Basal cells of the corneal epithelium in man and monkey

Variation in morphology associated with fixation procedures

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The cellular nature of the corneal epithelium was first described by van Leeuwenhoek (1693–1718), and it is now generally believed by workers who have carried out morphological studies that cells of an uncertain nature occur in the basal region of the corneal epithelium.

Ranvier (1881) described “wandering cells” that had migrated from blood vessels, but it was Virchow (1910) who first observed that some cells appeared darker than others apparently because of compression of the cytoplasm. A different view, however, was held by Egorow (1934), who believed them to be homologous with similar cells in the mammalian epidermis, and hence called them Langerhans cells. Binder (1951, 1952) described clear “matrix cells”, which remained permanently in the basal layer and which divided to give rise to young cells which were compressed forwards into the epithelium. Cogan (1951), however, stated that the dark cells probably represented the more mature basal cells which were in the process of migrating forwards.

The use of different techniques appears to have produced conflicting results. By staining with silver carbonate, Scharenberg (1955) was able to demonstrate “polymorph elements” which penetrated the epithelium and were intimately connected with the nerves, while Pau and Conrads (1957), using a similar technique, came to the conclusion that the so-called Langerhans cells were associated with nerve fibres and hence were analogous to Schwann cells.

The first electron microscopical study was carried out by Jakus (1954) on the rat cornea. She described finger-shaped processes on the surfaces of the basal cells, and these were subsequently named “basal layer branched cells” by Whitear (1960), who studied the mouse cornea electron microscopically and suggested that these cells were associated with the transport of metabolites. This was followed by an account of the rabbit cornea by Teng (1961), who a year later (Teng, 1962) described the human cornea. Because of the increased endoplasmic reticulum and tonofibril elements and the presence of osmophilic granules, he suggested that the more electron dense cells should be called “secretory epithelial cells”, since he believed that their apocrine secretion contributed to the formation of the basement membrane. He observed that these cells, usually few in number, increased in pathological conditions for reasons which were not fully understood at that time.

At the beginning of their extensive study, Sugiura and Wakui (1961) described "polygonal cells" staining deeply by silver impregnation techniques, and Sugiura, Wakui, and Kondo (1962) postulated that they could be non-pigmented melanocytes of neural crest origin. This was followed by an electron microscopical study by Segawa (1964), who described active and inactive melanocytes in the eyes of three Japanese children suffering from retinoblastoma, and believed that these were the "dendritic" cells described in histological preparations.

In the first electron microscopical description of the "polygonal cells", Wakui (1967) described them as being extremely electron dense, but he later suggested that the pale stained cells might be the metamorphosed melanocytes (Wakui, 1968).

Somewhat similar descriptions have also been made of pathological material. Stocker and Holt (1955), in describing a case of hereditary epithelial dystrophy, stated that the PAS-positive material deposited on the basement membrane was probably the product of the diseased epithelial cells in which the same staining properties could be demonstrated. They also noted that most of the nuclei were reduced to darkly stained granules. Grayson and Wilbrandt (1966) reported three cases of dystrophy of the anterior limiting membrane in which a similar deposition of PAS-positive material was noted between Bowman's membrane and the epithelium.

In describing the morphological features of lattice dystrophy of the cornea, Hogan and Alvarado (1967) attributed the extreme electron density of the basal cells to their degeneration, which subsequently led to their rupture and contributed to the development of the hyaline sheets seen on the surface of Bowman's membrane.

The nature of the "dark cells" was discussed fully by Rice, Ashton, Jay, and Blach (1968) in their study of Reis-Bücklers' dystrophy; they thought that in their cases the probable cause was chronic epithelial oedema.

Although the detailed descriptions vary, the criteria which characterize these cells may be summarized as follows:

- (1) Argyrophilia.
- (2) Supravital stainability with quinone-imine dyes.
- (3) PAS-positivity.
- (4) Basophilia in haematoxylin and eosin sections.
- (5) Dendritic or polygonal appearance.
- (6) Electron density.

Present investigations

Since the importance of fixation artefacts has hitherto been ignored, a study of the effects of fixation procedures was undertaken in an attempt to elucidate the nature of these "dark cells". It will be evident that certain morphological features which have previously given rise to speculation and differences of opinion may now receive a rational explanation.

Material and methods

Fixatives

- (1) 2.5 per cent. glutaraldehyde in Tyrode buffer (pH 7.4)
- (2) Zetterqvist's osmium tetroxide (pH 7.4)
- (3) 10 per cent. formol saline.

Specimens

Tangential and cross sections were made from all the specimens.

