MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Low-Intensity Infrared Laser on the Healing of Dermatome Wounds

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Changes in the microvessels, consisting in their dilatation and accelerated formation, and a shift of the peak of epitheliocyte proliferative activity are found to be the structural basis of the stimulating effect of infrared magnetic laser. Morphologic changes in connective-tissue cells determine both the proper development of the connective-tissue carcass, primarily of the fibroblasts, and the barrier protective and regulatory function of such cells as macrophages, neutrophils, plasma cells, eosinophils, and, last but not least, mast cells. Morphologic changes occurring during irradiation according to optimal schemes indicate activation of specific cell functions and are observed at earlier times.

Key Words: *low-intensity magnetic laser radiation; dermatome wounds; healing*

Low-intensity laser radiation is widely used in wound healing [2-6,8]. At the present time the effects of He-Ne laser have been studied most fully; persuasive evidence of a pronounced biostimulating effect of laser treatment at wavelength 630-640 nm has been obtained, and the optimal range of a stimulating dose has been determined [1,7,8]. The optimal schemes for low-intensity laser radiation in the infrared (IR) band are still to be established.

MATERIALS AND METHODS

The effects of a Lazur' gallium arsenide pulsed laser with a 40 mT magnetic system on the healing of experimental total-layer wounds were studied in rats. In 51 animals skin wounds of 0.95 cm^2 were daily irradiated in the morning for 10 days at frequencies 20, 80, 300, and 1000 Hz and magnet-exposed without laser for 0.5, 2, and 5 min. The wounds were morphologically examined by light and transmission electron microscopy, autoradiography, and stereophotometry on days 3, 5, 10, and 30 of laser therapy. Wound morphology was studied in histotopographic semithin slices involving all the wound zones with the adjacent skin sites, followed by "spot" electron microscopy. All the manipulations were carried out under ether narcosis. The animals were sacrificed by instant decapitation. For light and transmission electron microscopy the material was treated routinely. Wound size was measured planimetrically using scale macrophotographs at 5× magnification 1, 2, 3, 5, 7, and 10 days after the treatment was started. For studies of the proliferative activity, the animals were intraperitoneally injected ^3H-thymidine in a dose of 17 mBq 1 h before decapitation; paraffin slices were coated with type M

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photoemulsion. Morphometric data were processed by alternative and variational statistics.

RESULTS

Planimetric studies clearly indicate a dose-dependent stimulating effect of IR magnetic laser on the wound process. By day 3 the minimal size of wounds was 0.68 cm^2 at 80 Hz (5 min), 0.55 cm^2 at 300 Hz (2 min), and 0.58 cm^2 at 1000 Hz (0.5 min), *vs.* 0.85 cm^2 in the control. On day 10 the optimal schemes were 80 Hz (5 min): 0.35 cm^2 and 300 Hz (0.5 min): 0.23 cm^2, in comparison with 0.53 cm^2 in the control (Fig. 1, *a*), the reliability of differences being no more than 0.05.

Correlation analysis of planimetric data showed that IR magnetic laser appreciably accelerated wound healing (Fig. 1, *b*) and helped identify the optimal doses (frequency and time of exposure): 7 to 10 daily sessions for 2-5 min at 80 Hz, or 0.5-2 min at 300 Hz, or 0.5 min at 1000 Hz. Exposure to lower doses (0.5 to 5 min at 20 Hz or 0.5 min at 80 Hz) did not stimulate wound healing, whereas exposure parameters higher than the optimal could suppress the rate of wound healing (Fig. 1, *b*).

Some differences were noted in the effects of laser with various frequencies of pulse sequence for the same total doses, although the general processes of the stimulating action of IR magnetic laser radiation were stereotypical. For this reason, to simplify the presentation of our results, we singled out 3 groups of doses: subthreshold, optimally stimulating, and suppressive.

