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GROWTH OF THE MOUSE PANCREAS DURING POSTNATAL DEVELOPMENT

Gerson Francisco de Assis, Cristina Yuka Hassunuma, Sandra Marcia Hayasaki, Claudia Minami Kussano and Rumio Taga

Laboratory of Histology, Department of Morphology, Faculty of Odontology of Bauru, FOB-USP, Bauru, SP, Brazil.

ABSTRACT

The postnatal growth of the mouse pancreas was studied using stereological methods. The measurements obtained included gland mass, total cell number, the number and frequency of cells in each morphological compartment and the nuclear and cytoplasmic volume of the acinar cells. Pancreatic mass increased significantly (>10,000 fold) in the first 70 days of life and this was accompanied by an increase of 6,841% in the total cell number. The number of acinar, centroacinar, ductal and stromal cells increased by 9,241%, 7,027%, 4,864% and 3,360%, respectively. During the same period, the mean acinar cell volume increased by only 146%. These results showed that growth of the mouse pancreas during postnatal development, occurred through intense proliferative activity of all cell types and by an increase in the size of individual cells, notably the acinar cells.

Key words: Pancreas, acinar cell, centroacinar cell, duct cell, development, mouse, morphometry

INTRODUCTION

The terminal morphological and biochemical differentiation of the acinar cells of the rat and mouse pancreas occurs during prenatal life [11-17]. The molecular biology and genetic control of this development have been studied [2,4,22]. At birth, the exocrine pancreas of these rodents already possesses acini, intercalated ducts and excretory ducts immersed in an abundant stroma of loose connective tissue [3,19]. The acini are still small and immature, even though at this time their cells are already packed with zymogen granules produced during the final days of fetal life. During the first 30-40 days of postnatal development, the rat pancreas undergoes a significant increase in the mass, acinar cell volume, cell number and acinar cell synthetic capacity, and reaches the adult morpho-functional pattern by the end of this period [6,8,19,20].

While the postnatal development of the rat exocrine pancreas has been well studied quantitatively using light and electron microscopy, there have been few studies on the development of the mouse pancreas. In this report we present a detailed morphometric analysis of the mouse pancreas during the first 70 days of postnatal development. The data obtained were used to calculate the growth rate of the mouse pancreas during postnatal development.

Correspondence to: Dr. Rumio Taga
Faculdade de Odontologia de Bauru, USP, Alameda Dr. Octávio Pinherio Brisola 9-75, CEP 17012-901, Bauru, SP, Brasil. Tel: (55) (14) 235-8259, Fax: (55) (14) 223-4679, E-mail: gfassis@fob.usp.br

MATERIAL AND METHODS

Forty-two male Swiss mice 2, 7, 14, 21, 28, 35 and 70 days old were used (6 mice/group). The mice were obtained from the colony maintained by the Central Animal House of the Faculty of Odontology of Bauru. The pancreata were always collected between 8:00 and 10:00 a.m. to avoid circadian variations. After anesthesia by ethyl ether inhalation, the body mass of each animal was determined and the pancreas was carefully removed and immediately weighed. The pancreas was then fixed in Bouin solution for 3 h at room temperature, rinsed overnight in 70% ethanol, dehydrated in ethanol, cleared in xylene and embedded in Paraplast (paraffin plus plastic resin) melted at 58°C. Alternate sections 6 µm thick were obtained at intervals of 60 µm using a Jung-Leica 2045 Multicut microtome and stained by the Masson trichromic method.

Measurement of the processed pancreas volume

The volume of the processed pancreas (Vp) was estimated using the fresh gland mass (m), the gland density (δ) and a correction factor (Sf) for the shrinkage caused by histological processing, based on the formula Vp = (m/δ) x Sf. The gland density (δ) was determined in 70-day-old mice (n=12) by the method of Scherle [18] according to the recommendations of Mandarim de Lacerda [9]. The shrinkage caused by processing was evaluated in another group of 70-day-old mice (n=12) by the method of Taga and Sesso [24][1].

[1] Mean values obtained: δ = 1.12 mg/mm3 and Sf = 0.57.

Evaluation of the absolute cell number (Ni)

The absolute cell number of the pancreas and in each morphological compartment (acini, centroacinar cells, ducts and stroma) was determined by morphometric method II of Aherne [1]. In these counts, the endocrine cells were included in the stromal compartment.

