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Cystic fibrosis associated islet changes may provide a basis for diabetes

An immunocytochemical and morphometrical study

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Summary. The pancreases of 23 patients (mean age 10.5 years, range 5–22) years dying of cystic fibrosis (CF) were evaluated at autopsy by routine histology and immunostaining for changes in their endocrine cell compartment. The severely altered pancreatic tissues showed end stage CF, with either a fibrotic pattern (CF-FIB, $n=14$) or a lipoatrophic pattern (CF-LIP, $n=9$) prevailing. In all specimens, irrespective of the dominating pattern, the islet system was affected by marked periinsular and intrainsular sclerosis. Quantitatively, the volume densities (relative tissue components) of the parenchymal, fibrotic, fatty and total endocrine compartments as well as the four islet cell types (B, A, D, PP) were determined by point counting. Compared with controls, the CF patients (including two patients with overt diabetes and glucose intolerance, respectively) had a significantly decreased insulin (B)-cell ratio (from 64.4 to 34%) with a concomitant rise in non-B-cells (A-cells: 23.2 to 35%; D-cells: 10.4 to 22%; PP-cells; 2 to 9%). Comparison of endocrine cell ratios in CF-FIB pancreases with CF-LIP pancreases revealed no significant differences. The reduction of approximately 50% of insulin cells in CF patients with advanced disease supports the concept that destruction of exocrine tissue with concomitant fibrous disorganization of islets gradually changes the proportional distribution of the endocrine cells in favor of the noninsulin cells. This slowly ongoing process probably provides the basis for islet dysfunction, i.e. diabetes, increasingly observed in final stage CF.

Key words: Cystic fibrosis – Endocrine pancreas – Immunocytochemistry – Morphometry

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Introduction

Cystic fibrosis (CF) is a syndrome of apparent exocrine dysfunction resulting in obstructive lesions in multiple organs (Seifert 1984a). Its basic defect lies probably in pathways controlling chloride channel activity (Ming et al. 1988). Elevated serum concentrations of a "Cystic Fibrosis Antigen", showing significant homology with intestinal and brain calcium binding proteins, have been described. The gene to this protein has been mapped to chromosome 1 (Dorin et al. 1987). Markers tightly linked to the cystic fibrosis gene have been located on chromosome 7 (Collins et al. 1987). Cystic fibrosis is probably caused by a single locus but there is evidence for genetic heterogeneity (Tsui et al. 1985; Beaudet et al. 1988).

In the pancreas, CF primarily affects the exocrine compartment causing pancreatic insufficiency (Lebenthal et al. 1986); yet, the development of diabetes mellitus (DM) has also been noted (Schwachman and Grand 1978). DM occurring in conjunction with CF used to be rare (1–2%); however, presumably as a consequence of the increasing survival of CF patients, a raising incidence (8–13%) of overt DM has been reported in young adults suffering from CF (Lebenthal et al. 1986; Schwachman and Grand 1978).

As in the case of chronic pancreatitis (Klöppel et al. 1978), islet dysfunction in CF has been attributed to the progressive fibrosis of the exocrine pancreas (Handwerger et al. 1969; Klöppel 1984). Three recent studies based on quantiative immunocytochemical examinations demonstrated a loss of insulin cells from islets in advanced CF (Iannucci et al. 1984; Soejima and Landing 1986; Abdul-Karim et al. 1986). While, Soejima's (1986) and Abdul-Karim's group (1986) observed the decrease in insulin cells both in diabetic and non-diabetic

CF patients, other authors (Kopito et al. 1976) found this alteration in diabetic CF patients only.

Using the combined approach of immunocytochemistry and morphometry, the present study focuses on the issue whether in end stage CF changes in the ratios of endocrine cells in the pancreas are restricted to patients with diabetes, or are also found in non-diabetic CF patients.

In view of the apparent interindividual variation in islet changes (Iannucci et al. 1984) and dysfunction in CF we also attempted to answer the question whether there is any relationship between insulin-cell content of the islets and a fibrotic pattern or a more lipoatrophic change of the pancreas.

