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THE EFFECT OF SODIUM HYDROXIDE AND HYDROCHLORIC ACID ON HUMAN EPIDERMIS

An Electronmicroscopic Study

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Abstract. Electron microscopic study was done on biopsy specimens obtained from the volar surface of the forearm of 7 volunteers 15–180 minutes after application of 1 N sodium hydroxide or 1 N hydrochloric acid. The ultrastructural changes produced by these chemicals are different or similar in the following manner:

(*a*) In sodium hydroxide treated skin, the contents of horny cells were dissolved and only remnants of cell membranes were present in the superficial horny layer, whereas the superficial horny cells were usually preserved after treatment with hydrochloric acid, their cytoplasm presenting a porous pattern with circular profiles.

(*b*) The shortest time associated with recognizable changes in living (non-cornified) cells was 15 minutes with NaOH and 120 minutes with HCl. Intracellular edema was produced by both treatments.

(*c*) Tonofilament-desmosome complexes disappeared in NaOH treated sites but were present in HCl-treated skin.

(*d*) Increased numbers of lamellar granules on the outer surface of the uppermost granular cell layer were present in both experimental situations. Enlarged lamellar granules were seen only in HCl-treated skin.

(*e*) Keratohyaline granules appeared homogeneous after NaOH treatment, and filamentous in HCl-treated specimens.

Exposures to acids, alkalis, and various cleaning agents are common environmental and occupational hazards and consequently frequent causes of primary irritant contact dermatitis. It is the purpose of this paper to present our observations on the early electron microscopic changes produced by the application of normal solutions of sodium hydroxide and hydrochloric acid on healthy human skin. The results of this study will establish a baseline for comparison with other concentrations of acid and alkali and a variety of other agents and can provide a better understanding of fine structural changes appearing in contact dermatitis.

MATERIAL AND METHOD

Twenty lambda of 1 N sodium hydroxide or 1 N hydrochloric acid was applied to the flexor surface of the forearms of 7 healthy volunteers, aged 21–31, every 30 min to the end of the experiment. The subjects included Caucasian, Negro, and Japanese males and one Caucasian female. No prior cleansing of the test sites was done. Under 1% lidocaine local anesthesia, 4 mm punch biopsies were taken 15, 30, 45, and 60 minutes following the application of 1 N sodium hydroxide and 30, 60, 120, and 180 minutes following the application of 1 N hydrochloric acid. Control specimens were prepared from each subject.

One-half of each specimen was fixed in 10% formalin solution, embedded in paraffin and stained with hematoxylin and eosin for light microscopy. The other half was fixed in cacodylate-buffered osmium tetroxide for 2–3 hours, dehydrated with graded alcohol solutions and embedded in Maraglas 655 for electron microscopy. Staining was carried out with uranyl acetate and lead citrate after sectioning with glass knives. Thick sections were also prepared for light microscopy and stained with toluidine blue. Sections were examined with a RCA EMU-3F or EMU-3G electron microscope.

To eliminate the possibility that the presence of strong alkali or acid on the specimens might produce fixation artefacts, similar test sites were prepared on the forearms of a healthy 25-year-old Caucasian male. At 60 min, one of the alkali-treated and one of the acid-treated sites was rinsed four times with Millonig buffer (pH 7.3) using a cotton tipped applicator for gentle wiping. Biopsies were then obtained from rinsed and non-rinsed sites and a control site and handled according to the procedures described. At the end of 2 hours' fixation in

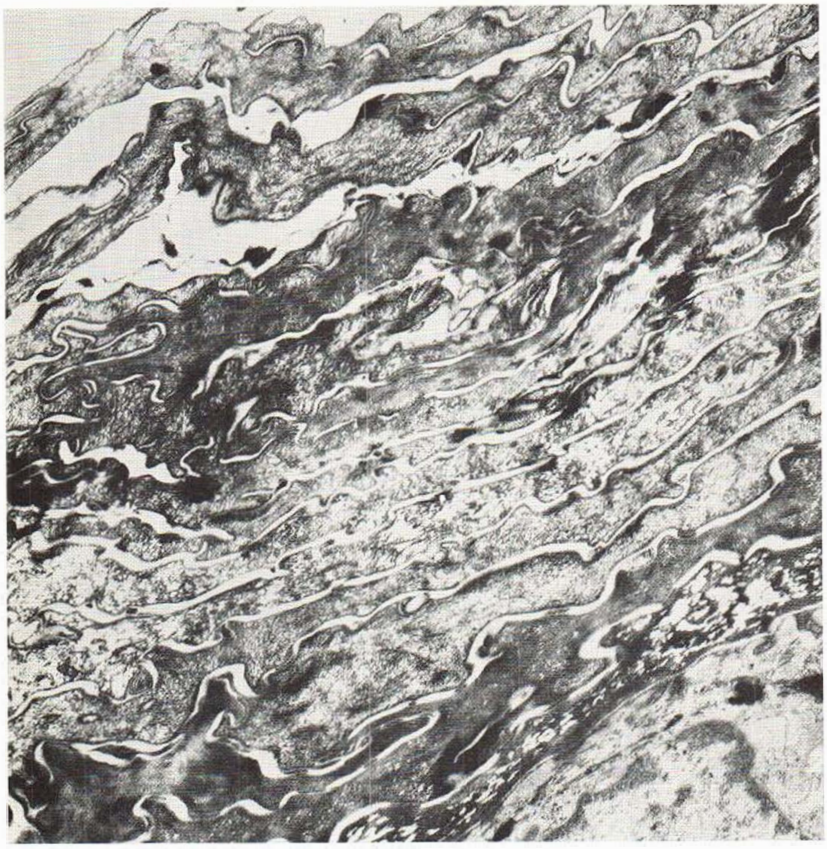

Fig. 1. Control skin—horny layer. × 13 600.

osmium tetroxide, the pH of all the solutions was measured and found to be 7.3 for rinsed as well as unrinsed specimens.