Fifty histological fields per mouse were selected by systematic sampling using an 8X Zeiss Kpl eyepiece containing a Zeiss II integration grid with 10 parallel lines and 100 points within a quadrangular area and a 100x Zeiss oil-immersion objective. For each field the number of cell nuclei (*n*) and the number of intersections (*c*) between nuclear images and grid lines were determined. The nuclei falling on the forbidden line were not counted in order to avoid over estimation [7]. Based on the total area examined (A), the distance between the grid lines (d), the thickness of the section (t), the ratio ($i = c/n$) and the processed pancreatic volume (Vp), the absolute cell number (Ni) was calculated using the formula: $Ni = \frac{2n \cdot Vp}{A(i \cdot d + 2t)}$.

The number of histological fields per mouse (50) was based on pilot experiments in which sample size was assessed using the multiple X^2 sample homogeinity test with a probability of 0.05 [10,26].

Determination of the nuclear volume (Vn) and cytoplasmic volume (Vcyt) of acinar cells

The orthogonal diameters of 50 acinar cell nuclei per mouse were measured using a Ramsden type Olympus 10x micrometer eyepiece and a 100x oil-immersion objective. Using the mean radius (r) of the nuclei for each mouse, the mean nucleus volume was calculated by the formula for the volume of a sphere: $Vn = 4/3\pi \cdot r^3$.

The nuclear volume density (ρn), i.e. the fraction of the cell volume occupied by the nucleus, was evaluated by point-counting volumetry [27] using the same 8X Zeiss Kpl eyepiece with a Zeiss II integration grid and a 100X oil-immersion objective. In 50 histological fields selected by systematic randomization for each mouse, the number of points over the nucleus (Pn) and the cytoplasm (Pcyt) of the acinar cells was scored. The volume density of the nucleus (ρn) was calculated using the relation: $\rho n = \frac{Pn}{Pn + Pcyt}$ (μm°).

The value thus obtained was overestimated because of the Holmes effect [27]. This overestimation was corrected using the correction factor (K_O) determined by the equation $K_O = \frac{1+3t}{2D}$ (μm°), where t = section thickness and D = mean nucleus diameter. Thus, the corrected nucleus volume density (ρn_{corr}) is: $\rho n_{corr} = \frac{\rho n}{K_O}$ and the corrected volume density of the cytoplasm is: $\rho cyt = 1 - \rho n_{corr}$. Knowing the volume density of the cytoplasm (ρcyt), the corrected volume density of the nucleus (ρn_{corr}) and the mean nucleus volume (Vn), the cytoplasmatic volume (Vcyt) was calculated by the equation: $Vcyt = \frac{Vn \cdot \rho cyt}{\rho_{ncorr}}$ (μm³).

Statistical analysis

The cell numbers and nuclear and cytoplasmic volumes of the various age groups were compared by analysis of variance (ANOVA). The comparisons between successive age groups were done by pairwise multiple comparison procedures (Student-Newman-Keuls method) using the Sigma Stat-Jadel™ Scientific Software for Windows and the level of significance was set at 0.05. The use of the terms "increase" or "decrease" in the text denotes that there was a significant difference ($P<0.05$) between sucessive age groups in the period, e. g. an increase in the period from day 2 to day 21 indicates differences between days 2 and 7, 7 and 14 and 14 and 21. On the other hand, the occurrence of no significant increase or stabilization implies that $P>0.05$ between two successive age groups. The total number of cells and the number of each cell type were analyzed by linear and exponential regressions using the same software. The goodness of fit was assessed using the coefficient of determination (r^2).

RESULTS

The changes in body mass, pancreatic mass, total cell number, absolute number and frequency of each cell category, and the nuclear, cytoplasmatic and cell volumes of acinar cells are shown in Figures 1 - 3. The body mass increased by 1,315% and by 44% from day 2 to day 28 and from day 35 to day 70, respectively. There was no significant increase between 28 and 35 days of age. Similarly, the pancreatic mass showed increases of 4,638% and 69% from day 2 to day 28 and from day 35 to day 70, respectively, but no significant increase between 28 and 35 days of age (Fig. 1).

