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The Endocrine Pancreas in Chronic Pancreatitis

Immunocytochemical and Ultrastructural Studies

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Summary. The endocrine pancreatic tissue from patients with severe primary chronic pancreatitis ($n=6$), secondary chronic pancreatitis due to duct obstruction by carcinoma ($n=6$) and non-diabetic, non-pancreatitic controls ($n=4$) was studied qualitatively and quantitatively using specific immunocytochemistry and electron microscopy. Grouping of variously sized islets in the sclerotic tissue (sclerosis islets), islet neoformation by ductuloinsular proliferation, and intrainsular fibrosis were the main qualitative findings. Immunocytochemical quantitation of the distribution of insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP) producing cells revealed a significant relative increase in the number of A cells and a decrease in the number of B cells of the sclerosis islets in primary chronic pancreatitis (B-44.1 ± 9.3% : A-38.3 ± 2.4% : D-8.6 ± 5.1% : PP-4.6 ± 4.1%) as well as in secondary chronic pancreatitis (B-38.0 ± 14.3% : A-38.4 ± 19.0% : D-9.1 ± 5.8% : PP-14.5 ± 23.4%) compared with controls (B-71.1 ± 8.1% : A-24.3 ± 5.5% : D-8.0 ± 2.8% : PP-0.5 ± 0.4%). The number of PP cells was significantly increased in primary chronic pancreatitis only. It is suggested that scarring of the exocrine pancreas affects islet composition, probably by impairment of the local circulation and of glucose diffusion, thus leading to reduction of the number and glucose sensitivity of B cells. The hyperplasia of A and PP cells appears to be a secondary phenomenon due to the loss of B cells.

Key words: Pancreatic diabetes – Endocrine pancreas – Islet composition – Immunocytochemistry – Ultrastructure.

Introduction

Carbohydrate intolerance is a frequent complication of chronic pancreatitis, leading to subclinical or overt forms of diabetes mellitus. It has been shown

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that in all these cases insulin release is defective (Anderson et al., 1970; Grabner et al., 1974; Bank et al., 1975). Since insulinopenia is associated with chronic pancreatitis it is likely that it results from damage to the B cells. However, morphological findings supporting this assumption are lacking.

The aim of this study is to provide data on the qualitative and quantitative histology and cytology of the endocrine pancreas in cases of severe primary and secondary chronic pancreatitis, using specific immunocytochemistry as well as electron microscopy. The quantitative distribution of insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP) containing cells in chronic pancreatitis is compared with the corresponding values in non-diabetic, non-pancreatic controls.

Material and Methods

Patients

Surgical specimens of the pancreas were obtained from 16 patients who underwent partial or total pancreatectomy. The most important clinical data are summarized in Table 1.

Group I: Four patients who did not suffer from pancreatitis nor from diabetes mellitus served as controls. Their pancreas or parts of it were removed because of carcinoma of papilla Vateri, gastric carcinoma, bile duct carcinoma or retroperitoneal sarcoma.

Table 1. Clinical data

Case No.	Age/Sex	Clinical Diagnosis	Radiological Calcification	Alcohol Abuse	Diabetes mellitus
Group I					
1	58 ♀	Carcinoma of papilla Vateri	–	–	–
2	34 ♂	Gastric carcinoma	–	–	–
3	69 ♂	Bile duct carcinoma	–	–	–
4	40 ♂	Retroperitoneal sarcoma	–	–	–
Group II					
5	40 ♂	Chronic relapsing pancreatitis	+	+	manifest
6	46 ♀	"	+	+	subclinical
7	36 ♀	"	+	–	subclinical
8	38 ♂	"	+	+	subclinical
9	39 ♂	"	–	+	manifest
10	40 ♀	"	+	–	subclinical
Group III					
11	40 ♂	Pancreatic carcinoma (head)	+	+	subclinical
12	75 ♂	"	–	–	–
13	70 ♂	"	–	–	–
14	54 ♂	"	–	+	manifest
15	59 ♂	"	–	–	manifest
16	66 ♂	Carcinoma of papilla Vateri	–	–	–

Group II: In six patients the diagnosis of severe primary chronic pancreatitis was established by a history of recurrent attacks of pancreatitis associated with raised serum amylase, by abnormal responses of bicarbonate and enzyme secretion to secretin/pancreozymin stimulation, by determination of fat excretion and by endoscopic retrograde cholecysto-pancreaticography. Diabetes mellitus was diagnosed by abnormal glucose tolerance tests, fasting hyperglcemia and/or glucosuria.

Group III: Six patients had secondary chronic pancreatitis following obstruction of the pancreatic duct by carcinoma in the head of the pancreas.

Light Microscopy

At least four tissue samples of the resected pancreatic specimens were fixed in Bouin's solution (for size of specimens see Table 2). Serial paraffin-embedded sections were stained with hematoxylin and eosin, periodic acid Schiff (PAS), aldehyde fuchsin (AF), phosphotungstic acid hematoxylin (PTAH) and Grimelius silver technique (Grimelius, 1968).

Immunocytochemistry

Insulin, glucagon, somatostatin and human pancreatic polypeptide (HPP) were localized on serial sections (5 µm thick) in order to visualize B, A, D and PP cells (Lausanne 1977 classification;

Table 2. Morphological data and diabetes mellitus

Case No.	Histological diagnosis	Pancreas specimen (size – cm)		Proportion of sclerosis %	A–B ratio[a]	Diabetes
Group I						
1	Normal pancreas	head	3×4×2	–	1:3.5	–
2	"	tail	6×4×2	–	1:2.9	–
3	"	head-body	8×4×2	–	1:3.5	–
4	"	total pancreas		–	1:3.0	–
Group II						
5	PCP with severe sclerosis	tail	4×2×2	40–60	1:0.9	manifest
6	"	head	3×2×1	60–80	1:0.7	subclinical
7	"	body-tail	5×2×1	80–90	1:1.0	subclinical
8	"	head-body	5×3×2	60–80	1:1.4	subclinical
9	"	body-tail	5×3×2	60–80	1:0.8	manifest
10	"	tail	3×2×1	50–70	1:1.4	subclinical
Group III						
11	SCP with severe sclerosis	body-tail	8×2×1	50–70	1:1.6	subclinical
12	"	head-body	7×4×3	60–80	1:1.7	–
13	"	total pancreas		60–80	1:1.1	–
14	"	head-body	8×4×2	50–70	1:0.9	manifest
15	"	total pancreas		50–70	1:0.4	manifest
16	"	head-body	7×2×2	50–70	1:1.4	–

