glucagon secretion from the A cells is most often well preserved (Kalk et al. 1974, 1975). The release of PP in patients with chronic pancreatitis and steatorrhea was recently found to be significantly reduced in response to a fat meal (Adrian et al. 1979). The apparent discrepancy between these pathophysiological and our morphological observations might be explained as follows. In spite of a relative PP cell hyperplasia in the remaining islets, the absolute number of PP cells may be reduced in chronic pancreatitis, particularly if the PP cell rich lobules in the head region are also destroyed. The occurrence of steatorrhea in chronic pancreatitis indicates a loss of acinar cell function of about 90% (Adrian et al. 1979) and, thus, an almost complete replacement of the parenchyma by connective tissue. In addition, extensive scarring of the exocrine parenchyma surrounding the islets may alter the responsiveness of PP cells to physiological stimuli by destruction of blood vessels and nerves. Moreover, the secretion of a postulated (humoral) signal, which is considered to be partly responsible for the release of PP (Schwartz et al. 1976), could be, and probably is, inadequate in severe chronic pancreatitis. The same may be true for the impaired B cell function.

Another type of PP cell hyperplasia, combined with an A cell and to a lesser degree with a B cell hyperplasia, was found along the advancing edge of pancreatic carcinoma. Apart from those islets which were localized in the midst of the cancerous tissue (Stobbe phenomenon) we observed regeneration and proliferation of endocrine tissue in close association with invading carcinomatous ducts in 3 patients. The endocrine cells (predominantly PP and A cells) formed small rows or clusters in close contact with carcinomatous duct cells, or were localized in their immediate vicinity. These features clearly distinguished this tpye of regional endocrine hyperplasia from the non-homogenous distribution of PP cells in the normal pancreas and from the type of hyperplasia seen in chronic pancreatitis.

The factor inducing this localized hyperplasia of PP cells is probably the spread of the ductular carcinoma along normal ducts. It is of interest that the proliferating cells are mainly PP- and A cells, while B cells and especially D cells are involved to a lesser degree.

Considering all these findings it is evident that in the human panreas one should be cautious in using the term PP cell hyperplasia. Its use is only justified if the normally irregular distribution of the PP cell is taken into account.

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Addendum: While this work was in progress there appeared another paper on regional islet cell distribution in the normal pancreas [Malaisse, F., Stefan, Y., Cox, J., Perrelet, A., Orci, L. (1979)]. The data given in this paper differ slightly from our results. Diabetologia 17:361–365

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