Patients, material and methods

Patients. Twenty-three patients with advanced CF were studied. The age ranged from 5 to 22 years (mean age 10.5 years), the male:female ratio was 1:1.3. All patients had pancreatic insufficiency on clinical investigations. Death was related to respiratory complications. One patient (aged 17 years) had clinically overt diabetes. Another patient (aged 12 years) had impaired glucose tolerance. No manifest diabetes was present in the other patients. Six normoglycemic patients with diseases unrelated to the pancreas (e.g. accident, congestive heart failure, pneumonia) served as controls (range 5–40 years, mean age 15 years, sex ratio M:F = 1:1.3).

Tissue processing. The pancreas was carefully dissected at autopsy, performed between 12–36 h p.m. Specimens were obtained from the body of the pancreas, fixed in Bouin's solution and processed for routine paraffin embedding. Serial sections, 3–5 μm-thick, were stained with Hematoxilin/Eosin (H&E) and periodic acid Schiff (PAS). Immunocytochemistry was performed on the subsequent sections using the avidin-biotin-complex method (ABC), with a monoclonal antibody against insulin (Biogenex via Camon, FRG, primary dilution 1:20) and polyclonal antibodies against glucagon (INC = International Nuclear Corporation, Stillwater, MN, USA, 1:5000), somatostatin (INC, 1:5000) and human pancreatic polypeptide (HPP, gift of Dr. R.E. Chance, Indianapolis, USA, 1:10000). Details of the staining procedure were described previously (Klöppel et al. 1978). To ascertain the staining specifity, appropriate controls were performed.

Qualitative histology. Slides from two blocks of each patient were assessed qualitatively regarding the predominant histological changes, i.e. fibrotic or lipoatrophic (Lebenthal et al. 1986; Abdul-Karim et al. 1986). A fibrotic pattern (CF-FIB) was considered if fibrous replacement of pancreatic parenchyma was the major finding and lipoatrophy moderate or minimal. The pattern was called predominantly lipoatrophic (CF-LIP), if the replacement of the exocrine parenchyma by fatty tissue was prevailing. In both instances, there was a subtotal or total loss of intact acinar tissue. In addition, the presence and extent of intrainsular fibrosis and nesidioblastosis was determined.

Morphometry. The different tissues and cell compartments of the pancreas were evaluated morphometrically. This was performed by point-counting using a 10 × 10 mm grid with 1 mm intervalls, which was inserted in the ocular tube of a ZEISS photomicroscope. Stage 1 was performed on a H&E stained section at a magnification of 160 × evaluating the parenchymal (acinar cells and ducts), fibrous, fatty and endocrine tissue components. Stage 2 was performed evaluating the different endocrine cells (B, A, D, PP) at a magnification 400 ×. At each stage, the tissue of at least one entire cross-sectional slide was evaluated. The scanning table was moved step by step. In that way, the test areas occupied by the grid in the eye-piece were adjacent to each other covering all the tissue for morphometric evaluation. Counts from each test field were added up for the entire section. Volume densities were calculated from the point counts of total endocrine, exocrine, fibrous, lipomatous (stage 1) and endocrine cell tissue (stage 1 and stage 2) (Oberholzer 1983; Oberholzer et al. 1987).

Statistical analysis. The statistical analysis was performed using a computer program system including the tests of Wilcoxon, Mann and Whitney as well as Kruskal-Wallis (Dixon 1983). For the statistical evaluation of paired values Wilcoxon's rank sum test was used. Values were considered to be statistically significant when expressing a two-sided level of probability $p < 0.05$ (Sachs 1984).

