THE MATURATION OF CONVOLUTED GRANULAR TUBULE CELLS FROM MOUSE SUBMANDIBULAR GLANDS DURING ITS POSTNATAL DEVELOPMENT. INCREASE IN THE CELL SIZE*

A MATURAÇÃO DE CÉLULAS DOS DUCTOS GRANULOSOS DE GLÂNDULAS SUBMANDIBULARES DO CAMUNDONGO DURANTE O DESENVOLVIMENTO PÓS-NATAL. EVOLUÇÃO DO VOLUME CELULAR.

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We studied the evolution of nuclear and cytoplasmic volume and of the cytoplasm-nucleus ratio of convoluted granular tubule secretory cells in the submandibular glands of male albino ***Swiss*** mice at 21, 28, 35, 42 and 56 days of age. Nuclear volume was measured on 0.25 µm sections by morphometric method of Bach and the volume densities of nucleus and cytoplasm were determined by point-count volumetry. The data were used to calculate the cytoplasm/nucleus ratio and cytoplasmic volume. The nuclear volume of convoluted tubule cells decreased by 44.6% over the study period, where as the cytoplasmic volume increased markedly by 126.2% from 21 to 35 days of postnatal life, with no statistically significant increases occurring thereafter. The cytoplasm/nucleus ratio increased by 331.3% over the study period.

Uniterms: Convoluted granular tubule; Submandibular gland; Morphometry.

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INTRODUCTION

The intralobular duct system of the submandibular glands of rodents exhibits a predominat and highly contorted segment called convoluted granular tubule. This ductal segment is formed by high, prismatic, crowded protein-secretin cells[2,6,8,14,15,16,18,19,20].

These secretory cells differentiate and mature during postnatal development[5,7,8,9,10,11,12,13,16,22,23]. Thus, ductal cells of the rat and mouse submandibular glands start to exhibit small apical granules during the 2nd and 3rd week of development. These cells continue to accumulate a growing number of granules, reaching a morphological pattern quite similar to that of adult animals around the

end of the 2nd month of postpartum development[8,9,11,12,16,20,22,23].

Since one of the events characterizing the maturation of convoluted granular tubules is the increase in cell volume, the objective of the present paper was to study the evolution of the nuclear and cytoplasmic volumes of these cells during the period from the 3rd to the 8th week of development.

MATERIAL AND METHODS

Histological procedures

Twenty-five male albino *Swiss* mice were obtained from the Central Animal House of the School of Dentistry of Bauru, University of São Paulo, Brazil at 21, 28, 35, 42 and 56 days of life in groups of 5 animals per age.

Gland were collected between 8:00 and 10:00 a.m. with the animal under ether anesthesia. The right submandibular gland was rapidly dissected, removed and cut into small fragments of ± 1 mm^3. Approximately 30 fragments per animal were routinely processed for histology as follows: the material was fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.3, for 3 hours, quickly washed in sodium phosphate buffer, postfixed in 1% osmium tetroxide in 0.05 M sodium phosphate buffer, pH 7.3, for 2 hours, quickly washed in sodium phosphate buffer, and stained with 0.5% uranyl + 106 mg sucrose/ml solution. All of the above steps were carried out at ± 4ºC. The material was then dehydrated in 70%, 80%, 90%, 95% and 100% ethyl alcohol, washed twice with propylene oxide (20 minutes per wash), placed in propylene oxide + Araldite resin (1:1) for 2 hours, placed in Araldite for 1 hours, and embedded in Araldite in an oven at 60ºC.

After 5-day polymerization in the oven, 10 fragments per animal were piched at random and trimmed and cut with a Porter-Blum MT-1 microtome. The sections (0.25 µm thick) were stained with a mixture of equal parts of methylene and azure II.

Morphometric evaluation of mean nuclear radius and calculation of mean nuclear volume

The mean radius of the convoluted tubule secretory cells for each animal was calculated by the method developed by BACH[3] in 1963 (for details, see ARCON et al.[1], 1980).

A total of 250 nuclear cross-sections selected at random were measured for the sections from each animals using an Olympus 8X Ramsden-type ocular micrometer and a 100x immersion objective. The mean radius of the nuclei was calculated from the frequency distribution of the radii from these cross-sections using the mathematical formulae of BACH[3].

These formulae were programed in PC-386 Microcomputer for rapid calculation.

Nuclear volume was calculated by the formula for the volume of a sphere:

$$VN = 4/3 \cdot \pi \cdot r^3$$

Morphometric evaluation of nuclear and cytoplasmic volume density. Calculation of the cytoplasm/nucleus ratio and of cytoplasmic volume

Nuclear volume density (Vvn) and cytoplasmic density (Vvcyt) of the convoluted tubule secretory cells was estimated by point-count volumetry (WEIBEL, [25]). Counts were performed with a Zeiss Kpl 8x ocular micrometer containing a Zeiss integration II grid with 100 points symmetrically distributed in a quadrangular area and a 100x Zeiss immersion objective. The number of points coinciding with the nuclear and cytoplasmic images of the cells under study were counted in 50 histological fields selected at random.

