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Electron Microscopic Study of Erythrocyte Shape after Ultraviolet and Red Coherent Extracorporal Irradiation of the Blood

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At present quantum hemotherapy has gained wide acceptance in clinical practice. The literature available definitely proves the importance and dissimilar action of different bands of the electromagnetic spectrum on the organism and, in particular, on the blood [1, 3-11].

The aim of the present study was to investigate the effect of ultraviolet (UV) and red coherent radiation on the shape of erythrocytes after extracorporal irradiation of the blood.

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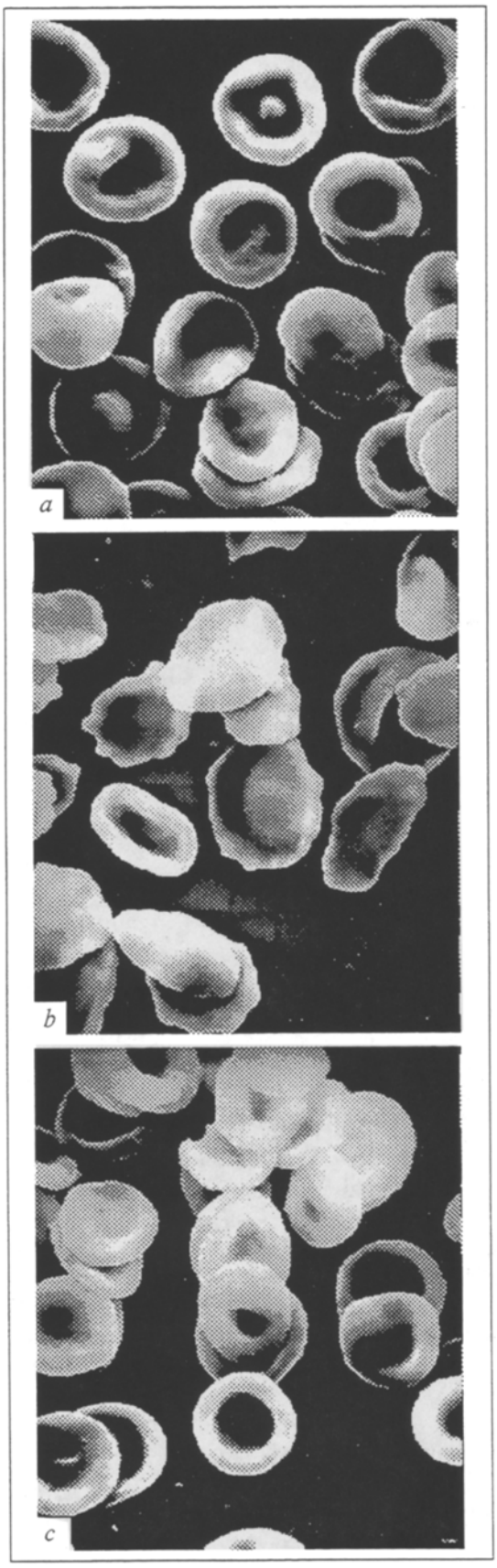
MATERIALS AND METHODS

Two experimental series involving extracorporal irradiation of the total blood volume (TBV) were performed on 23 adult male and female dogs weighing 15-18 kg, subjected to quarantine and veterinary examination. A helium-neon laser (HNL, red coherent irradiation, λ=632.8 nm, 1 mW) and a DRB-8 lamp (UV irradiation, λ=254 nm, 8 W) were used for irradiation of the blood. A single irradiation was performed during one hour during the blood flow through a PK 11-05 disposable system for blood transfusion: in the first series the irradiation was performed by a defocused laser beam over 10 cm of the tube; in the second se-



—**Fig. 1.** Erythrocytes after extracorporal TBV irradiation with HNL: *a*) immediately after; *b*) 5 days after; *c*) 15 days after exposure (×4000).

ries the blood was UV-irradiated while being passed through a quartz cuvette attached to the tube. The rate of blood flow through the system was 100 ml/min. In each experimental series blood samples were taken from a peripheral vien of the hind leg before, immediately after and then 5 and 15 days after the irradiation.

For investigation of the shape of the erythrocytes by means of scanning electron microscopy (SEM) the blood preparations were fixed with 2% paraformaldehyde in phosphate buffer saline (pH 7.4) and treated according to the method proposed by Krymskii *et al.* [2]. The preparations were examined with a JEM-100 CX II electron microscope (Jeol, Japan).

RESULTS

The investigation revealed the presence of deformed erythrocytes immediately after the extracorporal HNL irradiation of TBV (Fig. 1, *a*), suggesting injury to the erythrocyte membrane. This kind of irradiation induces an alteration in the biconcave shape of the erythrocytes: they become flatter, and, in some cases, thick finger-shaped processes appear on the cell surface.

Marked abnormalities of the erythrocytes are observed 5 days after exposure (Fig. 1, *b*). The deformed cells increase in number, the bulk being so-called "scalloped" erythrocytes, characterized by a wavy border together with a change in shape from normal biconcave to a flat disk. It can be assumed that the scalloped shape results from a disturbance in the energy potential of the erythrocyte membrane.

