dividuals in the frog population sample studied were almost equal, whereas the proportion of left-dominant individuals in the human populations is only between 1% and 7%.

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# Morphological Study of the Effects of Textile-Immobilized Enzymes on an Experimental Purulent Wound

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Morphological changes in an experimental purulent wound in a rat model is studied for application of surgical gauze with immobilized enzymes: trypsin, lysozyme, collitin, or co-immobilized trypsin and lysozyme. Comparison of the times of wound cleansing and healing shows that immobilized enzymes are more effective than native preparations, and the therapeutic effect of gauze with the enzyme complex is higher than that of gauze with individually immobilized enzymes. Morphological studies confirm that immobilized trypsin-lysozyme complex and collitin are the most efficient in hastening and potentiating reparative processes in a purulent wound.

**Key Words:** *purulent wound; healing; immobilized enzymes*

Proteolytic enzymes immobilized on naturally occurring materials have found a wide application in the management of purulent wounds. The therapeutic effects of trypsin immobilized on cellulose or polycaproamide (Dalcex-trypsin and Pax-trypsin, respectively) have been studied in detail [2,5]. New types of wound dressings with immobilized enzymes displaying complex therapeutic activity have been developed at the Institute of Textiles. Dalcex-trypsin-lysozyme is surgical gauze onto which the proteolytic enzyme trypsin and the bacteriolytic enzyme lysozyme are immobilized. This dressing has not only necrolytic but also bacteriolytic activity. Dalcex-collitin is gauze on which the proteolytic polyenzyme preparation collitin is immobilized. Collitin possesses trypsin-like, chymotrypsin-like, and elastase-like activities [4]. These materials have passed clinical trials; however, there is no available information regarding their effects on wound healing, and neither morphological studies nor comparison with other conventional dressings (for example, Dalcex-trypsin) have been performed.

This study is a morphological comparison of the efficiencies of different enzymes immobilized on surgical gauze in the treatment of an experimental wound.

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TABLE 1. Results of Treatment of Experimental Purulent Wounds (Each Series Included 25 Animals)

| Material and method of treatment | Time (days) of | | Acceleration of healing | |
|---|---|---|---|---|
| | wound cleansing | complete healing | days | % |
| Without treatment (control) | 14.9±0.81 | 27.2±0.21 | – | – |
| Surgical gauze (control) | 11.6±0.71 | 23.7±0.47 | 3.5 | 12.9 |
| Dalcex – trypsin | 7.1±0.52 | 19.1±0.52 | 8.1 | 29.8 |
| Dalcex – lysozyme | 7.1±0.29 | 19.3±0.35 | 7.9 | 29.0 |
| Dalcex – trypsin – lysozyme | 5.8±0.30 | 16.2±0.24 | 11.0 | 40.4 |
| Dalcex – collitin | 4.9±0.50 | 15.1±1.30 | 12.1 | 44.5 |
| Local application of 0.1% trypsin solution | 8.5±0.42 | 21.6±0.44 | 5.6 | 20.6 |
| Local application of 0.1% lysozyme solution | 7.2±0.26 | 21.5±0.53 | 5.7 | 20.9 |
| Local application of 0.1% collitin solution | 7.8±0.51 | 21.2±0.30 | 6.0 | 22.1 |

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150-200 g. Under hexenal anesthesia the fur was shaven in the scapular and neck areas. The area was swabbed with iodine, and a 2×2 cm piece of skin with the fascia was excised. A special stencil was used to outline the wound contours. The muscle base and edges of the wound were compressed with Kocher's clamp. For standardization of treatment and for the prevention of wound contraction due to its elasticity a plastic frame was sutured to the wound edges. A polyethylene "hood" was fixed on the frame to prevent drying and to facilitate the fixation of dressings. The wound was then flushed with 1 ml of suspension of a 24-h culture of pathogenic *S. aureus* (strain 209) containing 1 bln. microorganisms. Treatment was started 48 h after the development of purulent inflammation. Gauze soaked in normal saline was applied to the wound. During several days at the beginning of treatment the dressings were changed every day until the wound was completely clean. Gauze soaked with normal saline was then applied to the wound and changed daily. The following bandages were used for the treatment in the hydration phase: Dalcex-trypsin (proteolytic activity 0.2 PU/g material), Dalcex-lysozyme (bacteriolytic activity 100 U/g material), Dalcex-trypsin-lysozyme (proteolytic activity 0.2 PU/g material, bacteriolytic activity 100 U/g material), and Dalcex-collitin (proteolytic activity 0.2 PU/g material). Proteolytic and bacteriolytic activities were evaluated by hydrolysis of casein [1] and lysis of *Micrococcus lisodeicicus* [3], respectively. Surgical gauze or surgical gauze soaked in a 0.1% solution of the test enzymes (lysozyme, trypsin, or collitin) was used in the control groups. The times of wound cleansing from pus-necrotic detritus, times of complete wound healing, and the dynamics of the reparative process were estimated. Histological and histochemical studies were performed on biopsy material collected at different periods of wound healing. Sections (4-6 μ thick) were stained with hematoxylin and eosin or Van Gieson picrofuchsin, or impregnated with silver after Gomori. Glycosaminoglycans were visualized with toluidine blue, neutral mucopolysaccharides were revealed by the PAS reaction, and RNA was recovered with pyronine-methyl green after Brachet.

