Preliminary and Short Report

VISUALIZATION OF THE CELL LAYERS OF THE STRATUM CORNEUM*

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The anatomy of the horny layer is usually spoiled beyond recognition by tissue processing. It appears to be a loose, sloughing mass, whereas in fact it is a coherent, plastic membrane which serves as the "barrier" against the inward and outward movement of all substances including water.

By light microscopy of transverse sections, one can rarely make out that the unit of organization is a cell, with well defined cell membranes. During the process of keratinization, the cells are flattened so completely that their thickness is less than one micron, close to the limit of resolution of the light microscope. In consequence, so elementary a fact as the number of cell layers comprising the stratum corneum in various regions of the body, is not known. The electron microscope could easily provide this information, but a less cumbersome technic was selected for this task. Utilizing the knowledge that horny cells swell greatly in alkaline solutions, we have devised a simple light microscope technic for counting the cell layers of the stratum corneum. At the same time, some special morphologic features of certain areas can be visualized. These data are important in explaining the "barrier" function.

METHOD

From living subjects, horny layer sheets may be conveniently secured from different regions by the cantharidin-blister technic, or from autopsy-skin by separation with trypsin (1). The stratum corneum is then directly embedded in paraffin without fixation or dehydration, and sections cut at 5 microns, not more. These are dried at 60° C for one hour, de-paraffinized in xylol and mounted as usual. Alternatively, one can also start with 5 microns thick frozen sections of skin, ignoring the presence of dermis and epidermis. Fixed, dehydrated specimens are altogether unsatisfactory for this purpose.

The mounted specimen is cover-slipped and flooded with two to three drops of 0.4 N sodium hydroxide, followed immediately by a drop or two of 0.1% solution of methylene blue.

As one watches under the microscope, the horny layer begins to swell almost immediately. Indistinct laminae suddenly become recognizable cells. As the keratinized contents are solubilized by the alkali, the resistant cell membranes of the swollen cells become very clear and distinct, aided by an acquired avidity for the blue dye. After a few minutes, the cell layers can be easily counted (Figs. 1A and 1B). These developments are identical when the specimen consists of frozen sections of skin; one can additionally observe the swelling and dissolution of the epidermal cells, a technic of possible usefulness in other connections. The horny cells of the head, hands and feet swell the most, suggesting some fundamental structural difference (Fig. 2). These, interestingly, are the regions of highest cell turnover and keratin synthesis (2). Moreover, for equal thicknesses, these horny layers tend to be far more permeable.

Over most of the adult body, the horny layer is about 15 cells thick, exempting of course, special regions like the palms, soles, scrotum and the dorsum of hands and feet. This is at least twice the number of cell layers of the viable epidermis, although keratinized cells, being so extremely flattened, make up only about 10% of the cell population of the epidermis per unit area (3).

SUMMARY

By treating transverse sections of blister tops or frozen sections with 0.4 N sodium hydroxide, the horny layer swells, the resistant cell membranes become distinct and susceptible to dyeing with methylene blue.

The structural organization of the horny layer is thus revealed and the number of cell layers readily counted.

