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AN ELECTRON MICROSCOPIC STUDY OF HUMAN EPIDERMIS AFTER ACETONE AND KEROSENE ADMINISTRATION*

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ABSTRACT

An electron microscopic study has been done on normal human skin exposed either 30 or 90 minutes to two lipid solvents (acetone and kerosene) commonly encountered in occupational situations.

After topical application of acetone and kerosene, cellular damage was observed mainly in the stratum corneum and stratum spinosum. Keratinized cells, keratohyalin cells, spinous cells, and their nuclear and cell envelopes were affected in somewhat different ways by the two solvents. Marked intracellular edema of keratinized cells and vacuolation of spinous cells were seen after exposure to acetone, while large lacunae and disappearance of the keratin pattern in keratinized cells and evidence of cytolysis of spinous cells were found only after kerosene exposure. These cytoplasmic and nuclear degenerative changes were more pronounced after 90 minutes' than after 30 minutes' exposure. Many cell membranes were ruptured and ruthenium red localization showed that desmosomes and other junctional complexes were often disrupted.

Seventy-two hours after discontinuation of the solvent application, a high degree of restoration of the normal ultrastructural pattern was observed in superficial layers, while residual evidence of cell degeneration and evidence of early reactive changes were found in the viable portions of the epidermis.

The effects of various chemical agents on human epidermis have been studied in our laboratory for the past three years. These studies are being done for two reasons: (a) to analyze the intimate structural changes produced by different injurious agents in human epidermis, to compare their effects, and to obtain data regarding their action; (b) to provide a better understanding of the mechanism of primary irritant contact dermatitis which is frequently observed in industry as well as in the home. With the electron microscope, we can detect earlier structural changes than has been possible heretofore by light microscopy and we can follow the changes sequentially. In a previous paper, the ultrastructural alterations after topical application of a strong alkali (1N sodium hydroxide) and a strong acid (1N hydrochloric acid) were described [1]. A comparative study between a soap and a household detergent was recently carried out using electron microscopy and histochemistry.† Other workers have investigated the effect of a proteolytic enzyme (subtilisin [2]) and of mercuric chloride [3].

In the present study the ultrastructural changes after topical administration of the lipid solvents (acetone and kerosene) on human skin are presented.

MATERIALS AND METHODS

Topical application of acetone and kerosene was done on healthy skin of the volar surface of forearms of six male volunteers (two Caucasians and four Negroes). Two small glass tubes (5 mm diam) containing approximately 1 ml acetone were applied to two sites on the right forearm and maintained in vertical position by adhesive tape for either 30 minutes or 90 minutes. Kerosene was applied in the same manner for 30 or 90 minutes to the left forearm. The tubes were then removed. No cleansing of the test sites was done either before or after the procedure. Under 2% lidocaine local anesthesia, 4-mm punch biopsies were taken immediately from one of the test sites on each arm. The other sites were observed for 72 hours and biopsy specimens obtained at that time. Control specimens were taken from each subject before exposure. Immediately after removal, skin specimens were cut into small pieces, fixed in 3% sodium cacodylate-buffered glutaraldehyde (pH 7.3; osmolarity 300–330 milliosmols) for 2 hours, and postfixed in 1% osmium tetroxide in phosphate buffer for 2–3 hours [4]. Some specimens were fixed in 3% glutaraldehyde to which 0.5 mg per ml ruthenium red was added [5] for 1 hour,

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† Predeteanu C: The effect of soap and detergents on human epidermis. An electron microscopic study. (In preparation)

washed for 1 hour in buffer containing 0.5 mg ruthenium red per ml, and finally fixed in 1% osmium tetroxide in phosphate buffer for 2 hours. Half of the specimens were dehydrated through graded concentrations of ethanol and embedded in Epon-812; the remaining specimens were dehydrated through progressive series of acetone, then embedded in Vestopal-W or Araldite-502. Thick sections were stained with 1% toluidine blue for light microscopy. Thin sections were cut using LKB and Porter–Blum ultramicrotomes equipped with glass or diamond knives. Sections were stained with uranyl acetate and lead citrate, then examined under Hitachi HS-8 or RCA EMU-3G electron microscopes at 5,600–18,000X.

RESULTS

Early Changes

Clinically, only mild edema and hyperemia were observed following 90 minutes' exposure to acetone. Somewhat more intense hyperemia followed exposure to kerosene. Light microscopic study showed a reduction and disorganization of horny layers with intercellular edema following acetone exposure; dislocation and disruption of stratum corneum were more evident after kerosene application.

Electron microscopic observations revealed ultrastructural features which were quite specific for acetone or kerosene.

Acetone. Following acetone administration, marked changes were observed in the stratum corneum, as compared to the compact keratin pattern of control skin (Fig. 1). Intracellular edema with large and oval spaces containing a fine granular material (Fig. 2) were evident. The surrounding keratin layers were disrupted and ede-

