ON THE PATHOLOGY OF THE IRIDOCORNEAL-ENDOTHELIAL SYNDROME: THE ULTRASTRUCTURAL APPEARANCES OF 'SUBTOTAL-ICE'

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SUMMARY

The iridocorneal-endothelial syndrome (ICE syndrome) is characterised by corneal failure, glaucoma and iris destruction. Specular photomicroscopical and histological studies of the corneal endothelium in this disease show a population of abnormal cells named 'ICE-cells'. In many patients some areas of the endothelium are occupied by ICE-cells and others by normal cells, an appearance described as 'subtotal-ICE'. Specular photomicroscopical observations suggest that ICE-cells and normal endothelial cells may actively interact at the boundary zone where they meet. The purpose of this study was to examine the ultrastructural appearances of the boundary zone to gain insight into the cellular pathology of this region. Thirty-five corneas taken from patients with the ICE syndrome were examined by light, transmission and scanning electron microscopy. The subtotal-ICE appearance was demonstrated in four specimens. The morphology of ICE-cells at the boundary zone suggests that they are non-motile but also implies a general state of high metabolic activity. Many of the normal endothelial cells in this region are damaged, an appearance which may result from a toxic effect from the nearby ICE-cells.

The iridocorneal–endothelial syndrome (ICE syndrome) is characterised by a 'hammered-silver' appearance of the corneal endothelium, corneal failure, glaucoma and spectacular iris abnormalities which include nodules on the anterior iris surface, atrophy, distortion and synechiae formation.[1–7]

Endothelial specular photomicroscopy (ESP) of ICE syndrome corneas[8-15] has demonstrated a population of abnormal cells which have been named 'ICE-cells'.[10] ICE-cells are said to be unique to the ICE syndrome and observed in every patient except those whose corneal oedema is sufficiently severe as to preclude examination.[10] ICE-cells are larger and more pleomorphic than normal cells and their specular reflex shows 'light–dark reversal': the cell surface is dark instead of light, often with a central light spot, and the intercellular junctions are light instead of dark. Four patterns of ICE-cell distribution have been described.[10,11] In 'total-ICE' the normal endothelial mosaic is completely replaced by ICE-cells. In the two forms of 'subtotal-ICE', 25–75% of the endothelial surface is occupied by ICE-cells and the remainder by normal cells (Fig. 1); in some instances, 'subtotal-ICE(+)', the density of normal endothelial cells is greater than in age-matched healthy subjects, whilst in others, 'subtotal-ICE(−)', it is decreased. Finally 'disseminated-ICE' denotes ICE-cells scattered individually or in small clusters amongst the mosaic of normal endothelial cells.

Ultrastructural examination of specimens from patients with the ICE syndrome demonstrates two predominant cellular populations.[12,16–18] Cells with epithelial features are the histological equivalent of the ICE-cell seen by ESP, whilst cells which are similar to the endothelial cells of normal corneas represent the normal cells demonstrated by ESP alongside ICE-cells, in cases of subtotal- and disseminated-ICE.

ESP studies of subtotal-ICE have shown that changes may occur in the contour of the ICE-cell

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mass[10,11] and also that normal endothelial cells close to ICE-cells may be smaller than those some distance away.[13] These observations suggest that the 'boundary zone' at which ICE-cells and normal endothelial cells meet may be a site of active interaction between the two cell types. The purpose of this study was to examine the ultrastructural appearances of the boundary zone in order to gain insight into the cellular pathology of this region.

MATERIALS AND METHODS

Criteria for Inclusion of Patients in the Study

Patients were included in the study when they had clinical signs[1–7] and ESP appearances[8–15] typical of the ICE syndrome. All patients had the 'hammered-silver' appearance of the corneal endothelium as well as various combinations of corneal oedema often at normal or minimally elevated intraocular pressure, glaucoma and iris signs such as atrophy, synechiae and nodule formation. In addition ICE-cells were visualised by ESP in all patients except those with severe corneal oedema.

In order to avoid inadvertent inclusion of patients with other disorders such as posterior polymorphous dystrophy[19] or Fuchs' endothelial dystrophy[20,21] subjects were excluded if any one of the following criteria applied to them: (1) Onset at 10 years of age or less; (2) a family history of a similar disorder; (3) widespread guttate or vesicular/geographic lesions of the corneal endothelium; (4) any preceding major ocular disorder, surgery or penetrating/severe trauma (except filtering surgery necessitated by the ICE syndrome process itself).

