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THE ORDERED COLUMNAR STRUCTURE OF MOUSE FILIFORM PAPILLAE

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SUMMARY

Examination of carefully oriented 1-μm and 5-μm sections of mouse dorsal tongue together with scanning electron microscope observations indicates a high degree of cellular organization in the papillae. It has been suggested that each filiform papilla consists of 2 dominant and 2 minor columns of cells. Labelling patterns of the basal cells have been investigated in relation to these columns.

INTRODUCTION

The study of epidermal proliferation and differentiation was advanced by the work of Mackenzie (1969) and Christophers (1970, 1971) who demonstrated that the stratum corneum of mouse dorsal epithelium was composed of large, flattened, roughly hexagonal cells stacked into columns.

The structure and behaviour of the cells within these columns has been investigated by a number of people (Christophers, 1970, 1971, 1972; Mackenzie, 1972, 1975; Christophers, Wolff & Laurence, 1974; Goerttler, Reuter & Stahmer, 1973; Potten, 1974; Allen & Potten, 1974). Some of these studies have led to the suggestion that the dorsal skin and ear epidermis in mice and many other species may be considered to be composed of a number of discrete units of proliferation (epidermal proliferative units, EPUs) (Potten, 1974; Allen & Potten, 1974). Recent reinterpretation of earlier radiobiological work has further suggested that only a fraction (less than 10%) of the basal cells are stem cells (clonogenic) (Potten & Hendry, 1973). Similar experiments and conclusions have recently been reached on the intestinal mucosa (Potten, 1976*b*; Potten & Hendry, 1975; Cheng & Leblond, 1974). An apparent correlation exists between low cell production rates, thin flattened mucosa and ordered columnar stacking. Though distinct functional proliferative zones can be recognized in the intestinal crypts no detailed ordered structure has so far been observed.

It has been known for some years that a difference exists between the morphology of the anterior and posterior facing aspects of both mouse and rat tongue filiform papillae (Kutozov & Sicher, 1951; Cameron, 1966; Cane & Spearman, 1969; Farbman, 1970). The anterior aspect has a dense keratohyalin complex in the stratum granulosum and forms a 'soft' orthokeratin. In comparison the posterior aspect of the papilla has a stratum granulosum, is free from keratohyalin granules, is rich in ribosomes, tonofibrils

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and sulphydryl bonds and forms a 'hard' orthokeratin. Farbman (1970) described the electron-microscopic appearances of these different areas in the rat.

Earlier attempts have been made to clarify the arrangement of the filiform papillae (Cameron, 1966; Cane & Spearman, 1969).

This paper presents some evidence indicating that the tongue papilla is a simple modification and slight distortion of a few neighbouring epithelial columns. The consequences of this model for cell-kinetic interpretation are discussed.

MATERIALS AND METHODS

Autoradiographic data

Male DBA2 mice, 7–8 weeks old, were injected intraperitoneally with 50 μCi of tritiated thymidine (^{3}H-TdR)(5 Ci/mMol) at 10.00 hours (approx. 2·5 μCi/g) and killed 40 min later. The tongues were carefully dissected out and fixed for 20 min in Carnoy and then stored until required in 70% ethanol. Each tongue was carefully sectioned sagittally (represented as the *xy* axis on all figures) or transversely (represented as the *wv* axis) and the 5-μm-thick sections Feulgen stained. Autoradiographs were prepared by the dipping technique using Ilford K5 nuclear emulsion. The slides were developed after 10 days exposure at 4 °C in light-tight boxes, and counterstained with fast green. Only papillae from a 1-mm^2 of tissue just in front of the *sulcus terminalis* (the groove separating the proximal and distal aspects of the tongue) were scored. Extreme care was necessary in section orientation during embedding and sectioning in order to be able to relate basal cell activity on the dermal papilla to the complex 3-dimensional structure of the filiform papilla. Technically, sectioning of the papilla longitudinally was more difficult because of the hardness of the keratin tips. This, combined with the small size of each papilla rendered sectioning in a true plane difficult to achieve without a certain degree of distortion. Once the section was precisely oriented with regard to both the filiform and dermal papillae the basal cells were numbered along the basement membrane starting with cell position 1 adjacent to the papillary-interpapillary junction (Fig. 3, p. 152) which could be observed using phase-contrast microscopy. Only regions which contained sections with more than 5 cell positions up the sides of the dermal papillae were used (Figs. 3, 5). Providing papillae with deep dermal indentations and a clear cell position one were scored it was not essential that the filiform papilla itself appeared in complete structural detail (see later). Only papillae which showed the posterior column of cells to arise from the apex of the dermal papilla were scored. Obliqueness of sectioning could give the false appearance of the posterior column arising from the anterior aspect of the dermal papilla. The relative labelling patterns for the cell positions were determined by scoring a minimum of 50 papillae from each of four or more mice.

