Immunohistochemical Study of Purulent Wounds in the Rat after Application of Collagenase Isolated from the King Crab *Paralithodes Camtschatica*

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A high proteolytic enzyme activity elicited in the hepatopancreas of the king crab was demonstrated previously [4]. Elastase and two isoforms of collagenolytic protease proved to be the enzymes responsible for the proteolytic activity in this tissue [5,6]. These proteases were purified to homogeneity by chromatographic techniques, which allowed us to examine their molecular and catalytic properties [7,8,11].

A higher necrolytic activity of enzyme preparation isolated from the king crab (compared with that of trypsin, terrilytin, carypazim, etc. [3]) stipulated its use in the treatment of purulent wounds [1]. A comparison between the biological activity of the entire enzyme complex and its components has shown that isoforms A and C of collagenolytic protease play the major role in wound debridement. The enzyme complex was therefore designated crab collagenase. Here we report the results of an immunohistochemical study of experimental purulent wounds after application of crab collagenase.

MATERIALS AND METHODS

Collagenase was originally isolated from the hepatopancreas of the king crab *Paralithodes camtschatica* as described [2]. Polyclonal antibodies to the isozymes of crab collagenase were obtained after immunization of rabbits with homogeneous enzyme preparation in complete Freund's adjuvant. Anti-fibrinogen goat polyclonal antibodies were from Sigma. Murine monoclonal antibody IST-9 recognizing the ED-A sequence specific for cellular fibronectin was a generous gift of Dr. L. Zardi and anti-laminin polyclonal antibodies were kindly supplied by Dr. H. K. Kleinman. Antibodies to type I collagen of rats and plasma fibronectin of humans were affinity purified on collagen- and fibronectin-Sepharose, respectively, after rabbit immunization [10].

Purulent wounds were modeled in nonlinear albino rats (n=18) weighing 180-220 g. Animals were anesthetized by ether inhalation, and wounds were produced after depilation by incising a 2 cm^2 piece of skin. The wound was then left for 3 days without dressing until the granulation tissue formed. The wound bottom was cut with a razor blade and inseminated with 10^6 *Staph. aureus* (a 24-h culture, under an occlusive dressing). Crab collagenase preparation was applied on day 3 after the procedure. For convenience, the enzyme solution was preincubated with 50 mg Gelevin and applied as gel. The rats were divided into 3 groups, 6 animals in each. Group I animals were not treated with crab collagenase. Group II animals were treated with 250 μl of the enzyme solution (collagenase concentration 3 mg/ml) for 3 days: gel with detritus was removed with a moist swab, and

