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Histopathologic and Electron Microscopic Studies of the Effects of Solcoderm on Normal Epidermis and Superficial Cutaneous Tumors

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Abstract. Histologic and electron microscopic studies of Solcoderm-treated normal skin and human lesions were performed to help elucidate the mode of action of this new agent. Its fixative properties were readily demonstrated and generally proved to be adequate for histological diagnosis from the post-treatment scab. The degree of preservation of fine intracellular structures was particularly remarkable, since similar treatment with equivalent strengths of nitric acid alone destroys these substructures. Dermal lipids and collagen were found to be natural barriers against the penetration of Solcoderm applied to the skin surface. Mild mechanical disruption and the disturbed cellular continuity of pathologic tissue allows the solution to penetrate treated lesions more freely and enhances the likelihood of their complete destruction when an appropriate quantity of solution is properly delivered by an experienced therapist.

Introduction

The application of Solcoderm to the epidermis and to skin tumors induces immediate and delayed reactions which end with scab formation. In this study, the process has been followed histologically and by electron microscopy in order to help elucidate the mode of action of Solcoderm on normal and pathologic tissues and to determine whether it is possible to establish a precise diagnosis by histologic study of the resulting scab.

Material and Methods

Study of Animal Skin Specimens

The study was planned to evaluate the fixative effect of Solcoderm on normal skin and to compare treated normal skin with treated keratoses and related lesions [1] from hairless mice irradiated by ultraviolet (Westinghouse tubes) with suberythemal daily doses from age 1 month for up to 6 months. Biopsies were taken at regular intervals from treated and untreated mice. Some of the skin tumors which appeared at 4 months were treated with Solcoderm and studied. Punch biopsies were performed 3 min, 24 h, 48 h, 1 week, and 2 months after a single application of 0.05 ml of Solcoderm on the dor-

sum of hairless mice. For light microscopy, specimens were either unfixed or fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. For electron microscopy, the Karnowsky fixative was used, followed by osmium tetroxide postfixation. The small blocks were oriented and embedded in Epon.

Study of Human Specimens

98 crusts which spontaneously fell off after Solcoderm treatment were collected in small, dry wells and brought to the laboratory where they were kept as delivered for 1–8 weeks before being processed for study. The scabs were then carefully cut perpendicular to the surface into two parts with the aid of a magnifying lens. One portion was rehydrated for 24 h in cold sodium buffer for histology and then submitted to routine processing and hematoxylin and eosin staining for histologic light microscopy study. The other half was hydrated for electron microscopy, soaked in uranyl acetate and transferred to alcohols or postfixed in osmium tetroxide, dehydrated, and embedded in Epon.

Thick sections (1 μm) and silver grey sections were cut on a Reichert ultrotome with a diamond or glass knife. The thick sections were stained with toluidine blue. Thin sections were observed without staining or with lead citrate staining on a Philips 300 electron microscope. 20 scabs were processed and studied in detail by electron microscopy.

Solcoderm/Nitric Acid Comparative Study

1 patient volunteer with multiple seborrheic warts of similar size on the back presented an opportunity for a controlled comparative study under double-blind conditions of the response to Solcoderm, plain nitric acid (6.2 *N*), and a solution of similar acidity without nitric acid. 24 essentially identical lesions were divided into sets of three lesions randomly code labelled A, B and C. The initial color of each lesion was scored on a scale from 1 to 5 (light brown to black) to aid in creating matched sets. Clinical observations of red and white halo formation, lesion color change, and appearance at the site after fall off were recorded at 10 min, 1 day, and at 10-day intervals thereafter. When crusts formed, they were harvested just before their expected fall and processed according to the methodology described in 'Study of Human Specimens'.

The data generated were tabulated and, for each set of three, an overall opinion was recorded before the code was broken as to which treatment yielded more, less, or equally satisfactory results, i.e., each treated lesion was compared to two lesions with other treatment.

Results

Animal Skin Specimens

The normal skin of the hairless mouse was fixed as well by Solcoderm treatment as with routine histologic technique using formalin (fig. 1a, b). The staining qualities were the same. Biopsies taken at different times after a single Solcoderm application showed regeneration of new epidermis from skin appendages and adjacent lateral epidermis with a well-preserved 'old epidermis' (fig. 1c).

Ultraviolet irradiation induced lesions such as dyskeratosis (fig. 1d), benign horn (fig. 1e), papilloma (fig. 1f), or invasive squamous cell carcinoma. Small lesions were studied without further formalin fixation. Larger lesions, removed surgically, needed formalin fixation to insure proper identification of structures in the deeper tissues.

