MORPHOMETRIC EVALUATION OF THE VOLUME OF THE SPHERICAL AND NONSPHERICAL CELL NUCLEI IN SEMITHIN PLASTIC EMBEDDED SECTIONS.

AVALIAÇÃO MORFOMÉTRICA DO VOLUME DE NÚCLEOS ESFÉRICOS E NÃO ESFÉRICOS EM CORTES SEMIFINOS DE TECIDO INCLUÍDO EM PLÁSTICO.

Rumio TAGA *

Eliana Parisi ALVARES **

Antonio SESSO ***

ABSTRACT

The objective of present paper was to describe a method for the evaluation of the volume of nonspherical nuclei in 0.5 um thick araldite embedded sections, and to compare with the methods applied for the determination of volume of spherical nuclei in the same sections. Terminal tubule and acinar cell nuclei of submandibular glands from 15-day old rats, were studied. Radii of approximately spherical terminal tubule cell nuclei, were measured in 0.5 um semithin sections and the mean nuclear radius was estimated using Bach's method. The mean nuclear volume was calculated by the geometric formula for the volume of the sphere. For comparison, the nuclear volume of the same cells, was also evaluated by an indirect method, through estimation of the number of nuclei per unit glandular volume and, the uncorrected and corrected cell nuclear volume densities in the gland. The volume of nonspherical acinar cell nuclei was evaluated solely by the indirect method; the value thus obtained was also corrected.

UNITERMS

Morphometry; Nuclear volume.

---

* Laboratory of Histology, Department of Morphology, Faculty of Dentistry of Bauru, University of São Paulo; CNPq Research Fellow.
** Department of Histology, Biomedical Institute, University of São Paulo.
*** Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine of São Paulo, University of São Paulo.
This research was supported by grant of the FAPESP(Biológicas 75/1282).

INTRODUCTION

The evaluation of the volume of the spherical cell nuclei in semithin (0,5 um of thickness) or ultrathin (70 - 100 nm of thickness) plastic embedded sections of tissues, can be made in practice, by measures of the transection circle diameters of the nuclei in the sections. From the profile size distribution obtained, the mean nuclear diameter can be calculated by various methods (ref. in Weibel[7]). These methods can'not be applied in determination of the volume of the nonspherical nuclei; in this case, the volume of the nuclei may be estimated indirectly.

In the present paper, we described a method for the evaluation of the volume of nonspherical nuclei in 0.5 um plastic embedded sections and compared the results with that obtained in spherical nuclei by, both, the indirect and Bach"s method[3].

The approximately spherical nuclei of terminal tubule cells and nonspherical nuclei of acinar cells of the developing rat submandibular glands, were studied in this work. Terminal tubules are transient secretory structures found at the extremity of the intralobular ducts in submandibular glands of postnatal developing rats. During the first month of postnatal life, these terminal units, are gradually substituted by the sero-mucous acini(ref. in Alvares and Sesso[1]).

MATERIAL AND METHODS

Morphometric studies were realized on submandibular glands of 15day old male *Wistar rats*, obtained from the Central Animal House of the School of Medicine of São Paulo, University of São Paulo, Brazil, in group of four animals. Glands were collected between 8:00 and 10:00 hs A.M., with the animals under ether anesthesia. Fragments of the glands with approximately $1mm^3$, were fixed with 2% glutaraldehyde in 0.09M phosphate buffer (pH 7.2) for 2 hours, followed by immersion and fixation in 1% osmium tetroxide in 0.05M phosphate buffer (pH 7.2) containing 106 mg sucrose/ml, for 2 hours. The fragments were then dehydrated in 70%, 80%, 90%, 95% and 100% ethanol, washed with propylene oxide and embedded in Araldite. After five days of polymerization in the oven, 10 fragments per animal were cut with a Porter Blum Mt-1 microtome. The semithin sections (0.5 um thick) were stained with methylene blue + azur II (1:1).

Morphometric evaluation of the mean volume of spherical nuclei by the method of Bach[3].

The mean radius of approximately spherical nuclei of terminal tubule cells (Fig.1) was evaluated by the method of Bach[3]. Initially a total of 260 nuclear transections were measured from each animal, using an 8x Ramsden-type micrometric eyepiece and a 100X objective. The mean radius was obtained from the frequency distribution of the nuclear radii from the these transections, by the mathematical formulae of Bach[3]

$$Mo = Po.\sigma\,[\sqrt{2}\frac{\pi}{\delta}(r_o - \frac{h}{2}) + f(\sqrt{2}\frac{\pi}{\delta}.(r_o - \frac{h}{2})\,f(0)] + \frac{\sqrt{2}}{\pi}.\frac{1}{\delta}.\sum_{i=0}^{\infty} f(\sqrt{2}\pi\frac{ri}{\delta}).pi$$

$$mo = \frac{Po(r_o - {}^{h}/_{2})^2}{2.h.r_o} = \sum_{i=0}^{\infty}.pi$$

$$ml = \frac{Po(r_o - {}^{h}/_{2})^3}{3.h.r_o} = \sum_{i=0}^{\infty} ri.pi$$

$$R = \frac{M_1}{M_o} = \frac{1}{2}\frac{(mo}{Mo} - \delta^)$$

$$S^2 = \frac{2}{\pi}.\frac{(m_1}{Mo}\frac{-\delta.\bar{R}}{})-R^2$$

where,

ri = values distributed by class interval;

pi = frequency;

r_o = first distribution value;

po = frequency of the first value;

h = class interval;

δ= section thickness.

