Morphological changes in the human corneal epithelium associated with surgical corneal clouding

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SUMMARY Light microscopy and electron microscopy were used to define morphological changes occurring in human corneal epithelium during surgery. No correlation was established between preoperative medication or peroperative fluids and corneal epithelial clouding. It is suggested that the changes we observed in relation to epithelial clouding were the direct result of disturbance to endothelial pump functions.

Transient corneal clouding obscuring the view of intraocular structures during surgery has frustrated most ophthalmic surgeons. With the advent of closed intraocular surgical techniques observed through the operating microscope the effect has become more apparent. It has been known for many years that the obscuration is caused by the corneal epithelium. Surgeons have removed the epithelium of clouded corneas and restored a clear view. The effect seems more marked in diabetic patients, and Foulks et al. suggested basement membrane abnormalities were a causative factor.

The normal cornea is approximately 78% water. The state of hydration rests on the balance between the hydrating forces (swelling pressure of the proteoglycan stromal matrix and intraocular pressure) and the dehydrating forces (endothelial and epithelial pumps and barriers, and surface evaporation).

The contribution of each of these factors needs to be examined in terms of its potential contribution to the genesis of the corneal oedema. It should be possible to detect the sites of accumulation of oedema fluid and of any associated cell damage. If we can identify the most significant factors involved in corneal oedema we may then be able to suggest ways of reducing their effects.

We have carried out a study of the anatomical changes in the corneal epithelium in 10 patients undergoing ophthalmic surgery.

Materials and methods

Patients attending St George’s Hospital for ophthalmic surgery were assessed preoperatively. Previous ophthalmic surgery and intercurrent eye disease in addition to that for which surgery was planned were recorded. An inquiry was also made for systemic disease and topical and systemic medication. The patient’s age was noted. Careful slit-lamp examination was performed with particular attention to the cornea. The intraocular pressure was also recorded.

A record was kept of the preoperative ocular medication each patient received. At the time of surgery records were made of the nature of the skin preparation used, the duration of corneal illumination, the nature, osmolarity, and volume of fluid used to moisten the cornea and also of that used in intraocular infusion and of any peroperative problems. The degree of corneal clouding was recorded as nil, mild, moderate, or severe at the preoperative examination and also at the beginning of surgery, during surgery, and at the end of surgery.

Small specimens of peripheral corneal epithelium were taken at the beginning and end of the surgical procedure. These were immediately transferred to appropriate fixatives for later examination with light and electron microscopes. Samples were collected from 10 patients—seven undergoing extracapsular extraction with lens implant, two intracapsular cataract extraction, and one lens aspiration. Specimens were fixed in half-strength Karnovsky’s fixative pH 7.4 for 2–12 hours at 4°C. They were then washed briefly in several changes of 0.1 M sodium cacodylate-HCl buffer (pH 7.4 and postfixed in 2%
osmium tetroxide made up in the same buffer for 1 h at room temperature. Specimens were then washed in 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in ethanol and propylene oxide, and embedded in Spurr resin. All sections were cut on a Sorvall MT2-B ultramicrotome.

Thick sections (1 μm) for light microscopy were cut with a glass knife and stained with either toluidine blue or crystal violet. Thin sections for electron microscopy were cut with a diamond knife, stained with uranyl acetate and Sato’s lead, and examined in a Phillips 301 electron microscope.

Cryofreezing was used as a control to check that the fixative techniques were not producing significant changes.

**Results**

Our results show that changes are produced in the corneal epithelial morphology during the course of eye surgery. These changes occur throughout the thickness of the epithelium, those near the stromal surface differing from those near the tear surface. These changes can be described in terms of cytoplasmic changes, cell/cell junction changes, and changes in the cell nucleus.

