

The Human Anterior Lens Capsule—an attempted chemical debridement of epithelial cells by ethylenediaminetetracetic acid (EDTA) and trypsin

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SUMMARY The addition of edetic acid (EDTA) or trypsin to the infusion during a simulated extracapsular cataract extraction on cadaver eyes facilitates the removal of lens epithelial cells from the anterior capsule. Modification of the chemical composition of infusions used during extracapsular surgery may maximise lens epithelial cell removal and hence reduce the incidence of opacification of the posterior capsule after cataract extraction.

A major cause of a poor visual outcome following extracapsular cataract extraction, with or without intraocular lens implantation, is opacification of the posterior capsule. Quoted incidences vary from 15% to 35% at two years¹ and 50% at three years,^{2,4} and it is more frequent in children.^{5,7} The average time to opacification is 26 months, with a range of three months to four years.⁸

It has been suggested that the chief cause of opacification following extracapsular surgery is proliferation of residual lens epithelial cells.⁹ We have previously demonstrated that these cells are not removed during surgery but remain adherent to the anterior and equatorial capsule.¹⁰ If these cells could be completely removed during surgery, the incidence and degree of posterior capsule opacification, and the subsequent need for secondary capsulotomy procedures, would be significantly reduced.

The purpose of this study was to assess the effectiveness of chemical substances in facilitating a complete removal of the lens epithelial cells from the anterior and equatorial capsule during an extracapsular cataract extraction. Two substances were selected. Edetic acid (EDTA) is a calcium chelating agent which is used to dissociate epithelial cells from basement membranes in in-vitro studies.¹¹ Trypsin is a proteolytic enzyme that specifically hydrolyses the acyl groups of lysine and arginine, important in intercellular bonding, and is again used to prepare isolated epithelial lens cell suspensions.¹²

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Materials and methods

Extracapsular cataract extractions were performed under standard microscopic operating conditions on fresh (≥ 48 h post mortem) human cadaver eyes. A full thickness section of cornea was removed at the start of the procedure to act as a histological control in each case. Extracapsular extraction then proceeded with an anterior capsulotomy, nucleus expression, and as complete a removal of soft lens matter as possible by means of coaxial irrigation-aspiration cannula.

Six pairs of eyes were operated upon, the only variable being the composition of the irrigation fluid.

This consisted of:

Normal balanced saline solution	2 eyes
30 mM EDTA solution	2 eyes
15 mM EDTA solution	2 eyes
5 mM EDTA solution	2 eyes
2% Trypsin solution (Sigma, Poole, UK)	2 eyes
0.5% Trypsin + 5 mM EDTA solution	2 eyes.

The eyes and the control cornea were then fixed, sectioned, and stained as previously described.¹⁰

Results

Histological examination of the sections revealed that the epithelial cells of the lens remained attached to the anterior capsule when the infusion contained balanced saline only (Fig. 1). The cells have a normal morphological appearance and intercellular junctions are similarly undisturbed.

Sections from eyes that were infused with 15 mM

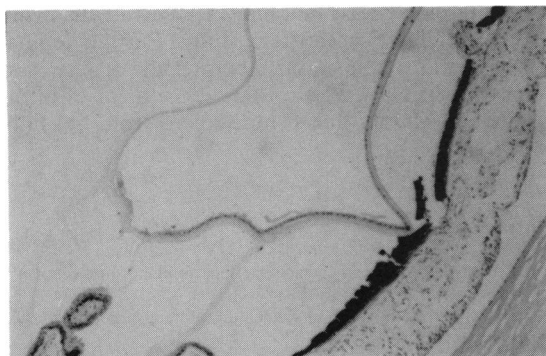


Fig. 1 Histological sections of the lens capsule in an eye irrigated with balanced saline only. The lens epithelial cells remain adherent to the capsule and their morphology is undisturbed. Haematoxylin and eosin stain. Field width 2.8 mm.

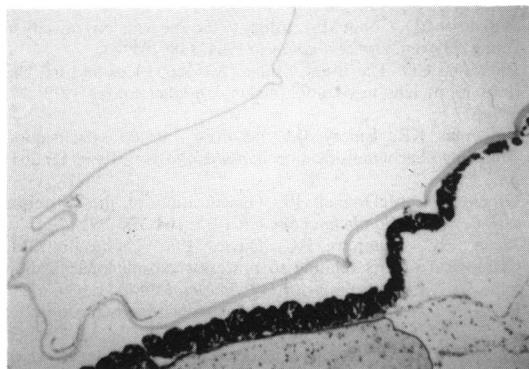


Fig. 2A

Fig. 2 Histological sections of the lens capsule in an eye irrigated with (A) 15 mM EDTA (field width 2.8 mm) and (B) 30 mM EDTA (field width 1.1 mm). The epithelial cells have separated from the lens capsule as a sheet. The lens zonules in these specimens (not shown in fig. 1) were intact.

