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STEROIDS, LYSOSOMES AND DERMATITIS

An Ultrastructural Study

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Abstract. Three different forms of dermatitis were treated with a new topical steroid to determine its mechanism of action on these diseases. Subsequent light and electron microscopic examinations revealed morphologic evidence of the stabilization of epidermal lysosomal membranes by the steroid.

In 1961, Weissman & Dingle (10) suggested that the anti-inflammatory activity of glucocorticoids is due in part to the stabilization of lysosomes. Recently, it has been shown that steroids have a biphasic effect on membranes (4), i.e., they stabilize membranes at relatively low concentrations and may actually labilize them at higher concentrations. The purpose of this study was to determine the effects of a new non-fluorinated topical steroid on the ultrastructural morphology of several forms of dermatitis, in particular its ability to stabilize lysosomal membranes.

MATERIALS AND METHODS

Serial biopsies were obtained from 3 patients, each with a different form of dermatitis. All biopsies were obtained using 1% Xylocaine® anesthesia. Specimens were fixed in cacodylate-buffered 1% osmium tetroxide at pH 7.3 for 2 hours, passed through a series of graded alcohols and embedded in Epon 812. Thin sections were cut on an LKB Ultratome, using a diamond knife, and stained with uranyl acetate and lead citrate. They were examined on an RCA EMU-3G Electron Microscope. Acid phosphatase studies were performed according to the technique of Bainton & Farquhar (1) on a specimen of acute contact dermatitis 5 days after therapy was begun, and on atopic dermatitis before therapy was initiated. Cacodylate-buffered 2.5% glutaraldehyde at pH 7.3 was the fixative in these instances. Light microscopy was performed on half of each specimen taken from the patient with atopic dermatitis.

1. Two-mm punch biopsies were taken from the arm of a 65-year-old white female with chronic contact dermatitis before topical steroid therapy and at 1, 2, 3, 5 and 7 days after treatment was started.

2. A 40-year-old white male, who had previously had only a mild dermatitis following contact with poison ivy, was exposed to the broken stem of a poison ivy plant on the right and left mid-back areas. One side was treated with the topical steroid preparation, the other was left untreated. The steroid preparation was applied to the treated side, beginning 16 hours after exposure, when erythema was becoming clinically noticeable. Two-mm punch biopsies were taken from the untreated side before therapy and at 4 and 10 days after experimental exposure. Two-mm punch biopsies were obtained from the treated side 6 hours after therapy was begun and at 3, 4, 5 and 10 days thereafter.

3. Paired serial biopsies were obtained from the forearms of a 9-year-old white male with chronic recalcitrant atopic dermatitis, before therapy and at 1, 4, 8 and 15 days. One side was treated with the steroid cream, the opposite side with the control cream.

With the exception of the untreated acute contact dermatitis and the control side of atopic dermatitis, all sites were clinically healed at the time of the final biopsies. The topical steroid used in this study was Desonide (Tridesilon®—Dome Laboratories) (7), and the control cream was the base alone (Acid Mantle Cream®).

RESULTS

Light microscopy of atopic dermatitis

Light microscopic examination of the untreated atopic dermatitis skin revealed an apparent replacement of the stratum corneum and stratum granulosum by a serous crust. Acanthosis, spongiosis and invasion of the epidermis by inflammatory cells were also seen (Fig. 1). In the upper dermis there was a round-cell infiltrate and edema. The final pair of biopsies showed, on the treated side,

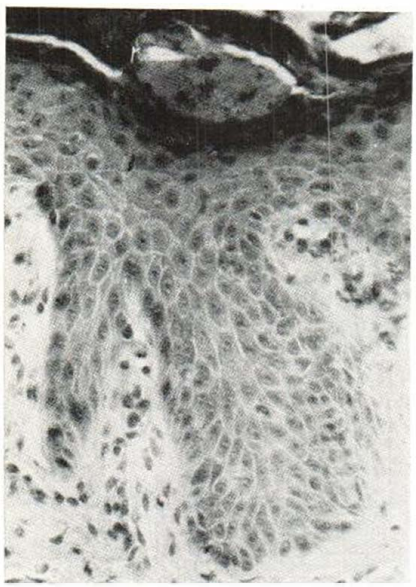

Fig. 1. Untreated atopic dermatitis. Note serous crust, acanthosis, spongiosis and inflammatory cells in dermis and epidermis. H & E, × 215.