HUMAN CORNEA, obtained from eyes enucleated for malignant melanoma of the posterior part of the choroid and known to be free from corneal pathology, was:

- (a) Fixed in formol saline, embedded in paraffin, and sectioned.
- (b) Fixed in glutaraldehyde, post-fixed in osmium, embedded in araldite, and sectioned.
- (c) Fixed in osmium, embedded in araldite, and sectioned.

MONKEY CORNEA was trephined and immediately:

- (a) Fixed in formol saline, embedded in paraffin, and sectioned.
- (b) Fixed in formol saline, post-fixed in osmium, embedded in araldite, and sectioned.
- (c) Fixed in glutaraldehyde, embedded in paraffin, and sectioned.
- (d) Fixed in glutaraldehyde, post-fixed in osmium, embedded in araldite, and sectioned.
- (e) Fixed in osmium, embedded in paraffin, and sectioned.
- (f) Fixed in osmium, embedded in araldite, and sectioned.

Paraffin sections

These were stained as follows: haematoxylin and eosin, periodic acid-Schiff (PAS) before and after treatment with diastase, toluidine blue, methylene blue, and silver impregnation using the Bielschowsky, and the Glee and Marsland techniques.

Araldite-embedded material

THICK SECTIONS were stained with toluidine blue and examined by light microscopy.

THIN SECTIONS, immediately following the thick sections, were stained for 20 minutes in 5 per cent. uranyl acetate in 1 per cent. acetic acid, followed by 0.4 per cent. lead citrate in N/10 sodium hydroxide for 5 minutes, and were examined with an AEI EM6 electron microscope.

Results

The distribution of the "dark cells" in tangential sections is summarized in the Table where the sections are classified according to the method of fixation.

Table Distribution of "dark cells" in tangential sections of monkey cornea

Sections embedded in	Fixation		
	Formalin	Glutaraldehyde	Osmium
Paraffin	++	++	++
Araldite	++	+	-

Paraffin sections of human cornea fixed in formalin

Haematoxylin and eosin

Dark basophilic cells were seen in the basal layer (Fig. 1, opposite) and were exactly similar to those obtained from corneal scrapings and stained with haematoxylin and eosin (Fig. 2, opposite). In tangential sections they appeared to form an interlacing network (Fig. 3, opposite).

FIG. 1 *Normal human corneal epithelium, showing "dark" basophilic cells and swollen pale-staining cells in the basal layer. Haematoxylin and eosin. $\times 440$*

FIG. 2 *Corneal scraping from the basal region, showing a columnar basophilic cell (arrow) similar to the "dark cells" illustrated in Fig. 1. Haematoxylin and eosin. $\times 490$*

FIG. 3 *Normal human cornea. Tangential section shows interlacing network formed by the "dark" basal cells. Haematoxylin and eosin. $\times 80$*

PAS

Dark cells appeared to be more PAS-positive (Fig. 4, overleaf), and similar granular PAS-positive material which was removed by diastase was seen in the intercellular spaces (Fig. 5, overleaf).

Methylene blue

The dark cells were clearly outlined because of the intense staining of the cytoplasm and the nucleus.

Silver impregnation

In tangential preparations the cell outlines were well defined (Figs 6 and 7, overleaf). Sometimes silver was deposited on the cell surfaces (Fig. 8, overleaf).

Thick araldite sections of monkey cornea

In the formalin-fixed material, there were numerous dark cells associated with pale swollen cells (Fig. 9, overleaf), while in the osmium-fixed material, there was uniform staining of all the cells (Fig. 10, overleaf). A few scattered dark cells were seen in the glutaraldehyde-fixed tissue.

FIG. 4 Normal monkey cornea, showing large pale cells compressing the "dark" PAS-positive cells. Periodic acid-Schiff. $\times 420$

FIG. 5 Normal human cornea, showing granular PAS-positive material within cells and in the intercellular spaces. Periodic acid-Schiff. $\times 420$

FIG. 6 Normal human cornea. Interlacing network of argyrophilic cells identical with "dark cells" seen in Figs 3 and 4. Silver impregnation. $\times 90$

FIG. 7 Normal human cornea. Aggregates of pale swollen cells are separated by rows of "dark" compressed cells. Silver impregnation. $\times 285$

FIG. 8 Normal human cornea. Silver deposits can be seen on the surface of some cells, simulating argyrophilic dendritic cells. Silver impregnation. $\times 420$

FIG. 9 Normal monkey cornea, showing numerous "dark" or "polygonal" cells and pale swollen neighbouring cells. (Formol saline/ OsO_4 /araldite/toluidine blue.) $\times 175$

FIG. 10 Normal monkey cornea, showing complete absence of the "dark cells" and minimal variation in cell size. (OsO_4 /araldite/toluidine blue.) $\times 190$

Ultrastructural features of the basal cells

(A) MONKEY CORNEA FIXED IN OSMIUM (Figs 11 and 12, overleaf)

The cells were uniformly stained and were of equal size; cohesion between the cells was maintained. The surfaces had convolutions, adjacent interdigitating cell membranes were attached by desmosomes, and hemidesmosomes were seen on the basal surface. The intercellular spaces were regular. The mitochondria, Golgi apparatus, and endoplasmic reticulum were clearly visible. The smoothly rounded nuclei were bounded by a double-layered nuclear membrane, the chromatin was well preserved, and nucleoli were frequently seen.

