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STEREOLOGICAL ANALYSIS OF THE GUINEA PIG PANCREAS DURING POSTNATAL DEVELOPMENT

Gerson Francisco de Assis, Renato Massaharu Hassunuma and Rumio Taga

Department of Biological Sciences (Histology), School of Dentistry, University of São Paulo(USP), Bauru, SP, Brazil.

ABSTRACT

The postnatal development of the guinea pig pancreas (*Cavia porcellus*) from 2 to 140 days old was studied stereologically by light microscopy. During this period, the pancreatic volume increased 805%. The compartmental volumes of the acini, intercalated ducts, excretory ducts, islets of Langerhans and stroma increased 756%, 1,372%, 1,591%, 3,393% and 793%, respectively, whereas the total external surfaces of the acini, intercalated ducts and excretory ducts increased 542%, 1,667% and 1,002%, respectively. This growth involved increases of 361%, 74%, 1,355% and 673% in the total number of acinar, centroacinar, intercalated duct and excretory duct cells, respectively. In the same period, the mean volume of acinar cells increased 210%. These results indicate that the growth of the acinar compartmental volume involves a significant proliferative activity of the acinar cells and an increase in the individual cell volume. Analysis of the surface-to-volume ratio during this growth showed that the acini increased significantly in size. On the other hand, the growth of the parenchymal structures was accompanied by a reduction on interacinar connective tissue spaces and by the final organization of the interlobar and interlobular septa.

Keywords: Development, guinea pig, pancreas, stereology

INTRODUCTION

The acinar pancreatic cells of guinea pigs have been used extensively as a model to study the basic mechanisms of synthesis, intracellular transport, condensation, storage and discharge of secretory proteins [3,4,7,13,15,18]. However, the pre- and postnatal development of the guinea pig exocrine pancreas has been poorly documented by quantitative light and electron microscopy. The few reports to date have focused solely on biochemical aspects [8,20].

In the present work, we evaluated the evolution of body mass and pancreatic volume, and the following parameters for each morphological compartment of the pancreas, during postnatal development in guinea pigs: a) volume density, b) total volume, c) surface density, d) total surface area, e) surface-to-volume ratio, and f) total cell number. The acinar cell volume was also determined. Mathematical analysis of these data allowed calculation of the growth rate postnatal development.

Correspondence to: Dr. Gerson Francisco de Assis
Departamento de Ciências Biológicas-Histologia, Faculdade de Odontologia de Bauru, Universidade de São Paulo (USP), Al. Dr. Octávio Pinheiro Brisolla 9-75, CEP 17.012-901, Bauru, SP, Brasil. Tel: (55)(14) 235-8259, Fax: (55)(14)223-4679, E-mail: gfassis@fob.usp.br

MATERIAL AND METHODS

Twenty-eight male guinea pigs (*Cavia porcellus*) reared and maintained by the Central Animal House of the Dentistry School of USP Bauru, were divided into groups aged 2, 7, 14, 21, 35, 70 and 140 days old. The litters were left with their dams up to the twenty-first day, when they were weaned. The glands were always collected between 10:00 a.m. and 12:00 a.m. to avoid circadian variations. The guinea pigs were anesthetized with ethyl ether and their body mass was determined using a Mettler P1000 balance. After an abdominal incision, the pancreas was exposed and carefully removed, and its fresh mass was rapidly determined on a Mettler P20 analytical balance.

Each pancreas was fixed in Bouin solution for 4 h at room temperature, rinsed overnight in 70% ethyl alcohol, dehydrated in ethyl alcohol, cleared in xylene and embedded in Paraplast (paraffin + plastic resin). Sections 6 µm thick were obtained at of 60 µm intervals using a Jung-Leica 2045 Multicut microtome and stained by the Gomori trichromic method.

The processed pancreatic volume

The processed volume of the pancreas (Vp) can be estimated from the fresh mass (m), the gland density (δ) and

the shrinkage factor (Sf) caused by histological processing, using the equation $Vp = {}^{m}/_{\delta.Sf}$.

The density value used for the pancreas was $\delta = 1.08$ g/cm^3, determined by Bolender [2] for adult guinea pigs. The shrinkage of the pancreas caused by histological processing was determined in guinea pigs aged 2, 21, 70 and 140 days old (four animals per group), by the method of Taga and Sesso [16]. The percentage of volume shrinkage after processing was expressed relative to the mass of the fresh pancreas. The mean volume shrinkage factor calculated for the four age groups was 0.52 with a coefficient of variation of 7.63%.

