An immunocytochemical and morphometric study of the rat pancreatic islets

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ABSTRACT

The rat pancreas has frequently been used as an animal model to study changes in islet cells in pathological conditions, such as diabetes mellitus and islet cell tumours, but detailed quantitative data on the islets are not available. This study was therefore undertaken to investigate (1) the volume density of pancreatic islets, (2) islet diameter, islet volume and islet cell number and (3) islet cell pattern, i.e. the distribution, volume and number of each cell type per islet. The study also investigated the possibility of differences in various pancreatic regions derived from the dorsal primordium. The rat pancreas was divided into 4 regions: lower duodenal (derived from the ventral primordium) and upper duodenal, gastric and splenic regions (derived from the dorsal primordium). Sections were stained immunocytochemically with anti-insulin (B cells), antiglucagon (A cells), antisomatostatin (D cells) and antipancreatic polypeptide (PP cells) antibodies, and were used for morphometric analysis. A total of 1292 islets was examined, 328 from the lower duodenal, 245 from the upper duodenal, 314 from the gastric and 405 from the splenic regions. The mean volume density of the islets per pancreatic tissue was found to be $2.6 \pm 0.1\%$, $2.3 \pm 0.1\%$, $2.9 \pm 0.2\%$ and $3.3 \pm 0.2\%$, in the lower duodenal, upper duodenal, gastric and splenic regions, respectively. The size–frequency distribution of the profile diameters of the islets showed an overall shift of all the size classes towards smaller sizes in the upper duodenal region, and towards larger sizes in the splenic region, as compared with the corresponding classes of the other regions. Two types of islets were identified, a PP-rich islet, characteristic of the lower duodenal region, and a glucagon-rich islet, characteristic of the upper duodenal, gastric and splenic regions.

A significant difference was observed between the upper duodenal and gastric and the upper duodenal and splenic regions in the mean volume density of the islets, mean islet diameters, mean islet volume, mean number of cells per islet and mean numerical densities of A, B and D cells per islet ($P < 0.05$). The results showed distinctive regional differences in the rat pancreas, not only between the pancreatic regions derived from the ventral and dorsal primordia, but also among the 3 regions derived from the dorsal primordium. These regional differences may reflect special functional adaptations.

Key words: Rat; pancreas; islets of Langerhans.

INTRODUCTION

Application of reliable and specific immunocytochemical techniques has allowed clear identification of the 4 major islet cell types: A cells (containing glucagon granules), B cells (containing insulin granules), D cells (containing somatostatin granules) and PP cells (containing pancreatic polypeptide granules). The structural organisation of these 4 cell types has a species difference which is considered to have considerable functional significance. In the human and rat pancreas, the B cells occupy the central portion of the islet, while A, D and PP cells are located peripherally (Goldsmith et al. 1975; Erlandsen et al. 1976; Orci et al. 1976, 1981; Sundler et al. 1977; Ito et al. 1978; Baetens et al. 1979; Nakamura et al. 1980; Unger & Orci, 1981; Stefan et al. 1982; Smith, 1983; Bani Sacchi & Bani, 1985). The arrangement differs in monkeys (Jones et al. 1980) and horses (Helmstaedter et al. 1976; Ito et al. 1978; Furuoka et al. 1989) where A cells are localised centrally and the other cells are arranged peripherally, whereas in snakes, B and A

cells are located centrally and D and PP cells are arranged peripherally (Rhoten, 1984). In sea bass, however, the central core of the islet is composed of B and D cells surrounded peripherally by A and PP cells (Carrillo et al. 1986; Lozano et al. 1991).

The relative proportions of the pancreatic islet cells show regional and species differences. Two distinct types of islets in the human and rat pancreas have been described, differing from each other in their cellular content: a PP-rich, glucagon-poor islet (PP islet) characteristic of the ventral lobe (derived from the ventral primordium) and a glucagon-rich, PP-poor islet (glucagon islet) characteristic of the dorsal lobe (derived from the dorsal primordium) (Orci et al. 1976; Pelletier, 1977; Sundler et al. 1977; Baetens et al. 1979; Gersell et al. 1979; Malaisse-Lagae et al. 1979; Nakamura et al. 1980; Unger & Orci, 1981; Orci, 1982; Stefan et al. 1982; Bani Sacchi & Bani, 1985). Other types of islet have also been described. Krause et al. (1989) reported 2 types of islet in opossum pancreas, depending on their interlobular or intralobular location. The islets of the dog pancreas were classified into polycellular and monocellular types, the monocellular type being composed only of B cells (Redecker et al. 1992). Jörns et al. (1988) described the islets of the rabbit according to their cellular composition as poly-, di- and monocellular. Bovine pancreatic islets were classified into large and small according to their diameter and location (Bonner-Weir & Like, 1980). The islets of the ostrich were classified into 5 populations according to their cell composition (Watanabe et al. 1991).

