Quantitation of Endocrine Cell Content in the Pancreas of Nondiabetic and Diabetic Humans

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SUMMARY

The application of immunofluorescence technique with anti-insulin, anti-glucagon, anti-somatostatin, and anti-pancreatic polypeptide (PP) antisera to sections of precisely sampled regions of the human pancreas allowed the quantitative evaluation of the total content of these four endocrine cell populations in 13 nondiabetics, in 2 insulin-dependent diabetics (IDDM), and in 2 non-insulin-dependent diabetic subjects (NIDDM) of various age and sex. In nondiabetic subjects, PP-cells appear sex-related. Male individuals have a significantly greater volume of PP-cells than female. In diabetic subjects, the only marked difference as compared with nondiabetics is the reduction of insulin cell volume in IDDM. Other small differences between individual endocrine cell volumes are detectable in both IDDM and NIDDM as compared with nondiabetics, but their significance is at present unclear. The qualitative changes of islet structure accompanying insulin cell reduction in IDDM were not considered in the present study. DIABETES *31*:694–700, August 1982.

The anatomical basis of the hormonal abnormalities that characterize the diabetic state in man has never been fully elucidated. While earlier studies from this and other laboratories have demonstrated abnormalities in individual islets obtained from small blocks of pancreatic tissue,[1,2] the implication of these findings is rendered uncertain by the inhomogeneity of islet composition in the pancreas.[3–8] In the following study, immunofluorescent staining of the four pancreatic hormones, insulin, glucagon, somatostatin, and pancreatic polypeptide, combined with the morphometric analysis of immunofluorescence in precisely sampled pancreatic regions, is employed to obtain for the first time quantitative data concerning the endocrine cell populations of the entire pancreas of nondiabetic and diabetic subjects.

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MATERIALS AND METHODS

Subjects. Table 1 summarizes the clinical and autopsy findings in all subjects studied. The 13 nondiabetic "control" cases had no clinical or postmortem evidence of pancreatic disease. One of the two insulin-dependent diabetics (IDDM) was a 30-yr-old female in whom the diagnosis of diabetes had been made 3 yr before death from ketoacidotic coma. The autopsy revealed a small (29 g), fibrous pancreas. The second IDDM subject was a 34-yr-old male with diabetes of 20-yr duration. He was hospitalized for hypoglycemic coma and renal insufficiency; death occurred following aspiration pneumonia. The autopsy revealed a moderately small pancreas weighing 46 g.

One non-insulin-dependent diabetic subject (NIDDM) was a 55-yr-old male Pima Indian; the diagnosis of diabetes was established 13 yr before death. The pancreas appeared grossly normal and weighed 117 g. The other case was a 47-yr-old Caucasian female with diabetes known for 14 yr, whose pancreas weighed 72 g.

Pancreatic sampling. Pancreases were sampled in 8 parts as previously described,[3] with or without attempting the separation of the PP-rich lobe.[6] The volume of each part was measured according to Archimedes' principle by immersing the sample in a graduated cylinder filled with saline and reading the increase of the level of the fluid; a 5-mm-thick slice of tissue was then taken in the center of each sample and fixed for 24 h in Bouin's fluid. Table 1 shows the postmortem delays for sampling and fixation (in most cases 3–11 h).

Immunofluorescence technique. After dehydration through increasing concentrations of ethanol, the fixed slices were embedded in paraffin and cut serially into 5-μm-thick sections. Consecutive serial sections were placed individually on glass slides and one of each stained with one of the following substances: (1) Hemalum-eosin (HE);

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TABLE 1
Sex, age, height, weight, and brief clinical characterization of the 17 subjects studied; subjects are ordered according to increasing age in each category