The volume, surface and total number cells of pancreatic structures and nuclear volume density of acinar cells

The morphometric measurements were obtained using a Zeiss 8 x Kpl eyepiece containing a Zeiss II integration grid with 10 parallel lines and 100 points in a quadrangular area, and a 100 x oil-immersion objective. The following data were obtained for 50 histological fields per animal, selected by systematic sampling[19]: a) number of points (Pi) above the images of each structure (i) and above the entire gland (P), b) number of points over the nucleus (Pn) and cytoplasm (Pcyt) of acinar cells, c) number of nuclei (n) for each pancreatic structure, d) number of intersections (Ii) of the contours of the structure with the grid lines, e) number of crossings (c) of the nuclei with the grid lines. Knowing the processed pancreatic volume (Vp), the distance between the grid lines (d), the total area examined (A), the total length of the lines of the grid (L) and the thickness of the histological section (t), the following morphometric dimensions were calculated[1]:

1) Volume density: $Vvi = {}^{Pi}/_{P}$,

2) Total volume: $Vti = Vvi.Vp$,

3) Surface density: $Svi = {}^{2Ii}/_{L}$,

4) Total external surface: $Sti = Svi.Vp$,

5) Surface-to-volume ratio: ${}^{S}/_{vi} = {}^{Svi}/_{Vvi} =$,

6) Total cells number: $Ni = \dfrac{2n.Vp}{A[(c/n.)d+2t]}$ and

7) Nuclear volume density of acinar cells:

$$\rho n = \frac{Pn}{Pn + Pcyt}.$$

The nuclear and cytoplasmic volumes of acinar cells

The orthogonal diameters of 30 acinar cells nuclei per animal were measured using a 10 x Ramsden type Olympus micrometer eyepiece and a 100 x oil-immersion objective. Using the mean nuclear radius (r) for each animal, the mean volume was calculated with the formula for the volume of a sphere: $Vni = {}^{4}/_{3}\pi.r^3$. The nuclear volume density (ρn), i.e., the fraction of the cell volume occupied by the nucleus estimated by point-counting method in thick histological sections, provides overestimates because of the Holmes effect [19]. This overestimation was corrected for using the correction factor (Ko) determined by the equation : $Ko = \frac{1-3t}{2D}$, where t = section thickness and D = mean nuclear diameter. Thus, the corrected volume density (ρn_{corr}) and cytoplasmic density (ρcyt_{corr}) was: $\rho n_{corr} = {}^{\rho n}/_{Ko}$ and $\rho cyt_{corr} = 1 - \rho n_{corr}$. Cytoplasmic volume (Vcyt) was calculated using the equation:

$$Vcyt = \frac{Vn.\rho cyt_{corr}}{\rho n_{corr}}.$$

Statistical analysis

The mean and standard error of the mean (SEM) were calculated for each dimension evaluated. All morphometric data for each age group were compared with those of the other groups by analysis of variance (ANOVA) and pairwise multiple comparison procedures (Bonferroni's method) using the SigmaStat-Jadel Scientific software package for Windows, version 1.0. The level of significance was set at $p<0.05$. Volume densities were compared after arcsin transformation of the original data. The data for the pancreatic volume, total acinar volume, total acinar surface, total stromal volume, acinar cell number and acinar cell volume were analyzed using linear regression (according the recommendations of Russ and Dehoff [12]), with the Arcus Professional Statistical Analysis software, version 2.0 XTc. The goodness of fit was assessed using the coefficient of determination (r^2). The growth rate for each dimension under study was calculated using the appropriate equations $Y = a + b X$.

RESULTS

The data for body mass, pancreatic volume and morphometric parameters are shown in Table 1. The evolution (change) of the more significant results was compared in percentages, except the volume density. The equations obtained by linear regression analysis and the corresponding calculated daily growth rate are given in the text.

Between 2 and 140 days of age, the body mass increased by 820% and the pancreatic volume by 805%. The equation for the increase in pancreatic volume during this period was $Y = 250.8 + 6.9 X$ ($r^2 = 0.90$) and the calculated growth rate was 6.9 mm^3/day.