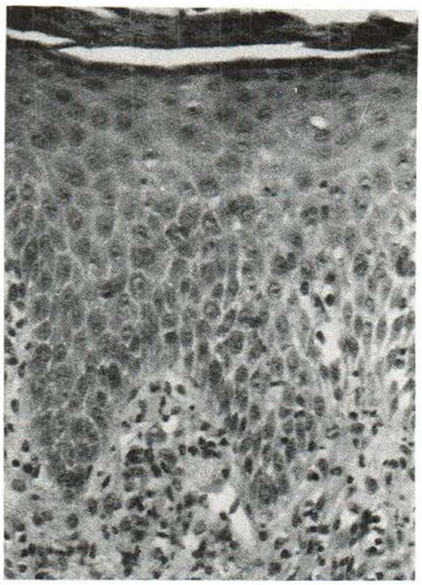

Fig. 3. Atopic dermatitis after 15 days therapy with the control cream. Parakeratosis, acanthosis, spongiosis and inflammatory cells in the epidermis and dermis are seen. H & E, × 215.

re-establishment of the normal architecture (Fig. 2), while the control skin maintained a picture similar to the untreated specimen (Fig. 3).

Electron microscopy of atopic dermatitis

Electron microscopy of the untreated atopic dermatitis revealed, as its most striking feature, the presence of large lysosome-like structures in the keratinocytes of the upper prickle cell layer, which became more numerous in the uppermost portion of the epidermis (Fig. 4). The contents of the lysosome-like structures were moderately electron dense. The stratum granulosum and stratum corneum were not present and appeared to have been replaced by a similar electron-dense substance, corresponding in position to the serous crust shown in Fig. 1. The lysosome-like structures demonstrated a positive reaction when the acid phosphatase test was performed (Fig. 5). Some of the keratinocytes had large clear, perinuclear, non-membrane-limited areas in their cytoplasm (Fig. 6).

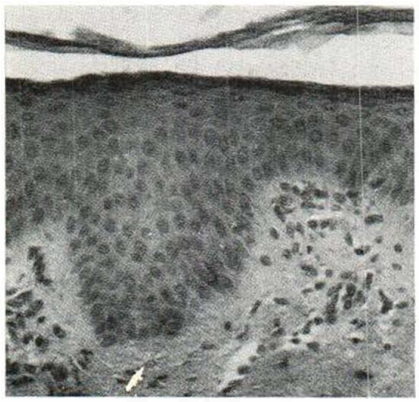

Fig. 2. Atopic dermatitis after 15 days therapy with the steroid cream. There is a return to near-normal architecture. H & E, × 170.

After 24 hours of treatment with the steroid cream, limiting membranes were noted about many of these perinuclear clear areas (Fig. 7). A parakeratotic horny layer and a granular layer were beginning to form. There was a decrease in the extracellular edema present between the keratinocytes in the upper portion of the epidermis.

After 4 days of treatment, extracellular edema was present only in the lower epidermal cells (Fig. 8). The keratinization process was proceeding in a more normal manner; however, nucleated cells were still present in the horny layer. The keratinocytes in the upper epidermis appeared to have returned to normal (Fig. 9); there were no lyso-

