THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 65:45–51, 1975

Vol. 65, No. 1
Printed in U.S.A.

ORDERED STRUCTURE OF THE EPIDERMIS

I. C. MACKENZIE, PH.D., B.D.S., F.D.S.R.C.S.

College of Dentistry, The University of Iowa, Iowa City, Iowa

Recent reports on the ordered structure of the epidermis are reviewed. The regular relationship between Langerhans cells and cell columns in the mouse is not typical of the epidermis of other animals. The pattern of mitotic activity previously described in mouse epidermis has also been demonstrated in the hamster, which suggests control of keratinocyte activity rather than the exclusion of keratinocytes from the central region beneath cell columns. These findings are discussed in relation to mechanisms which may be responsible for the formation of cell columns.

Alignment of the flattened keratinizing cells in the superficial strata of the greater part of mammalian epidermis leads to the formation of a series of ordered structural units [1–4]. The potential of keratinizing mammalian epithelia to form spatially organized units of structure is demonstrated in such structures as hair follicles [5] and tongue papillae [6]. Since many other tissues are also composed of small subunits of structure, it is not as surprising that a pattern of organization should exist in mammalian epidermis as that cell columns were not previously demonstrated.

The demonstration of structural units within the epidermis suggests two principal questions: (a) What is the function of this organizational pattern? (b) How are these units of structure established and maintained? Concerning the function of these units, very little is known. Possible effects on the function of the stratum corneum were discussed in a previous review [7]. Since that time (1972), so little new information has become available that scarcely anything more can be added. Formation and maintenance of these epidermal units probably involves the same mechanisms as those which lead to structural patterns in other tissues and may be related to factors which control differentiation and the rate of cell proliferation. These problems are central to biology, and the demonstration that the epidermis is yet another tissue with a precise spatial architecture does not add greatly to our understanding of them. The accessibility to and regularity of the structural units of the epidermis and their susceptibility to experimental alteration may, however, help to widen our knowledge of this field.

Current data on the morphology and distribution of ordered units of epidermal structure are briefly reviewed here. In addition, some recent findings on the relationship between these units of structure and the position of Langerhans cells and of cell division will be related to what little is known about how cell columns are established.

This work was supported by the John Hartford Foundation, Inc.

Reprint requests to: Dr. I. C. Mackenzie, College of Dentistry, The University of Iowa, Iowa City, Iowa 52242.

MORPHOLOGY OF EPIDERMAL CELL COLUMNS

Columnar units of epidermal structure are formed by the alignment of the greatly flattened cells of the upper epidermal strata. This cell alignment is most clearly seen in the stratum corneum of thin frozen sections of epidermis (Fig. 1) after expansion with buffered alkaline solutions [2,8] or by fluorescence microscopy after treatment with FITC and acetic acid [9]. After such treatment, the 10 to 30 layers of cells in the stratum corneum are clearly seen to form columns in which, for the greater part of their width, cells are in contact only with the cells immediately above and below. Laterally there is a slight overlap between the cells of adjacent columns and each cell interdigitates with its neighbors to form a regular steplike pattern. These regions of lateral interdigitation usually maintain a vertical alignment throughout the full thickness of the stratum corneum. The cell flattening associated with keratinization typically occurs at a level 3 to 5 cell

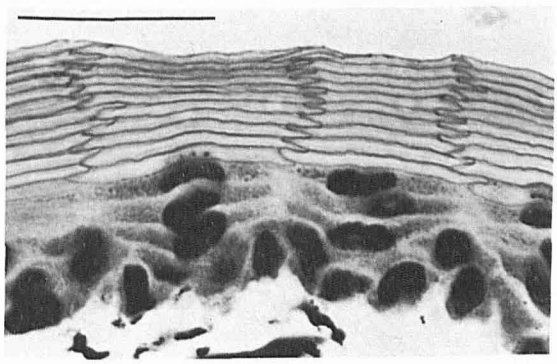

FIG. 1. Frozen section of hamster epidermis stained with methylene blue and expanded at pH 12. Beneath the regularly aligned and interdigitating cells of the stratum corneum are 3 to 4 layers of aligned nucleated cells. A number of smaller unflattened basal cells lie beneath each column. (Scale = 30 μm)

layers beneath the lowest cell in the stratum corneum. Therefore, in a thin epidermis such as that of the mouse, nearly all suprabasal cells are flattened and aligned (Fig. 1). Cells which have begun to flatten are occasionally seen to one or the other side of a cell column but, in normal circumstances, fully flattened cells are not seen out of alignment.