Previous studies on the rat pancreatic islets have concentrated only on finding differences between the relative numbers of each cell type per islet in the ventral and dorsal lobes (Wright & Carpenter, 1961; McEvoy & Hegre, 1977; Remacle et al. 1977; Baetens et al. 1979; Nakamura et al. 1980; Bani Sacchi & Bani, 1985). However, the possibility of differences between the pancreatic regions derived from the dorsal primordium was completely overlooked. Extensive quantitative data are available for human (Satio et al. 1978; Gersell et al. 1979; Stefan et al. 1982), monkey (Jones et al. 1980), horse (Furuoka et al. 1989), Chinese hamster (Carpenter et al. 1967), dog (Gersell et al. 1979; Bonner-Weir & Like, 1980) and cat (Furuzawa et al. 1992) pancreatic islets. Although the rat pancreas has frequently been used as an animal model in attempts to study changes in islet cell population following the induction of diabetes or islet cell tumours, detailed quantitative data concerning the pancreatic islets in this species have not been reported. The present study was therefore designed to

provide a detailed description of (1) volume density of the islets, (2) islet diameter, islet volume and islet cell number, and (3) islet cell pattern, i.e. the distribution, volume and number of each cell type per islet. The study also aimed to identify possible differences between the pancreatic regions derived from the dorsal primordium.

MATERIAL AND METHODS

Animal and tissue preparation

Six adult male Wistar albino rats, aged 60 d and weighing 250–300 g, were used. Animals were fed on a standard diet with water ad libitum. They were kept at a constant temperature of 24 °C and a light cycle of 12 h on/12 h off. Nonfasting blood glucose concentrations were measured to check that the animals were normoglycaemic. Rats were anaesthetised by ether inhalation, the abdominal aorta and inferior vena cava were incised distal to the pancreas and the animals were killed by exsanguination. In another group of rats India ink was injected either into the dorsal or ventral ducts to determine the ventral and dorsal lobes of the pancreas (Baetens et al. 1979). The duodenum, pancreas and spleen were removed from the rats and spread over a piece of hard paper and fixed in Bouin's solution for 12 h at 4 °C. After fixation, the pancreases were divided into 4 portions each: lower and upper duodenal, gastric and splenic (Fig. 1). Fixed tissue pieces from the central area of the 4 portions were dehydrated, embedded in paraffin wax and sectioned serially at 4 μm.

Immunocytochemical staining

Four sections, 20 sections apart were taken from the 24 specimens and stained by the following techniques.

Indirect immunoperoxidase (Sternberger, 1979). This was used to localise the insulin-producing B cells. The primary antibody was guinea pig antiswine insulin
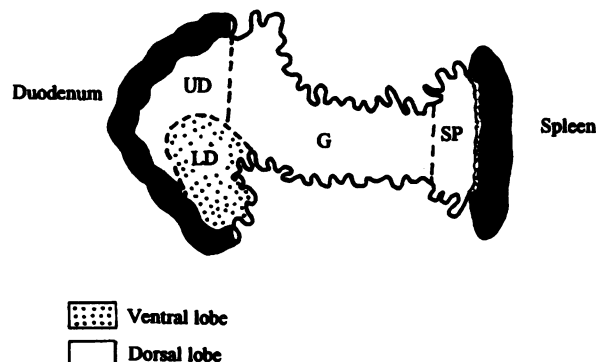

Fig. 1. Diagrammatic presentation of rat pancreas showing its 4 regions: lower duodenal (LD), upper duodenal (UD), gastric (G) and splenic (SP).

serum (optimal dilution 1:500). The secondary antibody was rabbit antiguinea pig immunoglobulin conjugated with peroxidase (dilution 1:200).

Avidin biotin complex (ABC) technique (Hsu et al. 1981). This was used to localise glucagon-producing A cells, somatostatin-producing D cells and pancreatic polypeptide-producing PP cells. The primary antibodies were rabbit antiporcine glucagon serum (optimal dilution 1:5000), rabbit antihuman somatostatin serum (optimal dilution 1:500) and rabbit antihuman pancreatic polypeptide serum (optimal dilution 1:5000). The secondary antibody was biotinylated swine antirabbit immunoglobulin (dilution 1:200). All sera and antisera were obtained from Dako Corporation, Carpinteria, CA, USA. The chromogen substrate used was 3,3'-diaminobenzidine tetrahydrochloride (DAB) from Sigma, St Louis, MO, USA. The counterstain was Harris' haematoxylin. Specificity controls were performed by replacing the primary antibody with non-immune serum, omitting the primary antibody and absorbing the primary antiserum with purified antigens.