(B) MONKEY CORNEA FIXED IN FORMALIN AND GLUTARALDEHYDE

(Figs 13 and 14, overleaf)

The cells showed varying degrees of electron density and in the "dark cells" the cytoplasm and the nucleus showed uniform density, although the cytoplasm contained a few vacuoles

FIG. 11 *Normal monkey cornea. Electron micrograph, showing uniform staining of basal cells, regular intercellular spaces (I), and differentiation of cytoplasmic organelles. (OsO₄/araldite/uranyl acetate/lead citrate.) × 7,800*

and osmophilic particles and cell organelles could not be distinguished. The nuclear membrane had a corrugated appearance and the chromatin was not unusually disturbed although it was extremely electron dense. Nucleoli were seen in some cells. The adjoining “pale cells” had irregular cell outlines caused by their bulging into the cytoplasm of the dark cells. The cytoplasm was granular and cell organelle details were somewhat obscured by the appearance of artificial vacuoles which were more numerous in the periphery of the cells.

(C) HUMAN CORNEA

Appearances were similar to those of the monkey cornea. No differences were observed between the central and the peripheral basal cells.

Discussion

This study clearly establishes an association between the appearance of the corneal epithelial cells and the fixatives used and there can be little doubt that the varied inter-

FIG. 12 Higher magnification of area shown in Fig. 11. Interdigitating cell membranes are attached by desmosomes (D), and intercellular spaces are regular. The cytoplasm contains numerous tonofibrils (T), and cell organelles are not distorted. (OsO₄/araldite/uranyl acetate/lead citrate.) $\times 22,000$

pretations of the histological appearances, themselves very varied, have been due to the different techniques used by the authors. The illustration of "polymorph elements" described by Scharenberg (1955) closely resembles the formalin-fixed human material

FIG. 13 Normal monkey cornea. Electron micrograph, showing scattered "dark cells" and adjoining "pale cells" with peripheral vacuoles. (Glutaraldehyde/OsO₄/araldite/uranyl acetate/lead citrate.) $\times 3,000$

illustrated in Figs 6 and 7, and the polygonal cells described by Sugiura, Wakui, and Kondo (1962) are constantly seen in formalin-fixed material and are similar to those seen in Fig. 9.

In general, during formalin fixation, cross links are formed between protein-end groups in such a way that escape of glycogen by solution in water is hindered (Baker, 1958) and the phospholipids in the cell membranes have a strong tendency to take up water. Hence there is an initial slight swelling of tissue during fixation and the subsequent process of dehydration and embedding leads to some shrinkage and distortion of the cells.

It is noteworthy that "dark cells" invariably have neighbouring swollen "pale cells", and this appearance suggests that at least part of their swelling is due to imbibition of water from the neighbouring "dark cells". Compression by swollen cells from several directions gives them a dendritic shape and their state of dehydration with concentration of the cellular elements makes them appear more PAS-positive. In fact they stain more intensely in all the preparations. Different appearances in any one section range from

FIG. 14 Higher magnification of the "dark cell" illustrated in Fig. 13. Note uniform density of cytoplasm and nucleus and corrugated nuclear membrane (arrow). (Glutaraldehyde/OsO₄/araldite/uranyl acetate/lead citrate.) $\times 24,300$

the large swollen cells through intermediate cells of varying size and density to the extremely electron dense small polygonal or dendritic cells. Since water forms 85 per cent. of the cell mass, it seems reasonable to assume that the cell size would be directly proportional to its water content.

In considering electron microscopical descriptions of "dark cells", fixation procedures appear to have been important. Wakui (1967) described them in formalin-fixed tissue, which was subsequently post-fixed in osmium. Hogan and Alvarado (1967) used glutaraldehyde fixation for their studies of lattice dystrophy, and it is a noteworthy feature in Reis-Bucklers' dystrophy reported by Rice and others (1968) that the epithelial debris, which was associated with the "dark cells", was seen only in the glutaraldehyde-fixed specimen and not in the osmium-fixed tissue.