PCP = primary chronic pancreatitis; SCP = secondary chronic pancreatitis

[a] In groups II and III only the A–B ratio in sclerosis islets is considered

Solcia et al., 1977). In order to eliminate pseudoperoxidase activity of erythrocytes the sections were exposed to 0.3% H_2O_2 in phosphate-buffered saline (PBS, pH 7.3) for 30 min. For the detection of insulin antiserum (dilution 1:1000), prepared in guinea pigs by subcutaneous injections of porcine insulin (Actrapid Novo) together with complete Freund's adjuvant, and a peroxidase-labeled rabbit anti-guinea pig-7′-globulin serum, were used as first and second layers respectively. Antisera to the other hormones were prepared in rabbits as described previously (Polak et al., 1975; Grimelius et al., 1976; Polak et al., 1976). These hormones were then localized by the unlabeled antibody enzyme method (Sternberger, 1974), using anti-glucagon (1:5000), anti-somatostatin (1:250) and anti-HPP serum (1:20,000) as first layer, sheep anti-rabbit Ig G (1:30) and soluble peroxidase-antiperoxidase complexes (1:30) as second and third layers respectively. For the histochemical peroxidase reaction the substrate of Graham and Karnovsky (1966) was used: 0.5 mg/ml of 3,3′-diaminobenzidine-tetrahydrochloride (Sigma) and 0.01% H_2O_2 in an 0.05 M Tris-HCl buffer (pH 7.6). After fixation in 1% OsO_4 in PBS (pH 7.3) the sections were dehydrated and mounted. Specificity of the reactions was determined by the following procedures: 1) non-immune rabbit serum as first layer, 2) specific antiserum absorbed with 50 µg of the antigen per ml diluted serum at 4° C for 24 h as first layer, 3) omission of 3,3′-diaminobenzidine-tetrahydrochloride or H_2O_2 from the incubation medium for the peroxidase reaction.

Quantitation

In serial sections of two blocks of each case the number and proportional distribution of insulin, glucagon, somatostatin and PP cells were determined as follows: In the controls (group I) 20 islets of different size (diameters ranging from 100 µm to 200 µm) were evaluated in each section. In the pancreatitis groups (groups II and III) two classes of islets were examined separately depending on whether they were localized (a) within the sclerotic tissue (sclerosis islets-SI) or (b) within the intact exocrine parenchyma (parenchymal islets-PI). Fifteen to 20 SI of varying diameters were evaluated in each section. The number of PI analysed was 5 to 10 depending on the degree of parenchymal destruction. In general 40 to 50 islets with about 1000 cells were examined in each case. The mean number ($\pm$SD) of B, A, D, or PP cells in the control islets and in the two islet types (SI and PI) of the pancreatitis groups was expressed as percentage (%$\pm$SD) of the relevant total number of islet cells counted.

Quantitative measurements were compared by the U-test of Wilcoxon, Mann and Whitney. For statistical evaluation of paired values (comparison of PI and SI in the same subject) Wilcoxon's rank-sum test was used.

Electron Microscopy

Fresh small samples of the pancreas from 3 of the 4 controls (group I), from 5 of the 6 cases with primary chronic pancreatitis (group II: cases 6, 7, 8, 9, 10), and from 3 of the 6 cases with secondary chronic pancreatitis (group III: cases 13, 15, 16) were examined. The time intervals between removal of the pancreas and fixation of the tissue in a buffered 2.5% glutaraldehyde solution ranged between about 1 min to no more than 15 min. After postfixation in cacodylate-buffered 1% OsO_4 the tissue samples were dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate, and viewed in a Zeiss EM 9 electron microscope.

Results

Group I (Controls)

The exocrine and endocrine pancreas was normal. The perisinusoidal spaces of the islets were small and contained virtually no connective tissue. The B

Table 3. Mean values (% of total islet cell number ±SD) of B, A, D and PP cells and their statistical comparison in normal pancreata, primary chronic pancreatitis and secondary chronic pancreatitis

Cell type	Normal pancreas ($n=4$)	Primary chronic pancreatitis ($n=6$)	Secondary chronic pancreatitis ($n=6$)
B cell (insulin)	71.1 ± 8.1	SI 44.1 ± 9.3 PI 60.5 ± 8.5	SI 38.0 ± 14.3 PI 58.6 ± 10.2
A cell (glucagon)	24.3 ± 5.5	SI 38.3 ± 2.4 PI 25.0 ± 9.1	SI 38.4 ± 19.0 PI 31.6 ± 12.4
D cell (somatostatin)	8.0 ± 2.8	SI 8.6 ± 5.1 PI 7.8 ± 4.6	SI 9.1 ± 5.8 PI 7.0 ± 4.9
PP cell (pancreatic polypeptide)	0.5 ± 0.4	SI 4.6 ± 4.1 PI 0.7 ± 0.6	SI 14.5 ± 23.4 PI 2.8 ± 4.2

SI = sclerosis islets; PI = parenchymal islets; ⌐¬ statistical significance $P < 0.05$

cells were well granulated and occupied the central part of the islets (Fig. 3a). Glucagon containing A cells lined the periphery and the sinusoids of the islets (Fig. 4a). Somatostatin containing D cells were also found at the periphery of the islets or were scattered in the islet parenchyma. PP containing cells were found at the periphery of the islets or between the acinar cells. The result of quantitative analysis are given in Table 3. At the ultrastructural level B, A and D cells were easily distinguished on the basis of the fine structure of their secretory granules. In addition we observed endocrine cells with small granules (120 μm to 250 μm), which occurred at the same frequency as the immunocytochemically stained PP cells. No subclassification of the small granule islet cells was attempted (Deconinck et al., 1971; Larsson et al., 1976; Capella et al., 1977), since it was considered to be of no relevance for this study.

Group II (Primary Chronic Pancreatitis)

The pancreata of all 6 cases displayed characteristic features of severe chronic pancreatitis with advanced peri- and intralobular scarring of the parenchyma. At least 50% of the parenchyma was replaced by connective tissue (Table 2). The remaining exocrine acini appeared atrophic. The sclerotic tissue contained ducts, groups of several islets and some scattered endocrine cell clusters, nerve bundles as well as infiltrates of lymphocytes, histiocytes and some plasma cells.