Morphometric analysis

Ninety-six immunocytochemically stained sections (16 from each rat, 4 sections per region) were used for morphometric analysis; 8–16 islet profiles were chosen at random from each slide for analysis, with a total of 42–65 from each specimen. A total of 1292 islets were examined at a magnification of $\times 1000$ (328 from the lower duodenal, 245 from the upper duodenal, 314 from the gastric and 405 from the splenic regions) to estimate: (a) the volume density of the islets per pancreatic tissue (V_{vi}); (b) the volume density of each cell type per islet (V_{vc}); and (c) the number of each cell type per islet. The point counting method of Weibel (1963) was used to estimate (a) and (b). For estimate (a) all the available sections (96) from the 4 pancreatic regions were examined at a magnification of $\times 400$. The numerical density of each cell type per islet profile (N_{Ac}, no./μm^2) was calculated by dividing the number of nuclei per islet profile by the mean islet area. The nuclear profiles were counted by direct counting method at a magnification of $\times 1000$. The numerical density of each cell type per unit volume of islet (N_{vc}, no./μm^3) was determined by a variant of DeHoff and Rhines formula (Williams, 1977),

$$N_{Vc} = \frac{N_{Ac}}{\overline{D}_n + t}$$

where N_{Ac} is the number of nuclear profiles per unit area observed in sections of thickness t and $\overline{D}_n$ is the

mean corrected nuclear diameter. The number of each cell type per islet (absolute number) was determined by multiplying N_{vc} by islet volume.

With a graticule of a calibrated linear scale the major (a) and minor (b), at right angle to (a), axes of the islets were measured. The profile diameter of the islets (d_i) was calculated from the equation $d_i = \sqrt{ab}$ (Williams, 1977). The size–frequency distribution of the profile diameters of the islets was plotted. The mean axial ratio of the profiles was calculated. Assuming that the islets are spheroid structures, the formula of Fullman (Williams, 1977), for the ungrouped profile range of sizes, was used to calculate the mean islet diameter ($\overline{D}_i$).

$$\overline{D}_i = \frac{\pi}{2} \times \frac{N}{1/d_i 1 + 1/d_i 2 \dots 1/d_i N}$$

where N represents the total profiles measured.

Similar steps were followed to measure the nuclear profile diameter (d_n). A total of 600 nuclei were measured at a magnification of $\times 1000$ (100 from each rat; 25 per region of the pancreas). The corrected mean nuclear diameter ($\overline{D}_n$) was calculated according to the Abercrombie method (Williams, 1977)

$$\overline{D}_n = d_n \times 4/\pi.$$

The mean corrected islet diameter ($\overline{D}_i$) and the mean corrected nuclear diameter ($\overline{D}_n$) were used to calculate the mean islet volume and mean nuclear volume (Williams, 1977)

$$V = 4\pi/3 \times (\overline{D}/2)^3.$$

Values are presented as means ± standard error of mean (S.E.M.). Data were analysed statistically by one-way analysis of variance (ANOVA) followed by Tukey's HSD (honestly significant difference) pairwise comparisons using a current SPSS statistical package, and the level of significance was determined to be less than 0.05 throughout the study.

RESULTS

Regional distribution of the islets in the rat

The mean volume density of the islets per pancreatic tissue (V_{vi}), the mean diameter of the islets ($\overline{D}_i$), the mean islet volume and the mean number of cells per islet in different regions of the rat pancreas are shown in Table 1. It was found that the islets were uniformly dispersed in the exocrine tissue of the pancreas, except that the V_{vi} varied from one region to another. The mean volume density of the islets per pancreatic tissue showed no significant difference between the upper

duodenal, lower duodenal and gastric regions. The upper duodenal was significantly different from the splenic and no significant difference was found between gastric and splenic regions (Table 1). It was also observed that the lower and upper duodenal regions were significantly different from the gastric and splenic regions in the mean islet diameter and mean islet volume, whereas no difference was found either between the lower and upper duodenal regions, or between gastric and splenic regions (Table 1). All 4 regions were significantly different from each other in their number of cells per islet (Table 1).

