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Keratinization of the oral epithelium

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Summary

The morphology of the keratinizing epithelia in the mouth is reviewed in the light of recent knowledge. There appears to be a spectrum of degrees of keratinization rather than distinct types, and the degree of keratinization is reflected in the degree of packing and orientation of tonofilaments. The role of keratohyaline and other granules in the process is discussed and it is suggested that modification of the cell membrane is an important part of keratinization. Although the potential of the various areas in the mucosa is genetically determined and appears early in fetal life, the connective tissue exerts an influence on the extent of keratinization of the surface in a manner which is not understood.

Introduction

Throughout the animal kingdom epithelial tissues play very varied roles in the protection of the underlying tissues. Hair, feather, horn, claw, hoof, and wool have as a common ingredient in their make-up the keratins, a macromolecular, resistant, insoluble protein class. The process by which the protein is made tough and insoluble is known as keratinization, a process that results in a wide range of products and whose nature is far from being fully understood. In the oral cavity the lining mucous membrane becomes keratinized to varying degrees in different animals and also in different areas of the mouth.

In man, in contrast to the common laboratory animals, the epithelium of the mouth has a surface which varies in its degree of keratinization from none at all, as in the cheek, through partial or parakeratinization, as found sometimes on the gingiva and parts of the palate, to full or orthokeratinization, as seen in most parts of the hard palate and overlying the gingiva where it is attached to bone.

In this review I shall consider the morphology of the oral keratinizing epithelium and compare it with the non-keratinizing mucosa and the epidermis. I also wish to examine some of the mechanisms which control and regulate keratinization and discuss briefly the clinical implications of these.

Orthokeratinization, in which the surface undergoes cornification as cells lose their staining characteristics and their nuclei, is found on the hard palate and on gingiva, especially where this is firmly bound down to underlying bone (Fig. 1). The epithelium lies on a basement membrane which separates it from the connective tissue. Above this there is a series of more or less well-defined layers. First comes the germinal layer, one or two cells thick, then the stratum spinosum, with its characteristic prickles, after which the stratum granulosum may appear, and finally the stratum corneum, which varies from a very thin layer perhaps only two cells thick to one

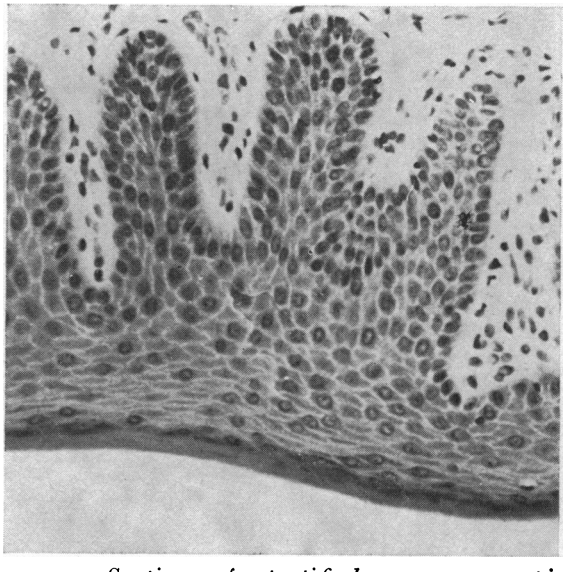

FIG. 1 *Section of stratified squamous epithelium fom the palate showing orthokeratinization. Haematoxylin and eosin.*

Arnott Demonstration presented on 2nd December 1975

of 10 or more cells in thickness. I will examine each of these in turn in the light of recent knowledge.

Basement membrane

This structure can be visualized with the light microscope, particularly after staining with the periodic-acid–Schiff (PAS) technique. There has been confusion over the relationship of the light microscope appearance to the structures seen in this area with the electron microscope (Fig. 2). The dense layer seen in electron microscope preparations is too narrow (of the order of 50 nm (500Å)) to be the structure seen with the light microscope (which has a dimension of approximately 1 μm). The electron-dense layer or lamina densa is separated from the basal cells by a clear zone of approximately the same width, and scattered along the length of the basal cell are thickenings of the cell membrane which resemble one-half of a desmosome and hence are called hemidesmosomes. Filaments have been described crossing this clear zone, but resolution of these filaments is difficult because of their size and lack of staining. On the connective tissue side of the basement membrane the collagen fibres form a lacework which is apparenty connected to the amorphous lamina densa by a series of anchoring fibrils[1] through which collagenous fibres run. The exact location of the area staining with PAS in the light microscope has not been identified at the ultrastructural level, though it would seem likely that the whole complex—namely, the clear and dense zones together with the anchoring filaments—would reach the width required for visualization with the light microscope, especially after complexing with the PAS reagents.