RESULTS

Clinical observations

All test sites developed slight erythema 25 to 30 min after the initial application of 1 N hydrochloric acid or 1 N sodium hydroxide. Later, acid produced dilated blood vessels within the test sites, whereas alkali produced "waxy" appearance in most sites, and in one subject (Caucasian female) produced a large flare at 60 min. Sodium hydroxide also produced prominent swollen hair follicles in several test sites.

Light microscopy

In H & E sections of skin from all subjects tested with hydrochloric acid there were no definite histologic changes until 120 min after the acid was applied. At that time, the horny layer was thinner and had an amorphous appearance. The prickle cell layer showed some intracellular and intercellular edema. After 180 min of contact, clefts and vesicles were present at the level of the granular layer and suprabasally. Dilated blood vessels were prominent in the upper dermis. There was no inflammatory infiltrate.

The application of 1 N sodium hydroxide produced changes more quickly. At 15 and 30 min, the horny layer was swollen and the prickle cell layer displayed a few pycnotic nuclei. At 45 and 60 min, intercellular edema was pronounced and cleft formation was seen in the middle and lower prickle cell layers. In several subjects, the entire epidermis was destroyed at 60 min.

Electron microscopy

Controls. The fine structural details of our sections are similar to those described by other authors (4). In particular, the superficial horny layer is composed of 3–8 layers of interdigitating cells, which are of two different types (Fig. 1). The contents of the uppermost cells consist of

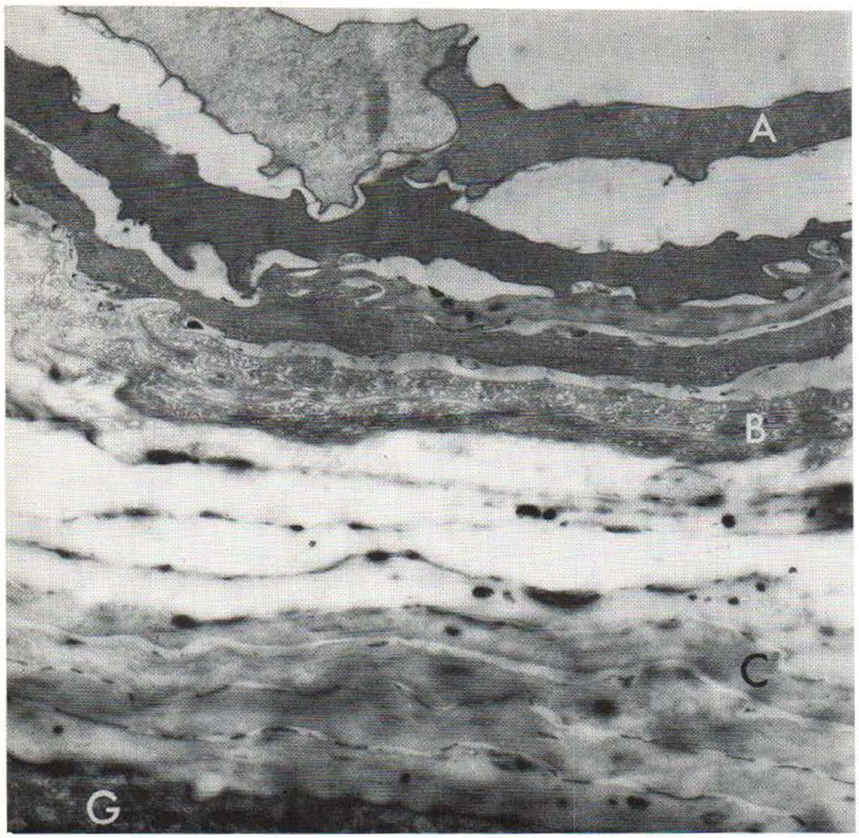

Fig. 2. The horny layer 30 min after application of 1 N sodium hydroxide. The uppermost 3 cells are swollen, appear homogeneous, and vary in opacity (A). Intercellular substances of varying shapes are seen. The cells (B) immediately beneath show a more or less fibrous structure. Other cells constituting the major part of the horny layer are not swollen (C). *G*, granular layer. × 11 000.

tightly packed fibrils while the lower cells have more loosely packed fibrous components and contain electron optically empty spaces of varying sizes and shapes. Layers of high opacity horny cells vary from 5 to zero with only loosely packed cells sometimes present on the skin surface. The intercellular spaces of the superficial horny layer tend to increase in width towards the surface, and contain intercellular substances of various shapes. Below an intermediate stratum of variable thickness, the basal horny layer consists of 4–12 cell layers. The cell contents show a keratin pattern (3) interrupted by electron-translucent spaces of varying sizes and shapes.

1. *Changes produced by 1 N NaOH*

(*a*) Horny layer: The application of 1 N NaOH reduced the horny cell layers at an inconstant rate. When the horny layer was present, the 1–3 uppermost cells were swollen to a varying extent and showed a homogeneous appearance but of differing degrees of opacity (Fig. 2). The cells below showed fibrous structure similar to that of the controls. Plasma membranes of swollen cells usually remained intact and became detached from their contents (Fig. 3). As the contents progressively dissolved, remnants of the membranes remained attached to those of adjacent cells to form double membranes. They are seen cut transversely, obliquely and tangentially. The intercellular spaces between swollen cells often were wider than normal and contained material (see Fig. 2) similar to that in the controls. Intercellular substances between the remnants of cell membranes were decreased in amount and density.