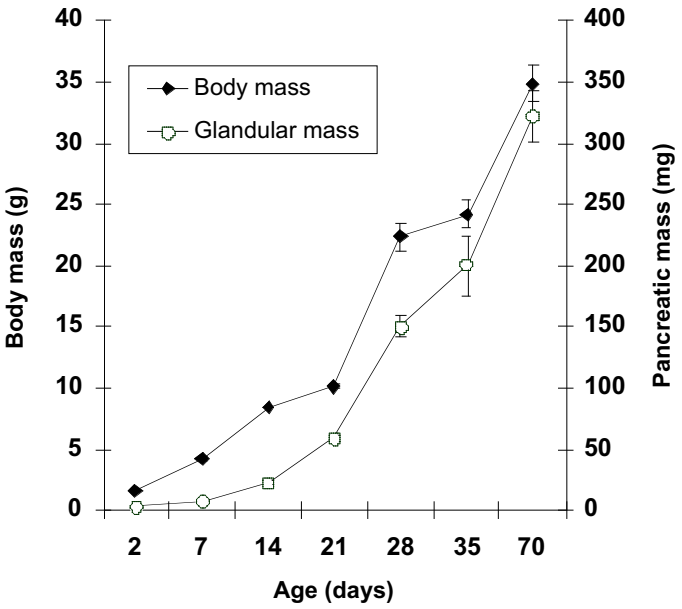

Figure 1. The body and pancreatic mass of mice during postnatal development. The points are the mean ± S.E.M. of 6 mice.

The total number of pancreatic cells also increased by 3,518% and 58% from day 2 to day 28 and from day 35 to day 70, respectively (Fig. 2A). The number of cells in all morphological compartments increased substantially in the same periods. Thus, in the first period the number of acinar, centroacinar, ductal and stromal cells increased by 4,615%, 2,817%, 2,796% and 2,014%, respectively, while in the second period, the increases were 57%, 82%, 59% and 60%, respectively.

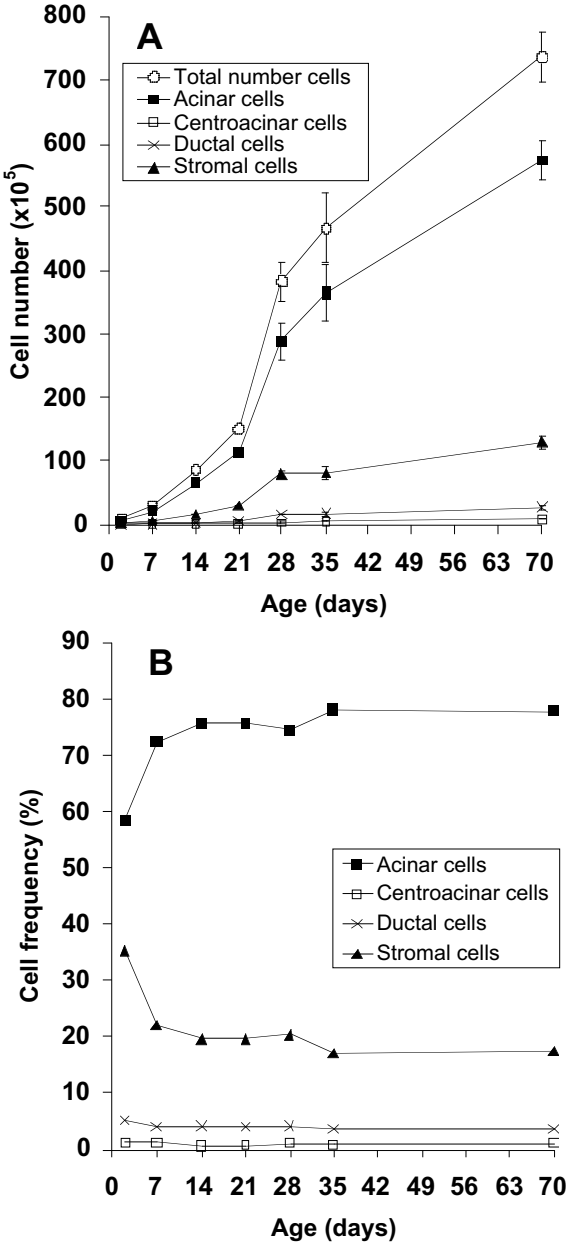

Figure 2. The total pancreatic cell number and absolute number of acinar, centroacinar, ductal and stromal cells (A), as well as the frequency of each pancreatic cell type (B) during postnatal development in mice. The points are the mean ± S.E.M. of 6 mice.

