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# Electron Microscopic Autoradiographic Investigation of Burn Injury Healing after Treatment with Levosin Ointment

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The modern local treatment of burn injuries includes multicomponent ointments with a primary hydrophilic component. The evaluation of the healing effect of ointments is, as a rule, conducted under clinical conditions, by assessing the results of bacteriological, cytological, and morphological observations [1-4,6]. However, these data give a general picture but do not disclose the mechanisms of the effect of the ointment on the injury. With this in mind we performed a study of burn injury granulation tissue during topical treatment, using one of the latest methods of structural-functional analysis, namely electron microscopic autoradiography.

## MATERIAL AND METHODS

The crust and granulation tissue samples were obtained from three patients with III-IV degree burns covering over 40-75% of the body surface, who were referred to the Burn Center of the A.V. Vishnevskii Institute of Surgery. In addition, three biopsy samples of different localization were taken from patient Ch. The tissue pieces to be analyzed were cut from biopsy samples taken during dressing. The crust and granulation tissue were monitored in their dynamics, before Levosin ointment treatment (control), and 10 and 20 days following the start of treatment. At these times an electron-autoradiographic and bacteriological study and a comparative quantitative analysis were conducted. The performance of the last included a light microscopic survey of 100 visual fields, within which the total number of fibroblasts, macrophages, and blood vessels, as well as the quantity of $^3$H-thymidine-labeled fibroblasts were counted. For the purpose of electron-autoradiographic analysis, pieces of approximately 1 mm$^3$ were cut from the granulation tissue and crust. The pieces were further incubated in medium 199 supplemented with $^3$H-uridine (100 μCi/ml, specific radioactivity 26 Ci/mM) or $^3$H-thymidine (20 μCi/ml, specific activity 21.6 Ci/mM). After 1.5-hour incubation at 37°C the unbound label was removed by washing with cooled medium 199 and phosphate buffer, at pH 7.4. The samples were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide solution, dehydrated in ascending grades of alcohol, and embedded in epon-araldite. Semithin sections prepared from each sample were covered with "M" photoemulsion, exposed for three days, and processed with D-19 developer. Semithin sections were stained with toluidine blue. After the analysis of these sections, the precise sites for ultratomy were formed, and the thin sections were covered with the mentioned emulsion by the loop method and exposed for 30-40 days. The developed autoradiographs were stained with uranyl-acetate and lead nitrate after Reynolds [7] and examined under a Philips CM 10 electron microscope.

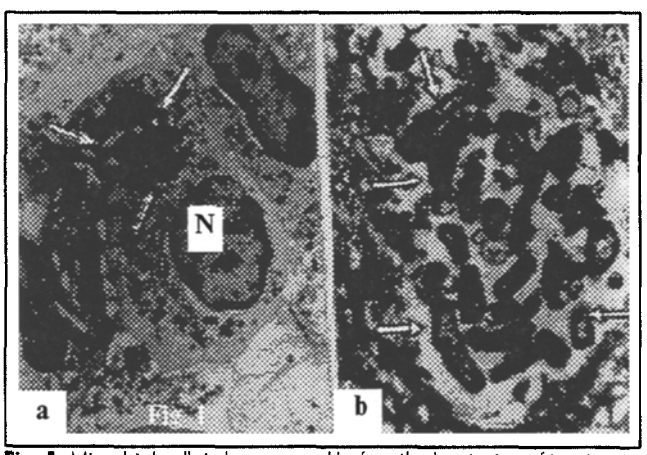


**Fig. 1.** Microbial cells in burn wound before the beginning of treatment with Levosin ointment. a) polymorphonuclear leukocyte with phagocytized microbes (arrows); incorporation of $^3$H-uridine (black grains of silver). N: leukocyte nucleus; b) microbes present in crust (arrows), intensely labeled with $^3$H-thymidine (black grains of silver over the cells). x12,000.

