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CLINICAL ARTICLES

STUDY OF PROTEOLYTIC ENZYMES, EPIDERMAL EXTRACTS* AND CANTHARIDIN IN PRODUCING CUTANEOUS BLISTERS.

By

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Ability of several enzymes in producing splits in the skin has been reported by several workers (Medawar, 1941; Stoughton, 1952; Hambrick and Blank, 1954; Goldblum et al, 1955, Fan, 1958; Burbach, 1959; Miller and Stoughton, 1960; Klein and Fitzgerald, 1962; Dobson and Bosley, 1963 and Einbinder et al, 1966) but no definite role has been assigned to them for the splitting seen in spontaneous diseases. This is because most of these enzymes were not traced to cutaneous origin. There is no report also of the capability of epidermal extracts in producing splits in the skin. Burbach (1959) failed to achieve any success with epidermal extracts.

The present investigation was undertaken to study the effects of intradermal injections of three proteases-trypsin, pepsin and chymotrypsin, and epidermal extracts in vitro and in vivo. Results of application of a 0.5% cantharidin ointment to skin are also reported.

MATERIALS AND METHODS

Experiments were conducted both on the living as well as the excised skin of dogs and humans.

Human skin was excised from amputated legs, while dog skin was removed from the abdomen immediately after killing the animal.

Enzyme solutions (pepsin, trypsin and chymotrypsin) of 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and 1.0% strengths were prepared in Ringer's solution. Epidermal extract was made by homogenising stretch separated human or dog epidermis in borate buffer pH 9.4 (Roe, 1958) in a pestle and mortar using neutral glass powder. The extract was kept at 4°C for 24 hours, centrifuged at 2500 rpm for 20 minutes in cold and the clear supernatent separated.

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In vitro experiments were conducted by injecting 0.1 ml of the enzyme solutions and epidermal extract intradermally and incubating the skin pieces in moist petri dishes at 37°C for 24 hours, at the end of which they were fixed in 10% neutral formalin.

Controls were taken by fixing one piece of skin immediately in formalin, incubating a second piece without any injection along with other pieces, injecting a third and a fourth piece with 0.1 ml of Ringer's solution and borate buffer respectively and incubating them with the experimental specimens.

In the in vivo experiments, intradermal injections were made with the solutions on the abdominal skin of dogs anaesthetized with pentobarbitone. The dog was killed with intravenous magnesium sulphate after 5 hours, the injected sites were excised and fixed in 10% neutral formalin.

In the humans, only 0.5% and 1.0% concentrations of the enzymes were used. Epidermal extracts were passed through a Seitz filter and tested for sterility before injecting. Biopsies were taken after 5 hours and fixed in formalin. Extracts of human epidermis were injected into humans, while those of dogs. For controls, Ringer's solution and borate buffer were used.

With cantharidin, the in vivo experiments were done by the method of Burbach (1961), while for in vitro studies, the skin was incubated at 37°C for 6 hours after application of 0.5% cantharidin ointment. The specimens were then fixed in formalin.

All the samples were embedded in paraffin, sectioned at 4-6 microns and stained with haematoxylin and eosin.

RESULTS

The pattern of changes produced by each of the enzymes and the epidermal extract was the same, although chymotrypsin produced markedly pronounced changes and these were better depicted in the human skin. The severity of changes was much less in the in vivo experiments as compared to in vitro studies. Macroscopic blisters were seen only in the in vivo studies and they were often haemorrhagic.

Appearance of vacuolated spaces at the dermoepidermal junction (Fig. 1) was the earliest change noticed, followed by extensive separation and appearance of inflammatory infiltrates in the perivascular areas (Fig. 2). The infiltrate was mononuclear in the experiments on the excised skin and was minimal, while in the in vivo studies, it was predominantly polymorphonuclear with an admixture of erythrocytes (Fig. 3). The infiltrate was massive with chymotrypsin.

Severer changes in the epidermis consisted of contraction of the nuclei, followed by their disappearance leaving behind vacuolated spaces in the epidermis which then gave an appearance of "honey-comb" (Fig. 4). This change though seen with all the three enzymes and the epidermal extract was best depicted by pepsin.

With yet severer changes, even the cytoplasm of epidermal cells disappeared leaving behind only stratum corneum (Fig. 5). In the dermis too, all the inflammatory as well as connective tissue cells disappeared.

Hair follicles, sweat and sebaceous glands underwent the same changes of separation from the basement membrane, 'honey-combing' and ultimately complete disappearance as seen in the surface epidermis.

Separation of epidermal cells from each other, individually as well as in masses was seen with pepsin and chymotrypsin (Fig. 6). Acantholysis seen with pepsin (Figs 7 and 8) resembled to a great degree that produced by cantharidin (Figs. 9 and 10). In addition to intraepidermal changes, cantharidin at times, produced dermoepidermal splits as well.

DISCUSSION

The aim of this investigation was to study the effects on skin, of some well known proteases which have their equivalents in intracellular cathepsins. Changes produced by trypsin had been reported by several earlier workers but pepsin and chymotrypsin had not yet been studied. The results obtained showed that all the three proteases produce almost similar effects, although the degree of changes varied with each enzyme. The pattern of changes suggests a non-specific proteolytic action on tissues, in the form of dissolution of all cellular components.

The ability of epidermal extracts in producing splits in the skin had been a controversial subject, because the extractants used for preparing the skin extract were also found to produce dermoepidermal splits. This was encountered in the present study as well, but extracts made in several other extractants were seen to possess similar activity and further, this activity was still present in the fraction consisting of precipitated proteins (unpublished observations). Further studies to isolate and identify the actual components responsible for splitting are in progress.

