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Pathology

Hypertrophy and hyperplasia of somatostatin-containing D-cells in diabetes

(glucagon/insulin/immunofluorescence/pancreas)

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ABSTRACT **Insulin-, glucagon-, and somatostatin-containing cells, identified by immunofluorescent staining, were quantitated morphometrically in sections of pancreas obtained from diabetic and nondiabetic humans and rats. Both the volume density and number of somatostatin- and glucagon-containing cells were significantly increased in the islets of juvenile-type human diabetics and of streptozotocin diabetic rats.**

Somatostatin, or a somatostatin-like immunoreactive material, has recently been identified in pancreatic islets (1–4) and localized to the D-cells (5–8). Previous reports of increased numbers of D-cells (or A_1-cells) in the islets of diabetic humans (9), rats (10) or guinea-pigs (11), together with demonstrations of somatostatin's potent inhibitory action upon insulin and glucagon release (12–22), prompted this morphometric comparison of somatostatin-, insulin-, and glucagon-containing cells in the pancreases of diabetic and nondiabetic humans and rats by means of immunofluorescent staining techniques§.

MATERIALS AND METHODS

Light Microscopy Methods. Pieces of tissue were fixed in Bouin's solution, dehydrated in alcohol, and embedded in paraffin. Sections of 5 μm thickness were stained with aldehyde fuchsin (23), phosphotungstic acid-hematoxylin (24), or hemalum-eosin, or processed by immunofluorescent techniques (see below).

Human Juvenile-Type Diabetic and Nondiabetic Pancreases. Pancreases were obtained from two juvenile diabetic patients at the Hôpital Cantonal de Genève within 35 min of death. One patient was a 32-year-old white male with severe juvenile-type diabetes of 18 years' duration who died of renal insufficiency. Grossly, the pancreas appeared atrophic. Conventional microscopic examination of an atrophic area in the head of the pancreas revealed few islets. These were composed mostly of A-cells stained with phosphotungstic acid-hematoxylin and were devoid of B-cells. In another fragment, in which islets were more numerous, no B-cells could be identified by aldehyde fuchsin staining, but numerous well-granulated A-cells were noted.

The other diabetic pancreas was obtained from a 31-year-old white female with a 29-year history of juvenile-type diabetes who died of renal insufficiency. Grossly, the pancreas was indurated and microscopic examination revealed acute pancreatitis. Islets were sparse and small, consisting largely of A-cells. No B-cells were noted.

The four nondiabetic pancreases were obtained from encephalographically dead kidney donors in Geneva and Dallas in accordance with local regulations.

Streptozotocin Diabetic and Nondiabetic Rats. Pancreases were obtained from four normal control rats and four rats with 16 months of diabetes produced by a single intravenous injection of 45 mg of streptozotocin per kg of body weight. These rats never required insulin treatment, but all were glycosuric at the time of sacrifice.

Immunofluorescent Staining Techniques. The indirect immunofluorescent technique of Coons *et al.* (25) was employed with use of a rabbit anti-somatostatin serum (a gift of Dr. M. P. Dubois) at a dilution of 1:50, highly specific rabbit anti-glucagon serum 15K at a 1:20 dilution, and a guinea pig anti-insulin serum (a gift of Dr. P. H. Wright) at a 1:50 dilution. Anti-rabbit or anti-guinea pig gamma globulin labeled with fluorescein isothiocyanate (Pasteur Institute, Paris) was employed as the second antibody.

Control experiments were performed using the specific antiserum absorbed with the corresponding antigen, 200 μg of cyclic somatostatin (a gift of Drs. J. Rivier and R. Guillemin), 50 μg of glucagon, or 2 units of insulin per ml of undiluted antiserum. After removal of the paraffin, sections were rehydrated and incubated for 2 hr with anti-somatostatin, anti-insulin, or anti-glucagon serum. After rinsing in phosphate-buffered saline, the sections were incubated with the fluorescein-labeled antibody for 1 hr, again rinsed in phosphate-buffered saline, counterstained with Evans blue (0.01%), and mounted in glycerol/phosphate-buffered saline.

