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CELLULAR ARCHITECTURE OF THE STRATUM CORNEUM*

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ABSTRACT

The formation of columns of cells in the horny layer has been confirmed using a new fluorescent staining technique. The inception of this orderly pattern was evident within 2–3 subcorneal cell layers. Germinative cells can be identified on the basis of their comparatively low dye uptake. The conspicuous staining of the cell membranes in the horny layer probably reflects the marginal band, which can otherwise only be seen in the electron microscope.

Despite extensive studies it has only recently been appreciated that the stratum corneum cells are arranged in columns and do not overlay each other in a random manner. This delay in what now seems to be an obvious detail stems from the fact that traditional histologic processing ruins the horny layer. Although in our earlier studies we observed the columnar arrangement with the alkaline swelling technique (1), it was Mackenzie who in 1969 used the same technique and showed conclusively that the cells were arranged in orderly stacks (2).

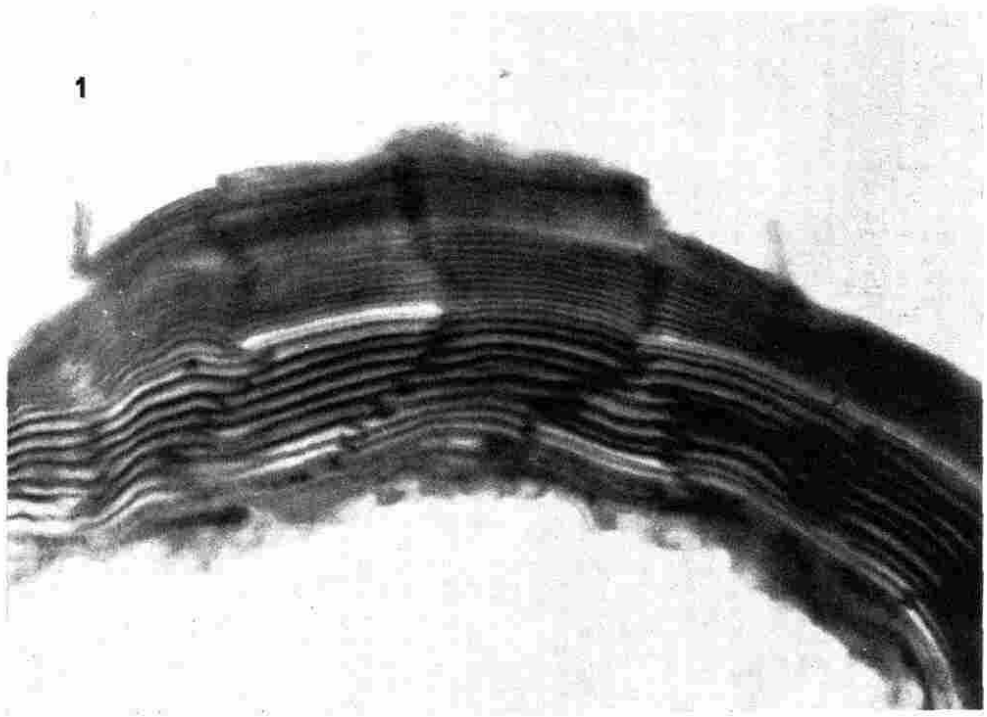

FIG. 1. Hamster ear epidermis with thick horny layer and very thin viable portion. The columns "sway" somewhat.

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The manner in which this architectural pattern develops is mysterious. The horny cells are dead and their position must be determined by cellular events taking place below the stratum

corneum. The current study had as a principle concern the clarification of this problem by using a new fluorescent staining technique. Unlike the alkaline swelling technique procedure, this preserves the viable epidermis and allows sharp visualization of the cell membranes of the horny layer (3).

MATERIALS AND METHODS

Samples were obtained from the ears and soles of feet of guinea pigs, rats, mice, hamsters and rabbits. Cryostat sections were cut vertically 4 μ thick and stored at 4° C. The staining technique has been described previously (3). A fresh solution (1:4000 to 1:10000) of fluorescein-isothiocyanate (Serva, Heidelberg) was placed over the sections for 2 minutes. After washing, the tissue was treated with 1% acetic acid and immediately examined under the Zeiss Fluorescent Microscope. Photographs were taken on color slide film (Kodak Ektachrome) and printed on Kodak Panalure F paper.