During this growth, the volume density of the acini varied from 73.5% on day 2 to a maximum of 80.4% on day 7 and a minimum of 69.6% on day 140; the volume density of the intercalated ducts plus excretory ducts varied little during the period studied, and remained around 2%. The islets of

Table 1. Data for body mass, pancreatic processed volume and various morphometric parameters obtained in guinea pig pancreas from 2 to 140 days of age. The percentages of the increase was calculated dividing the age data more old by less old.

Age	2	7	14	21	35	70	140
Body mass (g)	88.9±4.0	130.9±5.7*	215.7±13.6	255.5±7.8*	363.5±10.7*	559.9±10.71*	816.5±12.1
Processed volume (mm^3)	123.2±7.2	231.9±12.5	350.7±14.5*	490.9±25.3*	553.4±27.3*	873.4±34.0*	1115.2±40.2
Compartmental volume density (%)							
Acini	73.5±0.6*	80.4±0.9*	74.9±0.5	75.2±1.3*	79.3±0.4	76.9±0.7*	69.6±1.2
Intercalated ducts	0.8±0.1	1.2±0.1	1.5±0.3	1.3±0.2	1.3±0.1	1.2±0.3	1.3±0.1
Excretory ducts	0.9±0.1	0.8±0.1	1.2±0.1	1.4±0.2	1.7±0.1*	2.1±0.2*	1.5±0.2
Islets of Langerhans	1.1±0.1	1.3±0.2	1.1±0.1*	2.8±0.5	2.0±0.4	1.7±0.2*	4.3±0.5
Stroma	23.7±0.6*	16.4±0.8*	21.3±0.7	19.2±1.3	15.7±0.6	18.1±1.9*	23.3±0.9
Compartmental total volume (mm^3)							
Acini	90.6±5.7*	186.5±10.9	262.9±12.3*	368.4±13.9	439.3±23.6*	671.3±24.9*	775.4±25.3
Intercalated ducts	1.0±0.1*	2.8±0.3*	5.4±0.9	6.5±0.8	7.2±0.5	10.2±2.4*	14.7±0.2
Excretory ducts	1.1±0.1*	1.8±0.1*	4.1±0.3*	6.9±1.5	9.2±0.5*	18.4±2.3	17.3±2.5
Islets of Langerhans	1.4±0.1*	2.9±0.4	3.7±0.4*	13.7±2.7	11.5±2.5	15.1±1.9*	47.9±6.4
Stroma	29.1±1.4*	38.0±2.5*	74.6±2.7	95.3±11.0	86.2±1.2*	158.3±19.2*	259.8±15.7
Compartmental surface density (cm^2/cm^3)							
Acini	959.3±27.4	1047.9±48.7	969.2±22.9	834.7±15.1	886.2±9.6	877.2±52.7*	640.5±22.8
Intercalated ducts	16.6±2.6	26.8±1.9	31.9±4.7	23.6±4.2	29.4±3.2	28.2±6.9	32.1±2.4
Excretory ducts	8.7±0.9	7.5±1.6	13.5±1.5	11.9±1.4	12.8±0.4	13.4±0.6	17.6±1.7
Compartmental total surface (cm^2)							
Acini	118.5±9.3*	244.1±22.6	339.7±14.7	410.1±24.3	489.7±19.9*	761.2±24.7	713.4±30.2
Intercalated ducts	2.0±0.3*	6.2±0.6	11.2±1.8	11.4±2.0	16.4±2.3	24.1±5.3	35.7±2.5
Excretory ducts	1.1±0.1	1.8±0.4*	4.7±0.4	5.9±0.9	7.1±0.4*	11.6±0.3*	19.5±1.8
Surface-to-volume ratio (cm^2/cm^3)							
Acini	1305.8±41.0	1302.2±49.9	1293.9±32.7*	1110.5±28.2	1117.3±17.1	1139.9±62.9*	922.0±43.1
Intercalated ducts	1998.7±190.7	2228.7±44.8	2089.6±43.4	1730.3±145.5	2274.6±229.6	2389.1±47.9	2422.4±156.7
Excretory ducts	959.9±77.1	960.4±71.9	1171.7±111.5	898.7±113.3	770.7±39.9	661.9±83.2	1179.4±55.3
Total cell number ($x10^6$)							
Acinar	67.8±4.7*	108.0±7.8*	140.4±4.8*	167.3±5.6	187.6±15.9*	253.7±10.4*	312.8±11.9
Centroacinar	8.1±0.7	10.0±0.7*	12.9±0.9*	17.6±1.5	17.9±1.5	20.9±0.9	17.1±2.0
Intercalated ducts	1.1±0.1*	3.1±0.5*	5.4±0.8	6.5±1.0	7.9±0.9	11.5±2.4	16.6±1.1
Excretory ducts	0.9±0.1	1.2±0.2*	3.0±0.1*	4.6±0.5	6.3±1.0*	9.4±0.8	13.8±2.5
Acinar cell volume (μm^3)							
Nuclear volume	97.1±1.3	98.0±1.7	101.5±0.2*	132.1±3.3	137.3±1.1	142.7±1.5*	153.1±1.4
Cell volume	491.1±7.7*	706.2±14.4*	797.2±10.5*	1091.7±12.7	1224.5±77.9	1303.6±29.7*	1523.5±34.4