The size–frequency distribution of the profile diameters of the islets in the 4 pancreatic regions is shown in Figures 2 and 3. The size–frequency distribution of the profile diameters of the islets in the upper duodenal region showed an overall shift of all
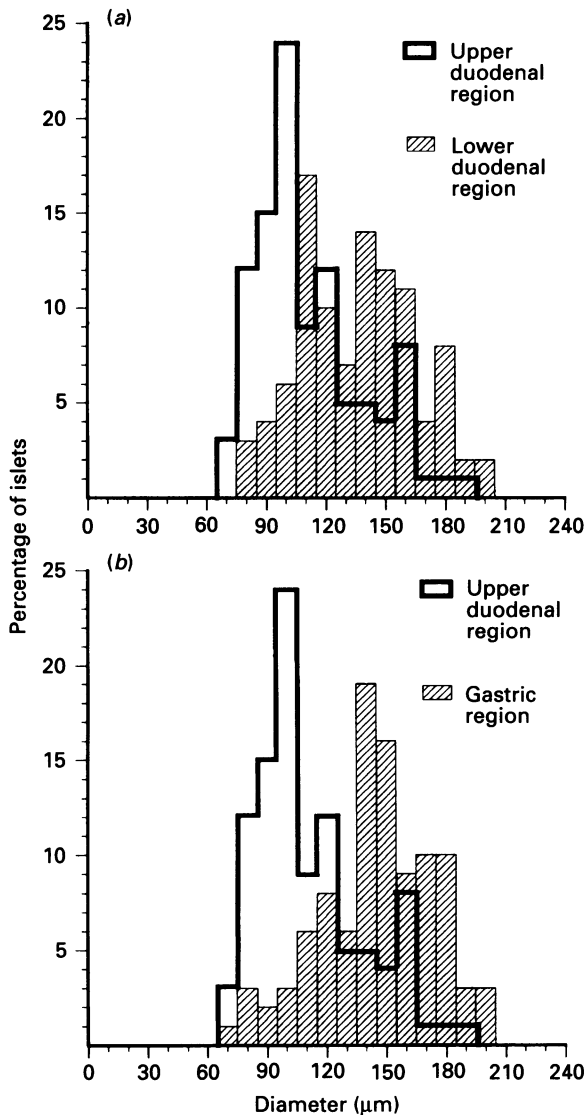

Fig. 2. Size-frequency distribution of the profile diameters of the rat pancreatic islets of the upper duodenal region compared with those of the lower duodenal (*a*) and gastric regions (*b*).
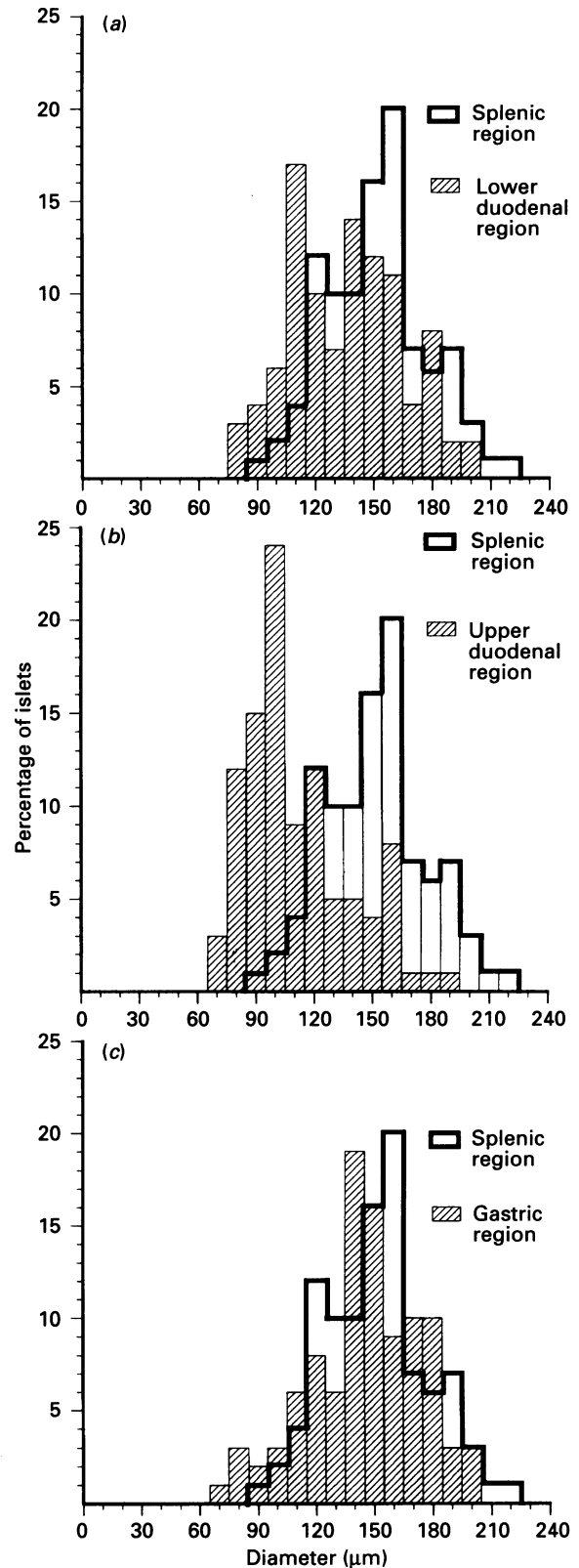