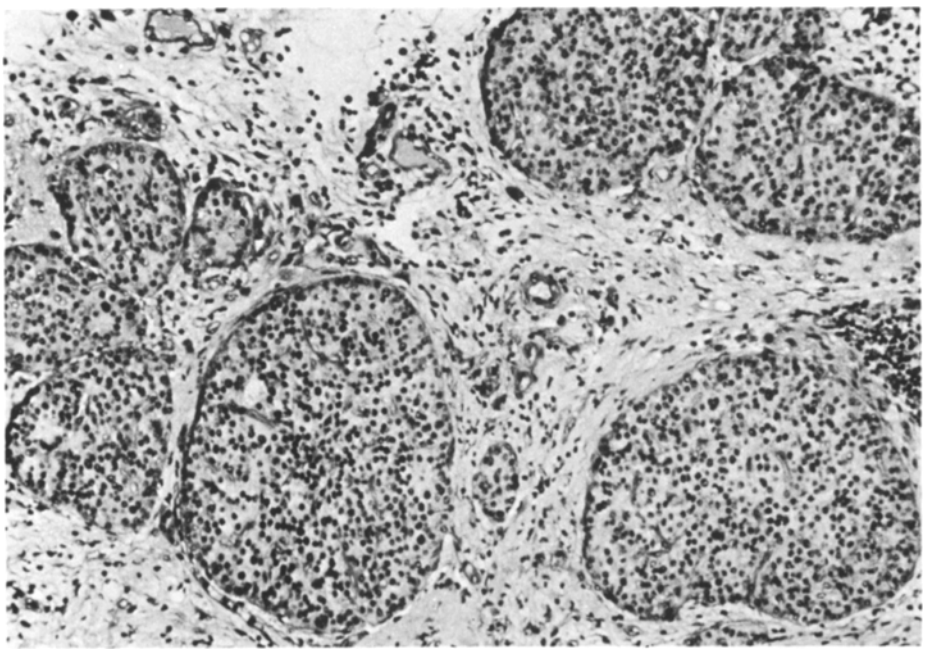

Fig. 1. Pancreas of patient with severe primary chronic pancreatitis: in the sclerotic tissue, replacing the exocrine parenchyma, a group of variously sized islets (sclerosis islets). PAS. × 120

The ducts were generally dilated and focally proliferated, and some were filled with partially calcified protein plugs. The duct epithelium adjacent to these plugs was flattened or destroyed, but squamous metaplasia or intraductal papillary proliferations were rarely seen.

The pancreatic islets showed an extremely variable distribution within the pancreatic tissue. Depending on whether they were found in the remaining parenchyma or in the sclerotic tissue, parenchymal islets (PI) and sclerosis islets (SI) were distinguished. The size of PI was quite constant (mean diameter 150 μm), and their B cells were well granulated. The size of SI was very variable (range of diameter 100 μm to 350 μm) and they were often arranged in groups within the dense connective tissue (Fig. 1). They often showed a distinct perisinusoidal fibrosis which sometimes split the islets into separate lobules (Fig. 3c). The B cells in the SI appeared, as a rule, to be less granulated than those of PI (Fig. 3). Occasional nesidioblastic processes with endocrine cells budding off from duct epithelium (ductulo-insular proliferation) and with formation of 'duct islets' (endocrine cell clusters arranged around mucin producing duct structures) were found in the areas of duct proliferations (Fig. 2).

The distribution pattern of the various cell types in PI, as revealed by immunocytochemistry, coincided with that of the controls. However, quantitation disclosed a slight, but statistically insignificant reduction of the proportion

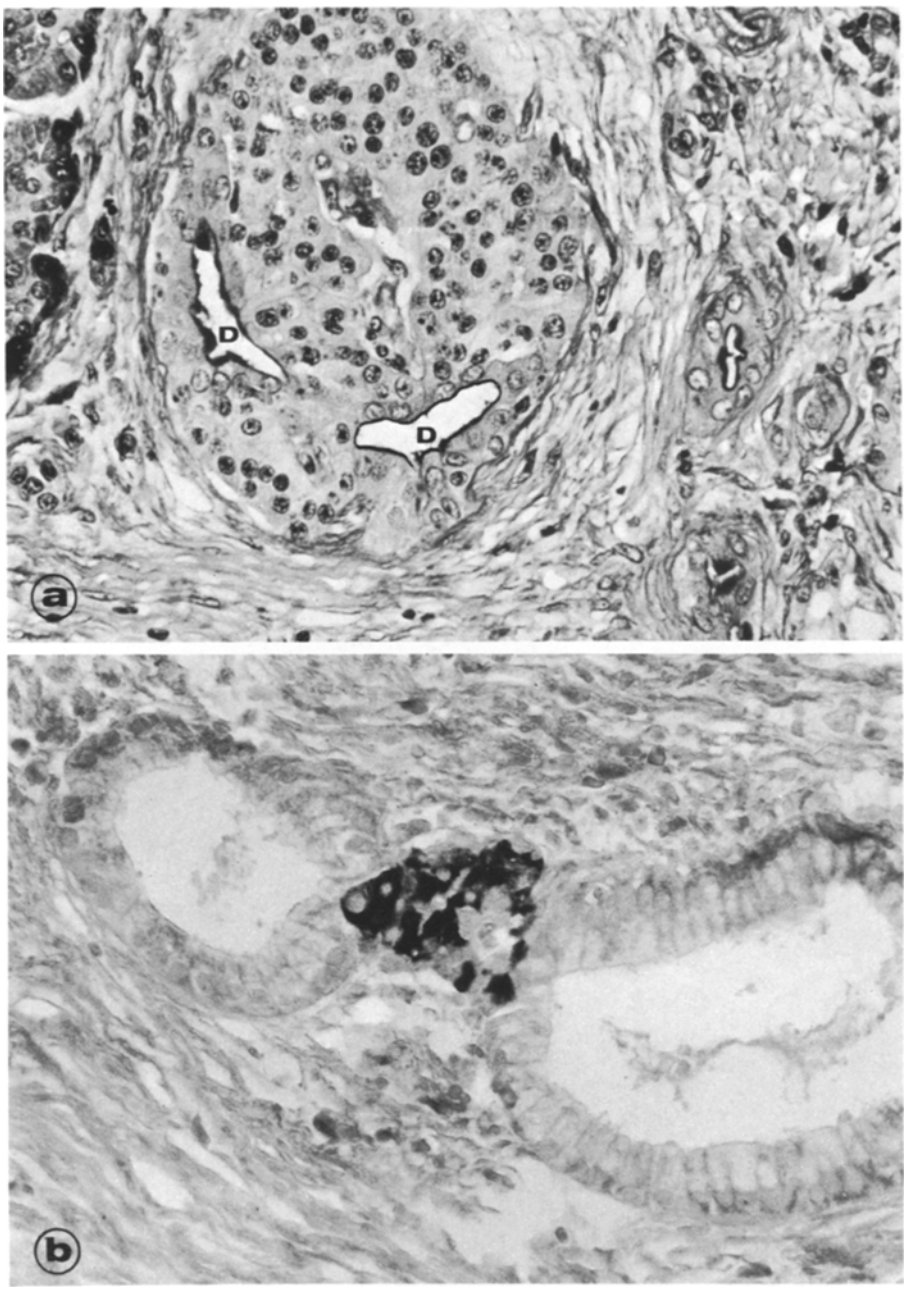

Fig. 2a and b. Nesidioblastosis in primary chronic pancreatitis. **a** Inside an islet ('duct islet') two small ducts (*D*) with mucin producing cells. PAS. ×300. **b** Budding of islet cells from a pancreatic duct. Immunocytochemical staining for insulin shows that B cells predominate in the endocrine cell cluster. ×300