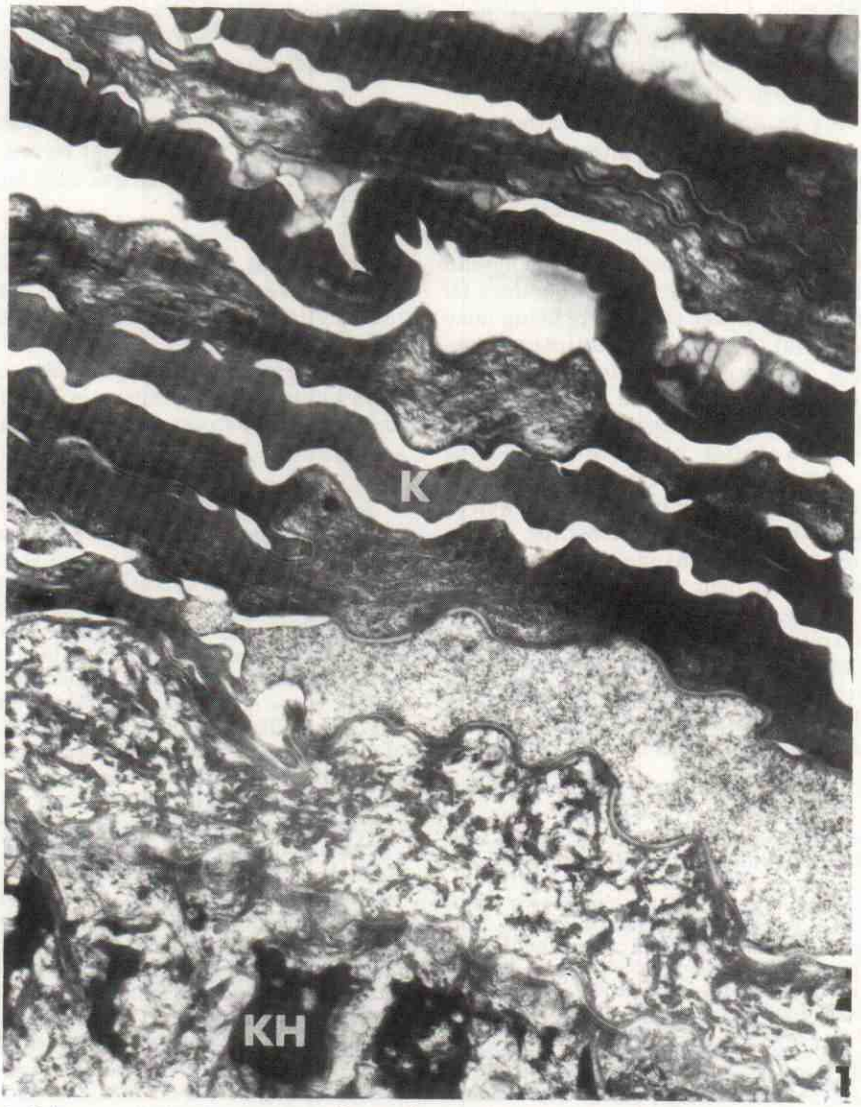

FIG. 1. Control human stratum corneum and granulosum. K—keratin layers; KH—keratohyalin granule. × 16,700.

matous. Fibrils and filaments were rarified and disrupted, and desmosomes between horny cells were broken. Ruthenium red deposits in the form of fine grains were increased and specifically localized to the outer membranes of corneocytes (Fig. 3b, as compared to control tissue, Fig. 3a). In the granular layer, the keratinocytes contained numerous large keratohyalin granules and small electron-dense bodies.

In the stratum spinosum, an interesting vacuolar transformation of the spinous cells occurred with the formation of paranuclear cisternae (Fig. 4). Usually the vacuoles contained membranous structures or a fine material in their lumina. Degenerative mitochondrial changes were also seen and tonofilaments were clumped. Endoplasmic reticulum was well developed. Nuclear chromatin was also clumped and peripherally located, as compared to control tissue (Fig. 5). The outline of the nuclear envelope was very irregular, giving a dentated pattern of the nucleus. Nucleolar damage only occasionally was seen. Intercellular spaces were enlarged and desmosomes disrupted. No ultrastructural changes were observed in the basal layer, Langerhans cells, or melanocytes.

Kerosene. Following exposure to kerosene, the ultrastructural changes were significantly different. Thus, the horny layers were reduced in number and the keratin pattern was disorganized. Large lacunar formations were present (Fig. 6); they contained fibrils in their lumina. Other horny cells were disrupted and disintegrated; plasma membranes appeared thick. Some keratinocytes were severely damaged, nuclei were pyknotic, cell membranes thick and disrupted, and tonofilaments clumped together. Advanced cell damage

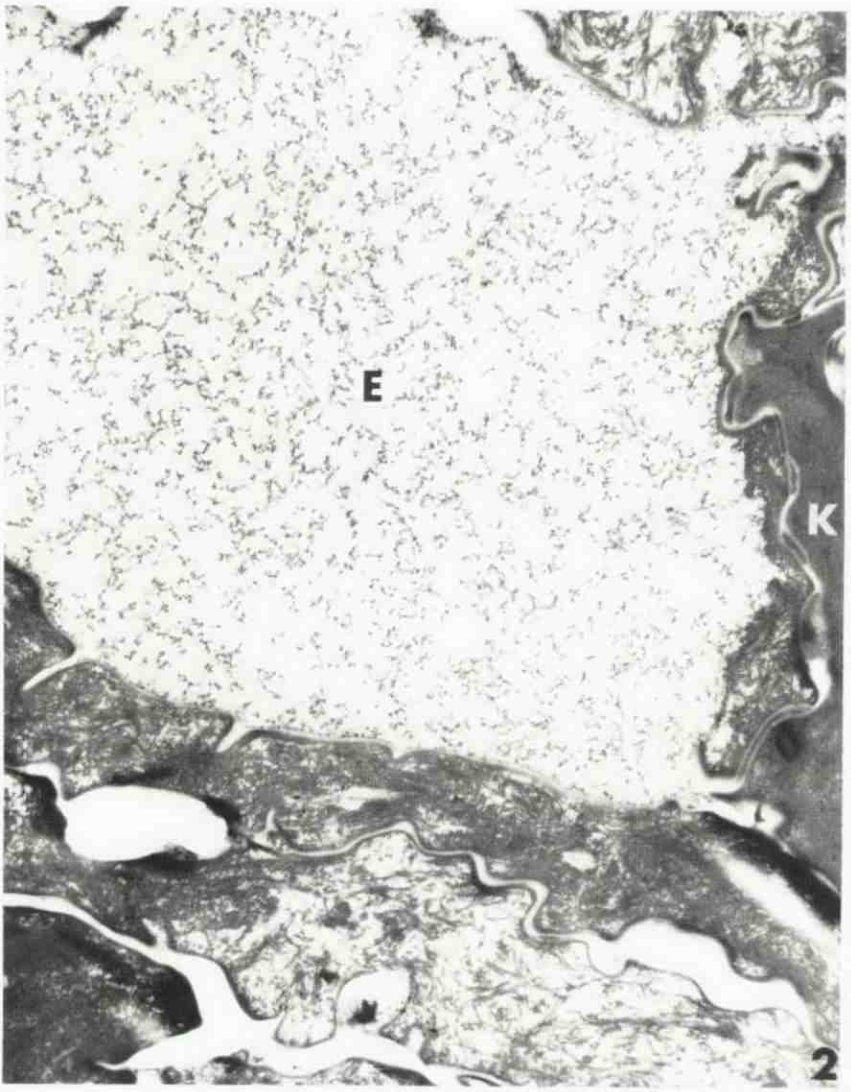

FIG. 2. Human stratum corneum following acetone administration (90 minutes). Marked intracellular edema (E); keratin layers (K) are disrupted. × 22,000.

