

# Morphofunctional Characteristics of Rat Ovarian Capillaries during Irradiation with Low-Intensity Laser

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Continuous irradiation with He-Ne laser light changes the structure of ovarian capillary bed in rats. These changes depend on the duration of exposure and class of follicles. Three stages in capillary response have been distinguished: 0.5-5 min (rapid increase in alkaline phosphatase activity in the capillary wall and increase in the specific compactness and diameter of capillaries); 5-30 min (relative stabilization of metric values); after 30 min (stable decrease in the activity of the enzyme and specific compactness of capillaries and increase in their diameter). Perifollicular capillary plexuses of 67-80- $\mu$ m follicles show the highest sensitivity to laser light.

**Key Words:** ovarian capillaries; alkaline phosphatase; He-Ne laser

High sensitivity of capillaries to photoexposure was noted at the beginning of this century [6]. However, the data on their reaction to electromagnetic radiation of optical range, including laser radiation, are scarce and contradictory [1,2,5]. The ovaries, possessing a well-developed plastic system of microvessels, are studied least of all.

We investigated the reaction of ovarian capillaries to low-intensity laser radiation of different duration.

## MATERIALS AND METHODS

The study was carried out on 56 adult outbred albino rats weighing 200-250 g. The animals were in the diestrus phase. They were irradiated with an LNG-105 He-Ne laser operating in the permanent mode ( $\lambda=632.7$  nm, 2  $\mu$ Wt, 0.76 Wt/cm<sup>2</sup>). The animals were irradiated under ether narcosis. Cutaneous bioactive points 0-1 XI involved in the regulation of ovarian function [4] were irradiated for 0.5, 1, 5, 15, 30, 45, and 60 min. The animals were decapitated immediately after the exposure (at least

6 animals per term). Intact controls were decapitated under ether narcosis at the same periods. The ovaries were removed, frozen, and 20- $\mu$ m cryostat sections were prepared. Capillaries were detected by a previously described method [3] for alkaline phosphatase (AP 3.1.3.1).

The enzyme activity was assayed in a Vickers-M50 scanning monochromatic densitometer. The diameter of follicles and capillaries was measured with an MOV-1-15 ocular micrometer. The total length of capillaries ( $L$ ) per unit of follicular area (specific density) was calculated. Capillaries located no further than 25  $\mu$ m from structural elements of the organ are directly involved in their blood supply [1,6]; and therefore, only the microvessels whose segments were situated in this zone projection were counted. The total length of capillaries was calculated from the following a formula:  $L=N/S_{25}$ , where  $N$  is the number of capillaries in the examined zone and  $S_{25}$  is the area of examined zone, calculated as  $3.14(25 \times DF + 625)$ , where  $DF$  is the follicle diameter.

Capillary bed of three classes of follicles was studied: I) 67-80  $\mu$ m in diameter (primary, not compact), II) 120-168  $\mu$ m (secondary, cavity), and

III 240-560  $\mu\text{m}$  (mature ready to ovulation). Each parameter was calculated for at least 6 consecutive ovarian sections and individually for each animal.

The data were analyzed using Student's *t* test.

## RESULTS

In the controls, the activity of alkaline phosphatase in histochemical reaction was assessed in the walls of follicles, small arteries, and capillaries (Fig. 1, *a*). Blood vessels with diameter 8  $\mu\text{m}$  were considered to be capillaries. Photometry revealed the differences in the enzyme activity in capillaries of different classes of follicles (Table 1). This parameter was the highest in capillaries delivering blood to cavity follicles. In the second and third class follicles the capillary diameters are more ( $p < 0.01$ ). The density of capillaries per area of follicles is the highest in class I follicles and the lowest in class III ones.

Stages in the transformation of perifollicular capillary network have been distinguished. The studied parameters rapidly increased between the 30th sec and 5th min of exposure (Table 1). The majority of parameters characterizing the function of capillary bed significantly increased during the first 5 min of laser irradiation. The increment in the enzyme activity and specific density of microvessels was 126-134%.

During the period of 5-30 min the changes were less pronounced in comparison with the controls. The diameter of capillaries slightly increased and the enzyme activity and specific compactness of capillaries stabilized. Capillaries were concentrated around follicles, forming vast vessel-free zones in the ovary (Fig. 1, *b*). Twisted microvessels with numerous branches and very high or very low activity of alkaline phosphatase appeared.

Prolongation of exposure over 30 min caused a stable dilatation of capillaries, while other parameters decreased to 70-80% of the control ( $p < 0.01$ ).

Another important feature of the ovarian capillary reaction to laser was the relationship between the degree of changes and class of follicles (Table 1). Capillary network of class I follicles was the most sensitive to laser. Excepting the diameter of capillaries, significant changes in the metric parameters were observed as early as during the first minute of irradiation. Changes in the parameters characterizing capillary status of class II and, even more so, class III follicles in response to laser light became significant during 5-15 min of exposure, being 115-125% of the initial value. The maximum deviations in the metric values of type I capillaries were 134-142%.