Results

Qualitative analysis

All 23 CF-patients, regardless of sex or age, showed end stage lesions of the pancreas. Exocrine parenchyma was either absent or present in minimal amounts. If present, it consisted largely of duct structures, usually showing cystic dilatation. Residual acinar cells were occasionally encountered. They were embedded in connective tissue and appeared to be atrophic. Of the 23 patients, 14 had a predominantly fibrotic histologic pattern (CF-FIB) (Fig. 1 A), 9 showed a predominantly lipoatrophic pattern (CF-LIP) (Fig. 1 B). In general, though not strictly, patients with a fibrotic pattern were younger than those with a lipoatrophic pattern. Irrespective of the prevailing histologic pattern, the islets were generally clustered within fibrous or lipomatous tissue that replaced the acinar compartment (Fig. 2). Islet aggregates were always embraced by connective tissue strands and in most instances accompanied by intrainsular fibrosis, with distinct perisinusoidal sclerosis. Intrainsular fibrosis was equally prevalent in both histological subgroups and for the most part of moderate degree. If intrainsular fibrosis was severe, the islets appeared to be split into several fragments. Occasionally, nesidioblastotic processes with endocrine cells budding off the duct epithelium were observed, in particular in the patients with the fibrotic pattern and not found in patients with the lipoatrophic pattern.

Morphometry of the exocrine pancreas

Pancreatic morphometry of the CF patients revealed a decrease of the parenchymal volume den-