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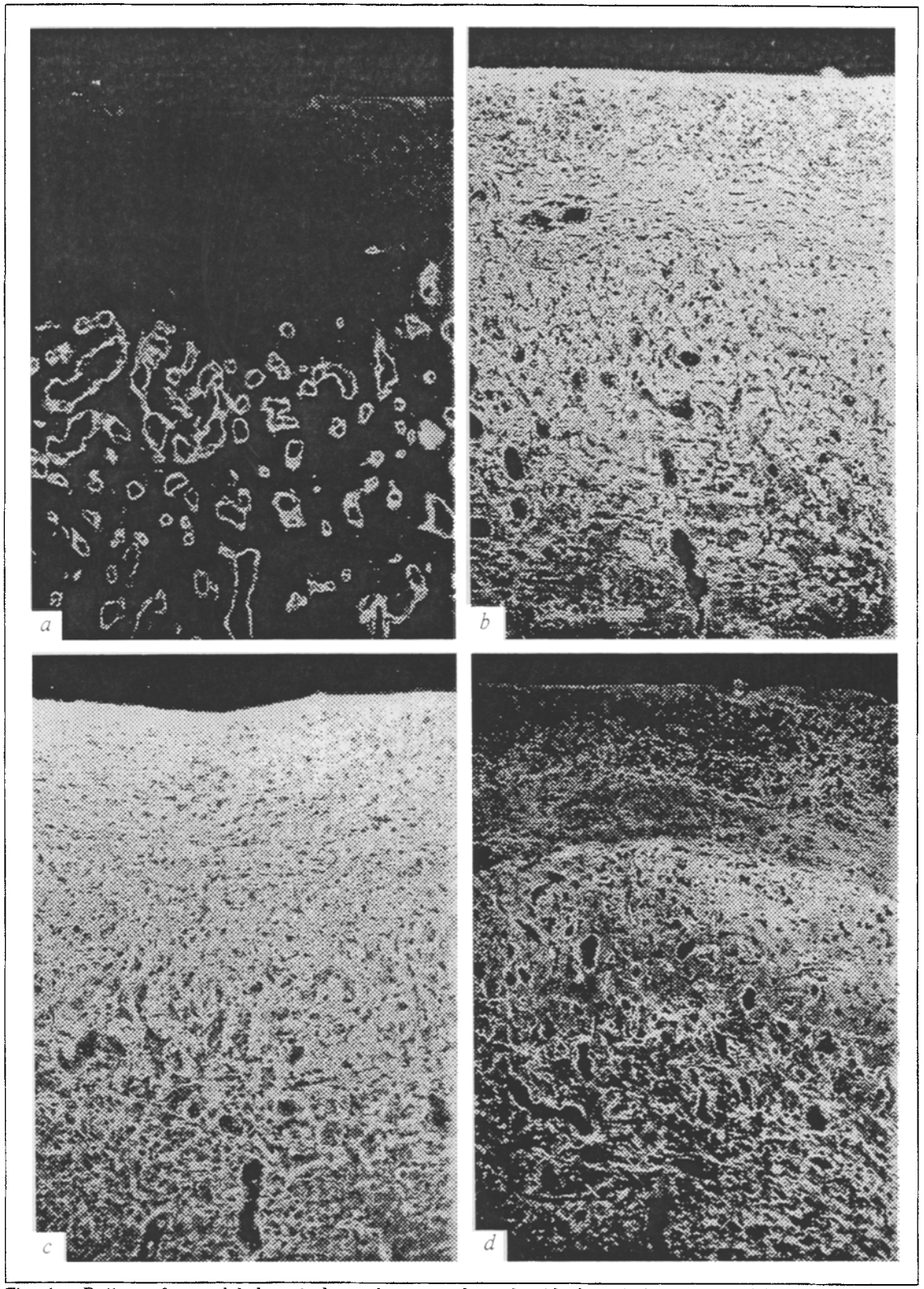

Fig. 1. Bottom of a modeled control purulent wound on the 4th day of observation. *a*) laminin in dilated capillaries of the granulation tissue: a thick necrotic layer is seen on the wound surface; *b*) fibrin in necrotic detritus and granulations; *c*) massive deposits of plasma fibrin in the granulation tissue; *d*) insignificant amounts of cellular fibronectin in granulations *in situ*. Immunofluorohistogram, ×100.

a new gel was applied to the wound. On day 4 the rats of groups I and II were sacrificed. Group III animals were sacrificed on day 2 after a single application of the same enzyme dose.

