EPIDERMAL PROTEASE*

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The object of this work was to discover something of the distribution of protease in the skin, and to work out a method for demonstrating proteolytic activity in small amounts of skin with the ideas of eventually studying some pathologic conditions.

In 1945, Beloff and Peters (2) described in the skin of animals a proteolytic enzyme active between pH 7.0 and 8.0. This protease could be partially extracted from fresh skin mince or from acetone dried skin using 5% KCl in phosphate buffer for extractions. Fruton (5) found that saline extracts of fresh rabbit skin yielded mostly peptidase, and a similar enzyme could be extracted from human skin. Neville-Jones and Peters (7) confirmed that fresh extracts of skin have a high dermopeptidase activity while extracts of acetone dried skin yielded mostly protease. The protease was found to digest casein, serum proteins, myogen and some proteins in the skin itself, and its pH optimum was 7.5. It was distinct from other proteolytic enzymes active at this pH in that it did not affect the specific substrates by which trypsin, chymotrypsin and dermopeptidase may be recognized, and it is distinct from the autolytic enzymes active in a more acid pH range. Beloff (3) found that serum albumin contained a factor which inhibited the protease of skin. With experimental burns in animals it was noticed that the protease would disappear from the skin (2), but that if the same amount of heat were applied to excised skin there was no loss of protease. Beloff and Peters (2) suggested that during burns protease was mobilized in the skin, but that it soon reached the blood stream where it would be quickly neutralized by protease inhibitor (3). Cullumbine (4) showed that products of digestion of protein by skin protease (as in vesicles from burns and vesicant gases) were active in producing inflammation in the manner of Menkin's leukotaxin (6). It seems that peptides of amino acid chain length between 8 and 14 would regularly produce inflammation by causing vasodilatation and leukotaxis and Spector (9) considered that leukotaxin was not a specific substance but that the effect could be produced by many polypeptides.

Thus the products of proteolysis may be of importance in burns and perhaps in vesication, and in other conditions where the skin is damaged.

METHODS

In earlier unpublished work casein was used as a substrate and the Sörensen formol titration as an index of proteolysis. With this method proteolytic activity could be demonstrated in extracts of acetone dried skin from baby rats, but the method was found to be unsuitable for small specimens of skin.

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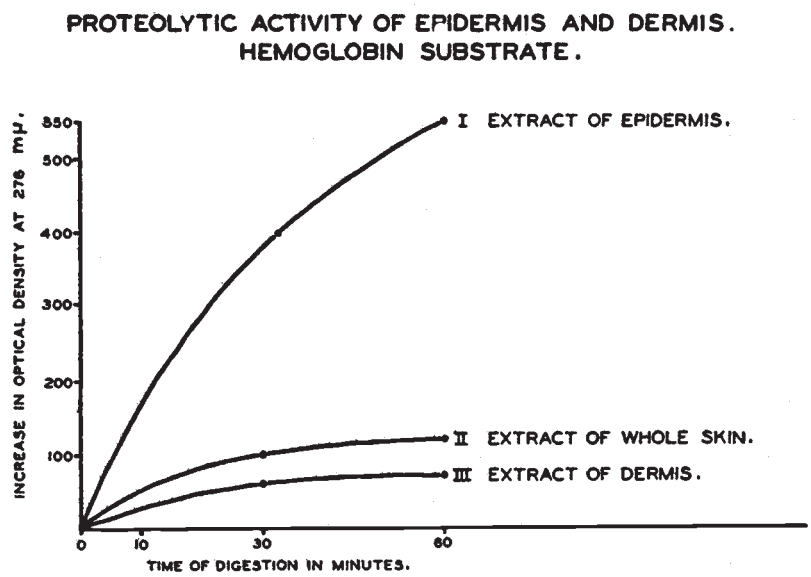