Thin sections and electron microscopy

Thin sections (1-μm) were prepared for light microscopy by fixing in 3% glutaraldehyde, postfixing in 1% osmium tetroxide in phosphate buffer and eventually embedding in an Araldite-Epon resin using standard techniques. Sections 1 μm thick were cut in a sagittal plane using a glass knife and stained with basic fuchsin and toluidine blue.

Similar blocks of tissue were thoroughly washed in Sorensen's buffer and fixed in the same way. These samples were dehydrated and critical point dried for study in a Cambridge stereoscan S4-10.

RESULTS

Scanning electron microscopy

The shape of the papillae can be seen in Figs. 1 and 2. The anterior surface is a large single cell that does not quite reach the tip which is composed of 3–4 progressively

overlapping (imbricated) posterior cells. The sides at the base of the papillae are varyingly overlapped and enclosed by additional cells. The surface structure of the cells is somewhat reticulate.

Light microscopy

The filiform papilla is a curved, tapering, cone-shaped body with a correspondingly complex-shaped cone of dermal tissue beneath it. Figs. 3 and 4 show sagittal sections of the papillae with their respective dermal projections, while Fig. 5 shows a transverse section. All these sections show clear evidence of a high degree of organization within the papillae (see particularly Fig. 4).

The scanning electron microscopy (SEM) and light microscopy suggest that the papillae consist of 4 or possibly 5 tilted, tapering columns of differentiating and differentiated cells – the boundaries of which are precise and well defined:

The anterior column (*a*) is continuous with the basal cells lining the anterior portion of the dermal papilla (*dp*). The dense keratohyalin granules enhance the appearance of a stack of cells tilted at an angle greater than 45°. There are easily defined junctions between these anterior cells and their interpapillary and posterior neighbours.