In areas where the tonofilaments in the noncornified epidermis were homogeneous (see under *b*) the basal 1–2 horny cell layers appeared dense

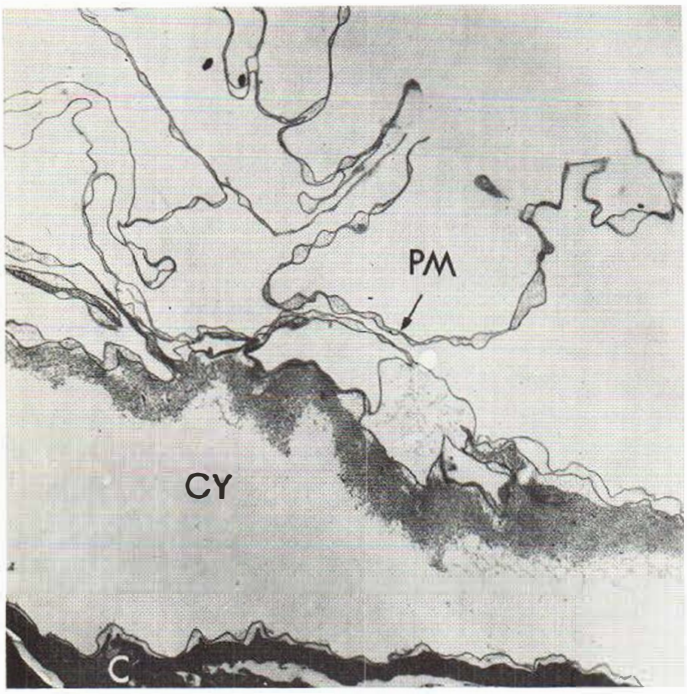

Fig. 3. Another area of horny layer 30 min after application of 1 N sodium hydroxide. Contents of superficial cells have disappeared, only remnants of their plasma membranes are seen (*PM*). The cell immediately below exhibits intense cytolysis (*CY*). Cell of the next lower layer is fairly well preserved. × 6 800.

(Fig. 4). These dense cells were seen with increasing frequency in 15, 30, 45 and 60 min specimens.

(*b*) Non-cornified epidermal cells: Areas of normal appearance, areas of initial changes, and areas of advanced changes were often observed in the same section as early as 15 min after application of NaOH. All stages were readily identified at the end of 1 hour.

The first detectable change was dissociation of tonofilament bundles into discrete filaments in the granular and upper spinous layers. As the changes advanced, the tonofilaments showed a homogeneous appearance (Fig. 4) and then they became indiscernible from the cell matrix (Figs. 4, 5). In the keratohyalin layer, the dense material of the keratohyalin granules formed variously sized and shaped dense bodies (Fig. 4). The first change of the desmosomes was the disappearance of the intercellular dense layer and attachment plaques, which preceded homogenization of tonofilaments and cytoplasm. The lamellar granules on the outer surfaces of the uppermost granular cells increased in number and opacity. Matrices of some mitochondria were dense (Fig. 6). Chromatin substance in the nucleus showed a peculiarly dispersed pattern similar to those observed in karyorrhexis and karyolysis (Fig. 4). The cytoplasm showed a homogeneous appearance at low magnification and a finely granular appearance at high magnification (Figs. 4, 6). Dense granules of unknown nature surrounded by electron-translucent areas (Figs. 4, 6) were seen throughout the non-cornified epidermis. These granules had a diameter of about 0.37–2 microns. In some instances, lamellar granules and dense granules were seen floating in a homogeneous cytoplasm.

The cell membranes and basal lamina were relatively resistant (Fig. 6). Interdigitation and protrusion of cell membranes disappeared and they became closely apposed (Fig. 4). The most advanced changes by the end of 1 hour included destruction of the entire horny layer and disappearance of almost all organelles except fragmented cell membranes in the non-cornified layers. Frequent interruption of the dermo-epidermal basal lamina was found (Fig. 7 A). Even in specimens showing severe destruction of other elements those cell membranes parallel to intact stretches of basal lamina were preserved.

2. *Changes produced by 1 N hydrochloric acid*

(*a*) Horny layer: The number of horny cell strata ranged from 13 to 23 and the superficial horny layer was preserved. The outermost 1–3 cells

