This seems to be due to the fact that the second blister was raised on exactly the same blistered site. Activation of collagenase is thought to occur after the first blister, and suggests a potential role of drugs known to inhibit collagenase. The hypothesis that tetracyclines might have an effect on connective tissue breakdown derived from the observation that minocycline inhibited pathologically excessive collagenase activity and collagen breakdown in skin and gingiva in both conventional and germ-free rats (5, 9). The interaction of laminin and heparin could contribute to the structural integrity of basement membranes (4). The measurement of suction blister time on animal skin may prove useful in measuring the effect of drugs on the DEJ. This was demonstrated in the present study, where increased suction blister time was shown after heparin or tetracycline therapy, in hairless rats.

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Human Epidermal Langerhans' Cells are Sensitive to Rapid Cooling by Ethyl Chloride

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Topical anesthesia with ethyl chloride spraying generates some remarkable reactive events in the human Langerhans' cell (LC) system. Many LC retract their dendrites and a considerable number move to the innermost layers of the epidermis within 15 minutes. This rapid motility supports the view that the interstices between cells in living epidermis are large. The LC cytomembrane is very susceptible to cold shock which causes the cytomembrane to superimpose upon itself forming abnormally shaped Birbeck granules. This process may consume too much of the cytomembrane to be compatible with cell survival. ***Keywords: Cytomembrane; Birbeck granules; Cell motility; Cell death; Intercellular space***

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We have demonstrated (1, 2) that extracellular factors can cause the cytomembrane of the human Langerhans' cell (LC) to superimpose upon itself thus forming plates of double membranes having the same morphology as Birbeck granules (BG). We have named this mechanism cytomembrane-sandwiching (CMS).

The LC cytomembrane is uniquely susceptible to some triggering stimuli and can show a response ranging from a formation of few BG to a CMS that is too extensive to be compatible with cell survival (3, 4). In this work, we report on the effects of rapid cooling achieved by spraying the skin with ethyl chloride.

MATERIAL AND METHODS

Spraying of volar forearm skin with ethyl chloride was performed at a distance of 30 cm for 30 seconds and punch biopsies (3 mm) were taken immediately (3 individuals) or

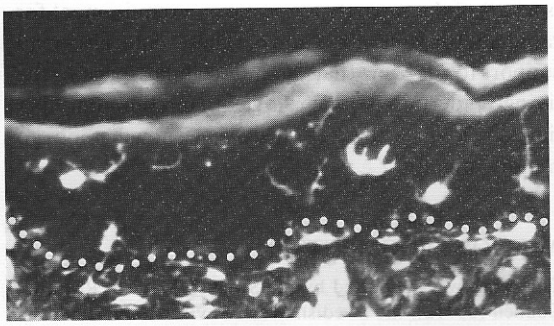

Fig. 1. Fluorescence microscopic picture of normal skin. Most Langerhans' cells have a pronounced suprabasal position and elaborate dendritic trees. Dotted line indicates the epidermal-dermal junction. ×270

after 15 and 30 minutes (6 individuals). Control biopsies were taken from the corresponding region of the other arm. The biopsies were divided into two parts. One was processed for electron microscopy (EM) (5); at least 30 serial sections were examined. The other part was incubated in 10^{-2} M l-dopa dissolved in Krebs-Ringer phosphate buffer (1 h, 37°C), washed in the same buffer (1 h, 37°C). Under these conditions, the LC take up (via a membrane transport mechanism; see ref. 6) and retain l-dopa and further processing for fluorescence microscopy (FM) according to the method of Falck & Hillarp (7) renders them highly fluorescent (Fig. 1). Around 30 serial sections were examined. Additional biopsies (3 individuals) were taken at 30 minutes after cold-exposure and immediately frozen for T6 labelling (DAKO-T6 from Dakopatts, Vectastain® ABC KIT from Vector Lab.) of the LC. 15–20 sections were obtained from each specimen.

RESULTS

At zero time FM revealed a pronounced effect on the LC dendrites. In two out of three specimens some LC lacked dendrites, others had a more or less reduced dendritic tree but normal-looking LC were not uncommon. There was no obvious loss of LC. Ultrastructurally, a surprising finding was that almost all of the presumably pre-existing BG were ring-shaped. Moreover, EM showed CMS of varying intensity from a moderate formation of long and undulating BG, often attached to the cytomembrane, to a total consumption of the cytomembrane combined with cytolysis. It is not known whether such damaged cells are visualized in FM. It can not be excluded that l-dopa is temporarily retained at cytoplasmic binding sites which are exposed at the loss of the cytomembrane. In the third specimen, the LC appeared unaffected in FM but EM disclosed the presence of numerous racket-shaped BG in some of them. Such vesiculation of BG have only been noted so far in LC deprived of Ca^{++} (4). Racket-shaped BG are rarely seen at EM of normal skin, provided a correct (iso-osmolar) glutaraldehyde-based fixative is used (8).

