

LITERATURE CITED

1. J. H. Ashe and B. Libet, *Brain Res.*, **217**, 93 (1981).
2. A. Biegon, R. Duvdevali, V. Greenberger, and M. Segal, *J. Neurochem.*, **51**, 1381 (1988).
3. J. M. East and J. R. Dutton, *J. Neurochem.*, **34**, 657 (1980).
4. R. Q. Evans, M. Watson, H. I. Jamamura, and W. R. Roeske, *J. Pharmacol. Exp. Ther.*, **235**, 612 (1985).
5. E.P. Fiedler, if J. Marks, and A.C. Collins, *J. Neurochem.*, **49**, 983 (1987).
6. H. I. Jamamura, M. Watson, J. K. Wamsley, et al., *Life Sci.*, **35**, 753 (1984).
7. L. M. Koval, V. A. Derkach, A. A. Selyanko, et al., *J. Auton Nerv. Syst.*, **6**, 37 (1982).
8. J. O. Rinne, *Brain Res.*, **404**, 162 (1987).

EFFECT OF DIRECT EXPOSURE TO LOW-INTENSITY LASER RADIATION ON MORPHOLOGY AND FUNCTION OF THE ZONA FASCICULATA OF THE ALBINO RAT ADRENAL CORTEX

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The action of a laser on biological tissue [2], and of skin wound surfaces [3] is known to promote healing of the damage tissue by intensifying repair processes. It has also been shown that the microcirculation in organs is increased under the influence of low-intensity laser irradiation [4]. It has been shown experimentally that with an increase in the rate of flow of the Ringer's solution used to perfuse the vascular system of the adrenal gland the function of its cortex is enhanced, and this is accompanied by corresponding morphological changes and by the release of corticosteroid hormones into the blood stream [7, 8]. These results served as the basis for the suggestion that local application of laser radiation to the adrenals would stimulate the microcirculation in them and would also bring about corresponding morphological and functional changes in the adrenal cortex.

The aim of this investigation was to study structural and functional changes in the zona fasciculata of the adrenal cortex in response to the direct action of low-intensity laser radiation on them.

EXPERIMENTAL METHOD

The zona fasciculata of the adrenal cortex of 30 noninbred female albino rats weighing about 200 g was studied. In the experiments of series I, under hexobarbital anesthesia, laparotomy was performed on 20 of the animals and the region of the left adrenal gland was subjected to superficial irradiation from a helium-neon laser (LG-75) for 30 min (total dose 11 J/cm², λ = 363 nm). The right adrenal was left intact and served as the control. In series II, 10 rats were anesthetized and subjected to direct irradiation of one adrenal gland from the dorsal aspect by means of an "Uzor" gallium arsenide laser for 8 min 32 sec (total dose $3.5 \cdot 10^{-2}$ J/cm², frequency 80 Hz, λ = 890 nm). The adrenals were fixed in a mixture of chromium salts and formalin. After fixation the gland was divided into two equal parts. One half was used to prepare frozen sections, 30 μ m thick, in which the degree of congestion of the capillaries was determined by the chromium method, and frozen sections 20 μ m thick, in which

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TABLE 1. Morphometric Parameters of Zona Fasciculata of Adrenal Cortex of Albino Rats during Direct Low-Intensity Laser Irradiation ($\bar{x} \pm S_x$)

Parameters	Control	Irradiation by helium-neon laser	Irradiation by gallium arsenide laser
Relative area of congested capillaries, in per cent <i>p</i>	10,6±1,4	14,9±1,6 <0,05	16,2±2,2 <0,05
Content of unsaturated lipids (coefficient of absorbance), % <i>p</i>	45,6±2,3	37,8±2,1 <0,02	42,5±3,6 >0,05
Volume of nuclei, μ^3 <i>p</i>	57,0±1,7	75,1±2,1 <0,001	72,9±1,4 <0,001
Volume of cells, μ^3 <i>p</i>	1555,9±60,7	1145,9±32,4 <0,001	1210,7±46,2 <0,001
Nucleo-cytoplasmic ratios, % <i>p</i>	4,2±0,2	7,4±0,3 <0,001	6,6±0,3 <0,001

Legend. *p*) significance of differences from values in control.

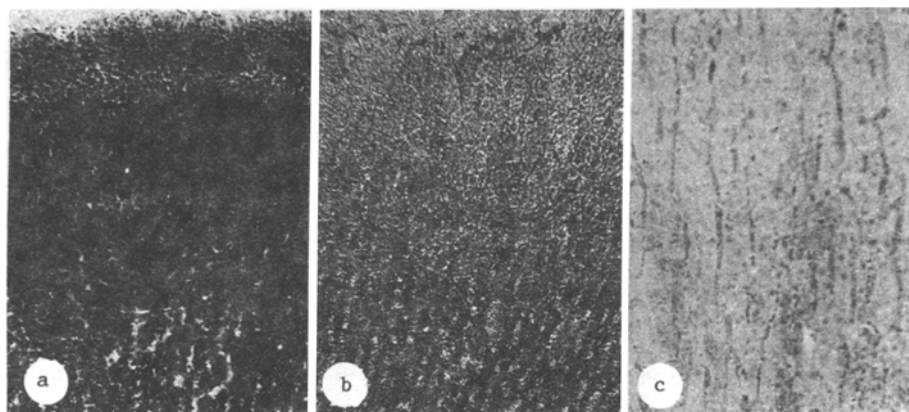


Fig. 1. Content of unsaturated lipids and congestion of capillaries in zona fasciculata of adrenal cortex of albino rats. a) control, b) congestion of capillaries and partial delipidization of outer layers of zona fasciculata, c) congestion of capillaries and decrease in lipid content in surface and deep layers of zona fasciculata. V. V. Yaglov's method. 80×.