Fig. 3. Size-frequency distribution of the profile diameters of the rat pancreatic islets of the splenic region compared with those of the lower duodenal (*a*), upper duodenal (*b*) and gastric regions (*c*).

Table 1. *Volume density of islets per pancreatic tissue, islet diameter, islet volume and number of cells per islet of rat* $(n = 6)$*

	Lower duodenal	Upper duodenal	Gastric	Splenic	ANOVA	
					F value	P value
Volume density of islets/panc (V_{vi})	0.0256±0.001	0.0228±0.001	0.0287±0.002	0.0328±0.002	7.2192	< 0.0018
Islet diameter (μm)	191±2.7	157±6.3	210±5.6	223±1.5	39.353	< 0.0001
Islet volume (μm³)	3649838±162598	2066068±243684	4851000±398846	5808820±106943	40.6937	< 0.0001
Number of cells/islet	11294±5091	5559±512	15285±839	18835±762	71.821	< 0.0001

* Values are presented as means ± S.E.M.

Table 2. *Nuclear diameter, nuclear axial ratio and nuclear volume in the 4 pancreatic regions of rat* $(n = 6)$* ·

	Lower duodenal	Upper duodenal	Gastric	Splenic	ANOVA	
					F value	P value
Nuclear diameter (μm)	6.297±0.14	6.182±0.10	6.341±0.15	6.389±0.12	0.4614	0.7123
Nuclear axial ratio	1.087±0.007	1.063±0.019	1.104±0.007	1.076±0.009	2.2841	0.1100
Nuclear volume (μm³)	131.72±9	124.02±5	134.75±10	137.53±9	0.5169	0.6754

* Values are presented as means ± S.E.M.

the size classes towards smaller sizes compared with the corresponding classes of the other regions (Figs 2a, b, 3b). Conversely, the islets of the splenic region of the pancreas showed an overall shift towards larger sizes compared with the corresponding classes of other regions (Fig. 3a, b, c). The profile diameters of the islets showed a mean axial ratio of 1.27±0.02, 1.39±0.02, 1.36±0.03 and 1.37±0.02 for the lower duodenal, upper duodenal, gastric and splenic regions, respectively, indicating that the islets could be treated as spheroids. The cumulative frequency distribution and the axial ratio of the profile diameters of the pancreatic islets from different regions of the rat pancreas showed that their population contained a range of sizes, justifying the use of Fullman's formula (Williams, 1977) to calculate the mean islet diameters $(\overline{D}_i)$. The mean corrected diameter of the islets was significantly lower in the upper duodenal than the other regions and no significant differences were found between gastric and splenic regions. It was also observed that the lower duodenal was significantly different from the other regions (Table 1).

All regions were significantly different from each other in their mean number of cells per islet. This mean number was found to be significantly lower in the upper duodenal and significantly higher in the splenic than the other regions (Table 1). The mean corrected nuclear diameter, mean nuclear axial ratio and mean nuclear volume showed no significant difference in the 4 pancreatic regions (Table 2).

Cellular pattern of rat islets

It was observed that A cells were arranged in clusters scattered at the periphery of the islets (Fig. 4a) and the B cells occupied the central portion of the islets (Fig. 4b). Most of the D cells were situated peripherally; only a few cells were found to occupy an intermediate position (Fig. 4c). PP cells were found to be arranged singly or in clusters at the periphery of the islet (Fig. 4d). No regional differences were detected in this pattern of cellular distribution in a total of 1292 islets examined. The mean volume density of each cell type per islet (V_{vc}) is shown in Table 3. The volume density of A cells was significantly lower in the lower duodenal than the other regions. In the upper duodenal, this volume density was significantly lower than the gastric and splenic regions, which showed no significant difference in their volume density of A cells (Table 3). It was also observed that the volume densities of B cells in the lower duodenal, gastric and splenic regions, were significantly higher than the upper duodenal region. No significant difference was found between lower duodenal, gastric and splenic regions. The volume density of D cells showed no significant difference in the 4 pancreatic regions (Table 3). The volume density of PP cells, was significantly higher in the lower duodenal than the other regions and no significant difference was found between upper duodenal, gastric and splenic regions (Table 3).