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Postmortem delay of sampling (h)	Diagnosis
Nondiabetics						
1	F	16(C)	173	52	15	Lymphoblastic leukemia.
2	F	18(C)	162	49	23	Recurrent asthma. Acute asthma crisis.*
3	M	29(C)	181	81	11	Myocardial infarction.*
4	M	35(C)	185	90	5	Traumatic paraplegia.† Aspiration pneumonia.
5	F	36(C)	170	70	6	Thrombosis left carotid.‡ Aspiration pneumonia.
6	F	38(C)	174	100	5	Myocardial infarction. Pulmonary embolism.
7	F	44(C)	165	56	9	Subarachnoid hemorrhage.*
8	M	45(C)	180	85	11	Liver cirrhosis. Upper digestive tract hemorrhage.
9	F	54(C)	158	64	12	Astrocytoma.
10	M	55(C)	180	80	6	Bronchial carcinoma. Cerebral metastases. Renal failure.
11	F	66(C)	153	59	6	Metastatic uterine carcinoma. Hydronephrosis. Pulmonary infarction.
12	M	72(C)	168	54	6	Pulmonary tuberculosis. Liver cirrhosis. Gastric ulcer. Gastrointestinal hemorrhage.
13	F	80(C)	157	48	6	Myeloblastic leukemia.
IDDM						
14	F	30(C)	167	67	3	Diabetes 3-yr duration. Acido-ketotic coma.
15	M	34(C)	187	60	6	Diabetes 20-yr duration. Hypoglycemic coma.
NIDDM						
16	F	47(C)	165	72	6	Diabetes 14-yr duration. Obesity. Neuropathy. Steinert's cardiomyopathy.
17	M	55(P)	166	68	6	Diabetes 13-yr duration. Hypertrophy of heart. Postoperative intraperitoneal hemorrhage.

C, Caucasian; P, Pima Indian.
* Sudden death.
† Traumatic paraplegia occurred 14 days before death.
‡ Thrombosis of carotid occurred 3 days before death.

(2) guinea-pig anti-insulin antiserum (Dr. P. H. Wright, Indianapolis, Indiana), 1:100 or 1:200 dilution; (3) rabbit anti-glucagon antiserum 15K (Dr. R. H. Unger, Dallas, Texas), diluted 1:20; or rabbit anti-glucagon antiserum K6563 (Dr. L. Heding, Bagsvaerd, Denmark) absorbed with human albumin, diluted 1:100; or rabbit anti-glucagon antiserum M107/8 (Dr. R. A. Donald, Christchurch, New Zealand) diluted 1:100; (4) rabbit anti-pancreatic polypeptide (PP) antiserum (Dr. R. E. Chance, Indianapolis, Indiana), 1:100 or 1:200 dilution; (5) rabbit anti-somatostatin (synthetic) antiserum (Dr. M. P. Dubois, Nouzilly, France) absorbed with human albumin, 1:100 or 1:200 dilution; or rabbit anti-somatostatin (synthetic) antiserum (Dr. S. Ito, Niigata, Japan) absorbed with human albumin, diluted 1:200. Dilutions were carried out with phosphate-buffered saline (PBS) and bound antisera were revealed by the indirect immunofluorescence technique[9] as follows: After an incubation of 2 h at room temperature with each antiserum, the sections were rinsed in PBS, exposed to anti-rabbit or anti-guinea pig IgG (1:20 dilution) labeled with fluorescein isothiocyanate (Pasteur Institute, Paris) for 1 h, rinsed in PBS, and counter-stained with Evans blue (0.01% in PBS). Each section was coverslipped in 25% glycerin diluted in PBS and finally observed for fluorescence in a Leitz Orthoplan microscope (Leitz Wetzlar, West Germany) equipped with a Ploemopak condensor.

The specificity of each immunofluorescent staining was tested by control experiments in which sections were incubated with the respective antisera adsorbed with their respective antigens. No staining was observed under such conditions.