FIG. 1

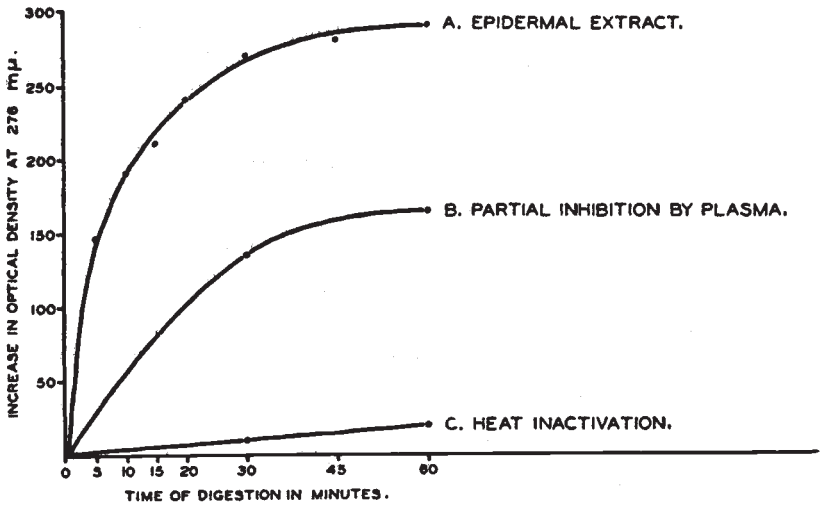

FIG. 2

The rather sharp rise in optical density in the first ten minutes of the incubation is usually seen in these experiments. Trypsin gives a similar digestion curve by this method. Trypsin is completely inhibited by soybean trypsin inhibitor but this same inhibitor has no effect upon the skin protease. Cysteine, heparin, treburon and polyethanol sulfonate had no inhibiting effect on the enzyme.

If in the process of extraction the epidermis is very finely divided sufficient protein is present in the extract to provide a substrate for the protease so that addition of denatured hemoglobin may not always be necessary.

The work has required relatively large amounts of raw material. About 1 gm. wet weight of epidermis would be required for initial extraction by 15 ml. of KCl in buffer. It has been difficult to obtain such amounts of skin especially as there is some loss of activity on prolonged storage.

Recently using microcuvettes we have been able to measure amounts of digest sample of the order of 0.4 ml. and initial extractions can be made with 100 to 200 mg. wet weight of epidermis.

DISCUSSION

The relatively large amounts of protease which may be extracted from epidermis suggest that a considerable part of the dermoprotease of Beloff and Peters may be derived from the epidermis. It is not surprising to find a proteolytic enzyme in the epidermis, an area that is actively proteoplastic. Living epithelial cells exert some proteolytic action on fibrin in the course of wound healing, and lysis of fibrin clot and digestion of egg albumen by epithelial cells in tissue culture has been demontrated by Santesson (8). Damaged epithelial cells may release proteolytic enzyme which in turn may produce inflammatory protein breakdown products such as may be of importance in vesication.

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DISCUSSION

Dr. Adolph Rostenberg, *Chicago, Illinois:* I would like to offer my congratulations to Dr. Wells and his colleague for this paper. I think pure research work such as this needs no justification but it is nice if one can make some clinical correlations.

Now what I am going to say is obviously somewhat speculative. The genesis of

many necrotic reactions of the skin is to a large degree unknown. One of the mechanisms that may play a part in their development is the Shwartzman phenomenon. Recently, primarily through the work of Thomas, Stetson and Good much additional information has been gathered about the pathogenesis of the Shwartzman reaction. It is essentially a two stage process. The first stage is a preparatory one in which it has been shown that the bacterial filtrates alter the metabolism of tissue into which they are put (the skin is usually that tissue), so that it is more vulnerable to proteolytic enzymes. Subsequently, when the eliciting dose is given intravenously the proteolytic enzyme inhibitors are depressed and consequently the tissue into which the preparatory dose was put is no longer protected against these enzymes. One of the problems has been, where do these enzymes come from? Dr. Wells' work offers at least a possible explanation for the source of these enzymes which, when the eliciting factors are introduced, cause the local necrosis. Interestingly, as he has shown you, there is a plasma inhibitor for his enzyme and Thomas, Stetson, et al, have shown that plasma inhibitors are markedly reduced by the secondary eliciting intravenous injection.

Dr. George C. Wells (in closing): I appreciate Dr. Rostenberg's remarks although it is a little unlikely that the enzymes, if such are released in the Shwartzman phenomenon, would actually be epidermal. We know little concerning the proteolytic activity of living epithelial cells except for the fibrinolysis caused by epidermal cells and this activity, is at the cell surface. Santesson's extensive studies of tissue cultures of mouse mammary epithelia are of great interest. It seems that proteolytic enzymes produced by living epithelial cells are important in relation to the normal cell environment, and it is noticed that this enzymatic activity is absent in most cultures of tumor cells.