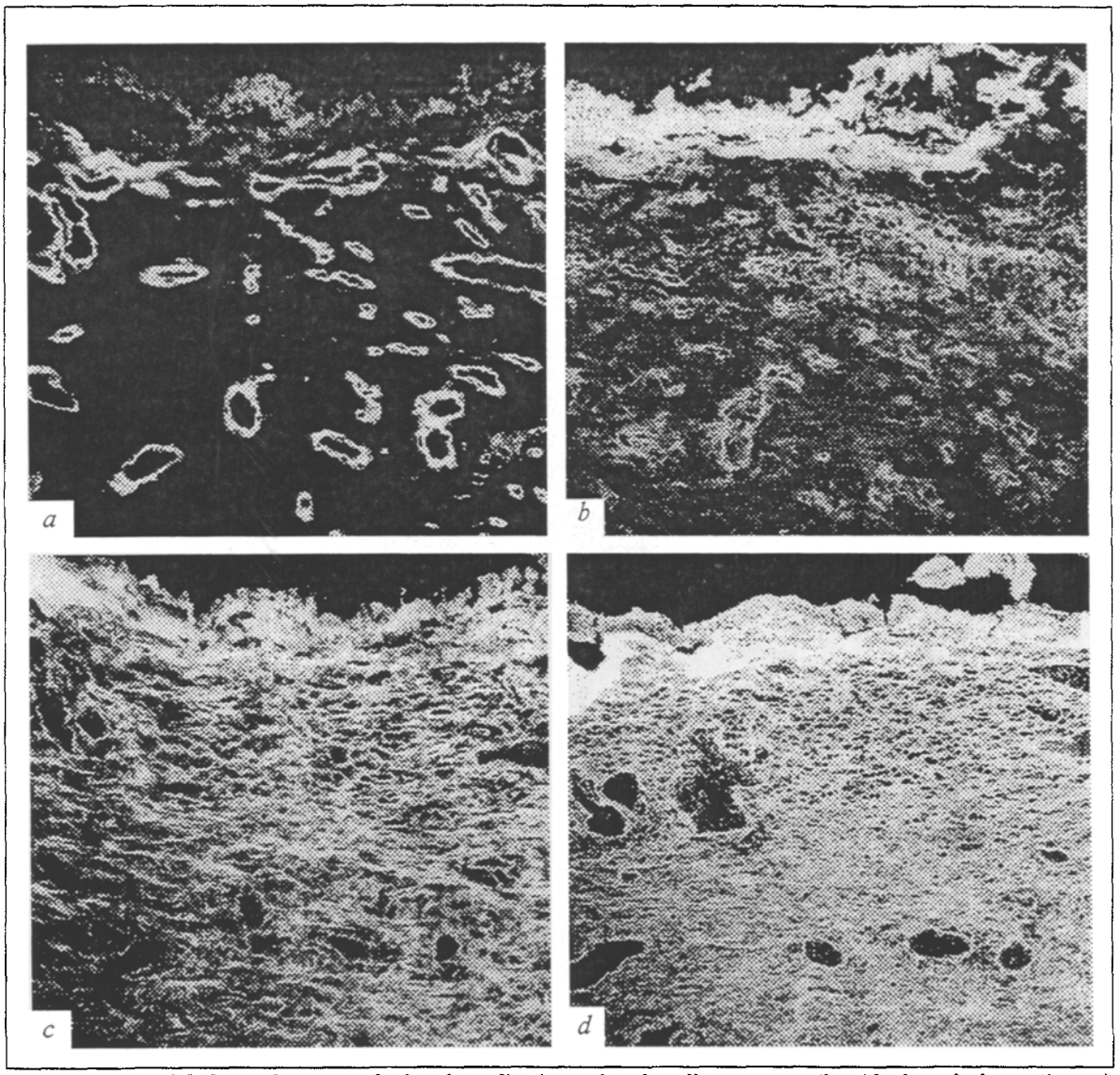

Fig. 2. Bottom of a modeled purulent wound after 3 applications of crab collagenase on the 4th day of observation. *a*) laminin in the surface layer of granulations; *b*) thin layer of fibrin located predominantly on the wound surface; *c*) considerable amounts of cellular fibronectin in the granulation tissue *in situ*; *d*) type I collagen in the granulation tissue. Immunofluorohistogram, ×100.

Immunohistochemical study was performed on nonfixed 5-μ thick cryostat sections. Sections were incubated with primary antibodies for 1 h at room temperature, washed with phosphate-buffered saline (PBS) for 30 min, and treated for 45 min with biotinylated antibodies to mouse, rabbit, or sheep IgG. After washing, the sections were incubated with FITC-conjugated streptavidin, washed with PBS for 45 min, and examined under an Orthoplan fluorescent microscope. Some sections were fixed with formaldehyde and stained with hematoxylin and eosin.

RESULTS

Previously it was shown that crab collagenase provides for a rapid debridement of purulent wounds [1]. The mechanism underlying this phenomenon is characterized in greater detail with the aid of immunohistochemical methods. On day 4, dilated capillaries located under a thick layer of necrotic detritus were identified with anti-laminin antibodies in the granulation tissue of collagenase-treated wounds (Fig. 1, *a*). Massive deposits of fibronectin and fibrin were seen in the detritus layer and granulation tissue (Fig. 1, *b, c*). As demonstrated with the use of the monoclonal antibody IST-9, *in situ* granulations contained small amounts of fibronectin, which reflected a moderate cellular synthesis of this protein (Fig. 1, *d*).