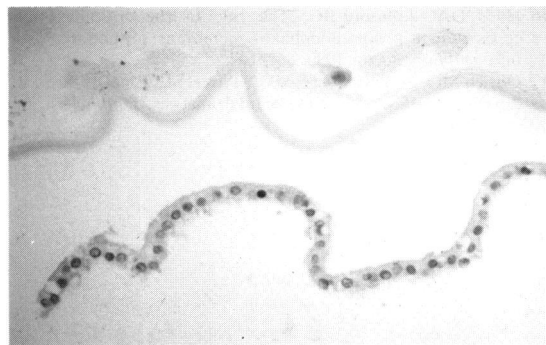


Fig. 2B

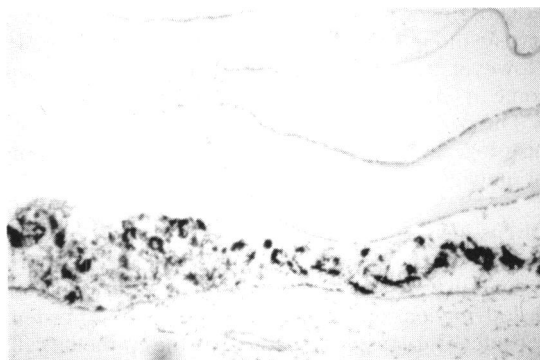


Fig. 3 Histological section of the lens capsule in an eye irrigated with 2% trypsin. The epithelial cells are partially stripped from the capsule but their morphology is altered and intercellular junctions are disrupted. There is much pigment deposition—presumably derived from the iris. Field width 1.1 mm.

and 30 mM EDTA (Figs. 2A, B) showed that the anterior epithelial cells had fallen away from the capsule as a sheet, while the intercellular junctions remained relatively intact. The zonule of the lens appeared relatively undamaged. While both infusions of 15 mM and 30 mM EDTA caused deepithelialisation of the capsule, 5 mM EDTA did not disturb the cell capsule attachments.

Sections from eyes infused with 2% trypsin showed that the epithelial cells had been stripped only in places from the capsule (Fig. 3), and there were marked morphological changes in the epithelial cells. The cytoplasm was swollen, cell borders were irregular, and intercellular junctions were broken, resulting in cells becoming separated from each other. There was extensive pigment deposition at the capsule, suggesting proteolytic damage to the iris. The zonules appeared fragmented, also indicating some damage.

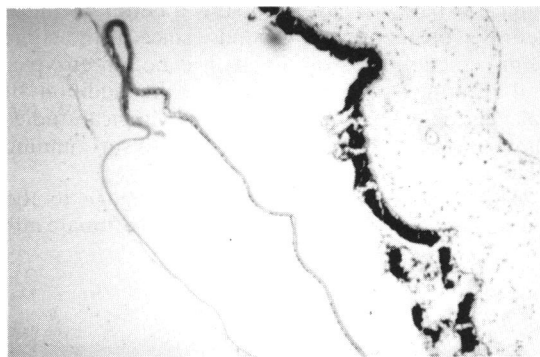


Fig. 4 Histological sections of the lens capsule in an eye irrigated with 5 mM EDTA and 0.5% trypsin. The epithelial cells remain adherent to the lens capsule. Field width 1.1 mm.

Those eyes infused with 5 mM EDTA and 0.5% trypsin did not show epithelial cell stripping (Fig. 4). However, there was pigment deposition indicating some iris damage.

The corneas in all these eyes were also examined histologically, and there was no evidence of stripping or damage to the endothelial cells.

Discussion

Irrigation and aspiration alone are incapable of dislodging lens epithelial cells from the anterior lens capsule. The capsule is secreted by the epithelial cells as their basement membrane and differs both chemically and ultrastructurally from the lens zonule¹³ to which it is attached.

The lens epithelial cells lie under the anterior and equatorial capsule, with their smooth bases apposed to the capsule and their apices facing the interior of the lens. Adjacent cells are attached to each other by a small zonula occludens at the apical borders and occasionally by a macula adherens. No cement substance or other extracellular material is seen between the base of the epithelium and the capsule or between the apices of the epithelium and the first layer of lens cells.¹³

EDTA in high enough concentrations is effective in disrupting the cell capsule bond and results in the cells falling away as a sheet. The intercellular junctions are not affected. Trypsin is effective in disrupting both cell capsule and intercell bonds but appears to inflict more damage on associated structures such as the iris in vitro—as would be expected from a proteolytic enzyme involved in digestion.

That the lens epithelial cells are the major contributor to opacification of the posterior capsule has been demonstrated by pathological⁹ in-vitro cell culture¹⁰ and animal¹⁷ studies. Any procedures that may maximise their surgical removal may be of great clinical benefit. EDTA is possibly a better chemical debrider on theoretical grounds, since in the short term its action is extracellular, it is not an enzyme, and its effects are reversible by the addition of calcium to the environment. Our histological studies show that EDTA exerts less damage to surrounding structures than does trypsin.

Although there was no obvious damage to the corneal endothelium, it is impossible to estimate any

potential side effects that may be incurred in a living eye from this post-mortem study. Further animal studies will be necessary before the safety and efficacy of EDTA as a routine constituent of intraocular irrigation fluids in humans can be fully assessed.

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