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LYSOSOMES IN KERATINOCYTES AFTER TAPE STRIPPING

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Abstract. Cellophane tape stripping of human epidermis was followed by an increase in extracellular space, much of which was filled with a moderately electron-dense material. Sixteen hours after stripping, large dense bodies containing material similar to that seen in the extracellular space appeared in keratinocytes of the upper epidermis. They attained a maximum volume after the extracellular space had begun to decrease. Some of the dense bodies contained acid phosphatase which indicated that they were lysosomes. At no time did the lysosomes appear to cause cell death, and thus they probably promote a healing process in the skin.

It has been demonstrated that human epidermal keratinocytes are phagocytic (1, 2). Others (3, 6, 7) have presented histochemical evidence that human keratinocytes possess hydrolytic enzymes in membrane-bound bodies and thus lysosomal activity. The formation and significance of large lysosomes present in keratinocytes of tissue taken 16 and 24 hours after stripping the skin with cellophane tape are discussed in this paper.

MATERIALS AND METHODS

Small areas of skin on the lower back of three Caucasian donors were stripped with cellophane tape (1). Tissue samples were taken at timed intervals up to 84 hours after stripping. Stripping operations were repeated to obtain additional 16, 24, and 48 hour tissue. Two specimens, one taken at 24 hours, and one taken at 48 hours after stripping, were fixed in cold glutaraldehyde and processed for the localization of acid phosphatase. Sections were cut with an LKB Ultrotome and examined with an RCA EMU-3G and a Forgflo EMU-4B electron microscope.

RESULTS

In the tissue taken immediately after stripping, the stratum corneum was one or two cell layers in thickness. Aside from this, the epidermis appeared normal. Eight hours after stripping the stratum corneum was still one or two layers thick. There was a considerable amount of extracellular space around melanocytes. The latter was the only discernible morphologic alteration in the epidermis at this time.

Sixteen hours after stripping, the stratum corneum had increased to a thickness of six to eight cell layers: of this, two layers comprised cells that had remained after stripping. The additional four to six layers consisted of cells that were somewhat flattened, showed incomplete keratinization and some contained pyknotic nuclei. These cells possessed some recognizable cell parts including large vacuoles, and they were somewhat more electron-dense than the underlying cells (Fig. 1). A granular layer with its associated keratinosomes and keratohyalin granules was absent. A few of the keratinocytes in the area just below the stratum corneum contained dense bodies which varied in size up to 10 μm in diameter (Fig. 2), were single membrane limited, and resembled lysosomes of phagosomes (Fig. 2 *b*). Melanosomes were the only cell components seen within these dense bodies. Noteworthy in the 16 hour tissue were numerous pinocytic vesicles and phagocytic vacuoles in keratinocytes of the upper epidermis (Fig. 3). Throughout the epidermis there was significant increase in extracellular space. A slightly dense osmophilic material was found in much of the extracellular space in the 16 hour tissue.

Twenty-four hours after stripping, the stratum corneum was increased to eight to ten cell layers. In the tissue processed for acid phosphatase localization, some of the large dense bodies first noted in the 16 hour samples demonstrated lead phosphate reaction product (Fig. 4). The dense