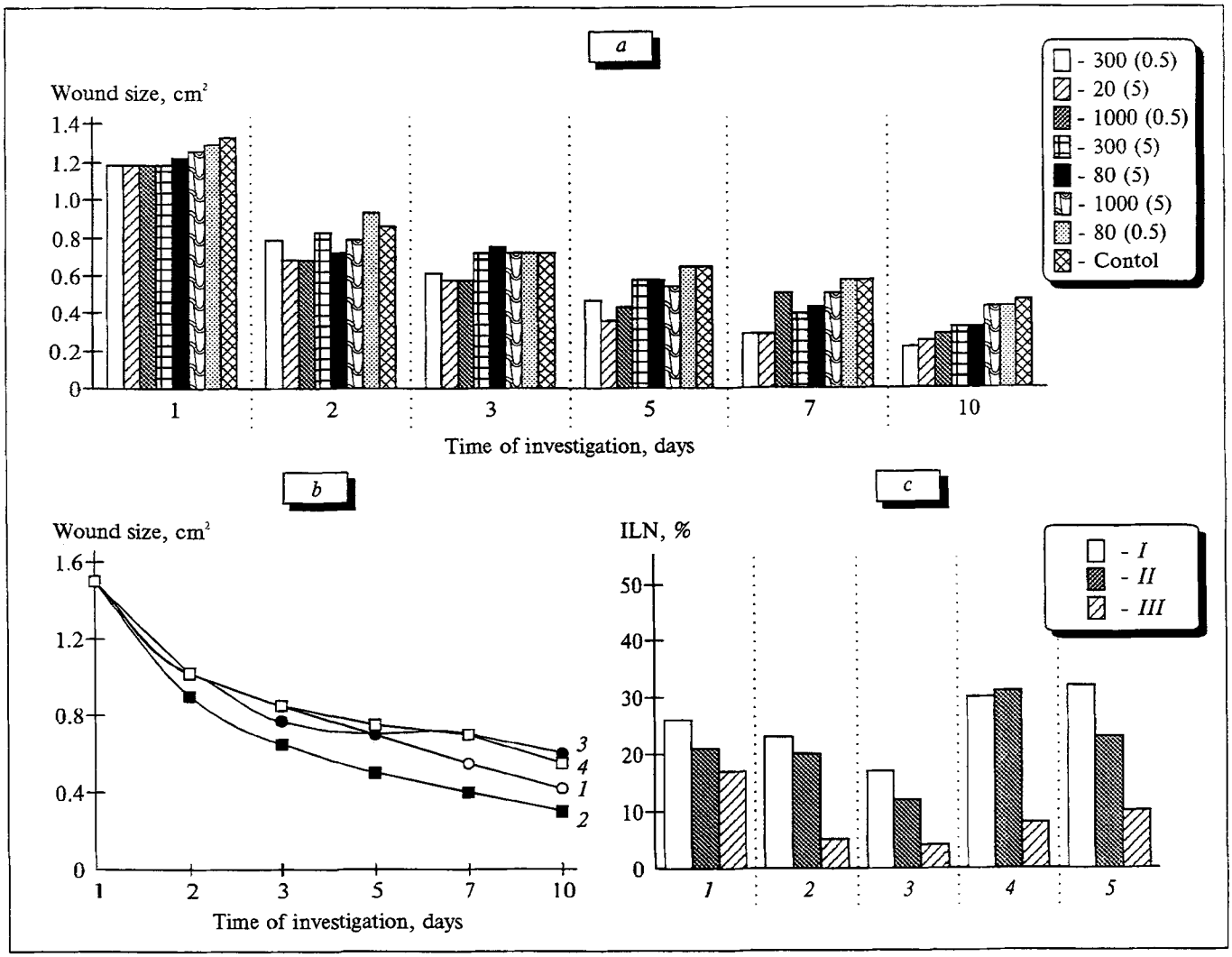

Fig. 1. Effect of low-intensity IR magnetic laser on the time course of healing and proliferative activity of cells in dermatome wounds of rats. *a*) size of dermatome wounds: first figure shows exposure frequency, Hz, with the exposure duration in min shown in parentheses; *b*) correlation between wound size and radiation dose; *1*) subthreshold doses; *2*) optimal stimulating doses; *3*) suppressive doses; *4*) control. *c*) proliferative activity of wound cells on day 10 of treatment: *I*) epitheliocytes near the wound; *II*) epitheliocytes far from the wound; *III*) fibroblasts of wound granulation tissue; *1*) control; *2*) 80 Hz, 5 min; *3*) 300 Hz, 0.5 min; *4*) 1000 Hz, 2 min; *5*) 1000 Hz, 5 min.