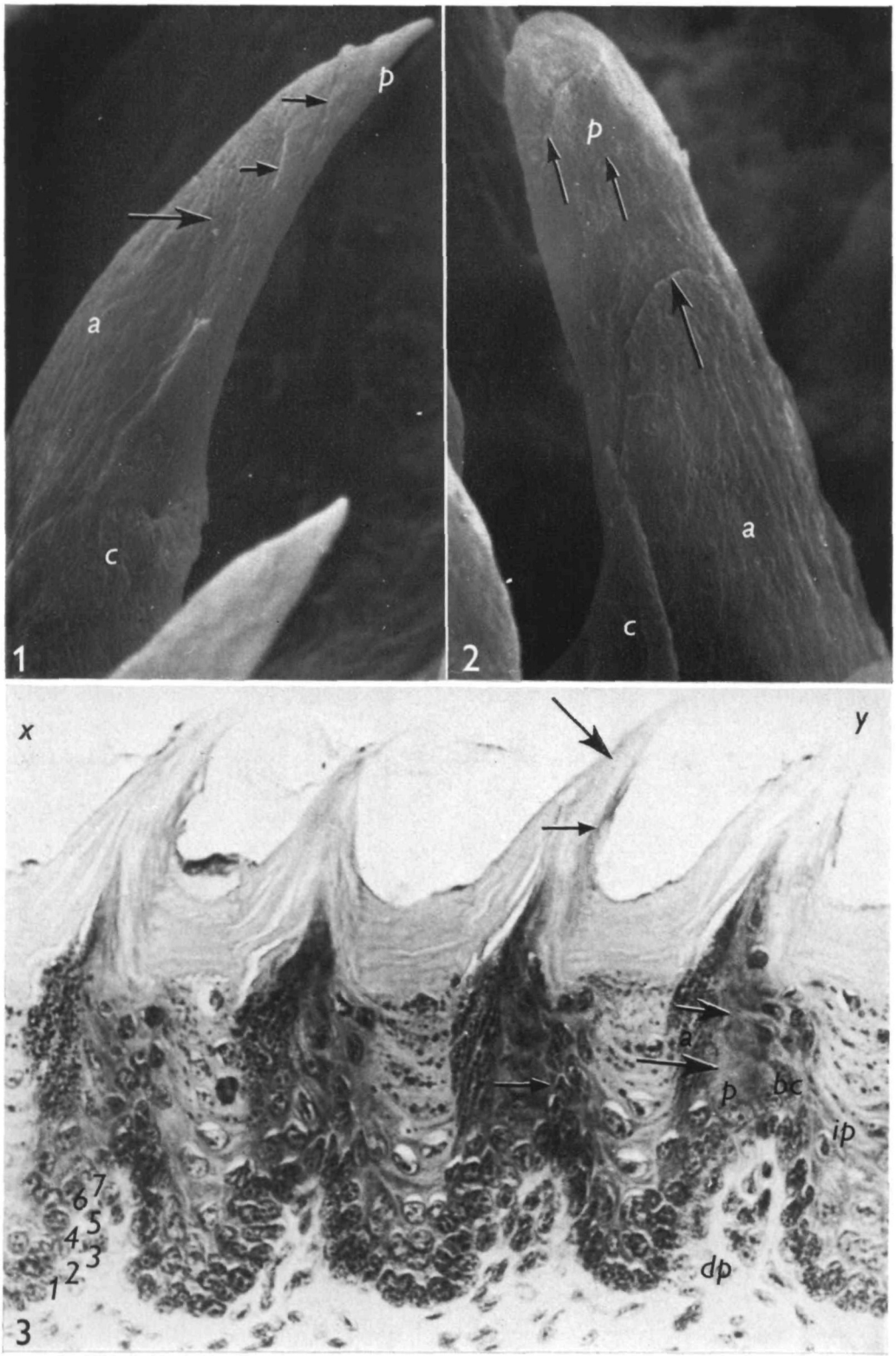
The posterior column (*p*) seems to arise from the apex of the *dp*. The column edges can be followed upwards through the stratum corneum, and can be observed on the surface (Figs. 1–3). The top of this column forms the tip of the papilla. The cell at the base of this column (overlying the dermal papilla apex) is less basophilic, with granular cytoplasm, and is a rounded cell with a larger nucleus than other cells situated in contact with the basement membrane (*bm*). As this column base cell occupies successively higher positions it inclines more steeply until it lies at the surface of the posterior column. It is suggested that this cell arises from the basal cells lining the posterior aspect of the *dp*.

The posterior buttress column (*bc*) is less well organized and interrelates superiorly with the 2 lateral columns (Figs. 1, 2) to form a collar of cells supporting the sides and rear of the filiform papilla, and at its base with interpapillary columns. The posterior buttress column seems to originate from the suprabasal cells posterior to the dermal papilla.

Generally speaking the cells of the posterior and posterior buttress columns tend to be smaller than those of the anterior column. This is reflected in the relative size of the surface cells of each column, those of the anterior regions being as long as 60 μm.

The posterior buttress column bridges the gap between the *interpapillary surface cell* and the *superficial cell* in the posterior column, having contact also with the lateral columns thus providing support for the posterior column. Columns of differentiating cells can also be observed in the interpapillary regions (Figs. 3, 6). Each column of the papilla and interpapillary epithelium consists of 19–20 cells. There are approximately 7–8 cell layers in the stratum corneum of the interpapillary epithelium and 8–10 layers in the papilla stratum corneum.

Labelling data. The labelling index (LI) data are presented at Table 1 and Fig. 7 as the percentage of labelled cells at each cell position for sections cut longitudinally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(*xy* axis) (anterior, posterior and *bc* columns) and transversely (*wv* axis) (lateral collar columns). As the dermal papilla tapers towards the apex a variable number of basal cells will be seen to border it, depending on the plane of section. This is why fewer cells were scored above position 7 in longitudinal sections and position 5 in the transverse sections. The LI is highest at position 1 and decreases with increasing position on the dermal papilla. Both lateral columns (*c*) show an approximately equal