Quantitative evaluation. Four steps were performed:
(1) Determination of the volume (μl) of the 8 parts of the gland obtained during sampling (see above).
(2) Determination of the volume (μl) of the PP-rich region: (a) by Archimedes' principle when the successful separation of this region was achieved at sampling; (b) on antero-poste-

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rior, 5-μm-thick sections of parts I–IV of the head[3] stained with anti-PP antiserum;[6] the contour of the PP-rich region was outlined and its surface expressed as a percentage of the total surface of the section (both surfaces were measured by planimetry). The percentage obtained was used to multiply the volume of the entire parts I–IV determined under step 1.

(3) Determination of the volume density (Vv) of the glandular (endocrine + exocrine) tissue in HE-stained sections of the PP-rich and glucagon-rich regions. This was performed by the point counting method[10] at a low magnification (×72), to subtract from the total volume density the volume occupied by nonglandular structures (e.g., fat, large vessels, connective septa). The volume (μl) of the glandular tissue in each region was obtained by multiplying the respective values of the volume density by the volume (μl) of these regions obtained according to steps 1 and 2 (Table 2).

(4) Determination of the volume density (Vv) of each immunofluorescent cell type within glandular tissue in three lobules of the PP-rich region of the pancreas and in three lobules of the glucagon-rich region (Table 3). The three lobules analyzed corresponded approximately to a surface of 5–9 mm² in the PP-rich region and 10–12 mm² in the glucagon-rich region.* The measurement of volume density was performed by the point counting method[10] at high magnification (×500). The expression of the volumes of immunofluorescent cells on a "per lobule" basis proved to be a more realistic expression of the endocrine cell content in the human pancreas than the "per islet" basis used in previous work on rodent pancreas,[1,11,12] since in the human PP-rich lobe, endocrine cells are grouped in ill-defined colonies. Moreover, the "per lobule" quantification allowed to take into account fluorescent cells dispersed outside the islets.

Point counting was performed directly on the fluorescence microscope equipped with a drawing tube through which the lattice of the test grid was projected over the fluorescent image seen in the eyepieces. The original formula for point counting[10] was modified[13] to take into account the overestimation of volume density of fluorescent cells due to the superposition of positive and negative structures within the thickness of the section. Thus, for each antiserum:[11]

$$\text{Vv (volume density)} = \frac{\text{P (cells)}}{\text{P (glandular tissue)}} - \frac{\text{T}}{2} \times \frac{\text{I (cells)}}{\text{2P (glandular tissue)} \times \text{d}}$$

P (cells) = number of intersection points of the test grid over immunofluorescent cells.

P (glandular tissue) = number of intersection points of the test grid over glandular tissue.

T = thickness of the section (5 μm).

I (cells) = number of lines (vertical and horizontal) of the test grid intersected by fluorescent cells or cell clusters (= correction for superposition).

d = distance between horizontal and vertical lines of the test grid.

The volume (μl) of each immunofluorescent cell population was obtained by multiplying the respective values of volume density (Vv) by the volume of glandular tissue calculated as detailed under step 3 (Table 4).

The immunofluorescent evaluation of endocrine cell populations requires a sufficient degree of preservation of cellular structures and of antigenicity of the hormones. We found that all pancreases fixed within 6 h after death, and most glands sampled 12 h postmortem, were suitable in both respects. Cold storage of the body allows for more extended preservation of the pancreas; case 1 sampled at 23 h postmortem presented satisfactory preservation of structure and immunofluorescent reaction.