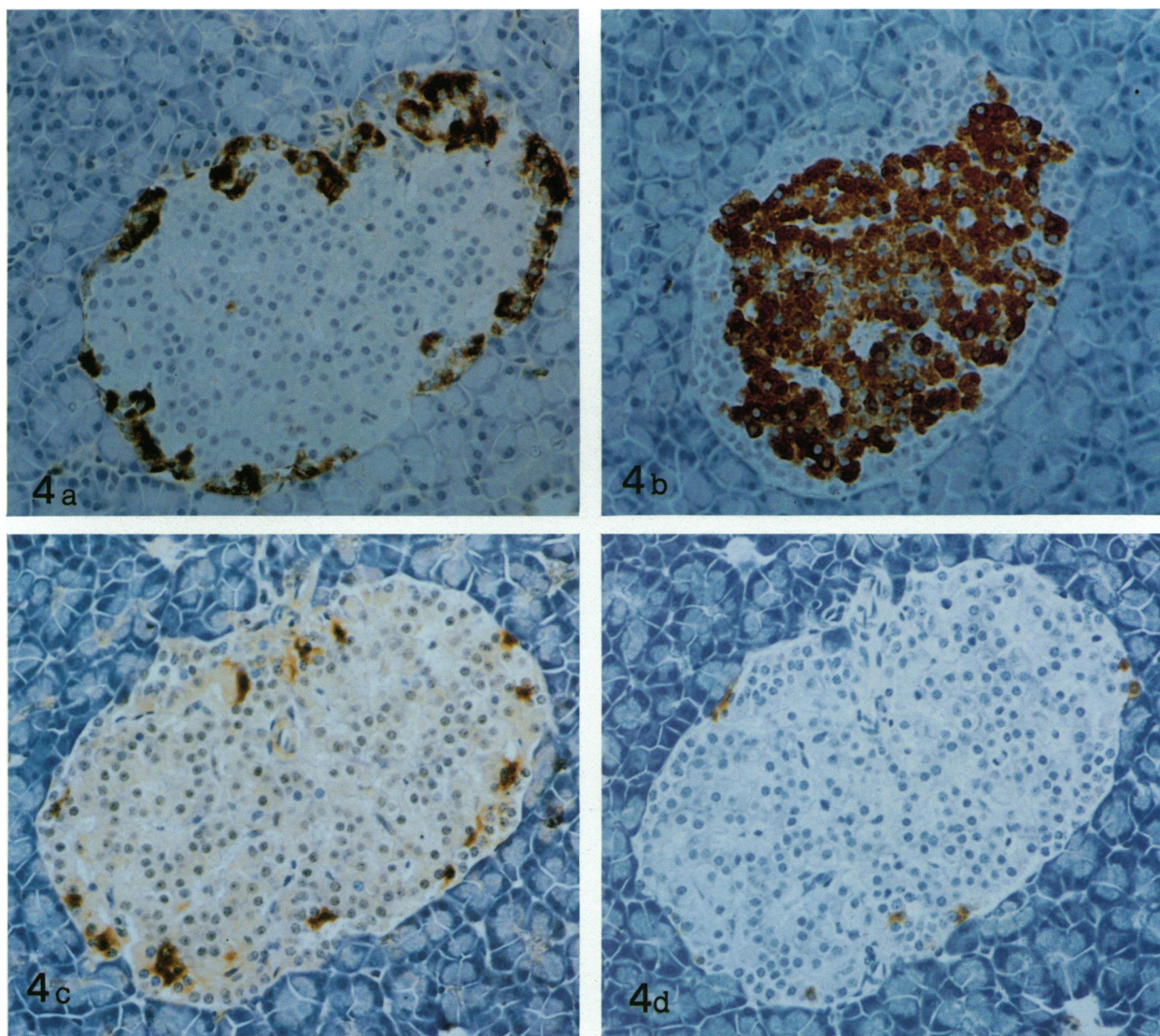The mean number of each cell type per islet in the

Fig. 4. Light micrographs of adjacent sections of rat pancreatic islet (splenic region) stained immunocytochemically with antiglucagon (*a*), anti-insulin (*b*), antisomatostatin (*c*) and antipancreatic polypeptide (*d*). × 200.