Acquisition of Corneas

Thirty-five corneas taken from patients with the ICE syndrome at the time of penetrating keratoplasty were processed for light, transmission and scanning electron microscopy. The methods used to obtain human tissue complied with the Helsinki Declaration.

Light and Transmission Electron Microscopy

Specimens were prepared for light microscopy and transmission electron microscopy either by fixation in glutaraldehyde and postfixation in osmium tetroxide followed by processing into Spurr's resin, or by fixation in paraformaldehyde followed by processing into Lowicryl K4M resin.

The 0.5 μm thick sections for light microscopy were stained with toluidine blue. The 70–100 nm ultrathin sections for transmission electron microscopy (TEM) were stained with saturated uranyl acetate in 50% ethanol and Reynolds' lead citrate and examined with a Philips EM 201 transmission electron microscope.

Scanning Electron Microscopy

Specimens were prepared for scanning electron microscopy (SEM) by fixation in glutaraldehyde and postfixation in osmium tetroxide, followed by dehydration, critical point drying, mounting on specimen stubs and gold sputter-coating. Examination was performed with a Cambridge Stereoscan 200 series scanning electron microscope.

RESULTS

The characteristics of the different cell types present on the endothelium of ICE syndrome corneas have been reported in more detail elsewhere.[18] Briefly, a population of well-differentiated cells with epithelial features such as abundant tonofilaments and desmosomes was observed in many specimens. These cells were larger and more pleomorphic than normal and were sometimes multilayered. They had many microvilli and conspicuous 'blebs'[22] (excrescences of the cell membrane filled with cytoplasm). They expressed a profile of differentiation markers which was consistent with their epithelial phenotype. These corresponded to the ICE-cells demonstrated by many ESP studies. A second population consisted of cells whose morphology and differentiation marker profile were similar to those of the endothelial cells from normal corneas. These corresponded to the normal endothelial cells seen by ESP in cases of subtotal-ICE.

The boundary zone between ICE-cells and normal endothelial cells was demonstrated in four specimens. The ICE-cells at the boundary zone formed a distinctive 'cuff' of bizarrely shaped cells (Fig. 2) which were usually monolayered (Fig. 3), although multilayering was sometimes observed (Fig. 4). These ICE-cells overlapped the surface of their normal neighbours (Figs. 3, 5, 6). Overlapping was restricted to the region of immediate contact between the two cell types (Fig. 3) and there were never extensive areas of coverage of normal cells by ICE-cells. The overlapping edge of the ICE-cells consisted of a sharply delineated ridge which was never seen to bear lamellipodia or ruffles.

Some of the structures which characterised the ICE-cell surface were exaggerated near the boundary zone. For example their microvilli often appeared taller and denser and sometimes formed exuberant frond-like clusters (Fig. 7). Other bizarre cellular configurations were also common (Figs. 8, 9). Blebs of the apical cell membrane (Figs. 10, 11) were more frequently observed in this region and were more varied and complex in their appearance than blebs seen elsewhere, which tended to be simple dome-shaped elevations sometimes with a ridged or granular surface.

Normal endothelial cells near the boundary were morphologically similar to cells of the same type

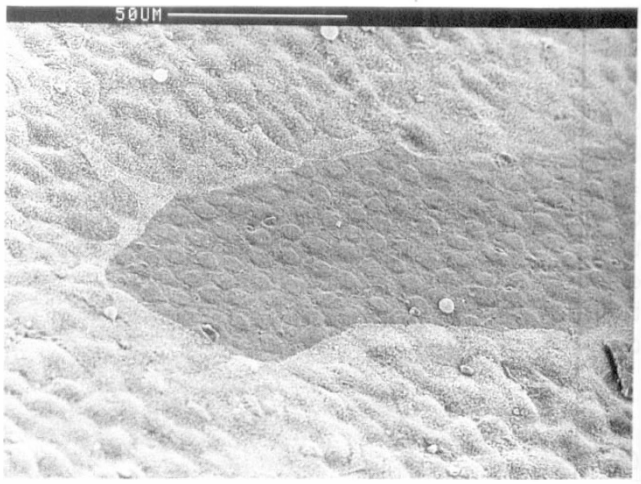

Fig. 1. *ESP of an ICE syndrome patient with the subtotal-ICE pattern of cell distribution. The cells on the right are typical 'ICE-cells' whilst those on the left resemble the cells of normal corneal endothelium. Compare with Fig. 3, which shows an SEM of subtotal-ICE in a different ICE syndrome patient. (ESP provided by Mr M. G. Kerr Muir, St Thomas' Hospital, London.)*

