Changes in the Volumes of the A-, B-, and D-Cell Populations in the Pancreatic Islets During the Postnatal Development of the Rat

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SUMMARY

The changes in the volumes of three of the principal islet cell types in the developing rat pancreas were quantitated from day 10 to day 210 of life. The insulin-, glucagon-, and somatostatin-positive cell populations of the islets were identified by immunocytochemical staining, and their volumes were determined by linear scanning. The insulin and glucagon content of the pancreata, and the concentrations of these hormones in plasma, were also determined at each age by radioimmunoassays. The volumes of the A- and D-cells reached their maximum values by day 50 and day 35, respectively. The content of glucagon within the pancreas reached adult levels by day 50. In contrast, the volume of B-cells and the insulin content were highest on day 210. The concentrations of both insulin and glucagon in the plasma reached adult levels by approximately day 50. There were no differences in the pancreatic parameters between male and female rats until day 25, when the wet weight of the male gland became significantly greater. The concentrations of the islet hormones and the percentages of the islet cell types within the pancreas did not differ between the sexes at any age. However, the greater weight of the pancreas resulted in a greater total content of islet cells and hormones in the male gland after day 25. The data suggest that, in the rat, the B-cell volume may continue to increase with age, while the A- and D-cells apparently do not. The physiologic consequences of these changes remain to be determined.

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Since the beginning of this century, there have been numerous attempts to estimate the total volume (mass, number) of the islets of Langerhans within the mammalian pancreas.[1–8] This has proved to be a difficult problem for several reasons. First, the volume of islets is small in relation to the rest of the pancreas. A large sample is required to insure statistical reliability. Second, the earlier studies were limited because of the nonspecificity and insensitivity of the tinctorial staining methods available. The development of immunocytochemistry and the availability of specific antisera to each of the islet peptide hormones have alleviated this problem. Third, since the early studies by Bensley,[3] a marked variability in the distribution of islets within the pancreas had been noted; the tail of the pancreas has relatively more islet volume than the head. More recently, Orci et al.[9] reported that differences also exist in the relative proportions of the cell types within the islets, depending on the location of the islet in the pancreas. Pancreatic polypeptide-positive cells were found primarily in the most caudal portion of the rat pancreas, which extends along the small intestine, whereas glucagon-positive cells were more prominent in the islets of the rest of the organ.

We have been interested in the factors that regulate the growth of the pancreatic islets both in vivo and in vitro. To provide baseline data, a survey of the growth of the islets during normal development has been undertaken. The development of the islets during the perinatal period (16 days postcoitum to 10 days postnatal) has recently been reported.[10,11] The present report details the changes in the volumes of the A-, B-, and D-cells of the rat pancreas from 10 days postnatal until 7 mo of age. The volumes of the specific islet cells (as determined by morphometric analysis after immunocytochemical staining) are correlated with changes in the insulin and glucagon contents of the pancreas.

MATERIALS AND METHODS

Animals and tissue sampling. Sprague-Dawley rats were used. The day of birth was taken as day 0. All litters were reduced to eight animals at birth and were weaned on day 21. After weaning, the animals were allowed free access to Purina rat chow and tap water. Animals from at least four different litters were killed, between 9 and 11 a.m. at 5-day in-

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tervals from day 10 through day 35, and on days 50, 70, and 210. The method of dissection and sampling has been detailed.[12] Briefly, the entire pancreas, including the portion extending along the jejunum, was removed and weighed. The pancreas was placed on dental wax and cut along the long axis into six to seven strips. Eight to ten small pieces were removed randomly from the strips, weighed, and fixed in Bouin's solution. These constituted approximately 10–15% of the gland by weight. The remainder of the pancreas was weighed, homogenized in at least 20 vol of acidified 70% ethanol (1.5% HCl), and extracted overnight at 4°C. In previous experiments, the concentrations of insulin, glucagon, and somatostatin in the pieces to be fixed were identical to those in the remainder of the pancreas.[12] Thus, the procedure yields a sample for morphometric analysis which accurately reflects the distribution of the islet cells within the pancreas.