Nuclear and cytoplasmic volume density was calculated by the following equations:

$Vvn = Pn/Pcel$ and $Vvcyt = Pcyt/Pcel$

where:

Pn = points over the nucleus, Pcyt = points over the cytoplasm, and Pcel = Pn + Pcyt.

The cytoplasm/nucleus ratio (Rc/n) that represents the number of the times that the cytoplasmic volume is larger than nuclear volume, was obtained by dividing cytoplasmic volume density by nuclear volume density:

$Rc/n = Vvcyt/Vvn$

Once nuclear volume (Vn) and the c/n ratio were known, the cytoplasmic volume (Vcyt) was calculated by the following simple multiplication:

$Vcyt = Vn \cdot Rc/n$.

RESULTS

Nuclear volume (Vvn) and cytoplasmic (vvcyt) densities and the cytoplasm/nucleus ratio (Rc/n) are presented in Table 1.

The evolution of the cytoplasm/nucleus ratio with the respective confidence limits is also presented graphically in Figure 1.

Analysis of Table 1 and of Figure 1 shows that the

TABLE 1 - Mean (± SEM) volume density (%) of nucleus and cytoplasm and cytoplasm/nucleus ratio for granular tubule cells of the submandibular glands from groups of 5 mice aged 21, 28, 35, 42 and 56 days of postnatal life

Age	Volume density (%)		Cytoplasm/nucleus
	Nucleus	Cytoplasm	Ratio
21	39.0 ± 0.82	61.0 ± 0.82	1.6 ± 0.06
28	29.7 ± 1.34	70.3 ± 1.34	2.4 ± 0.15
35	19.8 ± 0.74	80.2 ± 0.74	4.1 ± 0.20
42	16.1 ± 0.29	83.9 ± 0.29	5.2 ± 0.11
56	12.7 ± 0.28	87.3 ± 0.28	6.9 ± 0.18

cytoplasm/nucleus ratio of convoluted tubule cells grows linearly between 21 and 56 days of postnatal life, with an increase of 331.3%.

The evolution of nuclear, cytoplasmic and cell volume is presented in Table 2.

The data for the various age groups were compared by analysis of variance. For better visualization of nuclear and cytoplasmic volumes evolutions, the values given in the tables are presented graphically in Figure 2.

Analysis of Table 2 and of Figure 2 shows that the nuclear volume of convoluted tubule cells was sligthly, but not significantly, decreased from 133.5 to 113.9 μm^3 between 21 and 35 days of life, and markedly decreased from 113.9 to 74.0 μm^3 between 35 and 56 days of life.

Cytoplasmic volume increased substantially by 126.2% between 21 and 35 days of postnatal life. After this period, although a visually detectable increase ocurred, the difference was not statistically significant.

Cell volume increased from 341.9 to 585.4 μm^3 between 21 and 35 days of life, and stabilized thereafter.

DISCUSSION

The ductal phase of the postnatal development of the mouse and rat submandibular gland is characterized by the appearance and maturation of the convoluted granular tubule compartment[12,16]. According to JACOBY e LEESON (1959)[12], this phase involves three major processes: 1) ductal cell proliferation, 2) increased cell size, and 3) accumulation of secretory granules in the cells.

In the presence study we made an attempt to characterize the second process by studying the evolution of the nuclear and cytoplasmic volume of these cells. We used semithin sections of material embedded in Araldite resin because of two factors: a) the volumetric modifications caused by processing are negligible when this procedure is used[26] and b) in 0.25 µm sections, the overestimate of nuclear volume density due to the Holmes effect[23] is nonsignificant.

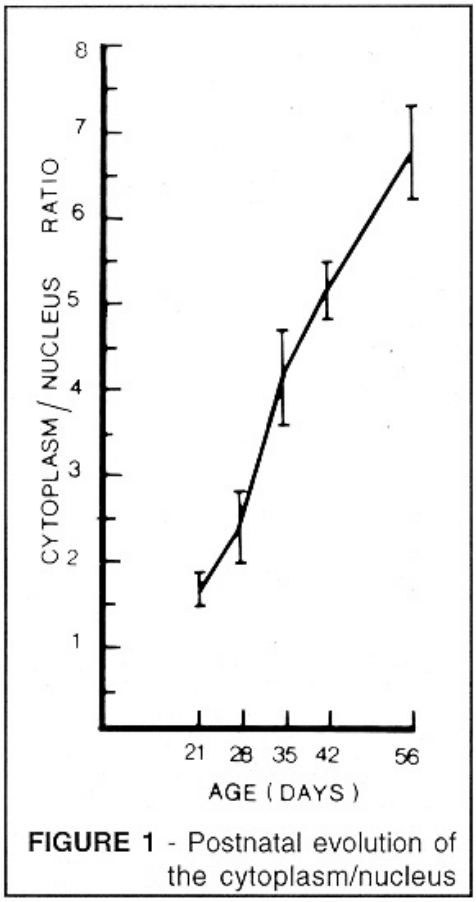

FIGURE 1 - Postnatal evolution of the cytoplasm/nucleus

Nuclear volume decreased linearly from 21 to 56 days of age. In this respect, it should be pointed out that SESSO[21] (1957), in a study of the evolution of nuclear volume in rat exocrine pancreatic secretory cells, detected an analogous decrease in the period from 1 to 121 days of postnatal life.