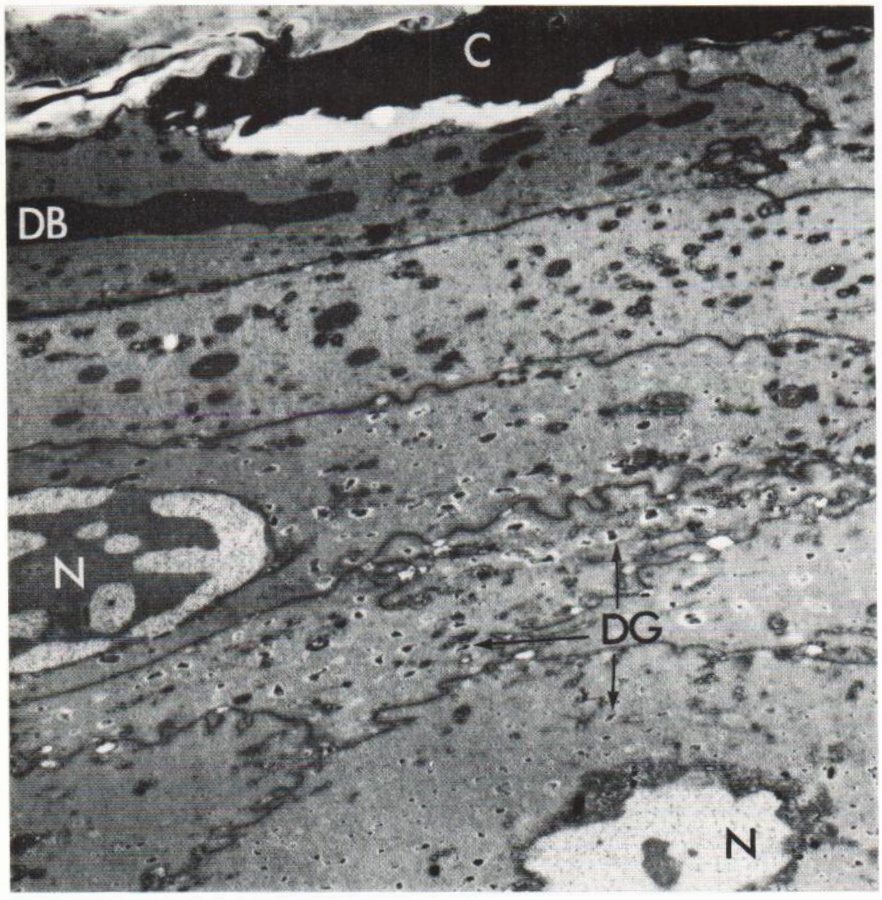

Fig. 4. Low magnified micrograph of the non-cornified epidermis 60 min after application of 1 N sodium hydroxide. Tonofilament bundles and desmosomes have disappeared and cytoplasm shows homogeneous appearance. Variously modified cell structures are seen: *C*, dense basal horny cell; *DB*, dense bodies in granular layer; *N*, nucleus showing peculiarly dispersed chromatin pattern; *DG*, dense granules surrounded by electron empty space. × 8 500.

showed densely packed fibrils in 1–3 hour specimens. Below these cells there were 1–5 layers whose cytoplasm increasingly showed a characteristic porous structure (Fig. 8 A). In 2–3 hour specimens, this porous structure was pronounced particularly along cell boundaries. Some cells lost their internal structures (Fig. 8 B), but the empty cell membranes encountered in sodium hydroxide treated skin were not seen. The width of intercellular spaces increased with time. Normal amounts of intercellular substances (Fig. 8 A, 8 B) were present.

The basal horny cells in 1 hour specimens showed no significant changes. Some of the basal horny cells in 2–3 hour specimens showed large areas of porous structure or large electron-transparent areas. Such cells were seen occasionally in control specimens but their number was increased in the test sites.

(*b*) Non-cornified layer: Specimens taken at 30 min and 1 hour did not show any morphologic changes. Two and 3 hour specimens showed intercellular and intracellular edema, especially in the lower granular and upper spinous layers (Fig. 9). The tonofilament-desmosome complexes were well preserved although stretched across the wide intercellular spaces. Focal disruption of the cell membranes was observed.

The lamellar granules in the border between cornified and non-cornified epidermis were increased in number as well as in density in the 2 hour specimens (Figs. 9, 10) whereas in the

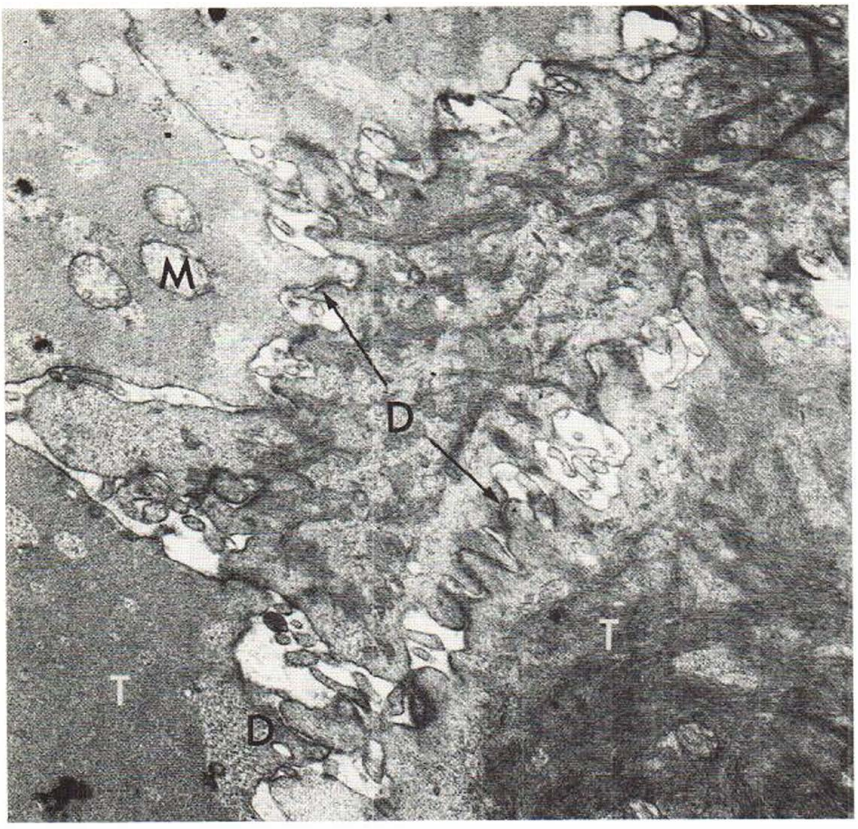

Fig. 5. Several cells of the spinous layer 45 min after application of 1 N sodium hydroxide show sequential changes of tonofilament bundles and desmosomes. The cells right of center of the picture contain disorganized tonofilament bundles (*T*). Tonofilaments (*T*) in the cells left of center appear homogeneous. *M*, swollen mitochondria with blurred cristae; *D*, desmosomes. × 20 000.