Table 1. *Labelling indices in mouse anterior filiform papillae*

Cell position	Anterior column	Posterior column	Left collar column	Right collar column
1	22·3 ± 3·7	25·2 ± 1·6	18·0 ± 3·6	22·6 ± 1·8
2	13·9 ± 3·8	20·2 ± 1·0	12·6 ± 2·3	11·5 ± 2·1
3	5·7 ± 1·7	11·5 ± 2·7	7·2 ± 1·2	9·8 ± 1·8
4	2·3 ± 0·8	7·1 ± 1·6	5·0 ± 1·3	4·4 ± 1·3
5	0·6 ± 0·6	6·7 ± 2·9	1·9 ± 0·8	1·0 ± 0·5
6	0·4 ± 0·4	2·7 ± 1·5	0·6 ± 0·6	0·6 ± 0·5
7	0·4 ± 0·4	2·7 ± 1·8	0	0
8	0·6 ± 0·6	3·7 ± 3·7	0	0
9	0·7 ± 0·7	1·1 ± 1·1	—	—
10	0	0	—	—
Column average	**5·9** (3426)	**9·3** (3399)	**7·3** (1331)	**7·8** (1403)
Interpapillary cells (*ip*)		17·3 (791) (Fig. 7A, *xy*)		20·8 (909) (Fig. 7B, *wv*)
Overall LI including *ip*		8·6 (7616) (Fig. 7A, *xy*)		10·9 (3643) (Fig. 7B, *wv*)

Labelling index calculated as $\frac{\text{No. of labelled cells at a given position}}{\text{Total no. of cells at that position}} \times 100$ and stated as % ± S.E.

The data for cells positions **1–5** in the lateral collar columns, and **1–7** in the anterior and posterior columns are based on a minimum of 50 counts per position per animal from at least 4 mice. Values for cell positions above these limits are based on fewer cell counts.

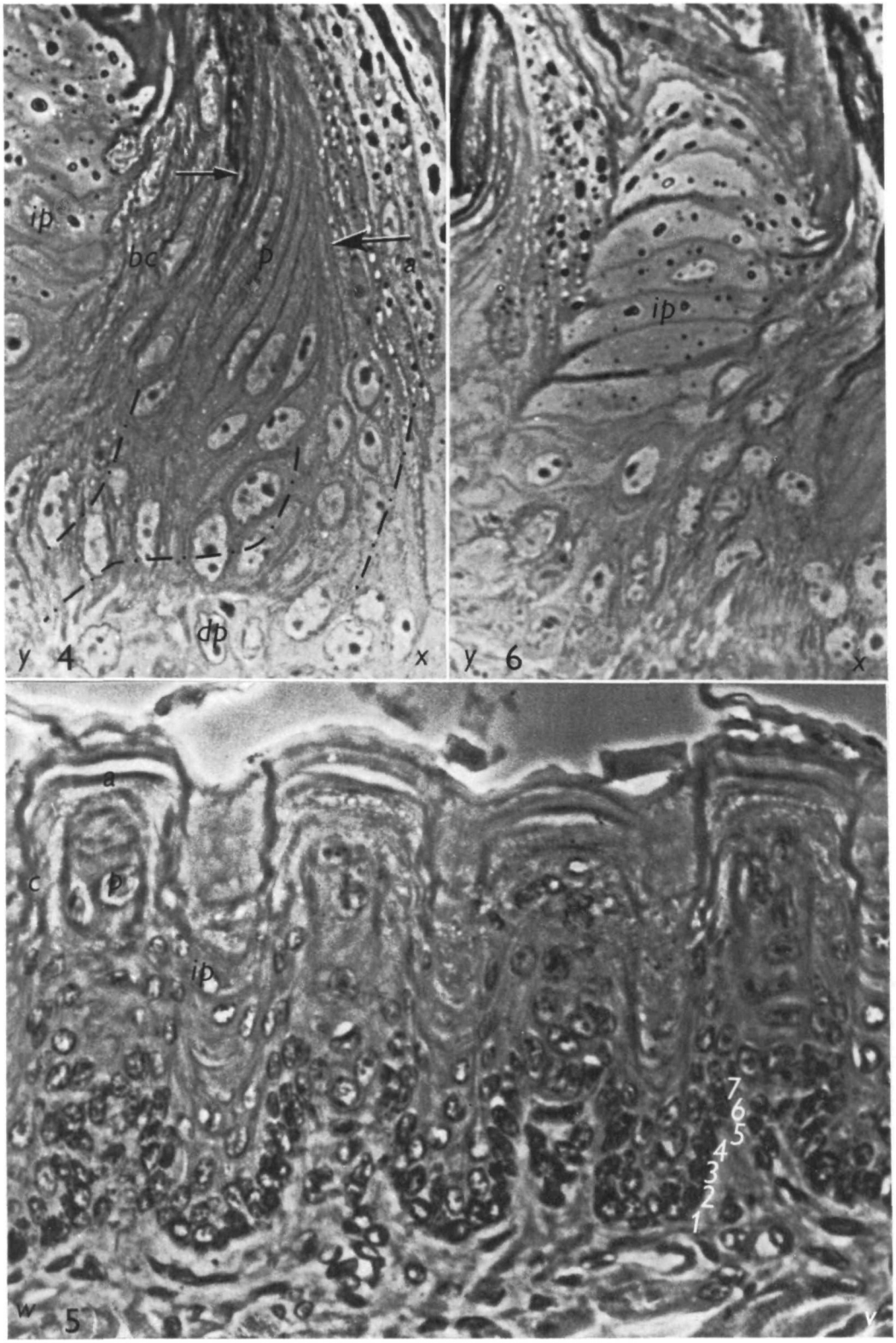
Figures in parentheses indicate the total number of cells counted for each column.