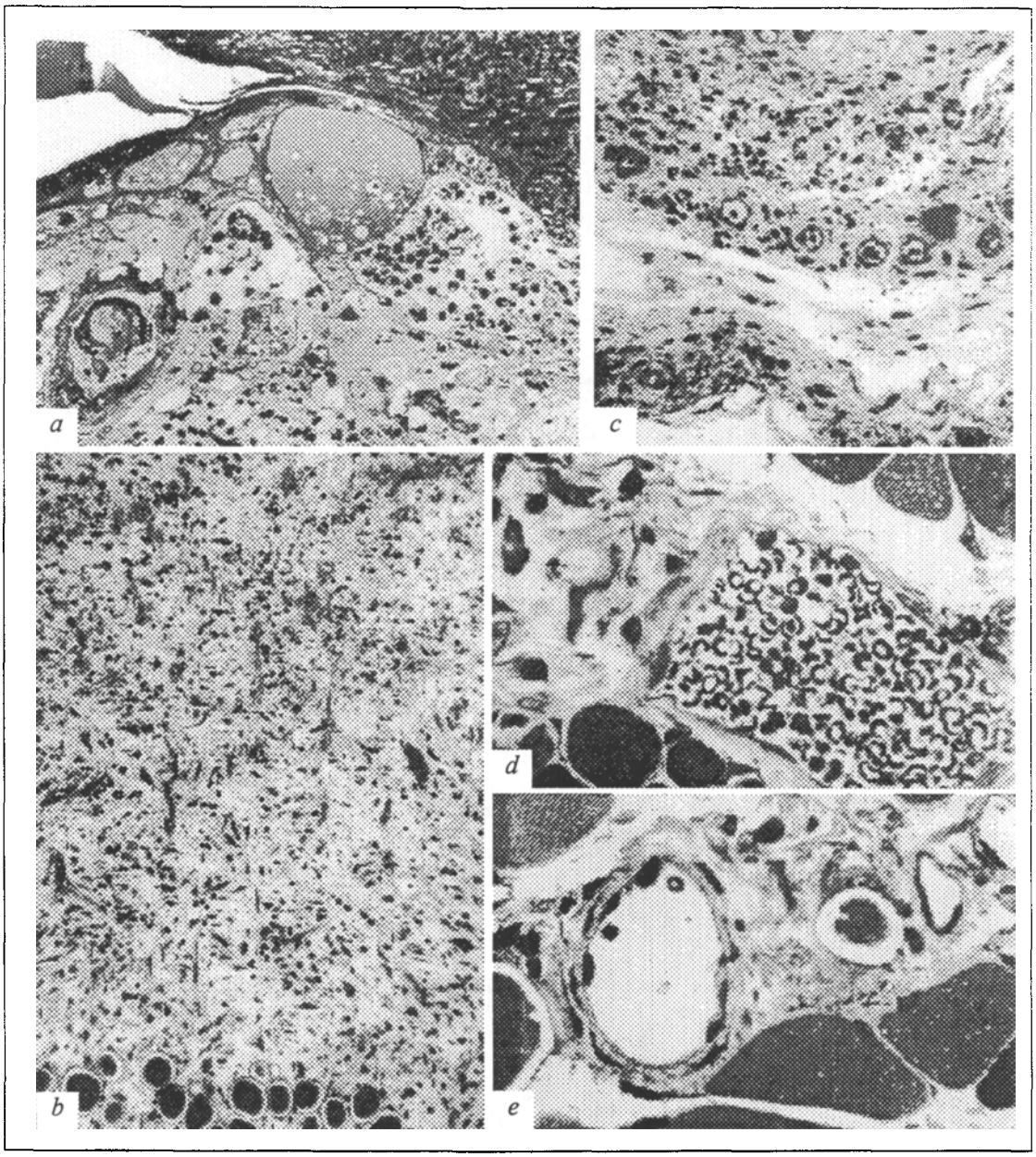

Fig. 2. Effect of low-intensity IR magnetic laser on wound process morphology. Semithin slices, staining with methylene blue-fuchsin. *a*) "tongue" of creeping epithelium, pronounced polymorphocellular infiltration; day 3, magnetic field exposure, 5 min, ×200; *b*) mature granulation tissue of wound with orderly arrangement of collagen fibers; day 3, 300 Hz, 2 min, ×100; *c*) dilated microvessels of subcutaneous fat far from the wound; day 3, 80 Hz, 5 min, ×100; *d*) dilated microvessel with numerous formed elements of the blood far from the wound; day 3, 300 Hz, 0.5 min, ×400: *e*) devastated dilated microvessel far from the wound; day 3, 300 Hz, 5 min, ×400.

