A CLINICAL METHOD FOR ASSESSMENT OF ENDOTHELIAL VIABILITY IN DONOR CORNEAE*†

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It is now well-established that endothelial viability of the donor cornea is essential for successful penetrating keratoplasty (Kaufman, Capella, and Robbins, 1966; Stocker, Eiring, Georgiade, and Georgiade, 1959). Unfortunately all the current methods of assessment render the donor eye unsuitable for a subsequent penetrating graft. These include:

- (1) Morphological studies of the endothelium of the intact cornea following:
 - (a) Histochemical staining of the cellular enzymes (Robbins, Capella, and Kaufman, 1965).
 - (b) Staining with a vital stain such as trypan blue (Stocker, King, Lucas, and Georgiade, 1966).
 - (c) Staining with nuclear stains.
 - (d) Staining with silver nitrate (Wilcox, Wood, Oh, Everett, and Evans, 1958).
- (2) Tissue-culture techniques, including embryonation of thin strips of endothelium placed on a chorio-allantoic membrane of a 9-day-old chick embryo and incubated for 48 hours to remove dead endothelial cells and preserve the living cells (Ocampo and Salceda, 1966).
- (3) Oxygen uptake of the isolated endothelium using a semi-micro respirometer (Leigh and Ridge, 1957).

At present donor eyes have become more plentiful in London; thus some attempt should be made at greater selectivity of material for penetrating grafts in the hope of reducing the incidence of graft failures. In this study we have examined with the slit lamp the posterior surface of the cornea of 400 eyes, and have described changes in the endothelium which indicate some measure of endothelial non-function as gauged by vital staining.

All the eyes were collected by the Westminster-Moorfields Eye Bank and were examined as soon after death as possible. The eyes were obtained from patients ranging in age from 24 to 85. They were enucleated using a sterile technique, with the minimum of trauma, and were placed in small, dry, air-tight sterile jars for transportation, surrounded by ice in the large polystyrene box (described by Mueller, O'Neill, and Trevor-Roper, 1965) which we have found to be the most satisfactory method of transportation.

The eyes were examined at varying periods after death, ranging from 3 to 48 hours, and the following factors were carefully noted:

- (1) Age of the deceased.
- (2) Cause of death.
- (3) Temperature at which the body was refrigerated.
- (4) Temperature at which the enucleated eyes were stored.

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All these factors have been shown to affect the corneal integrity (Archer and Trevor-Roper, 1967). Throughout the period of examination a mask was worn to minimize contamination, and the eyes, untouched by hand, were gently manipulated towards the open end of the glass jar. The slit-lamp beam was then directed through the open end of the glass jar and the distribution of any changes was recorded. Examination through the glass wall of the jar was found to be impractical. All the eyes which showed signs of either keratitis, dystrophy, or anterior uveitis were excluded from the survey, and eyes which had been subjected to surgery were also not included.

The changes in the endothelium which we detected were seen in the posterior band of the slit-lamp beam. Examination of the endothelium in the zone of the specular reflex is more difficult in donor eyes, since the eye must be kept steady by fixation; however, localized patches of swelling of the endothelial cells can be seen after 6 hours in the zone of the specular reflex (Hassard, 1965). The more general changes in the posterior band of the slit-lamp beam which we are describing probably indicate a greater degree of disfunction of endothelial cells. Our initial examination in eighty eyes showed the presence of round discrete dot-like opacities on the posterior surface of the cornea (Fig. 1). Initially they have a white outer rim with a colourless centre, but later tend to become uniformly white in colour. When donor corneae are stored at 4°C. and are repeatedly examined over 24 hours these changes are invariably seen to some degree. Unfortunately oedema of the stroma and folding of Descemet's membrane progress rapidly once the initial 18 hours after death has been passed, and these in turn obscure the endothelium, making the changes more difficult to observe. The endothelial opacities tended to be scattered in a random distribution, occurring either singly or in clumps of two or three, and they are seen in both the axial area and in the peripheral cornea. Occasionally they appeared in lines with as many as seven or eight small discrete opacities forming the line, and this appearance would seem

FIG. 1. — Slit-lamp painting of circular endothelial opacities in donor cornea.

to indicate gross involvement of the endothelium, for in these eyes careful biomicroscopy revealed multiple isolated opacities scattered over most of the posterior surface of the cornea. Forty eyes when initially examined fell into this category, and the lines appear to be related to folds in Descemet's membrane, lying close beside and parallel to one of these folds, and usually extending out towards the periphery in a radial fashion. All the endothelial opacities described above are flat lesions flush with the corneal surface. When both eyes from a donor were examined the degree of endothelial involvement in the two eyes was almost identical in 95 per cent. of cases.

