proteins on HAP columns. The 'releasing' activity of sodium bisulfite can be explained by its inhibiting a thiol protease insensitive to PMSF which produces protein fragments tending to form insoluble complexes. In spite of the absence of $NaHSO_3$ throughout the preparation of cell nuclei and chromatin we were able to recover the chromatin proteins almost completely, which would suggest that the protease is activated only when the chromatin complex has been dissociated.

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Proteases of Antarctic krill – a new system for effective enzymatic debridement of necrotic ulcerations

L. Hellgren, V. Mohr* and J. Vincent

*Department of Dermatology, University of Umeå, S-901 85 Umeå (Sweden), and *Department of Biochemistry, Norwegian Institute of Technology, University of Trondheim, Trondheim (Norway), 11 July 1985*

Summary. The Antarctic krill *(Euphausia superba)* possesses an 'over-dimensioned' digestive system, which is of vital importance for the survival of this euphaucean shrimp in the extreme marine environment. The isolated enzymes contain a well-balanced mixture of both endo- and exopeptidases, assuring fast and complete breakdown of proteinaceous material. These unique properties have now been shown to be extremely valuable for the effective removal of necrotic debris, fibrin or blood crusts in vitro. Therefore the krill enzymes should be considered as an important resource in the future management of necrotic wounds.
Key words. Antarctic krill *(Euphausia superba)*; proteases; enzymatic debridement in vitro.

Tissue necroses in secondary ulcers due to skin damage of diverse etiology such as burns, leg ulcers, decubitus is a common clinical problem. Although many factors are important in the healing of wounds, there is no doubt that the correct strategy in determinig the appropriate therapeutical approach must be first of all to attempt to identify and treat the main cause of the ulcers as well as the other underlying, negative factors. Adequate treatment of the systemic factors and disorders causing deterioration is thus a prerequisite for successful local wound care.

The local treatment of leg ulcers includes not only the removal of factors preventing healing (necroses, pus, fibrin, infections, etc.) but also the addition of those that improve the milieu of the healing wound, such as moisture.

The devitalized material, such as necroses, pus, blood crusts and fibrin, which accumulates in the wound delays the healing process. Therefore, one of the most important objectives of topical treatment is a rapid and efficient removal of necrotic debris, pus and fibrin so that a normal sequence of events, such as granulation and reepithelialization can take place.

Initially, the ulcerated area can be debrided either enzymatically or surgically. The main proteolytic enzyme preparations available at present in Scandinavia are stabilized crystalline trypsin (Trypure®, Novo), streptokinase-streptodornase (Varidase®, Lederle), bovine fibrinolysin combined with deoxyribonuclease (Elase®, Park-Davis) and collagenase (Iruxol®, Knoll). All of these enzyme preparations differ in their effectiveness as regards different substrates. However, it is generally agreed that their effect is not sufficient to permit a rapid debridement of the kind which is desirable in modern wound-care.

Previously we have reported in vitro and in vivo observations regarding the effect of stabilized, crystalline trypsin (Trypure®, and streptokinase-streptodornase (Varidase®) as debriding agents[1,2]. In connection with this work we also screened other enzymes of animal, bacterial and plant origin including some enzyme preparations of marine origin. One of these, obtained from Antarctic krill *(Euphausia superba)* was shown to possess outstanding debriding properties.

Krill is a marine crustacean which occurs in very large numbers in the Antarctic region. The common Antarctic krill, *E. superba*, attains a length of approximately 60 mm in the adult stage and lives at temperatures approaching that of freezing sea water. The distribution and biology of Antarctic krill have been reviewed by Everson[3]. Krill has developed a particularly efficient digestive

Table 1. The influence of proteolytic enzymes on different substrates originating from leg ulcers. The effects are expressed as percentages of the initial values (dry weight). The figures within brackets represent intermediate stages

– – – –	Weight decrease	76–100%
– – –	Weight decrease	51–75%
– –	Weight decrease	26–50%
–	Weight decrease	1–25%
0	No change	0%

Table 2. The influence of trypsin (Trypure®), streptokinase-streptodornase (Varidase®), krill enzymes and saline (control) on blood crusts isolated from leg ulcers. The data (dry wts) are expressed as percentages of the initial values, and ranked according to the scale in table 1

Enzyme	Concentration mg/ml	Time of exposure (h) 1	2	4
Trypsin	1	–	– –	– – (–)
	5	– –	– – (–)	– – –
	10	– – –	– – –	– – – –
Streptokinase-streptodornase	1	0	(–)	–
	5	(–)	–	–
	10	(–)	(–)	– (–)
Krill	1	– –	– – –	– – – (–)
	5	– – (–)	– – –	– – – –
	10	– – – (–)	– – – –	– – – –
Saline		0	(–)	(–)

system which ensures rapid food utilization and deposition of energy reserves during the short season when food is plentiful in the Antarctic. It has been demonstrated by several research groups that krill contains an array of proteolytic enzymes, including endo- and exopeptidases, effecting extensive breakdown of protein, with a consequent release of free amino acids in high yields[4,5].

