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DETERMINATION OF THE ABSOLUTE VOLUME OF DIFFERENTIATING CELLS IN THE EPITHELIUM OF THE HUMAN HARD PALATE

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The morphological examination of the normal epithelium of the human hard palate has suggested that a cell passing from the stratum basale to the stratum corneum increases in size. A knowledge of the absolute volume of single cells in the different strata is important for understanding the epithelial differentiation. Epithelial cell volume determination is difficult, because the cell shape changes drastically from the stratum basale to the superficial layer.

Recently, Loud et al. (1978) developed a stereological method to estimate the absolute cell volume of cardiac myocytes using heart tissue sections. The advantage of this method is its independence from cell shape and orientation. The aim of the present study was to test the applicability of this method for stratified oral epithelia and to estimate the absolute cell volume of differentiating cells in the normal epithelium of the human hard palate.

The tissue blocks originated from a previous stereological study of the normal epithelium of the human hard palate (Meyer and Schroeder, 1975). Ten biopsies, each represented by two tissue blocks were studied. From each block five sections were cut at microtome settings of 1, 2, 3, 4, and 5 µm, respectively. Differential staining of all nuclei within a section was achieved by epon extraction and subsequent staining with celestine blue (Snodgress et al., 1972). The 100 sections were photographed using 35 mm film. The depth of the microscope image-sharpness was adjusted to be about 5 µm. Positive contact copies were evaluated on a

projection surface at a final magnification of x1230.

The sampling sites remained the same as in Meyer and Schroeder (1975), except for the stratum basale from which only ridge regions were used. In the upper stratum spinosum and the stratum granulosum, the sample size was increased by pooling data for ridges and regions over connective tissue papillae. The evaluated areas were determined by point counting methods.

The formula of Loud et al. (1978) was used in the following reduced form

$$t = \overline{V}(c) N(n) - F$$

where t is the section thickness,  $\overline{V}(c)$  the average cell volume per nucleus, and N(n) the number of nuclei per epithelial area and F a constant.

The section thickness measurements indicated that the average thickness exceeded the corresponding microtome setting by 0.11  $\mu m$  with an average for the 5 standard deviations of 0.27  $\mu m$ .

For all three strata a linear correlation was observed between the number of nuclear profiles per area and the section thickness. The correlation coefficients ranged from 0.95 to 0.99. The estimated average cell volumes and the standard deviations were 930+150, 4,110+1,620, and 4,390+550  $\mu\text{m}^3$  for the stratum basale, the upper stratum spinosum, and the stratum granulosum, respectively.

It is concluded that (1) rather accurate volume estimation of epithelial cells is possible, (2) histologically different regions can be evaluated separately, e.g. epithelial strata, and (3) applying cell volume data to existing stereological data would allow calculation of all parameters per cell.

This study has already been published in full length: Müller-Glauser W. Absolute volume of differentiating cells in the epithelium of the human hard palate. Cell Tiss Res 1981; 221: 147-156.

## REFERENCES

Loud AV, Anversa P, Giacomelli F, Wiener J. Absolute morphometric study of myocardial hypertrophy in experimental hypertension. I. Determination of myocyte size. Lab

Invest 1978; 38: 586-596

Meyer M, Schroeder HE. A quantitative electron microscopic analysis of the keratinizing epithelium of normal human hard palate. Cell Tiss Res 1975; 158: 177-203

Snodgress AB, Dorsey CH, Bailey GWH, Dickson LG. Conventional histologic staining methods with Epon-embedded, osmicated tissue. Lab Invest 1972; 26: 329-337