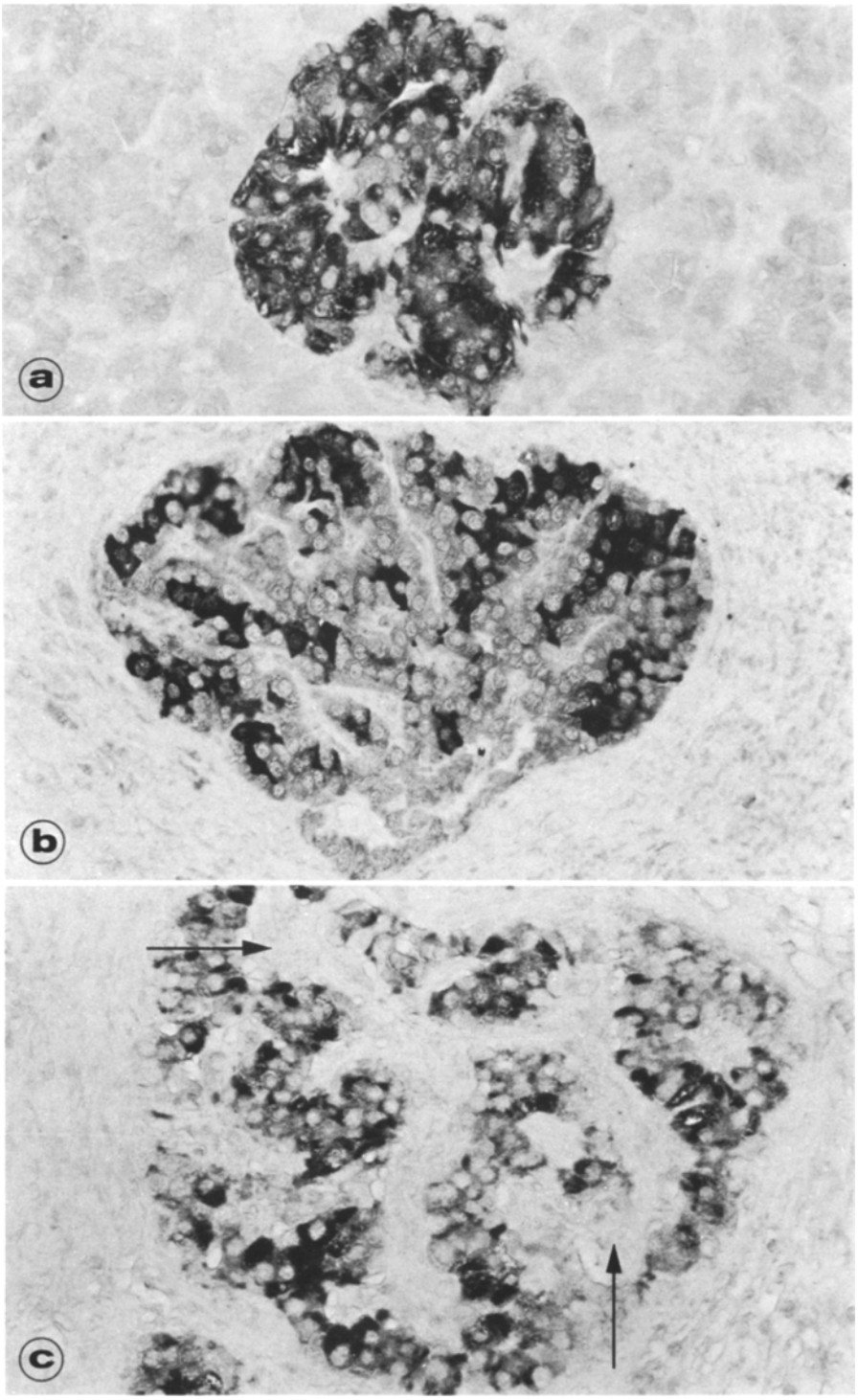

Fig. 3a–c. Immunocytochemical staining for insulin. × 300. **a** Normal islet from control is predominantly composed of well granulated B (insulin) cells. **b** and **c** Sclerosis islets from patients with primary chronic pancreatitis: the number of B cells and their staining intensity are moderately (**b**) or strongly (**c**) reduced. Note also in **c** the perisinusoidal fibrous bands dissecting the islet (arrows)

