Preparation of full-thickness flat mounts of rabbit cornea

A new approach

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The delicate endothelial layer of the cornea is easily damaged in the course of preparing a flat mount. Moreover, the techniques currently available necessarily cause some disruption to the normal corneal architecture. The purpose of this paper is to describe a new technique designed to avoid the disadvantages of previous methods.

Method

FLATTENING AND FIXATION

The device used to flatten the cornea (Fig. 1) consists essentially of a base plate with a central removable mounting ring 12 mm. in diameter, a clear Perspex perfusion chamber, and a piston.

The rabbit eye is excised with lids and conjunctiva attached and the entire corneal epithelium is removed. Using the technique of Maurice (1969) and Dikstein and Maurice (1972) as modified by Hodson (1971), the cornea is separated from the remainder of the eye while mounted on the ring. The advantage of this approach is that the endothelial surface is undisturbed and continues to be bathed in aqueous. The Perspex perfusion chamber is now placed in position over the cornea and tightened onto the exposed rim of sclera (Fig. 2). A constant flow infusion pump is connected to the chamber inlet and circulates the perfusion fluid (Dikstein and Maurice, 1972) through the chamber. The outflow is discharged at a height of approximately 20 cm. to maintain a positive pressure equivalent to intraocular pressure. This perfusion protects the endothelium and maintains the normal shape of the cornea while thinning occurs as a result of evaporation from the exposed external surface. After about 15 min. the piston is brought into contact with the apex of the cornea (Fig. 2a). Then, under direct vision from above, it is slowly advanced (Fig. 2b), flattening the cornea until the flattened zone coincides with a ring inscribed on the piston head (10 mm. in diameter). Concentric folds will appear if this process is too rapid or if the positive pressure within the chamber is lost. This folding cannot be reversed.

For fixation the perfusion fluid is replaced by 12 per cent. buffered formalin circulated at the same positive pressure for 1½ to 2 hours. At the end of this period the perfusion chamber can be removed and the mounting ring cut away, leaving a shallow cup of cornea with a flat base 10 mm. in diameter (Fig. 3).

STAINING (Ehrlich's haematoxylin and celestin blue)

- (1) Rinse specimen thoroughly in distilled water.
- (2) Fill the 'cup' successively and for periods of 5 min. each with 50, 90, and 50 per cent. alcohol.
- (3) Rinse again in distilled water leaving a film of water on the specimen.
- (4) Fill 'cup' with celestin blue and agitate gently for 30 min.
- (5) Rinse in distilled water leaving a film of water on the specimen.
- (6) Fill the 'cup' with Ehrlich's haematoxylin for 1 min.
- (7) Rinse in distilled water.

FIG. 2 Cut-away diagram shows cornea mounted on ring. The piston has been advanced and has flattened a disc 10 mm. in diameter. Positive pressure equal to intraocular pressure is maintained within the chamber during perfusion

a and b Mode of operation of piston

FIG. 1 Device used for preparing flat mounts of rabbit corneae.

A. Clear Perspex perfusion chamber
B. Corneal mounting ring C. Piston

FIG. 3 The fixed cornea has the shape of a shallow cup with a flat base. The disc is cut with a trephine

MOUNTING

The 10 mm. disc is trephined from the 'cup' (Fig. 4) and mounted in Farrant's medium under a coverslip. The preparation is suitable for examination at low power and at higher powers under oil (Figs 5 and 6).

FIG. 4 *Stained disc of cornea ready for mounting. The surface is completely smooth.*

FIG. 6 *High-power view of the same preparation as that in Fig. 5. The morphology of the nuclei has been well preserved $\times 150$*

AUTORADIOGRAPHY

With slight modification, this technique is suitable for autoradiographic studies of the corneal endothelium (Fig. 7).

FIG. 5 *Rabbit corneal endothelium on flattened disc. The whole field is in focus $\times 22.5$*

FIG. 7 *Autoradiograph of rabbit corneal endothelium on flattened disc. $\times 60$*

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After the endothelium has been labelled with the isotope (Mills and Donn, 1961), the specimen is flattened, fixed, and passed through alcohol as described above. Staining, however, is deferred. Instead the disc is trephined and autoradiographs are prepared using Kodak stripping film A.R.10. After the preparation has been developed, it is stained with celestin blue for 45 min. and Ehrlich's haematoxylin for 10 min. The process is completed by washing several times in acid alcohol 1 per cent. to destain the film.

Comment

Using this technique we have been able to produce, consistently, flat mounts that are smooth and devoid of folds. Although some distortion of the corneal stroma is inevitable as a result of compression and evaporation, this appears to be a minor disadvantage and is more than offset by the complete protection afforded to the endothelium during processing.

The maximum area that can be flattened in this way is about 75 sq. mm. It seems adequate for most purposes, including the examination of grafted corneae.

Summary

A completely new technique for the preparation of full-thickness flat mounts of the cornea is described.

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