Cytoplasmic volume grew perceptibly by 126.2% from 21 to 35 days of postnatal life, with no statistically significant increases thereafter. In this respect, GRESIK; Mac RAE[9] (1975) observed at the ultrastructural level that the highest accumulation of secretory granules occurred up to the 35th day of postnatal life. At the biochemical level, these investigators obtained an approximate increase of 300% in salivary amylase levels between 20 and 35 days, with a smaller and more gradual increase thereafter until adult levels were reached by 60 days.

TABLE 2 - Mean (± SEM) nuclear, cytoplasmic and cell volumes (μm^3) of granular tubule cells of the submandibular glands from groups of 5 mice aged 21, 28, 35, 42 and 56 days of postnatal life

Age (days)	Nuclear volume (μm^3)	Citoplasmic Volume (μm^3)	Cell (μm^3)
21	133.5 ± 7.25	208.4 ± 10.55	341.9 ± 16.94
28	125.7 ± 6.41	302.7 ± 26.53	428.4 ± 31.49
35	113.9 ± 5.33	471.5 ± 43.19	585.4 ± 48.38
42	95.1 ± 2.18	496.4 ± 12.56	591.5 ± 14.04
56	74.0 ± 4.99	509.5 ± 36.29	583.5 ± 40.94

Thus, we may infer that, even though cell volume increases little after the 35th day of postnatal life, there is a gradual increase in the amount of secretory granules, probably at the expense of a decrease in nuclear volume and the amount of other intracytoplasmic structures. In support of this inference, it should be pointed out that also renin[17] and the epidermal growth factor[24] accumulate at a higher rate during the first weeks of convoluted granular tubule development, but continue to increase in the cell up to a later age.

RESUMO

Na presente pesquisa estudou-se a evolução dos volumes nuclear e citoplasmático e da relação citoplasma-núcleo de células secretoras dos ductos granulosos de glândulas submandibulares de camundongos *Swiss*, machos e albinos, com 21, 28, 35, 42 e 56 dias de idade. O volume nuclear foi determinado por medidas realizadas em cortes histológicos de 0,25 µm de espessura pelo método morfométrico de Bach e as densidades de volume do núcleo e citoplasma foram avaliadas pela volumetria de contagem de pontos. Esses dados foram utilizados para calcular a relação citoplasma-núcleo e o volume citoplasmático. O volume nuclear das células dos ductos granulosos decresceu 44,6% no período estudado, enquanto que o volume citoplasmático au-mentou marcadamente 126,2% no período de 21 a 35 dias de vida pós-natal, após essa data o crescimen-to ocorrido não foi estatisticamente significante. Por outro lado, a relação citoplasma-núcleo aumentou 331,3% em todo período estudado.

UNITERMS: Ductos Granulosos; Glândula submandibular; Morfometria.

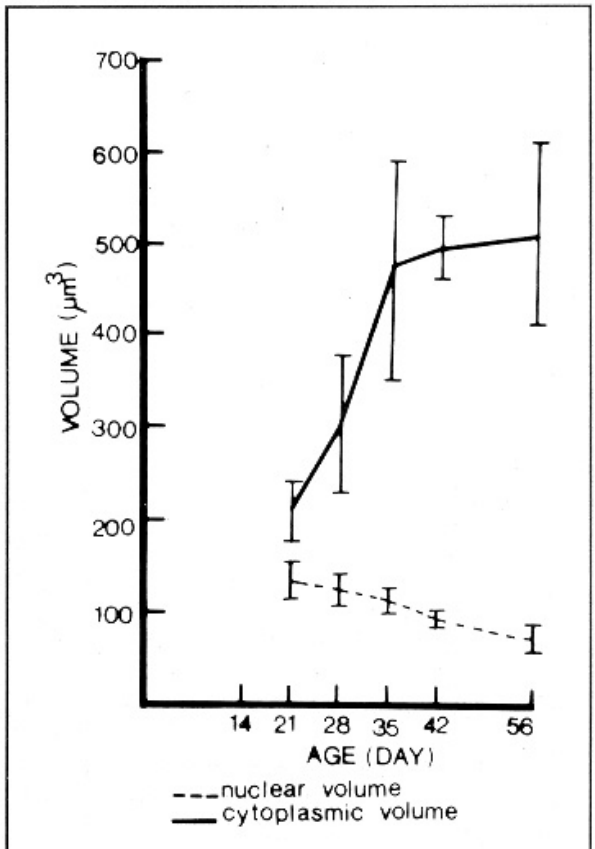

FIGURE 2- Postnatal evolution of the cytoplasmic and cell volumes

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