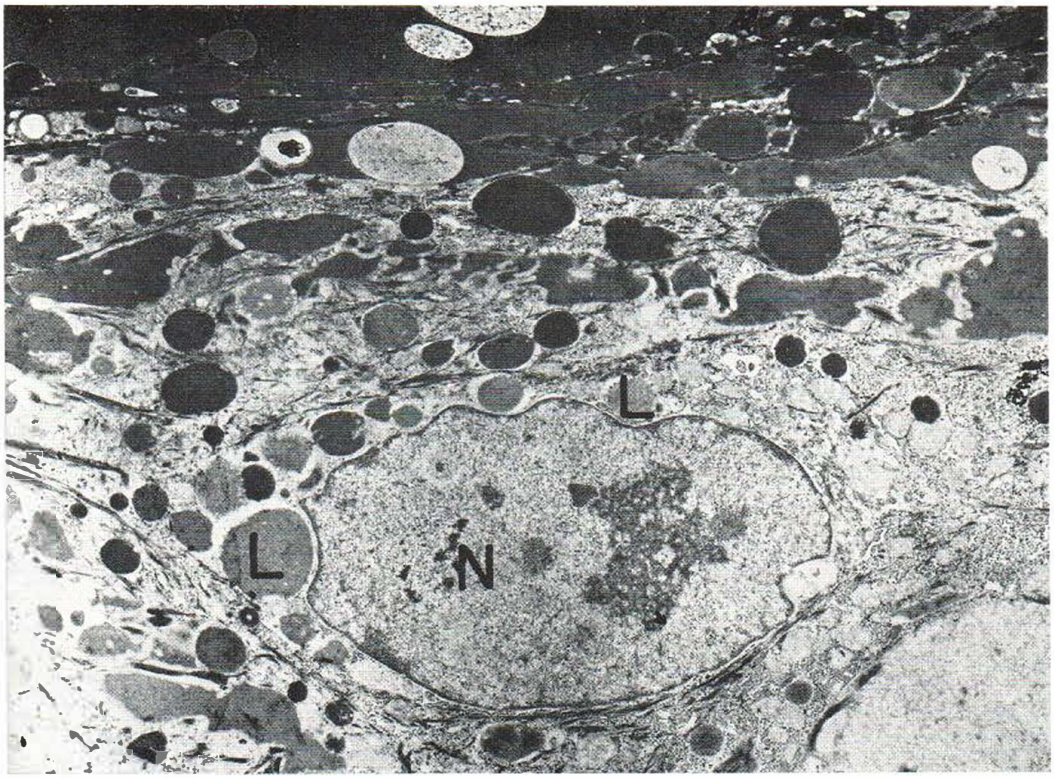

Fig. 4. Untreated atopic dermatitis. Note electron-dense lysosome-like structures (*L*) in keratinocytes of the upper epidermis. The dense transverse band in the upper portion of the micrograph corresponds to the serous crust in Fig. 1. *N*, Nucleus. × 8 400).

somes present and the keratinocytes were in close approximation to one another.

Following 8 days of treatment the morphology of the entire epidermis had returned almost to normal; a few small cytoplasmic vacuoles remained. On the 15th day, the treated epidermis was entirely normal, while control specimens retained lysosomal structures within keratinocytes (Fig. 10) and extracellular edema, as in the initial untreated specimens (Figs. 4 and 6).

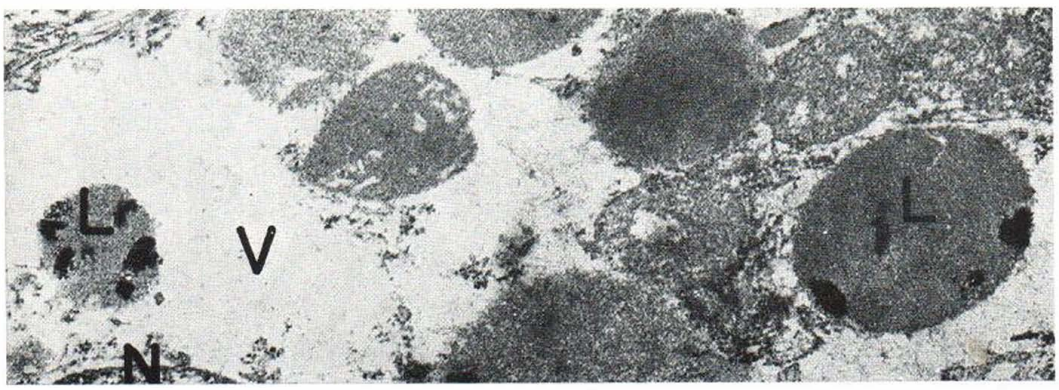

Fig. 5. Untreated atopic dermatitis. Lying within a perinuclear vacuole (*V*) are the moderately electron-dense structures (*L*) which demonstrate positive acid phosphatase reaction, establishing that they are lysosomes. × 31 500.