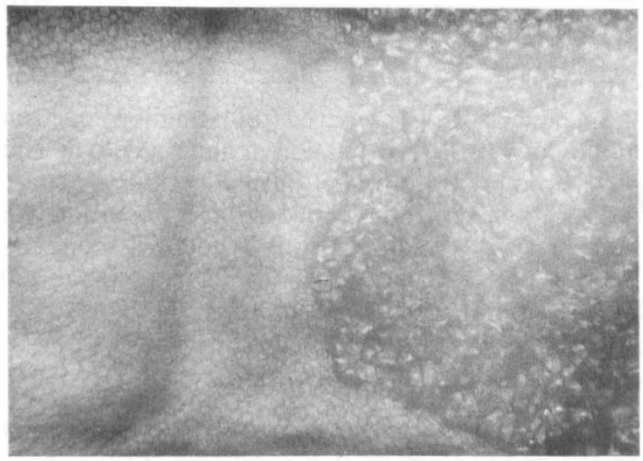

Fig. 2. *SEM showing subtotal-ICE. The ICE-cells are larger and their surface is covered by microvilli. The normal cells resemble those of normal corneal endothelium. They are devoid of microvilli and their surface shows a round elevation from the underlying nucleus.*

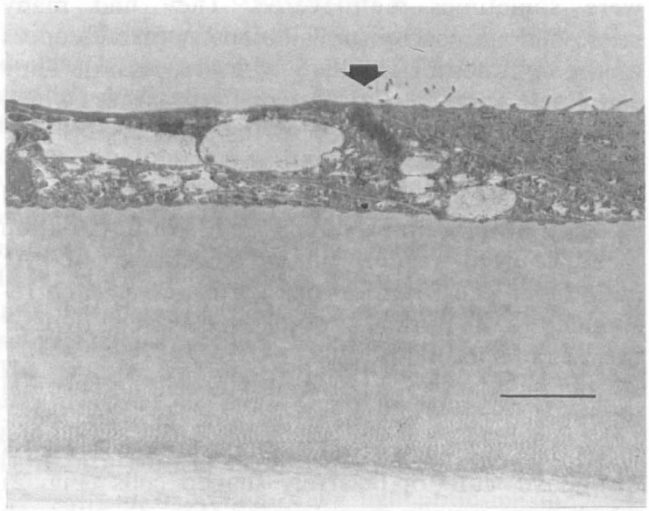

Fig. 3. *TEM of Lowicryl K4M-embedded tissue demonstrating the boundary zone (arrow) between ICE-cells (right) and normal endothelial cells (left). The ICE-cell with surface microvilli overlaps the normal cell, which contains large vacuolations. Scale bar represents 4 μm.*

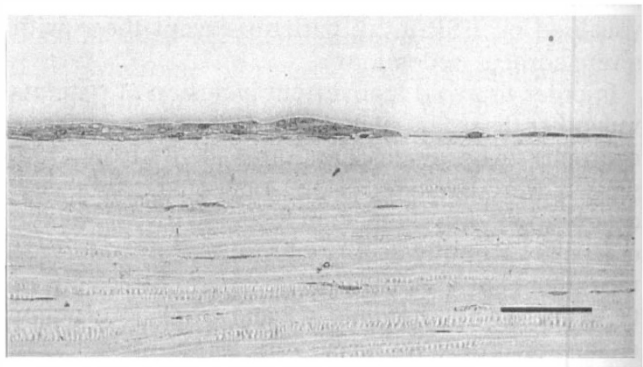

Fig. 4. *The boundary zone between ICE-cells (left) and normal endothelial cells (right). In this case the ICE-cells were multilayered. Light micrograph of Lowicryl K4M-embedded tissue. Scale bar represents 50 μm.*