Table 3. *Mean volume density* (V_{vc}) *of the 4 cell types in different regions of rat* (*n* = 6) *pancreas**

Cell type	Lower duodenal	Upper duodenal	Gastric	Splenic	ANOVA	
					F value	P value
A	0.0638 ± 0.002	0.2168 ± 0.003	0.2465 ± 0.004	0.2466 ± 0.002	1008.530	< 0.0001
B	0.6814 ± 0.003	0.6583 ± 0.005	0.6893 ± 0.005	0.6928 ± 0.007	8.595	< 0.001
D	0.0524 ± 0.004	0.0514 ± 0.002	0.0493 ± 0.001	0.0484 ± 0.001	0.7315	0.5453
PP	0.1858 ± 0.004	0.0264 ± 0.002	0.0279 ± 0.002	0.0280 ± 0.001	1297.833	< 0.0001

* Values are presented as volume fraction $\pm$ s.e.m.

different regions of the rat pancreas is shown in Table 4. The mean number of A cells per islet was found to be significantly lower in the lower duodenal region than the other pancreatic regions. In the upper duodenal region this number was significantly different from the gastric and splenic regions which showed no significant difference between each other (Table 4,

Fig. 5 *a*). The mean number of B and D cells per islet were found to be significantly different in the upper duodenal region compared with the other pancreatic regions. No significant difference was found between either the lower duodenal and gastric or the gastric and splenic regions (Table 4). D cells showed no significant difference in the mean number per islet in

Table 4. *Number of each cell type per islet in different regions of rat (n = 6) pancreas**

Cell type	Lower duodenal	Upper duodenal	Gastric	Splenic	ANOVA	
					F value	P value
A	474 ± 37	1272 ± 184	4278 ± 227	4887 ± 137	180.033	< 0.0001
B	8807 ± 915	3640 ± 620	9905 ± 1161	12815 ± 710	19.067	< 0.0001
D	487 ± 26	329 ± 24	595 ± 27	712 ± 48	24.939	< 0.0001
PP	1679 ± 168	143 ± 20	435 ± 32	405 ± 30	62.238	< 0.0001

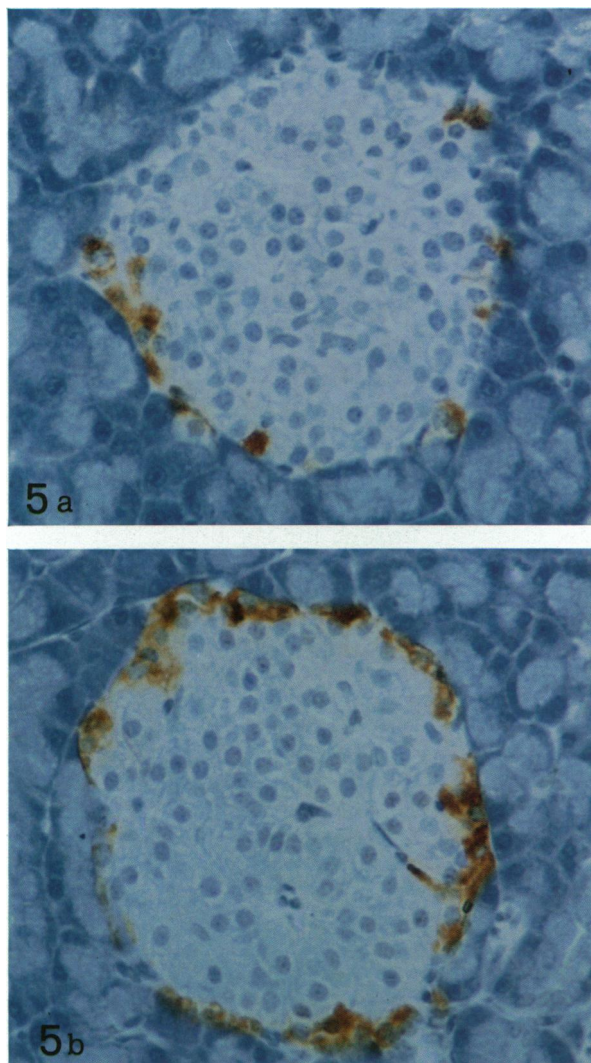* Values are presented as means ± S.E.M.

Fig. 5. Light micrographs of adjacent sections of rat pancreatic islet from the lower duodenal region stained immunocytochemically with antiglucagon (a) and antipancreatic polypeptide (b). × 320.

the 4 pancreatic regions. The mean number of PP cells per islet was significantly higher in the lower duodenal region as compared to the other pancreatic regions. The upper duodenal, gastric and splenic regions showed no significant difference in their mean number of PP cells per islet (Table 4, Fig. 5b).

