tion and irrigation of the anterior chamber, and although lens design with surface protuberances may be of some importance, it is clear that a biophysical interaction between methacrylate and the corneal endothelial surface produces extensive cell damage. It appears that the methacrylate adheres instantaneously to the endothelial surface, and with separation of the two surfaces the anterior membranes of the endothelial cells are torn off. The membranes are seen, by SEM, to remain adherent to the methacrylate surface.

Other substances such as glass and stainless steel are capable of producing similar endothelial damage. In contrast, the natural lens appears to cause no damage when it is touched to the endothelium. We feel, therefore, that this is a surface problem of these materials and is not due to the chemical makeup of the materials per se. When a portion of the glass sphere is covered with a soft lens, corneal endothelial damage occurs only in areas of direct contact between the endothelium and the glass.

If, in fact, the intraocular lens could be kept away from the endothelium and the chamber never lost, during lens implantation, the type of damage described in this investigation would not occur. It has been our experience, however, that placement of the intraocular lens into the eye with removal of the lens holder and suturing of the wound, is not always possible without brief touch between the endothelium and the lens. The risk of such touch is greatly increased when the vitreous face bulges forward. Because of the conclusive evidence that contact (no matter how brief) between lens and corneal endothelium can produce significant endothelial damage, we undertook a second investigation to see if we could modify the surface of the lens. We found that a variety of substances could prevent endothelial damage when the lens was dipped into the substance prior to being touched to the endothelium. Two patients have undergone uneventful lens implantation after the lens was dipped into protective solutions and cell loss was 11 per cent with a methylcellulose solution and 9 per cent with a polyvinyl pyrrolidone solution.6

The importance of endothelial damage at the time of surgery is not absolutely certain, but it is clear that if damage is extensive enough, corneal edema supervenes. Since the human corneal endothelium seems not to regenerate, it may be that if enough endothelial cells are lost at the time of surgery, even if the cornea clears, the death of additional endothelial cells with aging may result in an area of endothelial surface that can no longer be covered by the spreading cells. It seems likely, therefore, that late corneal edema may result in at least some of the patients. If endothelial contact with the methacrylate surface is a major cause of endothelial damage, and if such damage can be easily eliminated, it may not be necessary to withhold intraocular lens insertion from patients with mild endothelial changes. Also, the age criterion for lens insertion may become more flexible if further studies confirm our findings that intraocular lenses do not cause progressive damage to the endothelium and that the major cause of endothelial cell death at the time of surgery can be eliminated.


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A new technique for the vital staining of the corneal endothelium. DAVID J. SPENCE AND GHOLAM A. PEYMAN.

A new technique for the staining of the corneal endothelium combines the actions of the vital stain trypan blue and the intercellular stain alizarin red S. The technique is an improvement over former staining methods, because it defines both viable and nonviable cells and thereby permits an investigator to accurately quantify endothelial cell damage.

The vital stain trypan blue1-4 has been used for many years in the assessment of corneal endothelial viability. The technique is inexpensive and simple, and it is well suited for an overall determination of the endothelial status.

The disadvantage of the trypan blue method is that it is not as specific as is desirable. The dye enters and stains only those cells whose membrane permeability has been increased by cell wall damage. Healthy cells are therefore not delineated, and an accurate quantification of cell damage by grid counts is impossible. Using trypan blue, an investigator can only roughly estimate the...
degree of endothelial damage, and this is certainly a drawback.

This paper describes an improvement of the trypan technique, in which both viable and nonviable cells are delineated, and whereby accurate quantification of endothelial damage may therefore be accomplished.

**Methods.** New Zealand rabbits, weighing 2 to 3 kg., were killed by the intracardiac injection of a barbiturate, and the eyes were then removed intact. Some eyes were used immediately. Most, however, were stored in moist chambers at 4° C. before use. The storage period ranged from 1 to 6 days.

Before being stained, the corneas were carefully removed with a small rim of sclera and then separated into two groups. Corneas in the first group were placed in a room-temperature 0.9 per cent normal saline bath immediately following their excision. Some eyes were used immediately. Most, however, were stored in moist chambers at 4° C. before use. The storage period ranged from 1 to 6 days.

Before being stained, the corneas were carefully removed with a small rim of sclera and then separated into two groups. Corneas in the first group were placed in a room-temperature 0.9 per cent normal saline bath immediately following their excision. They were left to soak for 1 to 10 minutes and then were stained. Corneas in the second group were first exposed to a 2 hour period of temperature reversal, carried out at 37° C. in 10 ml. of tissue culture 199. They were then promptly transferred to a room-temperature 0.9 per cent saline bath, soaked for 1 to 10 minutes, and stained.