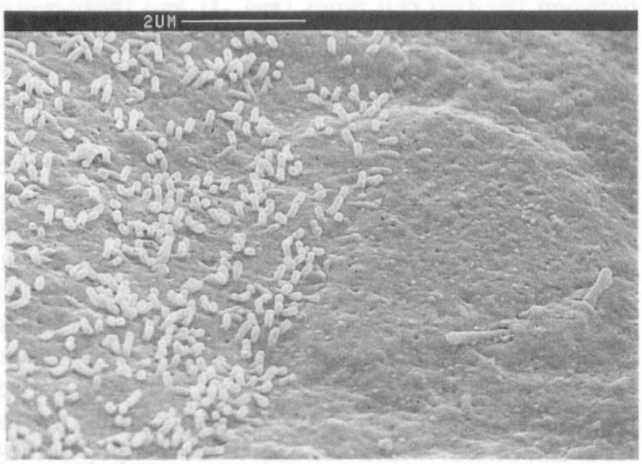

Fig. 5. *At the boundary zone this normal endothelial cell (right) is overlapped by the neighbouring ICE-cell (left). The round outline of the nucleus of the normal cell is partially obscured by the edge of the microvilli-covered ICE-cell. SEM.*

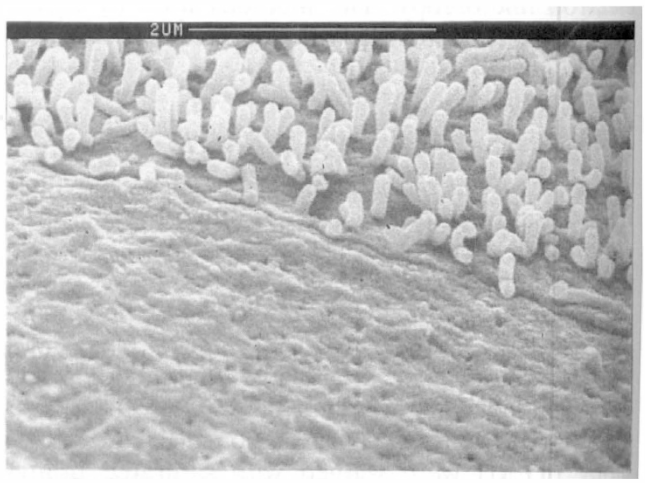

Fig. 6. *The ICE-cell shown in Fig. 5 (top) has microvilli on its surface. A ridge is seen where its edge overlaps the smooth surface of the normal cell (bottom). SEM.*

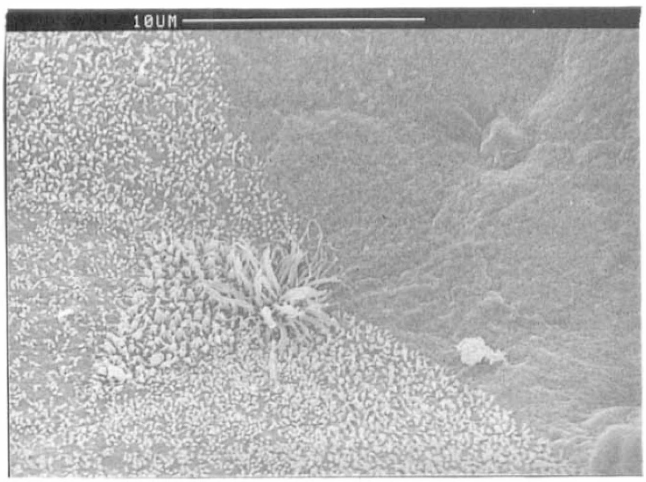

Fig. 7. *The microvilli on the surface of ICE-cells often appeared taller in the region of the boundary zone. Some have fallen onto the plain surface of the adjacent normal cell. SEM.*

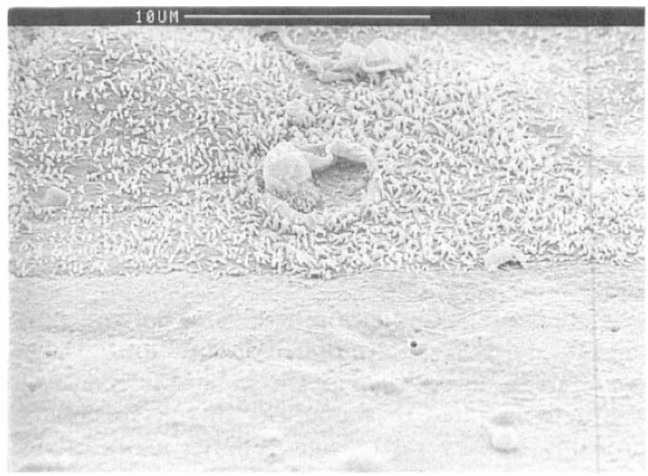

Fig. 8. *One of the bizarre structures demonstrated by ICE-cells at the boundary zone. SEM.*