ABBREVIATIONS IN FIGURES

a	anterior	*ip*	interpapillary region
bc	buttress column	*p*	posterior
c	lateral column	*wv*	direction of transverse section
dp	dermal papilla	*xy*	direction of longitudinal section

Figs. 1, 2. Scanning electron micrographs of an anterior filiform papilla from the lateral and supero-lateral aspects. The anterior, posterior, and lateral columns are indicated. Large arrows delineate the junction between anterior and posterior columns; small arrows indicate the cell boundaries of the superficial cells of the posterior column. × 1450.

Fig. 3. Sagittal (*xy*) (paraffin) section of tongue dorsal epithelium. Large arrows delineate the junction between anterior and posterior columns and small arrows the junction between posterior and posterior buttress columns. The cell positions are numbered on one dermal papilla.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distribution of labelling (Fig. 7B). The anterior column (*a*) shows a more rapid decrease in LI for ascending cell position than the posterior column. This trend is reflected in the column average LI values in Table 1.

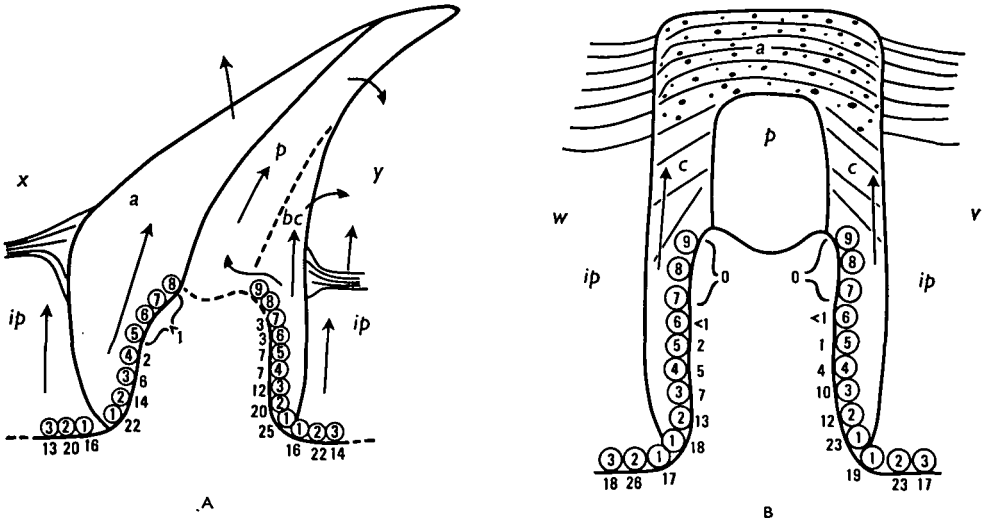

Fig. 7. Diagrammatic representation of longitudinal (A) and transverse (B) sections showing the labelling data (%) for each cell position. Arrows indicate probable flow patterns for the cells.