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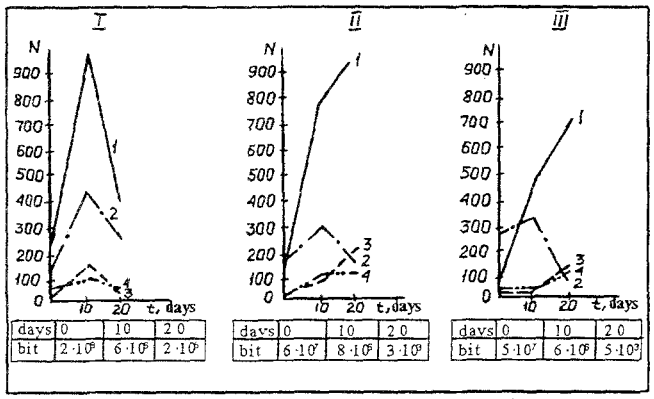


**Fig. 2.** Dynamics of fibroblasts, macrophages, and vessels in granulation tissue during healing of burn injuries of different localization (patient Ch.). I) upper arm; II) forearm; III) thigh. 1) total number of fibroblasts; 2) macrophages; 3) $^3$H-thymidine-labeled fibroblasts; 4) vessels. Abscissa: duration of wound healing (days); ordinate: number of cells. Microbial counts in burn wounds at examined periods presented in Tables.

## RESULTS

Before the start of Levosin ointment treatment, the burn wound was covered with suppurative-necrotic masses. The granulation was flabby or totally absent. The microbial dissemination was within $5x10^7$-$2x10^8$ per gram of tissue, the value significantly exceeding the critical level ($10^5$ ). The cellular repertoire was represented mainly by leukocytes, a small quantity of macrophages, and microbial bodies. The cellular elements were loosely arranged, with abundant cell detritus between them. Active phagocytosis of bacteria and destroyed cells by polymorphonuclear leukocytes (PMNL) was observed. Moreover, a pronounced intracellular degranulation of PMNL - conversion of granules into phagosomes - took place. The PMNL population was heterogeneous according to several morphological criteria, indicating differences in functional activity.

Microbial cells were disposed both in the intercellular space and in the phagosomes of PMNL and macrophages. Free microbes, as well as many phagocytized ones, incorporated $^3$H-uridine, *i.e.*, synthesized RNA (Fig. 1,a). The crust-located microbes were mostly enclosed in a cavity. These microorganisms not only incorporated $^3$H-uridine; many of them retained the capacity for multiplication (*i.e.*, they synthesized DNA) (Fig. 1,b).

A quite different picture was observed 10 days following the start of Levosin application. The surface of the wound was covered with granulation areas. The cellular elements were aranged more compactly and consisted mostly of fibroblasts, the number of which increased 4-7.5-fold, and of macrophages, the number of these increasing 2-3-fold. The number of vessels rose two- to threefold, in one observation even as much as 8 times (Fig. 2). PMNL appeared much less frequently than before treatment. There was a striking increase not only in the total number of fibroblasts, but also in the quantity of proliferating fibroblasts as well - 5-6 times over the initial level. Besides proliferation, the fibroblasts expressed a high functional activity, as evidenced from active nuclear RNA synthesis in many of them (Fig. 3,a) and, as a result, production of collagenous protein, which formed fragile fibers around the cells. As a rule, the proliferating (DNA-synthesizing) fibroblasts were localized near to the vessels (Fig. 3,b), which actively penetrated the granulation tissue. The vessel cells (endotheliocytes) also manifested a high functional and proliferative activity: the nuclear incorporation of $^3$H-uridine (RNA synthesis) and $^3$H-thymidine (DNA synthesis) was recorded (Fig. 3,b). DNA synthesis in the endotheliocytes attested to vascularization of the tissue.

The bacteriological data correlated with the results of the morphological quantitative analysis. The increment of macrophages, apparently related to the activation of phagocytic function, resulted in a decrease in the microbial population of the burn wound: 10 days following the start of Levosin treatment the number of microbes per gram of tissue decreased from $2\text{-}5x10^7$ to $6\text{-}8x10^6$. During this period of treatment, the granulation areas contained a negligible number of PMNL. The latter were characterized by extracellular degranulation, as multiple granules were seen in the intercellular space. Apparently, the application of Levosin ointment, which possesses antimicrobial activity, leads to the regulation of inflammation related to the release of active substances from the cells. It may be assumed that the extracellularly secreted enzymes exert a bactericidal and cytotoxic effect upon the microbes, thus also promoting a decrease in the microbial population of the burn wound. Commonly, wounds were infested with associations of various bacterial species: *Pseudomonas pyocyanea, Staphylococcus aureus, Bacillus coli,* and *Proteus.*