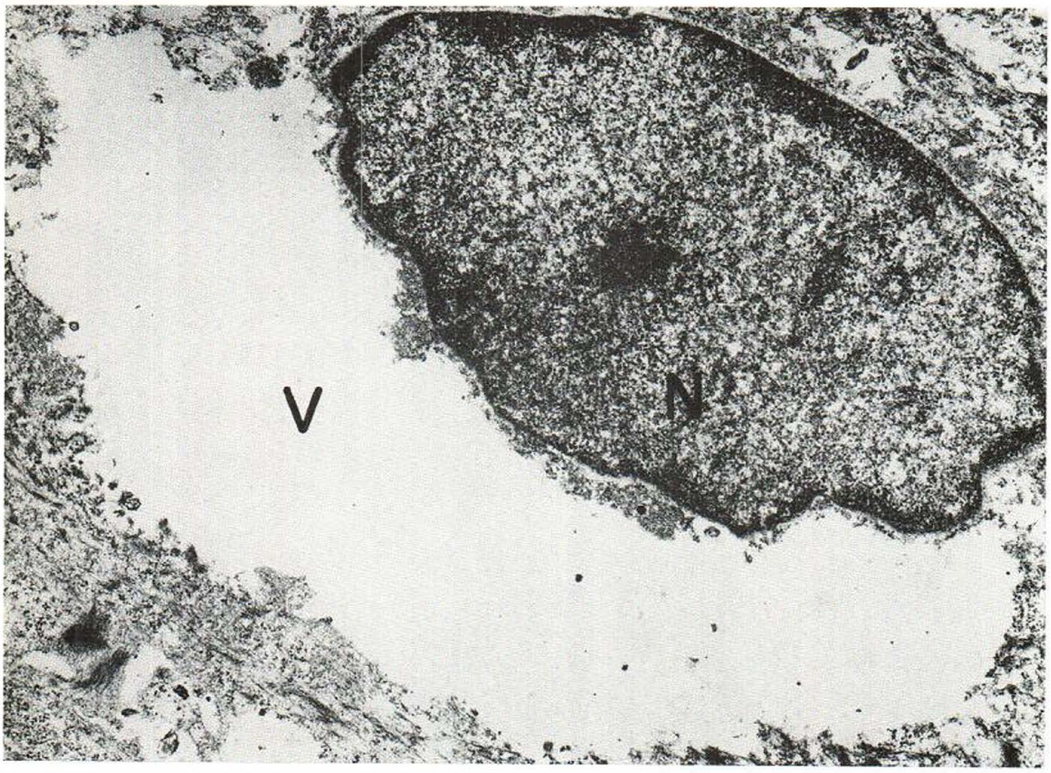

Fig. 6. Untreated atopic dermatitis. Note large clear perinuclear non-membrane-limited vacuole in cytoplasm of a keratinocyte. × 19 000.

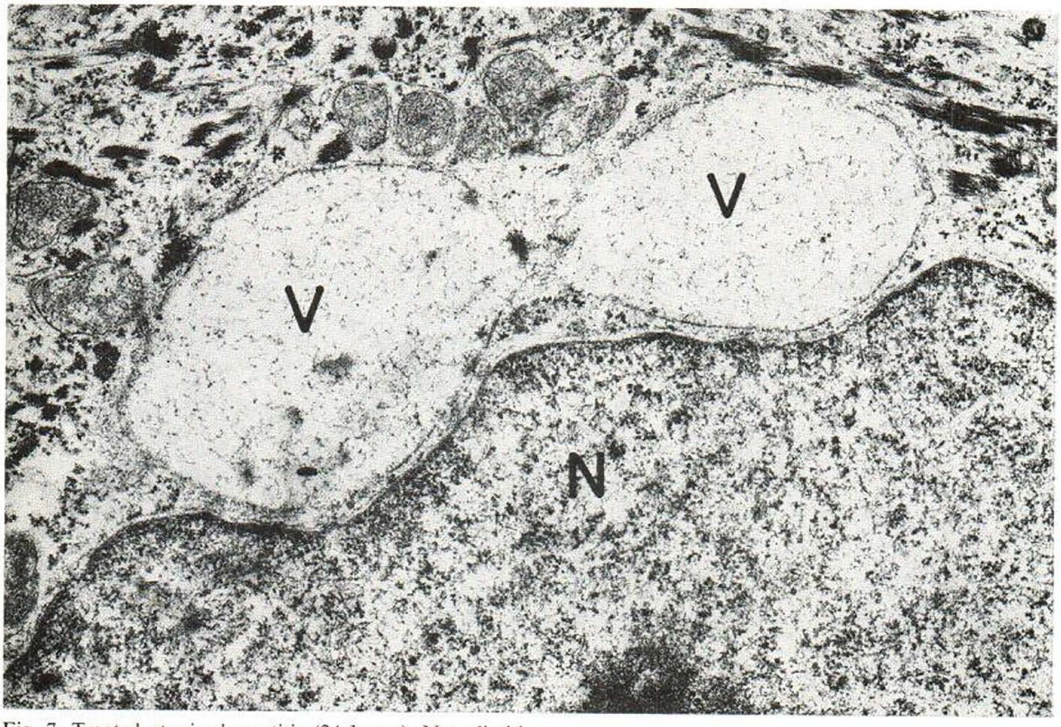

Fig. 7. Treated atopic dermatitis (24 hours). Note limiting membranes around the perinuclear vacuoles (*V*). × 25 850.