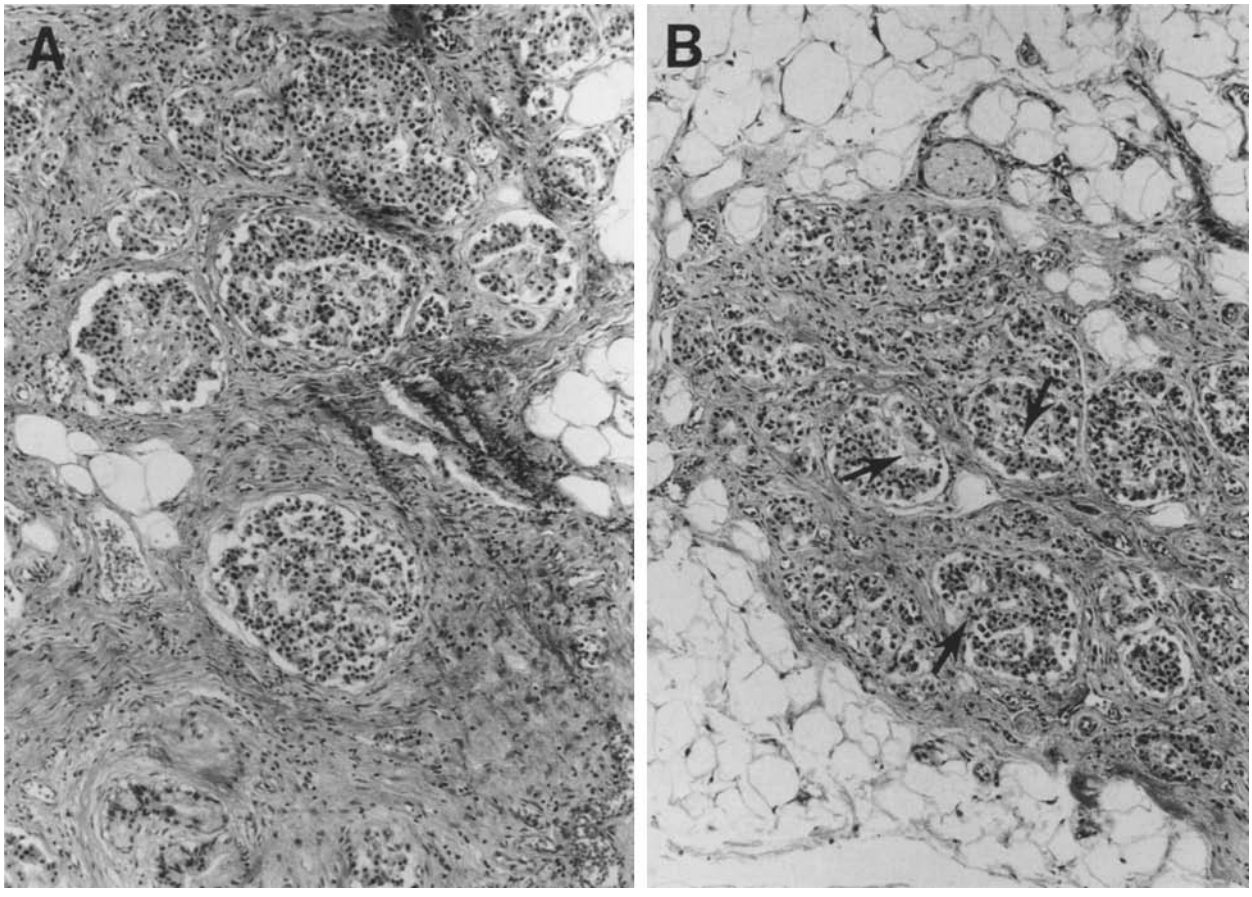

Fig. 1. (**A**) Pancreas from 19-year-old CF patient, showing a fibrotic histologic pattern: The acinar lobuli are replaced by fibrotic tissue. The islets are irregularly distributed and commonly show a marked intrainsular sclerosis. H&E, 125×. (**B**) Pancreas from 16-year-old CF patient, showing a lipoatrophic pattern: The pancreatic tissue is largely replaced by fatty tissue. Islets embraced by fibrotic bands cluster within fatty tissue. The *arrows* indicate intrainsular sclerosis. H&E, 125×

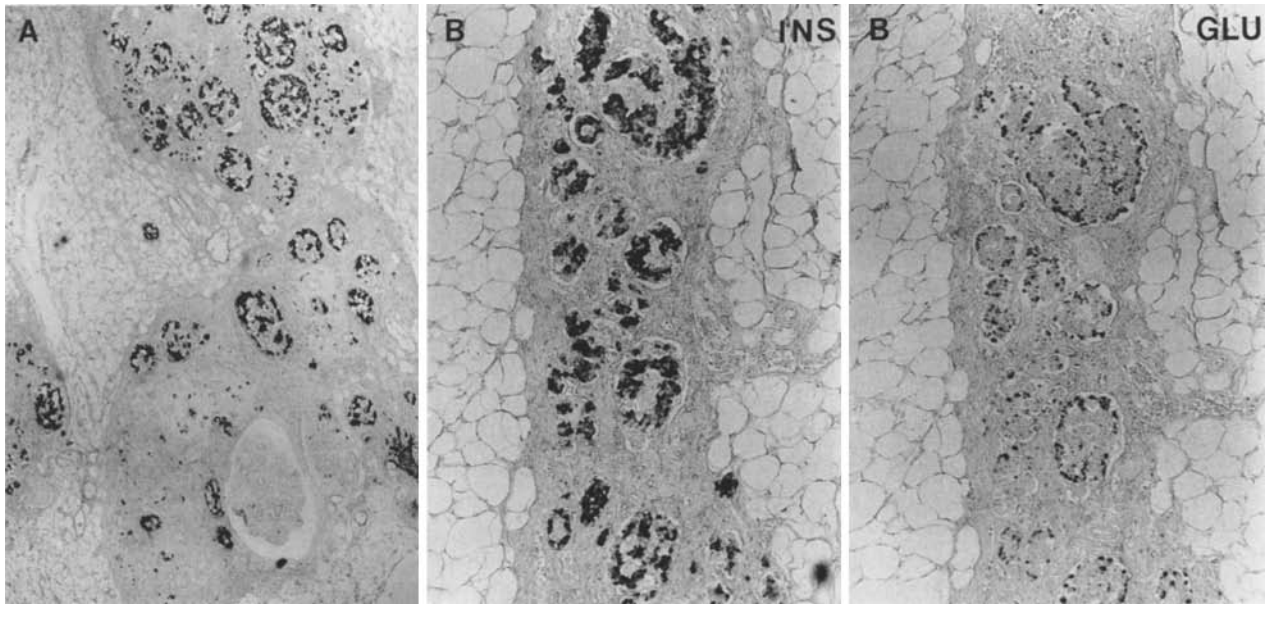

Fig. 2. Pancreas from 16-year-old CF patient, showing islets clustering in fibrous tissue. (**A**) Note the obvious reduction in immunocytochemically stained insulin cells. 40×. (**B**) Consecutive sections of islets with marked intrainsular fibrosis (*arrows*), immunochemically stained for insulin (INS) and glucagon (GLU), 125× (reduced to 67%)