Results of Study of Human Specimens

Macroscopic examination generally shows small disc-shaped specimens 2–5 mm in diameter and 0.5–1 mm in thickness. The borders are thin and translucent. Sections through a diameter are roughly ovoid.

Histologically, on the upper side of the crust, the old epidermis is recognized by the stratum corneum in continuity with Malpighian layer and basal cell layer. On the under side (i.e., detachment face) the dissociated portion of the stratum corneum of the

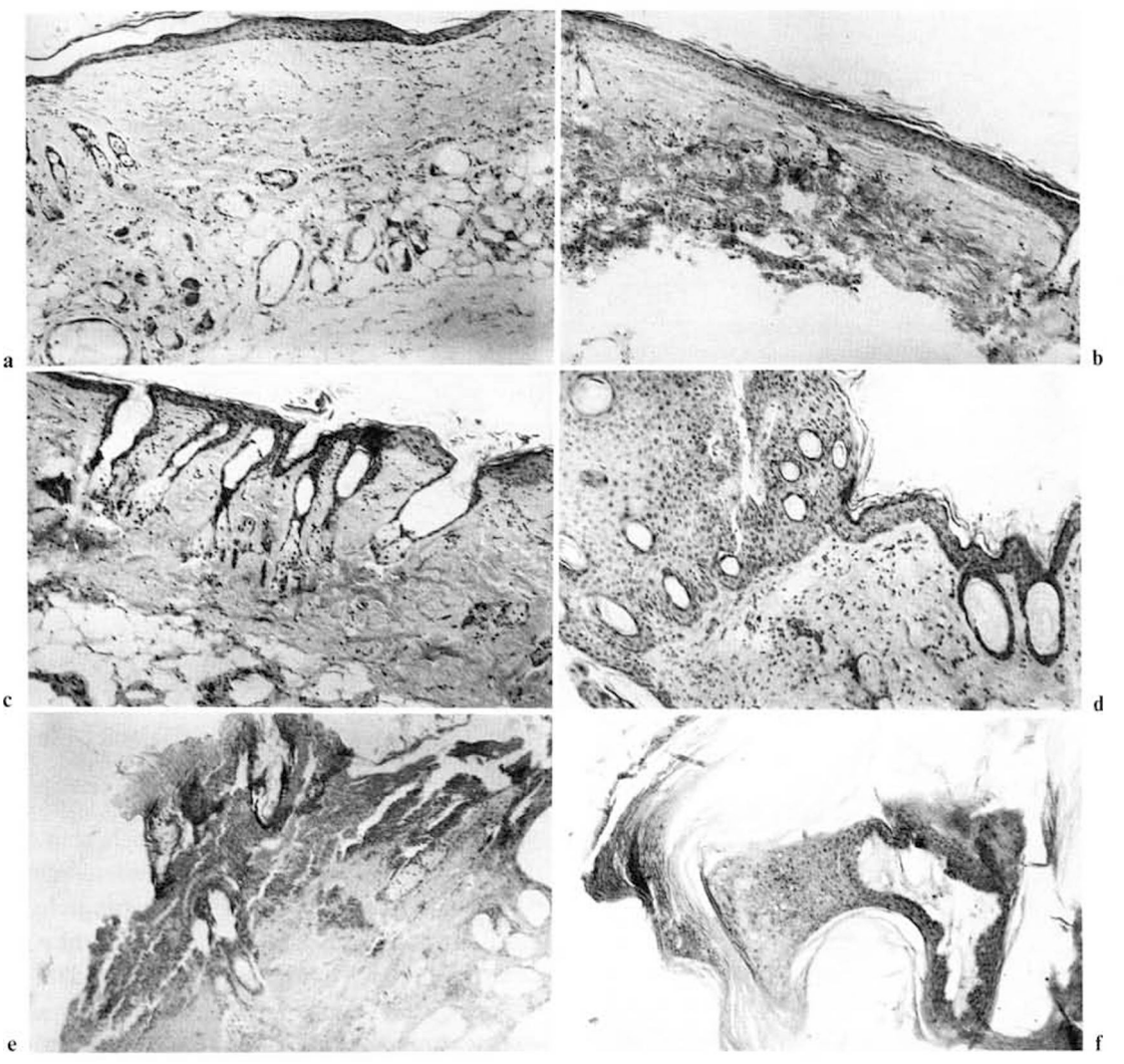


Fig. 1.a Mouse epidermis, formalin fixed. HE. **b** Mouse epidermis treated with Solcoderm and biopsied after 24 h. **c** Mouse epidermis treated with Solcoderm and biopsied after fall off of the scab. **d** Mouse epidermis showing *hyperkeratosis* treated with Solcoderm and excised after 1 week. **e** Crust of *benign horn* of ultraviolet irradiated mouse. **f** Crust of ultraviolet irradiation induced *papilloma* after treatment with Solcoderm and complete healing.