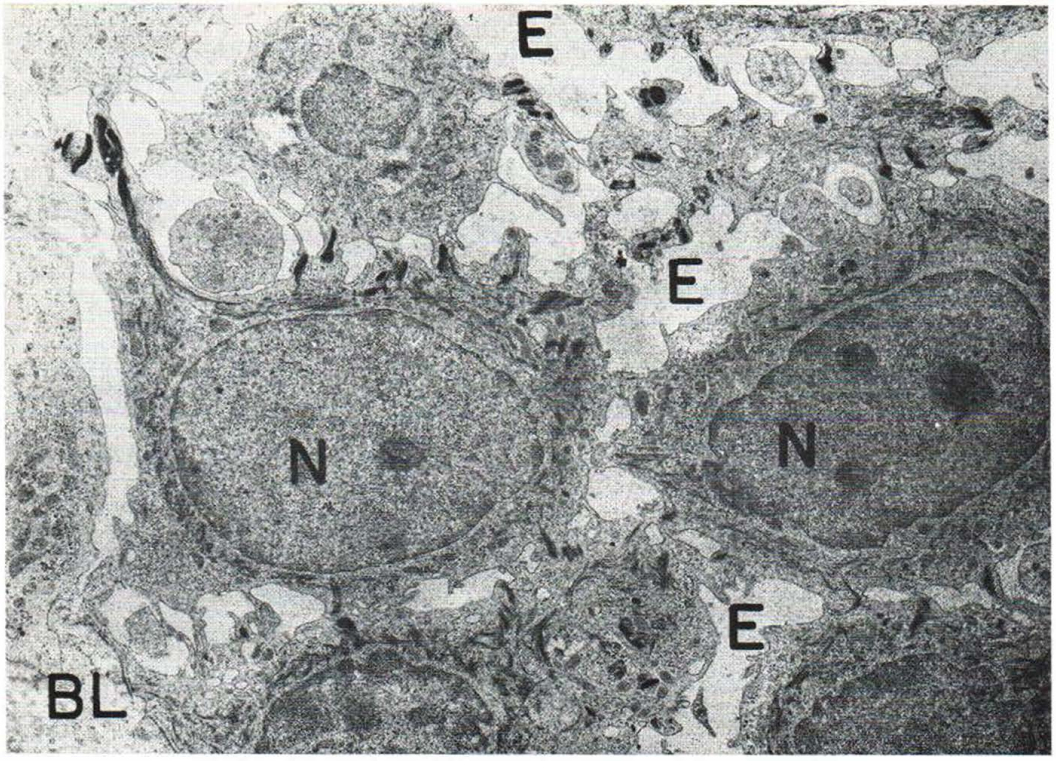

Fig. 8. Treated atopic dermatitis (4 days). Extracellular edema (*E*) is limited to the lower keratinocytes. *BL*, Basal lamina; *N*, nucleus. × 4 400.

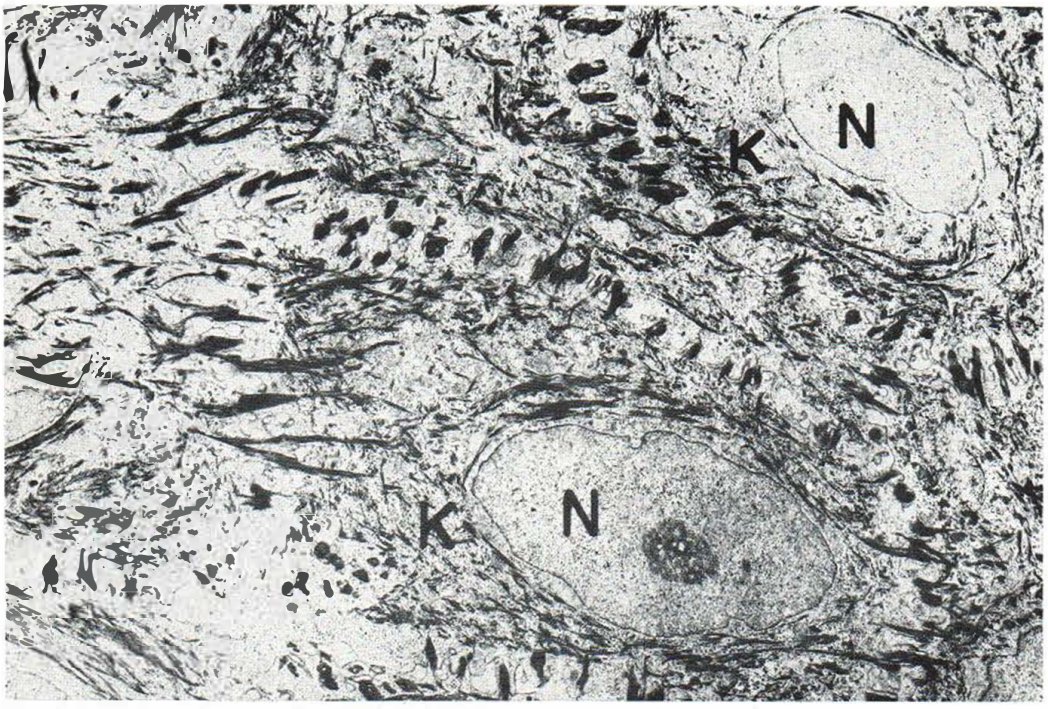

Fig. 9. Treated atopic dermatitis (4 days). Keratinocytes (*K*) in the upper epidermis have returned to normal. *N*, Nuc'eus. × 4 000.