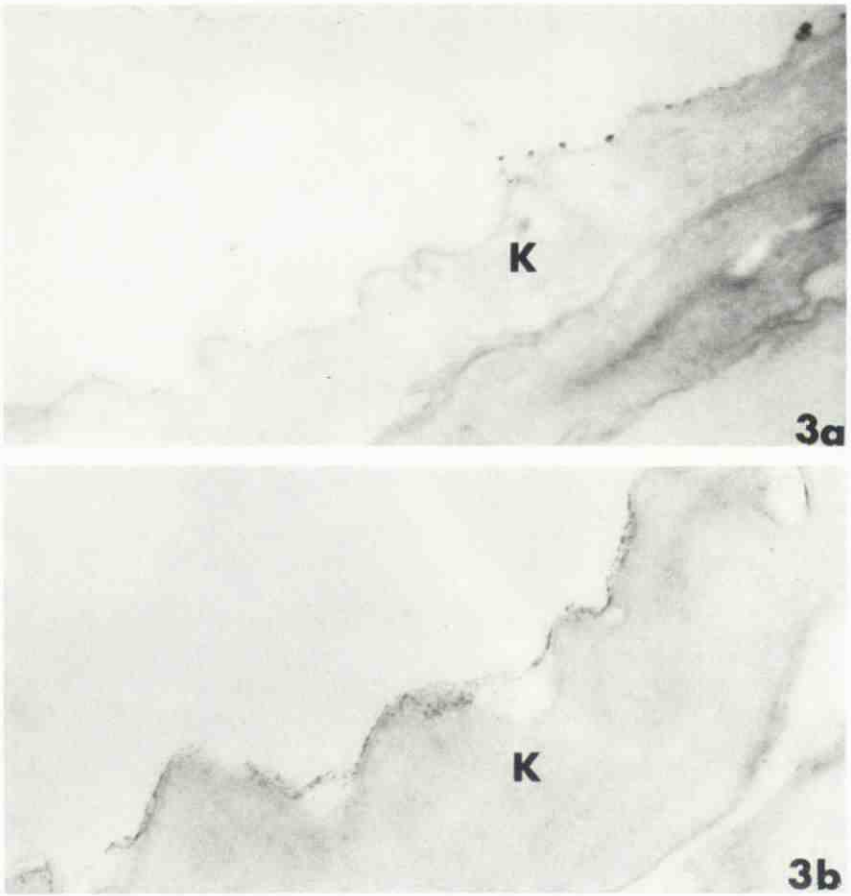

FIG. 3. a) Control—ruthenium red slightly stains the outer membranes of keratin (K) sheets. × 37,200. b) Ruthenium red stains specifically the outer membranes of the keratin sheets (K). Acetone administration (90 minutes). × 37,200.

was evident in the stratum spinosum; several spinous cells were undergoing cytolysis, the greater portion of the cytoplasm appeared as a homogeneous, structureless material (Fig. 7). Nuclei were shrunken and indented with peripheral chromatin. At higher magnification, the cytolytic process was more evident; severe mitochondrial changes were seen in the remaining cytoplasm. The cytoplasm was largely replaced by a fine granular material, the nucleus was elongated and poor in chromatin. A granular–globular structure was found within the nucleus.

Late Changes

At 72 hours following 90-minute exposure to acetone or kerosene, restoration towards a normal pattern was seen. Thus, the ultrastructural pattern of keratin was close to that of the controls: desmosomes and membrane-coating granules (keratinosomes) were present, plasma membranes were thick, and between keratin layers a homogeneous material was released from the membrane-coating granules (Fig. 8). In the stratum granulosum, a regenerative reaction was observed. Large keratohyalin granules, polyribosomes, and numerous membrane-coating granules appeared. Tonofilaments and desmosomes were present (Fig. 9). Some of the damaged spinous cells were degraded and partially resorbed, whereas others showed signs of regeneration; they had a well-developed Golgi complex, many dense bodies, myelin figures or lamellar whorl structures. The intercellular spaces were enlarged, appearing cyst-like. Cytoplasmic projections and desmosomes also were observed, but still some cytolytic areas remained. The ultrastructural pattern of Langerhans cells was well preserved (Fig. 10). No mitoses were seen in toluidine blue-stained sections by light microscopy or in thin sections under the electron microscope. Some spinous cells had 3–4 nucleoli (Fig. 11).

Changes After Short Exposure

After a shorter exposure (30 minutes) to acetone or kerosene, there was much less evidence of cell damage; few cells underwent cytolysis and vacuolation. Intercellular spaces were enlarged and desmosomes were disrupted (Fig. 12). The altera-

tions in the stratum corneum resembled those after 90 minutes' exposure, but were less pronounced.

DISCUSSION

In this study, electron microscopic examination revealed several types of cell damage, both at the level of the stratum corneum and the stratum spinosum. Ultrastructural changes were different for acetone and kerosene. Thus, after topical acetone application, the relevant changes were: intracellular edema with dislocation of keratin layers, an intense vacuolation within the cytoplasm of spinous cells, degenerative mitochondrial changes, and enlarged intercellular spaces in the stratum spinosum. Kerosene exposure induced large lacunae in the horny layers and advanced cytolysis and fragmentation of tonofilaments in the spinous cells. Both solvents induced marked nuclear changes, following 90 minutes' administration. At 72 hours a tendency for restoration of the ultrastructural pattern toward normal was observed.