For rapid calculation, these formulae were programmed in a Hewlett-Packard 9810A electronic calculator (Arcon et al.[2]). Nuclear volume was calculated by the formula for the volume of a sphere: $VN = 4/3\pi . R^3$.

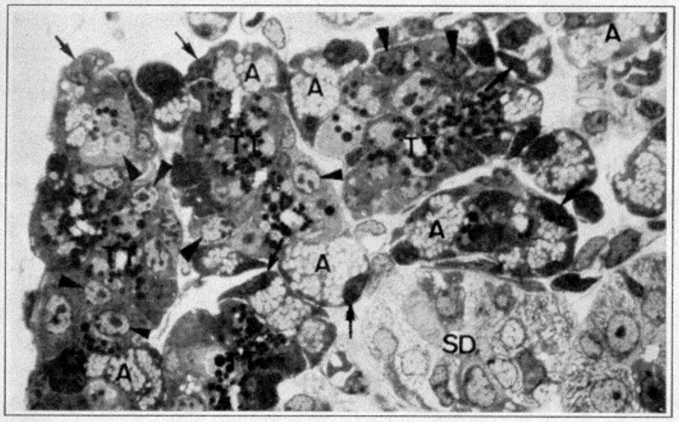

FIG. 1 - Terminal tubule (TT) and acinar (A) cells rat submandibular gland in 0.5 um thick section. 15th day of the post-natal development. Observe approximately spherical nuclei of terminal tubule cells (arrows heads), nonspherical nuclei of acinar cells (arrows) and striated duct (SD). 1,000X.

Morphometric evaluation of the mean volume of nonspherical nuclei and also of spherical nuclei by the indirect method.

The mean volume of the nonspherical nuclei from acinar cells (arrows heads in the Fig. 1) and of the approximately spherical nuclei Prom terminal tubule cells (arrows in the Fig. 1), were estimated indirectly by the number of nuclei of each cell type per unit glandular volume and of the volume density of the nuclei of each cell type in the gland.

In 25 randomly selected microscopic fields per animal, using a Zeiss integration II grid with 100 points simmetrically distributed in a quadrangular area and a 100X immersion objective, the number of nuclear transections within the eyepiece graticle square and the number of points over nuclei (Pi) and over glandular tissues (Pgl), were counted.

The volume density of the counted nuclei in the gland (V_{VNi})for each cell type, was calculated by the following equation: $V_{VNi} = Pi/P_{gl}$ (Weibel[6,7]); and the number of nuclei (N) of each cell type per unit salivary gland volume was estimated using the equation: $N = 1/\beta \ . \ n^{3/2} \ . \ V_{VNi}{}^{1/2}$ (Weibel & Gomez), where, n= number of nuclear transection per unit glandular area and V_{VNi} = volume density of the nuclei in the gland.

An indirect estimate of the mean nuclear volume V_{Ni} is obtained by the relation: $V_{Ni} = V_{VNi} \ . \ VT/N$, where VT is the unit glandular volume.

RESULTS AND DISCUSSION

The mean number of nuclei per cm^3 (N_{TT}) and nuclear volume density (V_{VNTT}) of terminal tubule cells are show in Table 1; with these data a mean nuclear volume of 113.3 ± 10.52 μm^3 was indirectely obtained.

TABLE I - Nuclear volume (Bach's method), number of nuclei, overestimated and corrected nuclear volume density in the gland, and overestimated and corrected nuclear volume evaluated by indirect method, in developing rat submandibular gland.

DIMENSION	15-day old rat submandibular gland	
	terminal tubule cell	Acinar cell
nuclear volume evaluated by Bach's method (in μm^3)	94.0 ± 12.15*	-
Nº of nuclei per cm^3 of gland (x 10^8)	3.94 ± 0.340	3.63 ± 0.304
Nuclear volume density in the gland (in cm^3)	0.044 ± 0.0037	0.033 ± 0.0012
Overestimated nuclear volume (in μm^3)	113.3 ± 10.52	93.8 ± 7,87
Nº of nuclei per cm^3 corrected for Holmes effect (x 10^8)	4.20 ± 0.365	-
Nuclear volume density in the gland corrected for Holmes effect (in μm^3)	0.039 ± 0.0035	-
Corrected nuclear volume (in μm^3)	94.0 ± 10.52	78.5 ± 6.58

* Mean ± standard error of mean

The mean nuclear volume of terminal tubule cells estimated by the Bach's method was 94.0 12.15 um^3 (Table 1).This volume is 17% lower than that evaluated through both the number of nuclei per unit glandular volume and the fraction of this volume occupied by the nuclei. Comparison of the terminal tubule cell nuclear volume estimates obtained by the two above procedures using a paired t-test[5] gave a value 2.45. This result indicates that the two mean values are different at the 9% probability level.