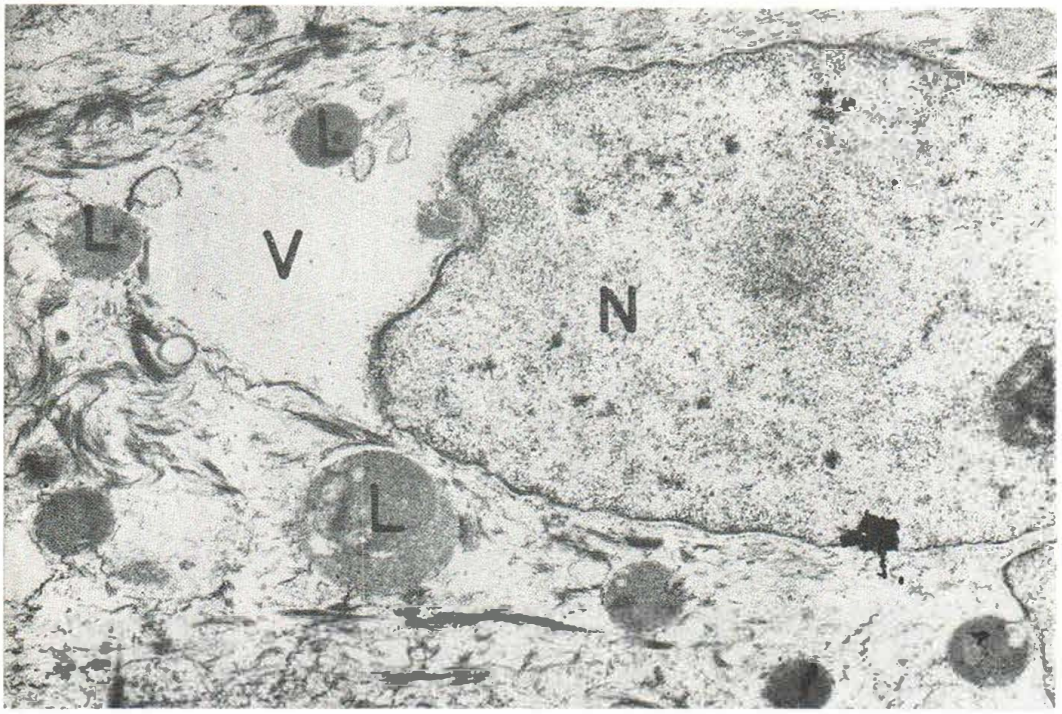

Fig. 10. Control atopic dermatitis (15 days). Observe lysosomes (*L*) and the perinuclear vacuole (*V*); compare with Figs. 6 & 7. These perinuclear vacuoles possessed limiting membranes in the steroid-treated specimen at 24 hours. × 16 400.