Since acetone and kerosene are lipid solvents, it is likely that their injurious influence on epidermal cells results mainly from removal of lipid components present in the stratum corneum. It is well known that lipid extraction severely affects the biophysical properties of the horny layer [6]. Kerosene, however, differs from acetone not only in its physicochemical characteristics as a solvent, but in its greater biologic activity as a skin irritant. It is, therefore, interesting to see that these two chemicals have different effects on the ultrastructure not only of the dead stratum corneum but also on the living cells of the stratum spinosum. Kerosene caused cytolysis, while acetone led only to a peculiar type of vacuolization. The latter change appears to be similar to that described by Braun-Falco [7] in guinea-pig epidermis after tape stripping and by Nix [8] in human skin after ultraviolet exposure. It may thus represent a less specific type of cell damage.

Intercellular edema and disruption of tonofilaments are, or course, seen in many types of epidermal damage, but the disruption of the keratin pattern may be a more specific effect. It was not seen in previous experiments in this laboratory when hydrochloric acid and sodium hydroxide were used. Topical application of 1N sodium hydroxide induced a homogeneous appearance and eventual dissolution of the cell contents, while 1N hydrochloric acid caused a porous structure of the stratum corneum [1]. Alterations seen

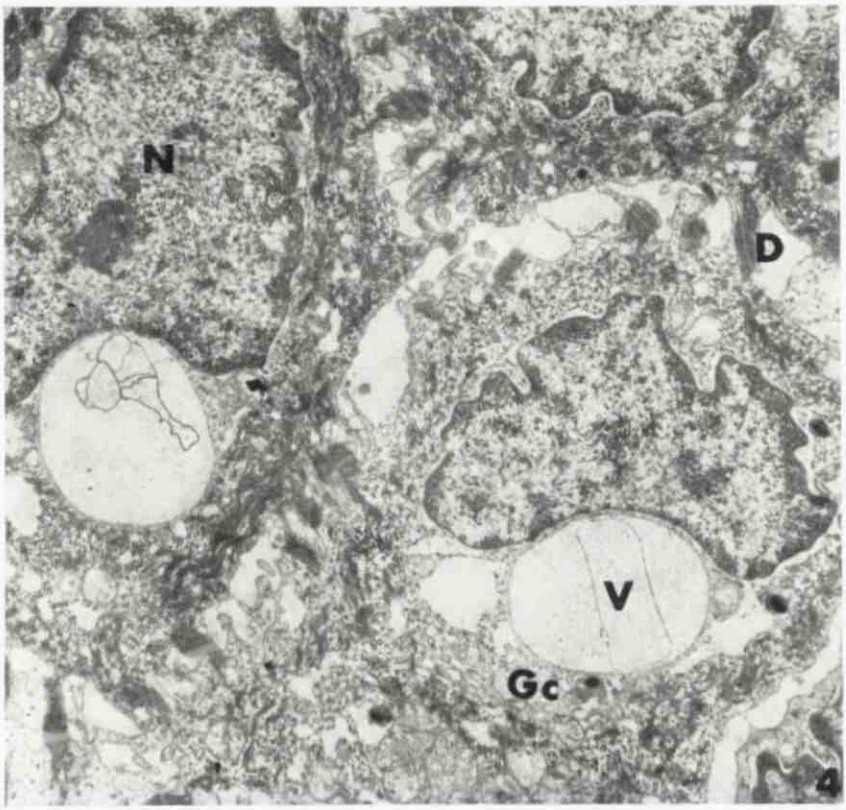

FIG. 4. Intense vacuolation in the stratum spinosum after acetone administration (90 minutes). Large paranuclear vacuoles (V) containing membranous structures in their lumina. N—nuclei with altered and irregular envelopes; Gc—Golgi complex; D—desmosomes. × 10,416.

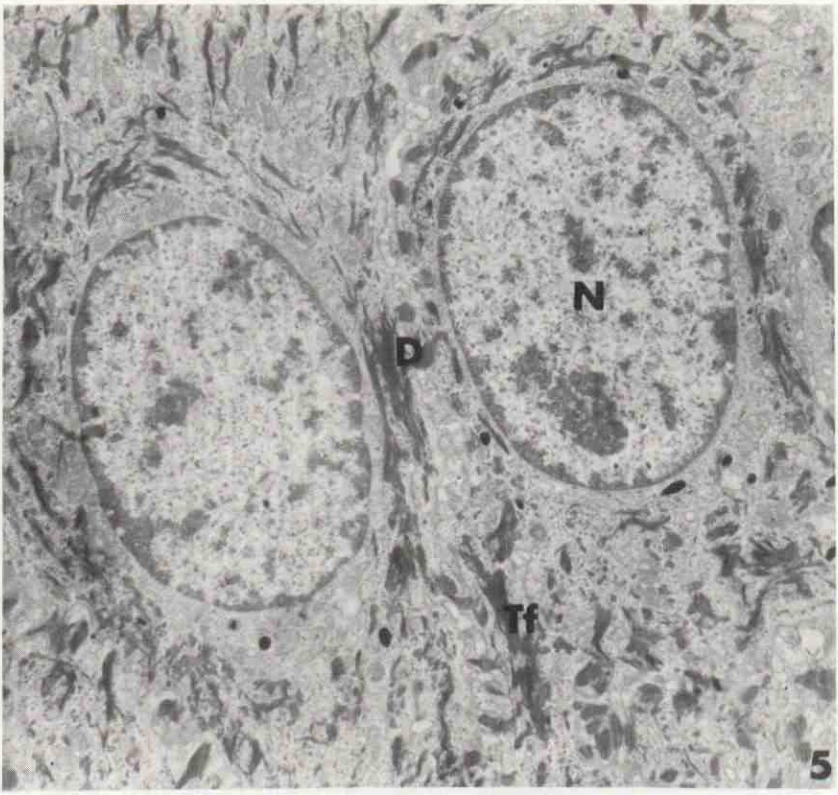

FIG. 5. Control human stratum spinosum. N—nucleus; D—desmosome; Tf—tonofilament. × 10,416.

by us in the present experiments also are different from those produced by a proteolytic enzyme [2]. It is possible that the changes produced by acetone and kerosene result from the disruption of lipoproteins in the stratum corneum.

Of considerable interest are the observations regarding the ultrastructural nuclear changes developed in the stratum spinosum, following application of both solvents. Nucleolar damage occurred only occasionally in our investigations. Interesting nuclear and nucleolar changes were recently described in human epidermis after exposure to ultraviolet light [9] and in baboon kidney cells following Actinomycin D administration [10]. However, the present findings are different from those described in these previous studies.