The basement membrane region is obviously important, as all material passing into or out of the epithelium must cross it. Very little, however, is known about its role in limiting the passage of materials. The thickness of the basal lamina—that is, the lamina densa—has been reported to be inversely related to the degree of keratinization, suggesting an inherent property of the epithelium to keratinize or not, but I will return to this later. In disease states the basal lamina has been reported to become duplicated and broken up and to show variations in density and continuity[2], but a study of such changes is difficult in that the plane of section in normal epithelium often produces apparent changes in its character.

Basal layer

This layer, known as the stratum germinativum or stratum basale, has cells which undergo division to make up for the cells shed off from the surface. The attachment of the cells to the basement membrane by way of hemidesmosomes has been mentioned. They are also in contact with their neighbours by desmosomes and between these there is a variable amount of amorphous material in the intercellular space. Basal cells of human buccal epithelium show few differences compared with those of keratinizing epithelium. Their contents of tonofilaments, their size, and their cellular organelles are similar in the epithelia from the two areas. Where there does appear to be a difference is in the morphology of the connective-tissue–epithelial junction. In some cases the interface consists of a series of ridges and grooves rather than pegs as in the classical description. The ridges may be narrow, and this is more commonly associated with keratinization, or broad,

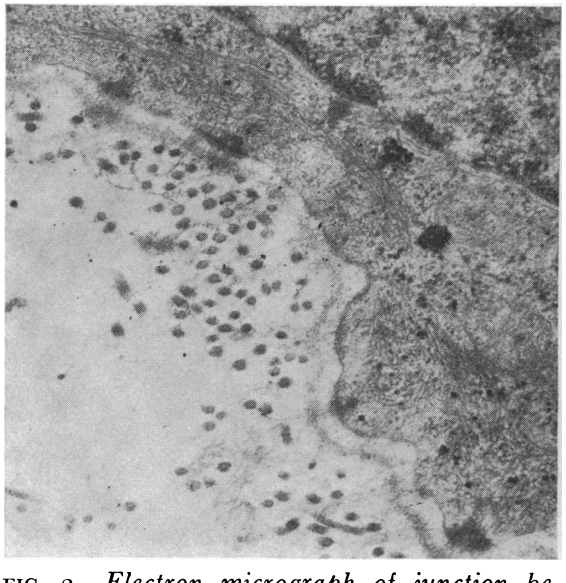

FIG. 2 *Electron micrograph of junction between epithelium and connective tissue. The lamina densa is separated from the epithelial cells by a clear zone. Hemidesmosomes are found at intervals on the basal cell membrane.*

as in non-keratinizing epithelium[3]. The effect of the ridges and pegs, when they occur, is to increase the surface area either for attachment or for exchange and on these grounds the interface is stronger in masticatory keratinizing mucosa than in the lining or non-keratinizing type.

The complexity of the interface has led to confusion in relating mitotic activity in the basal layer to the turnover of the epithelium. Studies of cellular proliferation have been made easier by the use of tritiated thymidine, a radioactive precursor incorporated exclusively into the DNA of dividing cells. The daughter cells retain radioactive DNA and thus it is possible to follow daughter cells resulting from a cell division which occurred shortly after administration of this material. With this technique attempts have been made to relate cellular proliferation rates to keratinization potential in the mouths of animals and man. Generally speaking, the non-keratinizing tissues have a higher turnover rate than the keratinizing epithelia, and the time taken for a dividing cell to differentiate and pass through to the surface in the mouse palate is 6–7 days[4]. On the cheek the rate is $3\frac{1}{2}$–$4\frac{1}{2}$ days and for comparison it has been calculated that turnover in skin is of the order of 30 days, though in diseased states such as psoriasis this may be speeded up dramatically. Man has not been studied in any detail as yet. An interesting aspect of this problem is what determines which cell is to be released from the pool of dividing cells. This is apparently not simply a case of one daughter cell being pushed upward, but some evidence suggests that both daughter cells may at times be retained and a resting cell pushed into the maturation pool[5].