In the group exposed to subthreshold doses no appreciable differences in wound morphology from that in the control were observed. A weakly stimulating effect at 20 Hz exposure for 2 or 5 min was due to the effect of a permanent magnetic field, this being confirmed by the results in the control group, in which the wounds were exposed only to a permanent magnetic field: the counts of mast cells and neutrophils in the wound reliably increased, and a moderate dilatation of the lumens of capillaries was observed.

In all the periods tested, the optimal stimulating doses led to an appreciable increase of the relative volume of capillaries, this evidently improving the microcirculation. The number of immunocompetent cells, mainly phagocytes, in the wound tissue increased in all sites of the wound and around it, as did the number of mitoses in the granulation tissue and epidermis and the count of lipocytes (Fig. 2).

When exposed to suppressive doses, the skin had a waxy appearance, with hair growth slowed

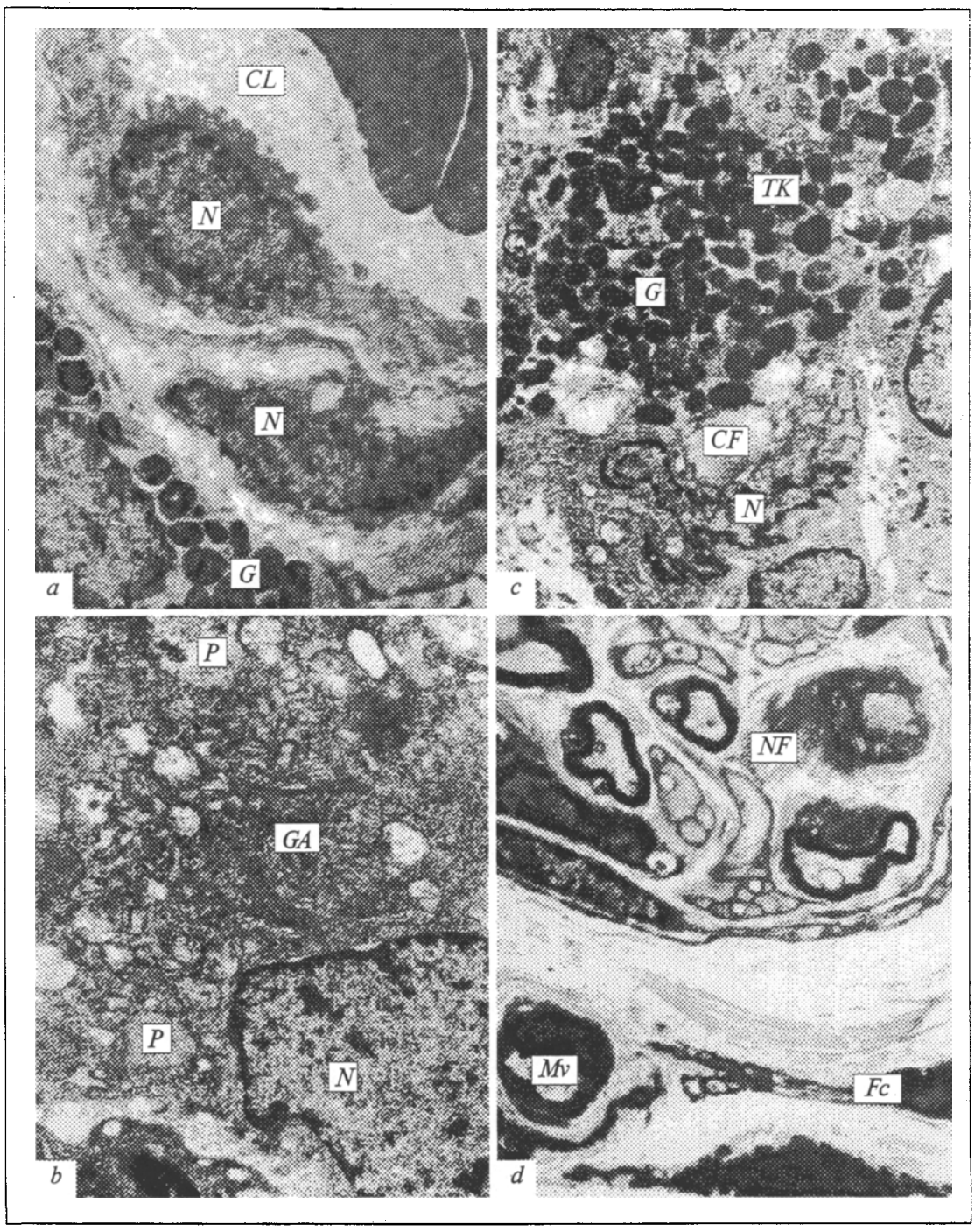

Fig. 3. Ultrastructural features of the stimulating effect of low–intensity IR magnetic laser radiation. Transmission electron microscopy. *a*) dilated capillary lumen (*CL*), thickening of endotheliocyte cytoplasm, and increased number of granules (*G*) in a mast cell. *N*: nucleus. Day 3, 80 Hz, 5 min, ×1000; *b*) macrophage with numerous phagosomes (*P*) in the cytoplasm. *GA*: Golgi apparatus. Day 10, 1000 Hz, 2 min, ×12,000; *c*) accumulation of collagen fibers (*CF*), release of mast cell (*MC*) granules into intercellular space. Day 10, 1000 Hz, 2 min, ×5000; *d*) a nerve fiber (*NF*) surrounded by collagen fibers and fibrocytes (*Fc*). *Mv*: microvessel. Day 10, 80 Hz, 5 min, ×6000.