DISCUSSION

Cellular organization

The SEM, the phase-contrast and normal light microscopy of 1-μm Epon-embedded and 5-μm paraffin-embedded material all indicate the existence of a considerable degree of organization in the filiform papillae. The SEM and some transmission electron microscopy (TEM) (data not shown) indicate a regular imbrication of the cells at the papilla tip. Phase-contrast microscopy shows clear evidence of a tilted columnar arrangement of the cells on the posterior aspect of the papilla and clear evidence of columns of cells in the interpapillary regions. The significant differences in keratinization patterns between the anterior and posterior aspects together with their rigid column boundaries and the organization present in the cornified layers indicate an autonomy and spatial independence of these 2 regions.

Fig. 4. Slightly oblique, longitudinal (*xy*) (1-μm) section showing the posterior columns, the dermal papilla and interpapillary areas. Phase contrast. Dotted lines represent cell flow lines. × 1000.

Fig. 5. Transverse (5-μm, paraffin) section showing interpapillary areas consisting of a single column of cells, with 4 papillae. One dermal papilla has the cells numbered. Phase contrast. × 500.

Fig. 6. Section (1-μm) showing an interpapillary column of cells. Phase contrast. × 900.

It is clear that once a cell begins keratinization it is committed in terms of its position and final appearances. The columns of differentiating cells can be traced to within one or two cell layers of the basement membrane (*bm*) and unless there is considerable intercolumn cellular exchange within the first one or two suprabasal layers the columns must be separate almost to the level of the *bm*. The shape and complexity of structure is diagrammatically presented in Fig. 8. Fig. 9 suggests a possible sequence

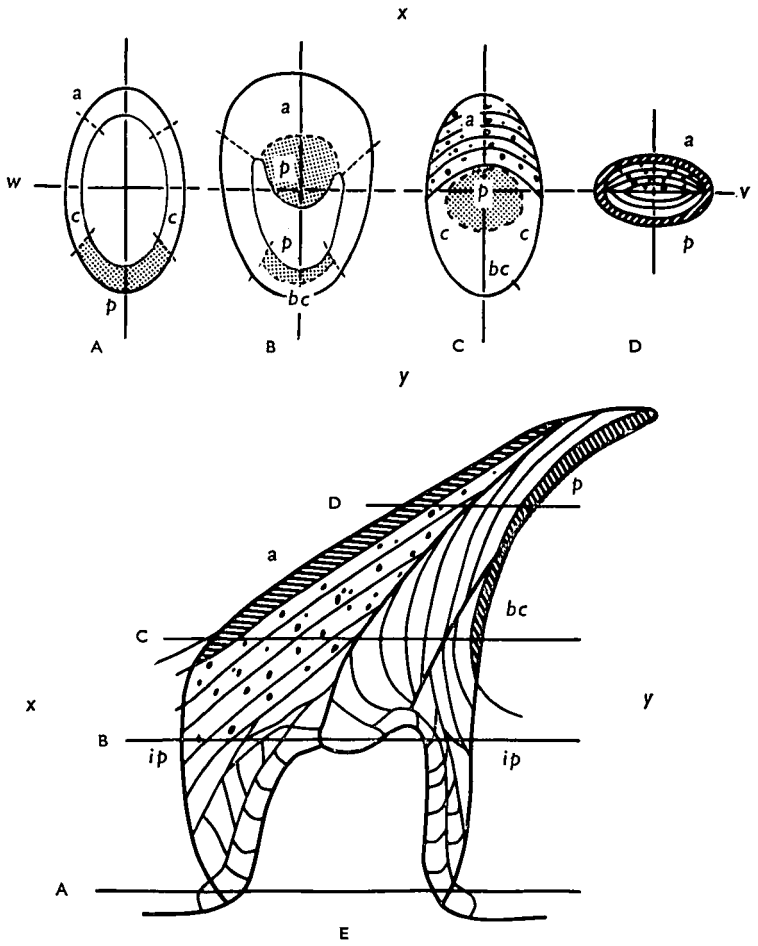

Fig. 8. Schematic representation of a longitudinal section (E) of a filiform papilla indicating its ordered structure with its cross-sectional configuration at various levels (A–D). The shaded portion in (A–C) represents the area occupied by the cells which form the posterior column. The shaded areas in (E) indicate the next cell to desquamate in each column. The anterior column contains keratohyalin.

of events by which simple papillae might be formed from the basic columnar organization seen in some flat epithelia. This sequence of events may be seen to some extent on the infero-lateral surface of the tongue where the transition from the ventral mucosa to the dorsal mucosa occurs.

