The phacoemulsification procedure.
II. Corneal endothelial changes

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The effect of phacoemulsification, with the Cavitron-Kelman instrument, on the corneal endothelium of rabbit and cats was studied by scanning electron microscopy and nitroblue tetrazolium staining. The various steps of the procedure were examined separately. Irrigation of the anterior chamber of the eye with balanced salt solution (Plasma-Lyte) for ten minutes caused no cell damage. Ultrasound and irrigation alone for four to six minutes caused increased permeability to NBT. Edema of endothelial cells and cell junction disruption occurred after eight minutes of anterior chamber irrigation with Plasma-Lyte. Uncomplicated phacoemulsification produced moderate cellular edema with scattered loss of endothelial cells. Destruction of endothelial cells was frequent after phacoemulsification, it appeared to be due to lens nucleus manipulation in the anterior chamber, instrumentation, and needle contact. From two to five days postoperatively, intercellular edema, altered cell morphology, and mosaic pattern were seen. However, it gradually recovered and seven to ten days later the endothelium appeared normal.

Aspiration and irrigation of infantile and juvenile cataracts through a modified hypodermic needle is a well-known surgical procedure. This technique, however, could not be used readily in adult or senile lenses due to the presence of a hard nucleus. Kelman in 1967, described a technique of extracapsular lens removal by means of an irrigating-aspirating needle which could be made to vibrate in a piston fashion at ultrasonic speed in order to break up hard lens material, a technique which he termed "phacoemulsification." Evaluation of a large series of cataracts removed by this technique indicated that the rate of complications was not different from that seen in cataracts removed by conventional techniques.

This new method of cataract removal requires that the surgeon be an accomplished operator through the surgical microscope and that he master the phacoemulsification technique in order to avoid trauma to the corneal endothelium, iris, or rupture of the posterior capsule, with projection of lens material into the vitreous. Kelman has published guidelines for the selection of patients, one of the most important of which is that the cornea be anatomically normal as judged by biomicroscopy.
Phacoemulsification is now a widely accepted surgical procedure and in many places is part of the formal surgical training for residents. Even though potential corneal complications have been mentioned, the histopathology of clinical or experimental cases has not been described. Corneal endothelial alterations due to prolonged irrigation with balanced salt solutions is the subject of another study, however, since anterior chamber irrigation is part of this procedure, it is also evaluated in this investigation. This paper describes corneal endothelial changes that could occur during the surgical procedure and the pattern of healing in experimental animals. Damage was usually due to manipulation of lens nucleus and instruments in the anterior chamber.

Materials and methods

Adult (3 kilograms) albino rabbits and adult mongrel cats were used for these studies. The instrument was the Cavitron-Kelman phacoemulsification machine, Model 7007. The following parameters were investigated: (1) the effect of irrigating saline or a balanced salt solution on the corneal endothelium, (2) the effect of ultrasonic vibration and irrigation of the anterior chamber on the endothelium, (3) endothelial changes following a complete phacoemulsification procedure. The times for steps 1 and 2 did not exceed five minutes, but the time for procedure 3 averaged 10 minutes. In general, we tried to duplicate an uncomplicated clinical situation where the length of time for the lens extraction averages 10 minutes. It is obvious that results of these experiments are based on the skill of the operators, controls are difficult to setup, however, they were arranged as follows. For Experiment 1, control eyes had no procedures done. Eyes from Experiment 1 were controls for Experiment 2 and these for Experiment 3. In this form alterations found in Experiment 3 could be separated from those which occurred in the two previous studies. Whenever a procedure had complications, the animal was separated from the study, except in one case described in Experiment 3.