3 hour specimens they were decreased in number. In the lower granular and upper spinous layer the lamellar granules were "ballooned out" (Fig. 9 Inset), their average long diameter being enlarged from 0.18 in the control to 0.27 microns. Lamellar structure was more obvious than in the control specimens and the granules consisted of 2–3 subunits surrounded by a common membrane. Most of the keratohyalin granules were of low density. Some of them showed a fibrillar structure (Fig. 10). The interfilamentous spaces of the tonofilament bundles were widened in the spinous and granular layers and each filament was discernible (Fig. 9). The chromatin substance of the nuclei tended to be displaced toward the well preserved nuclear envelope. The basal lamina was less dense and more vague than that of the controls (Fig. 7 B). The space between it and the cell membrane became narrower.

DISCUSSION

Primary irritant cutaneous injury is an extremely common dermatosis. However, the biologic nature of the irritant injury and the sequence of events resulting in cutaneous change are not well defined. Little electron microscopic work has been done to clarify the mechanism of injury in primary irritant contact dermatitis. Relatively strong concentrations of acid and alkali were used in this study with the intention of producing drastic changes with which the effects of the more common exposures to milder irritants could be compared.

Our electron microscopic findings on normal human epidermis agree generally with those reported in the literature (4). Minor variations may be due to differences in fixing and staining methods.

As regards the influence of NaOH and HCl

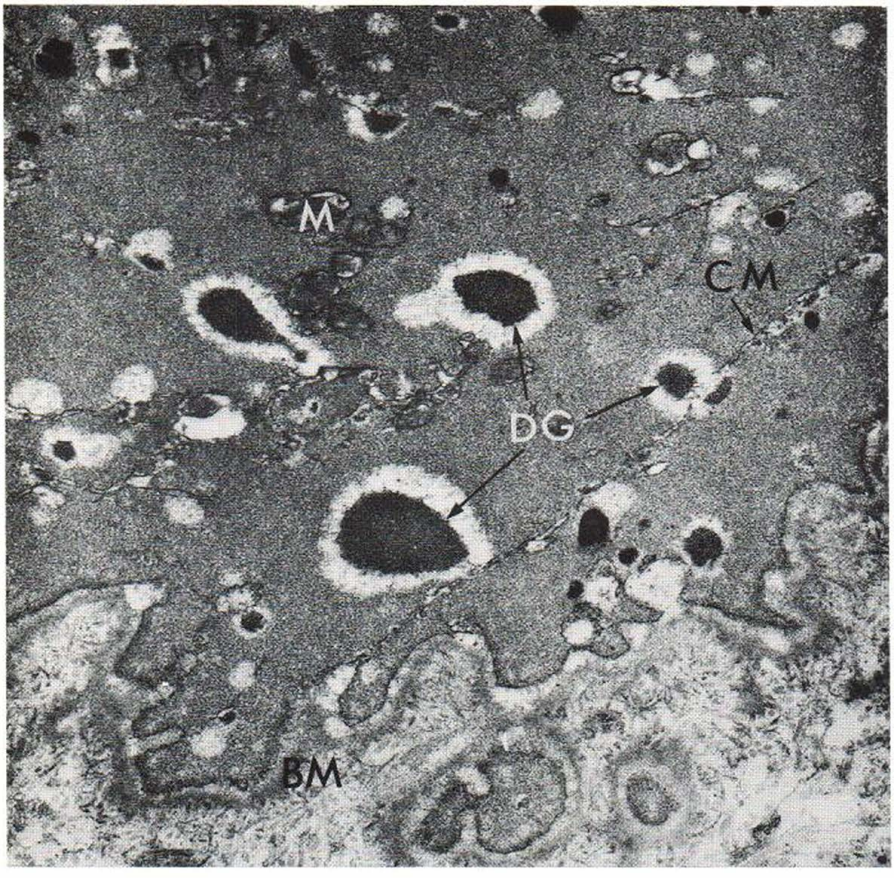

Fig. 6. Basal layer of non-cornified epidermis 30 min after application of 1 N sodium hydroxide. Higher magnification of the dense granules (*DG*) surrounded by clear spaces. The matrices of mitochondria are dense. *BM*, Basal lamina; *CM*, cell membranes. × 20 000.

on fixation, we considered the possibility that shifts in pH caused by the presence of alkali or acid on the experimental biopsy specimens might influence fixation and lead to artefacts. The results of our control experiment, in which the test solutions were rinsed off before biopsy was taken, demonstrated that pH of the fixing solutions was not significantly affected even without rinsing. The sections obtained from rinsed and unrinsed specimens showed esentially similar changes (Figs. 11, 12). Moreover, alterations generally proceeded with time from the surface downward with one exception which will be discussed in the next paragraph.