down at sites where the hair had been shaved. Light microscopy showed stasis in the large vessels in the early periods, with the capillaries dilated but containing no formed elements of the blood; capillaries around the wound were likewise devastated, with the number of neutrophil leukocytes, macrophages, and lipocytes decreased (Fig. 2). The relative volume of hair follicles and sebaceous glands was reduced.

In the control, the index of labeled nuclei (ILN) of epitheliocytes increased 5-6-fold in com-

parison with that in intact skin. Proliferative activity of the epidermis and, especially, of the "tongue" of creeping epithelium and proliferation of the granulation tissue cells reached its peak by days 3-5, after which it declined.

Exposure to subthreshold doses did not lead to a reliable increase of ILN or of the mitotic index (MI) in comparison with the control at any of the times tested. The optimal stimulating doses brought about an earlier attainment of the peak of cell proliferative activity (ILN and MI) (Fig. 1, *c*), although the absolute values of the peak did not increase significantly. In later periods these parameters were lower than in the control, the extent of reduction being in proportion with the rate of wound healing. For example, epitheliocyte ILN near the wound was 26.1% in controls, that in animals exposed to 80 Hz for 5 min 23.2%, and in those exposed to 300 Hz for 0.5 min 17.5%, the wound size being 0.53, 0.32, and 0.23 cm^2, respectively. After exposure to suppressive doses the peak of proliferative activity was observed later (Fig. 1, *c*). For example, by day 10 the ILN in epitheliocytes near the wound was 31.1% at 1000 Hz and 2 min and 33.1% at 1000 Hz and 5 min, the wound area being, respectively, 0.51 and 0.49 cm^2.