Table 1. Volume densities (Vv) and percentages (%) of various tissue compartments of the exocrine and endocrine pancreas in end stage cystic fibrosis

Morphometric Parameters	Controls	Controls vs CF-ALL	CF-ALL	CF-FIB	CF-FIB vs CF-LIP	CF-LIP
	$n=6$ x±SE	$P<$	$n=23$ x±SE	$n=14$ x±SE	$P<$	$n=9$ x±SE
Vv(END/PAN)	0.045±0.01	NS	0.043±0.001	0.041±0.002	NS	0.052±0.002
Vv(FAT/PAN)	0.001±0.0001	0.001	0.280±0.053	0.150±0.039	0.010	0.517±0.087
Vv(MES/PAN)	0.046±0.002	0.001	0.470±0.06	0.550±0.079	0.050	0.311±0.069
Vv(PAR/PAN)	0.903±0.049	0.010	0.207±0.044	0.259±0.067	0.050	0.120±0.007
Vv(A/END)	0.19 ±0.029	0.020	0.23 ±0.021	0.22 ±0.029	NS	0.24 ±0.017
Vv(B/END)	0.32 ±0.086	0.010	0.22 ±0.031	0.23 +0.035	NS	0.21 ±0.063
Vv(D/END)	0.05 ±0.012	0.010	0.15 ±0.015	0.16 +0.021	NS	0.14 +0.02
Vv(PP/END)	0.01 ±0.002	0.050	0.05 ±0.01	0.06 +0.013	NS	0.05 ±0.01
%(A/Cells)	23.2		35.0	33.0		37.0
%(B/Cells)	64.4		34.0	34.0		33.0
%(D/Cells)	10.4		22.0	24.0		22.0
%(PP/Cells)	2.0		9.0	9.0		8.0

$V_{V(END/PAN)}$	Volume density of endocrine tissue to pancreatic tissue
$V_{V(FAT/PAN)}$	Volume density of fatty tissue to pancreatic tissue
$V_{V MES/PAN)}$	Volume density of mesenchymal tissue to pancreatic tissue
$V_{V(PAR/PAN)}$	Volume density of parenchymal tissue to pancreatic tissue
$V_{V(A/END)}$	Volume density of A-cells to islet cell tissue
$V_{V(B/END)}$	Volume density of B-cells to islet cell tissue
$V_{V(D/END)}$	Volume density of D-cells to islet cell tissue
$V_{V(PP/END)}$	Volume density of PP-cells to islet cell tissue
$\%_{(A/Cells)}$	Percentage of A-cells of the total islet cells
$\%_{(B/Cells)}$	Percentage of B-cells of the total islet cells
$\%_{(D/Cells)}$	Percentage of D-cells of the total islet cells
$\%_{(PP/Cells)}$	Percentage of PP-cells of the total islet cells
CF-FIB	CF patients with predominant fibrous pancreatic tissue
CF-LIP	CF patients with predominant lipoatrophic pancreatic tissue

sities ($V_{v(PAR/PAN)}$) (Table 1). This was accompanied by a rise in the volume densities of the fibrous and fatty tissue portion ($V_{v(MES/PAN)}$, $V_{v(FAT/PAN)}$) (Table 1). The CF cases, distinguished by a predominantly fibrotic pattern (CF-FIB), showed higher parenchymal volume densities that CF-LIP pancreases. In the CF-FIB pancreas, the mean fatty tissue proportion amounted to 15%, compared to 52% in the CF-LIP pancreas (Table 1).

Morphometry of the endocrine pancreas

The mean volume density of the endocrine cell compartment, i.e. the islet area ($V_{v(END/PAN)}$), was almost the same in all CF patients and not significantly different from the controls. However, the ratio of the endocrine cells to the pancreatic parenchyma ($V_{v(END/PAN)} : V_{v(PAR/PAN)}$) increased from 1:20 in the controls to 1:5 in the CF pancreases.