new epidermis is more or less clearly seen below a 'necrotic area'. At each extremity of the section the old epidermis and new stratum corneum are in direct contact. The structures recognized between the old and new corneum vary according to the nature of the pathological condition. The cellular elements are generally pale pink with hematoxylin and eosin stain. The extracellular compartment does not take the stain. The

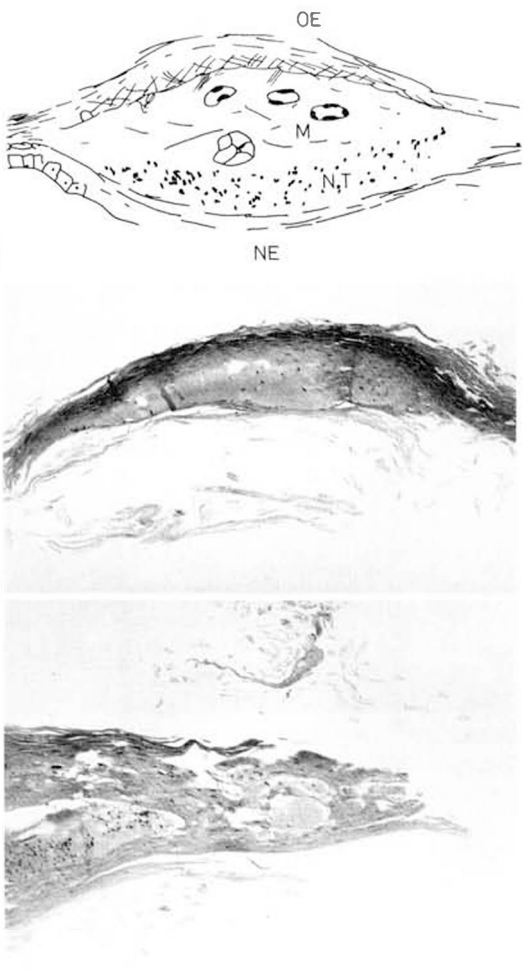


Fig. 2. a Scheme for the crust processed in routine histology. OE = Old epidermis; M = papillary dermis with capillaries and skin appendages; NT = band of necrotic tissue; NE = Stratum corneum of the new epidermis. **b** Scab of hyperkeratotic epidermis treated with Solcoderm. The crust was recovered after spontaneous fall off. **c** Details of the lateral aspect of the scab.

thickness of this band may vary from a few micrometers to 0.5 mm (fig. 2a, b).

The major architectural features of the epidermis and its appendages are well identified. The dermal vessels, hair roots, and sweat glands are clearly seen. However, cytologically the cell limits and the nuclear borders are not precisely defined, unless the lesion is edematous or retracted. Anisocytosis, anisokaryosis, and polychromatophilia, the usual landmarks for cytologists, cannot be observed and described.

Tumor Type Studies

Seborrheic Keratosis. This condition is particularly suitable for treatment by Solcoderm, since it is a pure proliferative benign epidermal cell lesion as illustrated in figure 1. The proliferation is well limited, and the arrangement pattern involves corneum cysts (fig. 3a).

Compound Nevus. With Solcoderm treatment the nests of melanocytes in the epidermis are not well preserved and the pigment is still apparent. In the dermis, nests and bundles of nevus cells are well distinguished from the surrounding normal tissues. In some instances the necrotic zone is well below the deepest nevus cell, indicating complete removal of the lesion. In other instances, the 'necrotic band' contains nevus cells which may be suspected of incomplete destruction. However, no local repigmentation (recurrence) has been observed to date (figs. 3b, c).

Common Warts. The classical 'column' of the virus-infected cells are recognized. Only the epidermal part of the wart is mummified by Solcoderm; the dermis is unaltered (fig. 3d).

Basal Cell Epithelioma. The diagnosis of superficial basal cell epithelioma treated with Solcoderm can usually be confirmed by the histologic pattern of the keratinocyte in the epidermis and the papillary dermis of the 'scab'. However, the diagnosis is not always possible strictly from the sections of scab.