No significant ultrastructural changes of melanocytes and Langerhans cells were observed in spite of considerable cytolysis in neighboring keratinocytes. Electron microscopic and histochemical studies of human epidermis after topical application of mercuric chloride [3] showed degenerative changes not only in the keratinocytes, but also in Langerhans cells and melanocytes.

The origin and function of the vacuoles in the stratum spinosum is unknown. It is possible that they are formed by invaginations of the plasma membranes which engulf material from the disintegrated cytoplasm. The prominent cytolysis which occurred in our experiments suggests protein degradation following kerosene application. The cellular degenerative changes become more pronounced the longer the exposure to acetone and kerosene.

As to the fate of severely damaged cells in the stratum spinosum, it is possible that some of them are engulfed by phagocytotic activity of their less severely damaged neighbors. There was no evidence of massive elimination of damaged cells through exfoliation.

It is notable that regenerative activity of the epidermis at 72 hours is much less vigorous after damage by lipid solvents than it is after tape stripping. Mechanical removal of the stratum corneum, which is followed by dehydration damage to superficial layers of the living epidermis, produces a mitotic burst within 48 hours and leads to elimination of damaged cells and restoration of the keratohyalin layer within 72 hours. Even partial removal of the stratum corneum without obvious light microscopic damage to living strata is followed by a proportional increase of mitosis [11]. The application of acetone or kerosene does remove part of the horny layer, and causes damage to superficial spinous cells. However, in our 72-hour specimens no mitotic figures were observed, and only the presence of 3–4 large nucleoli in some cells suggested early stages of the process leading

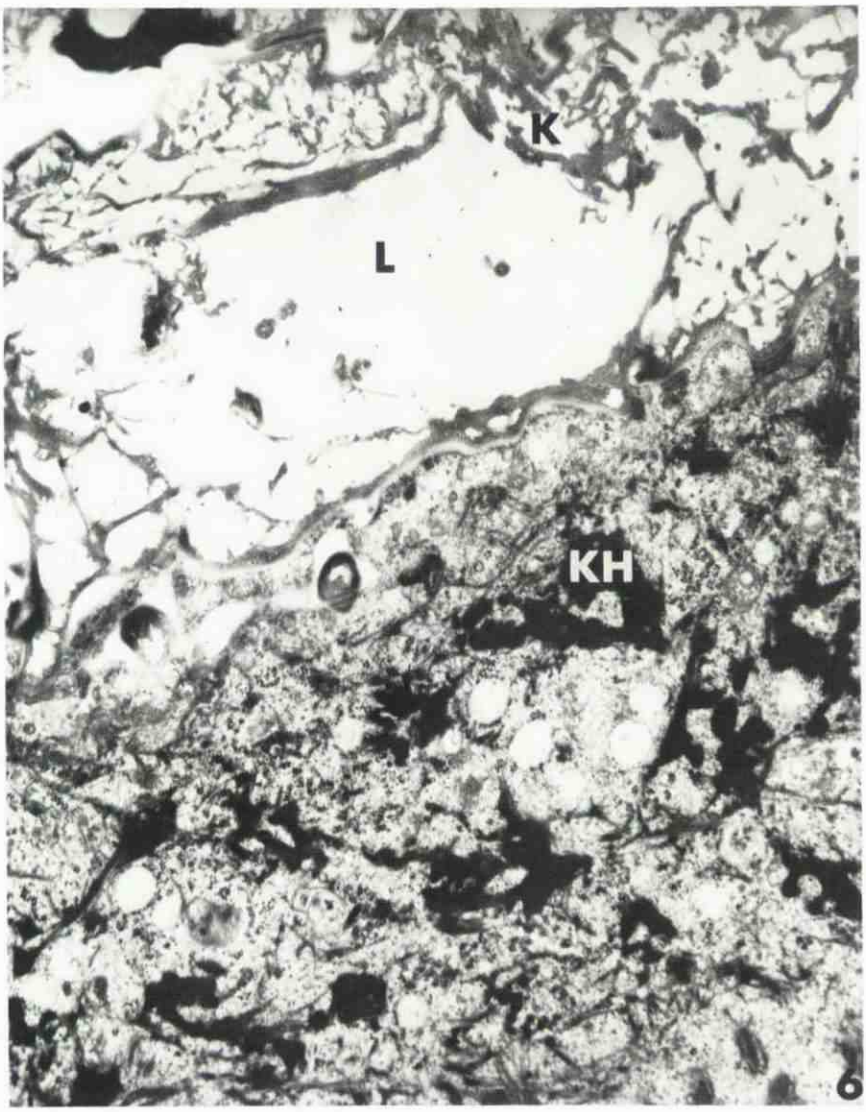

FIG. 6. Human stratum corneum and granulosum following kerosene administration (90 minutes). Large lacunae (L) and disorganization of keratin (K). KH—keratohyalin granules. × 22,000.