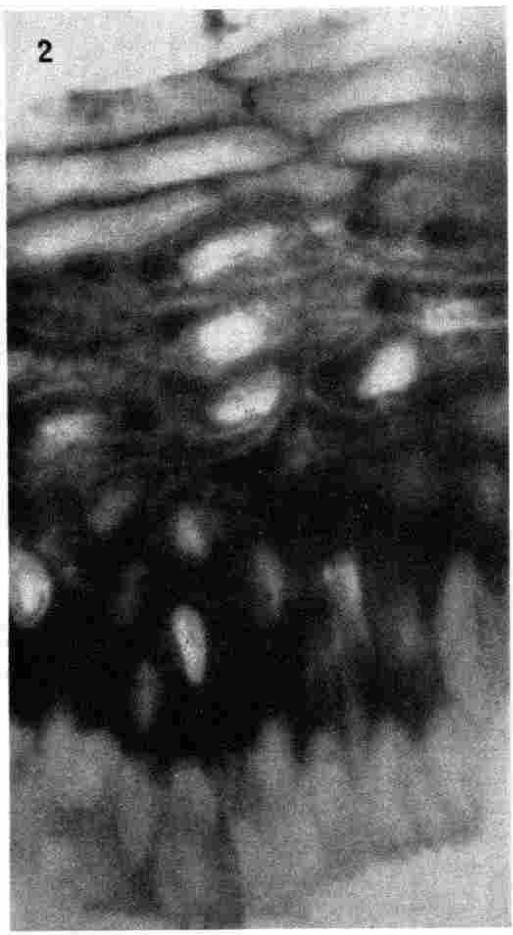


FIG. 2. Guinea pig ear epidermis. The basal layer stains faintly. Three membrane-negative cells are stacked in the subcorneal zone of the thick rete Malpighii.

TABLE

The number of cell layers in different laboratory animals

	Number of stratum corneum cells	Number of stacked Malpighian cells	Number of non-stacked Malpighian cells
Mouse	10–12	3	1
Rat	12	2–3	1
Hamster	10–15	2–3	1
Guinea pig	9–12	3	3–4
Rabbit	10–15	3	1

RESULTS

The results were quite similar in the different species and the observations can be given together.

The most striking finding was the differences in dye uptake in the various compartments of the epidermis. The cells of the germinative layer usually stained weakly or failed to stain. By contrast, the cytoplasm of all cells in the Malpighian layers took up the stain avidly. In the flattened cells of the horny layer however, only the cell membranes were strongly fluorescent (Figs. 1 and 2). A hitherto undescribed cell type was seen in the basal layer of the mouse ear epidermis. This was distributed at irregular intervals and stained quite deeply unlike the surrounding cells. Such cells were attached to the basement membrane by a thin stalk and were expanded distally into a balloon-like swelling (Figs. 3 to 6). These peculiar cells will henceforth be called "pedunculated cells". Particularly noteworthy was their location which was predominantly at the junctions between adjacent stacks.

The stacking so manifest in the horny layer was already evident in the subcorneal region. The distribution of stacked cells in various layers of the epidermis is shown in the Table. There were many more cells in the horny layer, ranging from 9–15. The number of stacked Malpighian cells was quite uniform: 2 or 3. With the exception of the guinea pig ear, which has a thick epidermis, the non-stacked Malpighian cells were always one cell layer thick. Some spe-

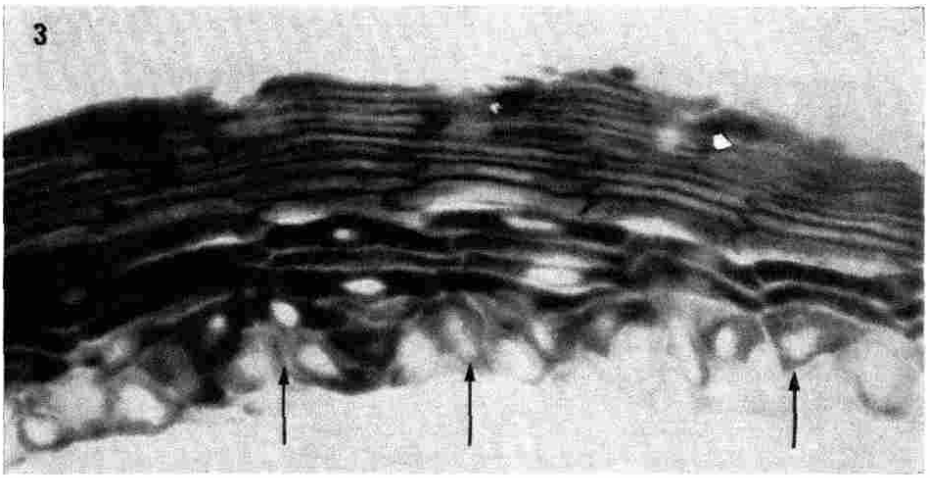
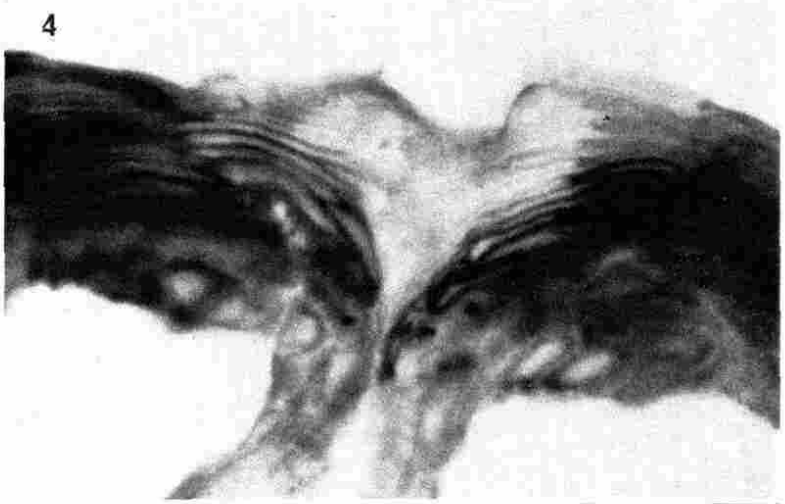