Because of the extreme flattening of the aligned suprabasal cells, some 2 to 4 smaller basal cells lie within the region beneath the cut edges of each column seen in sections of tissue. In thin epithelia, scalloping of the dermoepidermal junction often causes the appearance that basal cells beneath the center of the column are being pushed towards the connective tissue. However, clear demarcation of basal cells to form units of cells beneath each column is not seen.

Thicker epidermis, such as that of man (Fig. 2), shows a similar pattern of organization in the upper epidermal strata but several unflattened and apparently randomly positioned suprabasal cells lie between the basal layer and the level at which cell column formation is first detectable. Compared with rodent epidermis, human epidermis usually has a deeper and more irregular interdigitation between the columns of cells in the stratum corneum and fewer layers of aligned and flattened cells in the underlying granular layer.

The surface appearance of cell columns can be demonstrated in separated sheets of epidermis after impregnation with silver [8]. In these preparations (Fig. 3), the cell columns usually conform to a regular hexagonal outline and have a uniform degree of overlap with the cells of adjacent columns. The alignment and outline form for all cells within a particular column are similar.

Both transmission [1,7,10] and scanning [4,11] electron microscopy have confirmed and extended the information about the structure of epidermal cell columns obtained by light microscopy. Menton and Eisen [4] have demonstrated villous processes

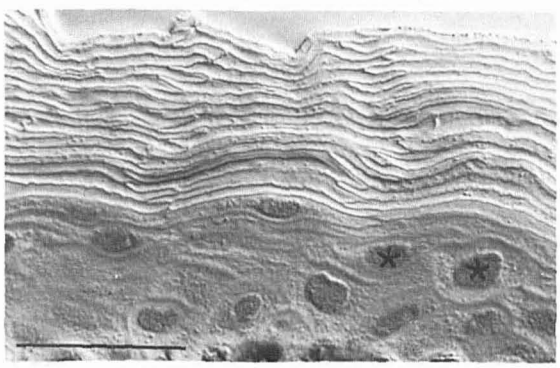

FIG. 2. Human abdominal epidermis. Alkaline expansion and Nomarski interference microscopy. A distinct column of cells is seen in the stratum corneum but the alignment and depth of the lateral interdigitations between cells are less regular than in rodent epidermis. Only 1 or 2 layers of nucleated cells are aligned, and unflattened cells (*) lie suprabasally. (Scale = 30 μm)

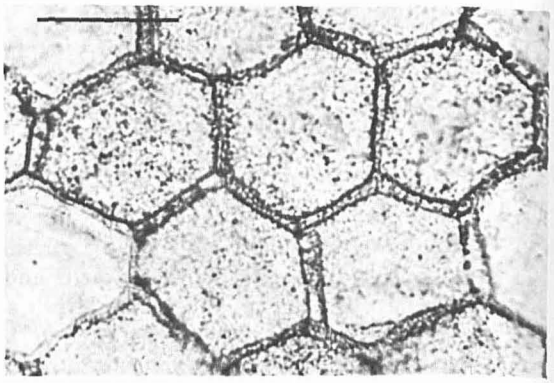

FIG. 3. Sheet of mouse epidermis impregnated with silver. The hexagonal outline form and the regular overlap between cells of adjacent columns are clearly demonstrated. (Scale = 30 μm)

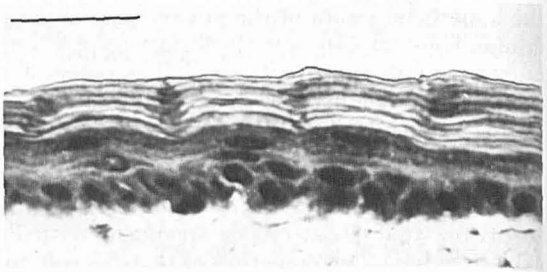

FIG. 4. Expanded specimen of body skin from a hen showing epidermal columns very similar to those of rodents. (Scale = 30 μm)

on the surface of stratum corneum cells which are apparently related to cell attachment and they have also shown differences between the thickness of stratum corneum cells from volar surfaces and those from regions with an ordered structure. Around the boundaries of the hexagonal cells from the ordered regions, they have also described a decrease in cell thickness and the formation of a steplike depression which apparently allows the margins of the horny cells of adjoining columns to interdigitate without increasing the thickness of the junctional region. Allen and Potten [10] have described the ultrastructural appearance of epidermal units in some detail and, of particular functional interest, have described a modified form of desmosomal attachment between the overlapping edges of the cells of adjoining columns. In this region, desmosomal attachments appear to differ from the roughly circular, platelike desmosomes of the lower strata in that an unbroken desmosomal ring, or desmosomal bands at least several μm in length, run adjacent to the cell periphery.