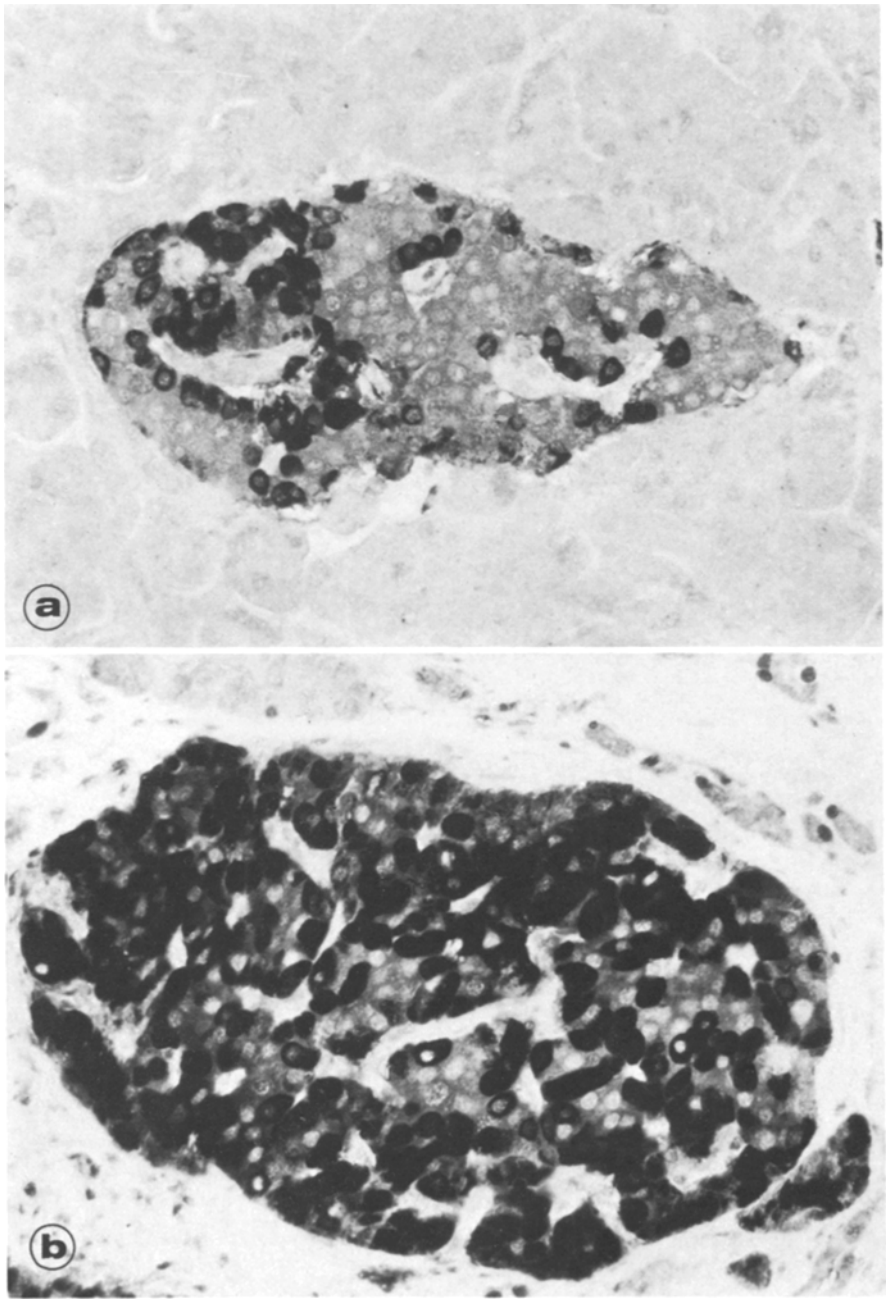

Fig. 4a and b. Immunocytochemical staining for glucagon. ×300. **a** Normal islet from control with peripheral or perisinusoidal localization of A (glucagon) cells. **b** A cell hyperplasia of a sclerosis islet in primary chronic pancratitis with heavily staining for glucagon.

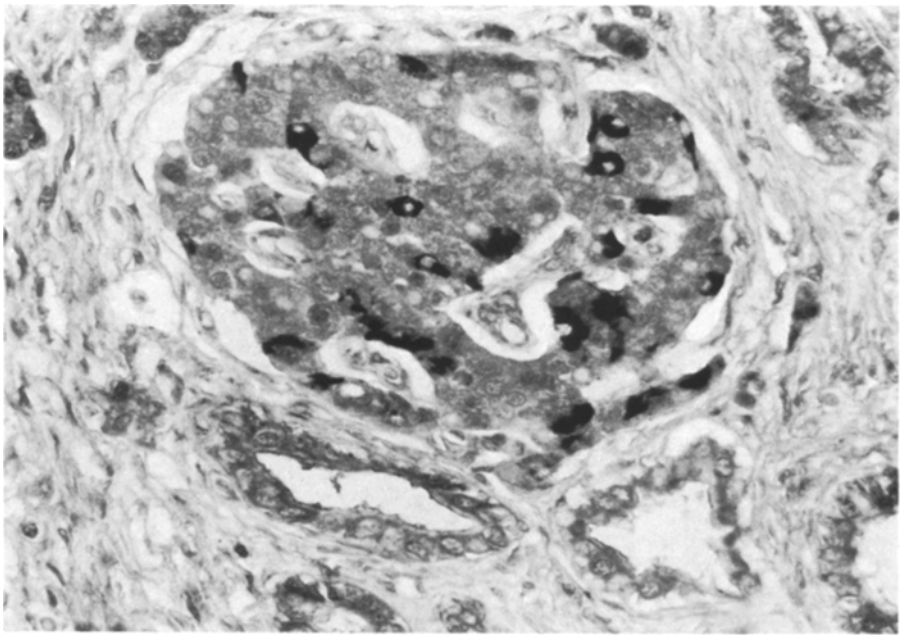

Fig. 5. Immunocytochemical staining for somatostatin in chronic pancreatitis. The number and distribution of D (somatostatin) cells in the sclerosis islet is comparable to that of normal islets. × 300

of the B cells when compared with controls. The mean proportion of A, D or PP cells was within normal ranges (Table 3).

In contrast, the immunocytochemical pattern of SI was qualitatively and quantitatively different from that of control islets and PI (Table 3). The B cells were split up into small groups (Fig. 3) and their proportion had diminished significantly, whereas the relative number of A cells had significantly increased. These frequently formed continuous cell ribbons along the periphery and perisinusoidal spaces, and had invaded the central parts of the SI along the sinusoids (Fig. 4). The number and distribution of D cells were in keeping with the findings in the controls (Fig. 5). The number of PP cells per islet varied a great deal, but their average relative number had statistically increased, whilst their peripheral localization in the islets was usually maintained. Single PP cells occurred in high numbers in the duct epithelium and around ducts, without forming real clusters (Fig. 6). In addition PP cells, together with A and B cells, took part in ductulo-insular proliferations.

On electron microscopy the SI revealed no consistant alterations of endocrine cells (5 cases). In particular, the B cells did not show any degenerative lesions, although the number of their secretory granules appeared to be somewhat reduced. Apart from B and A cells, which were the prevailing cell types in the islets, small numbers of D cells and small granulated cells were present. The perisinusoidal spaces were frequently enlarged and contained numerous bundles of collagen fibres between the basal membranes separating the sinusoids from the islet cells (Fig. 7).