After application of crab collagenase, in group II animals necrotic detritus was readily removed from the granulation tissue. In contrast to the case with control rats, newly formed capillaries were

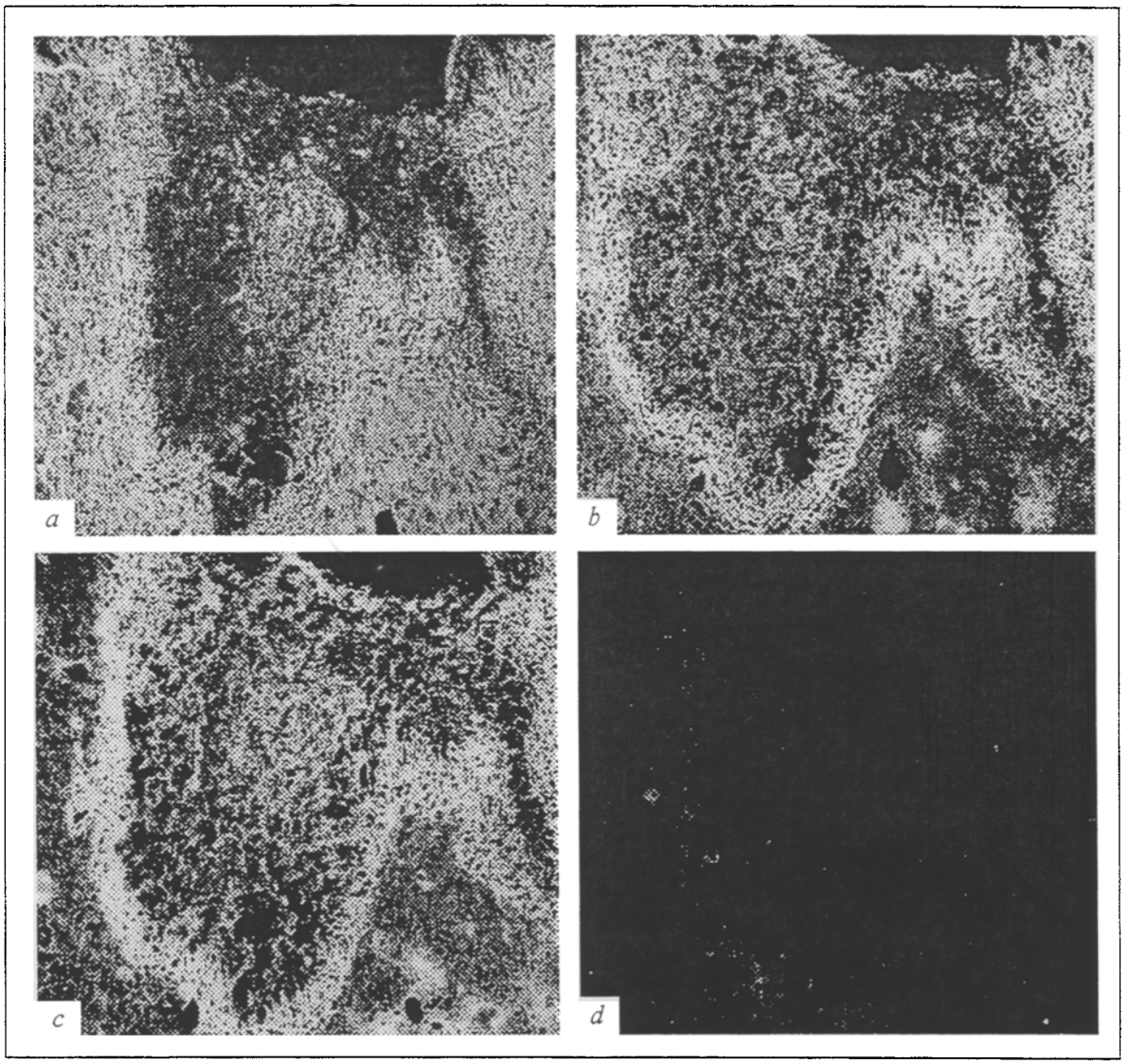

Fig. 3. Bottom of a modeled purulent wound after a 24-hour exposure to crab collagenase. *a*) fibrin in the granulations under the necrotic tissue layer; *b*) isozyme A is identified predominantly in the surface fibrin layer at the border with necrosis; *c*) analogously localized isozyme C is identified; *d*) no specific fluorescence is seen in the control section after replacement of immune sera with nonimmune rabbit IgG. Immunofluorohistograms. ×100.

localized throughout the entire thickness of the granulation tissue right up to the wound surface (Fig. 2, *a*). Extremely small amounts of fibrin were identified as a thin layer on the surface of vascularized granulations (Fig. 2, *b*). This fact of fibrin lysis on the wound bottom and surface tissues is of paramount importance, since the proinflammatory effect of fibrin and the necessity of fibrinolysis are well documented [9,12]. Cellular fibrin was identified both with polyclonal antibodies and monoclonal antibody IST-9 recognizing the ED-A sequence, which indicated an intense synthesis of this protein *in situ* (Fig. 2, *c*). The amount of type I collagen in the extracellular matrix of the granulation tissue was consistent with the stage of maturation of the tissue (Fig. 2, *d*).