TABLE 2
Volume (μl) of glandular tissue (endocrine + exocrine) and relative proportion (%) of PP-rich and glucagon-rich regions in the pancreas of each subject identified and numbered as in Table 1; the total volume of the pancreatic glandular tissue is obtained by summing up the values of the two regions

			PP-rich region		Glucagon-rich region	
No.	Sex	Age (yr)	Volume (μl) glandular tissue	%	Volume (μl) glandular tissue	%
Nondiabetics						
1	F	16(C)	3,140	6.2	47,330	93.8
2	F	18(C)	3,110	9.5	29,660	90.5
3	M	29(C)	4,570	9.2	45,010	90.8
4	M	35(C)	10,670	11.8	79,950	88.2
5	F	36(C)	10,980	14.2	66,100	85.8
6	F	38(C)	10,380	8.0	119,650	92.0
7	F	44(C)	3,930	10.4	34,040	89.6
8	M	45(C)	6,800	11.2	53,980	88.8
9	F	54(C)	7,380	11.5	57,000	88.5
10	M	55(C)	6,730	9.6	63,200	90.4
11	F	66(C)	3,650	11.0	29,670	89.0
12	M	72(C)	4,220	10.1	37,670	89.9
13	F	80(C)	5,450	8.5	58,470	91.5
IDDM						
14	F	30(C)	2,760	14.4	16,350	85.6
15	M	34(C)	5,790	16.2	29,880	83.8
NIDDM						
16	F	47(C)	7,820	15.0	44,220	85.0
17	M	55(P)	14,590	17.2	70,270	82.8

RESULTS

The clinical and autopsy findings in all subjects are shown in Table 1.

NONDIABETIC SUBJECTS

PP-rich region. This region contains nearly all the PP-cells of the pancreas where they represent the most important cell type in both relative (volume density, Vv: Table 3) and absolute (μl: Table 4 and Figure 1) volumes; insulin cells rank second; somatostatin cells follow in the third position, while glucagon cells are in exceedingly low volume and in

* Series of immunostained sections were performed in all 8 parts of the pancreas obtained during sampling. When the immunofluorescent pattern elicited with the four antisera was comparable among the 4 parts (V–VIII) of the body and tail, the glucagon-rich region was considered homogeneous and the complete morphometric evaluation performed only in a series of sections from either part V or VI. The same procedure was applied to the four parts (I–IV) of the pancreatic polypeptide-rich region and when homogeneous, the morphometric evaluation was carried out on either part I, II, or III.

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TABLE 3
Volume density (Vv) and relative percentage (%) of insulin-, glucagon-, somatostatin-, and PP-immunofluorescent cells in three PP-rich lobules of either part I, II, or III of the pancreatic head and in three glucagon-rich lobules of either part V or VI of the body of the pancreas. The values of Vv represent relative volumes which, multiplied by the volumes in μl shown in Table 2, allow the determination of total immunofluorescent cell content of each pancreatic region (see Table 4). To improve the presentation of the table, the values of volume density, all less than 0.01, have been multiplied by 10^3. Identification and numbering of subjects is identical to Table 1