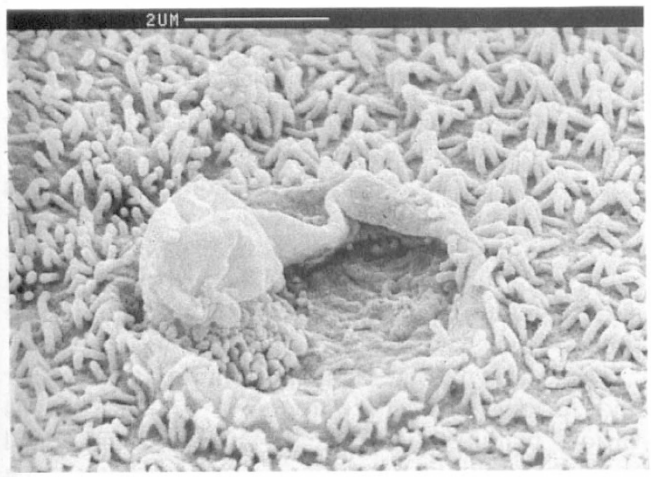

Fig. 9. *Detail of the ICE-cell structure shown in Fig. 8. SEM.*

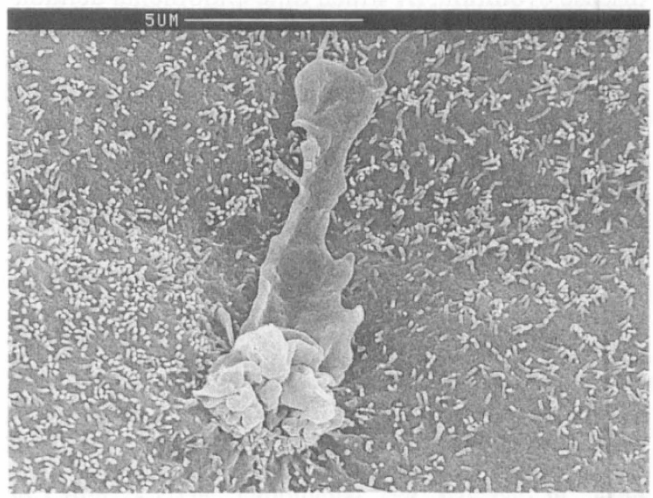

Fig. 10. *SEM demonstrating the complex morphology of a bleb on the apical surface of an ICE-cell in the region of the boundary zone.*

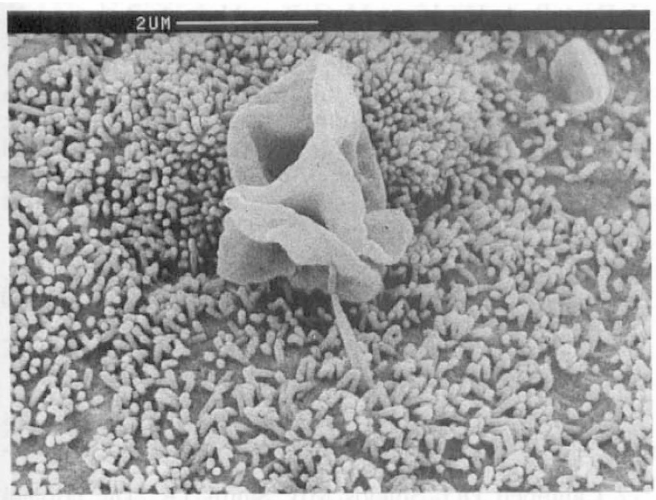

Fig. 11. *Another complex bleb on the apical surface of an ICE-cell in the boundary zone. SEM.*

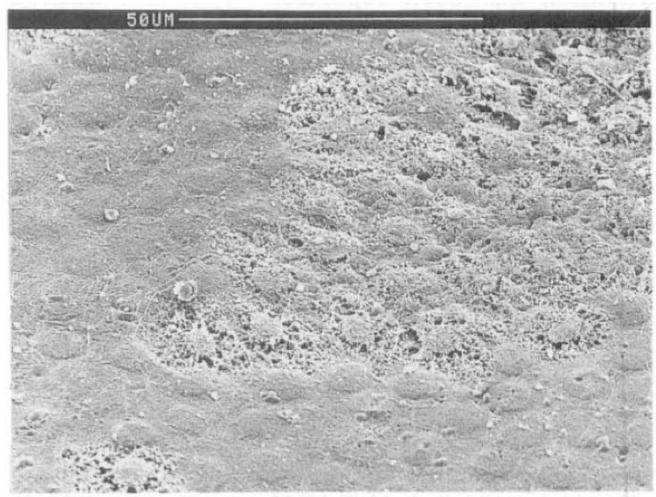

Fig. 12. *SEM of normal endothelial cells close to the boundary zone. Many cells are necrotic. In these cases membrane lysis reveals the remnants of the nucleus and cytoplasm.*

further away from this zone except that they often demonstrated multiple large intracellular vacuoles (Fig. 3) and many were frankly necrotic (Fig. 12).