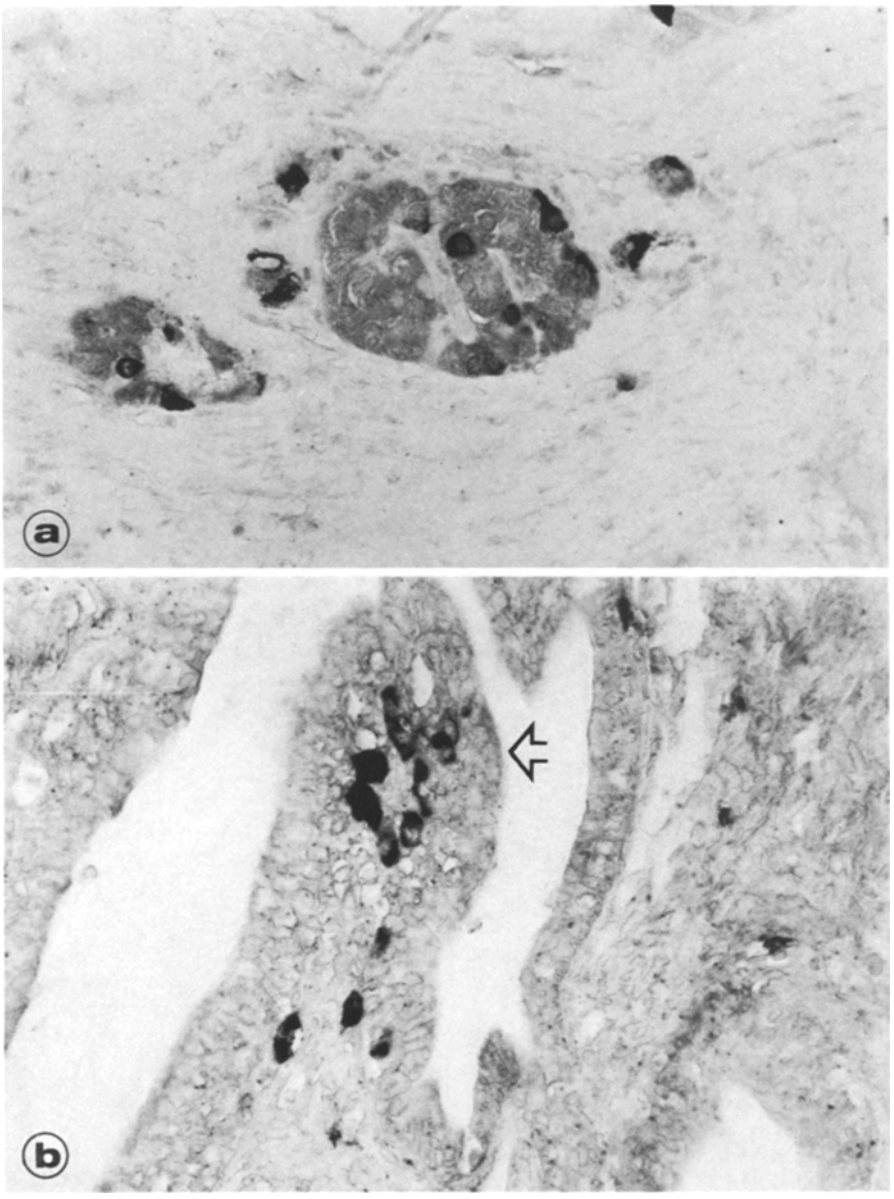

Fig. 6a and b. Immunocytochemical staining for pancreatic polypeptide (*PP*) in primary (**a**) and secondary (**b**) chronic pancreatitis. Increased numbers of PP cells in sclerosis islets (**a**) and in periductular areas (**b**). Close contact of PP cells with the duct epithelium (*arrow*). ×300

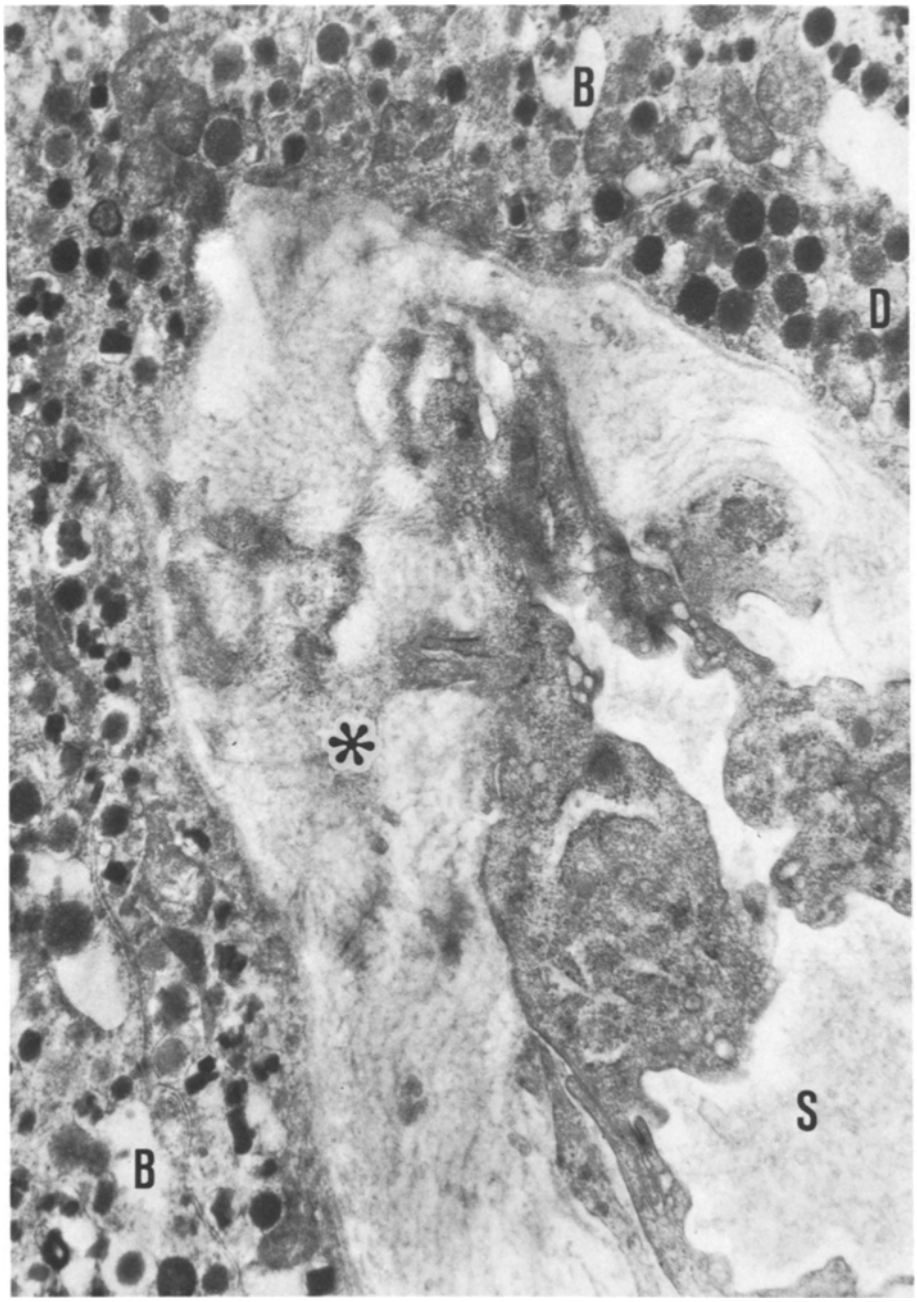

Fig. 7. Electron micrograph of a sclerosis islet in primary chronic pancreatitis: conspicious increase in collagen fibres in the perisinusoidal space (*asterisk*). Sinusoid (*S*). B cells (*B*). D cell (*D*). ×20,400