There is some doubt whether or not cell division occurs above the basal layer in the normal adult epithelium (Fig. 3). Mitotic figures and tritium-labelled cells are certainly found there, but this could be due to the section plane passing tangentially through a peg of connective tissue. Suprabasal mitoses make up a very small percentage of the whole dividing population in the mouth. In skin suprabasal mitoses have been estimated by Halprin[6] as making up one-third of the total number.

The control of mitotic behaviour in the basal layers might be thought to determine the degree of keratinization, since it has been seen that non-keratinizing areas have a higher turnover rate. A feedback mechanism has been described in skin, in which it was shown that stripping off the superficial layers with adhesive tape could dramatically increase basal proliferation rates some 40 times[7]. Bullough and Laurence[8] discussed the mode of action of chalones produced by the epithelium and demonstrated that in combination with adrenaline they could inhibit mitosis. If increased loss of epithelial cells occurs owing, for example, to abrasion, then an increase in mitotic activity when released from inhibition makes up for this loss. This activity will eventually increase the level of chalone production and thus the mitotic activity will again be inhibited as normal thickness is reached. The precise nature of the chemical process involved is not known, nor has the effect of the chalones on keratinization been investigated.

Stratum spinosum

The next layer is the stratum spinosum or prickle-cell layer. This layer, in contrast to the basal layer, is many cells thick and makes up the bulk of the epithelium. The cells are characterized by the development of intercell-

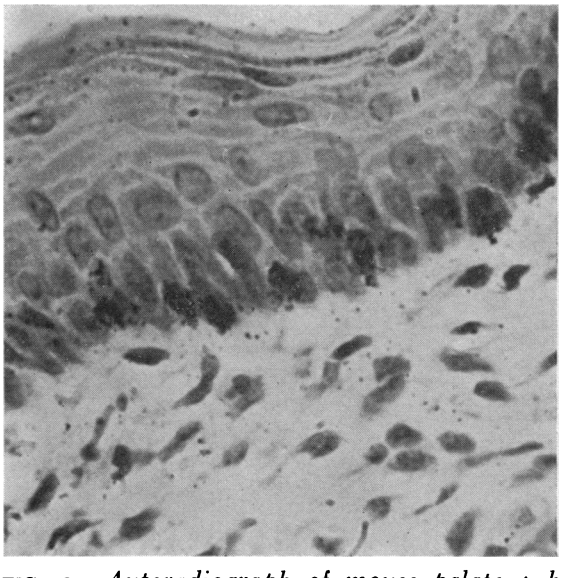

FIG. 3 *Autoradiograph of mouse palate 4 h after injection of tritiated thymidine. Labelled cells are found in the basal layer and sometimes appear in the layer above this. Haematoxylin.*

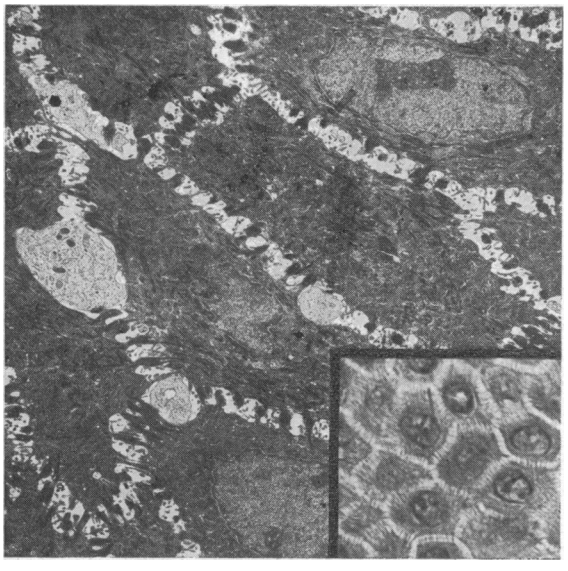

FIG. 4 *Electron micrograph of stratum spinosum. Cells meet each other across the intercellular spaces at desmosomes. Insert—light-microscope section of this region for comparison. Haematoxylin and eosin.*

ular space and by spinous processes or prickles all around their periphery (Fig. 4). Electron microscopy has revealed that the cell contents are not confluent where the spinous processes touch, but here the cells are attached to each other by desmosomes.