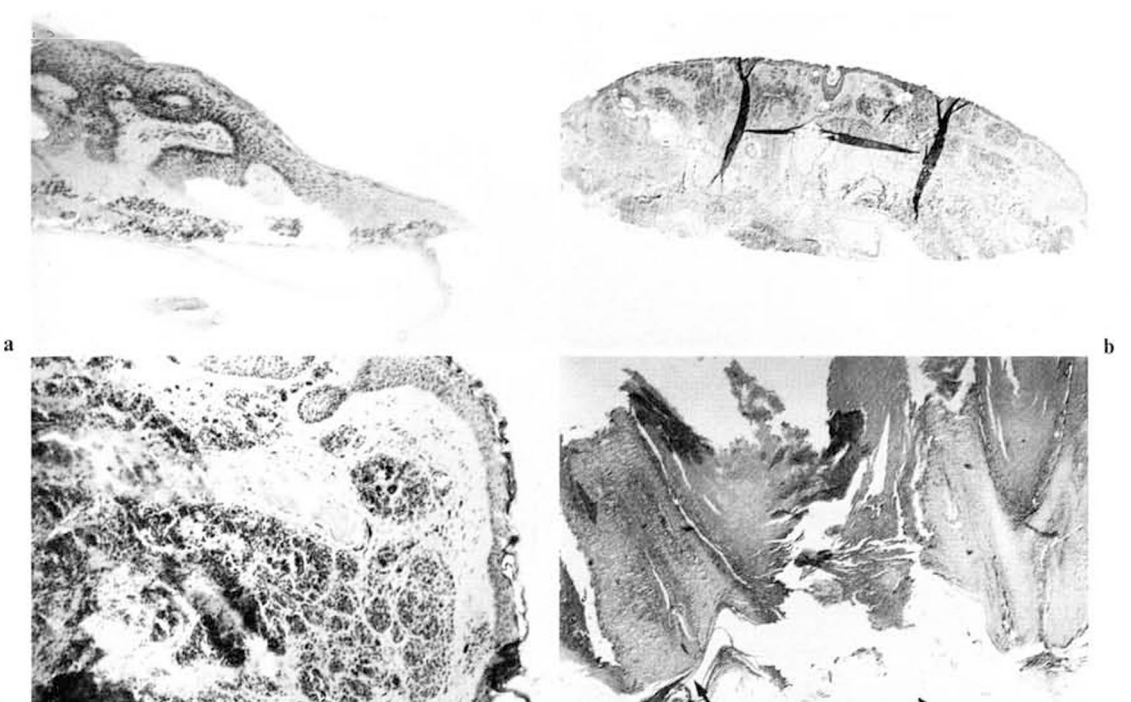


Fig. 3. **a** Seborrheic keratosis: crust after Solcoderm application. **b** Compound nevus: low magnification of the complete scab showing the nevus cells, the skin appendages, and the necrotic zone. No nevus nest is observed in the lowest part of the scab. **c** Compound nevus: higher magnification of the upper part of the same section shown in **b**. The differentiation of the nest is particularly well preserved. **d** Common wart: the new epidermis (arrow) is in contact with the virus-infected basal layer.

Solar Dyskeratosis. Solar-induced dyskeratoses characteristically change color rapidly when treated with Solcoderm. After a few days, thin scabs may be rescued and processed for histologic examination. They contain only stratum corneum and the thin epidermis (fig. 2b).

Dubreuilh Melanosis. A large number of clinically thin pigmented lesions were treated with Solcoderm after initial punch biopsy processed for routine histologic diagnosis. This superficial, intradermal proliferation of melanocytes is difficult to identify in the crust. While characteristic hyperpigmentation, melanophages, and inflammatory cells are observed, the presence of potential invading malignant cells cannot be ruled out.

Electron Microscopy

By electron microscopy, the fine structure of epidermal cells is very well preserved, at least for membranes and major cytoplasmic organelles. Delicate structures such as cytoplasmic membranes, keratinosomes, and tonofilaments are easily observed without further fixation (fig. 4a, b). The stratum corneum shows attachment plaques without edema between the layers. In the stratum

