# EXPERIMENTAL MORPHOLOGIC STUDY OF THE ACTION OF COLLAGENASE OF THE CRAB *Paralithodes camtschatica* ON WOUND HEALING

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Research into the effect of trypsin, chymotrypsin, terrilytin, and other proteases on wound healing has been published [4, 6, 7]. A new and highly active proteolytic enzyme, namely collagenase, has recently been isolated from the hepatopancrehs of the Kamchatka crab *Paralithodes camtschatica* [5, 8]. Crab collagenase (CC) had previously been successfully used to obtain cultures of endotheliocytes [3]. The aim of the present investigation was to study the morphologic features of the action of CC on experimental wound healing.

## EXPERIMENTAL METHOD

Experiments were carried out on models of septic wounds in noninbred male rabbits weighing 2500 g. The wounds were formed as follows: after depilation and treatment of the skin, full-thickness flat skin wounds 2.5 cm in diameter were inflicted under local anesthesia in the animal's dorsal region. The wound edges were sutured circumferentially to the underlying tissues and the floor of the wounds was incised in order to obtain regions of ischemic necrosis. After 3 days, $10^9$ bacterial cells of the 24-h culture of *Staphylococcus aureus* was introduced into the wounds.

The test preparations began to be applied to the resulting septic-necrotic foci after another 3 days. Various methods of applying proteolytic enzymes to wounds are known. In the present investigation, cellulose gauze and the polyvinyl sorbent Gelevin were used for this purpose. The animals as a whole were divided into eight groups, with six rabbits in each group. In group 1 (control) enzyme therapy was not given. In the rabbits of group 2, 0.25 mg of native CC was applied to the wounds by soaking towels consisted of three layers of gauze and measuring 4 $cm^2$ with a solution of the enzyme. Animals of groups 3, 4, and 5 received applications of enzyme with the aid of 50 mg Gelevin, which was sprinkled on the wound surface, and saturated with solutions of CC containing 0.25, 0.05, and 0.75 mg of the enzyme. The same quantities of CC on Gelevin were applied to the rabbits of groups 6, 7, and 8, but the wounds of these animals were not infected. Signs of inflammation on the dressings were assessed and planimetric investigations carried out. On the 3rd, 5th, 9th, and 14th days pieces of tissue 3-5 mm thick were taken from the floor of the wounds, fixed in 10% formalin solution, and embedded in paraffin wax. Histologic sections 7 $\mu$ thick were stained with hematoxylin-eosin and picrofuchsin.

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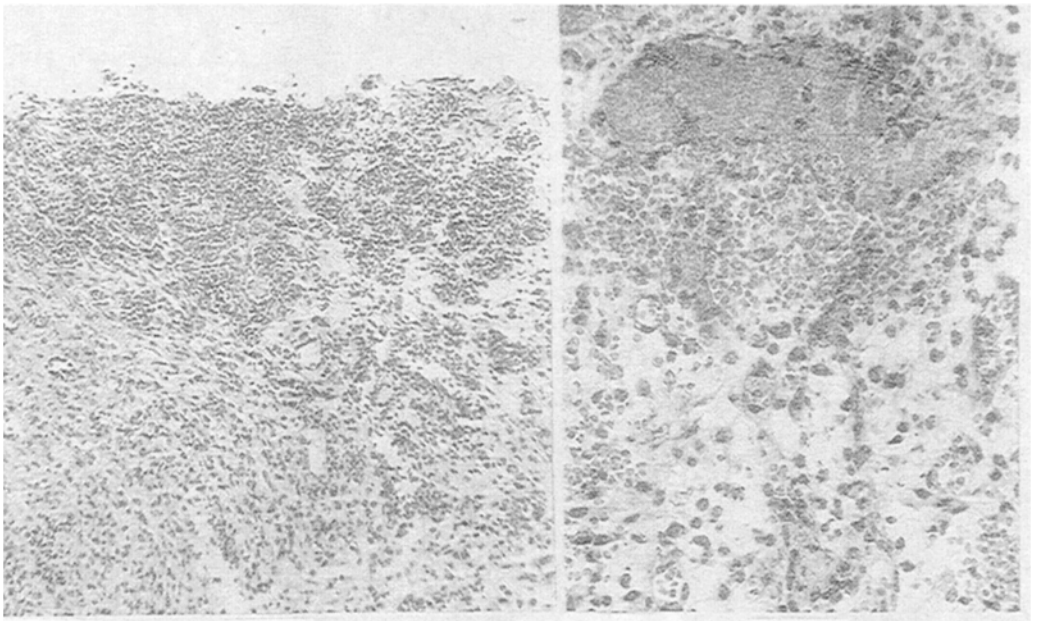