Morphometric Analysis. Following staining of sections for somatostatin, glucagon, and insulin, immunofluorescent cells were analyzed morphometrically in the first 20 islets encountered in sections of each pancreas. A total of 60 islets per subject and per animal were thus examined. The volume density per islet of each immunofluorescent islet cell type was determined by the point counting method of Weibel (26). The percent of the total number of immunofluorescent cells per islet was also calculated for each cell type.

In addition to the above calculations, the availability of the entire pancreas in the rats permitted calculations of total islet volume, total cell number, and total cell volume per pancreas.

§ Preliminary results of this work have been presented at the CIBA Foundation Symposium no. 41, London, June 30–July 3, 1975, and at the International Symposium of the Gastro-Entero-Pancreatic Endocrine System, Kyoto, Japan, August 31–September 3, 1975.

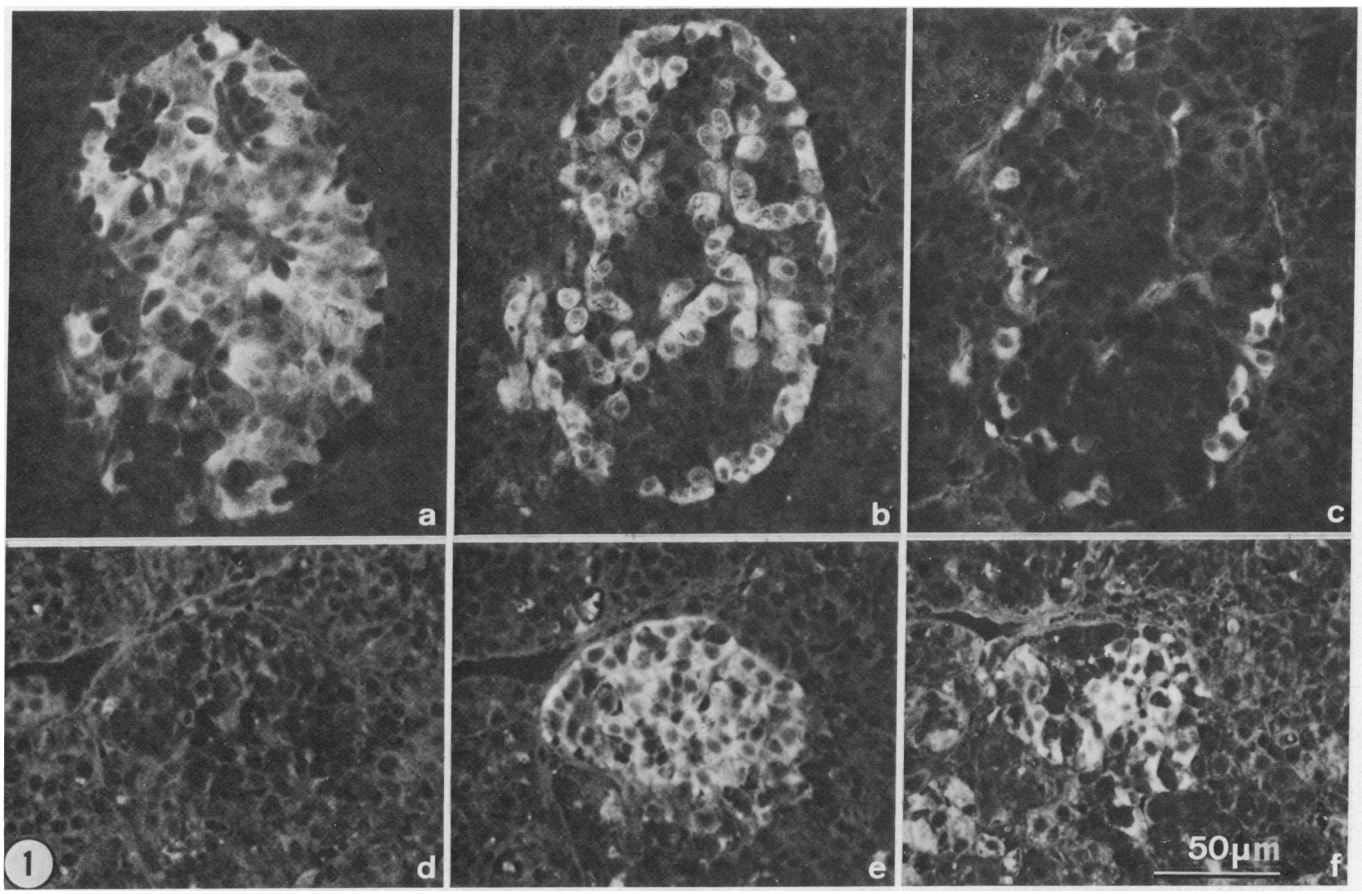

FIG. 1. (a–c) Distribution of B-, A-, and D-cells on serial sections of an islet from an adult nondiabetic subject, determined by the indirect immunofluorescent technique against insulin (a), glucagon (b), and somatostatin (c). (d–f) Islet of Langerhans in a chronic juvenile diabetic (patient C.C.). Serial sections treated with the indirect immunofluorescent technique against insulin (d), glucagon (e), and somatostatin (f). The only detectable immunofluorescent cells within the islet are the numerous glucagon- and somatostatin-containing cells. ×200.

RESULTS

Studies in human juvenile-type diabetics and nondiabetics

In the islets of the four normal humans (Fig. 1a) more than half of the cells were insulin-immunofluorescent. These were located in the central portion of the islets. Approximately one-third of the cells were glucagon-immunofluorescent cells (Fig. 1b), and these were situated both in the periphery of the islet and along the main capillary axes. Somatostatin-immunofluorescent cells made up about 10% (Fig. 1c) and were proximal to the A-cells. Morphometric analyses are recorded in Table 1.