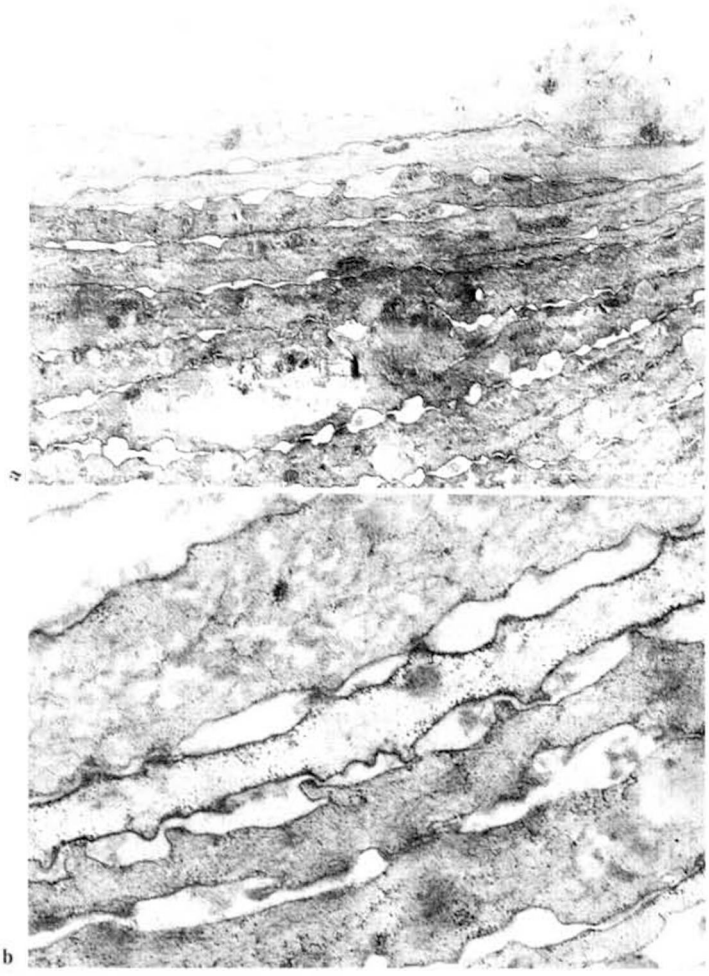


Fig. 4. **a** Solcoderm-treated human normal skin. Stratum corneum as observed at the electron microscope. Cytoplasmic membranes of the cells are well preserved. **b** Details of the stratum corneum. Spaces between the stratum corneum cells are well defined. In the absence of mechanical modifications of the stratum corneum during the application of Solcoderm, the relation between cells is well preserved.

Malpighi, the desmosomes hold their pentalamellar structure, and the relations between cells are usually maintained. Keratinosomes with their characteristic multilamellar patterns are observed close to the cytoplasmic membrane. Since these bodies (Odland bodies) are highly susceptible to spontaneous and rapid autolysis by their hydrolytic enzymes, their persistence indicates a rapid and excellent fixation by Solcoderm. Ordinarily they are only observed when glutaraldehyde formalin fixative is used for electron microscopy. The basal layer of keratinocytes is either well preserved or shows pericellular edema with detachment of cells (fig. 5b).

With regularity, the nuclear structures are completely destroyed. The chromatin is lost or homogenized, and the nuclear membrane is circumvoluted or fragmented. Nucleoli are not observed. Tonofilaments are dispersed in a homogenized cytoplasm where