Fig. 1



Fig. 2

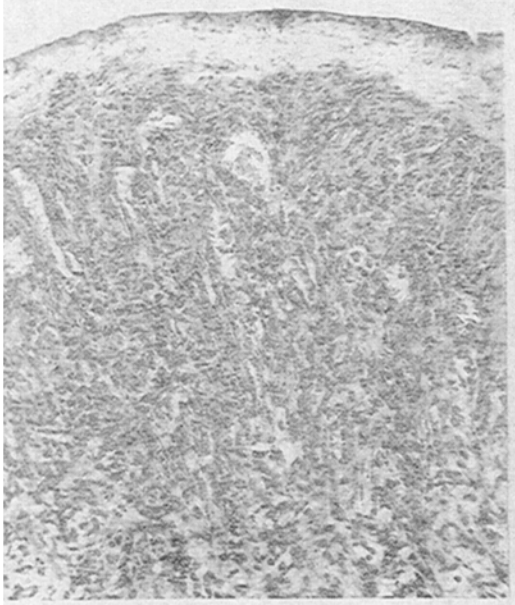

Fig. 3

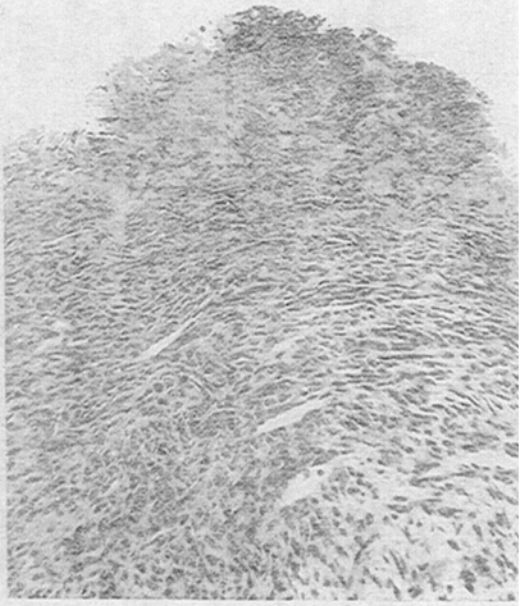

Fig. 4

Fig. 1. Congestion of blood vessels of granulation tissue and diapedesis of erythrocytes after exposure of 3 days to 0.25 mg collagenase and to gauze on septic wound. Hematoxylin-eosin; 90×.

Fig. 2. Fibrin thrombiin lumen of capillaries of granulation tissue on 9th day of continuous exposure to 0.25 mg collagenase and gauze on septic wound. Hematoxylineosin; 160×.

Fig. 3. Drying of surface layers of granulation tissue on 5th day of daily applications of 0.75 mg collagenase, with Gelevin present on septic wound. Hematoxylin-eosin; 90×.

Fig. 4. Increased cell density of granulation tissue due to fibroblasts and fibrocytes after 3 days of exposure to 0.05 mg collagenase and Gelevin applied to aseptic wound. Hematoxylin-eosin; 90×.

TABLE 1. Contraction of Experimental Wounds after Application of Crab Collagenase and Gelevin

| Quantity of collagenase applied to wound, mg | Contraction of area of wound after begin | | | |
|---|---|---|---|---|
| | on 9th day | | on 14th day | |
| | septic wound | aseptic wound | septic wound | aseptic pound |
| 0,05 | 59,3 | 75,4 | 84,7 | 96,2 |
| 0,25 | 79,5 | 56,7 | 91,3 | 95,1 |
| 0,75 | 88,7 | 35,3 | 99,4 | 72,2 |

## EXPERIMENTAL RESULTS

Before application all the wounds were characterized by inflammation with the classical clinical and morphologic features. When CC was applied to the gauze during the 1st redressing of two animals, on the 3rd day, partial cleansing of the wounds from the debris covering them, congestion of the granulations, and the appearance of marginal epithelization were observed. Histologic investigation after the enzyme had been present on the floor of the wounds for 3 days revealed granulation tissue without edema and with focal infiltration with polymorphonuclear neutrophils, and covered in places with septic-necrotic debris. Marked congestion of blood vessels with signs of diapedesis of erythrocytes also was observed, possible evidence of the improvement of the transport function of the microcirculatory system in the floor of the wounds (Fig. 1). Compared with the control, many young and mature fibroblasts were observed in the sections. Thereafter the wounds were dressed every 2-3 days. Complete cleansing of the wounds occurred by the 9th day, after which applications of CC were discontinued. The area of the wound surfaces at this time was reduced by 71%, and on the 14th day by 76% of its original value. Histologically, on the 9th day the granulations in the upper layer were clean and juicy, their vessels were congested, with diapedesis of erythrocytes in some places. Extensive epithelization of the wound edges was observed. The inflammatory infiltration was focal in character. Fibrin thrombi were found in the lumen of some capillaries, possibly as a result of prolonged exposure to CC (Fig. 2). In the remaining animals of this group, dressing was carried out daily. In this case cleansing of the wounds occurred on the 5th-6th days. Contraction on the 9th and 14th days amounted to 74 and 85% respectively. A study of sections of the floor of the wounds on the 5th day as a rule showed complete cleansing. The surface of the granulations was juicy due to congestion of capillaries and diapedesis of erythrocytes. After discontinuation of CC application, further maturation of granulation tissue was observed on the 9th day, with predominance of mature over young fibroblasts and with active synthesis of collager in the absence of any visible reaction of the microvascular bed.