THE DISTRIBUTION OF EPIDERMAL CELL COLUMNS

Columnar units of structure similar to those in mammalian epidermis are also found in the epidermis of other phyla, e.g., the frog [12]. An expanded specimen of chicken epidermis shows columnar units not easily distinguishable from those of a rodent (Fig. 4). Despite the absence of any system-

atic study of cell columns in nonmammalian epidermis, it appears probable that columnar patterns of organization occur quite frequently.

In the keratinizing epithelia of some mammals—e.g., in man, the rhesus, and the rodent—there appears to be a consistent pattern of distribution of columnar units which in general corresponds to the distribution of hair. Such a pattern of organization cannot be demonstrated in such specialized regions as plantar and palmar surfaces, the areola of the nipple, the lip, and oral mucosa [3,4,7,8,13–15].

POSITION OF LANGERHANS CELLS IN RELATION TO EPIDERMAL CELL COLUMNS

Dendritic ATPase-positive cells are numerically and spatially related to cell columns in sheets of mouse epidermis (Fig. 5) and these cells have a marked tendency to occupy a central position beneath each cell column [7]. Electron microscopical investigations suggest that most of these cells are Langerhans cells [7,10] and this spatial relationship to epidermal columns has suggested a relationship to the control of keratinocyte activity [10,16].

More recently, using similar methods [7] to study ATPase-positive dendritic cells in separated epidermal sheets of other species, we have found marked species differences in the morphology and distribution of Langerhans cells. In the hamster, for example, ATPase-positive cells are too large and too few to be regularly related to the overlying cells columns (Fig. 5), and in the guinea pig and the monkey, they do not seem to occupy a regular position although their number per unit area corresponds approximately to the number of cell columns present. The absence of a regular position of ATPase-positive cells in the epidermis of other species therefore suggests that their relationship to cell columns in mouse epidermis is atypical and is not important to the establishment of cell columns.

CELL PROLIFERATION AND EPIDERMAL CELL COLUMNS

Two aspects of cell proliferation are related to the formation of epidermal cell columns: (a) a slow rate of epidermal regeneration, and (b) the position of the cell division.

Rate of Cell Proliferation

Epidermal regions which form cell columns generally have a slower rate of cell proliferation than regions, such as plantar and palmar surfaces, which have a nonordered structure of the stratum corneum [13,17]. Moreover, some regions of the guinea-pig ear in which cells in the stratum corneum are randomly positioned have a higher labeling index than closely adjacent regions in which the cells are regularly aligned into columns [18]. Stripping the epidermis with adhesive tape increases mitotic activity for 2 to 3 days [19] during