The peptide hydrolases of krill are easily obtained by extraction in aqueous media, and can be further purified by chromatographic methods. The presence of both endo- and exopeptidases have been demonstrated, including trypsin-like activity, carboxypeptidase A and B and aminopeptidase (for review see Osnes[6]).

This paper aims to present some basic data suggesting that krill proteases may represent a unique enzyme composition which is highly suitable for the debridement of skin ulcerations.

Material and methods. Krill enzymes: Antarctic krill *(E. superba)* was obtained from commercial catches. The krill was frozen on board the vessel and kept at −20 °C. A preparation of krill peptide hydrolases was isolated in the form of an aqueous extract which was defatted, gel chromatographed and subsequently freeze-dried.

Debridement properties of krill enzymes in vitro: Materials originating from acute leg ulcers were used as substrates: necroses, fibrin and blood crusts. The materials were dissected into small pieces of similar weight and pre-weighed. The pre-weighed material was then introduced into test tubes containing krill enzymes at different concentrations. For comparison dilutions of stabilized, crystalline trypsin (Trypure®, Novo) and streptokinase-streptodornase (Varidase®, Lederle) were used. Physiological saline solution served as control. For each enzyme concentration at least seven replicates were run. The test tubes were incubated at +33 °C, and the readings were performed after various periods of time. The gravimetric evaluation of the changes in wet/dry wts was made using a high-precision balance (Mettler), connected to a computer. The changes were expressed as a percentage of the initial values. The effects were assessed according to a ranking scale (table 1).

Results and discussion. Removal of necrotic tissue from the wound surface is one of the basic factors in the healing process. The use of proteolytic enzymes as debriding agents diminishes the risks associated with surgery, such as enlargement of the ulceration area or destruction of viable adjacent tissue. However, the cleansing properties of the present enzymatic debriding agents are insufficient, resulting in incomplete or delayed debridement of the ulcer surface. Consequently the development of granulation tissue is delayed and the treatment period becomes unacceptably long.

Our study, carried out on materials originating from leg ulcers, provides clear evidence that krill enzymes are superior to crystalline trypsin (Trypure®) and streptokinase-streptodornase (Varidase®) on all substrates tested.

The figures are based on dry weight determinations, so as to obtain data which are independent of the hydration of the tissue. Wet weights, especially in the initial stage of the exposure of the samples to enzymes, reflect different degrees of tissue hydration and may not always correlate with the dry weight figures.

The blood crusts were rather rapidly dissolved by all enzymes tested. However, the krill enzymes, followed by trypsin (Trypure®) had the most pronounced effect. The degrading capacity of streptokinase-streptodornase (Varidase®) was rather weak (table 2). A similar pattern was observed for the next substrate examined: fibrin. This substrate was decomposed only to a limited extent by streptokinase-streptodornase, while krill enzymes, and also trypsin, showed a high fibrinolytic activity (table 3). Further biochemical comparisons of krill (as an enzyme mixture containing trypsin-like enzymes) and Trypure® (as pure trypsin) determined with casein as a substrate, confirmed the superior degrading properties of krill enzymes (unpublished data).

The decomposition of necrotic material was followed for a period of up to 24 h. There is no doubt that even after 6–12 h krill enzymes were superior to both enzymatic preparations tested (table 4).

Thus, the results presented show that the digestive enzymes of krill provide a highly effective system for wound debridement. The krill enzymes are distinctly more effective than the preparations previously employed for enzymatic debridement, which are based on the application of a single or a few enzymes with a limited debridement effect. The effectiveness of krill enzymes is probably due to a cooperative action of the naturally occurring endo- and exopeptidases in the digestive system of the krill, which are specifically designed to accomplish a complete breakdown of proteinaceous biological material.

Further studies and clinical experiments are needed to confirm the unique efficacy of krill enzymes for rapid and efficient cleansing of necrotic tissue.

Table 3. The influence of trypsin (Trypure®), streptokinase-streptodornase (Varidase®), krill enzymes and saline (control) on fibrin isolated from leg ulcers. The data (dry wts) are expressed as percentages of the initial values, and ranked according to the scale in table 1

Enzyme	Concentration mg/ml	Time of exposure (h) 1	2	4
Trypsin	1	−−	−−−	−−−(−)
	5	−−−	−−−(−)	−−−−
	10	−−−	−−−−	−−−−
Streptokinase-streptodornase	1	0	0	0
	5	0	0	(−)
	10	0	(−)	−
Krill	1	−−	−−−(−)	−−−−
	5	−−−	−−−−	−−−−
	10	−−−(−)	−−−−	−−−−
Saline		0	0	0

Table 4. The influence of trypsin (Trypure®), streptokinase-streptodornase (Varidase®), krill enzymes and saline (control) on necrotic material isolated from leg ulcers. The data (dry wts) are expressed as percentages of the initial values, and ranked according to the scale in table 1

Enzyme	Concentration mg/ml	Time of exposure (h) 6	12	24
Trypsin	1	0	0	−
	5	(−)	−	−(−)
	10	(−)	−−	−−−
Streptokinase-streptodornase	1	0	0	(−)
	5	0	(−)	(−)
	10	(−)	−	−(−)
Krill	1	(−)	(−)	−
	5	−	−(−)	−−
	10	−(−)	−−(−)	−−−
Saline		0	0	(−)

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