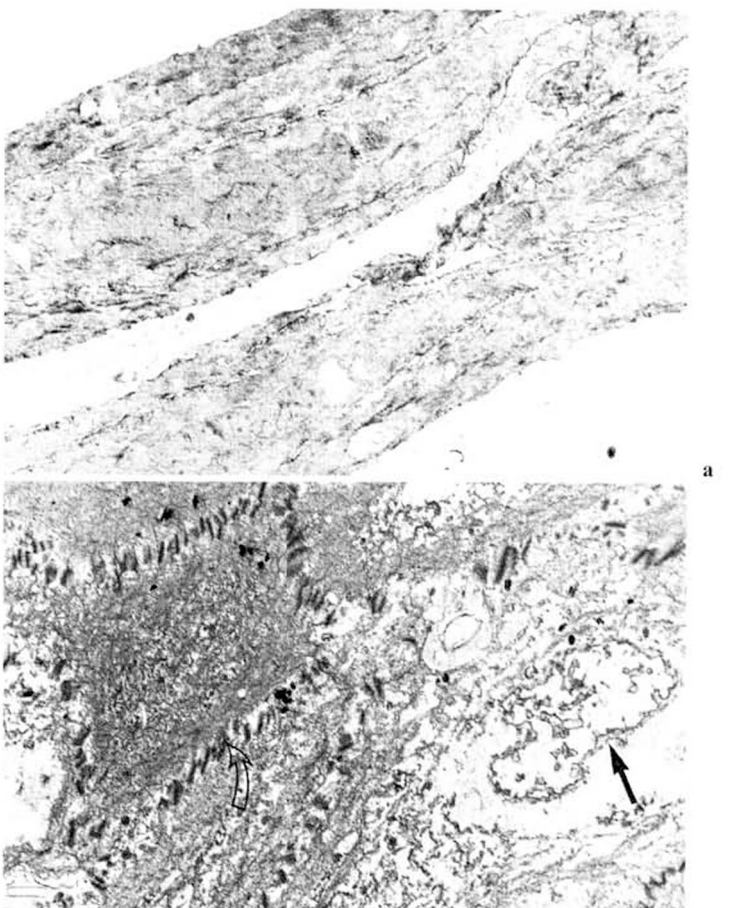


Fig. 5. **a** Human solar keratosis treated with Solcoderm. The ultrastructure, well preserved, shows how dyskeratotic cells of the stratum corneum are spontaneously detached in packages. **b** Normal human epidermis basal layer. The ralationship of desmosomes (white arrow) to adjacent cell membranes is intact. The nucleus (dark arrow) is deeply damaged. It has a characteristic pattern not observed with classical fixation or formalin fixation.

melanosomes are always well identified (fig. 6a).

The basal lamina shows a granular and fibrillar structure, but the anchoring filaments are lost. Hemidesmosomes are observed. The dermis is generally not fixed as well as the epidermis. The delicate collagen network and the elastic fibers are homogenized, and papillary or reticular dermis have the same appearance. In the papillary dermis, the capillaries are greatly modified. The basal lamina is observed as a finely granular layer surrounding retracted endothelial cells. All capillaries are dilated, and intravascular coagulation is apparent. Red blood cells fill the lumen mixed with fragments of platelets and endothelial cells (fig. 7a, b).

In the band of necrotic tissue located at the deepest portion of the biopsy, the cell structures are homogenized, and only circumvoluted nuclei are recognized in a mixture of destroyed tissue (fig. 8). The