			PP-rich lobules part I, II, or III[3]									Glucagon-rich lobules part V or VI[3]								
			Insulin		Glucagon		Somatostatin		PP		Total Vv endocrine	Insulin		Glucagon		Somatostatin		PP		Total Vv endocrine
No.	Sex	Age (yr)	Vv	%	Vv	%	Vv	%	Vv	%		Vv	%	Vv	%	Vv	%	Vv	%	
Nondiabetics																				
1	F	16(C)	5.89	31.6	0.27	1.5	0.38	2.0	12.07	64.9	18.61	8.61	79.3	1.75	16.1	0.42	3.9	0.08	0.7	10.86
2	F	18(C)	8.73	41.7	0.38	1.8	0.46	2.2	11.35	54.3	20.92	7.64	87.8	0.51	5.9	0.43	4.9	0.12	1.4	8.70
3	M	29(C)	2.34	11.9	0.10	0.6	0.26	1.3	17.00	86.3	19.70	5.79	84.8	0.75	11.0	0.25	3.7	0.04	0.6	6.83
4	M	35(C)	2.76	12.7	0.15	0.7	0.29	1.3	18.61	85.3	21.81	5.29	67.7	1.91	24.5	0.54	6.9	0.07	0.9	7.81
5	F	36(C)	1.25	6.3	0.03	0.2	0.18	0.9	18.43	92.7	19.89	7.59	81.9	1.21	13.1	0.35	3.8	0.12	1.3	9.27
6	F	38(C)	1.30	12.8	0.08	0.8	0.14	1.4	8.67	85.1	10.19	4.10	78.2	0.84	16.0	0.12	2.3	0.18	3.4	5.24
7	F	44(C)	6.44	41.8	0.07	0.5	0.34	2.2	8.56	55.5	15.41	9.14	87.3	0.81	7.7	0.49	4.7	0.03	0.3	10.47
8	M	45(C)	2.44	12.9	0.20	1.1	0.21	1.1	16.12	85.0	18.97	6.71	84.1	0.94	11.8	0.17	2.1	0.16	2.0	7.98
9	F	54(C)	3.34	15.7	0.01	0.1	0.33	1.5	17.66	82.8	21.34	10.03	89.7	0.68	6.1	0.44	3.9	0.03	0.3	11.18
10	M	55(C)	2.93	5.7	0.07	0.1	0.24	0.5	48.41	93.7	51.65	7.30	81.8	1.05	11.8	0.44	4.9	0.13	1.5	8.92
11	F	66(C)	6.93	22.0	0.09	0.3	0.47	1.5	24.02	76.2	31.51	12.59	85.1	1.68	11.4	0.48	3.2	0.04	0.3	14.79
12	M	72(C)	5.12	5.5	0.19	0.2	0.76	0.8	86.26	93.4	92.33	17.98	85.1	2.17	10.3	0.86	4.1	0.11	0.5	21.12
13	F	80(C)	1.47	8.7	0.05	0.3	0.28	1.7	15.11	89.4	16.91	4.37	70.4	1.50	24.2	0.25	4.0	0.09	1.4	6.21
IDDM																				
14	F	30(C)	0.00	0.0	0.04	0.3	0.28	2.0	13.76	97.7	14.08	<0.01	0.0	6.00	92.2	0.43	6.6	0.08	1.2	6.51
15	M	34(C)	0.00	0.0	0.12	0.8	0.80	5.4	13.98	93.8	14.90	1.96	25.4	5.21	67.4	0.41	5.3	0.15	1.9	7.73
NIDDM																				
16	F	47(C)	2.88	15.9	0.52	2.9	0.48	2.6	14.24	78.6	18.12	5.19	70.2	1.63	22.1	0.46	6.2	0.11	1.5	7.34
17	M	55(P)	1.32	11.4	0.12	1.0	0.07	0.6	10.07	87.0	11.58	2.27	63.1	1.13	31.4	0.14	3.9	0.06	1.7	3.60

some cases virtually absent (Table 3: relative volume; Table 4 and Figure 1: absolute volume).

Glucagon-rich region. This part of the pancreas contains nearly all the glucagon cells of the gland: in absolute volume (μl), glucagon cells represent from 5.9 (case 2) to 24.5% (case 4) of the total endocrine cell content of this region (Table 4); this large variation could not be related to either age or sex in the cases studied. Glucagon cells are preceded in volume by insulin cells, which rank first in both relative (Table 3) and absolute (Table 4) volumes, and

TABLE 4
Total volume (μl) of insulin-, glucagon-, somatostatin-, and PP-immunofluorescent cells in the PP-rich and in the glucagon-rich regions of the pancreas. The summing up of the values from the two regions gives the total pancreatic content of each immunofluorescent cell type. Identification and numbering of subjects is identical to Table 1