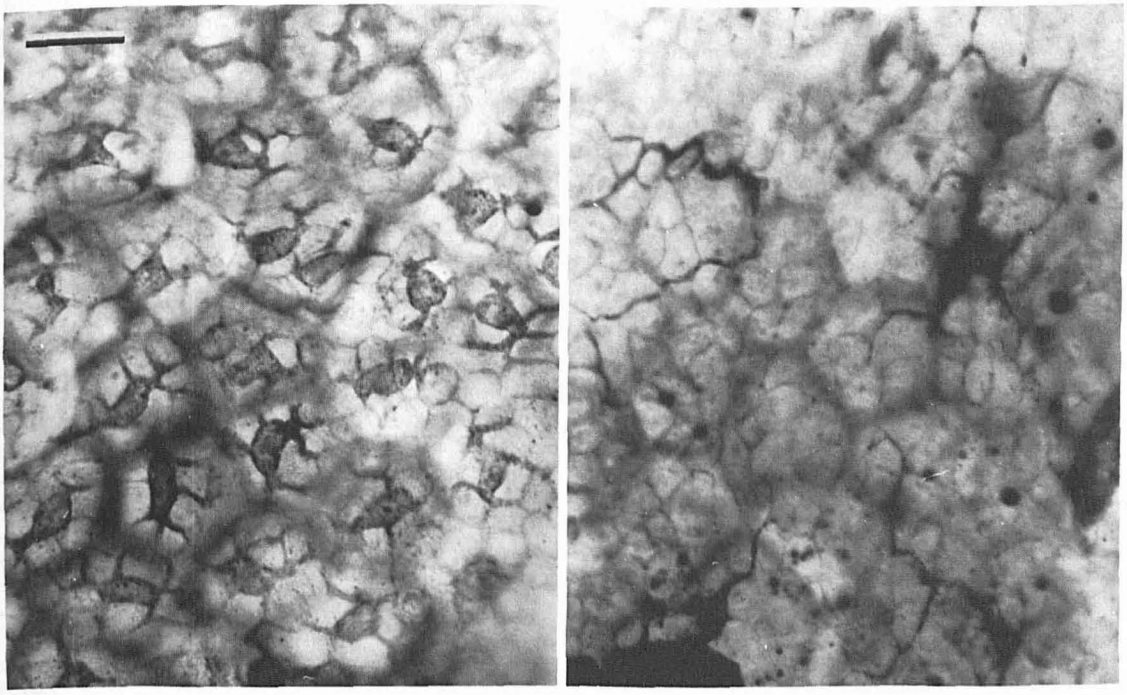

FIG. 5. Epidermal sheets processed to demonstrate ATPase activity and stained with Sudan black B to outline cell columns. *Left:* Mouse epidermis showing plump ATPase$^+$ dendritic cells with a marked tendency to occupy a central position beneath the overlying cell columns which are seen as out-of-focus hexagonal outlines. *Right:* Hamster epidermis at the same magnification showing widely spaced ATPase cells with long wandering dendrites. These cells are too few and too large to show a regular position in relation to overlying cell columns. (Scale = 30 μm)

which time cells are randomly added to the stratum corneum; the columns are re-formed only when the mitotic rate subsides to previous low levels. Evidence that mitotic rate is related to column formation has also been presented by Menton and Eisen [11] who showed that a columnar pattern of organization of the stratum corneum was absent in hyperkeratotic conditions of human epidermis associated with increased mitotic activity but present in hyperkeratotic conditions associated with little or no increase in mitotic activity. Thus, it has been suggested that slow maturation of epidermal cells enables them to become arranged in stacks [18]. However, other factors besides the rate of proliferation may be involved because the ordered structure is lost when the rate of cell proliferation rises after damage to the stratum corneum but is retained when the rate of proliferation increases as a result of pressure [20].

Position of Cell Division

Goerttler and co-workers [21,22] have confirmed earlier reports that cell division occurs principally in the basal cells lying beneath the periphery under each cell column [1,7]. Potten and Hendry [23] and Potten [24], who examined the number and distribution of mitotic and DNA-labeled cells under the cell columns, have postulated that these cells form an "epidermal proliferative unit" whose central cells act as "clonogenic cells."

These findings that patterns of basal cell activity are related to the overlying cell columns raise two problems about the position of cell division in the establishment of epidermal structure. First, all of the reported studies have used mouse epidermis in which dendritic cells, probably with very low mitotic activity [25], occupy the central region beneath cell columns. The observed tendency for mitosis to occur beneath the periphery of the cell columns could therefore result not from some mechanism that controls keratinocyte activity but simply from the displacement of keratinocytes from the central region. Second, if the units of basal cells beneath each cell column form "epidermal proliferative units," each group of interphase basal cells could be aligned within the periphery of the overlying column to which it is functionally related, and this preexisting alignment would influence the observed position of mitosis.

In hamster epidermis there is no regular relationship between the position of dendritic cells and epidermal cell columns (Fig. 5). Hamster epidermis was therefore used to investigate whether the greater frequency of mitosis beneath the outer edge of cell columns in mouse epidermis could be due to the occupation of the central region by nonmitotically active dendritic cells. The method used was essentially the same as that previously used to demonstrate patterns of mitotic activity in the mouse [2]. Adult male hamsters were injected with vinblastine sulfate to arrest mitotic cells in metaphase and 4 hr later the ears were removed. Frozen sections of metaphase figures were photographed to measure the position of each metaphase figure in relation to its overlying cell column. If all basal cells have an equal probability of division, the observed position of metaphase figures would be randomly distributed in equal lengths of the basal regions beneath the cell columns. If, on the other hand, there is a central region beneath each cell column in which mitotic activity occurs relatively infrequently, sections of cell columns would tend to show greater mitotic activity in the regions beneath the junctions between adjacent columns. This pattern of distribution would, however, be seen only in sections which pass through the central mitosis-free area, not in those which pass through the edges of columns [2]. These two types of sections can be distinguished, since the apparent width of a column sectioned through its edge will, in general, be less than one sectioned through the central region.