Experiment 1. The effect of "Plasma-Lyte 148" solution (pH 7.4), which is normally used with the Cavitron-Kelman machine, was evaluated for periods of four to five minutes (flow rate 25 ml per minute). In two eyes 0.9 per cent more saline was used instead of Plasma-Lyte for a period of five minutes. Rabbits or cats whose pupils had been previously dilated with 10 per cent neo-

synephrine, were anesthetized with intravenous pentobarbital (30 mg. per kilogram). Under the operating microscope a 2.5 to 3.0 mm. limbal incision was made for the introduction of the titanium tip of the Cavitron-Kelman Phacoemulsifier. At the end of the specified period of irrigation, the needle was withdrawn, the eye was closed with one suture, and then a similar procedure was done in the opposite eye. After completing this procedure the animal was sacrificed, the eyes were removed, and the corneas carefully excised. One of the corneas was immediately incubated in a solution containing nitroblue tetrazolium (NBT 0.3 mg. per milliliter and DPNH 0.3 mg. per milliliter). The second cornea was fixed in cold, buffered 2.5 per cent glutaraldehyde solution for 24 to 48 hours. It was then postfixed in 1 per cent osmium tetroxide and processed for scanning electron microscopy (SEM). Sixteen eyes were studied.

Experiment 2. Ultrasound irrigation. After the rabbit or cat had been anesthetized and placed under the surgical microscope, the phacoemulsifier tip was introduced into the anterior chamber through a small limbal incision and the needle was made to vibrate at maximum power for five minutes, trying to keep the tip of the needle in the center of the anterior chamber closer to the lens than to the endothelium. At the end of the designed time, the needle was withdrawn, the anterior chamber reformed, and the eye was closed with one 8-0 silk suture, the procedure was repeated in the opposite eye. When the experiment was completed on the second eye, the animal was sacrificed, the eyes were removed and the corneas excised. Each pair of corneas was processed for NBT staining scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The only irrigating solution used in these experiments was Plasma-Lyte. A total of sixteen eyes were studied.

Experiment 3. Phacoemulsification procedure—the procedure used in cats (8 eyes) and rabbits (20 eyes) was similar to that used in humans and the steps will be briefly described: (1) limbal incision, (2) air injection into the anterior chamber, (3) capsulotomy and luxation of nucleus into the anterior chamber with a cystotome, (4) phacoemulsification with irrigation-aspiration of cortical remnants. Times and power settings varied in different cases (average 10 minutes), but in general the high power settings (9-10) were used. Cats and rabbits were sacrificed at daily intervals to study the process of endothelial healing and regeneration. One cornea of each animal was stained with nitroblue tetrazolium (NBT) and the fellow eye was processed for scanning electron microscopy (SEM) or transmission electron microscopy (TEM). The experiment was terminated at ten days when it was observed that
Fig. 1. Rabbit corneal endothelium after five minutes of anterior chamber irrigation with Plasma-Lyte and ultrasonic vibration for five minutes. There is mild cellular swelling, variations in cell morphology, but cell junctions appear tight. (SEM x2,000.)

Clinically, as well as histologically, the corneas in all cases had fully recovered.

Experiment 4. Controls. Four rabbit eyes had no procedure performed. The corneas were removed immediately after sacrifice. Twenty rabbit eyes had luxation of the lens into anterior chamber following cystotomy capsulotomy through a limbal incision. No irrigation or aspiration was performed and the corneas were excised immediately after luxation after previously refilling the anterior chamber with Plasma-Lyte.

Electron microscopy. Samples for scanning electron microscopy were dehydrated in a graded series of alcohol-acetone after being fixed in 2.5 per cent buffered glutaraldehyde and 1 per cent osmium tetroxide. They were then dried in a critical point Bomar machine, mounted on aluminum stubs and coated with gold-palladium. They were examined in a scanning electron microscope (Stereo-Scan, Cambridge, England) at 10 or 20 kv. Pictures were recorded on Polaroid P/N material. Selected specimens were processed for transmission electron microscopy following fixation in glutaraldehyde (2.5 per cent) and osmium tetroxide (1 per cent). Samples were examined in a Zeiss (EM9-S) microscope.

Results

These experiments indicate that the extent and degree of endothelial changes increase with each succeeding step in the phacoemulsification procedure, it is directly related to the length of the operation and particularly to anterior chamber instrumentation and lens manipulation.