Thus, there are two important regularities in the reaction of ovarian capillary bed to He-Ne laser: 1)

Table 1. Changes in Morphometric Parameters of Ovarian Capillary Bed in Rats Exposed to Laser (M $\pm$ m)

Parameters	Follicle class	Control	Laser exposure, min						
			0.5	1	5	15	30	45	60
Enzyme activity, arb. units	I	21.0±1.05	23.3±1.12	24.61±1.05*	28.14±1.41*	28.9±1.54*	28.0±1.12*	27.5±0.96*	14.7±0.32*
	II	32.01±1.16	32.96±1.64	34.25±1.81	37.76±1.74*	38.9±2.1*	38.6±1.1*	37.9±2.0*	28±09*
	III	36.5±1.58	37.7±1.84	38.01±1.94	42.47±1.9*	45.05±2.0*	46.21±2.0*	44.2±1.5*	30±1.5*
Diameter of capillaries, μm	I	5.48±0.16	5.53±0.11	5.64±0.14	6.23±0.04*	6.14±0.03*	6.02±0.03*	6.07±0.02*	6.13±0.02*
	II	5.92±0.15	5.98±0.05	6.04±0.05	5.86±0.06*	6.51±0.02*	6.63±0.1*	6.99±0.14*	7.16±0.12*
	III	6.62±0.12	6.48±0.12	6.69±0.1	7.09±0.04*	7.11±0.12*	7.41±0.09*	7.52±0.16*	7.86±0.14*
Summary length of capillaries per unit of follicular area (specific density), mm×10 <sup>3</sup>	I	3.68±0.19	4.12±0.2	4.38±0.22*	4.64±0.25*	5.19±0.26*	5.09±0.22*	4.95±0.24*	2.96±0.12*
	II	3.29±0.17	3.39±0.17	3.42±0.19	3.52±0.19*	4.01±0.3*	4.18±0.2*	4.22±0.19*	3.09±0.14*
	III	2.52±0.12	2.59±0.11	2.61±0.13	2.72±0.14	3.24±0.15*	3.18±0.16*	3.02±0.16*	2.12±0.11*

Note. \* $p < 0.01$  vs. the control.

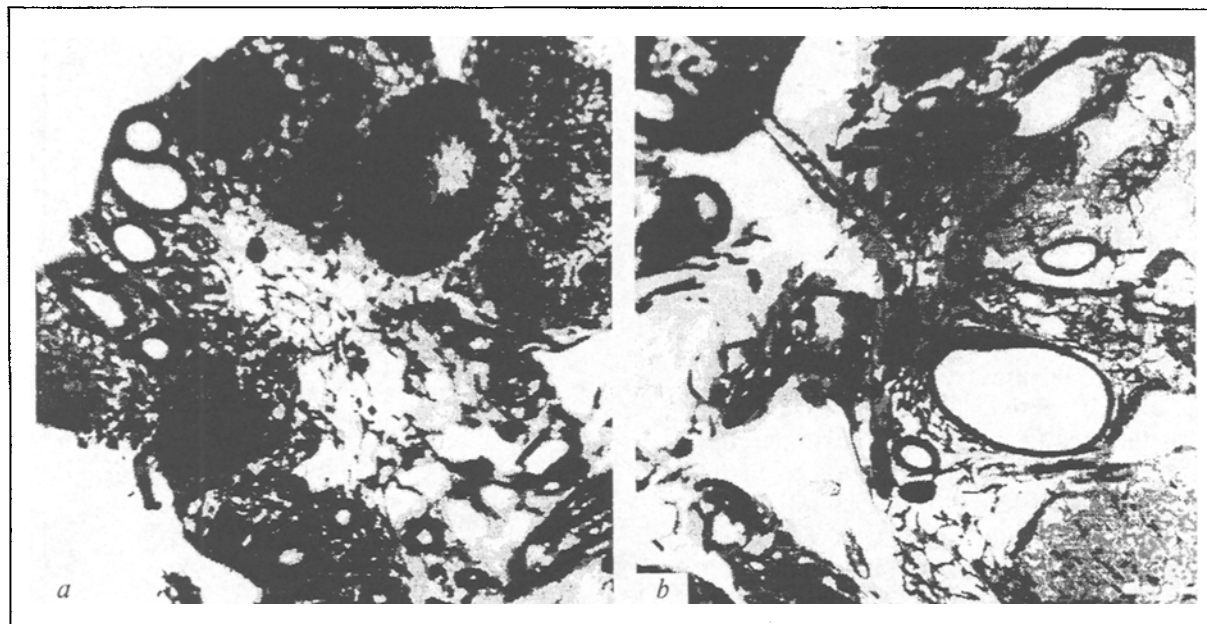


Fig. 1. Ovarian capillary bed in intact rats (a) and rats irradiated with laser light for 5 min (b). Gomori staining,  $\times 140$ .

staged transformation of capillary network and 2) relationship between the degree of changes in perifollicular plexuses and in the size (class) of follicles.

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