The Table and Figure 6 show the results when the distribution of mitosis beneath cell columns in hamster epidermis was compared with that in mouse ear epidermis. In the former, the number of metaphase figures lying beneath the junctional regions between cell columns is significantly higher (χ^2, $p < 0.01$) than those beneath the central regions. An attempt was made to distinguish, on the basis of column width, between those sections passing through the edge and those passing through the center of cell columns. In the former the observed position of metaphase was randomly distributed, in the latter the nonrandom distribution was even more marked. These findings for hamster are quite similar to those for mouse [2]. The comparatively fewer central mitoses in mouse epidermis (Fig. 6) may represent the influence of centrally positioned dendritic cells, but because of improved methods of processing and the greater number of observations made with hamster mate-

TABLE. *Distribution of 1015 metaphase figures in inner and outer quarters of the basal region beneath cell columns*

The distribution beneath all column sections shows a 25% greater number of metaphases beneath outer quarters. Sections judged, on the basis of length, to pass through a postulated central low-mitosis area showed a greater proportion of mitoses beneath the junctional region. The distribution found in the hamster is similar to that found previously in the mouse [7].

	Sections passing through:		
	Center of column	Edge of colmn	All sections
Outer quarters	352	212	564
Inner quarters	261	190	451
Outer/inner (hamster)	1.35	1.12	1.25
Outer/inner (mouse)	1.52	1.01	1.26

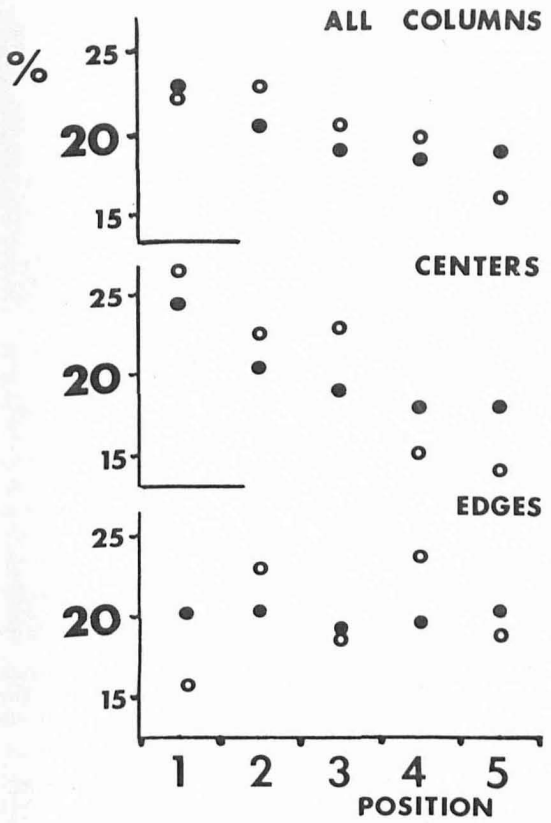

FIG. 6. Distribution of metaphase figures in five regions of equal length from the edge (*1*) to the center (*5*) of sectioned columns in hamster (●) and mouse (○) epidermis. *All columns:* Distribution of mitoses beneath all columns examined; fewer mitoses are found beneath the central region. *Centers:* Distribution of mitoses beneath columns judged to be sectioned through the central region; the distribution becomes more marked. *Edges:* Distribution of mitoses beneath columns judged to be sectioned through the edge; the distribution is essentially random.

rial, a direct comparison of these two sets of data is probably not justified.

The finding of a pattern of mitotic activity related to the periphery of cell columns in a tissue such as hamster epidermis, in which Langerhans cells do not occupy a central position, indicates that this pattern is somehow related to the control of cell proliferation rather than to the exclusion of keratinocytes from the central regions of cell columns. However, the accuracy of any study of the position of cell division in sections of epidermis is hampered by uncertainty about the plane of section in relation to a particular cell column. Intact sheets of mouse epidermis were therefore used to study more closely the distribution of both mitotic and interphase basal cells beneath cell columns. Adult male Balb/C mice were injected with colchicine 4 hr before death to arrest dividing cells in metaphase, and intact sheets of ear epidermis were obtained by treatment with buffered EDTA [26]. The sheets of epidermis were fixed in Bouin's solution, washed, stained with hematoxylin, and mounted flat on microscope slides. Nomarski interference microscopy showed that both metaphase and interphase basal cells and the outlines of cell columns were clearly visible (Fig. 7).

Any hexagon can be divided, by a series of similar concentric hexagons, into 10 concentric regions of equal area by dividing a measured major radius (r_{10}) into lengths r_1, $r_2 \cdots r_{10}$ so that $r_1 = r_2/\sqrt{2} \ldots r_{10}/\sqrt{10}$. Tracings were therefore made of the position of over 3,000 blocked metaphase figures in relation to the outlines of overlying hexagonal cell columns, and the length of the radius passing from the center to the edge of the column through each metaphase figure was measured (Fig. 8). The region in which the center of each metaphase figure lay was then calculated by a simple computer program, and the distribution of metaphase figures within each of 10 regions from the center to the edge of the columns was examined with the expectation that random distribution of metaphase figures within the basal layer would result in an equal probability of occurrence of metaphase figures in each area. The pattern of distribution of all the basal cells lying beneath more than 100 cell columns was also examined by the same method.