Routes of penetration

By the end of 1 hour after application of sodium hydroxide and 2 hours after hydrochloric acid, living epidermal cells showed severe changes regardless of whether the horny layer had been destroyed or not. On clinical observation, some of the subjects developed prominent perifollicular papules during the exposure to sodium hydroxide. It is plausible that some of the irritants reached the living cells by way of the follicular route as first suggested by Sulzberger and his group (7). Scheuplein (9) who recently analysed the absorption pathway mathematically, concluded that "shunt diffusion" (follicular) is dominant in the initial stages of percutaneous absorption. The fact that alterations of horny cells proceeded downward with time suggests that the chemicals did not penetrate without leaving evidence of their action. However, if all cell layers of the stratum have a barrier or retarding function, a view expressed by Kligman (6) and recently by I. Blank

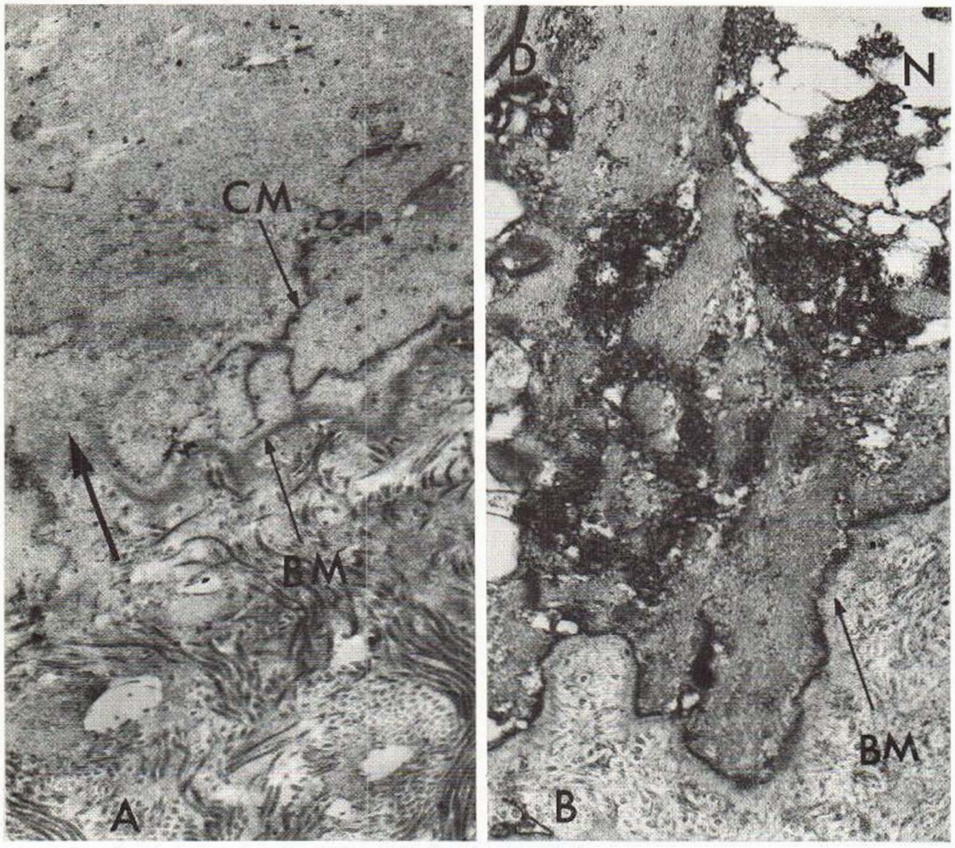

Fig. 7. The dermo–epidermal junction. (A) 60 min after application of 1 N sodium hydroxide. (B) 120 min after application of 1 N hydrochloric acid. In (A) only amorphous cell contents and fragmented cell membranes are seen in this most advanced stage of epidermal destruction. Interruption of dermo-epidermal basal lamina (*BM*) is indicated by large arrow. *CM*, Cell membranes. × 14 500. In (B) the basal lamina (*BM*) is less dense and more vague than that of the control. Intermembranous space is narrow. Nuclear chromatin substance is attached peripherally at the nuclear envelope which is well preserved. *D*, Desmosome. × 20 000.

(1), one must consider the possibility that the partially neutralized irritants which failed to cause visible change in the relatively insoluble lower horny cells still maintained sufficient strength to affect the living cells of the stratum malpighii.

Dissimilarities and similarities between the ultrastructural changes caused by sodium hydroxide and hydrochloric acid

The ultrastructural changes produced in the horny cells differed widely. Sodium hydroxide caused homogeneous appearance and later complete dissolution of cell contents, while hydrochloric acid produced a porous appearance with circular profiles. We were not able to detect, at the magnification achieved, which cellular component (filaments or matrix) of the keratin pattern was originally affected and cannot decide the question whether acid and alkali affected different protein components or the same component in different ways.

Thus, in HCl-treated skin, owing to intercellular edema, desmosomal attachments were pulled out into the intercellular spaces, but did not dissolve. Separation between cells occurred through breaks in the membrane of one cell permitting the desmosome to retract toward the opposite cell. Somewhat similar changes have been observed within 4–8 hours after the mechanical injury of tape stripping (8). Na●H, in contrast, caused disappearance of the intercellular dense layer and at-