			PP-rich region				Glucagon-rich region			
No.	Sex	Age (yr)	Insulin	Glucagon	Somatostatin	PP	Insulin	Glucagon	Somatostatin	PP
Nondiabetics										
1	F	16(C)	18.5	0.8	1.2	37.9	407.5	82.8	19.9	3.8
2	F	18(C)	27.2	1.2	1.4	35.3	226.6	15.1	12.8	3.6
3	M	29(C)	10.7	0.5	1.2	77.7	260.6	33.8	11.3	1.8
4	M	35(C)	29.4	1.6	3.1	198.6	422.9	152.7	43.2	5.6
5	F	36(C)	13.7	0.3	2.0	202.4	501.7	80.1	23.1	7.9
6	F	38(C)	13.5	0.8	1.5	90.0	490.6	100.5	14.4	21.5
7	F	44(C)	25.3	0.3	1.3	33.6	311.1	27.6	16.7	1.0
8	M	45(C)	16.6	1.4	1.4	109.6	362.2	50.7	9.2	8.6
9	F	54(C)	24.6	0.1	2.4	130.3	571.7	38.8	25.0	1.7
10	M	55(C)	19.7	0.5	1.6	325.8	461.4	66.4	27.8	8.2
11	F	66(C)	25.3	0.3	1.7	88.3	373.5	49.8	14.2	1.2
12	M	72(C)	21.6	0.8	3.2	364.0	677.3	81.7	32.4	4.1
13	F	80(C)	8.0	0.3	1.6	82.3	255.5	87.7	14.6	5.2
IDDM										
14	F	30(C)	0.0	0.1	0.8	38.0	<0.2	98.1	7.0	1.3
15	M	34(C)	0.0	0.7	4.6	80.9	58.6	155.7	12.3	4.5
NIDDM										
16	F	47(C)	22.5	4.1	3.8	111.4	229.5	72.1	20.3	4.9
17	M	55(P)	19.3	1.8	1.0	146.9	159.5	79.4	9.8	4.2

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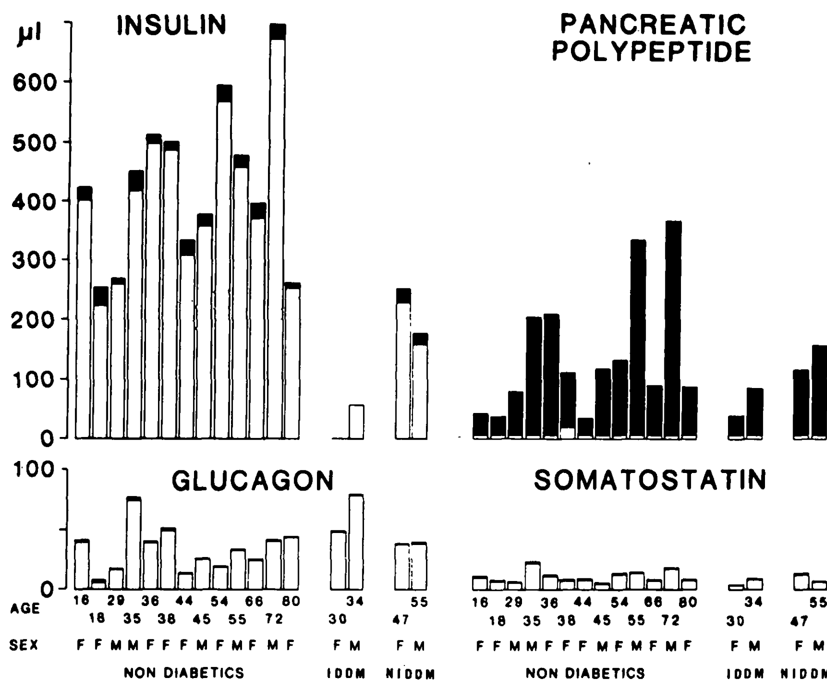

FIGURE 1. Cumulative histogram of the volume (μl) of immunofluorescent cell populations in the pancreas of nondiabetic and diabetic subjects. In the four panels, each column corresponds to one subject identified by sex and age at the bottom of the columns. In each column, the white part represents the volume of immunofluorescent cells in the glucagon-rich region of the pancreas and the black part represents the volume of the same cell type in the pancreatic polypeptide-rich region. For pancreatic polypeptide, except in case F38, the contribution of the glucagon-rich region (in white) to each column was drawn arbitrarily at the limit of visibility permitted by the photographic reduction. For the actual values, see Table 4. This applies also to some black bars at the top of columns in glucagon and somatostatin panels, which, drawn to scale, would not have been visible. IDDM = insulin-dependent diabetes mellitus; NIDDM = non-insulin-dependent diabetes mellitus.

followed by somatostatin cells and PP-cells (the latter in very small volumes) (Tables 3 and 4).