The results of this investigation are shown in Figure 9. Mitotic activity was found to be low not only beneath the central region of cell columns but also immediately beneath the columnar junctions. This pattern of distribution appeared similar in all specimens examined and the centers of metaphase figures occurred with greatest frequency per area at a mean distance of 4.4 ± 0.5 μm within the periphery of the overlying cell columns. The pattern of distribution for all the basal cells lying beneath cell columns did not, however, differ significantly from a random distribution. Apparently, therefore, there is no evidence for a preexisting alignment of interphase cells within the periphery of cell columns to account for the observed position of mitotic activity.

DISCUSSION

Units of epidermal structure are the typical configuration of much of mammalian epidermis. The presence of such units in amphibia and birds suggests that this pattern of organization is fairly basic for keratinizing epithelia. As a general rule, cell columns are found only in relatively thin epithelia but, in view of the suggested relationship between the rate of cell proliferation and epithelial thickness [27], this may well be a reflection of the rate of cell proliferation [13,17]. Whether corresponding patterns of spatial organization are completely lacking in thicker epithelia in which no morphologic pattern has been demonstrated is not yet clear.

The relationship between Langerhans cells and cell columns found in mouse epidermis appears not to be typical; therefore, Langerhans cells may not

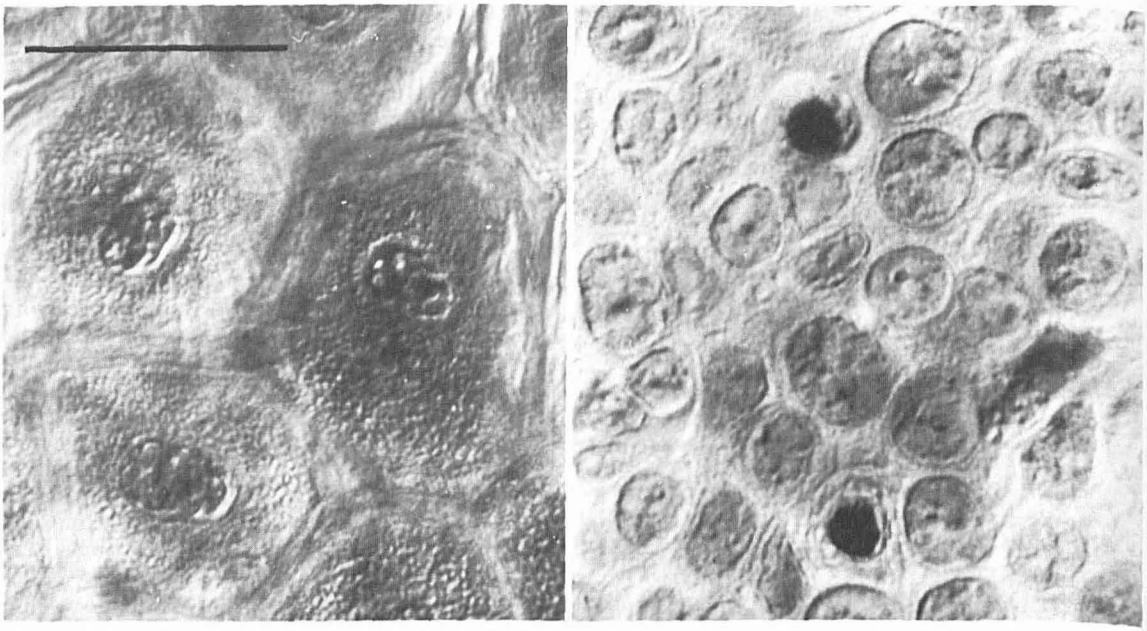

FIG. 7. Sheet of mouse epidermis, separated with EDTA and stained with hematoxylin, viewed by Nomarski interference microscopy. *Left:* With plane of focus at a level between stratum granulosum and stratum corneum, the outlines of cell columns are clearly visible. *Right:* Changing plane of focus to the basal layer demonstrates the position of metaphase figures in relation to overlying cell columns. (*Scale* = 30 μm)