The characteristic feature of erythrocytes 15 days after irradiation is the restoration of the shape changes that were induced by HNL radiation and observed in the earlier stages. The scalloped pattern of the cell border becomes smoothed out, the central process disappears, and the erythrocytes regain their normal biconcave shape.

UV irradiation of the blood, in comparison with HNL irradiation, causes a more pronounced increase in the number of abnormal erythrocytes (Fig. 2, *a*). This manifests itself in the by lacking of the biconcave shape in many cells, together with irregular outlines. Some erythrocytes have thick finger-shaped processes on the surface. Simultaneously, scalloped erythrocytes with a more pronounced waviness of the borders and a flatter shape are observed.

In the blood samples taken 5 days after irradiation in comparison with those drawn immediately the erythrocytes exhibit smoothing of the wavy borders as well as the appearance of a central notch; however, erythrocytes with a thick finger-shaped process still occur in the blood (Fig.2, *b*).

The tendency toward the normalization of erythrocyte shape remains observable 15 days after extra-

corporal UV irradiarion (Fig. 2, *c*). Scalloped erythrocytes are few in number and negligible amounts of other types of abnormal cells (cells with finger-shaped and crest-shaped processes as well as flatened cells) occur in the blood.

Thus, our results confirm the sensitivity of erythrocytes to UV and red coherent radiation. The examination of the erythrocyte preparations at different time points after irradiation reveals considerable differences in the shape of the cells depending on the type of radiation and the interval after it (a study of the dose-dependent effect is beyond the scope of the present work). In the irradiated blood in the early stages of the experiment the number of abnormal cells is elevated, suggesting a disturbance in the molecular structure of the cell membrane. It should be noted that UV more than red coherent irradiation affects the shape of erythrocytes. Both types of irradiation used produce reversible changes in erythrocyte shape; however, the inertness of the restoration process is different for UV and red coherent irradiation: in the first case, erythrocytes with central processes occur even 15 days after exposure , whereas in the second case, this time interval is enough for complete restoration of the erythrocyte shape. The changes in erythrocyte shape may be assumed to be connected with injury to the structure of the erythrocyte membrane caused by direct influence of the irradiation on the cell or by photoinitiation of free-radical oxidation in the erythrocytes. Simultaneously, an inhibition of of glycolysis and the of pentose cycle and an impairment of the action of actomyosin-like contractile protein probably occur, which in turn lead to inhibition of the "work" of the membrane ATPases and to a decrease of the energy potential required for maintaining cell shape.

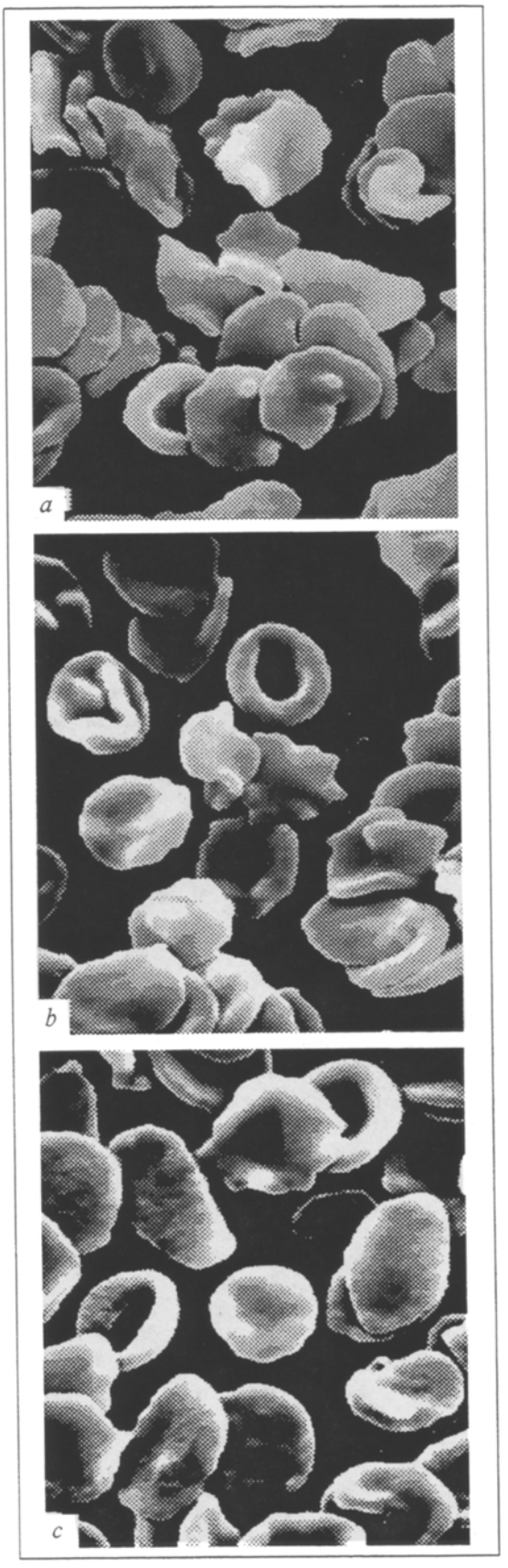


Fig. 2. Erythrocytes after extracorporal UV irradiarion of TBV: *a*) immediately after; *b*) 5 days after; *c*) 15 days after exposure (×4000).

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