At these 'spot welds' there is very close contact of the cell membranes, and the inner leaflet of the cell membrane is thickened. Tonofilaments abound in the cells and many run into the dense plaque of the desmosome. It may be that they link up with other plaques, but it is not clear if any filaments actually cross the gap between cells at the desmosome. Although the numbers of desmosomes are much greater in the stratum spinosum than in the stratum basale, it is rare to find desmosomes in the process of formation. If desmosomes are attachment plates, then they must break down and re-form as individual cells move past one another, as the autoradiographic evidence would suggest takes place, but no reports of such breakdown and reformation in oral epithelium have been found. Tonofilaments tend to accumulate more in the keratinizing tissue, but in the stratum spinosum the differences are not striking and the situation varies from one animal species to another.

Stratum granulosum

This layer is so called because of the granules of keratohyalin it contains, and these can be seen with the light microscope in palatal or tongue epithelium. Chemical analysis of isolated keratohyaline[9] indicates that this is a sulphur-rich amorphous precursor of the horny cell content rather than a side product of the keratinization process. The ultrastructural characteristics of the keratohyaline granules have complicated rather than simplified the picture in relation to their function. At least two types of granule exist[10]. One type, seen in highly keratinized areas, is irregular in shape, highly electron dense, and associated with tonofilaments (Fig. 5). These are also seen in skin epithelium. This type is not found in non-keratinizing areas of the human mouth but may occur in parakeratinized areas of the palate and gingiva. A second type is more regular in outline and has been described by Jessen[11] in both keratinizing and non-keratinizing epithelia. It does not appear to be asso-

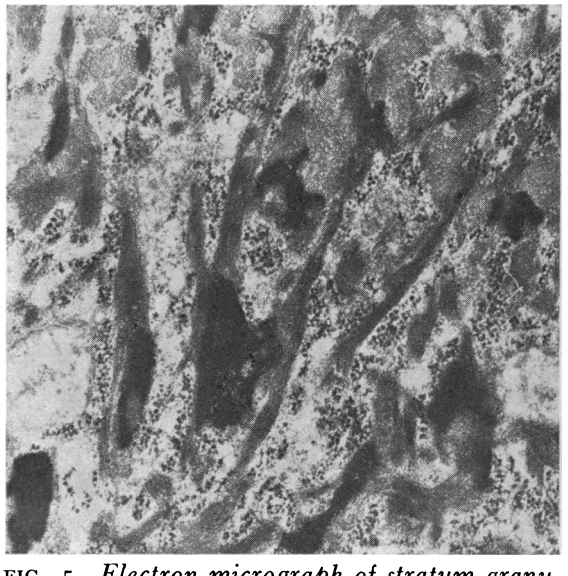

FIG. 5 *Electron micrograph of stratum granulosum in keratinizing epithelium with typical keratohyaline granule associated with tonofilaments. (Reproduced from Chen and Meyer[10] by courtesy of Charles C Thomas, Publishers, Springfield, Illinois.)*

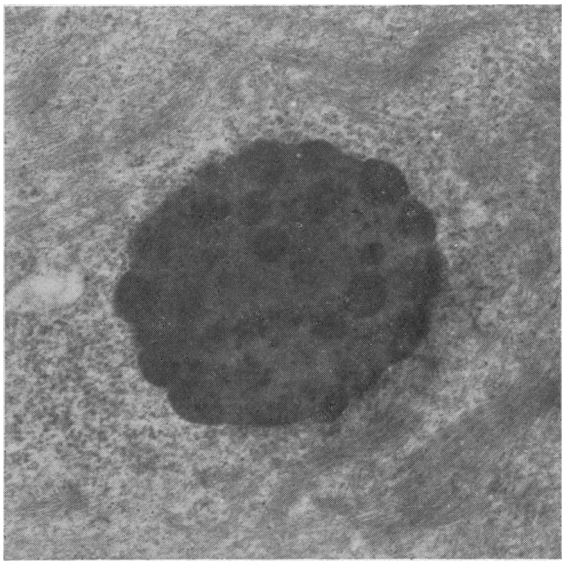

FIG. 6 *Electron micrograph of cell from stratum granulosum showing the second type of keratohyaline granule. Small granules are embedded in an electron-dense matrix.*