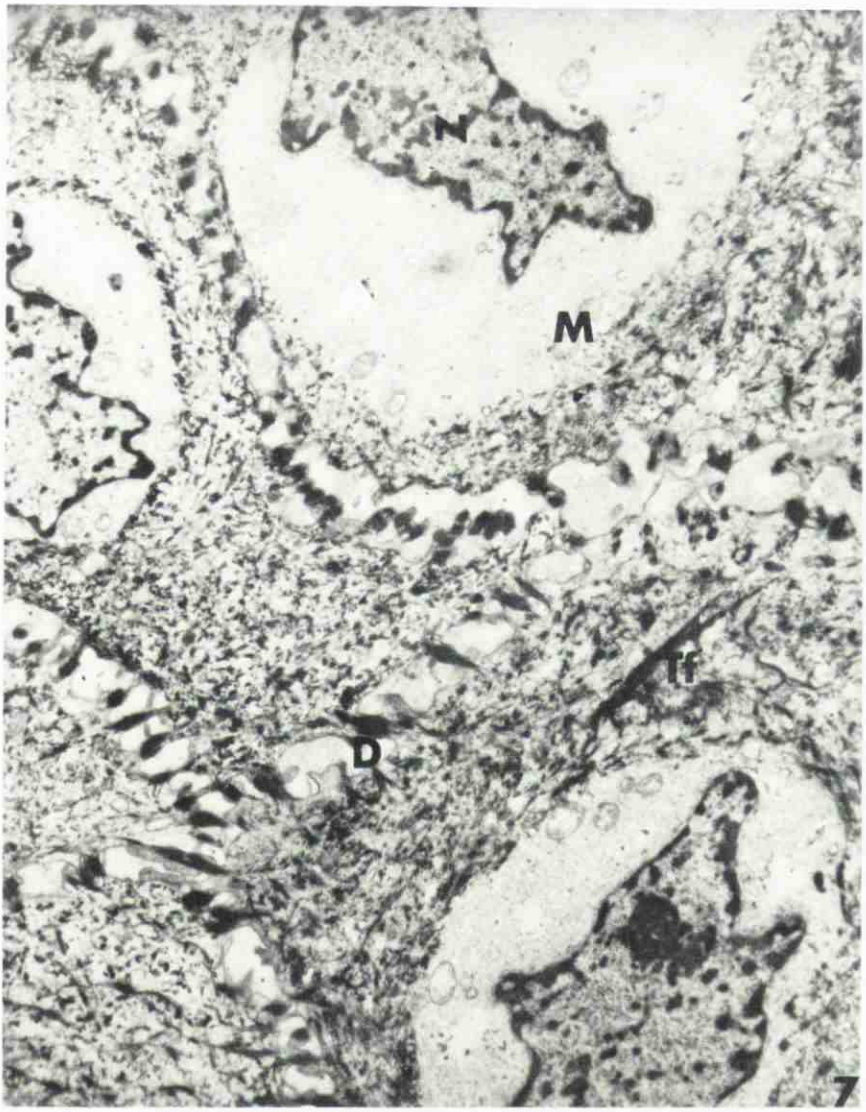

FIG. 7. Advanced cytolysis in stratum spinosum following kerosene administration (90 minutes). N—nuclei; D—desmosomes; Tf—tonofilaments; M—mitochondrion. × 10,000.

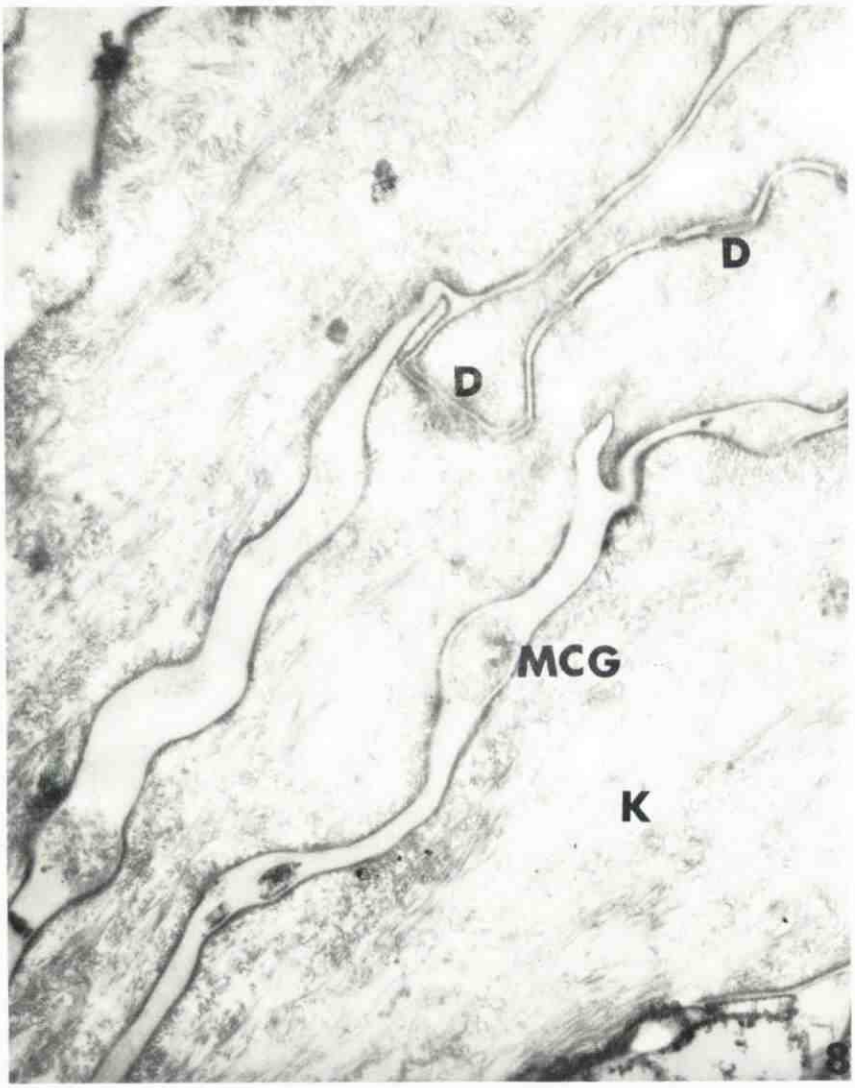

FIG. 8. Human stratum corneum 72 hours after acetone administration. Well-defined keratin pattern (K); D—desmosomes; MCG—membrane coating granules. × 33,000.