the lipid content was determined [6]. The other half of the adrenal was embedded in paraffin wax, sections were cut to a thickness of 5 μm , and these were stained with hematoxylin and eosin for karyometry and cytometry. The diameter of the nuclei and the length and width of the cells were measured by means of an MOV-1-15x ocular micrometer. The volume of the cells was calculated by the formula $V = V^2 \cdot \beta$, the volume of spherical nuclei by the formula $V = D^3 \cdot 0.52$, and of ellipsoidal nuclei by the formula $V = (L + \beta)^3 \cdot 0.07$ [5], where V denotes the volume of the nucleus or cell, D the diameter of the nucleus, and β the short diameter of the ellipsoidal nucleus or width of the cell, and L the long diameter of the nucleus or length of the cell. The nucleocytoplasmic ratios were expressed as percentages, i.e., the volume of the nucleus was divided by the volume of the cytoplasm and the results multiplied by 100. Changes in the content of unsaturated lipids in the adrenal cortex were judged by the value of the coefficient of absorbance, determined by a cytospectrofluorometric method and expressed in percent. The relative area of the blood capillaries was determined in percent as the ratio to the area of parenchyma of the adrenal cortex, by means of a stereometric grid [1]. The results were subjected to statistical analysis on an "Elektronika BZ-34" microcalculator, using known programs.

EXPERIMENTAL RESULTS

The experiments showed that during irradiation of the adrenals by a helium—neon laser congestion of the capillaries of the outer part of the zona fasciculata was increased from 10.6 ± 1.4 to $14.9 \pm 1.6\%$ (Table 1; Fig. 1b). At the same time the coefficient of absorbance was reduced from 45.6 ± 2.3 to 37.8 ± 2.1 , indicating a significant ($p < 0.02$) fall in the level of

unsaturated lipids in the superficial layers of the zona fasciculata (Fig. 1). Parallel with this the dimensions of the adrenocortico-cytes were statistically significantly reduced and the volume of their nuclei increased ($p < 0.001$), leading to a significant increase in the nucleocytoplasmic ratios ($p < 0.001$).

During exposure of the adrenals to radiation from a gallium arsenide laser, capable of penetrating deeper into the tissue and working on pulsed mode, total changes were observed in the zona fasciculata of the adrenal cortex. Throughout the depth of the adrenal cortex congestion of the capillaries was increased from 10.6 ± 1.4 to $16.2 \pm 2.2\%$ (Table 1; Fig. 1c). In sections through the cortex of several adrenal glands a decrease in the content of unsaturated lipids could be detected from the surface to the deep layers of the zona fasciculata (Fig. 1c). The dimensions of the adrenocortico-cytes were significantly reduced ($p < 0.001$) and the volume of the nuclei ($p < 0.001$) and the nucleocytoplasmic ratios ($p < 0.001$) were significantly increased. The increase in volume of the nuclei and in the nucleocytoplasmic ratios and the decrease in the content of unsaturated lipids following laser irradiation, combined with hyperemia of the adrenal cortex, are signs of increased functional activity of their zona fasciculata [5]. Morphological and functional changes found after direct irradiation of the adrenals in the zona fasciculata point to the fact that radiation from a low-intensity laser can exert a stimulating effect on the function of these glands.

The results are in agreement with observations made by other workers [2-4], who found increased congestion of the microcirculatory bed of healthy skin, after laser irradiation, in areas of skin damage and in gastric and duodenal ulcers, and this, together with other factors, helped to speed up the healing of wounds and ulcers. Increased congestion of the capillaries and the accompanying morphological signs of increased functional activity of the zona fasciculata after low-intensity laser irradiation indirectly confirm data obtained by other workers [7, 8] to the effect that increasing the flow rate of Ringer's fluid used to perfuse the adrenals enhances adrenocortical function.

The results now obtained are evidence that the differences in the action of helium—neon and gallium arsenide lasers are due, first, to the greater depth of penetration of laser radiation in the infrared range, and also the pulsed mode of working of the semiconductor laser, giving rise to a marked effect on the microcirculatory bed in not only the superficial, but also the deep layers of the adrenal cortex. The results indicate good prospects for the use of laser irradiation of the adrenal region in clinical practice to stimulate adrenocortical function.

LITERATURE CITED

1. G. G. Avtandilov, *Morphometry in Pathology* [in Russian], Moscow (1973).
2. N. F. Gamaleya, *Lasers in Experimental and Clinical Medicine* [in Russian], Moscow (1972).
3. A. S. Kryuk, V. A. Motovnikov, I. V. Khokhlov, and N. S. Serdyuchenko, *Therapeutic Efficacy of Low-Intensity Laser Radiation* [in Russian], Minsk (1986).
4. V. I. Kozlov, F. B. Litvin, and O. A. Terman, *The Use of Lasers in Surgery and Medicine* [in Russian], Vol. 1, Moscow (1988), pp. 525-528.
5. Ya. E. Khesin, *Dimensions of the Nuclei and Functional State of Cells* [in Russian], Moscow (1967).
6. V. V. Yaglov, *Arkh. Anat.*, No. 6, 81 (1969).
7. J. P. Jiinson, G. P. Vinson, and B. J. Whitehouse, *J. Endocr.*, **111**, 391 (1986).
8. G. P. Vinson, J. P. Hinson, and B. J. Whitehouse, *J. Endocr.*, **112**, Suppl. 16 (1987).