**Cytoplasmic Changes**

The layers of cells close to the tear surface show a reduction in cytoplasm and a separation of cell membranes, though the desmosomes and tight

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**Figs. 1-4**  Progressive destruction of the superficial layers of the corneal epithelium from Fig. 1 at the beginning of an operation to Fig. 4 at its end. st = Stroma (or stromal surface). s = Surface. v = Vesicle. d = Desmosomes. n = Nucleus.
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Junctions do not separate. The extreme of this condition is to have a stack of cell membranes separated by the remains of the cytoskeleton resting on the underlying cells (Figs. 1–4). The innermost layer of the epithelium, that is, the layer adjacent to the stroma, shows surprisingly different changes. Here the cytoplasm appears to remain intact, but there is an accumulation of vesicles within the cells (Fig. 5). These vesicles have electron lucent contents and are possibly membrane bound. They occur in large numbers. As the number of vesicles increase so the number of layers that contain them increase, starting at the basal layer and progressing upwards through the epithelium to the surface layers. These changes are initially more severe in the dark cells (Fig. 5), but as the number of vesicles increases both light and dark cells become equally affected (Fig. 6). (Usually nuclear collapse does not occur widely until the light cells have begun to accumulate significant numbers of vesicles—Fig. 7.)

**Nuclear Changes**

The nuclei too respond differently according to their location in the epithelium. In the basement layer of cells the nucleus and nuclear membrane are collapsed away from the cytoplasm, thus leaving the outer layer of the nuclear envelope attached to the cytoplasm (Fig. 6). Some of the nuclear pores remain attached

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**Figs. 5–8**

Fig. 5: Vesiculated dark cell of innermost layer of corneal epithelium with intact nucleus. Fig. 6: Nuclear collapse in the innermost epithelial layers. The outer layers still contain intact nuclei. Fig. 7: Exclusive dark cell vesiculation with intact nuclei in the innermost layer of the epithelium. Fig. 8: Outermost layer of corneal epithelium showing the desmosomes resisting cell separation. Symbols as in Figs. 1–4.
to the inner layer of the nuclear envelope. In the more superficial layers the nuclei are better preserved.

**INTERCELLULAR SPACES**

Contrary to some reports, we found that there is very little separation of the cells, and the expansion of the intercellular spaces seems to be limited by the desmososomal junctions (Fig. 8). This is in contrast to chronic oedema and to the oedema associated with infection where there is considerable expansion of the intercellular spaces (Dilly N, personal observations). In some specimens there appears to be herniation of some of the cell cytoplasm out of the cells. This occurs in epithelia that are not supported by stroma, and the herniation probably occurs through that part of the cell membrane that was attached to the underlying basement membrane by hemidesmosomes. It is possible that this change represents the mechanical trauma of stripping off the epithelium from the underlying stroma while the biopsy is taken.

We could not show any relationship between the observed morphological changes and the factors recorded preoperatively. Neither was there any apparent relationship to the type of surgery, type of skin preparation, peroperative intraocular or corneal fluids, or the nature or duration of the surgery or the surgeon.

**Discussion**

The human corneal epithelium has a great capacity for recovery from many insults, but not infrequently it fails to maintain its transparency during ophthalmic surgery. The transparency of the cornea is dependent on many factors, a very significant one of which is the state of hydration of the stroma and epithelium. The water within the cornea is in dynamic balance. The factors involved in this balance must include: evaporation from the surface, the swelling pressure of the cornea, the intraocular pressure, and the efficiency of the intracellular pumps. For oedema to occur this balance must be upset, so that water accumulates. This means that either water uptake is increased or water loss is decreased.

The major factor in the uptake of water is the stromal swelling pressure. The normal stromal swelling pressure is 40–50 mmHg. It will, of course, decrease as the oedema worsens because of the high water binding capacity of the proteoglycan stromal matrix. During surgery this must be the major factor responsible for the uptake of water into the cornea, as the contribution of the intraocular pressure will be to some extent eliminated by the surgical decompression of the globe. The swelling pressure is a result of the matrix between the collagen bundles of the stroma, and it seems improbable that the surgical procedures can interfere with its activity. The factors that oppose this hydration are surface evaporation and the endothelial and epithelial pumps. Surface evaporation plays a minor role but may become important in compromised corneas. Surface evaporation will result in an increased tear tonicity and a resultant forward movement of water through the cornea. This osmotic extraction is thought to account for only 4% of the total dehydrating forces.