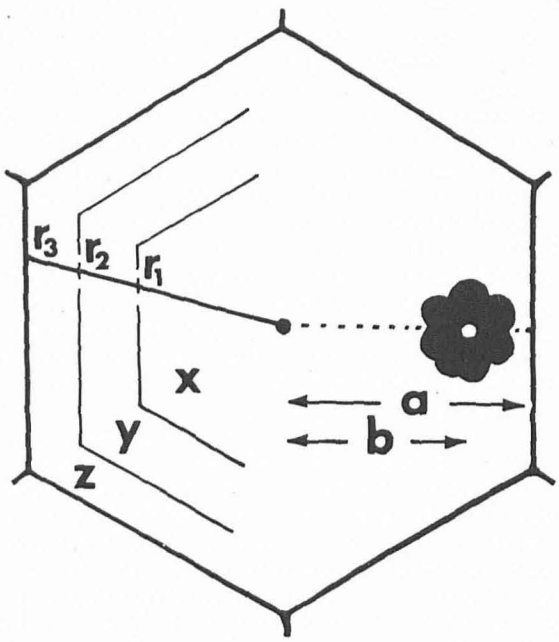

FIG. 8. Diagram of the method of examining the distribution of metaphase figures beneath cell columns. A hexagon can be divided into concentric regions of equal area (x, y, z) by division of the distance (r_3) from the center to the edge of the hexagon into lengths (r_1, r_2) such that $r_1 = r_2/\sqrt{2} = r_3/\sqrt{3}$. The distribution of metaphase figures within 10 such concentric regions of each column was calculated from the ratio of the distance from the center of the column to the center of the metaphase (a) to the length of the radius passing through the metaphase (b).

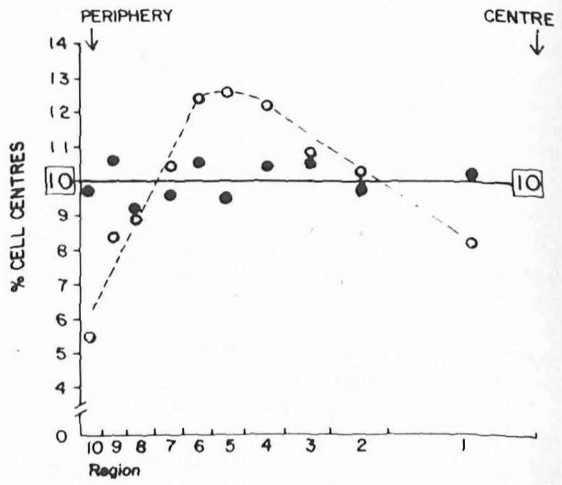

FIG. 9. The distribution of the centers of metaphase and interphase basal cells in concentric regions of equal area beneath cell columns. The distribution of interphase basal cells (●) conforms to a random distribution with approximately 10% of the cells being found in each area. A significant deviation (χ^2, $p < 0.001$) from a random distribution of metaphase figures (○) was found with metaphase figures occurring with maximum frequency per area 4–5 μm within the periphery of the columns.

be directly associated with the establishment of this pattern of organization. There is now strong evidence that, in thin epithelia at least, the position of cell division is related to the columnar structure of the overlying strata. Demonstration of this pattern in hamster epidermis indicates that the position of mitosis in mouse is not simply the

result of an exclusion of keratinocytes from the central region by the peculiar position of Langerhans cells. A more detailed examination of the position of mitosis in mouse epidermis had demonstrated that mitotic activity is found with maximum frequency just within the periphery of overlying cell columns, but there was no evidence for grouping basal cells in the units beneath each column which might have provided evidence of "epidermal proliferative units." The reason for the observed position of mitosis is therefore unclear.

The ordered alignment of cells in the upper epidermal strata indicates that the maintenance of normal epidermal structure involves more than a control of the type of cellular synthetic activity and a balance between rates of cell formation and maturation [27]. A further mechanism for controlling spatial organization of cells has also to be considered, but it is not yet known at which level this spatial information is first expressed. It could be that age differences between the cells situated at different levels in the epidermis are associated with differences in the cell surface properties of older cells which, being recognized by cells undergoing flattening, result in cell alignment [7]. Cells appear to emigrate from the basal layer beneath the peripheral regions of the columns [3,10] and some such mechanism appears to be necessary to account for their subsequent alignment. Patterns of mitosis beneath cell columns, which could be due to a feedback mechanism [2], would then presumably be secondary to column establishment. In this case, the mechanism establishing ordered epidermal structure would be entirely "intraepidermal" in origin. In view of the important role played by dermal tissues during embryogenesis, this degree of independence in adult epithelia is questionable, but information on this question might be obtained by studies of patterns of mitotic activity in a thicker epidermis and of spatial organization in separated epidermal sheets after transplantation to suitable recipient sites.