The posterior aspect of the papilla would appear to be composed of 2 regions

(*p* and *bc*). It is not clear at present whether this represents a bifurcating column or 2 merging columns but the higher LI for the basal cells lining the posterior aspect of the dermal papillae may suggest the former (Table 1).

The number of interpapillary nuclei seen in sections cut transversely (*wv* axis) is $4{\cdot}4 \pm 0{\cdot}2$ (LI = 20·8%) while at right angles to this it is $5{\cdot}9 \pm 0{\cdot}8$ (LI = 17·0%). This is what might be expected on the basis of the model shown in Fig. 9. A further point regarding section orientation is the fact that the probability of observing good papilla profiles is less in the longitudinal axis (*xy*) than the transverse. As can be seen from the model (Fig. 9) this is partially due to the slightly greater IP distance and also due to the oblong shape of the papilla.

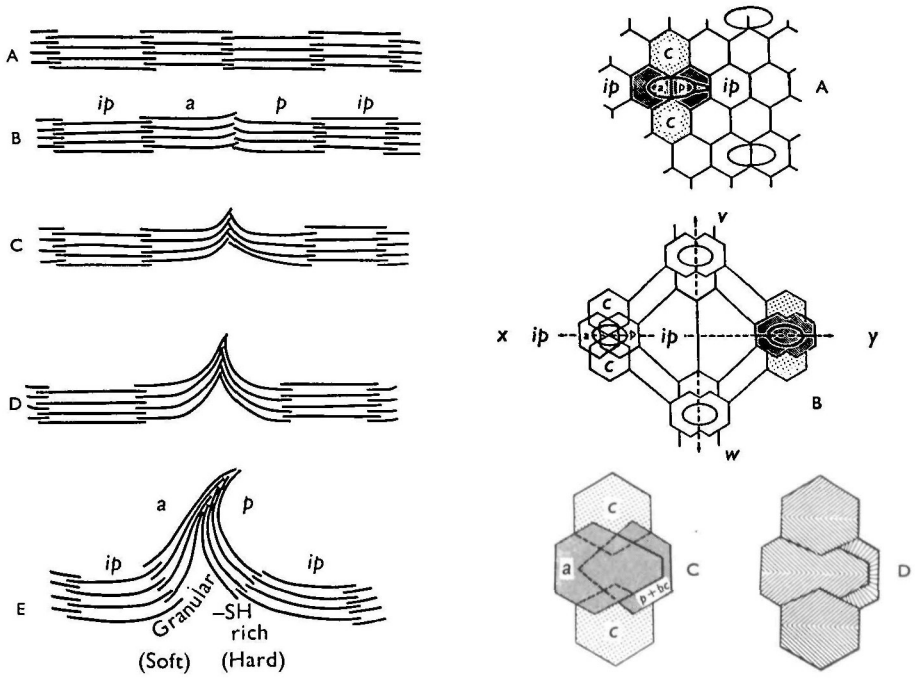

Fig. 9. Left, hypothetical representation of filiform papilla formation by progressive distortion of columnar stacks of epidermal cells.

Fig. 9. Right, surface view of filiform papilla formation described in the left-hand figure. The presence of the oval dermal papilla below the originally flattened hexagonal columns (A) leads to progressive distortion and overlapping of columns *a*, *p*, *c* and a 'stretching' of *ip* columns (B). As the distortion is of unequal proportions, columns *a* and *p* emerge dominant to columns *c* (C) which overlap *a* and *p* (D). Column *bc* is assumed to originate from the same basal cell complement as column *p* (C). (See also hexagonal grid systems in Potten & Allen, 1975.)