In both of the two diabetic pancreases, islets were clearly less numerous than in the normal pancreases. No insulin-immunofluorescent cells could be identified in the diabetic islets (Fig. 1d), which consisted largely of glucagon-immunofluorescent cells (Fig. 1e); somatostatin-immunofluorescent cells were also abundant (Fig. 1f). Morphometric analyses revealed both cell types to be significantly increased above the control values (Table 1).

Studies in normal and streptozotocin-diabetic rats

In 60 islets examined in the pancreases of four nondiabetic rats, morphometric analyses (Table 2) of the three types of immunofluorescent cells (Fig. 2a–c) were rather similar to the findings in the four nondiabetic humans. In the four diabetic rats (Fig. 2d–f) insulin-immunofluorescent cells were sharply reduced both in volume density and percent of total immunofluorescent cells per islet, whereas glucagon-immunofluorescent and somatostatin-immunofluorescent cells were significantly increased (Table 2).

Calculations for entire pancreases revealed a reduction in total islet volume in the diabetic pancreases to approximately half the control values (Table 3). The volume and number of insulin-immunofluorescent cells per diabetic pancreas were reduced significantly below the control values. The total volume and number of glucagon-immunofluorescent cells per pancreas did not differ significantly from normal, whereas the total volume of somatostatin-immunofluorescent cells per pancreas was 2.5 times normal and the total number was almost twice normal (Table 3).

DISCUSSION

Current knowledge of the pathology of islets of Langerhans in human diabetes is based entirely on routine autopsy material with use of conventional staining methods (27, 28). The present studies of portions of two relatively fresh human diabetic pancreases by immunofluorescent techniques revealed a total absence of insulin-immunofluorescent cells and an abundance of glucagon-immunofluorescent and somatostatin-immunofluorescent cells in the 60 randomly chosen islets examined when compared to islets of nondiabetic subjects. However, in view of the sparsity of islets in most of the sections of the two diabetic pancreases studied, an absolute increase in the total pancreatic population of the latter cell types seems unlikely.