Additional immunochemical studies were undertaken to find out how deep crab collagenase penetrates into the wound tissues during 24 hours. Sections cut from collagenase-treated wounds were successively incubated with anti-fibrin and anti-isozyme A and anti-isozyme C antibodies. As expected, the detritus on the wound surface, particularly at the border with necrotic tissue, contained considerable amounts of fibrin (Fig. 3, *a*). Both isozymes of collagenolytic protease were localized in this area, i.e., they were co-localized with fibrin (Fig. 3, *b, c*).

Under the action of crab collagenase on modeled purulent wounds the wound surface is almost entirely freed of the fibrin-containing necrotic detritus, and rapidly and the properly purified wound

bottom is well prepared for the next stage of the wound healing process. At the same time, during a 24-hour exposure the enzyme does not penetrate into granulations and, consequently, has no negative effect on the newly formed tissues. The results obtained indicate that these processes are responsible for the potent therapeutic effect of the crab collagenase preparation.

REFERENCES

1. A. A. Adamyan, S. P. Glyantsev, I. Yu. Sakharov, and T. V. Savvina, *Byull. Eksp. Biol. Med.*, **114**, № 12, 660-663 (1992).
2. A. A. Artyukov and I. Yu. Sakharov, Patent № 4806987 (1991).
3. S. P. Glyantsev and A. G. Annaev, in: *Intensive Care of Severe Burns* [in Russian], Moscow (1992), pp. 67-69.
4. F. E. Litvin, A. A. Artyukov, and I. Yu. Sakharov, in: *Biologically Active Compounds of Hydrobionts - Novel Therapeutic, Therapeutic-Preventive, and Technological Preparations* [in Russian], Vladivostok (1991), pp. 8-9.
5. I. Yu. Sakharov, F. E. Litvin, A. A. Artyukov, and N. N. Kofanova, *Biokhimiya*, **53**, № 111, 1844-1849 (1988).
6. I. Yu. Sakharov, A. V. Dzhunkovskaya, N. V. Zakharova, and A. A. Artykov, in: *Biologically Active Compounds of Hydrobionts - Novel Therapeutic, Therapeutic-Preventive, and Technological Preparations* [in Russian], Vladivostok (1991), pp. 8.
7. I. Yu. Sakharov, F. E. Litvin, and A. A. Artyukov, *Biokhimiya*, **57**, № 1, 40-45 (1992).
8. I. Yu. Sakharov and F. E. Litvin, *Ibid.*, pp. 61-69.
9. M. Aberg, U. Hedner, S. Jacobsson, and U. Rothman, *J. Plast. Reconstr. Surg.*, **10**, 103-105 (1976).
10. G. L. Idelson, V. R. Muzykantov, E. E. Cheknena, A. A. Shnyra, B. V. Shekhonin, and S. P. Domogatskii, *Collagen Rel. Res.*, **7**, 383-397 (1987).
11. I. Yu. Sakharov and F. E. Litvin, *Comp. Biochem. Physiol.*, **97B**, 407-410 (1990).
12. H. Wokalek and R. Heidner, *Errr. Med. Pract.*, **5**, 35-42 (1984).

Lipid Spectrum of the Skin in Psoriasis

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Lipids determine cell sensitivity to hormones and other biologically active substances [5]. Disturbances of lipid metabolism are observed in psoriasis [1], but the published data are incomplete and contradictory.

Comparative analysis of the lipid spectrum of both intact and pathologically altered skin in dermal psoriasis may be used for elucidating the individual pathogenetic stages, as well as for the development of effective and goal-directed treatment of this severe and puzzling disease. The aim of the present research was to study the lipid composition along with the activity of one of the enzymes of lipid metabolism, phospholipase A_2 (PLA-2), in the skin of patients with psoriasis.

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MATERIALS AND METHODS

Biopsy specimens obtained from psoriatic plaques (PP) and from visually unaltered portions of the skin (VUS) of patients with psoriasis served as the object of investigation.

Lipids were extracted from skin homogenate [4] after Folch [8]. The fractional content was determined by thin-layer chromatography in the

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