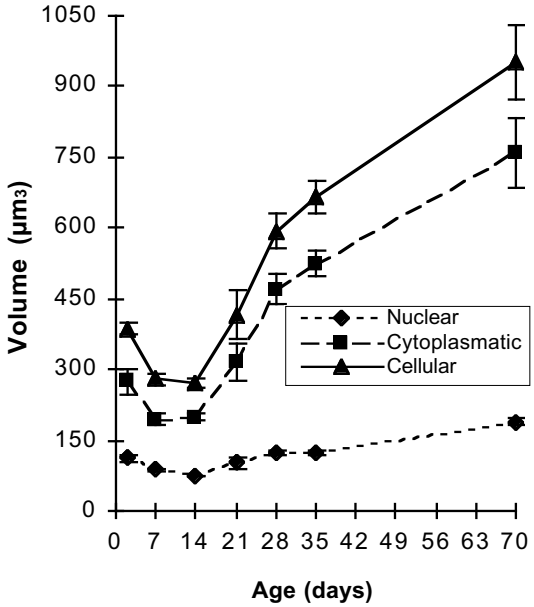

Figure 3. The nuclear, cytoplasmic and cell volumes of mouse pancreatic acinar cells during postnatal development. The points are the mean ± S.E.M. of 6 mice.

The frequency of acinar cells increased from 58.4% on day 2 to 72.5% on day 7. The stromal cells showed a significant decrease in frequency from 35.3% on day 2 to 19.6% on day 14. The centroacinar cells decrease from 1.3% on day 2 to 0.7% on day 14. From day 14 onwards, the frequencies of all cell types remained relatively stable (Fig. 2B).

The mean nucleus volume of acinar cells decreased by 33% from day 2 to day 14, increased by 66% from day 14 to day 28, remained stable from day 28 to day 35 and increased by 53% between 35 and 70 days of age (Fig. 3). On the other hand, the mean acinar cell volume decreased by 27% from day 2 to day 7, stabilized from day 7 to day 14 and increased by 249% between 14 and 70 days of age.

Analysis of the increase in body mass, pancreatic mass, total cell number and cell number in each morphological compartment of the pancreas indicated that there was exponential growth in all these parameters in the period from 2 to 28 days of age. In contrast, from 28 to 70 days, the changes in these parameters were best expressed by linear regression (Table 1).

DISCUSSION

The pancreas of the rat and mouse grows rapidly during the first two months of postnatal life [3,8,19,21,23]. The pancreatic mass of the mice in this

study increased by 10,054% from day 2 to day 70 of postnatal life. This increase occurred in two phases, from day 2 to day 28 and from day 35 to day 70, with a mean growth rate of 5.65 mg/day and 3.47 mg/day, respectively. No significant increase was observed from 28 to 35 days of age.

Table 1. Exponential and linear regression equations for the total cell number and absolute number of acinar, centroacinar, ductal and stromal cells for the periods from 2 to 28 days and 28 to 70 days of postnatal life.

Parameter	Exponential regression 2 to 28 days	Linear regression 28 to 70 days
Total cell number	$y = 100.51 . e^{(0.1328 x)}$	$y = 83.13 x + 1545.02$
	$r^2 = 0.967$	$r^2 = 0.754$
	$F = 819.84 (P < 0.001)$	$F = 37.12 (P < 0.001)$
Acinar cells	$y = 62.95 . e^{(0.1412 x)}$	$y = 65.44 x + 1187.69$
	$r^2 = 0.949$	$r^2 = 0.704$
	$F = 516.43 (P < 0.001)$	$F = 38.05 (P < 0.001)$
Centroacinar cells	$y = 1.09 . e^{(0.1196 x)}$	$y = 1.09 x + 6.29$
	$r^2 = 0.924$	$r^2 = 0.628$
	$F = 334.37 (P < 0.001)$	$F = 26.96 (P < 0.001)$
Ductal cells	$y = 4.69 . e^{(0.1265 x)}$	$y = 2.74 x + 78.87$
	$r^2 = 0.968$	$r^2 = 0.528$
	$F = 840.27 (P < 0.001)$	$F = 17.88 (P < 0.001)$
Stromal cells	$y = 29.39 . e^{(0.1155 x)}$	$y = 12.54 x + 402.94$
	$r^2 = 0.979$	$r^2 = 0.544$
	$F = 1301.37 (P < 0.001)$	$F = 19.12 (P < 0.001)$