No cells with features intermediate between ICE-cells and normal cells were seen. Mitotic figures were not observed in either population.

DISCUSSION

ESP studies suggest that up to 40% of patients have the subtotal-ICE pattern of cell distribution.[12,15] The boundary zone between ICE-cells and normal cells was demonstrated in a minority of our specimens, probably for several reasons. Firstly, many of our cases were known to be total- or disseminated-ICE, rather than subtotal-ICE, from pre-operative ESP examination. Secondly, corneal grafting was of necessity not performed until an advanced stage of disease evolution, by which time endothelial denudation was often the predominant appearance. Endothelial cell loss with baring of Descemet's membrane is seen in the end-stage of all corneal endotheliopathies.[23,24] Thirdly, in some instances the boundary zone may have been located outside the area encompassed by the corneal graft which was of relatively small size (about 7.25 mm) compared with total corneal diameter (about 12 mm).

The shape adopted by ICE-cells at the immediate border with normal endothelial cells suggests a state of immobility. These cells were widely spread[25,26] and lacked organelles such as lamellipodia or ruffles which are associated with movement.[26-28] Moreover, they were never seen to have entirely overgrown their normal neighbours. This interpretation of the ICE-cells' morphology is consistent with evidence from other sources. Corneal failure in the ICE syndrome is often only slowly progressive and follow-up of many years without significant deterioration has been reported.[3,7,8,11–13,29,30] Such a prolonged course implies that rapid changes in the status of the endothelium resulting from movement of ICE-cells either do not occur or occur only intermittently. ESP studies of subtotal-ICE have demonstrated many patients in whom cell movement at the boundary zone did not occur.[10] Also, the hypothesis that epithelial and corneal endothelial cells can exist alongside each other for prolonged periods is supported by experimental evidence. In organ culture of corneal explants in the human,[31] rabbit[32,33] and dog,[34] corneal epithelial cells migrated over the cut stromal surface until they reached the corneal endothelium, at which point epithelial migration ceased. Epithelial and endothelial cells were subsequently maintained without significant changes in either population. However, some aspects of ICE-cell morphology imply a state of cellular 'activation' rather than quiescence. The microvilli and blebs which characterised the apical surface of ICE-cells were exaggerated near the boundary zone, suggesting that cells in this region may have been particularly active metabolically[22,35] even if not actually engaged in mitosis or movement.

These cells were often damaged or necrotic at the boundary zone, suggesting that ICE-cells may have a toxic effect on neighbouring normal endothelial cells. Extensive overgrowth by ICE-cells was never seen, so that the death of normal endothelial cells cannot be explained by this mechanism. Glaucoma may damage normal corneal endothelium[36–41] but elevated intraocular pressure would presumably have a widespread, rather than localised, noxious effect.

In healthy corneas, the endothelial cells maintain transparency by active transport of fluid and solutes out of the stroma.[42] Continued loss of endothelial cells eventually overwhelms the capacity of those remaining to deturgesce the cornea and oedema ensues.[43] The number of necrotic endothelial cells seen at the boundary zone implies that this may be a significant site in the ultimate development of corneal failure.

The morphology of the specimens in this study differs somewhat from the recent case of Lee *et al.*[17] but that case showed extensive endothelial changes very early in the course of the disease, before other manifestations became apparent clinically. The relatively frequent finding of lymphocytes in the endothelium may only be seen early in the disease process.

The ICE syndrome is pleomorphic in its behaviour and further studies are required to elucidate its various manifestations. Ideally *in vivo*, longitudinal studies should be performed. The advent of the confocal scanning microscope may make this possible.

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Key words: Corneal endothelium, Endothelial specular photomicroscopy, ICE-cell, ICE syndrome, Iridocorneal-endothelial syndrome, 'Subtotal-ICE'.

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