ciated with tonofilaments (Fig. 6). Jessen, by using oxidation and pepsin digestion, concluded that this second type was made up of two components, 'single' granules and a dense matrix in which they were embedded. The 'single' granules he believed to contribute to the thickening of the cell membrane which is seen in keratinizing cells, and the matrix was thought to become dispersed between the tonofilaments. However, there does seem to be considerable species variation in the morphology of the granules, and the function of similar granules in non-keratinizing epithelium is problematical.

Another type of granule is found in the cells of the upper layers of the stratum spinosum and in increasing numbers in the cells of the stratum granulosum. These granules, visible only with the electron microscope, measure about 0.1–0.2 μm across and have been the subject of many investigations. They were called 'membrane-coating granules'[12] as it was considered that they produce the thickening of the cell which is seen in keratinized epithelia They have also been named microgranules, keratinosomes, and lysosomes. They have a circular or elongated profile and contain either amorphous or stacked lamellated material (Fig. 7). Martinez and Peters[13] believed that the lamellated granules contained discs with a five-layered structure and that after discharge into the intercellular space they were applied to the outer cell surface. This modification strengthened the superficial surface of the cells and perhaps contributed to the barrier function of the epithelium. Electron histochemistry reveals acid phosphatase within the granule, and the granules are thought to shed their contents into the intercellular spaces[14] since acid phosphatase activity is found both in the space and in intercellular structures which resemble the stacked membranes. The acid phosphatase is considered by some workers to act on the desmosomes, thus facilitating the release of cells from the surface[15].

The differing appearance between the microgranules of keratinizing epithelia, where the lamellated type are found, and those of non-keratinizing epithelia, in which the internal structure of the microgranule is usually amorphous, suggests different functions in the two situations. Hashimoto[16] believed that the amorphous granules contributed to the intercellular cementing substance. They have been described in large numbers in developing oral mucosa, where they apparently contribute to

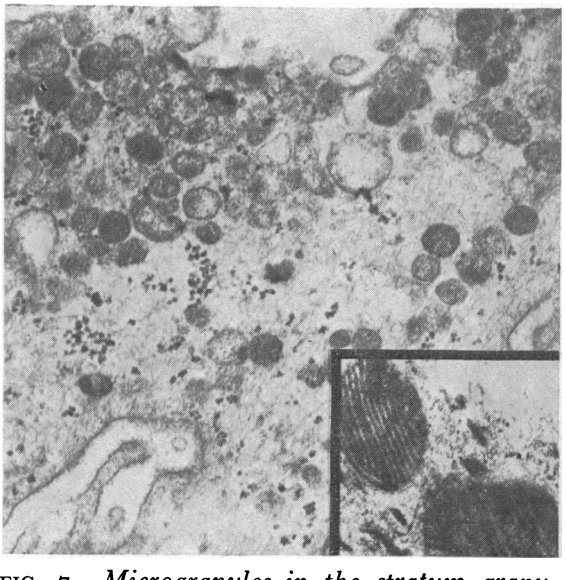

FIG. 7 *Microgranules in the stratum granulosum. They are spherical and sometimes have an amorphous content. Insert—lamellated microgranule.*

the extracellular 'fuzz'. Another interpretation is that the granules are not separate vesicles but inpouchings of the cell membrane[17]. The cellular interdigitations are so complex that in places they are sectioned transversely and may appear to be within the cell. Support for this suggestion comes from the disposition of the granules near the superficial periphery of the cell. It is further suggested that the granule contents are similar to the contents of the intercellular space because they are part of it. The acid phosphatase appears to be inside the microgranule only because it is present in the intercellular space.

