

# INVESTIGATION OF THE CAPILLARIES OF THE RAT PAROTID GLAND DURING A 3-h SECRETORY CYCLE

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Principles governing changes in diameter of the lumen and area of the endothelium of capillaries in the parotid salivary gland of rats with an experimentally established 3-h secretory cycle, and also during circulatory ischemia of the organ for 5 min, were studied. The diameter of the lumen and area of the endothelium of the capillaries changed very little with the phase of the secretory cycle. Occlusion of the common carotid artery for 5 min likewise did not change the diameter of the lumen or the area of the endothelium of the capillaries. The results suggest that the increase in the transcapillary blood flow during secretion can be explained by the recruiting of more capillaries into the circulation.

**KEY WORDS:** Parotid salivary gland; capillaries – diameter of lumen; area of endothelium; occlusion of carotid artery; secretory cycle.

The process of formation and discharge of the secretion in the parotid salivary gland is closely bound up with the characteristics of the transorgan blood flow. These characteristics are peculiar in that during the secretory cycle there is a redistribution of blood between the channels of the shunting and nutritive blood flow. The facts show that despite an abundance of juxtacapillary communications [5, 7, 9, 11], the transcapillary blood flow is considerably intensified during periods of secretion [6, 8]. Since an increase in the transorgan blood flow through the capillaries can be produced in various ways (by an increase in the diameter of the lumen of the capillaries, recruiting of a previously nonfunctioning "closed" capillary into the circulation, and also a change in the pressure gradient in the microcirculatory system), comparisons of data obtained by physiologists for the level of the transorgan blood flow in the gland during liberation of secretion with the morphological characteristics of the capillaries can shed light on the concrete mechanism of the increase in transcapillary blood flow in the parotid salivary gland.

The object of this investigation was to study changes in the diameter of the lumen and area of the endothelium of capillaries of the parotid gland in rats with an experimentally established 3-h food cycle.

## EXPERIMENTAL METHOD

A 3-h food cycle was established in male rats weighing 140–150 g in accordance with Brodskii's recommendation [2]. Material was taken before feeding (0), immediately after feeding (A), and 10, 20, 40, 60, and 120 min after feeding. Four animals were investigated in each phase of the secretory cycle. To produce circulatory ischemia of the organ, a ligature was applied to the common carotid artery of two rats under pentobarbital anesthesia, the ligature was tied and material taken from the ischemic gland 5 min later. The experimental material was fixed with 2% glutaraldehyde solution and subsequently treated by the usual methods of electron microscopy, then embedded in Araldite. Ultrathin sections were examined and photographed in the Hitachi 11-2 electron microscope with an accelerating voltage of 75 kV. Quantitative analysis of the results was carried out in accordance with the basic principles and methods of stereology [1, 3].

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TABLE 1. Diameter of Lumen and Area of Endothelium of Capillaries in Parotid Gland of Rats at Various Phases of the Secretory Cycle ( $M \pm m$ )

Times after feeding (in min)	Capillary divided at level of marginal zone of endothelial cells		Capillaries divided at level of nuclei of endothelial cells	
	diameter of lumen	area of endothelium	diameter of lumen	area of endothelium
0	$3,86 \pm 0,36$	$5,23 \pm 0,55$	$4,2 \pm 0,3$	$15,22 \pm 1,47$
A	$4,61 \pm 0,22$	$6,6 \pm 0,54$	$3,92 \pm 0,4$	$15,68 \pm 1,84$
10	$3,95 \pm 0,4$	$5,7 \pm 0,68$	$3,96 \pm 0,51$	$17,05 \pm 1,55$
20	$3,88 \pm 0,15$	$5,07 \pm 0,4$	$3,44 \pm 0,2$	$13,11 \pm 1,23$
40	$3,63 \pm 0,39$	$6,64 \pm 0,39$	$3,99 \pm 0,57$	$14,2 \pm 1,54$
60	$3,82 \pm 0,2$	$6,71 \pm 0,64$	$4,27 \pm 0,4$	$13,54 \pm 1,01$
120	$3,99 \pm 0,28$	$6,1 \pm 0,63$	$3,91 \pm 0,41$	$15,9 \pm 1,9$