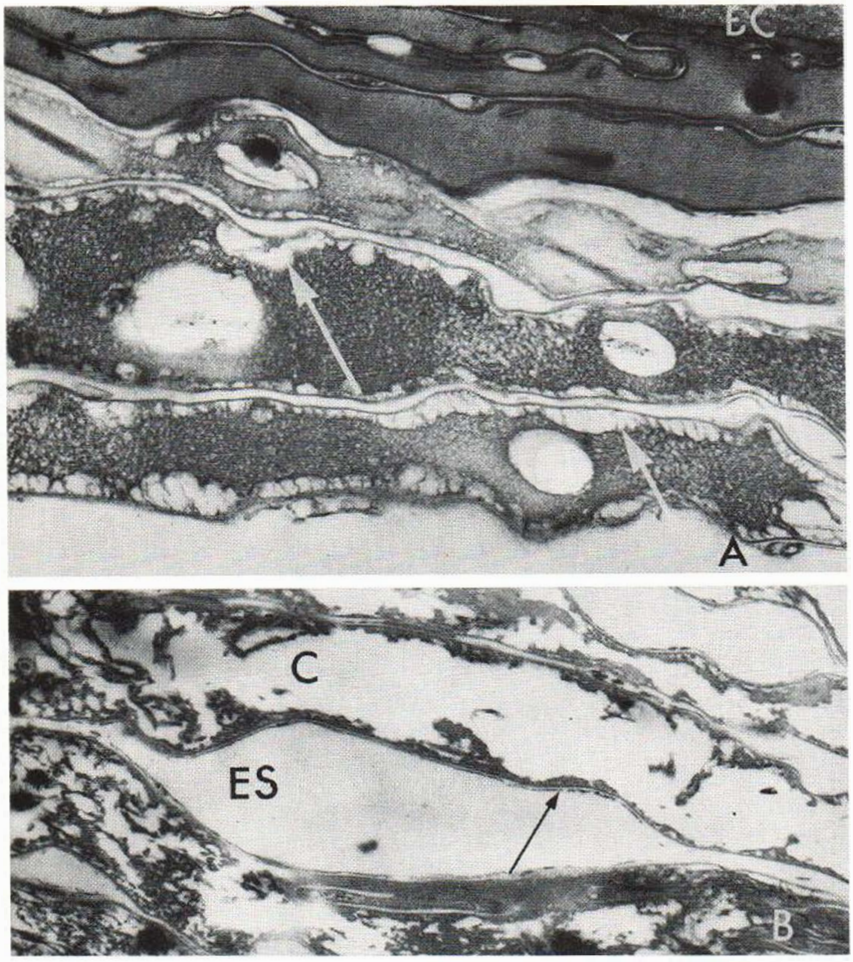

Fig. 8. (A) Superficial horny layer 180 min after application of 1 N hydrochloric acid. Lower half of the picture shows cells characterized by marked porous appearance (*arrows*) with circular profiles especially at the cell boundaries. *BC*. Part of red blood cell resting on skin surface. × 20 000. (B) Other area of superficial horny layer 180 min after application of 1 N hydrochloric acid. Disorganization of cellular pattern. Some cells (C) contain only fragments of dense material. Intercellular substances (←) and wide intercellular space (*ES*) are visible. × 27 000.

tachment plaques at the same time when tonofilament bundles were split into filaments.

In the specimens treated with hydrochloric acid, enlarged lamellar granules were observed. Many granules were composed of 2–3 subunits of packets of lamellae. Some investigators (2, 5, 10) have described different *main* orientation of packets of lamellae within an individual granule. Our observations suggest that this difference of orientation is due to the presence of tightly packed subunits, which under the application of hydrochloric acid become more clearly visible.

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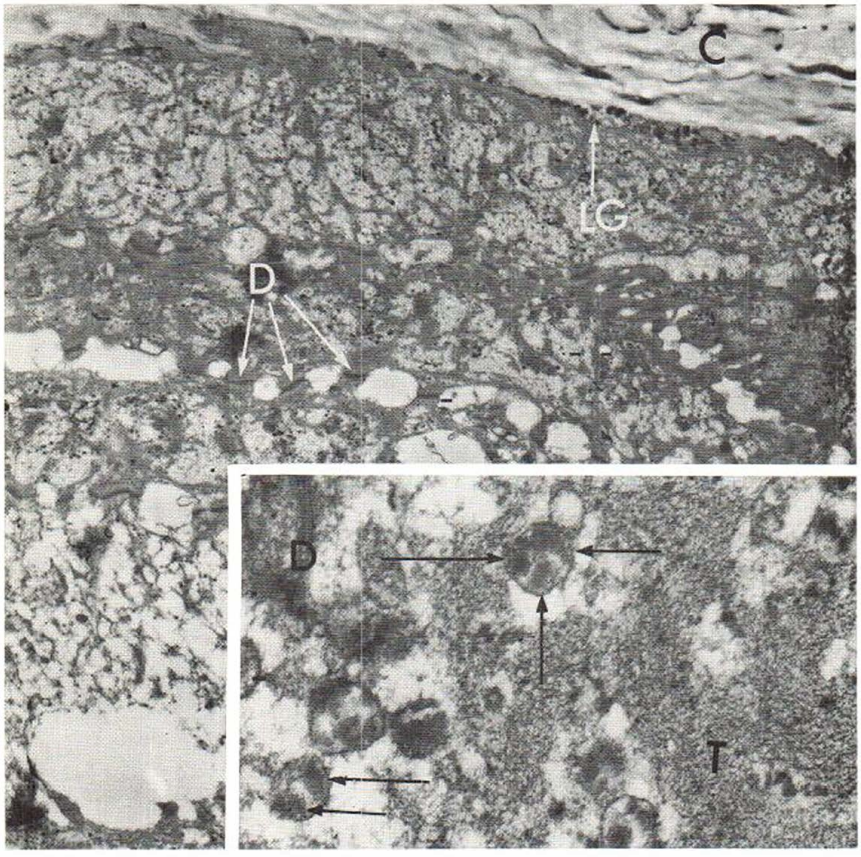

Fig. 9. Low magnified micrograph of 120 min after application of 1 N hydrochloric acid. Inter- and intracellular edema is prominent in the spinous layer. Desmosomes (*D*) are stretched between cell bodies. Increased number of lamellar granules (*LG*) in the border between cornfied (*C*) and non-cornified epidermis. × 8 500. *Inset* depicts enlarged lamellar granules 120 min after application of 1 N hydrochloric acid. Arrows point to subunits of the individual granule. The lamellar structure is more obvious than in control specimens. Tonofilament bundles (*T*) are disorganized. *D*, Desmosome. × 40 000.