There was a relative decrease in B-cell ($V_{v(B/END)}$) from 0.32 to 0.22 ($p<0.01$) and a relative increase in A-cells ($V_{v(A/END)}$) (0.19 to 0.23, $p<0.02$), D-cells ($V_{v(D/END)}$) (0.05 to 0.15, $p<0.01$) and PP-cells ($V_{v(PP/END)}$) (0.01 to 0.05, $p<0.05$) (Table 1). Therefore, the relation to the endocrine cells within the islet changed accordingly (Table 1). The distribution of the endocrine cells within the islet, i.e. B-cells in the center and non-B-cells in the periphery, was indistinguishable in both controls and the two CF groups. No significant difference in the endocrine cells could be detected between the CF-FIB and CF-LIP nor with respect to the degree of changes in the exocrine pancreas in relation to the endocrine pancreas. No distinction could be made between the sexes with regard to the predominant histologic pattern or the changes in the exocrine pancreas.

CF patients with diabetes

One CF patient (17 year old male) who had overt diabetes, showed predominantly fibrotic changes of the pancreas. The decrease of B-cells in this patient was remarkable but did not exceed the range

of values obtained from the non-diabetic CF patients. A similar change of the B-cell volume density was observed in another patient with a disturbed glucose metabolism. This 12 year old boy had impaired glucose tolerance for 2 years presenting with peak blood glucose values of 135 mg% (7.5 mmol/L) 2 h after oral glucose load (75 g). The predominant histological pattern in this patient was lipoatrophic. The liver of the patient with overt diabetes showed moderate portal fibrosis, while the patient with glucose intolerance had macronodular liver cirrhosis, which was also noted in two other patients.

Discussion

The final stage of cystic fibrosis is characterized by an obstruction of the pancreatic ducts by viscous secretion leading to complete acinar atrophy accompanied by fibrosis and lipomatosis (Lebenthal et al. 1986; Schwachman and Grand 1978; Kopito et al. 1976). Our morphometric analysis of these pancreatic lesions in 23 patients older than 5 years and suffering from final stage CF revealed that, compared with controls, the proportion of the connective and fatty tissue had increased by about 10 times and 200 times, respectively, whereas the exocrine parenchyma (including acini and ducts) was reduced. Similar results were also obtained by Kopito et al. (1976).

Despite these severe alterations of the exocrine pancreas in the present series of patients with CF, the volume density of endocrine tissue was found to be within normal limits. Yet, the volume densities of immunocytochemically identified endocrine cells were significantly altered. The volume densities of the endocrine cell types were changed in relation to each other, showing a relative decrease of B-cells and a concomitant increase in non B-cells compared to the controls. Similar findings have been reported by several other groups (Iannucci et al. 1969; Soejima and Landing 1986; Abdul-Karim et al. 1986) and these are substantiated by the present study. However, while Iannucci et al. (1984) found the loss of insulin cells and the concomitant rise in non-insulin cells restricted to diabetic patients, Soejima and Landing (1986) and Abdul-Karim et al. (1976) identified these changes both in CF patients with and without diabetes. Our results agree with those of the latter authors, since the two patients with disturbed glucose metabolism included in our series, showed endocrine cell ratios within the range of the normoglycemic CF patients. These findings in connection with the data of the two other groups support the view that a shift in the endocrine cell ratio secondary to a loss of insulin cells precedes the development of diabetes in CF.

Differences between the aforementioned studies and our work are noted concerning the percentage of insulin cells which varied from study to study, ranging from 28.3 to 47.4% in CF patients and from 53.4 to 64.4% in the normoglycemic control groups. Similar variations were also noted for the non B-cell ratios. Glucagon cells, for instance, were either found to be normal in number (Abdul-Karim et al. 1986) or distinctly increased (Soejima and Landing 1986). Since quantitative evaluations of the endocrine pancreas are difficult, the above mentioned discrepancies may be attributed to the various morphometric methods used in the different studies. While our analysis was based on the evaluation of at least one entire cross-sectional slide of the body of the pancreas, including more than 50 islets on each stained section, in Iannucci's study (1984) the figures were obtained from 12 islets/patient and in Abdul-Kamrim's study (1976) from 30 islets/patient.