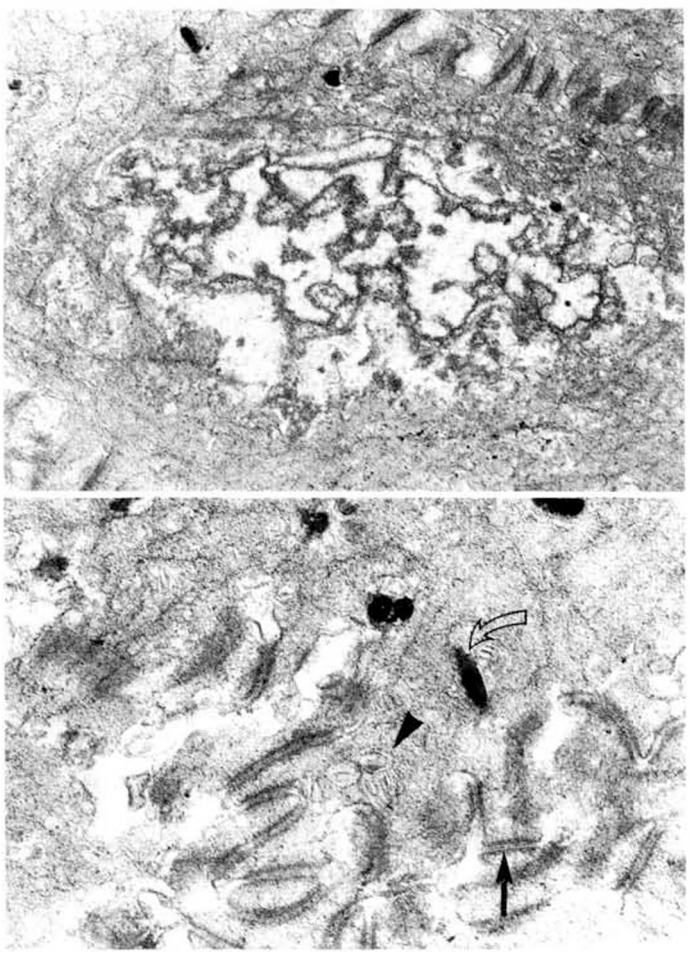


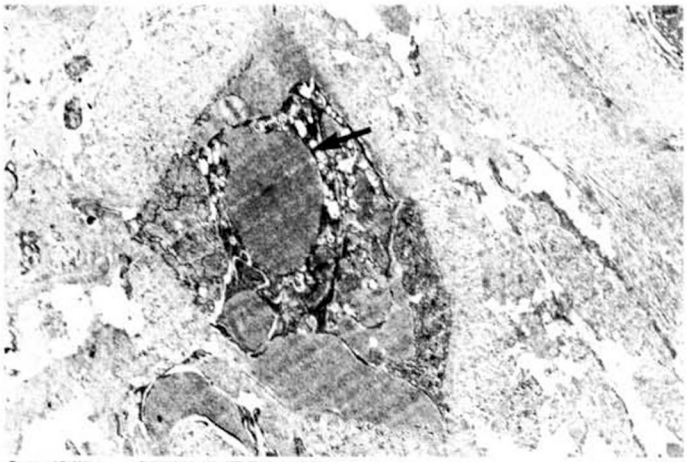
Fig. 6. a Solcoderm-treated normal skin. Electron microscopy. Detail of the characteristic pattern of the nucleus. Chromatin and nucleolar structures cannot be indentified, and the nuclear membrane is circumvoluted. In the surrounding cytoplasm, fibrillar and granular materials are present. **b** Details of the intercellular content. The pentalamellar structure of the desmosomes is clearly observed (dark arrow). Also the lamellar pattern of keratosomes is preserved (arrowhead). The dark bodies represent melanin granules (white arrow).

structure of the new stratum corneum is normal.

Solcoderm/Nitric Acid Comparative Study

On breaking the code, it was found that by overall evaluation the preparation without nitric acid was rated superior to either of the nitric acid preparations in only one instance. In contrast, nitric acid was rated better in 9 of the 16 comparisons, and Solcoderm was better in 7 of the 16.

Thus, there was no significant difference between plain nitric acid and Solcoderm in the scoring of the noted parameters. However, there was a clear difference in crust formation. A crust was noted at 10, 20, and 30 days in only 6 of 23 entries for nitric acid compared to 14 of 21 for Solcoderm (26 vs. 67%), a difference which is statistically sig-



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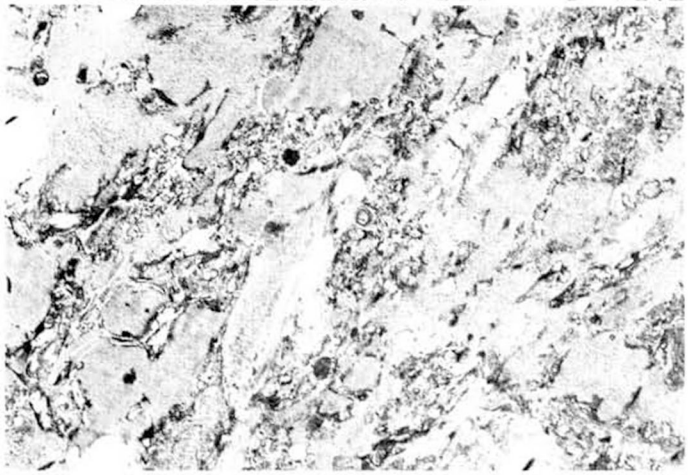

b

Fig. 7. **a** Capillary vessel in the papillary dermis of treated human normal skin. Electron microscopy. Solcoderm induces a rapid coagulation of the capillary. The arrow points to a red blood cell embedded in a platelet thrombus. The surrounding collagen is preserved. **b** Same material as above. Reticular dermis. Collagen and elastic tissue are identified.

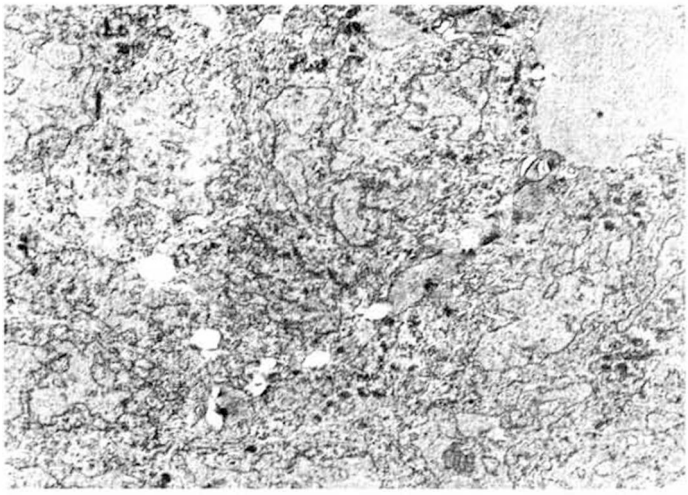

Fig. 8. Ultrastructure of the 'necrotic band' which separates the pathological tissue from the 'new' epidermis. Only cell ghosts are present. Solcoderm has not penetrated to that area, and organelles cannot be identified.