A very recent scanning electron microscope study[18] of the surface characteristics of the epithelial cells indicates that there are pits in the surface of the cells as well as microvilli, and this suggests that at least some of the appearances of the microgranules may indeed be due to the plane of section.

Stratum corneum

In this layer there is a dramatic change in the structure of the cells. The nucleus disappears, the granules are lost as well as all the organelles, and the cell appears to be packed with tonofilaments. Changes are seen also in the intercellular space. Desmosomes lose their intermediate layer and many are lost altogether since the numbers are reduced. Often there is simply a dense zone between the cells. As indicated earlier, the cell wall thickens by an increase in thickness of the inner cell lamina and the profile of the cell becomes much less complicated as many of the microvilli are lost. The internal architecture of the cells is highly electron dense (Fig. 8), which has led to difficulties in investigating the keratinizing process. Packing of tonofilaments appears to vary randomly among the cells in a similar way to the variations reported in skin keratin[19].

Meyer[10] has investigated the tonofilaments in keratinizing and non-keratinizing epithelia and concludes that the packing and orientation of these fibrils within the cytoplasm are the most distinctive differences between the two types. She points out, however, that there would appear to be a spectrum ranging from non-keratinizing epithelia through parakeratinization to orthokeratinization.

As the cells become condensed and flattened a distinct thickened cell membrane appears. Thus keratinization is not a change that is confined to the cell contents but includes modification of the cell membrane and the intercellular material. This modification means that although the cell membrane is physically weaker than keratin, chemical processes are resisted more than with keratin and thus the cornified layer could be regarded as an efficient, well-integrated system of protection to the underlying tissues.

The mystery of the disappearing nucleus and other organelles at the beginning of this layer has not been solved. No trace is found in light or electron microscope studies. Perhaps the fixatives we use dissolve out the partially disintegrated and soluble products of their breakdown. There is a variation in the degree of disintegration depending on the degree of keratinization, but Omanski[20] sums up the situation by saying: 'Little is known about the breakdown other than the fact that it occurs'.

Another interesting feature of the stratum corneum is the passage of cells through it. No longer do we see the random migration of individual cells, but all cells apparently migrate as a sheet. The evidence for this comes from autoradiographic studies with labelled protein. The radioactivity is found in a band

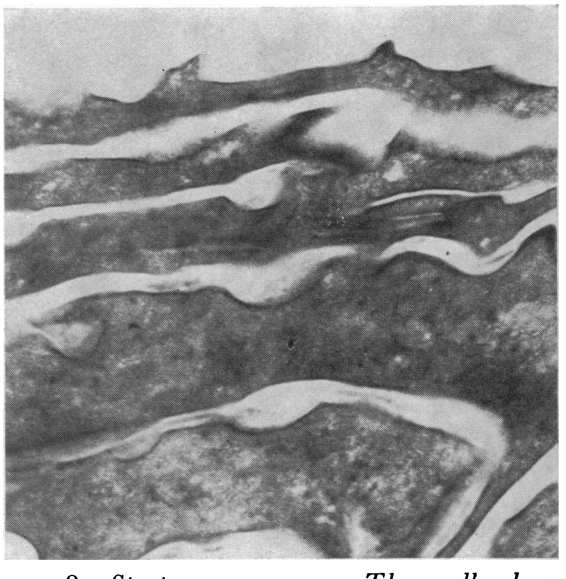

FIG. 8 *Stratum corneum. The cells have thickened cell membranes and contain dense bundles of tonofilaments.*

which moves up through the stratum corneum uniformly. At the surface, rather than individual cells being shed, sheets of the superficial layer are lost, this loss being a reflection of the degree of wear and tear on the surface. Changes in desmosome structure may be associated with the loss, but there are conflicting reports on these structures and the release mechanism has still to be explained.

During keratinization the water content of the cells decreases dramatically in spite of the fluid environment in the mouth. Thus it is not just desiccation of the surface layers, as may occur in skin. Work is done in expelling the water from between the bundles of tonofilaments, though our knowledge of this process is scanty. It has been estimated[21] that in passing from the basal layer to the surface of the granular layer a cell increases its dry weight 29-fold. The observations were based on the buccal cells in the rat and this is similar to the rate of protein synthesis in the pancreas.

