The responses of hairless mouse epidermis to tape stripping, ethanol and vitamin A acid were compared using electron microscopy and cytochemistry. Stripping the skin 4 times with cellophane tape removed most of the stratum corneum and caused the development of enlarged intercellular spaces and dense intramitochondrial inclusions. These changes began within an hour, reached a maximum by 24 hr, and subsided by about 4 days. There was also some accumulation of glycogen and the development of occasional basal lamina breaks and a few intracellular lipid droplets by 24 hr. Topically applied ethanol (95%) produced a similar response, though less damaging. In addition, ethanol induced the formation of numerous lipid droplets in the cytoplasm of keratinocytes by 24 hr and often caused sloughing of the stratum corneum. Vitamin A acid (3%) was dissolved in propylene glycol for application to the skin. We found the application of propylene glycol alone to produce no epidermal changes. Vitamin A acid produced mild damaging effects with small intercellular spaces and intramitochondrial inclusions, but no lipid droplets or glycogen were detected. Vitamin A acid also caused dramatic inhibition of keratinization by 24 hr. Intramitochondrial inclusions were digested by protease, indicating a protein component and they also stained with silver methenamine, indicating the presence of glycogen or glycoprotein.

Stripping the skin with tape removes corneocyte layers and produces a burst of mitotic activity in basal keratinocytes resulting in the regeneration of the corneocyte layer [1–3]. Ethanol applied to the skin also increases epidermal mitotic activity [4] and light microscopic studies have shown ethanol to cause some swelling of the epidermis, but no cellular damage was detected [5]. No ultrastructural studies of these ethanol effects have been reported.

Vitamin A acid has recently been found useful as a topical treatment for several skin diseases [6]. In addition, synthetic analogs of vitamin A have shown promise in the prevention and treatment of certain epithelial cancers [7]. When vitamin A acid has been applied to the skin, it has caused inhibition of keratinization [8], and excess vitamin A in tissue culture has not only inhibited keratinization but transformed embryonic skin to a mucus-producing and even ciliated epithelium [9,10]. Vitamin A acid application can also cause the skin tumor keratoacanthoma to produce mucus [11], but it is not yet certain whether it can also cause normal skin in vivo to produce mucus. The mechanisms of vitamin A activity in the epidermis are not well understood and the ultrastructure of the epidermal response is not fully described. Thus, we compared this response to that caused by stripping and by ethanol to distinguish those effects which were merely damaging from those peculiar to vitamin A activity.

MATERIALS AND METHODS

Vitamin A acid (all-trans-retinoic acid, Eastman) was obtained from Fisher Scientific Company. Protease from Streptomyces griseus, repurified type VI, 6.5 units/mg solid, was obtained from Sigma Chemical Company. Hairless mice used in this study were from a cross of C3H hairless (hr/hr) mice (Jackson Laboratories, Bar Harbor, Maine, U.S.A.). Only hairless males 6 to 10 mo old from the F₂ progeny of a back cross of hairless males with the F₁ (hr/C3H) females were used.

An area of posterior dorsal skin was stripped 4 times with cellophane tape as described by Finkus [1]. Vitamin A acid (3%) was dissolved in propylene glycol and applied once to the posterior dorsum by pipet; propylene glycol and ethanol were applied in the same way. Mice were killed by neck fracture, skin specimens surgically removed and sliced into 1-μm by 2-mm pieces, fixed in Dalton's fixative [12], dehydrated in ethanol and embedded in Epon-Araldite. Ultrathin sections were cut and stained with uranyl acetate and lead citrate or with silver methenamine; some were incubated in protease before staining. Sections were examined in an RCA EMU 3H and a Philips EM 201 electron microscope.

Silver methenamine stain was prepared as described by Movat [13]. Because Dalton's fixative contains potassium dichromate, we were able to omit the usual ten minute incubation in 1% periodic acid and transferred sections directly to the silver stain. Protease digestion was performed according to the method of Monnerson and Bernhard [14].

RESULTS

Stripping 4 times caused no visible damage to the live epidermal cells but the epidermis reacted a short time later with widened intercellular spaces and microvillus projections (Fig 1). Dense inclusions formed within many mitochondria in keratinocytes as the spaces developed (Fig 2). These changes began within an hour, reached a maximum by 24 hr, then subsided over the next few days. Glycogen accumulated around the nuclei of some keratinocytes (Fig 2) and few breaks in the basal lamina were found to develop by 24 hr poststripping. The dense intramitochondrial inclusions were digested by protease (Fig 3), indicating they contain a protein component. A few lipid droplets were observed in keratinocytes 18 hr after stripping.

Ethanol caused the formation of smaller intercellular spaces than those caused by stripping; they also formed at a later time (18 hr). Dense intramitochondrial inclusions occurred to a lesser extent than with stripping. These inclusions stained with silver methenamine (Fig 4) indicating they contain glycogen or glycoprotein. Ethanol also caused accumulation of glycogen in a perinuclear location. These glycogen deposits were identified cytochemically with silver methenamine (Fig 4). No basal lamina breaks were found but basal lamina material was dispersed into the dermis (Fig 5). Another effect of ethanol was sloughing of the stratum corneum (Fig 6). Ethanol also induced the...
formation of many lipid droplets (Fig 6); some contained small darker staining areas of unknown significance (Fig 7). Attempts to stain lipid droplets with silver methenamine resulted in a nonspecific spotted pattern of silver precipitation (Fig 8), indicating an absence of glycogen or glycoprotein.