An allometric analysis of the increase in pancreas mass relative to body mass, in the first 70 days of postnatal development in mice, revealed a monophasic growth pattern with the coefficient K = 1.56, indicating positive allometric growth, i.e., the unit increase of pancreatic mass was greater than that of the body mass [23].

The ontogenetic growth of an organ may result from an increase in the absolute cell number through mitotic activity or via an increase in cell volume [5,11,25]. Enesco and Leblond [5] studied the participation of these two mechanisms in the growth of the rat pancreas during postnatal development. In the first 17 days, growth occurred through rapid mitotic activity, mainly in the acinar cells, whereas between 17 and 48 days of age, an increase in acinar cell volume predominated. Thereafter, both mechanisms diminished gradually, to stabilize at very low levels. Subsequent studies in rats using autoradiographic [19] and morphometric [6,8] methods have confirmed the involvement of these two mechanisms in the growth of the pancreas.

Dore *et al.* [3] reported a biphasic pattern for the postnatal development of the mouse pancreas. During the first phase, from birth to day 15, proliferation and growth of the endocrine cells occurred, while in the second phase, from day 15 to day 30, there was proliferation and growth of the exocrine cells. However, these authors did not assess the role of cell mitosis or increase in cell volume in the observed growth.

As shown here from day 2 to day 28, when the pancreatic mass increased by 4,638%, the total cell number and absolute number of acinar cells, centroacinar cells, ductal cells and stromal cells increased by 3,518%, 4,615%, 2,817%, 2,796% and 2,014%, respectively. The observation that the percentage increase in pancreatic mass was greater than that of the total cell number indicated that the increase in cell volume also had an important role in this growth.

In the same period, the mean volume of acinar cells, the predominant pancreatic cell type (representing on average 73% of the cells), decreased by 27% from day 2 to day 7 and then increased by 118% from day 7 to day 28. From day 35 to day 70, the pancreatic mass, total cell number and acinar cell volume increased by 61%, 58% and 43%, respectively.

Analysis of the absolute cell number and acinar cell volume indicated that from day 2 to day 28, proliferative activity and an increase in cell volume, contributed significantly to pancreatic growth, with a marked predominance of the first mechanism. Although there was a significant decrease in the activity of both mechanisms from day 35 to day 70, increases in cell size predominated over proliferative activity.

Analysis of the curves for the increase in cell number in each morphological compartment of the pancreas suggested exponential growth for these cell populations from day 2 to day 28. A least squares fit-exponential regression analysis provided an optimum fit of the data to the equations. The high correlation of the data to the equation for each cell type supported the hypothesis of exponential growth. This conclusion agrees with Sesso *et al*. [19] who using continuous

labeling with ^{3}H-thymidine, demonstrated that rat pancreatic acinar cells grow exponentially from birth until postnatal day 20-23.

Using the equations in Table 1, the growth rate was estimated by calculating the doubling time (the time necessary for the number of cells in a compartment to double) using the relationship: TD = ln2/k, where TD = duplication time, ln2 = natural logarithm of 2 and k = exponent of the exponential equation [19]. For a cell population that grows exponentially without cell death, the duplication time (TD) is equal to the generation time (TG) of these cells, i.e., the duration of their cell cycle [21].

The duplication times calculated for the acinar, centroacinar, ductal and stromal cell populations in mouse pancreas from 2 to 28 days of age were 4.9, 5.8, 5.5 and 6.0 days, respectively. For comparison, Kachar *et al.* [8], using the same methodology to study growth of the rat pancreas from 2 to 20 days of age, obtained the duplication times of 4.7, 4.8 and 4.9 days for the acinar, centroacinar and intercalated duct cells, respectively.

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