Electron microscopic examination of the wounds showed that the maximal differences in cell ultrastructure, regarded as the morphological basis of their function activation, were observed at the optimal stimulating doses. These differences were most pronounced on days 3-5 of laser therapy, involving primarily the fibroblast ultrastructure. The profiles of granular endoplasmic reticulum containing electron-dense material were more expanded in the fibroblast cytoplasm, in comparison with the control, a considerable area of it being occupied by structures of the Golgi apparatus. The cytoplasmic membrane contained numerous outgrowths and invaginations in which accumulations of fibers with varying degrees of striation were discernible. During the first 3 days of exposure the ultrastructure of polymorphonuclear neutrophil leukocytes was indicative of activation of their phagocytic function. The considerable number of secretory granules in the cytoplasm of plasma cells, the well-developed Golgi apparatus, and the intensive release of secretory granules in the intercellular space attested to active secretion of these cells. The microvessel lumens were rather wide, the endotheliocyte cytoplasm thickened (Fig. 3). Numerous outgrowths were seen at the lumen surface, particularly near the perikaryon. The nuclei were rather large, with euchromatin predominating. All this pointed to an extremely high functional activity of endotheliocytes. Mast cells, as a rule, were adjacent to microvessels. Their cytoplasm contained numerous rather polymorphic secretory granules, with large ones predominating, and the nuclei and, especially, the nucleoli were increased in size. By day 5 the number of macrophages in the granulation tissue had increased. They contained a variety of phagocytic structures in the cytoplasm. Fragments of phagocytosed cells, fibrin, and detritus were often seen in the vacuoles. Microvessel lumens were dilated, and there were numerous outgrowths and microvilli on the lumen surface of endothelial cells. Many vacuoles were present in the cytoplasm. Endotheliocytes undergoing mitosis were frequently observed. "Budding" of capillaries was commonly noted. By days 7-10 the granulation tissue cells became more compactly arranged, closely adhering to each other (Fig. 3). The cell-to-cell zones were filled with accumulations of collagen fibers. Fibroblasts with signs of a high degree of maturation and differentiation were generally seen. A considerable number of nerve elements, notably of large myelin fibers, were discernible in the wound in later periods. Nerve fibers were surrounded by collagen fibers and numerous fibroblasts (Fig. 3). This indicated that IR magnetic laser stimulates the processes of innervation repair in dermal structures.

Moderately pronounced cell alterations were observed for exposure to suppressive doses (Fig. 4). Marked swelling of mitochondria was observed in the fibroblasts, and in the basal and prickle-cell layer cells of the wound edge epidermis a moderately expressed perinuclear edema was observed along with an increase of the number of tonofilaments and intercellular processes with desmosomes. Rather large vacuoles were forming in some cells of the perinuclear zone. Pronounced alterations and swelling both in the wound and in the adjacent tissues were observed in later periods. Although this was paralleled by intensive formation of collagen fibers, there were large gaps between the fibers, indicative of edema. Appreciable ultrastructural changes were observed in the epidermis. Along with numerous tonofibrils, a variety of vacuoles with low electron density appeared in the epitheliocyte cytoplasm. In the nuclei autochromatin, numerous nuclear pores, and rather large nucleoli predominated.

Hence, the structural basis of the stimulating effect of IR magnetic laser radiation consists, first of all, in changes of the microvessels, presenting as their dilatation and accelerated new formation due to enhanced proliferative activity of endotheliocytes. Morphological changes in connective-tissue cells de-