**Solutions.** The solution of 0.25 per cent trypan blue in 0.9 per cent normal saline contained 0.25 gm. of trypan blue and 100 ml. of 0.9 per cent normal saline. The combination of alizarin red S and normal saline contained 0.20 gm. of alizarin red S, 70 ml. of 0.9 per cent normal saline, and 30 ml. of 0.1 per cent ammonium hydroxide. In the ammonium hydroxide-saline solution were 0.1 ml. of ammonium hydroxide (chemically pure 28 per cent) and 100 ml. of 0.9 per cent normal saline.

**Staining technique.** The corneas were removed from the normal saline bath and placed in a corneal cup, endothelial side up. Enough trypan blue dye was dropped onto the tissue to cover the entire endothelial surface. After 1½ minutes, the corneas were picked up and the dye dropped off. The tissues were then immediately passed through two successive baths of 0.9 per cent normal saline. Very gentle agitation removed the excess trypan blue.

The tissues were again transferred to a corneal cup, and enough alizarin solution was applied to cover the entire endothelial surface. After 45 seconds, the corneas were picked up, the dye dropped off, and the tissues passed through a single bath of 0.9 per cent normal saline.

**Results.** Trypan blue used alone stained only
the damaged cells (Fig. 1). With the addition of alizarin red, both damaged and healthy cells were delineated (Fig. 2). The healthy cells were outlined by a hexagonal intercellular border, and the contents of these cells remained clear. In contrast, the damaged cells, which were still attached to Descemet's membrane, showed the alizarin-stained outlines plus the trypan-stained nuclei. Damaged areas in which cells had become dislodged from Descemet's membrane showed diffuse staining with both trypan and alizarin.

The staining remained very distinct for approximately 2 hours, provided the tissues were kept in a solution of 0.9 per cent normal saline. The results were identical with and without temperature reversal.

Discussion. The improvement offered by the proposed technique can be appreciated by a comparison of Figs. 1 and 2. It is apparent that the precise quantification of endothelial damage is possible only when normal cells are defined, as in Fig. 2.

Consistently good results were obtained, provided a few aspects of the staining procedure and solution preparation were followed carefully. For example, the length of exposure of the endothelial sheet to the staining solutions was strictly adhered to. Also, most of the saline solution was allowed to drop off the endothelium before proceeding to the next step.

Most importantly, in the preparation of the alizarin solution, the alizarin was completely dissolved in the saline mixture before the ammonium hydroxide was added. Failure to add the base at this time often resulted in the formation of a heavy precipitate. Even though the shelf-life of the intercellular stain appeared to be about 3 weeks, we prepared a fresh solution each day. Fresh solution seemed to improve the results.

We believe the proposed technique is a significant improvement in corneal endothelial staining. The procedure is simple and inexpensive, yet it allows for an accurate measurement of cell damage by grid counts. The method is not intended to supplant other means of endothelial assessment. Rather, it is offered as an additional useful evaluative tool.

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Key words: corneal endothelium, vital stain, intercellular stain, trypan blue, alizarin red.

REFERENCES


Electrical parameters of the isolated cornea of the dogfish, Squalus acanthias. OSCAR A. CANDIA, C. ADRIAN M. HOBGEN, AND PHILIP I. COOK.

The electrical potential difference and electrical resistance of the nonswelling cornea of the dogfish, Squalus acanthias, were examined. It was found that routine procedures used in the procurement of fish invariably produce damage to the corneal epithelium which affects electrical measurements and possibly composition of the aqueous humor. We found no electrical evidence of ionic pumps in the corneal epithelium of this elasmobranch. The electrical resistance of corneas with apparently well-preserved epithelium was 300Ω cm.² (compared to 300Ω cm.² in corneas with damaged epithelium).

It is well documented that the corneas of elasmobranchs do not swell when isolated and placed in distilled water or salt solutions. The nonswelling property of these corneas has been explained morphologically by the presence of sutureal fibers which bind the anterior and posterior limits of the corneal stroma. Because of the restriction imposed by the length of these fibers, the thickness of the corneal stroma cannot increase.

Corneal swelling which leads to opacity of this tissue is prevented in other species by the operation of metabolically dependent "pumps," presumably located in the endothelium and possibly in the epithelium. It has been shown in the rabbit cornea by Maurice and other investigators that the endothelium possesses a very active fluid pump that can account by itself for the maintenance of normal hydration. In the frog, Zadunaisky and