REFERENCES

1. Mackenzie IC: Ordered structure of the stratum corneum of mammalian skin. Nature (Lond) 222: 881–882, 1969
2. Mackenzie IC: Relationship between mitosis and the ordered structure of the stratum corneum in mouse epidermis. Nature (Lond) 226:653–655, 1970
3. Christophers E: Cellular architecture of the stratum corneum. J Invest Dermatol 56:165–169, 1971
4. Menton DN, Eisen AZ: Structure and organization of mammalian stratum corneum. J Ultrastruct Res 35:247–264, 1971
5. Cohen J: Dermis, epidermis and dermal papillae interacting, Advances in Biology of Skin, vol IX, Hair Growth. Edited by W Montagna, RL Dobson. Oxford, Pergamon, 1969, pp 1–18
6. Cameron IL: Cell proliferation, migration and specialization in the epithelium of the mouse tongue. J Exp Zool 163: 271–284, 1966
7. Mackenzie IC: The ordered structure of mammalian epidermis, Epidermal Wound Healing. Edited by HI Maibach, DT Rovee. Chicago, Year Book, 1972, pp 5–25
8. Mackenzie IC: An examination of cellular organization within the stratum corneum by a silver staining method. J Invest Dermatol 61:245–250, 1973
9. Christophers E: Eine neue Methode zur Darstellung des Stratum corneum. Arch Klin Exp Dermatol 237:717–721, 1970
10. Allen TD, Potten CS: Fine structural identification and organization of the epidermal proliferative unit. J Cell Sci 15:291–319, 1974
11. Menton DN, Eisen Z: Structural organization of the human stratum corneum in certain scaling disorders. J Invest Dermatol 57:296–307, 1971
12. Komnick H, Stockum W: Die Feinstruktur der Epidermisoberflache an der Extremitaten des Krallenforsches. Arch Histol Jap 32:17–40, 1970
13. Christophers E: Die epidermale Columnarstruktur. Voraussetzungen und moglicher Entstehungsmechanismus. Z Zellforsch Mikrosk Anat 114:441–450, 1971
14. Plewig G: Grosse Form und anordung Verhornender schleimhautzellen. Arch Dermatol Forsch 242:30–42, 1971
15. Mackenzie IC, Nuki K, Loe H, Schiott CR: A study of the stratum corneum of human oral mucosa after exposure to chlorhexidine. J Periodont Res (in press)
16. Sagebiel RW, Clarke MA, Hutchins LH: Dendritic cells in oral epithelium, Current Concepts of the Histology of Oral Mucosa. Edited by C Squier, J Meyer. Springfield, Ill, Thomas, 1971, pp 143–166
17. Christophers E, Wolff HH, Braun-Falco O: Zelldifferenziurung im Stratum granulosum: Elektronenmikroskopische-autoradiographische Einbaustiudien mit ^{3}H-Histidin. Arch Dermatol Forsch 242:179–190, 1972
18. Christophers E: Correlation between column formation, thickness and rate of new cell production in guinea pig epidermis. Virchows Arch [Zellpathol] 10:286–292, 1972
19. Christophers E: Kinetic aspects of epidermal healing, Epidermal Wound Healing. Edited by HI Maibach, DT Rovee. Chicago, Year Book, 1972, pp 53–69
20. Christophers E: The architecture of the stratum corneum after wounding. J Invest Dermatol 57:241–246, 1971
21. Karatschai M, Kinzel V, Goerttler K, Suss R: Geography of mitoses and cell divisions in the basal cell layer of mouse epidermis. Z Krebsforsch 76:59–64, 1971
22. Goerttler K, Reuter M, Stahmer HE: Morphologische Untersuchungen zur Proliferationskinetik der Mausehaut. Z Zellforsch Mikrosk Anat 142:131–146, 1973
23. Potten CS, Hendry JH: Clonogenic cells and stem cells in epidermis. Int J Radiat Biol 24:537–540, 1973
24. Potten CS: The epidermal proliferative unit (EPU). The possible role of the central basal cell. Cell Tissue Kinet 7:77–88, 1974
25. Mackenzie IC: Langerhans cell population kinetics. J Dent Res 52 (Suppl):141, 1973
26. Scaletta LJ, MacCallum DK: A fine structural study of divalent cation-mediated epithelial union with connective tissue in human oral mucosa. Am J Anat 133:431–454, 1972
27. Bullough WS: The control of epidermal thickness. Br J Dermatol 87:187–199, 1972