Morphology and morphometry. The pieces from each pancreas were embedded together in paraffin and the block was serially sectioned (4 μm). Adjacent sections at a fixed interval through the block (every 18th section) were mounted on separate slides and stained immunocytochemically for insulin, glucagon, or somatostatin. The number of sections which were quantitated for each hormone varied from 16 to 60, increasing with the age and wet weight of the pancreata. A modification[13] of the unlabeled antibody enzyme technique of Sternberger et al.[14] was used. Guinea pig anti-insulin and rabbit anti-pancreatic glucagon antisera were raised in this laboratory. Guinea-pig anti-somatostatin antiserum was the generous gift of Dr. Jonathan A. Parsons (Department of Anatomy, University of Minnesota). After the immunocytochemical procedure, the sections were lightly counterstained with hematoxylin. The volumes of the pancreas and of the hormone-positive cells were estimated using a linear scanning technique.[15] Every 18th section through each tissue block was scanned at a transverse interval of 75 μm. Adjacent sections, stained for each of the three hormones, were scanned to determine the volumes of each of the three cell types. The data are expressed as millimeters of scan. Although these units are unconventional, a correlation between the linear distance obtained by such a systematic scan and the wet weight (volume) of the tissue is predicted by stereological formulae,[16] and has been empirically demonstrated using rat pancreas.[17] In addition, the continued use of these units permits comparison with several previous communications from this laboratory. The total volume of each of the islet cells within the pancreas was obtained by multiplying the volume obtained by scanning the portion which had been fixed by the ratio of the total wet weight of the pancreas to the wet weight of that fixed portion.

Hormone assays. The concentrations of insulin and glucagon in the extracted pancreata, and in heparinized plasma obtained at the time of killing, were determined by radioimmunoassays. The details of the methods for extracts[18,19] and plasma[20,21] have been published. Primary antisera were similar to those used for immunocytochemistry. For the insulin assay, all extracts were diluted to that portion of the standard curve where crystalline rat insulin standards and extracts of rat pancreas give similar results.[22,23,17] The total contents of insulin and glucagon in each pancreas were calculated by multiplying the concentration determined by radioimmunoassay by the wet weight.

Statistical analysis. At each age interval, the islet cell volumes and hormone contents were compared with those of the next younger age and with adult pancreas (210 days) using Student's *t* test.

RESULTS

The changes in the total islet cell volumes during development are presented in Table 1. Since there were no differences at any age between males and females in terms of the percentage of the islet cells within the pancreas, the data from both sexes were pooled. There were no differences in pancreatic wet weight ($P > 0.05$) between males and females until day 25 (Table 2), so the data from both sexes

TABLE 1
Changes in the percentages and total volumes of the insulin-, glucagon-, and somatostatin-positive cells of the pancreatic islets during development of the rat

Age (days)	Sex	N	Insulin-positive		Glucagon-positive		SRIF-positive	
			(%)	(mm/Gland)	(%)	(mm/Gland)	(%)	(mm/Gland)
10	Both	23	2.3 ± 0.1§	82.9 ± 9.7§	1.3 ± 0.2§	45.8 ± 5.2§	0.6 ± 0.1§	22.0 ± 4.0§
15	Both	16	1.5 ± 0.1†	90.1 ± 8.1§	1.1 ± 0.1§	52.5 ± 3.1§	0.5 ± 0.1‡	20.8 ± 3.1§
20	Both	19	1.1 ± 0.1*	93.1 ± 6.1§	0.6 ± 0.1‡	62.0 ± 2.3§	0.2 ± 0.1	28.1 ± 3.9§
25	Male	11	0.7 ± 0.1*	122 ± 14§	0.4 ± 0.1	82 ± 5†§	0.2 ± 0.1	35 ± 4§
	Female	11		104 ± 8§		65 ± 3§		29 ± 2§
30	Male	10	0.9 ± 0.1	152 ± 14§	0.6 ± 0.1‡	106 ± 11§	0.2 ± 0.1	41 ± 3§
	Female	10		133 ± 9*§		93 ± 10*§		36 ± 2§
35	Male	8	0.8 ± 0.1	185 ± 16§	0.5 ± 0.1	109 ± 9§	0.4 ± 0.1	76 ± 7†
	Female	8		163 ± 12§		97 ± 9		70 ± 8†
50	Male	8	0.8 ± 0.1	328 ± 22†§	0.4 ± 0.1	192 ± 17†	0.2 ± 0.1	102 ± 9
	Female	8		272 ± 24†§		160 ± 14†		84 ± 7
70	Male	8	0.9 ± 0.1	621 ± 58†§	0.4 ± 0.1	190 ± 10	0.1 ± 0.1	99 ± 8
	Female	8		459 ± 41†§		141 ± 17		74 ± 4
210	Male	8	1.1 ± 0.1	1027 ± 79†	0.2 ± 0.1	189 ± 12	0.1 ± 0.1	86 ± 6
	Female	8		820 ± 64†		145 ± 12		66 ± 7

All data are mean ± SEM; SRIF = Somatostatin.
* Different from next younger age, $P < 0.01$; † $P < 0.001$.
‡ Different from adult (day 210), $P < 0.01$; § $P < 0.001$.