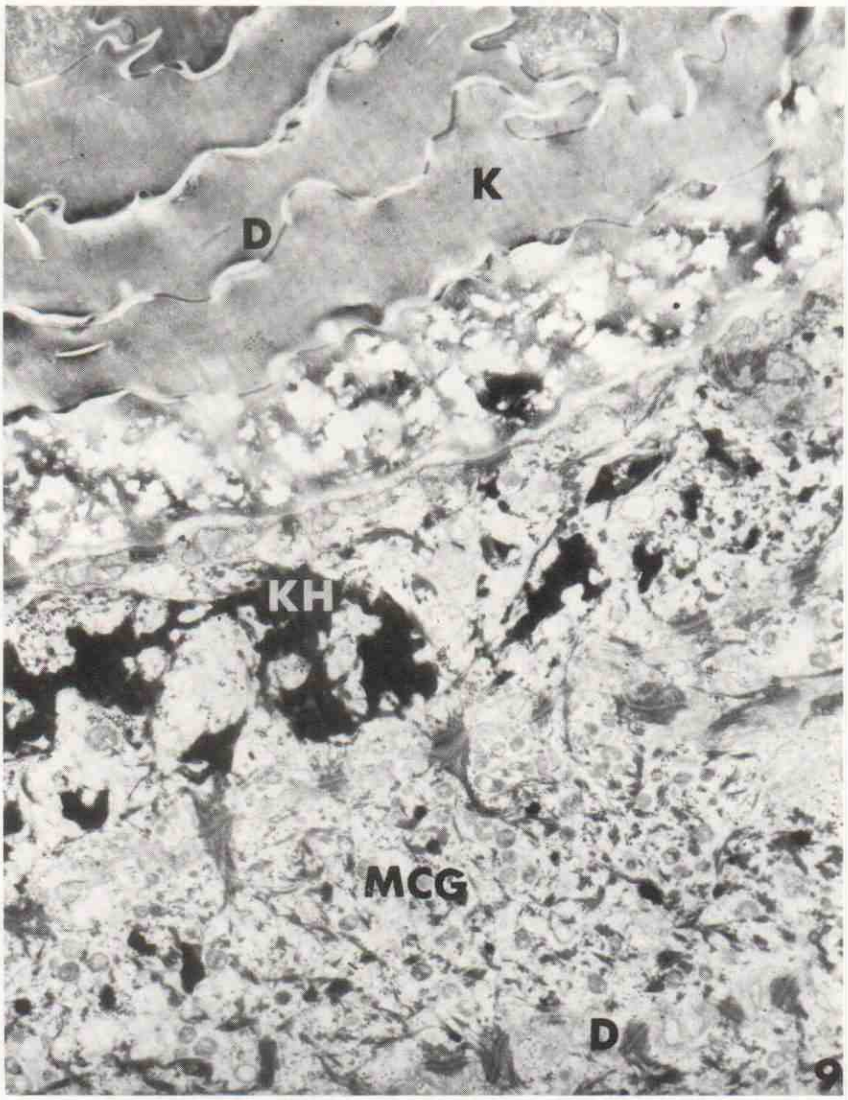

FIG. 9. Regenerative reaction in stratum corneum and granulosum 72 hours after acetone administration. KH—keratohyalin granule; MCG—several membrane coating granules; K—keratin layers; D—desmosomes. × 16,700.

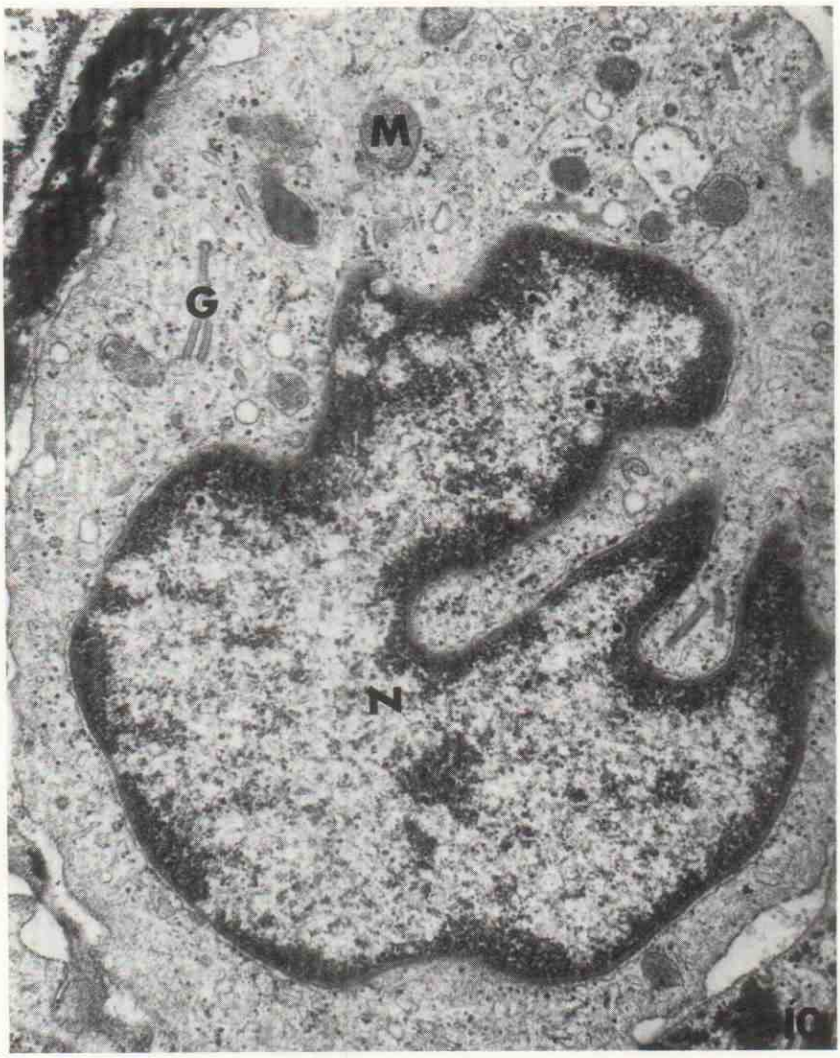

FIG. 10. Langerhans cell after acetone administration (72 hours). No significant changes occur. G—characteristic granule; M—mitochondrion; N—nucleus. × 33,000.