FIG. 3. Mouse ear epidermis. The difference in stainability of the basal cells is clearly visible in cells leaving the basal layer in the intercolumnar areas (arrows).



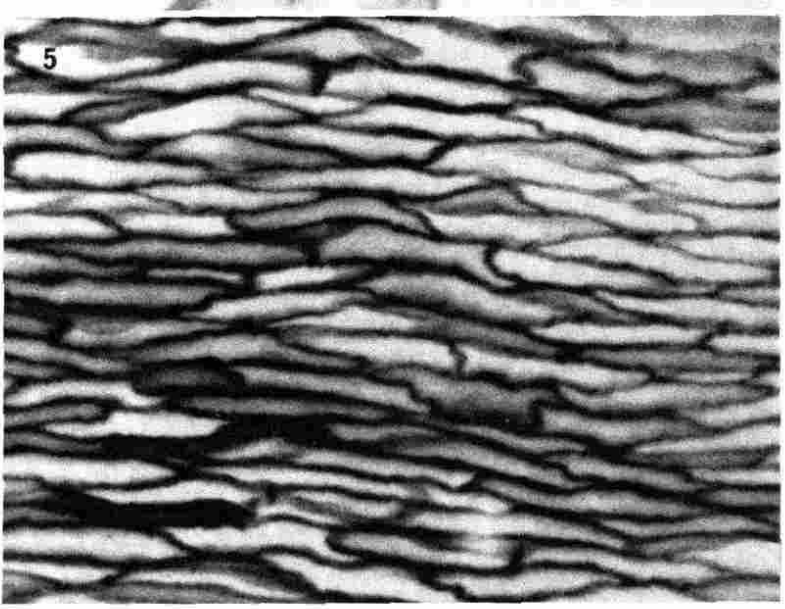


FIG. 4. Mouse ear epidermis, cross section through a follicular orifice. Note decreased dye-binding by the infundibular horny cells.

Fig. 5. Guinea pig foot pad. The cells of the str. corneum are randomly distributed

cial details are worthy of note. Horny cells derived from the follicular infundibulum failed to take up the dye. It was not possible therefore to visualize the horny mass in the follicular orifice.

In rat and in mouse epidermis the subcorneal zone occasionally showed granular fluorescence, whereas in other species the staining was more uniform. Stacking of horny cells was never seen in the epidermis from the soles of the feet. No "pedunculated" cells were observed.

Finally the basal layer was uniformly weakly stained. Sole epidermis is of course very much thicker than ear, with many more cells layers in the horny and Malpighian layers.

DISCUSSION

The fluorescent dye technique has permitted some new observations which may be useful in studying the process of differentiation in keratinizing epithelia.