We have investigated the nature of these changes by histochemical staining. Eyes showing marked changes were selected and the total distribution of the endothelial opacities was carefully mapped out. In these eyes, the position of suitably distinct and characteristic linear opacities in relation to the limbus was marked by cautery burns of the sclera. The anterior segment of the eye was then separated with a scalpel from the posterior three-quarters of the globe, the iris and lens being removed from the former with forceps. The cornea with a rim of remaining sclera was then incubated at 37°C. for 90 minutes in a balanced salt solution, which contained the soluble colourless salt, para-nitro-blue tetrazolium and the substrate beta-hydroxy butyrate buffered at pH between 6.9 and 7.1. Under these conditions the para-nitro-blue tetrazolium is reduced to its insoluble prussian blue diformazan only in the presence of lysed mitochondria, which are found in irreversibly damaged cells (Rhodin, 1954). Thus degenerate endothelial cells stain blue while the viable cells remain colourless (Fig. 2).

FIG. 2.—Non-viable endothelial cells, darkly stained, after incubation with para-nitro-blue tetrazolium. $\times 100$.

The posterior surface of the cornea can also be examined using a Specular Microscope and this has revealed that each circular opacity is really a clump of two or three non-viable cells (Fig. 3, opposite). It was also apparent after staining that, in a cornea which showed these clumps of non-viable cells, there were many more single non-viable cells which could not be seen with the standard slit-lamp magnification. This demonstrated that, in a cornea which showed marked endothelial opacities on biomicroscopic examination, the whole endothelium was in a very degenerate condition.

FIG. 3.—Endothelial cells (individual) of donor cornea viewed with specular microscope.
(a) Normal cells. × 375.
(b) and (c) Normal cells plus clump of non-viable endothelial cells.

It is now generally agreed by most authorities that donor eyes should be enucleated within 10 hours of death to give a reasonable chance of a successful penetrating graft. However, we have detected marked endothelial opacities in at least 7 per cent. of eyes examined within this period. This further stresses the importance of careful slit-lamp examination of donor eyes immediately before grafting. The suitability of eyes for penetrating keratoplasty should not be based on clinical judgement of macroscopic appearance, aided by arbitrary time limits for the intervals between death and enucleation, and death and eventual grafting. Donor eyes should be assessed individually and neither used purely because they are fresh and from a healthy young donor, nor discarded if the donor is elderly.

In general these changes were found to be more marked when there had been a delay greater than 10 hours before enucleation. They were particularly obvious in hypotonic eyes with a tendency towards corneal indentation, and thus were seen very frequently in the eyes of the elderly. This further stresses how often donor material from bequeathed eyes is disappointing; at least 65 per cent. of such donors in our records were over 70 years of age.

However, since these changes were also visible in the eyes of young adults, other factors such as debilitating diseases (particularly chronic renal failure) must also play a part in their formation. Lack of refrigeration in mortuaries and careless enucleation and transportation of donor eyes all tended to accentuate these changes.

An attempt has been made to follow up the results of penetrating grafts in which donor corneae showing these endothelial opacities have been used. The assessment of these has proved difficult for many reasons, particularly variations in surgical technique and in the state of health of the recipient corneae. However, it is certain that a few isolated opacities do not appear to influence the eventual clarity of a penetrating keratoplasty. On the other hand, doubt has arisen over wisdom of using corneae which display large patches of these lesions, and it is felt that such corneae should be used only for lamellar grafting.

Summary

Four hundred eyes collected by the Westminster-Moorfields Eye Bank were assessed in an attempt to obtain some useful information as to the state of their endothelial viability. Examination of the posterior surface of many donor corneae with the slit lamp has revealed the presence of dot-like opacities, which have been shown by histochemical means to be aggregates of non-viable endothelial cells. It is suggested that the proportion of these opacities may prove a valuable yardstick in assessing the general level of endothelial viability in any particular donor cornea.

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