Total pancreas. When absolute volumes (μl) of the respective cell populations are considered with respect to the entire pancreas, the following facts are detectable (see Table 4 and Figure 1): (a) insulin cells always represent the most abundant cell type with 52.8% (cases 4 and 10) to 80.7% (case 7) of the total endocrine cell volume; (b) in spite of the relatively small contribution of the PP-rich region to the total glandular tissue (6.2% to 14.2%, Table 2), PP-cells are the second most frequent cell type with 7.3% (case 1) to 36.6% (case 10) of the total pancreatic endocrine volume occupied; this rather wide variation seems to be due to the fact that unlike other endocrine cell types, PP-cells are not equally distributed according to sex and age. The mean calculated on all female subjects yields a total PP-cell volume of 93 μl ± 21.0 (the large SEM is due to case 5 with an unusually high PP-cell content), while in male subjects, the mean reaches 220.8 μl ± 57.1 (female versus male: $P < 0.025$). When the respective proportions of PP-cells to the other endocrine cell types is calculated, the difference between female and male becomes even more significant: 15% ± 2.1 (female) versus 26.5% ± 3.2 (male), $P < 0.005$. In addition, when the ages of all nondiabetic subjects are considered, older subjects (≥54 yr) appear to have a larger PP-cell volume than the younger (≤ 45 yr) (202.2 ± 61.5 versus 104.9 μl ± 25.0, $P < 0.05$). This supports the previous suggestion that the proportions of endocrine cells in the pancreas may not be constant throughout life.[14] With a total absolute volume varying from 16.3 (case 2) to 154.3 μl (case 4) (5.0% to 18.0% of total endocrine volume), glucagon cells rank third while somatostatin cells, with a volume of 10.6 (case 8) to 46.3 μl (case 4) (1.9% to 5.4%) appear the least abundant cell type in the adult pancreas (Table 4).

IDDM SUBJECTS

As compared with the nondiabetic subjects, the PP-rich region of the 2 IDDM shows no insulin cells; glucagon and PP-cell volumes (μl), on the other hand, are comprised within nondiabetic limits, while somatostatin cell volumes appear slightly lower in case 14 and slightly higher in case 15 than in any of the nondiabetics. In the glucagon-rich region, case 14 has a barely detectable insulin cell volume, while case 15 has only one-fourth of the lowest nondiabetic value; glucagon cell volume (μl) of case 15 is very slightly above the highest nondiabetic volume, somatostatin cell volume of case 14 slightly below the lowest nondiabetic, while PP-cell volumes are comprised within nondiabetic limits (Table 4 and Figure 1).

NIDDM SUBJECTS

In the PP-rich region, insulin and PP-cell volumes (μl) of the 2 NIDDM subjects are within nondiabetic values; glucagon cell volumes of both cases, however, are above the highest nondiabetic, more than twice for case 16; somatostatin cell volumes, on the other hand, are respectively higher (case 16) or lower (case 17) than any of the nondiabetics. In the glucagon-rich region, insulin cell volume of case 17 is smaller than the lowest nondiabetic; all other values are within nondiabetic limits (Table 4 and Figure 1). In both IDDM and NIDDM subjects, the proportion of the total glandular pancreatic tissue contributed by the PP-rich region is slightly higher than in any of the nondiabetics (Table 2).