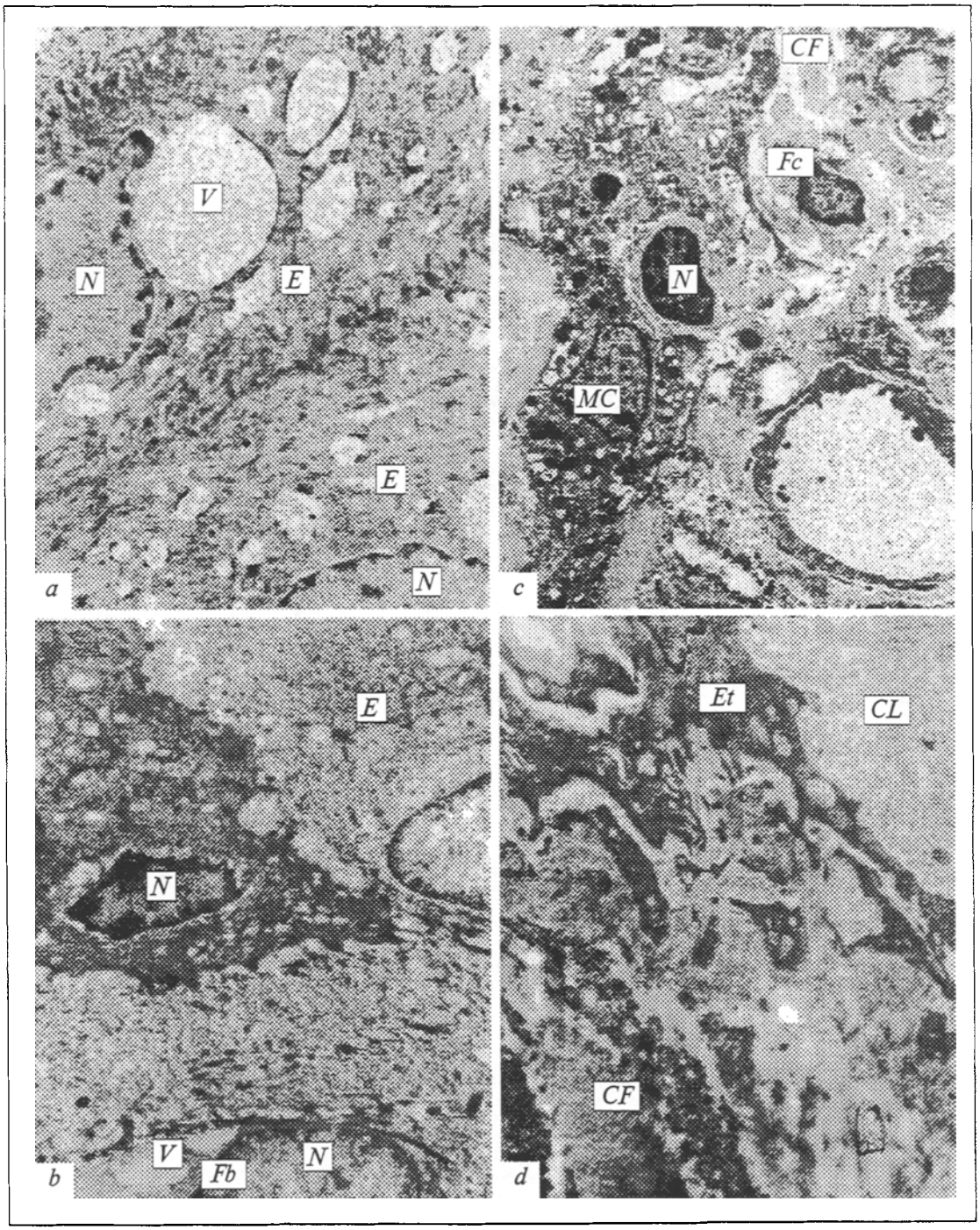

Fig. 4. Ultrastructural features of altering effect of low–intensity magnetic laser radiation. Transmission electron microscopy. *a*) large vacuoles (*V*) in epidermocyte (*E*) cytoplasm and nuclei (*N*) with euchromatin predominance. Day 10, 1000 Hz, 5 min, ×7500; *b*) vacuoles (*V*) in fibroblast (*Fb*) cytoplasm and large perinuclear vacuoles in epidermocyte of wound edge. Day 3, 300 Hz, 5 min, ×1000; *c*) pronounced reduction of granules in mast cells (*MC*) and numerous phagosomes in fibroclasts (*Fc*). *CF*: collagen fibers. Day 10, 1000 Hz, 2 min, ×3000; *d*) edema in the derma, swelling of mitochondria, and uneven thickness of endotheliocyte (*Et*) cytoplasm, *CL*: capillary lumen. Day 10, 1000 Hz, 5 min, ×7500.

termine both the sound development of the connective-tissue carcass, primarily of fibroblasts, and the barrier protective and regulatory functions of such cells as macrophages, neutrophils, plasma cells, eosinophils, and, especially, mast cells.

The above changes attest to an activation of specific functions of cells that is observed earlier, on days 3-5, if the optimal exposure schemes are used. In cases of overdosage these changes are observed later. Hence, IR magnetic laser, while not

qualitatively altering the wound process, has the ability to speed it up or slow it down.

The optimal schemes of wound irradiation using the Lazur' magnetic laser are 2 to 5 min at 80 Hz, 0.5 to 2 min at 300 Hz, and no more than 0.5 min at 1000 Hz. The optimal duration of a course of therapy is 7 to 10 days.

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