Propylene glycol alone caused no epidermal changes but vitamin A acid in propylene glycol produced small intercellular spaces by 24 hr (Fig 9). Intramitochondrial inclusions also appeared along with the spaces (Fig 9). The basal lamina was normal and there was no glycogen, lipid droplets nor sloughing of the stratum corneum. Overall, the damaging effects of vitamin A acid were less than after stripping or ethanol. The elements of keratinization, namely, keratohyalin granules and tonofilaments, were reduced by 24 hr (Fig 10). Keratohyalin

Fig 1. Intercellular spaces (arrows) 24 hr after stripping. D, dermis (× 10,000).

Fig 2. Intramitochondrial inclusions (arrows) and glycogen (G) in a keratinocyte, 18 hr after stripping. N, nucleus (× 27,000). Inset: Intramitochondrial inclusions (arrows) 1 hr after stripping (× 50,000).

Fig 3. Protease digested intramitochondrial inclusions (arrows), 18 hr after stripping. After half an hour of protease digestion, these inclusions stain less densely (× 25,000).

Fig 4. Silver methenamine stained section showing glycogen (G) and intramitochondrial inclusions (arrows), 24 hr after ethanol. N, nucleus (× 15,000). Inset: Intramitochondrial inclusions (arrows) from the same tissue (× 60,000).

Fig 5. Basal lamina (BL) showing some dispersion (arrows), 24 hr after ethanol. C, collagen (× 25,000).
DISCUSSION

Stripping produced the typical epidermal response to wounding with the formation of intercellular spaces [3]. These spaces are a common morphological finding in epidermis stimulated to regeneration by a variety of means. The present study has revealed an early development of these spaces, within 15 min after stripping. The mechanisms involved in the formation of

granules were decreased in size and numbers and appeared round instead of irregular in shape; also, they were not associated with tonofilaments. Instead of keratinizing elements, there were increased amounts of ribosomes, endoplasmic reticulum and Golgi apparatus, even in the uppermost layers of the epidermis.
spaces are unknown; the fact that spaces form so early may be useful in discovering these mechanisms. The tearing of plasma membranes during the formation of spaces suggests that cell separation is not due to just a lack of adhesiveness between cells but may involve fluid pressures.

Intramitochondrial inclusions found in damaged epidermal cells appear to be part of the epidermal response to injury since they accompany the formation of spaces. Morphologically similar inclusions have been found in the epidermis or in the epithelial cells of mucous membranes after various damaging treatments [15]. Rupec and Bruhl [15] have accepted the name "corpora intra cristam" for this type of inclusion as originally proposed by Frié and Sheldon [18], and have demonstrated digestion of these inclusions in sections incubated in protease. We have confirmed protease digestion of corpora intra cristam and found that they also stain with silver methenamine. Together, these 2 findings suggest a glycoprotein component in corpora intra cristam. Rupec and Bruhl also found these inclusions to be extracted by chloroform/methanol and concluded they contain a lipid component as well. Although a few lipid droplets were found after stripping, that does not necessarily mean stripping induced their formation; such droplets appear occasionally in normal epidermis as well [17].

In this study, topical application of ethanol caused considerable epidermal changes; intercellular spaces and intramitochondrial inclusions were formed, glycogen accumulated, the stratum corneum was sloughed off and lipid droplets appeared. Bruckner and Guess [18] also found intercellular spaces and broken cell membranes in the epidermis after injecting ethanol into the dermis. The skin's response to ethanol damage appears to be similar to that after tape stripping in that there is increased epidermal mitotic activity after stripping [3] and after the application of ethanol [4]. Considering these effects, ethanol appears unsuitable as a solvent for studies of compounds applied to the skin; some previous work using ethanol may have to be re-evaluated.

The ultrastructural effects of vitamin A acid applied to normal adult mammalian skin have been studied in guinea pig ear skin by Wolff, Christophers and Braun-Falco [8]. They found greater effects with several daily applications of 3% vitamin A acid in acetone than we found with a single application of 3% vitamin A acid in propylene glycol; for example, Wolff et al found glycogen accumulation and moderately dense droplets, whereas we found none. The single application of vitamin A acid in acetone can cause no epidermal damage. Wolff et al reported unpublished preliminary findings that the moderately dense droplets stained with silver methenamine. Since the single application of vitamin A acid reported here produced no moderately dense droplets, we were unable to confirm or deny that vitamin A acid can cause adult mammalian epidermis to produce mucus or glycoprotein droplets.

Wolff et al found that vitamin A acid inhibited keratinization and the present study confirms this finding. Logan [20] suggested there was no inhibition of keratinization in mammalian skin in vivo but the ultrastructural studies by Wolff et al and ourselves show specific inhibition of the production of keratinization elements. This inhibition of keratinization was the only change we found that was exclusively associated with vitamin A activity, since the other changes are typical of a variety of damaging treatments.

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