THE EFFECT OF KERATINASE ON HUMAN EPIDERMIS*

RICHARD L. DOBSON, M.D. AND LARRY BOSLEY, M.D.

Keratinase (Merck), an enzyme derived from *Streptomyces fradiae*, is used in the leather industry in the preparation of hides. Its effect on human skin has not been previously reported.

METHODS

a) 0.1% solutions of keratinase in tris buffer, pH 8.0, were prepared. 4 mm. punch biopsy specimens of skin from the back of a normal young man were incubated at 37° C. in 0.1% keratinase for 15 minutes to 2 hours.

b) Gauze saturated in 01% keratinase and covered with plastic film was applied to the normal skin of the back for 2 and 8 hours. 4 mm. punch biopsy specimens were obtained after treatment.

c) After cellophane stripping of areas of the skin of the back (5, 10 and 26 strips), gauze saturated with 0.1% and 1% keratinase was applied to the stripped areas for 2 or 4 hours. 4 mm. punch biopsy specimens of these areas of skin were obtained after treatment.

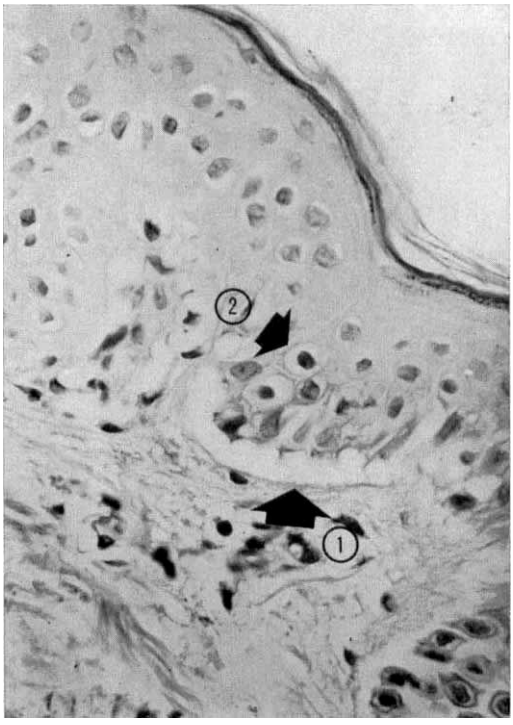

FIG. 1. Epidermis after 30 minutes incubation in 0.1% keratinase. There is dermal-epidermal separation (Arrow 1). Some cells of the prickle cell layer have a prominent perinuclear halo (Arrow 2) H & E. × 236.

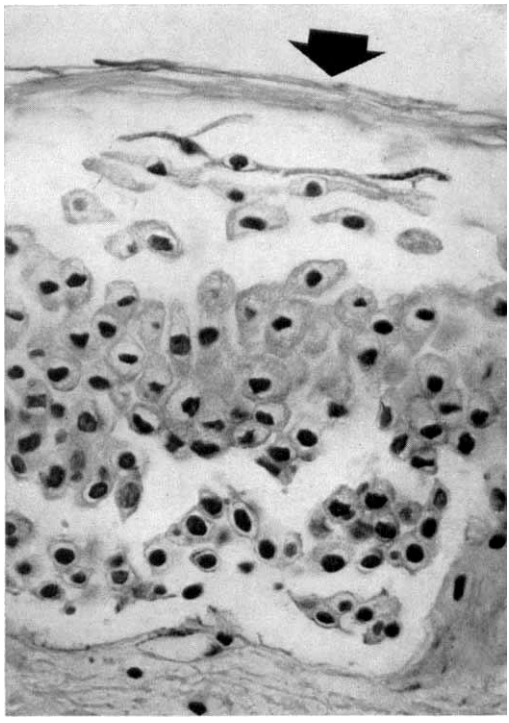

FIG. 2. After 2 hours of incubation in 0.1% keratinase-clusters of cells are floating freely. The stratum corneum, however, is intact (Arrow) H & E. × 378.

d) All skin specimens were fixed in 10% formalin and stained with hematoxylin and eosin.

RESULTS

After 30 minutes of incubation in 0.1% keratinase, skin biopsy specimens showed dermal-epidermal separation. A few cells of the lower stratum spinosum had prominent perinuclear halos (Fig. 1). With further incubation these

* From the Division of Dermatology, University of Oregon Medical School, Portland 1, Oregon.

This study was supported by the Office of Surgeon General, U.S. Army Medical Research and Development Command, Research Contract DA-49-193-MD-2184 and by a PHS Training Grant 5 T1 AM 5300-03.

Received for publication December 1, 1962.

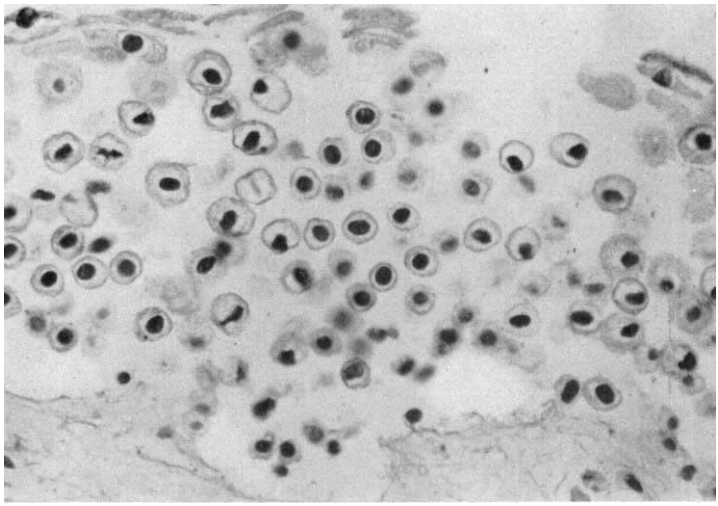

FIG. 3. After 2 hours of incubation some areas of epidermis show complete dissolution. Numerous discrete acantholytic cells can be seen. H & E. × 378.