TABLE 2
Changes in weight, insulin, and glucagon content of the rat pancreas during development

Age (days)	Sex	N	Wet weight (mg)	Insulin (mU/mg)	Insulin (mU/gland)	Glucagon (mU/mg)	Glucagon (mU/gland)
10	Both	56	46.0 ± 1.3§	3.41 ± 0.22§	149 ± 5§	44.9 ± 4.5§	2.00 ± 0.20§
15	Both	21	75.7 ± 3.1*§	4.62 ± 0.50§	343 ± 39†§	37.1 ± 4.1§	2.81 ± 0.30§
20	Both	25	90.2 ± 2.4†§	3.64 ± 0.23§	320 ± 20§	28.5 ± 2.0§	2.57 ± 0.17§
25	Male	20	346 ± 20†§	0.94 ± 0.04†‡	325 ± 40§	8.1 ± 0.5†§	2.80 ± 0.21§
	Female	11	295 ± 18†§		271 ± 31§		2.39 ± 0.18§
30	Male	17	412 ± 19§	1.12 ± 0.19	429 ± 51§	6.5 ± 0.7‡	2.69 ± 0.19§
	Female	10	420 ± 27§		401 ± 29‡		2.31 ± 0.21§
35	Male	12	474 ± 38§	0.81 ± 0.06§	419 ± 28§	5.1 ± 0.6	2.71 ± 0.28§
	Female	8	420 ± 27§		330 ± 25		2.4 ± 0.25§
50	Male	10	814 ± 19†§	1.43 ± 0.12*	1175 ± 100†‡	5.7 ± 0.7	4.64 ± 0.49*
	Female	10	676 ± 20†§		960 ± 80†‡		3.86 ± 0.41*
70	Male	10	1200 ± 144*	0.84 ± 0.06	1100 ± 117‡	4.9 ± 0.9	5.92 ± 0.11
	Female	10	887 ± 35†		817 ± 59		4.23 ± 0.80
210	Male	8	1164 ± 55	1.54 ± 0.19*	1772 ± 212*	4.6 ± 0.7	5.34 ± 0.32
	Female	8	919 ± 46		1427 ± 119†		4.22 ± 0.29

All data are means ± SEM.
* Different from next younger age, $P < 0.01$; † $P < 0.001$.
‡ Different from adult (day 210), $P < 0.01$; § $P < 0.001$.

prior to that age were also pooled. The insulin-positive cell volume of the developing pancreas was relatively constant at the earliest ages examined in this study. There was no statistically significant increase between consecutive age groups until days 25 (males) and 30 (females). There were substantial increases in the B-cell volume at 50, 70, and 210 days in both sexes. On day 70 the B-cell volume was only 70% of that on day 210. In contrast, by 50 days of age, the glucagon-positive cell volume was not significantly different from that of the mature adult (day 210). The somatostatin-positive population was even more precocious, reaching the day 210 volume by day 35 postnatal. The islet cells comprise a relatively large percentage of the pancreas early in life (over 4% on day 10). The increase in the volume of islet cells was relatively slow between 10 and 25 days of age, whereas the wet weight of the gland rose rapidly during this interval. Consequently, the percentages of each of the islet cells fell to approximately the adult value by days 20–25.

Changes in the insulin and glucagon content of the developing pancreas are shown in Table 2. Early in the neonatal period, both the insulin and glucagon concentrations in the pancreas were quite high. The total content of insulin rose significantly on day 15 and then stayed relatively constant until day 50. The increase in the wet weight of the pancreas during this interval resulted in a fall in insulin concentration. Subsequently, there was a rise in the pancreatic insulin content. Between days 70 and 210, the increase in the total content of insulin within the gland was due in large part to the increase in its concentration. The glucagon content of the pancreas did not rise significantly from day 10 until day 50, when it reached its maximum. The rapid rise in pancreatic wet weight during the period resulted in a dramatic decrease in pancreatic glucagon concentration.

The concentrations of insulin and glucagon in the plasma during this period are shown in Table 3. The variation in the concentrations of both hormones was considerable in all age groups as indicated by the large standard deviations. The plasma insulin concentration at the younger ages tended to be below that of the adult (day 210), but stabilized at the adult level by day 50. In contrast, plasma glucagon concentration was higher in the younger rats and fell gradually, also approximating the adult level by day 50.