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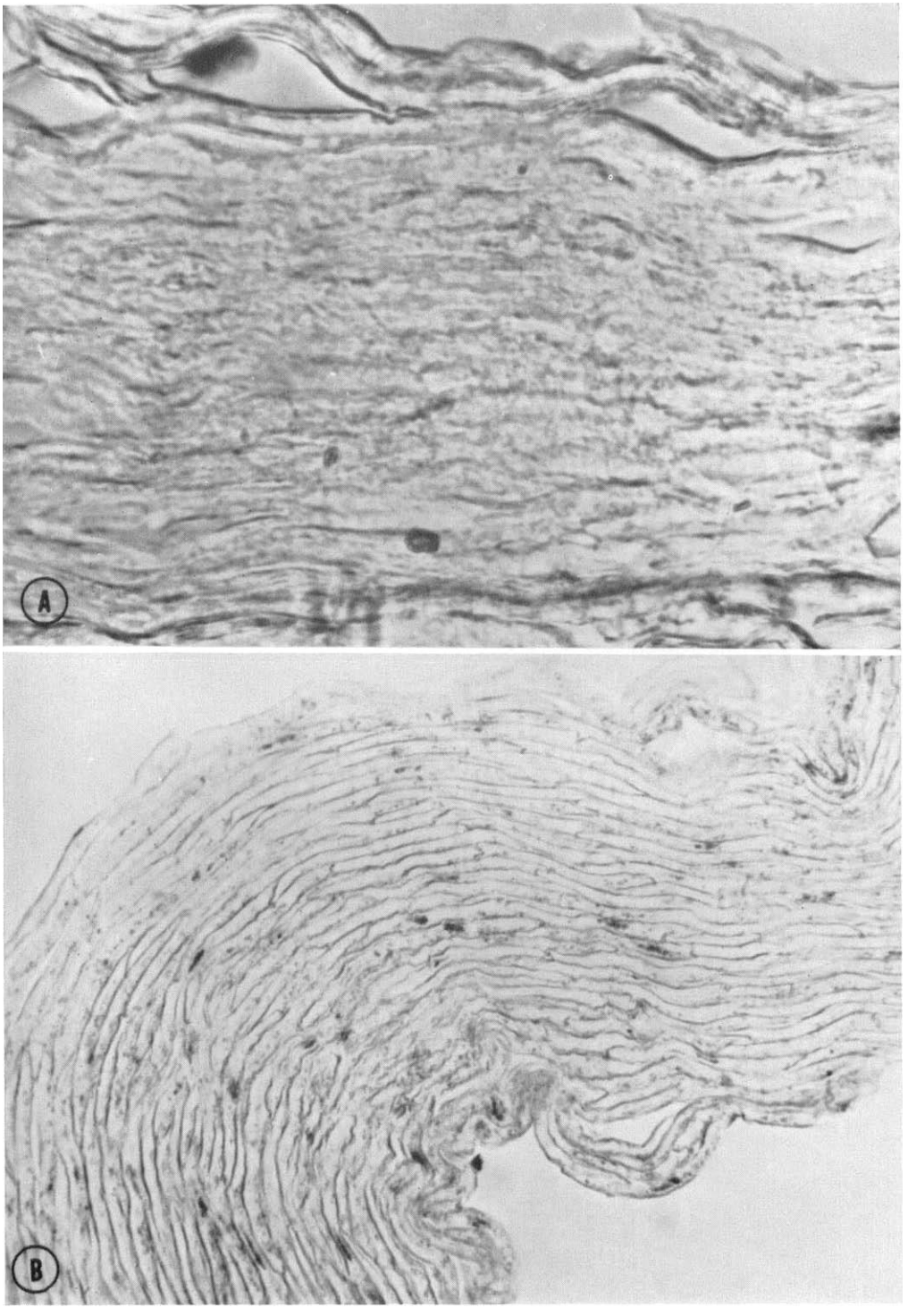

FIG. 1A. Blister top horny layer from dorsum of hand, mounted in water. Individual cells cannot be made out (× 946).

FIG. 1B. Same specimen treated with sodium hydroxide and methylene blue. The resistant membranes of the swollen cells stand out clearly and the cell layers can be easily counted (× 731).

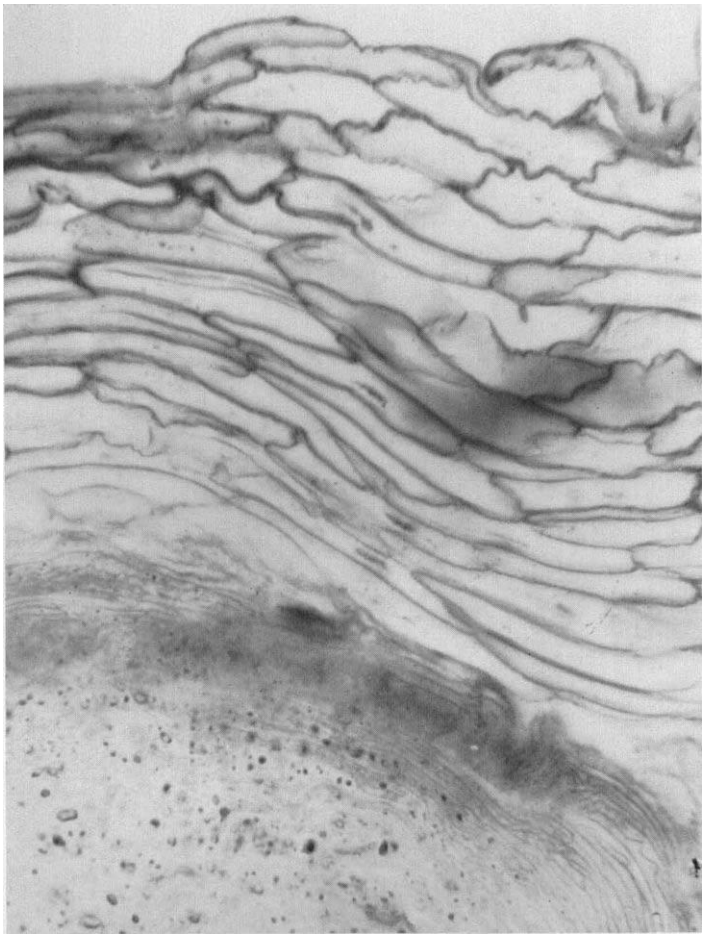

FIG. 2. Blisters from scalp treated with sodium hydroxide and methylene blue. The horny cells of the head, hands and feet exhibit greater swelling than elsewhere. The cell membranes become deeply dyed after post-treatment with sodium hydroxide (× 902).