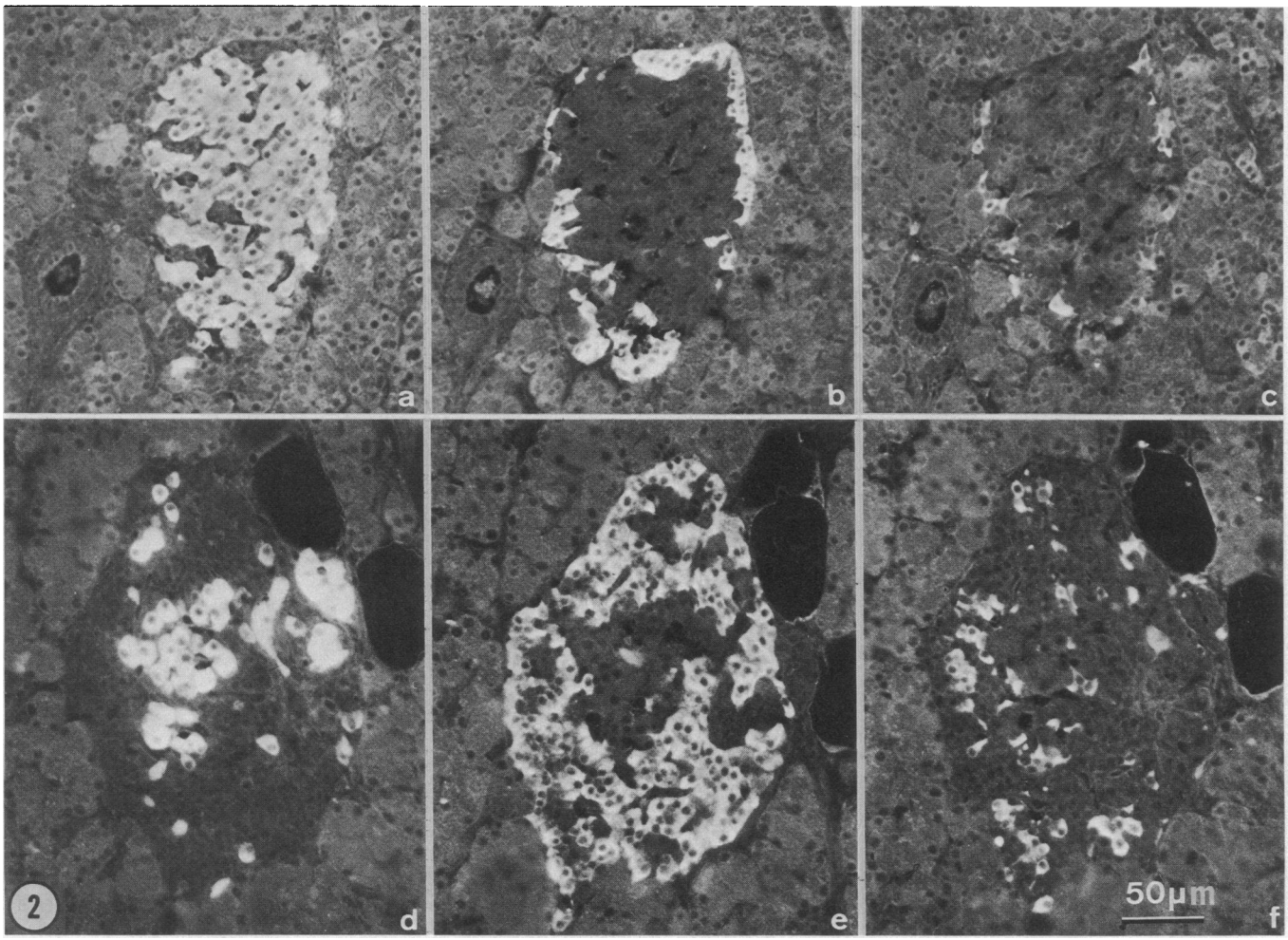

FIG. 2. (a–c) Distribution of B-, A-, and D-cells on serial sections of an islet from a normal rat determined by the indirect immunofluorescent technique against insulin (a), glucagon (b), and somatostatin (c). Both glucagon-fluorescent cells and somatostatin-fluorescent cells are located at the periphery of the islet forming the so-called mantle islet around the centrally located B-cells. (d–f) Distribution of B-, A-, and D-cells on serial sections of an islet from a 16 month streptozotocin-diabetic rat determined by indirect immunofluorescent technique against insulin (d), glucagon (e), and somatostatin (f). Most of fluorescent cells are glucagon- and somatostatin-containing cells, which are now seen both at the periphery and in the inner portion of the islet. ×200.