The adenosine triphosphate (ATP) dependent endothelial pump accounts for the bulk of the corneal fluid flow, pumping water from the cornea into the anterior chamber. Both the epithelium and the endothelium act as semipermeable membranes and bar the flow and diffusion of electrolytes but not the diffusion of water. The epithelium has a much poorer pump function but is a better barrier than the endothelium. Whatever the pump mechanisms, ions are pumped out and water follows. The surgical decompression of the globe will enhance this pumping mechanism. One of us (GT) has observed a correlation between the density of corneal clouding and the use of closed intraocular infusion during surgery. Clouding occurs shortly after infusion begins and clears when the instruments are removed. The closest part of the cornea to the infusion is the single layer of endothelial cells lining the inner surface of the stroma. It is tempting to postulate temporary disturbance of endothelial cell ion pumps as being responsible for the clouding. Since clouding of the cornea usually occurs after irrigation of the anterior chamber it is reasonable to speculate that the irrigation is interfering with pump function. Since the cornea is almost totally permeable to water, any failure of the electrolyte pumping mechanism will result in an accumulation of fluid within the cornea.

It is also necessary to consider other contributory factors such as the heat of the operating lights, preoperative ocular medications, and skin preparations.

We consider it is unlikely that the skin preparation affected the corneal epithelium adversely, since care was always taken to avoid any spillage into the conjunctival sac. MacRae et al. found in testing five commonly used skin preparations that only povidone iodine solution (without detergent) was non-toxic to the cornea. The majority of our patients received povidone iodine skin preparation.

During intraocular surgery saline or balanced saline solutions are frequently used to replace the tear film. During surgery for retinal detachment some surgeons have protected the cornea with gelatin sponge. The use of hyaluronic acid, possibly combined with a contact lens in closed vitrectomy, has
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been advocated by Pruett et al. and Spencer et al. Norn studied peroperative protection in 221 consecutive cataract extractions and concluded that Healon was the most effective in preventing epithelial damage as assessed by vital staining with 1% lissamine green. However, four or five instillations of saline per half hour also satisfactorily prevented stromal deturgescence and associated corneal clouding. Methyl cellulose, polyvinyl alcohol, and a soft contact lens did not give any added benefits. However, we could not prevent corneal clouding by saline instillations alone. Our observations would suggest that most if not all of these procedures are irrelevant to the control of corneal clouding, though of course they may protect the corneal epithelial cells from other damage.

When we altered the osmolarity of the tear film substitute we did not observe any difference in the morphological changes we have described. We did record a 4°C temperature increase on the surface of the cornea in one patient between the beginning and the end of surgery. Other subjects showed a smaller rise. Again we found no correlation with the degree of corneal clouding. We believe that it is unlikely that the changes in epithelial morphology are due to light and/or heat damage caused by the operating microscope, since such temperature changes would produce very little extra evaporation from the eye surface.

The surprising finding from our work was the absence of large expanded extracellular spaces that contained oedema fluid. Such fluid filled spaces are well documented. In chronic epithelial oedema Lohman et al. reported intercellular fluid accumulation in the basal layers. This has also been reported as a very early finding by Tripathi and Bron. Our findings probably represent the changes associated with the very recent onset of an acute oedema, not the more long-term changes previously reported. Presumably the conditions we describe could progress to mimic these descriptions.

Our report of these acute changes shows that the cells of the cornea can suffer severe morphological changes and yet recover their function. We believe that our description is the first published report of the morphological changes occurring in the human corneal epithelium during surgery. Our findings have not shown any single factor to be responsible for these changes. Previous investigations of this subject have concentrated on direct toxic effects to the epithelium of changes in osmolarity, preoperative drops, or skin preparation. Attempts to protect the corneal epithelium should perhaps be focusing on protecting the functional integrity of the endothelium. The reason for this is that we suspect that transient changes in endothelial function are of greater significance.

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References


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