In the present study of human and monkey cornea, the variation in electron density and cell size is minimal when the tissue is fixed in osmium alone. Similar findings have

been reported by Teng (1961, 1962) and Jakus (1962), who have not described the extremely electron dense cells in their osmium-fixed material. It is reasonable to suggest that there would be minor morphological differences between the cells which would be due to their differences in age and metabolic activity at the time of fixation, and this would no doubt be seen in electron micrographs as variations in electron density. These differences would represent insignificant variations. The morphological evidence for the attribution of these differences to the degree of hydration of the cells is very striking. The interdigitating cell membranes are pushed away from the paler cells and into the darker cells, but there is no distortion between cells of an equal degree of electron density (Fig. 15).

FIG. 15 *Normal monkey cornea. Electron micrograph, showing cell membranes of "pale cells" (P) bulging into "dark cells". There is no distortion between cells of an equal degree of electron density (arrow). (Glutaraldehyde/OsO₄/araldite/uranyl acetate/lead citrate.) × 7,500*

Electron density in osmium-fixed preparations may be due to the presence of reducing substances or to the concentration of intracellular elements in general. The electron micrographs of the "dark cells" in formalin-fixed material also show corrugated nuclear membranes indicating a partial loss of intranuclear contents due either to dehydration, with transfer of fluid to the neighbouring cells as stated earlier, or to rupture of cell and nuclear membranes (not seen in the plane of section) with release of their contents. The uniform nature of the electron density and the presence of swollen neighbouring cells is presumptive evidence that the first process takes place, and it would also seem probable that in some cells the second process occurs, for an increased tension exerted by the swollen cells would lead not only to compression of their neighbours but also to rupture of their cell membranes. However, it seems more likely that the "dark cells" were initially extremely swollen and ultimately ruptured and hence were compressed by the less swollen neighbouring cells with intact cell membranes. Because of the attachment of the cellular epithelium to the curved and rigid Bowman's membrane, mechanical factors must also be considered, and any alteration in the curvature of Bowman's membrane would exert tension on the basal cells of the epithelium. This would also explain their predilection for this region. Whatever the exact mechanisms of their evolution, caution must be exercised in interpreting "dark cells" as primary physiological or pathological entities.

Artefactual vacuoles and other artefacts seen in the "dark cells" need to be distinguished from swollen mitochondria resulting from autolysis, and from the morphological features associated with cell death, *viz.* swollen mitochondria, dilated endoplasmic reticulum, ruptured lysosomes with the formation of autophagic vacuoles, disaggregation of ribosomes, and irregularity of nuclear and cytoplasmic membranes associated with clumping of nuclear chromatin. The animal material used in this study was fixed immediately after removal so that autolytic changes would be prevented.

Since the "dark cells" in the normal cornea vary in number and degree of density according to the state of hydration of the cells, which itself varies according to the fixative used, it is reasonable to assume that they would be more evident in pathological states associated with corneal oedema (Rice and others, 1968).

Melanocytes and Langerhans cells similar to those of the skin (Charles and Ingram, 1959; Breathnach, Silvers, Smith, and Heyner, 1968) were not seen in the normal human

FIG. 16 Vascularized human cornea, showing dendritic melanocytes (arrows) in the basal layer. Haematoxylin and eosin. $\times 420$

cornea. But it is an old observation which has recently been proved experimentally by Henkind (1965) that dendritic melanocytes migrate into the cornea after its vascularization. Corneal melanocytes obtained from such an eye are shown in Fig. 16 (see p. 603).

Summary

The effect of fixation procedures on the structural and ultrastructural appearances of the basal cells of the corneal epithelium in man and monkeys is described for the first time. An association between the fixative used and the appearance of the cells is established. Fixation artefacts should be considered when interpreting histological and electron microscopical appearances. They could be minimized in electron microscopical studies by osmium fixation of fresh tissue. The need for the application of standard techniques of fixation when interpreting the significance of comparable morphological appearances, and for the use of normal controls when comparing the pathological features of differently fixed materials, is stressed.

There is sufficient evidence to attribute the morphology and staining characteristics of the "dark cells" to their state of hydration.

The literature contains diverse opinions and considerable confusion in nomenclature. There is no evidence for the interpretation of descriptive terms such as polygonal cells, polymorph elements, dendritic cells, clear matrix cells, dark secretory cells, and pale epithelial cells in terms of special cell types, and apparently none exists in the living cornea. Cells which could be morphologically characterized as Schwann cells, Langerhans cells, and amelanotic melanocytes were not seen in the present study. Melanocytes have been demonstrated in the cornea after its vascularization.

It is therefore concluded that the normal human corneal epithelium consists of cells of one lineage only.

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