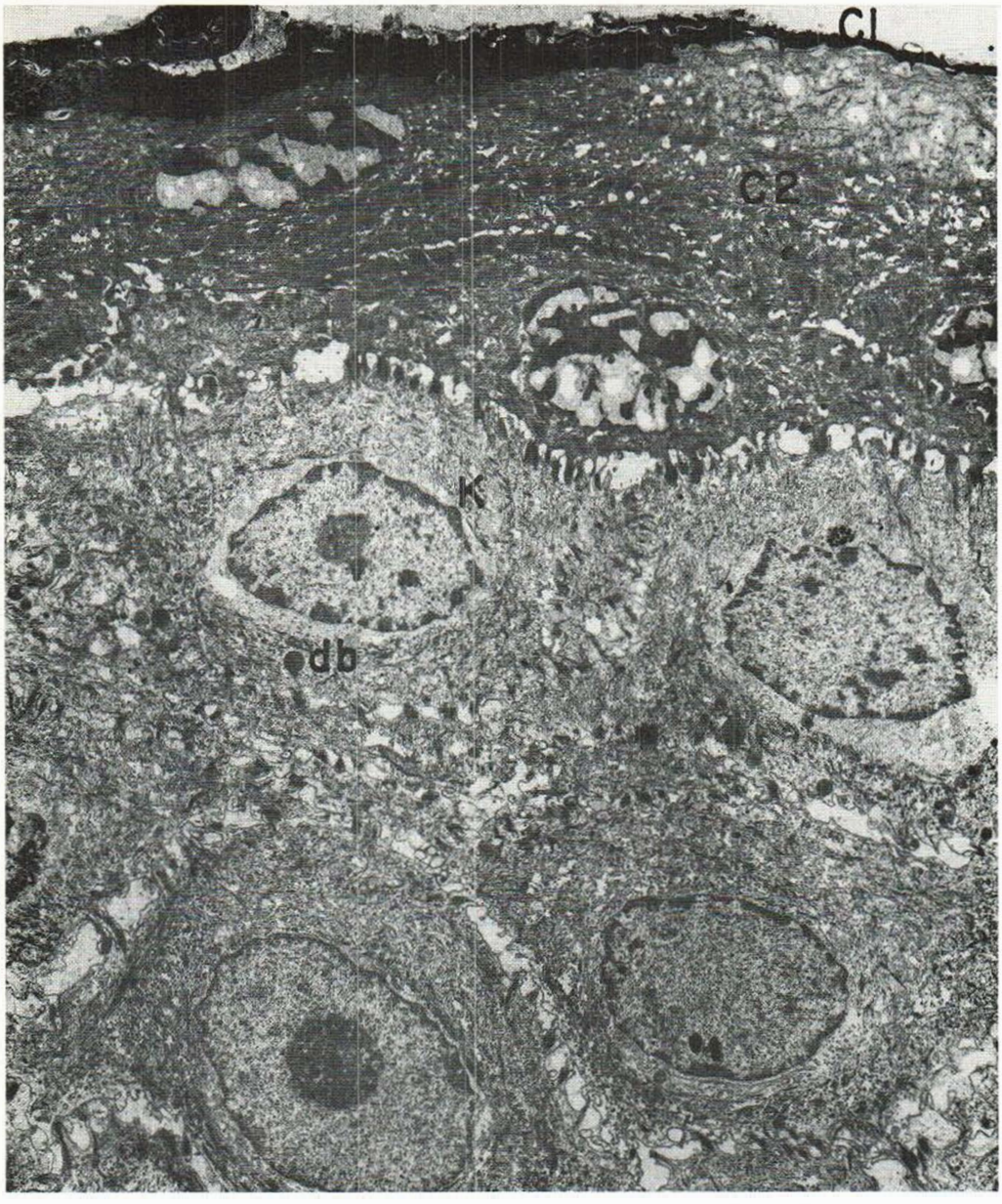

Fig. 1. Sixteen hours after stripping, the corneum was increased to six to eight cell layers in thickness and appeared to consist of two layers (C_1 C_2). (C_1) is that part of the corneum left after the stripping; (C_2) is the new portion replaced in the time interval between the 8 hour and the 16 hour biopsies. Note the lack of a normal-appearing granular layer. *K*, keratinocytes; *db*, dense bodies. × 2 600.

bodies or lysosomes were also more numerous at this time. Large vacuoles were present within the cytoplasm of the keratinocytes, possibly the result of a degeneration of the contents of the dense bodies (Fig. 5). However, many of the lysosomes persisted and were intact in the stratum corneum.