TABLE 2. Diameter of Lumen and Area of Endothelium of Capillaries in Parotid Gland during 3-h Secretory Cycle and after Circulatory Ischemia of the Gland for 5 min

Index	3-h food cycle		Circulatory ischemia		Student's t-criterion	P
	number of observations	$M \pm m$	number of observations	$M \pm m$		
Diameter of lumen	300	$3,98 \pm 0,08$	30	$3,61 \pm 0,22$	1,58	$> 0,05$
Area of endothelium of anuclear fragments of capillaries	181	$5,98 \pm 0,22$	20	$6,2 \pm 0,83$	0,06	$> 0,05$
Area of endothelium of nuclear fragments of capillaries	119	$14,99 \pm 0,56$	10	$13,46 \pm 1,87$	0,6	$> 0,05$

**Note.** The absence of a statistically significant difference between the diameters of the blood capillaries divided at the level of nuclei of the endothelial cells and of capillaries divided at the level of the marginal zones of the endothelial cells makes it possible for them to be pooled for comparative analysis. The relative invariance of the parameters studied at the different phases of the secretory cycle meant that for comparative analysis it was possible to use the mean value of these parameters calculated after pooling of all measurements irrespective of the phase of the secretory cycle.

All measurements were made on a morphometric apparatus, using a square grid with a step of  $1 \text{ cm}^2$ . The area of the lumen of the capillary and the area occupied by endothelium were measured on survey photographs of sections through the capillaries. The diameter of the capillary lumen was calculated from the corresponding area by the equation

$$D_{\text{lumen}} = 2 \sqrt{\frac{S_{\text{lumen}}}{\pi}}$$

Since the planimetric characteristics of capillaries divided at the level of the nucleus-containing zone of the endothelial cells and divided at the level of the marginal zone of the endothelial cells differ considerably, the corresponding quantitative data were analyzed separately.

## EXPERIMENTAL RESULTS

The results (Table 1) suggest that the diameter of the capillary lumen is only very slightly dependent on the presence or absence of a nucleus in the section, although the area of parts of the endothelium containing nuclei is much greater than the area of anuclear factors of the endothelium. The mean values of the diameter of the nucleus and area of the endothelium varied almost independently of the phases of the secretory cycle. The variability of these parameters also remained unchanged and the coefficient of variation in most cases did not exceed 50%.

It is difficult to harmonize these results with observations of physiologists indicating a tenfold increase in the transcapillary blood flow during liberation of the secretion [8]. Since no definite changes could be found in the diameter of the capillary lumen in the present experiments in the various phases of the secretory cycle of the salivary gland, the increase in the nutritive blood flow can be explained on the assumption that the area of the capillary lumen is relatively independent of the intensity of the capillary hemodynamics and is determined by other factors [4, 10]. Hence, it follows that the diameter of the lumen of capillaries perfused with whole blood ought not to change when the blood flow through them stops.

To test this hypothesis experiments were carried out in which the circulation in the parotid gland was completely blocked for 5 min. Since the blood flow through the capillaries was completely stopped, they could be regarded as nonfunctioning ("closed"). Because of the experimental conditions the development of intracellular edema in the endothelium of the "closed" capillaries, one of the first signs of ischemic cell damage, should have been expected. However, analysis of the experimental material showed no ischemic intracellular edema manifested as translucency of the matrix of the cytoplasm. This was conclusively confirmed by the absence of statistically significant differences between the area of the endothelium and the diameter of "closed" and functioning capillaries in the parotid gland (Table 2). Consequently, occlusion of the common carotid artery for 5 min does not cause ischemic damage of the endothelial cells and enables the effect of a change in the level of the hemodynamics on the parameters to be analyzed. There are also grounds for considering that the diameter of the capillary lumen is independent of the intensity of the blood flow in them. The increase in the transcapillary blood flow during the period of discharge of the secretion by the parotid salivary gland cells can therefore be explained by the recruiting of a larger number of capillaries into the circulation.

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