DISCUSSION

The results of this study showed that the islets were dispersed evenly in the rat pancreas, but with distinct regional differences in their volume densities. The volume density of the islets was found 2.6%, 2.3%, 2.9% and 3.3% in the lower duodenal, upper duodenal, gastric and splenic regions, respectively. Previous studies on other mammals reported figures for the volume density of the islets in the whole pancreas, ranging from 1 to 2.5% (Overholser, 1925; Wright & Carpenter, 1961; Petersson, 1966; Carpenter et al. 1967; Remacle et al. 1977; Volk & Wellman, 1977; Kaung & Elde, 1980; Telford & Bridgman, 1990). Reddy et al. (1986) observed that there was no difference in the total number of islets per unit area between the duodenal and splenic regions in possum pancreas. Krause et al. (1989) reported similar findings in opossum pancreas. Furuzawa et al. (1992), however, found a regional difference in the distribution of the pancreatic islets in the cat (2% duodenal, 3.3% gastric, 3.2% anastomotic and 3.5% in splenic portions). The significance of the regional differences in the distribution of the pancreatic islets is not clear; it may, however, reflect special functional adaptations in the species.

The present work also showed presence of regional difference in the mean islet diameter, the mean islet volume and the mean number of cells per islet in the 4 pancreatic regions of the rat. We could not find in the available literature any study on the size, volume and cellular number of the rat pancreatic islets and their regional distribution. Satio et al. (1978), in their study on the normal human pancreas, reported differences in size distribution, number and volume of islets, but the head and its uncinate process were not examined. Furuzawa et al. (1992) reported regional differences in islet distribution and islet diameter in the 4 pancreatic regions in the cat.

The present finding of no significant difference in the mean nuclear diameter and mean nuclear volume in the 4 pancreatic regions is suggestive of similarity of

the size of the islet cells in the rat pancreas. The larger the islet, the greater is the number of cells. The size and cellular composition of the pancreatic islets not only showed an interspecies difference, but also an intra-species difference (Bonner-Weir & Like, 1980; Jörns et al. 1988).

In the present study, the cellular pattern of the rat islets showed a central core of B cells with peripherally arranged A, D and PP cells. This is in agreement with previous reports (Goldsmith et al. 1975; Sundler et al. 1977; Ito et al. 1978; Baetens et al. 1979; Bani Sacchi & Bani, 1985). The cellular pattern of the islets has a species difference that may reflect a functional significance. Pipeleers et al. (1982) have shown that the relationships of islet cells to one another can have a dramatic effect on secretion. It was found that in diabetic animals and man there is marked disruption of the normal histological relationships (McEvoy & Hegre, 1977). Orci et al. (1981), working on the human pancreas, reported that there is an obligatory association between D and A cells for appropriate control of secretion from pancreatic endocrine cells. Moreover, Hunderson et al. (1981) reported that the function of the acinar pancreas is affected by somatostatin and glucagon. Also the core-to-mantle pattern of islet cell perfusion (from B cell to A cell and thence to the D cell), which was proposed by Stanger & Samols (1990), might have an explanation of the functional significance of the cellular pattern of the islets.

The results have also demonstrated the presence of 2 types of islets in the rat pancreas, glucagon-rich, PP-poor (glucagon islet) in the upper duodenal, gastric and splenic regions and PP-rich, glucagon-poor (PP islet) in the lower duodenal region. This is in agreement with previous studies (Orci et al. 1976; Baetens et al. 1979; Bani Sacchi & Bani, 1985) which relate these regional differences to developmental backgrounds.

The present study has shown significant differences in the number of A, B, D and PP cells per islet in the 4 pancreatic regions that might indicate species differences and functional adaptations. It is interesting that the number of B and D cells per islet showed a significant decrease in the upper duodenal region as compared with other regions ($P < 0.05$). Moreover, we observed a significant decrease in the number of A cells per islet in the upper duodenal region compared with the gastric and splenic regions ($P < 0.05$). The regional differences of the islet cells in the rat pancreas, observed in this study, demonstrate the importance of knowing the area of the pancreas sampled for quantitative studies.

From the findings of the present study, it is concluded that distinct regional differences exist in the rat pancreatic islets. These differences existed not only between the ventral (lower duodenal region) and dorsal lobes, but also between the regions derived from the dorsal pancreatic lobe (upper duodenal, gastric and splenic regions). Baetens et al. (1979) attributed the regional differences in the rat pancreatic islets to their development from two different pancreatic primordia, ventral and dorsal. Another explanation of the regional differences postulates two different arterial systems for irrigation of pancreatic islets for the ventral and dorsal lobes (Orci et al. 1976; Syed Ali, 1984). However, both hypotheses are insufficient to justify the differences between the regions of the dorsal pancreatic lobe, as observed in this study. Further investigations are required to elucidate the reasons for these differences in various regions of the dorsal lobe of the pancreas.

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