The results in rats with insulin-independent streptozotocin diabetes of long duration were similar to the humans at the "within-islet" level. A highly significant decrease in the number and volume of insulin-immunofluorescent cells per pancreas and an increase in the total number and volume of somatostatin-immunofluorescent cells per pancreas were observed in the diabetic rats. But the number and volume of glucagon-immunofluorescent cells per pancreas did not differ significantly from the controls despite the significant increases per diabetic islet.

We interpret these findings as evidence of D-cell hypertrophy and hyperplasia in diabetes, the physiologic and pathophysiologic significance of which remains to be determined. A change in the numbers and/or volume of cells

Table 1. Insulin-, glucagon-, and somatostatin-immunofluorescent cells in the islets of chronic juvenile-type diabetic subjects, and of nondiabetic subjects

	Insulin	Glucagon	Somatostatin
Controls (n = 4)	0.624 ± 0.022 [0.573 ± 0.033]	0.240 ± 0.024 [0.322 ± 0.033]	0.092 ± 0.012 [0.104 ± 0.012]
Diabetics (n = 2)	0 [0]	0.688 ± 0.029* [0.736 ± 0.027]*	0.238 ± 0.024* [0.264 ± 0.027]*

Mean ± SEM volume density and percent of the total number of immunofluorescent cells (in brackets).
* $P < 0.001$ that value is the same as for controls.

Table 2. Insulin-, glucagon-, and somatostatin-immunofluorescent cells in the pancreatic islets of control and streptozotocin-diabetic rats

	Insulin	Glucagon	Somatostatin
Controls (n = 4)	0.558 ± 0.012 [0.628 ± 0.035]	0.161 ± 0.025 [0.276 ± 0.032]	0.051 ± 0.005 [0.096 ± 0.007]
P	<0.001 [<0.001]	<0.005 [<0.005]	<0.001 [<0.001]
Diabetics (n = 4)	0.145 ± 0.031 [0.125 ± 0.024]	0.405 ± 0.040 [0.535 ± 0.037]	0.288 ± 0.015 0.341 ± 0.025

Mean ± SEM volume density and percent of the total number of immunofluorescent cells (in brackets). P is that control and diabetic values are the same.

Table 3. Volume of pancreas and endocrine pancreas, and volume and number of insulin-, glucagon-, and somatostatin-immunofluorescent cells per pancreas in control versus diabetic rats

	Pancreas, mm^3	Endocrine pancreas, mm^3	Insulin		Glucagon		Somatostatin	
			mm^3	Cells $\times 10^{-6}$	mm^3	Cells $\times 10^{-6}$	mm^3	Cells $\times 10^{-6}$
Controls ($n = 4$)	1450 ± 104	11.2 ± 1.0	6.3 ± 0.7	5.7 ± 0.6	1.7 ± 0.2	2.4 ± 0.2	0.6 ± 0.1	0.9 ± 0.1
P	N.S.	<0.005	<0.0005	<0.0005	N.S.	N.S.	<0.005	<0.02
Diabetics ($n = 4$)	1450 ± 155	5.1 ± 0.5	0.8 ± 0.2	0.6 ± 0.1	2.1 ± 0.3	2.5 ± 0.3	1.5 ± 0.2	1.7 ± 0.2

Values are mean ± SEM. The diabetic rats had streptozotocin diabetes of 16 months' duration. P is that the values are the same for controls and diabetics. N.S., not significant.

containing hormone-like immunoreactivity does not permit conclusions with respect to secretory activity. Yet this demonstration of absolute hyperplasia of D-cells in diabetes, a disorder in which relative or absolute hyperglucagonemia is uniformly present (15, 19), raises the possibility of a compensatory D-cell response, perhaps to the excessive glucagon secretion of diabetes (22, 29). Large numbers of somatostatin-immunofluorescent D-cells have also been observed in a glucagon-secreting A-cell tumor (L. Orci, R. Rufener, and A. E. Lambert, unpublished observations). Conceivably, D-cell hyperplasia in diabetes reflects a generally unsuccessful effort to correct glucagon hypersecretion through increased local release of somatostatin.

Note Added in Proof. Using a radioimmunoassay, Patel and Weir have recently demonstrated an augmentation of both somatostatin and glucagon in isolated islets from streptozotocin-diabetic rats (30).

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