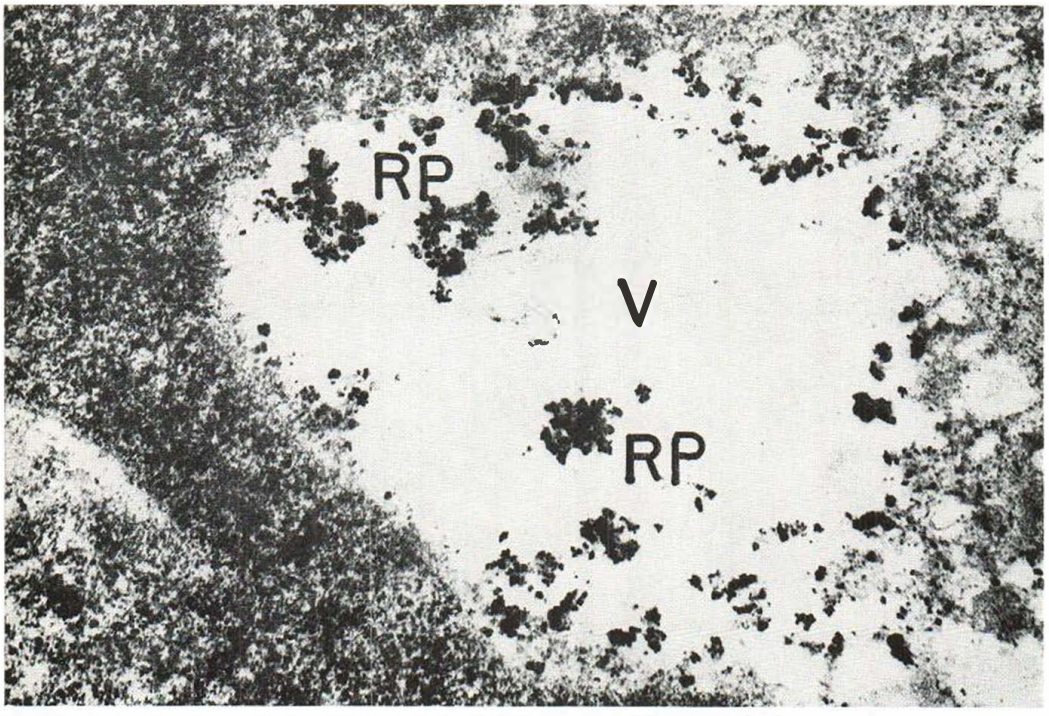

Fig. 11. Untreated acute contact dermatitis. Acid phosphatase reaction product (*RP*) is seen in perinuclear clear area (*V*), which in later specimens possessed a limiting membrane. × 41 000.

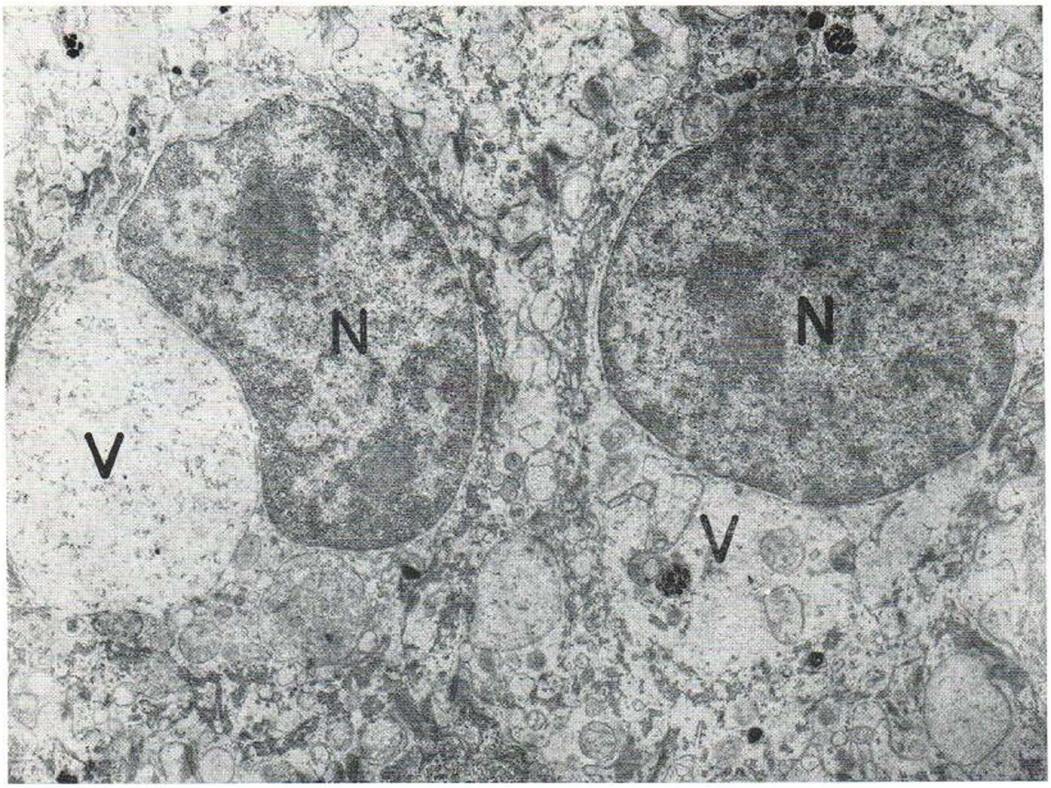

Fig. 12. Untreated acute contact dermatitis. Cell on left demonstrates perinuclear vacuole (*V*) with broken membrane. Vacuole (*V*) in cell on right possesses no limiting membrane. × 11 100.

Electron microscopy of acute and chronic contact dermatitis

1. *Untreated.* The contact dermatitis specimens showed large intracytoplasmic vacuoles, with a positive acid phosphatase reaction (Fig. 11) identifying them as lysosomes. In the untreated acute contact dermatitis these lysosomes showed either broken or no limiting membranes (Fig. 12). At 10 days, lysosomes had enlarged or coalesced producing large clear cytoplasmic spaces in dark shrunken cells with pyknotic nuclei (Fig. 13).