Although terminal tubule cell nuclear profiles are lighter than the azurophilic cytoplasm in semithin sections, some these transections represent small nuclear caps which in 0.5 um thick sections, are enveloped and topped by cytoplasm. In point counting volumetry, this cytoplasm is not scored as such causing over estimations of the nuclear volume density (V_{VNi}) in the gland. These over estimations increase the nuclear volume values indirectly estimated.

In order to estimate the height K of a nuclear cap from a nucleus with radius R, with diameter 2RF in 0.5 um thick araldite section, the following relation was used : $R^2 = RF + (R - K)^2$ (Floderus, 1944, ref. in Eranko[4]). In terminal tubule cells which possess, according to evaluation carried out by Bach's method[3], a mean nuclear radius (R) of 2.86 um, the profiles of radius (RF) less than 1.62 um may be from caps with K less than 0.5 um. The smallest caps measured 1.12 um. Analysis of 1,040 nuclear transection radius values showed that 12.3% of the transections, had radii between 1.62 and 1.12 um. Overestimated nuclear volume density in the gland (V_{VNTT}) may be corrected when the nuclei are spherical, by Holme's factor (Weibel,[6]). Corrected nuclear volume density (V_{VNTTC}) is calculated by $V_{VNTTC} = V_{VNTT}/K_o$, where $K_o = 1 + 3t/2D$, t = section thickness (0.5 um) and D = mean nuclear diameter (evaluated by Bach's method).

Using the corrected nuclear volume density, in the Weibel e Gomez's relation[8], a new cell number estimate was obtained (Table 1). From the latter value and the correct volume density, a new mean nuclear volume of 94.9 ± 10.52 um^3 was obtained which is close to the mean value obtained by the Bach'procedure[3] and 16.3% lower than that evaluated through of the over estimated data by the indirect method.

In view of the previous results it seemed necessary to correct the indirect estimation of the acinar cell nuclear volume for the Holme's effect. We assumed that, as in the terminal tubule cells, the uncorrected acinar cell nuclear volume indirectly estimated would be 16.3% greater than the actual nuclear volume. Introducing this correction, a nuclear volume of 78.5 um^3 was obtained (Table 1).

Running Tittle: Morphometric determination of nuclear volume

O objetivo deste trabalho foi de apresentar um método para a avaliação do volume de núcleos não esféricos em cortes semifinos de 0,5 um de espessura, e de realizar uma comparação com os métodos aplicados à determinação de volume de núcleos esféricos, no mesmo tipo de cortes. Núcleos de células dos túbulos terminais e dos ácinos de glândulas submandibulares de ratos com 15 dias de idade, foram estudados. Ráios de transecções de núcleos aproximadamente esféricos dos túbulos terminais foram mensurados em cortes semifinos de 0,5 um e o ráio nuclear médio foi estimado pelo método de Bach. O volume nuclear médio foi calculado pela fórmula geométrica do volume da esfera. Com o objetivo de comparação, o volume nuclear dessas mesmas células foi também determinado por um método indireto, através da avaliação do número de núcleos por unidade de volume glandular e das densidades de volume não corrigida e corrigida dos núcleos na glândula. O volume médio dos núcleos não esféricos de células acinosas foi avaliado, somente pelo método indireto; o valor assim obtido, também foi posteriormente corrigido.

UNITERMOS

Morfometria; Volume nuclear.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Antonio dos Reis Batista and Mr. Hélio Correa for technical assistance and to Ms Beonildes Teresinha Ruiz Correia for typing the manuscript.

REFERENCES

1- ALVARES, E.P.; SESSO, A. Cell proliferation, differentation and transformation in the rat submandibular gland during early post-natal growth. A quantitative and morphological study. Arch. histol. jap., v.38, n.3, p. 177-208, 1975.

2- ARCON, L.C. et al. Applications of the Gunter Bach methods to estimate the mean radius of spheres after measurements of the radii of circles in histological sections. Ciência e Cultura, *v.32*, 1941 - 1953, 1980.

3- BACH, G. Uber die Bestimmung von charakteristischen Grossem einer kugelverteilung aus der Verteilung der Schnit tkreise. *Z.* wiss. Mikrosk., v.65, p.285-291, 1963.

4- ERANKO, O. Quantitative methods in histology and microscopic histochemistry-karger, Basel, New York, 1955.

5- LISON, L. Statistique appliquée a la biologie expérimentale. Gouthiers- Villars, Paris, 1958.

6- WEIBEL, E.R. - Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol., v.26, p.235-302, 1969.

7- WEIBEL, E.R. Stereological methods. Volume 1: Practical methods for biological morphometry. Academic Press Inc., London, 1979.

8- WEIBEL, E.R.; GOMEZ, D.M. A principle for counting tissue structures on random sections. J. Appl. Physiol., v.17, p.343-348, 1962.

Correspondence to:

Dr. Rumio Taga

Dep. Morfologia, Fac. Odont. Bauru - USP

Bauru, CEP 17043-101 - C. P. 73, SP, Brasil.