The presence of inflammatory infiltrates in the in vivo studies indicates the activation or release of a leucotaxine-like substance under the action of the proteases as suggested by Duthie and Chain (1939) and Burbach (1960). Demonstration of leucotaxine in thermal burns (Cullumbine and Rydon, 1946) further supports this belief.

Absence of macroscopic blisters and the paucity of inflammatory infiltrates in the in vitro experiments could be attributed to the absence of circulation in excised skin and further supports the views of Burbach (1960), Kandhari and Pasricha (1965) and Sulzberger et al (1966), that the blister fluid is derived chiefly from blood.

Milder changes seen in the in vivo experiments could be attributed to a rapid absorption and removal of the injected substances from the site of action or to their inactivation by some inhibitory influence of blood.

Acantholysis produced by cantharidin has been studied extensively. Among other views Stoughton and Bagatell (1959) believed that cantharidin activates proteolytic enzymes which are in turn responsible for acantholytic changes. Bagatell and Stoughton (1964) isolated an active principle having properties of an enzyme from skin to which cantharidin had been applied. Resemblence of acantholytic cells produced by pepsin to those seen with cantharidin in the present study further supports the above contention and it is possible that cantharidin also activates intracellular cathepsins which mimic the activity of pepsin or other allied enzymes. Cantharidin only triggers the process as many unknown stimuli are supposed to do.

SUMMARY

Results of a study undertaken to investigate the effects of intradermal injections of trypsin, pepsin, chymotrypsin and epidermal extracts and application of cantharidin ointment to skin of humans and dogs in vivo and in vitro are reported. Dermo-epidermal separation with progressive loss of all cellular elements of epidermis and dermis were seen with all the proteases and the epidermal extracts. The effects possibly represented a non-specific proteolytic activity of the enzymes. Epidermal extracts were also seen to produce similar changes. Acantholytic changes seen with pepsin resembled those seen with cantharidin and it is postulated that the action of cantharidin is mediated by a pepsin like cathepsin. Severity of changes in the in vivo studies was much less, possibly due to absorption or inactivation of the enzymes by the circulation. Absence of macroscopic blisters and the paucity of inflammatory reaction in the excised skin were attributed to absence of circulation.

REFERENCES

1. BAGATELL, F. and STOUGHTON, R. B.: Vesication and Acantholysis: in The Epidermis, editors, W. Montagna and W. C. Lobitz, Chapter XXXI p. 601. Academic Press Inc., New York, 1964.
2. BURBACH, J. P. E.: Experiments on blister formation. I. Experiments with proteolytic enzymes and hyaluronidases. Dermatologica, 118: 379, 1959.
3. BURBACH, J. P. E.: Experiments on blister formation. II. Contents of blisters. Dermatologica, 120: 345, 1960.
4. BURBACH, J. P. E.: Experiments on blister formation. IV. The action of cantharidin. Dermatologica, 123 42; 1961.
5. CULLUMBINE, H. and RYDON, H. N.: A study of the formation, properties and partial purification of leukotaxine. Brit. J. Exp. Path., 27: 33, 1946.
6. DOBSON, R. L. and BOSLEY, L.: Effect of keratinase on human epidermis. J. Invest. Derm., 41: 131, 1963.
7. DUTHIE, E. S. and CHAIN, E.: A polypeptide responsible for some of the phenomena of acute inflammation. Brit. J. Exp. Path., 20: 417, 1939.
8. EINBINDER, J M.; WALZER, R. A. and MANDL, I.: Epidermal-dermal separation with proteolytic enzymes. J. Invest. Derm., 46: 492, 1966.
9. FAN, F.: Epidermal separation with purified trypsin. J. Invest. Derm., 30: 271, 1958.

10. GOLDBLUM, R. W.; LINDGREN, V.; PIPER, N. and CAMPBELL, A. : The enzymatic separation of human epidermis and dermis. Plastic and Reconstructive Surgery, 15: 68, 1955.
11. HAMBRICK G. W. Jr. and BLANK, H. : Whole mounts for the study of skin and its appendages. J. Invest. Derm., 23: 437, 1954.
12. KANDHARI, K. C. and PASRICHA, J. S. : Study of proteins and electrolytes in serum and blister fluid of pemphigus. J. Invest. Derm., 44: 246, 1965.
13. KLEIN, M. and FITZGERALD, L. R. : Enzymatic separation of intact epidermal sheets from mouse skin. J. Invest. Derm., 39: 111, 1962.
14. MEDAWAR, P. B. : Sheets of pure epidermal epithelium from human skin. Nature, 148: 783, 1941.
15. MILLER, R. F. and STOUGHTON, R. B.: Enzymatic vesication in vivo. I. Effects of papain on human skin. J. Invest. Derm., 35: 141, 1960.
16. ROE, D. A. : Application of paper electrophoresis to the diagnosis of psoriasis. A study of psoriatic scales extracts. Ann. N. Y. Acad. Sci, 73. 977, 1958.
17. STOUGHTON, R. B. : Enzymatic cytolysis of epithelium by filterates of feces from patients with ulcerative colitis. Science, 116: 37, 1952.
18. STOUGHTON, R. B. and BAGATELL, F. : The nature of cantharidin acantholysis. J. Invest. Derm., 33: 287, 1959.
19. SULZBERGER, M. B.; CORTESE, T. A. Jr ; FISHMAN, L. and WILEY, H. S.: Studies on blisters produced by friction. I. Results of linear rubbing and twisting technics. J. Invest. Derm., 47: 456, 1966.