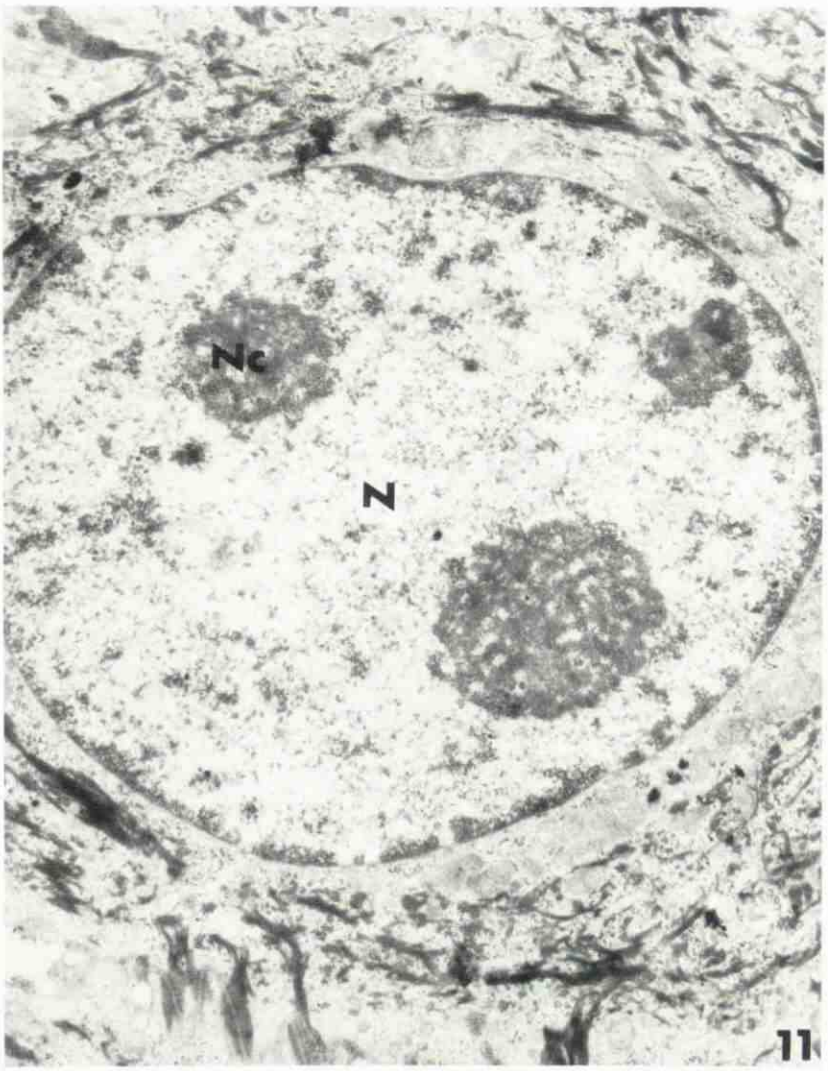

FIG. 11. Human stratum spinosum following kerosene administration (72 hours). Cells having 2-3 nucleoli (Nc) are frequently seen; N—nucleus. × 16,700.

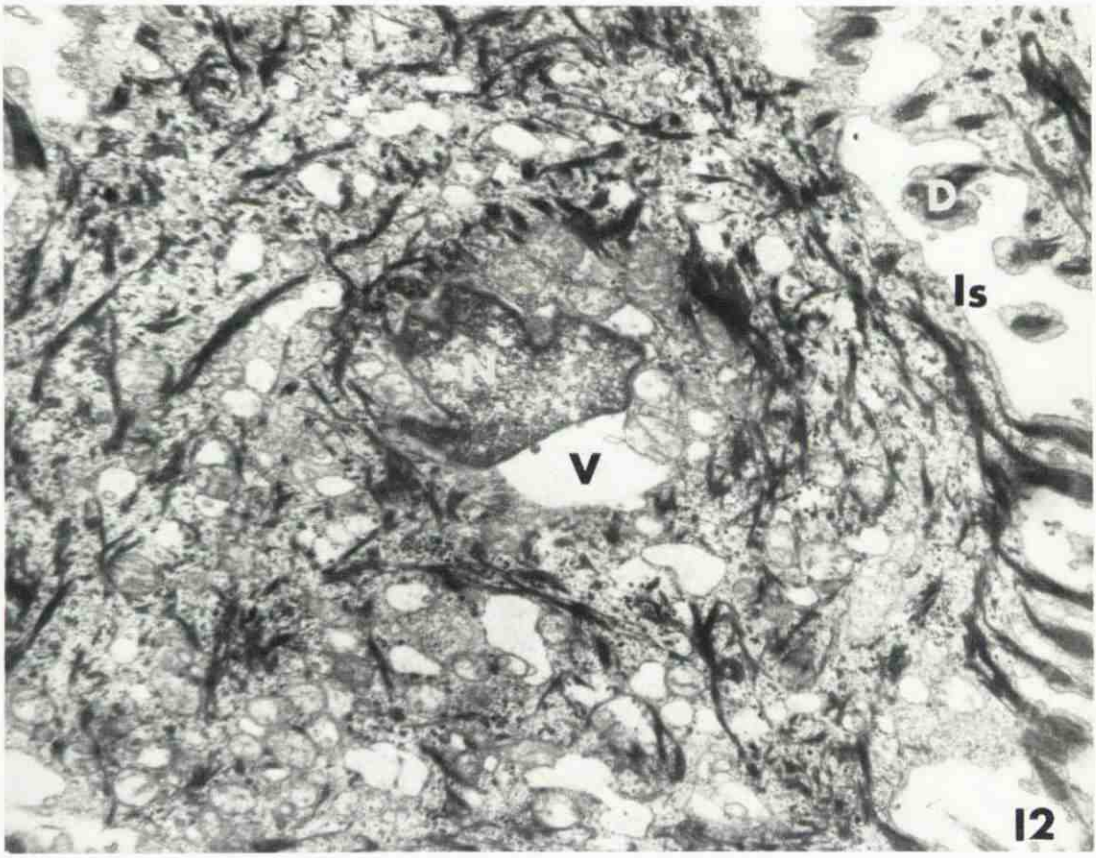

FIG. 12. Human stratum spinosum after a shorter (30 minutes) acetone exposure. Large intercellular spaces (IS) with disrupted desmosomes (D); few vacuoles (V); N—nucleus. × 16,700.

to mitosis. The reasons and mechanisms for these striking differences in regeneration activity remain to be investigated in future studies which combine ultrastructural investigation with biochemical and autoradiographic procedures.

Our ultrastructural findings, when compared with those of other authors and those made in our own laboratory using other substances, begin to reveal the spectrum of action of different chemical agents on the epidermis, the characteristic pattern of damage exerted by specific agents on the several components of epidermis, and the details of cell movement in the injured human epidermis.

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