## RESULTS

Analysis of the times of wound cleansing from pus-necrotic detritus and the times of complete wound healing showed that immobilized enzymes are much more effective in the treatment of such a wound than are native enzymes. This is explained by the fact that native enzymes are rapidly inactivated and washed out by exudate, while immobilized enzymes are more stable and have a prolonged effect [6]. Dalcex-trypsin-lysozyme and Dalcex-collitin proved to be the most effective of the tested dressings. In contrast to Dalcex-trypsin and Dalcex-lysozyme, these materials have complex therapeutic activities: necrolytic and antibacterial (Dalcex-trypsin-lysozyme) and complex proteolytic (Dalcex-collitin). This complex activity allows the latter to more effectively degrade the necrotic elements in comparison with trypsin.

Histological and histochemical studies showed that by the 3rd-5th day reparative processes were slightly activated in animals treated with Dalcex-trypsin and Dalcex-lysozyme. A fairly thick leukocytic-fibrinous layer with scattered microbial colonies was preserved on the wound surface. Individual vertical blood vessels were seen in the forming granulation tissue. Tissue edema was pro-

nounced. Microabscesses and foci of secondary necroses were seen. The macrophagal reaction was weak. By the 3rd day, animals treated with Dalcex-trypsin-lysozyme, and especially those treated with Dalcex-collitin had developed a vigorous reparative reaction. Islets of granulation tissue with vertical blood vessels and actively proliferating fibroblasts were formed in the wound. The wound surface was becoming rapidly freed of tissue detritus. Tissue edema was becoming less pronounced. In animals treated with Dalcex-collitin a well-developed layer of fibroblasts was formed by the 5th day together with vertical blood vessels. Indications of vigorous synthesis of acid glycosaminoglycans and collagen were revealed in this layer. Signs of epidermis regeneration were evident at the edges of the wound. Microabscesses and secondary tissue necroses were much less frequent than in animals treated with Dalcex-trypsin and Dalcex-lysozyme.

Maturation of the granulation tissue was progressing mature by the 7th-10th day, particularly in the groups treated with Dalcex-trypsin-lysozyme and Dalcex-collitin. In the latter, the layer of horizontally oriented fibroblasts was rapidly growing, this being accompanied by the gradual collapse of the vessels and the disappearance of the layer of vertical vessels. The number of neutrophils in the lower layers of maturing tissue was decreasing, and fibroblasts prevailed, some of them converted to low-activity fibrocytes. Inflammatory alterations in granulation tissue were becoming less pronounced. There were no secondary necroses and abscesses. Most noticeable in these animals was "creeping" of the regenerating epidermis onto the surface of the granulation tissue.

On days 15-20 day remodeling of connective tissue progressed: the fibrous tissue became thinner, the number of macrophages in it increased, and metachromasia of the ground substance was intensified. By the 20th day the wound was completely epithelialized. In some animals treated with Dalcex-collitin complete epithelialization was achieved by the 15th day of treatment. Newly formed epidermis was confined to the PAS-positive basal membrane; epidermal cells were completely differentiated and no longer contained any glycogen granules. Fibrous tissue was located under the epidermis. Fuchsinophilic fibers prevailed with low-activity fibrocytes between them. Occasional neutrophils were seen under the newly formed epidermis. Vertical blood vessels were not detected.

Thus, Dalcex-collitin has the most potent therapeutic effect, while the effect of Dalcex-trypsin-lysozyme is weaker; less effective are Dalcex-trypsin and Dalcex-lysozyme.

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