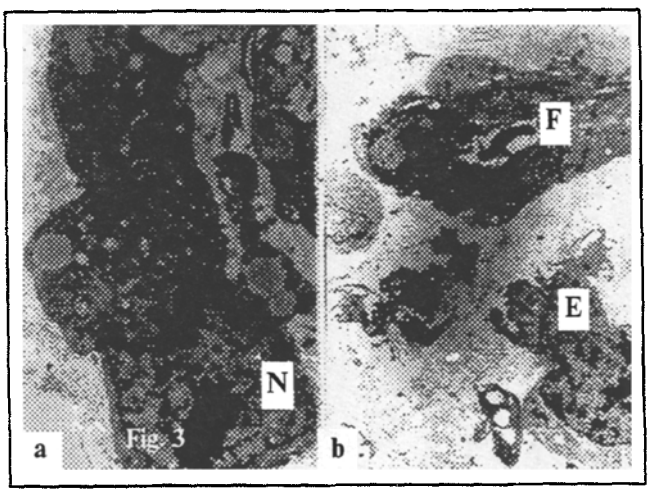


**Fig. 3.** Cells of burn wound granulation tissue 10 days following beginning of Levosin treatment. a) RNA synthesis in fibroblast nucleus (N) (black grains of silver), x7000; b) DNA synthesis (black grains of silver) in nuclei of endotheliocyte (E) and fibroblast (F), localized next to the capillary. Erythrocyte seen in the lumen (L) of the vessel.

Twenty days after the start of Levosin treatment, the granulation tissue was characterized by a further increase in the number of fibroblasts, on average twofold as compared to the former time, and 2-12 (in one observation 21) times over the initial numbers (*i.e.*, before treatment). The vascularization proceeded more gradually. The number of macrophages drastically fell, and the microbe count of the burn wound also decreased. Fibroblasts became the predominant cellular elements in the wound. The presence of multiple collagen fibers, interspersed with fibroblasts and vessels, reflected the maturity of the granulation tissue. The increase in the number of proliferating fibroblasts correlated with the increase of vascularization at all assayed periods. This is consistent with the hypothesis earlier suggested by D.S. Sarkisov et al. [5] regarding the histogenetic relationship between fibroblasts and small vessels of granulation tissue.

One should stress the difference between the courses of the wound process in various body areas of the same patient. Analysis of biopsy material obtained from three different injured sites of patient Ch. (upper arm, forearm, thigh) 20 days after the start of treatment with the ointment, revealed a marked drop in all studied cellular parameters at one particular site (upper arm). Bacteriological analysis also revealed only a slight decrease in microbial dissemination in this region (only by one order of magnitude). Thus, in this particular wound region a delay in healing was registered. This leads us to conclude that the regeneration process in extensive burn wounds is of a heterogeneous character.

Thus, electron-autoradiographic analysis has shown that topical application of Levosin ointment activates the healing of burn injuries, as demonstrated by an increase in the total quantity of fibroblasts and vessels of the granulation tissue, as well as activation of the functional and proliferative capacities of fibroblasts. In addition, the destruction of bacterial bodies and a decrease in their number take place.

## LITERATURE CITED

1. B.M. Datsenko, B.M. Kostyuchenok, I.M. Pertsev, and V.N. Kalinichenko, *Khirurgia*, No. 1, 136 (1984).
2. B.M. Datsenko, L.A. Blatun, I.M. Pertsev, *et al.* Local Treatment of Wounds [in Russian], (1991), p. 20.
3. V.N. Kalinichenko, Use of Levomycetin-Containing Multicomponent Ointments with a Primary Hydrophilic Component in the Treatment of Purulent Wounds [in Russian], Ph.D. Thesis, Khar'kov (1983).
4. M.I. Kusin and B.M. Kostyuchenok. Wounds and Wound Infection [in Russian], Moscow (1990).
5. D.S. Sarkisov, E.G. Kolokol'chikova and A.A. Pal'tsyn. *Byull. Eksp. Biol.*, No. 6, 730 (1987).
6. T.I. Tamm. Enhancement of Dioxidine Antibacterial Activity in the Local Treatment of Purulent Wounds [in Russian]. Ph.D. Thesis, Khar'kov (1984).
7. E.S. Reynolds. *J. Cell Biol.*, **17**, 208 (1963).

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# Changed Permeability of Cardiomyocyte Sarcolemma after Short-Term Total Ischemia

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Changes in the membrane system of myocardial cells [2,4] contribute much to the pathogenesis of postischemic involvement of the myocardium developing after heart and respiratory arrest and resulting in impairment of its contractility [3]. Sarcolemma structural integrity is known to determine to a great extent such characteristics of the myocardium as excitability, conductivity, and contractility, and it is of crucial importance for recovery of the structure and function of the ischemic cells [4,6,7]. Recent research has revealed the possibility of injury to the cell membranes under conditions of reperfusion and reoxygenation [2,4,9]. The status of the myocardial membranous system after total ischemia of the whole

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