By 48 hours the stratum corneum had increased to 12 to 16 cell layers and the extracellular space appeared more nearly normal. A granular layer with keratinocytes containing the normal complement of keratinosomes and keratohyalin granules was again evident. An outstanding feature of this

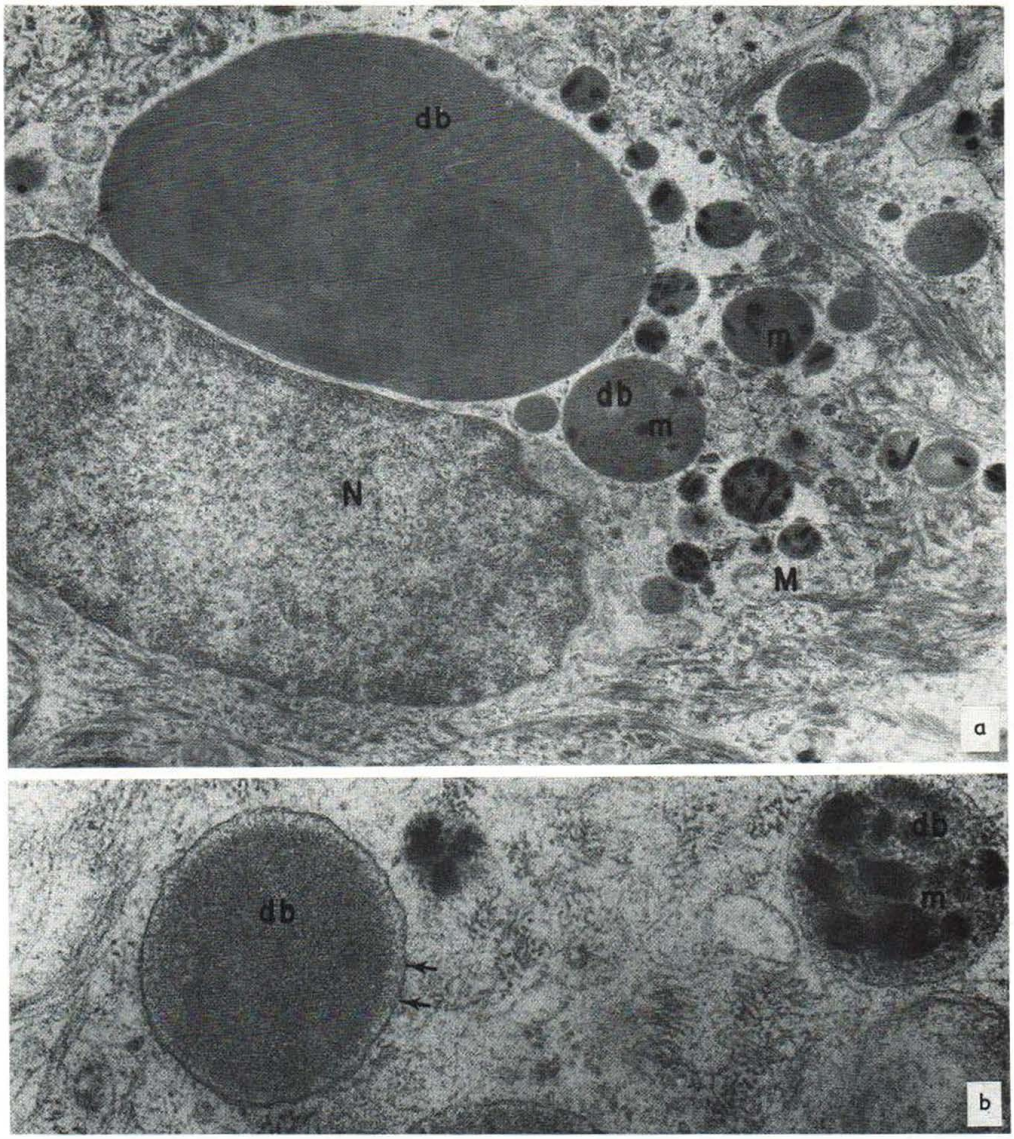

Fig. 2 (*a*). The dense bodies (*db*) vary in size up to 10 μm and many of them contain melanosomes (*m*). *M*, mitochondria; *N*, nucleus. × 12 600.

(*b*) Dense bodies (*db*) in 16 hour tissue. The limiting membrane is evident (→). *m*, melanosomes. × 65 000.

time interval was the proliferation of keratinocytes with many of the basal keratinocytes in some stage of cell division. The lysosomes seen in the 16 and 24 hour tissues were almost completely absent in the 48 hour tissue.