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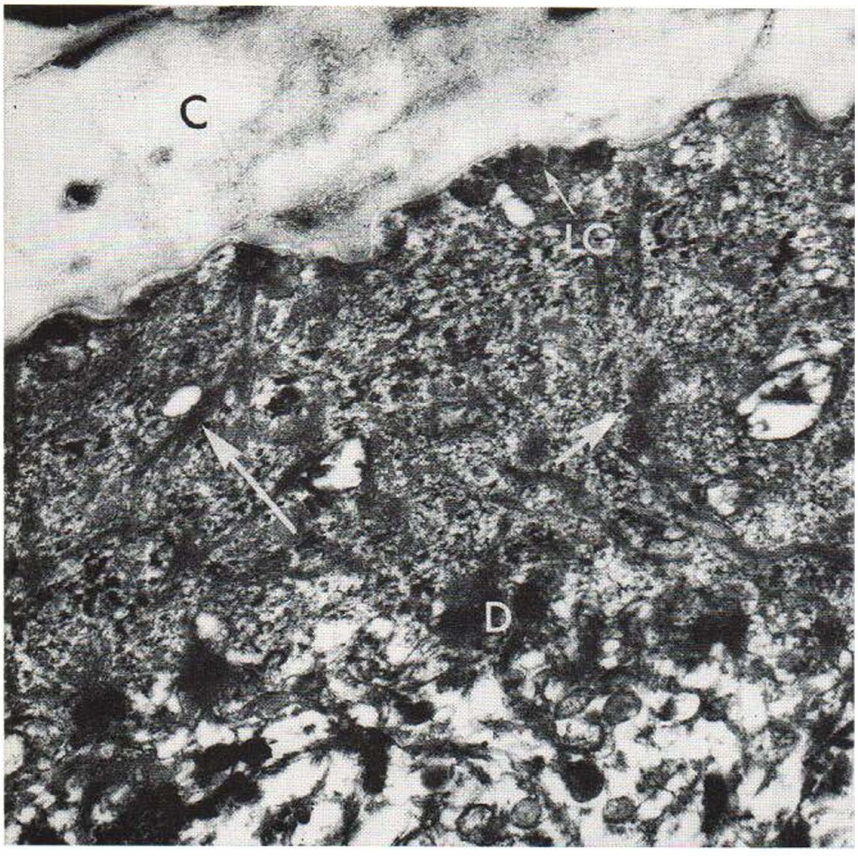

Fig. 10. Granular layer 120 min after application of 1 N hydrochloric acid. The keratohyalin granules show fibrillar structure (←) resulting from loss of their original characteristic dense substance. Increased lamellar granules (*LG*) are seen in border between cornified and non-cornified layer. *D*, Desmosomes; *C*, cornified layer. × 27 000.

Fig. 11. The horny layer 60 min after application of 1 N sodium hydroxide. × 13 500.

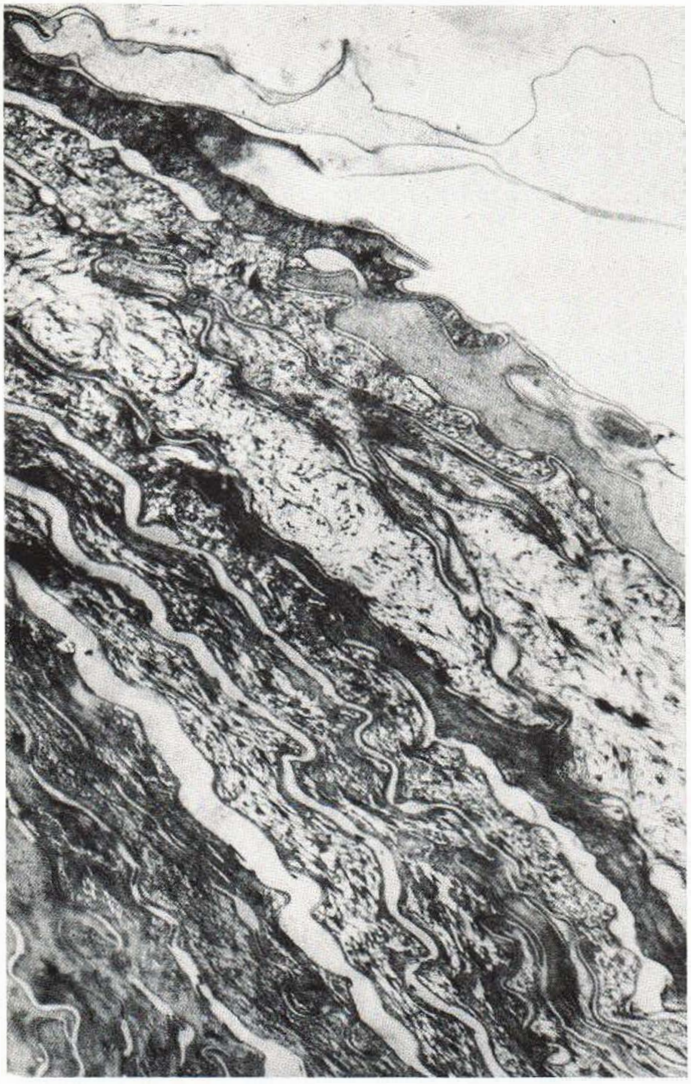

Fig. 12. The horny layer 60 min after application of 1 N sodium hydroxide. This specimen was not rinsed before biopsy was taken. The sections obtained from rinsed (Fig. 11) and unrinsed specimens showed essentially similar changes. × 13 500.