Keratin in the mouth remains relatively translucent as compared with skin, probably because it is kept moist and because the cornified layers are normally compact. In skin the superficial layer of the stratum corneum, the stratum disjunctum, a layer of loosely knit bundles of cornified cells, gives the skin its opacity. In the mouth if the layers of keratin become broken up or if abnormal keratinization occurs there is a loss of translucency and the area appears white, perhaps because of the spaces in the cornified layer.

Regulation of keratinization

The morphology of keratinization in the mouth having been dealt with, it is pertinent to look at the factors that govern the process and that might lead us to methods of controlling or altering this mysterious property of the epithelium. The degree of keratinization—that is, the thickness of the keratin layer and whether para- or orthokeratinization occurs—is dependent on several factors.

The first of these is genetic. The epithelium of the cheek and the palate show differences as early as 15–16 weeks in utero. Periderm cells which are associated with keratinization of skin during development are found in the oral cavity only on those sites that are destined to become keratinized. Further keratinization occurs on the palate before birth, indicating an independence of functional stimuli.

Attempts to alter the degree of keratinization have met with ambiguous results. Toothbrushing and other methods of producing friction will not induce the normally non-keratinizing epithelium of either the alveolar mucosa or the lining mucosa of the gingival sulcus to keratinize. On the other hand gingival mucosa which shows parakeratinization will change towards orthokeratinization with frictional stimuli. It is interesting in this respect to note that inflammation may produce a change from ortho- to parakeratinization, and toothbrushing may simply remove the irritant that is inducing the inflammation. With transplantation experiments it is difficult to know how long the transplanted epithelium survives, but recent elegant experiments have shown the importance of the underlying connective tissue. Grafts from the palate stripped of all epithelium and implanted on the alveolar bone became covered by keratinized epithelium after healing, whereas similar connective tissue grafts from the buccal sulcus had non-keratinizing epithelium covering them on healing[22]. A similar result has been achieved with mouse skin. Spearman[23] transplanted morphologically distinguishable mouse ear epidermis, after combining it with tail dermis, into a prepared bed in the tail of a second mouse. After 30 days the transplanted epidermis was altered from the ear type to the tail type.

It has also been suggested that systemic factors affect the degree of keratinization. A recent sequential cytological study[24] over 2 months showed no differences between young men and women in the variation of the cornification of the palatal epithelium. Another study on the keratinization of the gingivae during pregnancy and afterwards has likewise shown that any changes that occur are more likely to be the result of local inflammatory conditions than of any systemic hormonal variations[25].

Dendritic cells

In addition to the keratinoblasts in the basal layer and the keratinocytes in the functional layers, dendritic cells of two types have been distinguished. These are called melanocytes and Langerhans' cells. They resemble each

other in structure but differ in cell content and in position. Melanocytes are found among the basal cells and contain granules of melanin. They are present in all races and are probably of neural crest origin. The Langerhans' cells are found in more superficial layers and contain characteristic 'tennis-racket' organelles. These cells were thought to be effete melanocytes, but recently it has been suggested that they may control keratinization, since they are more numerous in keratinizing epithelia[26]. An association with chalone production has also been postulated.

Conclusion

In summary, then, the oral epithelium is seen to show a wide variation in the degree to which the surface cells become keratinized. The basement membrane and the connective tissues below this seem to exert an influence on this process of keratinization. Basal cells proliferate more slowly in oral keratinizing regions but faster than in epidermal sites. During keratinization tonofilaments accumulate and are arranged in dense bundles. The bundles are embedded in an amorphous cement which may have its origin in the keratohyalin material. Modification of the cell membrane is an essential part of keratinization and this occurs by thickening of the inner leaflet and addition of material to the outer aspect of the cell. This latter material appears to be derived from the microgranules, which may also be concerned in loosening the cells before desquamation.

The whole process appears to be genetically determined and the underlying connective tissue may play a role in this process in the adult. Local factors can alter to a limited extent the degree of keratinization of the surface cells. It can be seen, therefore, that keratinization is an intriguing, complicated, and important process whose true nature still awaits to be explained. This explanation, I believe, will come only through the collation of information from biochemical, physiological, and morphological studies.

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