DISCUSSION

Cellophane tape stripping of human epidermis is a simple method of removing enough of the stratum corneum to simulate minor injury and to induce a more rapid than normal cell turnover in the epidermis. We have found that tissue fluids brought to the surface during the stripping procedure reduce the efficiency of the tape which probably accounts in part for our failure to remove all of the stratum corneum.

With the exception of a considerable amount of space around the basal melanocytes, tape stripping results in minimal changes in the epidermis in

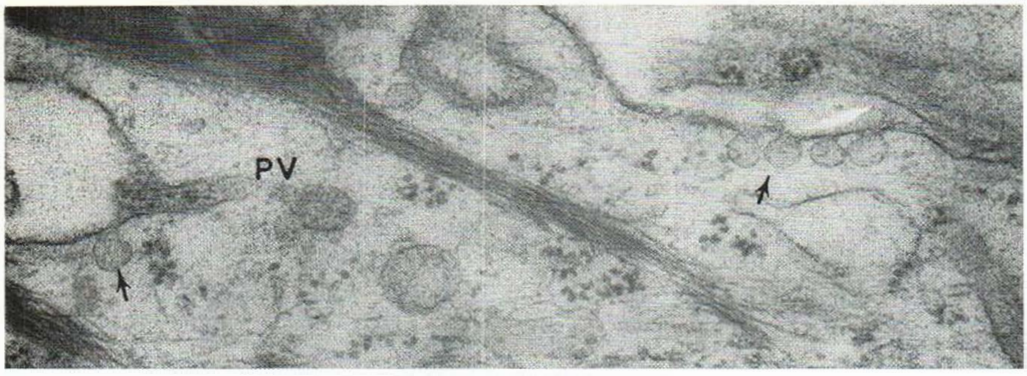

Fig. 3. This keratinocyte which is high in the epidermis has many pinocytic vesicles (→) and phagocytic vacuoles (*pv*). × 53 000.

the first 12 hours after stripping. At 16 hours there is a rapid movement of keratinocytes toward the surface of the skin manifested by the lack of a normal granular layer and the development of an abnormal-appearing stratum corneum in the interval between the 8 hour and the 16 hour biopsies. In addition, in the 16 hour tissue some of the keratinocytes in the layer just below the stratum corneum were still perpendicular to the basal lamina and the desmosome-tonofilament complexes were intact. These facts tend to indicate that a mechanism exists which results in an almost immediate replacement of the stratum corneum at the expense of the overall thickness of the epidermis. Prose et al. (6) were the first to demonstrate that lysosomes were present within keratinocytes of the stratum granulosum and the stratum corneum in lesions of infantile eczema. In some of the lysosomes there were melanosomes and "cytoplasmic fragments". The lysosomes in eczematous skin were found to be stable and it was felt that they probably "served an autodigestive function rather than a suicidal or keratinocidal one".

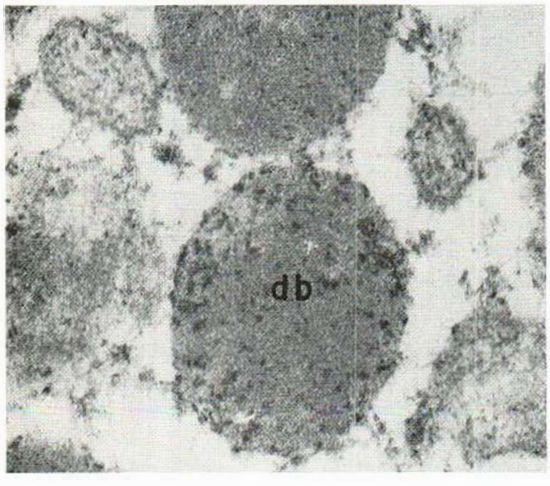

Fig. 4. Lead phosphate reaction product is demonstrated in this dense body (*db*). × 72 000.