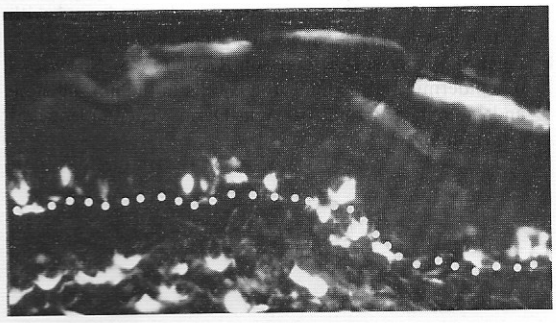

Fig. 2. 30 minutes after topical anesthesia with ethyl chloride the Langerhans' cell have migrated to a basal position. Note the almost complete lack of dendrites. ×270

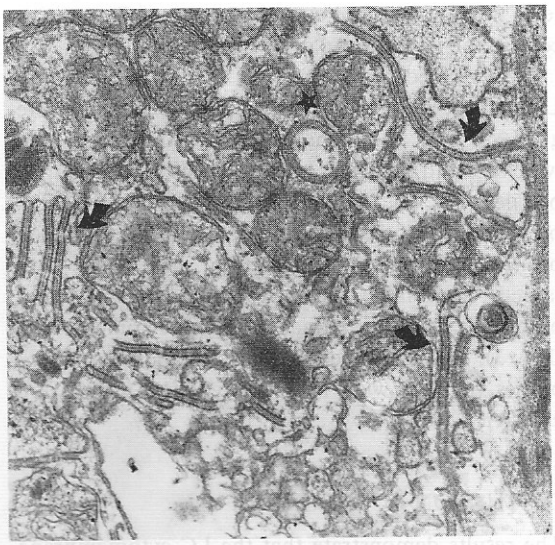

Fig. 3. Electron micrograph of a Langerhans' cell 30 minutes after exposure to ethyl chloride. Asterisk indicates a ring-shaped profile of a Birbeck granule, and arrows abnormally shaped Birbeck granules still attached to the cytomembrane. ×25.000

A most striking finding at 15 and 30 minutes after cold-exposure was that in 5 out of 6 specimens quite a few LC had moved to the innermost epidermal layers (compare Figs. 1 and 2). The dendritic trees of the LC were reduced as at zero time (Fig. 2). The question whether this cell movement took place in situ or required the additional 2 hrs of incubation which is performed before snap-freezing, was answered using immunohistochemistry: in two out of three volun-

teers there was a pronounced increase of T6-positive cells in the innermost part of the epidermis.

EM revealed some effects of cold-exposure in all LC but the variations between the specimens were great. Also in these specimens normal-looking BG were rare and there was an abundance of ring-shaped profiles (Fig. 3). In some cells, numerous BG displayed some degree of unzipping, from racket-shaped profiles to complete vesiculation. Other cells contained abnormally shaped (long, curved and branched) BG often attached to the cytomembrane (Fig. 3), and still other LC had lost their cytomembranes (partly owing to extensive CMS) and were cytolytic.

DISCUSSION

The results demonstrate that the LC cytomembrane and the BG, which derive from the cytomembrane, are very susceptible to rapid cooling. Moreover, cold shock can fatally injure the LC, perhaps precisely because they possess the unique CMS mechanism that is easily triggered. While it is known that certain substances can trigger CMS, it is not known if membrane proteins and/or lipids are involved in the mechanism behind generation of CMS. Sodium lauryl sulphate produces CMS but has both denaturing and detergent properties (4). Digitonin is a powerful trigger (1) indicating that perturbation of membrane lipids evoke CMS. This possibility finds some support in the present findings, since much experimental evidence points to particular involvement of membrane lipids in cold shock injury, although it is conceivable that rapid cooling can initiate protein denaturation as well.

The remarkably rapid withdrawal of LC (within 15 min) towards basal layers following rapid cooling of the epidermis was an unexpected effect. We have observed (unpublished results) that topical application of certain toxic substances can cause the LC to retire to the basal layer. The migration of LC conveys the impression that the cells are capable of escaping a damaging milieu. Whether the retraction of dendrites is a part of the capacity to escape or a requirement for rapid motility, is not known. These data also lend support to the view that the intercellular space in the living epidermis is much larger than commonly believed (8), since the LC must have enough room to rapidly migrate.

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