All values are the mean ± S.E.M. of 4 animals - * $p < 0.05$ compared to the next highest age interval for each parameter.

Langerhans exhibited two phases of significant increases in volume density, from 14 to 21 days (1.0% to 2.8%) and from 70 to 140 days of age (1.7% to 4.3%). The stromal volume density fluctuated throughout the study, with a maximum value of 23.7% on day 2 and a minimum of 15.7% on day 35.

The compartmental volume of the acini, intercalated ducts, excretory ducts, islets of Langerhans and stroma grew substantially from 2 to 140 days of age, with increases of 756%, 1,372%, 1,591%, 3,393% and 793%, respectively. The linear equations for the growth of the acini and stroma were $Y = 204.1 + 4.7\ X$ ($r^2 = 0.85$) and $Y = 39.7 + 1.6\ X$ ($r^2 = 0.96$), respectively, and the calculated growth rates were 4.7 mm^3/day and 1.6 mm^3/day, respectively.

The surface density of the acini remained stable from day 2 to day 35 and decreased 28% at 140 days of age. No significant differences were observed for the intercalated ducts among the age groups, while the surface density of the excretory ducts increased 80% from day 7 to day 14 and stabilized thereafter.

The total external surface for the acini from day 2 to 70, of the intercalated ducts from day 2 to 140 and of the excretory ducts from day 7 to 140 increased by 542%, 1,667% and 1,002%, respectively. The equation obtained for the growth of the acini was $Y = 178.2 + 8.7\ X$ ($r^2 = 0.96$) and the calculated growth rate was 8.7 cm^2/day.

The surface-to-volume ratio of the acini decreased by 14% and 19%, from day 14 to 21 and from day 70 to 140, respectively. For the intercalated ducts and excretory ducts, there were no significant differences among the age groups.

The total number of acinar cells increased 361% from 2 to 140 days of age, in two phases, between days 2 and 21 and days 35 and 140 with increases of 147% and 67%, respectively. The equations obtained for growth during the first and second phases were $Y=64.7 + 5.1\ X$ ($r^2 = 0.96$) and $Y=158.1 + 1.1\ X$ ($r^2 = 0.94$), respectively, and the rates of cell accumulation were 5.1 x 106 cells/day and 1.1 x 106 cells/day, respectively. The number of centroacinar cells from day 7 to 21, of intercalated duct cells from day 2 to 140, and of excretory duct cells from day 7 to 70 increased by 74%, 1,355% and 673%, respectively.

The mean acinar cell volume increased by 210% from day 2 to 140, in two phases, from day 2 to 21 (122% increase) and from day 21 to 140 (39% increase). The equations obtained for each phase of growth and the corresponding daily increase in cell volume were $Y = 447.7 + 29.4\ X$ ($r^2 = 0.92$) and $Y = 1{,}063.8 + 3.3\ X$ ($r^2 = 0.92$), and 29.4 μm^3 and 3.3 μm^3, respectively.

DISCUSSION

The guinea pig pancreatic volume increased of 820% from day 2 to day 140 of postnatal life, with a mean daily accumulation of 6.9 mm^3. The greatest increase in volume occurred from day 2 to day 21 (around four-fold). Joekel *et al*. [8] observed that the pancreatic mass of guinea pigs almost doubled during the first week of life. In comparison, the pancreatic mass of the hamster, rat and mouse grows 4,360%, 5,928% and 10,246%, respectively, between 2 to 70 days of age [6,14,17]. The significantly greater growth in these animals probably reflets the fact that they were born slightly less developed, i.e., they are all altricial mammals, while the guinea pig is born much more mature, i.e., it is a precocial mammal.