DISCUSSION

The growth of the islet cells differs from that of the exocrine pancreas. Early in postnatal life, the exocrine pancreas actually decreases in volume,[24] while the islet volume continues to increase.[7,8,10] This differential growth results in a relatively high islet/exocrine ratio during the first days of life. As indicated in the present study, at about weaning, the wet weight of the pancreas increased dramatically, sexual dimorphism first appeared, and the islet volumes remained stable for a time. These factors resulted in a reduction in the percentage of islet in the pancreas (as estimated by the volumes of the B-, A-, and D-cells) to that of the adult by day 25.

The increases in the A-, and D-cell volumes in the developing pancreas were essentially completed by days 50 and 35, respectively, whereas the volume of B-cells continued to increase. The selection of day 210 as the end of this study

TABLE 3
Developmental changes in plasma insulin and glucagon

Age (days)	N	Insulin (μU/ml)	Glucagon (pg/ml)
10	39	44 ± 24	407 ± 225†
15	25	36 ± 20*	367 ± 240†
20	49	53 ± 28	236 ± 119†
25	50	35 ± 28*	277 ± 219†
30	36	37 ± 12*	137 ± 96
35	72	34 ± 17*	224 ± 136*
50	30	54 ± 22	147 ± 60
70	25	47 ± 10	128 ± 40
210	75	56 ± 17	164 ± 61

Data are mean ± SD.
Males and females did not differ.
* Different from day 210, $P < 0.01$.
† Different from day 210, $P < 0.001$.

was arbitrary, and it remains unknown if the B-cell volume had plateaued by this age, and if so, when the plateau had been reached. These data complement those of Haist and Pugh[6] which, based on a small number of rats, indicated that the islet volume continued to increase with increasing body weight. Since Overholser[5] reported that the number of islets in the rat pancreas did not rise after approximately day 50, the increase in the total islet volume could only be accomplished by an increase in the size of individual islets. The data in the present communication suggest that the increase in islet volume is due primarily to increase in the B-cell volume. Unfortunately, this extrapolation from the available data was not confirmed directly, since the morphometric technique used in the present study does not quantitate the number of islets. Consequently, the changes in the volumes of individual islets was not determined.

The continued increase in the B-cell volume could be interpreted as indicating that the demand for their secretory product, insulin, increases with increasing age or weight. If this assumption proves correct, then the lack of continued growth by the A- and D-cells may indicate that the demand for their counterregulatory hormones does not increase. Linear regression analysis was used to compare the changes in the mean insulin and glucagon contents with the volumes of the B- and A-cells in all of the age groups. Males and females were considered as separate data points at each age. There was a highly significant correlation between pancreatic insulin and B-cell volume ($r = 0.84$, $P < 0.001$) and pancreatic glucagon and A-cell volume ($r = 0.82$, $P < 0.001$). This would indicate that hormone assays could be used to rapidly approximate differences in cell volumes between experimental groups. This is a relevant point, since the determination of hormone content by radioimmunoassays is easier and faster than the morphometric quantitation. Unfortunately, a radioimmunassay for somatostatin was not available at the time this study was performed so a correlation between somatostatin and D-cell volume could not be made.

The patterns of change in the plasma concentrations of insulin and glucagon during development (Table 3) are similar to those reported by others.[25,26] These investigators have discussed, in detail, the possible mechanisms of the changes in plasma hormone concentrations during development as well as the possible physiologic significance of the changes.

Finally, some mention of the pancreatic polypeptide (PP) cells of the pancreas is in order. The work of Orci et al.[9,27] and others[28] has demonstrated that the distribution of the PP cells is unequal in the pancreata of the rat, dog, and human. Unfortunately, the volumes of the PP cells were not determined in the present study. It should be emphasized, however, that while the PP cell volume may be of interest, the omission of such quantitation does not affect interpretation of the data on the other cell types. The sampling procedure ensured that segments of tissue from all portions of the gland were quantitated. The data are relevant to the gland as a whole, although they certainly do not reflect the heterogeneity between anatomically different portions of the pancreas. Such differences can be quite marked, as indicated in a recent report by Milner et al.[29] in which the characteristic hypertrophy of the B-cell population in human infants of diabetic mothers was more marked in the PP-poor areas of the gland. Any quantitative, morphologic approach to the study of changes in the islet cell volumes must have a carefully controlled sampling procedure to ensure that the nonrandom distribution of the islet cell types does not bias the results.

In summary, during the development of the rat pancreas, the B-cell volume continues to increase at least through day 210 while the A-, and D-cell volumes remain constant after day 50 and 35, respectively. There was a close correlation between the insulin and glucagon content of the pancreas and the B- and A-cell volumes.

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