In addition, in contrast to the studies by Brown and Magde (1971), Iannucci et al. (1984) and Soejima and Landing (1986), who encountered nesidioblastosis in about 37% of their CF cases, this was a rare observation in our cases, in particular in the patients with impaired glucose metabolism, and the degree of nesidioblastosis was minimal. We therefore hesitate to consider this finding to contribute to a major extend to the metabolic regulation and being a key phenomenon in the diabetic CF patients.

From chronic pancreatitis it is known that islets embedded in sclerotic tissue have a reduced number of insulin cells while islets surrounded by intact acinar parenchyma retain their normal endocrine cell ratio (Klöppel et al. 1978). This implies that scarring of the pancreas alters the endocrine cell composition and thereby probably induces islet dysfunction. Similarly in CF, Handwerger and associates (1969) suggested that insulinopenia, found in 42% of their patients at variable degrees, is a consequence of islet disorganization and disruption by severe pancreatic fibrosis. Conversely, lipoatrophy of the exocrine pancreas, as seen in Shwachman's syndrome (Shwachman et al. 1978; Seifert 1984b), seems not to be associated with any changes of the islet structure and function. As in advanced CF a predominantly fibrotic change of the pancreas (CF-FIB) can be distinguished from a lipoatrophic pattern (CF-LIP) (Oppenheimer and Easterly 1975; Vawter and Shwachman 1979), it was of interest to compare the endocrine cell

content of the islets in advanced CF in relation to these two dominating histological changes. However, this analysis failed to reveal any significant differences between both CF subgroups regarding their endocrine cell ratios. In particular, there was similar reduction in the proportion of B-cells in both subgroups. This finding was somewhat unexpected in view of the preserved islet system in true lipoatrophy of the pancreas. An explanation, however, may be supplied by the observation that, even in CF pancreases with the most extreme lipoatrophic pattern, the islets that clustered together were encapsulated and partly distorted by fibrous bands. This again emphasizes the important role of pancreatic fibrosis in the development of islet dysfunction.

While there is apparently a good correlation between islet disorganization due to pancreatic fibrosis and impaired insulin secretion, the causal link between these lesions remains elusive. "Strangulation" of the islet vasculature with subsequent hypoxia due to perisinusoidal sclerosis is the factor primarily discussed (Klöppel et al. 1978; Handwerger et al. 1969). If this is true, this raises the question whether the impaired insulin secretion is a result of the diminuished insulin cell number or a functional insulin cell defect without visible morphological alterations. As there is yet no study reporting on changes of the total islet cell volume in CF pancreases, we can only speculate on the total reduction in insulin cells. Presumably, however, the decrease in insulin cells does not exceed 80–90%, even with the lowest density of islets found in CF patients with diabetes (Iannucci et al. 1984; Soejima and Landing 1986). This assumption is supported by the observation that, in CF patients, abnormalities of insulin secretion are usually much milder (Handwerger et al. 1969; Kjellman and Larson 1975; Lippe et al. 1977) than in patients with recent onset type 1 diabetes, whose loss in insulin cells is estimated to be 80–90% (Klöppel et al. 1984). Diabetes in CF is therefore probably a result of an acquired functional abnormality of the insulin cells in islets affected by infiltrating fibrosis rather than a consequence of their moderate numerical decrease. Alike insulin cells and contrary to glucagon cells (Lippe et al. 1977), PP cells also appear to be susceptible to the effects of fibrosis, because late-stage CF patients exhibited severely impaired PP secretion rates (Adrian et al. 1980; Allen et al. 1983; Nousia-Arvanitakis et al. 1985).

In summary, our morphometric analysis shows that advanced CF of the pancreas induces islet changes which may provide the basis for the glucose intolerance and overt diabetes eventually developing in some CF patients. As not all patients with advanced CF become diabetic, other late complications of CF such as liver cirrhosis with reduced insulin degradation, subsequent hyperinsulinism and peripheral insulin resistance (Johnston et al. 1977) might contribute to the changes in glucose metabolism seen in CF patients.

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