The growth of the guinea pig pancreas occurs through an increase in the absolute volume of all morphological compartments (acini, intercalated ducts, excretory ducts, islets and stroma). The fact that the volume densities of various structures showed little variation with age, indicated that the growth of the various compartments was proportional in order to maintain the relationship among organs seen at the beginning of the study.

The volume of the acini increased 756% from day 2 to day 140 of age, with a mean growth rate of 4.7 mm^3/day. In the hamster and mouse, the total volume of the acini showed a much greater percentage increase in the first 70 days (10,411% and 13,384%, respectively) [6,17]. The total external surface of guinea pig acini increased 542% from 2 days to 70 days of age, whereas in this same period, the increase in total volume was greater (641%). This observation suggested that there was an augmentation in the size of individual acini but not in their number, as confirmed by the decrease in the surface-to-volume ratio.

The observation that the pancreatic volume growth rate was 46.8% greater than that of the compartmental acinar volume, suggested that increases in other structures contributed to the organ volume growth. Analysis of the evolution of compartmental volumes indicated that the principal structure involved was the stroma. The volume of the stromal compartment increased 793% from 2 days to 140 days of age, with a growth rate of 1.6 mm^3/day. From 70 to 140 days, part of this increase involved the appearance of adipose cells in the interlobar and interlobular connective tissue. This accumulation of adipose cells may not be peculiar to the pancreas of adult guinea pigs, since it also occurs in human parotid and submandibular glands [10].

Because of their small total volumes, the intercalated ducts, excretory ducts and islets contributed little to the total organ growth, but, individually, exhibited significative morphometric changes during postnatal development. Thus, from 2 to 140 days of age, the total volume and external surface of intercalated ducts increased 1,372% and 1,667%, respectively. For the excretory ducts, the total volume from 2 to 70 days and the total external surface from 2 to 140 days of age grow 1,591% and 1,757%, respectively. The relative stability of the surface-to-volume ratios suggested that these structures maintained their morphological shape and diameter throughout the study. Since the diameter and shape did not change significantly, the increases in compartmental volume and surface must have occurred exclusively through growth in duct length, i.e. cell proliferation. In this respect, it should be noted that during the period from 2 to 140 days for intercalated ducts and 7 to 70 days for excretory ducts, the number of cells increased by 1,355% and 673%, respectively. Kachar *et al.* [9] reported increases of 1,539% and 1,213% in the number of intercalated duct and excretory duct cells, respectively, in rat pancreas from 2 to 33 days old.

Between 2 and 140 days of age, the compartmental volume of guinea pig islets increased 3,393%, with greatest growth from 14 to 21 days of age. Thus, at the end of third week, islets with larger diameters are already observed. In the mouse at birth, the islets are small and grow during early postnatal life, acquiring an adult pattern after the fifteenth day [4]. In contrast, in hamsters, the islets start to grow during the first 14 days of postnatal life and continue to increase in size until the end of the first year [11].

Clearly, a large part (53.2%) of the increase in the pancreatic volume occurred through growth of the acinar morphological compartment. As noted elsewhere [5], the growth of an organ or its components may involve an increase in the absolute number of cells through proliferation or an increase in the volume of individual cells.

Our results for the guinea pig pancreas show that the acinar cell population grows in two phases, from 2 to 21 days and 35 to 140 days of age, with increases of 147% and 67%, and cell accumulation rates of 5.1 x 106 cells/day and 1.1 x 106 cells/day, respectively. Thus, the proliferation of acinar cells is slightly greater in the first phase.

Subjective morphological analysis showed that the acinar cells increased individually in size during morphological and functional maturation. This increase also occurred in two phases, from 2 to 21 days and 21 to 140 days of age, with increases of 122% and 39% and a mean cell volume gain of 29.4 μm^3/day and 3.3 μm^3/day, respectively.

These results show that, between 2 and 21 days of age, which corresponds to the suckling period of the animals, as well as between 21 to 140 days of age, the proliferative activity and the increase in volume of acinar cells play an important role in the growth of the guinea pig pancreas.

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