## MORPHOLOGY AND PATHOMORPHOLOGY

EFFECT OF LASER IRRADIATION ON TISSUE BASOPHILS OF THE DURA MATER: HISTOPHYSIOLOGICAL STUDY

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There is already a considerable literature on the biostimulating action of laser radiation. Low-intensity radiation from a helium-neon laser (HNL) has been shown to have a beneficial effect in diseases associated as a rule with disturbances of nutrition of organs and tissues. The beneficial effect of laser radiation has been explained by the stimulating action of monochromatic coherent red light on cell function [1, 2, 12]. Some workers consider that during irradiation for 30 min cell activity increases proportionally to the duration of exposure to HNL [3] whereas others claim that the response of cells within this time interval is virtually independent of the duration of action of the laser [12]. There is also a third point of view, which is that low-intensity laser radiation with an exposure of up to 1 min and over 30 min has no biostimulating action on cells [2, 12].

## EXPERIMENTAL METHOD

Experiments were carried out on 108 mature noninbred albino rats weighing 170-180 g. The source of radiation was the GNL-108 HNL with wavelength of 632.8 nm and power density of 0.76 mW/cm<sup>2</sup>. Animals of the experimental group received a single dose of irradiation in the right parietal region with an exposure of 0.5, 1, 10, and 30 sec; 1, 5, 15, and 30 min; and 1 and 3 h. The control consisted of an unirradiated group of animals with dissected skin-muscle flap, kept under the same animal house conditions as the experimental rats. The dura meter was removed immediately after death of the rats by decapitation. Tissue basophils (TB) were revealed by staining with 0.5% methylene blue solution and by the methods of Furness and Costa [10] for catecholamines, of Luppa [11] for succinate dehydrogenase and cytochrome oxidase, of Wachstein and Meisel [13] for Mg+-ATPase, and of Gomori for alkaline phosphatase [8] and for acid phosphatase [9]. The state of TB function was judged by calculating the total number of TB per square millimeter of dura in the right and symmetrical part of the left parietal region, and the relative percentages of intact and degranulated forms of TB. To determine the level of TB activity more accurately, a degranulation coefficient [5] was calculated: T = N/n, where n is the total number of TB and N the number of degranulated forms of TB.

## EXPERIMENTAL RESULTS

TB in the dura mater of the control animals were distributed along the course of the blood vessels and in the intervascular regions, they were round, oval, or fusiform in shape and measured from 9 to 20  $\mu$  (Fig. 1: a, b, c). Histochemically, several cell populations could be distinguished among TB. The most numerous consisted of TB detectable with methylene blue; there was a somewhat smaller concentration of TB giving a positive reaction for cytochrome oxidase and catecholamines. Cells labeled with alkaline and acid phosphatase were less numerous than the others. Differences in the concentration of a particular TB population in the right and left parietal regions of the dura of these rats were not significant (p > 0.05). Independently of the method used to study and localize TB, several functional forms could be distinguished among them: I) nondegranulating, conventionally considered to be intact; II) weakly degranulating, around which single granules were observed; III) moderately degranulating; IV) actively degranulating with mass release of granules

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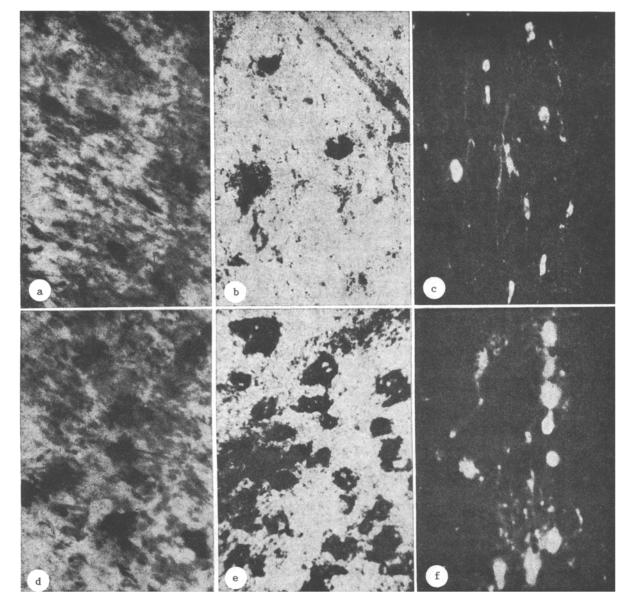


Fig. 1. TB in dura mater of rats of control (a, b, c) and experimental (d, e, f) groups. Stained: a, d) with methylene blue, b, e) for succinate dehydrogenase, c, f) with glyoxylic acid. Magnification 112.

(Fig. 1: a, b, c). Counting showed that the relative numbers of intact and degranulating (I, II-IV) forms of TB largely depended on the method of staining of the preparations (Fig. 1: a, b). For instance, cytochrome oxidase and acid phosphatase were detected mainly in intact TB, succinate dehydrogenase and ATPase mainly in degranulating TB.

The reaction of TB to 15-min irradiation with HNL can be interpreted as an increase of their functional activity. This was shown, first, by an increase in the concentration of TB in the dura (Fig. 1: c, e), second, by an increase in the number of degranulated forms of TB (Fig. 1: d, e, f), and third, by an increase in the degranulation coefficient.

Different responses of TB depending on the method of study and the localization of the cells are illustrated in Fig. 1: c, d, which shows that the total number of TB increased by the greatest degree in the cell population giving a positive reaction for succinate dehydrogenase and MG<sup>++</sup>-ATPase (p < 0.001). In preparations stained with methylene blue the increase in the total number of TB was less significant (p < 0.05), but the concentration of degranulated forms was almost 4.5 times greater than in the control. These differences in the response of TB were found also during analysis of data obtained in a study of the left (unirradiated) side of the dura mater. However, the level of functional activity of TB was rather lower in the dura on the left than on the right (irradiated) side.

Results obtained by studying the state of function of TB in the dura after different exposures to HNL can be summarized as follows. TB react distinctly to irradiation by HNL. After only 0.5 sec the number of degranulating forms of cells was increased by almost 2.5 times (p < 0.01), but between 15 min and 1 h of irradiation their concentration reached a maximum (p < 0.001). The concentration of intact TB fell correspondingly. Meanwhile the total number of TB in the regions of the dura studied did not change significantly after exposure to laser radiation. Only after 5 min of irradiation by HNL did the increase in the number of cells become significant (p < 0.05), but after 30 min of continuous exposure to the laser the total number of TB fell to the control value (p > 0.05), and after 3 h of irradiation it was only 75% of the initial value (p < 0.001). In this connection the coefficient of degranulation — the parameter of the degree of activity of TB [5] — rose sharply during the first few seconds of exposure to HNL, for the next 5 min it remained high and relatively constant with only small fluctuations, but with a further increase in the dose of irradiation it rose again, although more slowly.

The response of TB on the left (unirradiated) side of the dura largely repeated the pattern observed for cells directly exposed to HNL radiation. This suggests that the combined response of the total TB population of the dura is of the nervous reflex type, as other workers also have postulated [6, 7].

Three important conclusions can be drawn from the results of these investigations: first, TB of the dura mater, which are local regulators of tissue homeostasis [4, 6, 7], provide a convenient model with which to study the mechanisms of action of laser radiation; second, changes in functional activity of TB under the influence of laser radiation are easily detected by the use of histochemical and morphometric methods; third, the biostimulating action of HNL on TB depends on the dose of irradiation: the first peak of activity of the cells is observed during uninterrupted exposure to the radiation for 30 sec, the second peak during exposure of between 15 min and 1 h.

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