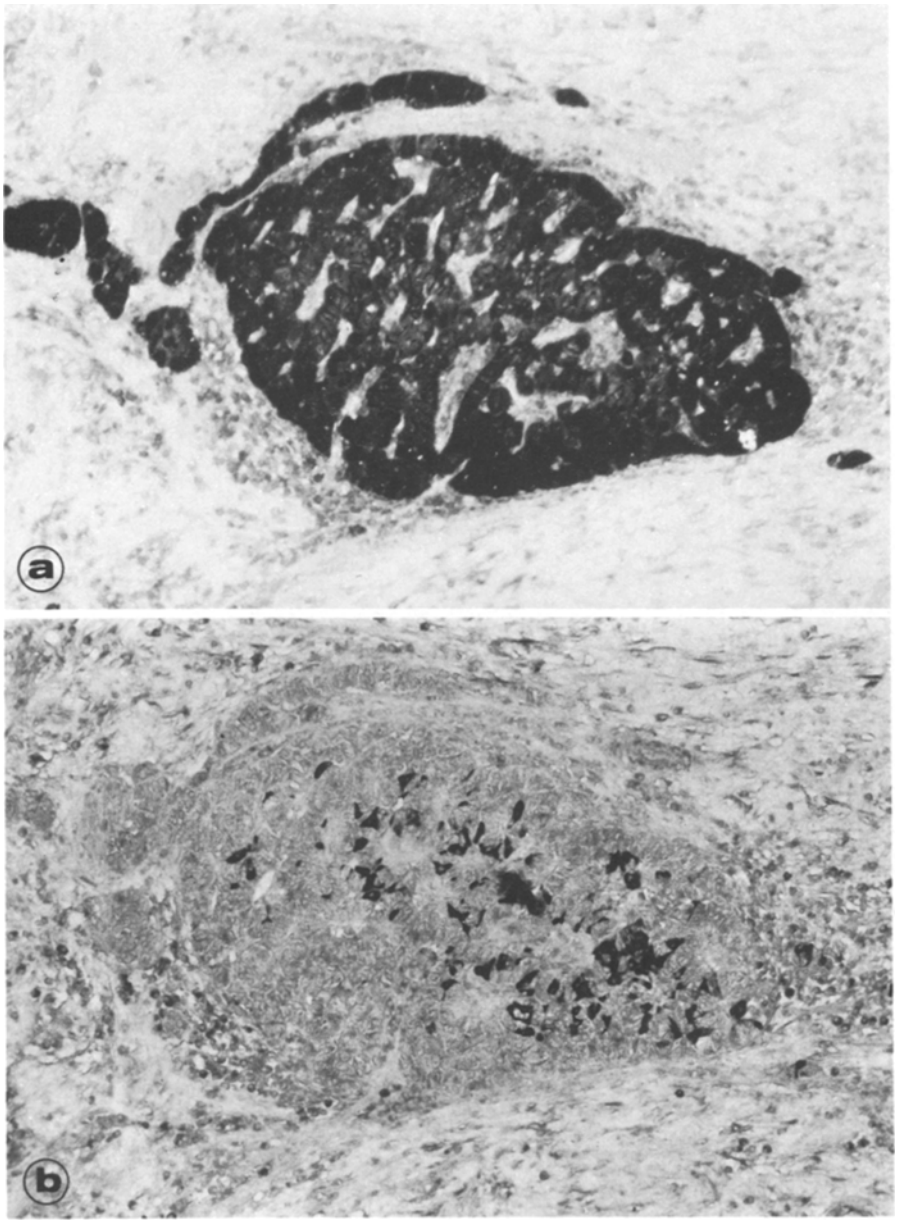

Fig. 8a and b. Immunocytochemical staining for pancreatic polypeptide (*PP*) and insulin. ×120. **a** A large sclerosis islet in secondary chronic pancreatitis is almost exclusively composed of PP cells. **b** The same islet on a consecutive section merely contains scattered B (insulin) cells. ×120

Group III (Secondary Chronic Pancreatitis)

In all 6 cases a circumscribed adenocarcinoma in the head of the pancreas or a carcinoma of papilla Vateri obstructed the main pancreatic duct. All pancreatic ducts and their branches showed marked prestenotic dilatation. Papillary proliferations or squamous metaplasia of the duct epithelium were frequently seen. Around the main ducts marked concentrical sclerosis had developed, associated with severe atrophy of the exocrine pancreas. The sclerotic tissue was moderately infiltrated, mainly by lymphocytes and hsitiocytes. The quantitative relationships of connective tissue and remaining parenchyma is indicated in Table 2. The remaining acinar tissue contained normally distributed medium sized PI. In the sclerotic tissue SI of varying size were grouped together or scattered, some were invaded and dissected by fibrous septa along the sinusoids.

The immunocytochemical results in PI and SI were virtually identical with those in primary chronic pancreatitis. However, the proportion of PP cells in SI was not significantly different from that of controls (Table 3). The mean percentage of PP cells appeared to be increased, but their number varied to such extent from one islet to another and from case to case that the values obtained were not statistically significant. Some islets were almost exclusively composed of PP cells (Fig. 8).

The ultrastructure of all endocrine cell types in SI (3 cases) was normal. As in primary chronic pancreatitis the perivascular tissue was increased in SI.

Discussion

The overall incidence of subclinical (chemical) or manifest (overt) diabetes mellitus secondary to chronic pancreatitis varies between 40% and 70% (Marks and Bank, 1963; Sarles and Sarles, 1964; Strohmeyer et al., 1974; Bank et al., 1975). If only chronic calcific pancreatitis is considered, the frequency of diabetes increases to 90% (Bank et al., 1975). From this can be deduced that chronic pancreatitis per se affects the function of the B cells.

Functional studies, using various tests for insulin secretion, have shown that in almost all cases with chronic pancreatitis the insulin reserve is depleted (Anderson et al., 1970; Grabner et al., 1974; Bank et al., 1975). This depletion is most severe in chronic calcific pancreatitis. However, although the functional state of the endocrine pancreas has been thoroughly examined (Peters et al., Bank et al., 1968; Joffe et al., 1968; Raptis et al., 1971; Grabner et al., 1974; Kalk et al., 1974; Kalk et al., 1975; Ebert et al., 1976; Häcki et al., 1977), little is known of the histo- and cytopathology of the islets in this disease and hence of the pathogenesis of pancreatic diabetes.