Labelling data

The average interpapillary LI is 17–21% while the overall average value for all cells adjacent to the *bm* is 9–11% which is similar to other overall LI values for rats or mice (Toto & Dhawan, 1966; Barakat, Toto & Choukas, 1969; Cameron, 1966; Cutright & Bauer, 1967; Blenkinsopp, 1968; Toto & Ojha, 1962; Messier & Leblond,

1960). Somewhat similar values to these *ip* results can be obtained for the ventral surface (Laurence, 1973; Sharav & Massler, 1967). Many of these values do not distinguish between proximal and distal papillae or papillary and interpapillary regions. The data presented here clearly show that the LI varies considerably depending on position on the dermal papilla (from virtually zero to 25%) with the highest values tending to be found at the very base of the dermal papilla.

These LI values represent only one point in the diurnal cycle (10.00 hours). Significant diurnal variations are suggested by the data of Blenkinsopp (1968) and Messier & Leblond (1960).

Cell movement

The presence of the highest LI at the base and the overall high degree of organization suggests flow patterns such as those indicated in Fig. 7. The data could be explained by assuming that cells at position **2** are derived from those at position **1**, position **3** from position **2** and so on. It is however clear that some cells move suprabasally where in our experience, when section orientation is taken into account, label is rarely found. Many apparently suprabasal labelled cells can be observed on sections of the anterior dorsum which are due to the plane of section in this region. These cells we believe are basal cells of the lateral columns. An alternative mode of proliferative organization might be for all initial migration to occur in a plane at right angles to the *bm* instead of predominantly parallel to it. Our preliminary observations favour the latter model, i.e. a sequential ageing of cells as they move up the dermal papilla with the predominant, but not exclusive, movement of cells being parallel to the *bm*. This may also be suggested embryologically (Baratz & Farbman, 1975). If this is true then tongue papillae, and possibly other epithelia where the basal layer has infolds, represent a situation intermediate between the organization of a simple flat epithelium (Potten & Allen, 1975; Potten, 1976*a*) and that of a more complex arrangement such as seen in small intestinal crypts (Potten, 1975*a*, 1976*b*). In the latter case all cell movement is along, or with, the *bm* whereas in dorsal epidermis much of the cell movement is away from the *bm*. Once a cell loses close contact with the *bm* some commitment to maturation may result.

Cellular turnover and cell production

The presence of a series of columns within the papilla requires that the cell production rates for each column within (*a* and *p*), and also those immediately around (*c* and *ip*) each papilla be roughly constant, otherwise cornified cells have constantly to make and break cellular contacts with their neighbours. Since they are dead, metabolically inert cells this seems unlikely.

Attempts have been made to correlate epithelial thickness, cell production rate and a high degree of organization (Potten, 1975*b*). The LI values seen in the tongue epithelium seem to suggest that for tongue this correlation does not hold. The epithelium is thick, has a high production rate, based on the limited LI data, and yet has a high degree of organization.

The interpapillary regions appear to have cell production rates of about 2·6 per

100 cells per h which can be compared with similar data for other regions presented elsewhere (Potten, 1975*b*).

Assuming the average length of S (T_s) over a 24-h period was within the values 6·5–8·0 h and that the single LI values obtained are representative for the system, the data are consistent with a turnover time of 65–80 h. The average values for the epithelium obtained in this study are similar to the average obtained by Blenkinsopp (1968) and Messier & Leblond (1960), taking into account some of the diurnal variations. The present data suggest turnover times of 34–42 h for the interpapillary regions where the average LI is 17–20 %, slightly lower than some previously published values for rats (Hamilton & Blackwood, 1974). Our model suggests that the cellular turnover times for some cell positions might be even more rapid (28–36 h for cell position 1).

CONCLUSIONS

The anterior filiform papilla of the tongue represents a simple modification and distortion of a small number of epithelial cell columns. The complex shape of both the papilla and its dermal component has to be accounted for in the evaluation of sections whether from the point of view of the internal organization or proliferative indices. Once the sample and its section have been oriented, labelling patterns can be obtained for individual cell positions which range from 0 to 25 % for the LI. A model has been proposed suggesting a considerable degree of autonomy for each of the columns of cells and the associated basal cells. This model further suggests that the basal cells are arranged in order of increasing maturity (decreasing proliferative potential and activity) with increasing cell position (upwards). This model implies that there is a presumptive stem cell zone at the base of each dermal papilla for each of the 4 major columns of cells.

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