Odland & Ross (2), in a study of wound healing, reported similar inclusions in keratinocytes. Although their investigation did not include histochemical tests for localization of acid phosphatase, they did discuss the phagocytic capabilities of keratinocytes in human epidermis. Wolff & Schreiner (7) injected a protein marker into the skin of human volunteers. These authors were able to establish (with the electron microscope) that the protein material was ingested by keratinocytes into membrane-limited phagosomes which they felt probably fused to form larger vacuoles. Their cytochemical tests established that most of the phagosomes in the later stages had been transformed into phagolysosomes. The lysosomes seen in the upper epidermis of our tissue taken 16 and 24 hours after stripping were similar in appearance to those found in the aforementioned studies. Our 24 hour tissue incubated to localize acid phosphatase demonstrated moderate lead phosphate deposits in many of the dense bodies, thus indicating that they also were phagolysosomes. We were unable to locate any keratinocytes which we felt were damaged or destroyed by an autophagic process. The lysosomes might therefore serve a bene-

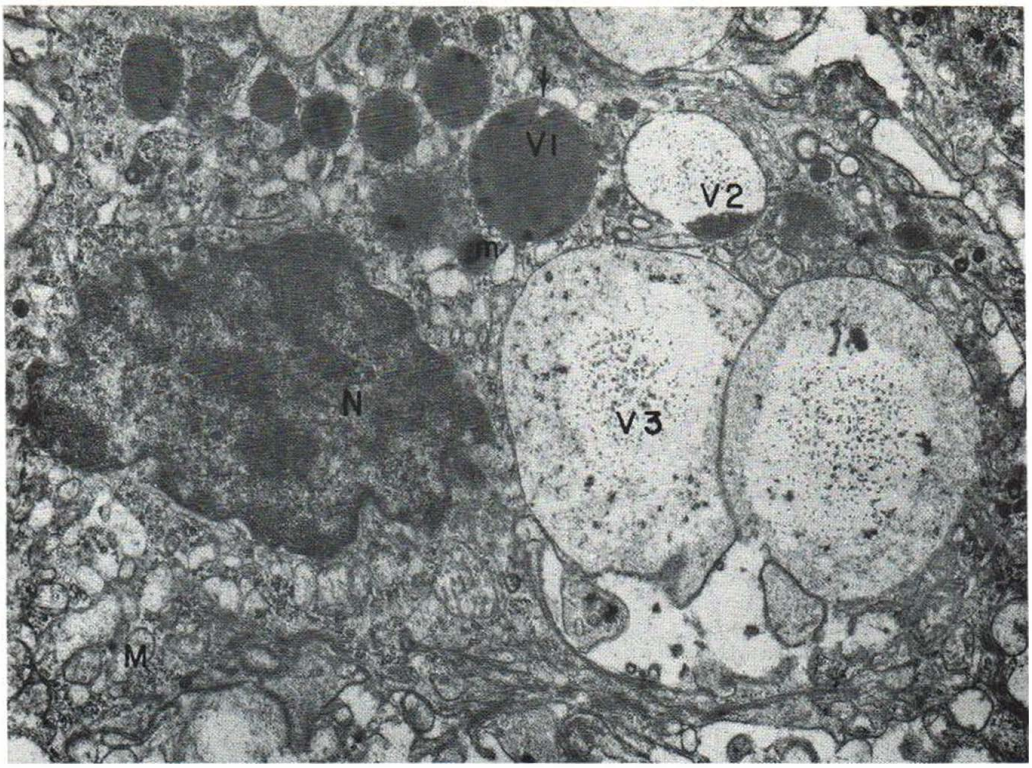

Fig. 5. A few of the keratinocytes in the 24 hour tissue contain large vacuoles (*V*) which give the impression of degradation of their contents. Note the clear area (→) in vacuole (*V1*) and the small amount of dense material remaining in vacuole (V_2). *m*, melanosome; *M*, mitochondria; *N*, nucleus. × 15 700.

ficial function in healing. There was a delay between the initial removal of the horny layer and the time at which mitotic activity increased. The greatest mitotic activity was noted approximately 48 hours after stripping (4, 5). An increase of mitotic activity in the basal layer began the normal replacement of cells to restore the thickness of the epidermis. This restoration was made evident by the presence of 12 to 16 cell layers in the stratum corneum and the reappearance of the stratum granulosum.

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