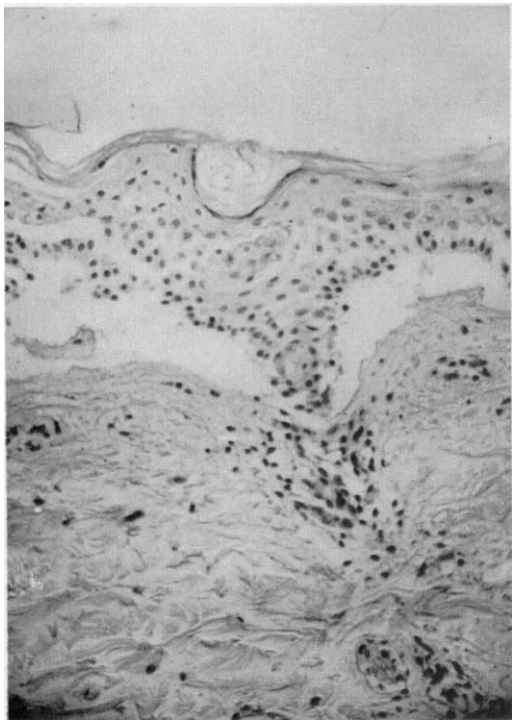

FIG. 4. After 2 hours of incubation the epidermal sweat duct unit contains some acantholytic cells but tends to remain intact. H & E. × 94.

changes progressed so that by 2 hours complete epidermal separation had occurred. Between the intact stratum corneum and the dermis, clusters of cells with clear perinuclear halos floated freely (Fig. 2). In some areas only discrete, rounded, acantholytic epidermal cells could be seen (Fig. 3).

Similar changes were also noted in the external root sheath of the hair folicle and the basal cells of the sebaceous gland. Lipid-containing sebaceous cells, however, were unaffected. The epidermal sweat duct unit was somewhat resistant to keratinase and tended to remain intact (Fig. 4). The eccrine secretory unit and duct were unaffected.

Application of keratinase to intact skin for up to 8 hours was without effect because of the resistance of the stratum corneum. Partial removal of the stratum corneum by means of cellophane tape stripping similarly was without effect. With complete removal of the stratum corneum, however, the application of both 0.1% and 1% keratinase produced marked changes. Numerous free-floating acantholytic cells were present and in some areas the epidermis was entirely gone (Fig. 5). A 0.1% concentration of keratinase applied for 2 hours produced maximal changes.

DISCUSSION

The inability of keratinase to digest stratum corneum would imply that the action of this enzyme is not primarily on keratin. Perhaps a more appropriate designation would be "acanthase" since the primary action of this enzyme appears to be a dissolution of epidermal prickles.

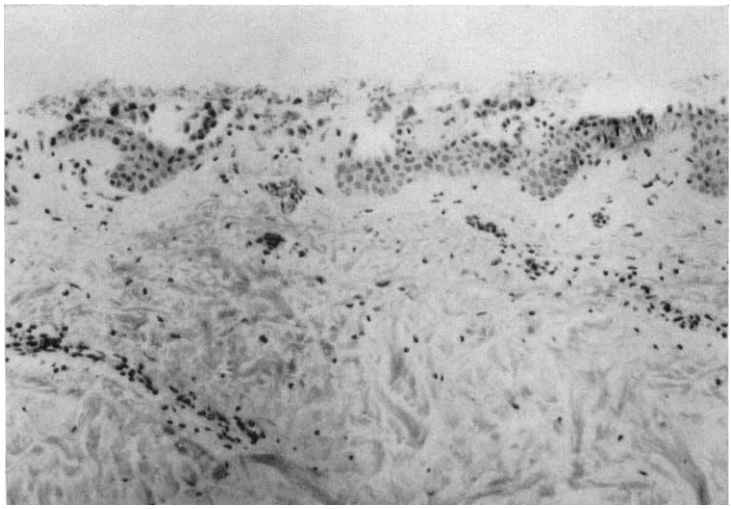

FIG. 5. The epidermis after 2 hours of contact with 1% keratinase *in vivo*. The stratum corneum has been removed by stripping. The changes are identical to those produced *in vitro*. H & E. × 59.

Whether this is a direct effect on tonofibrils or on the desmosomes is unknown.

It is of interest to note that all layers of the epidermis (excluding the stratum corneum) as well as the external root sheath of the hair follicles and the basal cells of the sebaceous gland are similarly affected. The inability of keratinase to produce separation of lipid-containing sebaceous cells suggests that the areas of attachment of these cells are qualitatively different than those in the epidermis. This applies also to the secretory cells of the eccrine gland since these cells appeared unchanged after 2 hours of incubation *in vitro*.

It is well known that the "epidermal sweat duct unit" has biologic properties which differ from those of the adjacent epidermis (1). The relative resistance of this structure to keratinase is an additional facet of the unique biological properties of this unit.

SUMMARY

A preliminary study of the action of keratinase on human skin has shown that the primary action of this enzyme is acantholytic. With the exception of the stratum corneum all layers of the epidermis, the external root sheaths of the hair follicle and the basal cells of the sebaceous gland are equally affected. The eccrine gland and the epidermal sweat duct unit are relatively resistant to the action of keratinase. Because of the failure of keratinase to lyse mature keratin it is suggested that "acanthase" would be a more appropriate appellation.

REFERENCE

1. LOBITZ, W. C. JR., HOLYOKE, J. B. AND MONTAGNA, W. The epidermal eccrine sweat duct unit: A morphologic and biologic entity. J. Invest. Derm., **22:** 157, 1954.