The present morphological study of six cases with primary chronic pancreatitis, all showing subclinical or manifest diabetes mellitus, disclosed distinct qualitative and quantitative changes in the endocrine pancreas, in addition to the well known lesions of the exocrine parenchyma (Doerr, 1964; Seifert, 1966; Böcker and Seifert, 1972; Becker, 1973). The most obvious qualitative changes were the focal accumulation of SI of variable size, occasional neoformation of islets by ductuloinsular proliferation (nesidioblastosis) and perisinusoidal fi-

brosis in SI. The accumulation of SI probably results from progressive collapse of the exocrine parenchyma. However, the occurrence of ductuloinsular proliferation indicates that some neoformation of endocrine pancreatic tissue must take place. Perisinusoidal fibrosis, which was most obvious on electron microscopy, was observed in SI but was rare in PI and virtually absent from control islets. We feel that since only a limited number of cases could be examined by electron microscopy the finding of perisinusoidal fibrosis must be confirmed by future studies. The fine structure of all endocrine cell types of the pancreas, particularly that of the B cells, appeared to be normal in chronic pancreatitis.

The proportional distribution of insulin (B), glucagon (A), somatostatin (D) and human pancreatic polypeptide (PP) containing cells varied very little in the controls. For B, A and D cells our results correspond closely to the data from other studies (Orci et al., 1976; Gepts et al., 1977). PP cells, which were irregularly distributed, represented only about 0.5% of the endocrine cell mass. In chronic pancreatitis the proportion of B cells was reduced to about 60% of the control values. This loss of B cells was most marked in SI. In contrast to the B cell reduction there was a significant increase of A and PP cells in SI. The increase of the number of A cells confirms earlier findings of A cell hyperplasia in chronic pancreatitis (Eder, 1955; Wacjner, 1965, Paloyan et al., 1967). The number of D cells remained unchanged.

The fact that the changes of the histological and cytological composition of the islets in chronic pancreatitis were most marked in SI, but considerably lower in PI, suggests a strong dependence of these islet lesions and their functional disturbances on the progressive process of scarring in the pancreas. Evidence in support of this suggestion is given by the results obtained in the 6 cases of secondary chronic pancreatitis with severe sclerosis of the pancreas due to chronic duct obstruction by a carcinoma. Islet morphology in this group, in which three patients had developed diabetes, was almost identical to that found in primary chronic pancreatitis. Eder (1955) who observed analogous islet changes in pancreatic sclerosis following carcinomatous duct obstruction, reported diabetes in two of three cases. Carbohydrate intolerance was found in ten of fifteen dogs with pancreatic sclerosis after chronic duct ligation (Idezuki et al., 1969). Mucoviscidosis, which also causes severe sclerosis of the pancreas, is ten times more frequently associated with diabetes than would be expected from the normal population incidence (Weber, 1974). It can therefore be concluded that any scarring of the pancreas results in changes in structure and function of the islets. The mode of action of pancreatic sclerosis on islet composition and function is uncertain; one may speculate that trophic factors from the surrounding acinar tissue, essential for islet integrity, are lost by the parenchymal destruction, or that sclerosis affects islet integrity by disturbing local circulation and glucose diffusion (Bommer et al., 1976; Seifert et al., 1977). In support of the latter assumption is the perisinusoidal fibrosis of the SI observed in chronic pancreatitis.

The reduction of the B cell mass and the increase in A cells manifest itself in a shift of the A–B ratio from 1:3.0–3.5 in controls to 1:0.4–1.7 in chronic pancreatitis (Table 2). Whether the decrease in B cells and the increase in A cells is absolute cannot be settled by this work, as only the relative distribution of the endocrine cells was determined. However, from the A–B ration and

taking into account the widespread parenchymal destruction, it can be inferred that the absolute B cell number is reduced. Thus the B cells seem to be more sensitive to trophic disturbances affecting the islets than any other islet cell type. These results are in accordance with data demonstrating a reduced insulin reserve in almost all cases with chronic pancreatitis (Grabner et al., 1974). However, from experiments (Martin and Lacy, 1964) and from investigations in patients with partial pancreatectomy (Nieschlag et al., 1974) it is evident that a diabetic state will develop only after removal of at least 80% to 90% of the pancreas. It is, therefore, likely that in chronic pancreatitis there occurs not only a numerical decrease of B cells but also that the B cells lose their optimal responsiveness to glucose, possibly by impaired perisinusoidal diffusion. Bank et al. (1975) showed that in this disease the B cells respond better to oral glucose than to i.v. glucose, suggesting that the entero-insular pathway is intact, whereas the glucose receptor may be defective.

A cells seem to be more resistant to pancreatitic sclerosis than B cells. Glucagon release was frequently found to be normal or even inappropriately high for the circulating glucose concentration (Kalk et al., 1974, 1975). An inadaequate response of the A cells to blood glucose levels may be directly related to pancreatic destruction but it has been experimentally demonstrated that the suppressive effect of high glucose levels on glucagon release is abolished in the absence of insulin (Müller et al., 1971). It is therefore conceivable that, by analogy with genuine diabetes (Unger, 1975), the relative hyperglucagonaemia and A cell hyperplasia in chronic pancreatitis are due to the insulin deficiency per se, and are thus a secondary phenomenon.

Quantitation of the D cells in chronic pancreatitis revealed no abnormalities compared with controls. This finding contrasts with the results in juvenile diabetics, in whom an A cell and D cell hyperplasia has been demonstrated in addition to an almost complete B cell loss (Orci et al., 1976).

The number of PP cells was found to be increased in chronic pancreatitis. However, PP cell hyperplasia only proved to be of statistical significance in primary chronic pancreatitis due to the variability of PP cell increase in the secondary form of the disease. Although in single cases of both groups islets largely composed of PP cells were observed, the findings were not as conspicious as has been reported in juvenile diabetics (Gepts et al., 1977) and in other pancreatic diseases (Larsson, 1977). The frequent occurrence of PP cells in close contact with duct epithelium in chronic pancreatitis indicates that the increase in these cells probably represents a regenerative phenomenon due to pancreatic injury. Recently it has been shown that secretion of PP is impaired in chronic pancreatitis, suggesting a functional defect of the hyperplastic PP cells (Häcki et al., 1977).

In *conclusion,* the present study offers the following concept for the pathogenesis of diabetes in chronic pancreatitis. The prime cause of the relative (and probably also absolute) reduction of the B cell number and impairment of the B cell responsiveness to glucose seems to be the progressive sclerosis of the pancreas, with involvement of the islets. In most cases the degree of sclerosis determines the severity of the insulin deficiency and the resulting diabetic state (Seifert and Klöppel, 1974). In those cases showing only mild chronic pancreatitis but overt diabetes, an additional genetic disposition to diabetes might be present.

A cell hyperplasia and PP cell hyperplasia are most probably secondary to the B cell loss and/or pancreatic injury and seem, therefore, not to be involved in the pathogenesis of diabetes in chronic pancreatitis.

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