By contrast, on the wound surface ln animals of the control group, an area of necrosis remained for a long time firmly bound with the underlying tissues, both clinically and morphologically. On the 9th day contraction of the wounds amounted to only 37%, and on the 14th day to 54% of the initial area. The results thus indicate that CC is a highly effective agent for enzymic wound cleansing. It was observed that, unlike other proteases, under the influence of which necrolysis of the wound debris took place tangentially, under the influence of CC the debris as a rule separated from the wound surface in the form of a continuous layer. In our opinion the phenomena observed in the capillary bed require further study.

As a dressing material gauze is known to be traumatic and to be deficient in sorptive properties [7]. In the search for a more active and less traumatic material, we tried Gelevin [4], one of a new generation of absorbent dressing materials, and studied the effect of CC on wound healing in its presence. During daily applications of 0.25 mg CC to wounds, a more marked healing effect was noted than after application of the same quantity of the enzyme together with gauze. Cleansing of the wounds from septic-necrotic deposits took place by the 4th-5th day, accompanied by quite active contraction of the wounds on the 9th and 14th days (Table 1). In sections on the 5th day dried granulation tissue was observed, distinguished by an increased cell density on account of fibroblasts and many collagen fibers. This phenomenon can evidently be explained by the much higher absorbent activity of Gelevin than

of gauze, and also by the very slight degree of exudation from the experimental septic wounds in rabbits. Moderate diapedesis of erythrocytes also was observed outside the boundaries of the vascular bed, and due to the effect of CC.

To determine the optimal quantity of CC necessary for effective necrolysis, the action of different concentrations of protease on the septic wound was studied. With the daily applications of 0.75 mg of the enzyme cleansing of the wounds occurred as early as on the 2nd-3rd day. Treatment was continued until the 5th day. At this time contraction was already considerable, and on the 14th day the wounds were completely healed. Incidentally, immediately after cleansing of the floor of the wounds from debris drying of the wounds began on the 3rd day, and on the 5th day the upper layer of granulations had dried completely with the formation of foci of coagulation necrosis (Fig. 3), but nevertheless, this did not disturb the favorable course of wound healing. Marked congestion of capillaries also was recorded histologically on the 3rd day, with diapedesis of erythrocytes on the 5th day, with the formation of hemorrhages. On the 9th-14 day signs of necrobiosis of the surface layer of the granulations disappeared and the microcirculatory changes were reduced in intensity. On application of 0.05 mg CC to the wound surface cleansing of the wounds began rather later, on the 7th-8th day, and contraction of the area of the wounds took place more slowly. Dehydration of the granulations and changes in the microcirculation were not present.

To assess the effect of CC on the 2nd phase of wound healing, its action on granulation tissue formation in aseptic wounds was studied. In these observations, by contrast with the previous series of experiments, the quantity of CC introduced into the wound showed negative correlation with repair. For instance, the earlier (on the 2nd-3rd day) appearance of granulations and more energetic contraction were observed in wounds to which a smaller quantity of enzyme was applied (Table 1). Histologically, in sections through the floor of the wounds of these animals, mature granulations with many fibroblasts, fibrocytes, and much collagen could be observed as early as on the 3rd day, but with a very small number of vertical vessels (Fig. 4). In wounds to which 0.75 mg of CC had been applied on Gelevin, granulations appeared later (on the 5th-7th days), they were pale in color, poorly vascularized, with considerable drying of the surface layers and increasing edema of the deep layers. Long-term application of collagenase also promoted the vascular changes described above, and their severity depended on the content of CC in the dressing.

Collagenase of the crab *P. camtschatica* is thus a highly effective remedy for cleansing septic wounds. The use of Gelevin as carrier, compared with gauze, greatly enhances the therapeutic activity of the enzyme. In order to obtain a maximal necrolytic and cleansing effect on wounds and to minimize the inhibitory effect of CC of newly formed granulation tissue, it is recommended that the dose of the protease be reduced as the wounds become cleansed of necrosis. It can be concluded from these results that for clinical use of CC, the indications for and times of collagenase wound therapy must be determined on a strictly individual basis.

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