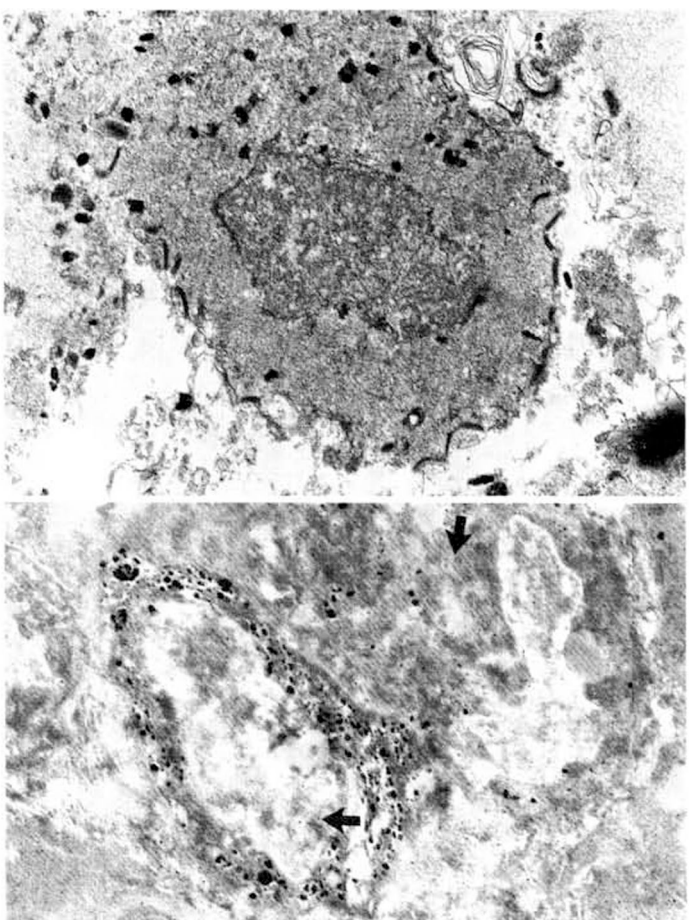

Fig. 9. **a** Electron microscopy. Solcoderm-treated human skin. The basal cell is detached from basal lamina or surrounding keratinocytes. Peripheral desmosomes do not project into the cytoplasm of the cell, indicating that a mechanical destruction during the application had previously altered the normal cell relations. **b** Electron microscopy. Normal human skin treated with plain nitric acid. Compared with previous pictures after Solcoderm treatment, it is obvious that cell organelles and desmosomes as well as the nucleus are not preserved (arrows). Only the melanin granules may be identified.

nificant ($p < 0.02$) by χ^2 analysis. This index of superior mummification with Solcoderm as compared to plain nitric acid is consistent with the results reported elsewhere [2].

Histology and electron microscopy revealed that diagnosis and fine-structure preservation were of excellent quality in Solcoderm-treated specimens, while specimens treated with the other acid preparations were unsuitable for diagnosis, and organelles could not be recognized (figs. 9a, b).

Discussion

One of the most important findings is the preservation of the fine structures of cells, even though 'classical' fixatives were not used and a long time elapsed between the application of Solcoderm and the processing for electron microscopic studies. This phenomenon indicates that Solcoderm causes an immediate fixation of tissue which it penetrated, corresponding to the change of

color of the tissue observed macroscopically. The fixative effect is limited to the extent of penetration, i.e., to the basal lamina, if no mechanical force is applied. The liquid follows the intercellular spaces, and its penetration is stopped by the papillary dermis. The cell nuclei are not fixed in the same way as are the proteins and lipids of cell membranes. The nuclear structures are destroyed. Nevus cells in nests in the dermis or basal cell epithelioma cells in bundles in continuity with the surface of the treated lesion are rapidly fixed.

These observations indicate unique fixative properties for Solcoderm, since tissues treated by plain 6.2 *N* nitric acid are necrotic without preservation of organelles or membranes. Since the cells involved in the application of Solcoderm are immediately fixed and capillary vessels coagulated, there is little basis for concern that such treatment might cause dissemination of pathologic cells.

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