DISCUSSION

The present paper provides the first quantitative evaluation of insulin, glucagon, somatostatin, and pancreatic polypeptide cells in the whole pancreas of 13 nondiabetic, 2 insulin-dependent diabetic, and 2 non-insulin-dependent diabetic humans. Quantitative data from random sections of the

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pancreas of 4 nondiabetics and 2 insulin-dependent diabetics, expressed on a "per islet" basis, were previously published.[1] The data reported in relative volume (Vv) showed a significant increase in somatostatin cells; we pointed out, however, that in view of the limited sampling and sparsity of available islets an absolute increase of somatostatin cells in the entire pancreas was considered unlikely. This caveat appears now fully justified by the present data.

The results of this study must be interpreted within the limitations of postmortem studies and the heterogeneity of the pathologic conditions and therapies. In addition, the inference of quantitative cell evaluation on the hormone profile of the subject must be viewed with equal caution: bright cellular immunofluorescence allowing the cells to be quantitated implies that the secretory granule content of these cells is high; it is thus impossible to determine the amount of degranulated cells, i.e., not immunofluorescent by our standards, which could nevertheless actively secrete and contribute to the hormonal status in vivo.

Besides the significant sex-related variation in the volume of pancreatic polypeptide cells in the group of nondiabetic "control" subjects, there were several differences between individual endocrine cell volumes (μl) of diabetic subjects compared with nondiabetic values of the same cell type. However, given the very limited number of diabetic cases studied and the wide variations presented by the nondiabetic "control" subjects, we feel at present unable to attribute any significance to the difference noted, except for the extreme reduction (although one case still maintained an appreciable level) of insulin cell volume in IDDM subjects. This quantitatively confirms the long-known observation of scarcity or absence of such cells in insulin-dependent diabetes.[15,16] On the contrary, the hyperplasia of pancreatic polypeptide cells previously reported in this condition[2] was not confirmed. This may be explained on the basis of a sampling bias. In either the diabetic or nondiabetic PP-rich region, pancreatic polypeptide cells will always appear more frequent than any other endocrine cell type. The marked increase in the relative proportion of glucagon cells found in the pancreas of IDDM subjects (although the absolute volume of this cell type was barely above nondiabetic range in only one case), imposed extensive modifications on the topographic relationships between the various endocrine cells of the islet. This modification is especially important when considered with respect to the hypothesis that the arrangement and relative numbers of endocrine cells are not random but that the topographic order serves an integrated islet function.[17,18] On the contrary, the relative and absolute volumes of immunofluorescent cell types in the NIDDM cases, all within or close to nondiabetic range, do not cause apparent topographic alteration in the islets.

The limited number of diabetic cases available precluded statistical comparison with the nondiabetic "controls"; it is possible, however, that the quantitation of further diabetic pancreas will disclose significant changes besides the decrease of insulin cell volume in IDDM. Moreover, it should be mentioned that this paper did not consider the wide spectrum of structural changes characterizing IDDM[1,15,16] and, to a lesser extent, NIDDM islets (unpublished data). Once quantitated, these changes should contribute, together with the present data on absolute volumes, to a more complete picture of the diabetic pancreas.

In summary, the present study has shown the feasibility of a quantitative analysis of endocrine cells in precisely sampled pancreas of adult humans and has established the upper and lower limits of different endocrine cell content in a limited population of subjects of different age and sex and in a few cases of diabetes. Other recent studies reported on pancreatic endocrine cell content in neonates and infants.[19,20]

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Note added in proof. While the manuscript was in press, a further case of NIDDM could be evaluated (male, 78 yr, Caucasian). The following volumes (μl) of endocrine cells were found (cf., Table 4): (1) PP-rich region: insulin = 6.1; glucagon = 0.3; somatostatin = 0.5; PP = 65.3; and (2) glucagon-rich region: insulin = 186.0; glucagon = 100.7; somatostatin = 5.8; PP = 4.6.

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