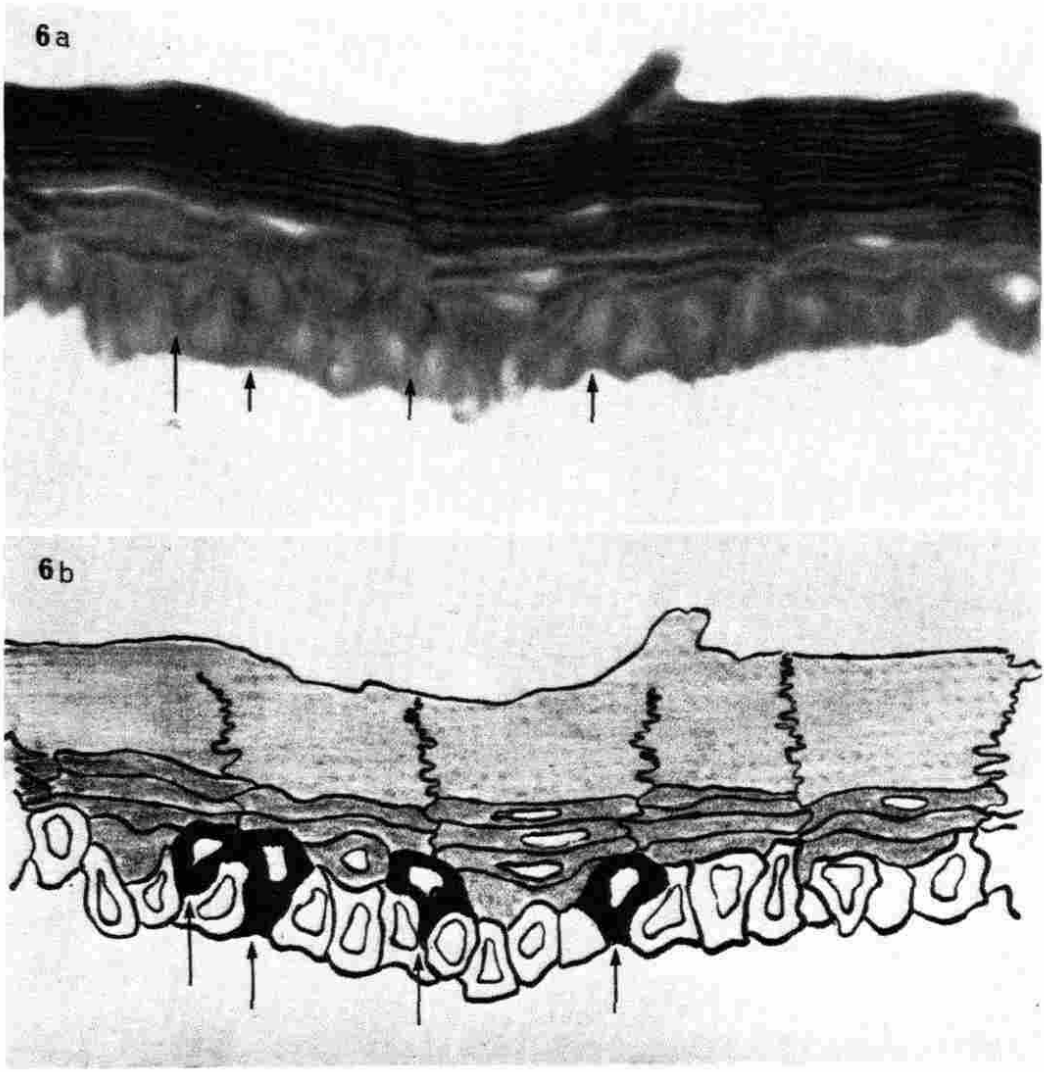


FIG. 6a. Mouse ear epidermis. Orderly arrangement of the horny layer with strong staining of the cell walls and absence of membrane staining in the Malpighian cell layers. Arrows indicate ascending basal cells.

FIG. 6b. Camera lucida drawing of a. The black cells are likely to leave the basal layer. Note that they are located between the borders of adjacent columns.

In the horny layer the dye concentrates in relation to the cell membranes, while in the Malpighian layer the cytoplasm stains with no accentuation of the borders. This difference might be only relative: prolonged exposure does in fact result in dye uptake by the corneal cytoplasm. This probably reflects the fact that the dense packing of fibrous proteins retards diffusion of the dye. On the other hand, the conspicuous staining of the cell membrane region suggests that a different product is visualized. Electron microscopy has revealed a layer here, which Hashimoto has called the marginal band (4, 5). This is a thickening of the inner layer of the plasma membrane and it's distribution corresponds exactly to the fluorescent staining.

In the species studied so far the cells of the germinative cell layer have stained poorly. Hence, undifferentiated, proliferating cells can be identified. Differentiating cells which are in transit to the horny layer have good cytoplasmic staining. It will be interesting to test this aspect in psoriatic epidermis where the reproductive pool constitutes the bottom 2–3 cell layers.

The forces which cause the Malpighian cells to be sorted out into columns make an interesting question. It is clear that cell stacking begins within the living epidermis shortly after differentiation starts. Thus, the Malpighian layer has a greater orderliness than suspected previously. It seems certain that desmosomal attachments are not permanent and that spatial relationships between cells can be altered in the lowermost regions.

Finally, the "pedunculated" cells seem to be too specifically placed to be ignored. Unlike germinative cells, they stain brighter and they are predominantly found between adjacent cell stacks. This peculiar positioning may have some connection with Mackenzie's finding of increased mitotic activity in these "intercolumnar" areas (6), from which newly formed daughter cells are supposedly directed into the columnar pathways.

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