2. *Treated.* In treated acute and chronic contact dermatitis, the cellular architecture of the granular and upper prickle cell layers returned to normal by 4 days; by 10 days the entire epidermis appeared histologically normal. At 4 and 5 days the number of lysosomes was greatly reduced, with none being seen in the granular and upper prickle cell layers. By 10 days all evidence of extra- and intracelilular edema had disappeared from the keratinocytes of the treated specimen, and none of the dark pyknotic keratinocytes were observed.

DISCUSSION

The electron-dense structures seen in atopic dermatitis (Fig. 5) and the large clear perinuclear vacuoles in acute and chronic contact dermatitis (Fig. 11) are lysosomes, as confirmed by the acid phosphatase reaction. Prose (8) showed similar acid phosphatase positive lysosomes in his study of infantile eczema. The large perinuclear intracytoplasmic vacuoles in skin with acute and chronic contact dermatitis have also been seen in epidermal cells in cases of dermographism (2), epidermolysis bullosa (6) and in normal skin following ultraviolet irradiation (3, 5).

In untreated acute contact dermatitis at 4 days, there was a breakdown of lysosomal membranes, with an extension of clear areas around the nucleus. After 10 days, some of the keratinocytes demonstrated striking cellular breakdown, with

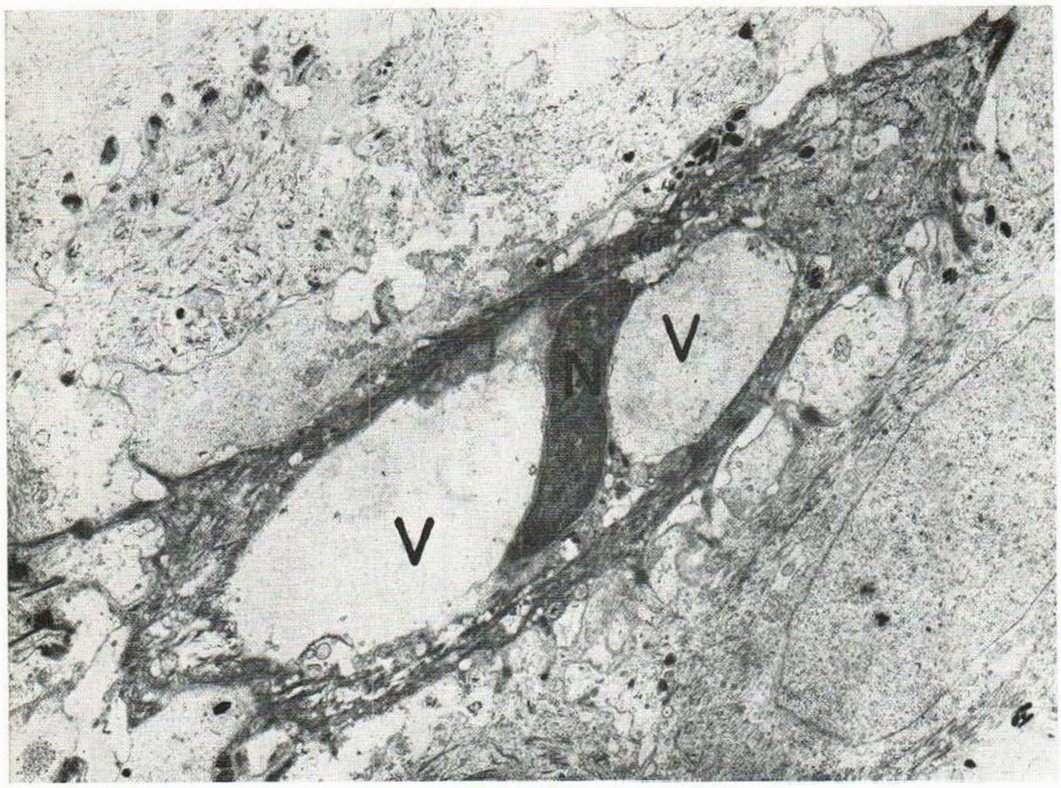

Fig. 13. Untreated acute dermatitis (10 days). Note the dark shrunken cell with pyknotic nucleus (*N*) and large clear vacuoles (*V*). × 8 450.

large clear areas in the cytoplasm and pyknotic nuclei (Fig. 13). (Weissmann, in a review of lysosomes (9), discussed in detail this process of lysosomal breakdown and subsequent cellular destruction.)

In the three forms of dermatitis studied, the steroid-treated skin showed the formation of unbroken membranes around the lysosomes within 24 to 48 hours, followed by disappearance of lysosomes from the keratinocytes by 10 to 15 days.

ACKNOWLEDGEMENTS

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