Experiment 1. The Plasma-Lyte solution induced loss of microvilli, changes in cell shape, stretching of cell junctions, and slight swelling of the cell body of cat and rabbit endothelium (Fig. 1). In the five-minute period of continuous irrigation the cell junctions did not rupture. In the preceding paper we showed that this solution did not cause cell damage when run up to twenty minutes. The corneal endothelium of rabbit corneas which were irrigated intracameraliy with normal saline solution showed cell disruption, intercellular vacuolation, rupture of cell junctions and cell death (Fig. 2).

Experiment 2. Activation of the ultrasonic tip in the anterior chamber (plus irrigation) produced two types of alterations: increased permeability of the endothelial cells to the nitroblue tetrazolium stain and intercellular vacuolation. Corneal endothelium showed a diffuse deep blue staining of the endothelial layer (Fig. 3), in addition to scattered cytoplasmic vacuolation and intercellular vacuoles. Normal
Fig. 2. Rabbit corneal endothelium after five minutes of continuous irrigation of anterior chamber with 0.9 per cent saline solution. There is cell destruction, intercellular and cytoplasmic vacuolation. (SEM x2,000.)

Fig. 3. a, Flat endothelial preparation showing increased NBT staining, cytoplasmic and intercellular vacuolation after anterior chamber irrigation and ultrasound (5 minutes). b, Control shows minimal NBT staining. (Nitroblue tetrazolium stain, x200.)
Fig. 4. Central portion of rabbit cornea showing large areas of endothelial loss (*) after Experiment 3. These abrasions were caused by lens rubbing or instrument contact. (SEM x100) (En = endothelium, Des = Descemet's membrane.)

Fig. 5. Rabbit endothelial cells showing cytoplasmic vacuoles and ruptured membrane (x4,800). (AC = anterior chamber, Des = Descemet's membrane.)

endothelium stained with NBT shows minimal cytoplasmic staining, except at the cellular junction. The endothelium of some cat corneas showed less penetration of NBT; however, a mild degree of vacuolation in and around cells was observed. Scanning electron microphotographs of corneal endothelium exposed to five minutes of vibration (Power No. 7) and irrigation demonstrated mild to moderate endothelial edema. This may have been caused by the continuous irrigation.

Experiment 3 (Phacoemulsification-irrigation-aspiration procedure). Immediately after phacoemulsification multiple areas of cell destruction and areas of bare Descemet's membrane were observed (Fig. 4). Areas of cell destruction were round, oval, or elongated and varied from 9 to 12 μ to several millimeters in size. The smaller lesions were obviously not related to areas of needle contact to the cornea; other areas, however, suggested that the tip of the needle had touched the endothelium baring Descemet's membrane. Isolated defects in the endothelial surface which involved two or more cells were likely caused by projected lens fragments or air bubbles. These changes were also seen in cross-sections of the endothelial layer (Fig. 5). Fig. 6 is
an NBT-stained preparation showing a large endothelial defect probably caused by needle contact to the cornea or abrasion by lens material.

One of the most severe complications that may occur during this procedure is caused by overheating of the ultrasonic tip (Fig. 7). This occurred in a cat eye in which the irrigating solution was inadvertently cut off for several seconds. In addition to tissue overheating at the limbal incision, this eye showed areas of endothelial destruction as can be seen in Fig. 8, as well as multiple, small (10 to 30 μ), scattered areas of endothelial destruction which could have been caused by bubbling if not by projection of lens particles. This procedure was completed with maximal ultrasonic power and lasted 5 minutes.

24 to 48 hour stage. All of these corneas were clinically clear and of apparent normal thickness; however, no measurements were made. NBT preparations showed cytoplasmic staining in the area surrounding an injury. Cells of large size, at times binucleated with cytoplasmic vacuoles or large intercellular spaces, were often seen. The surrounding corneal endothelium showed staining along the intercellular space. Intracytoplasmic and intercellular vacuoles were still present in rabbit corneas, but cat corneas had almost normal endothelial structure as seen in transmission electronmicrographs (Fig. 9) even though NBT staining was present.

Scanning electron microphotographs of these clear rabbit and cat corneas showed that the endothelial layer was intact for the most part, but the regular mosaic-like arrangement of endothelial cells was altered and replaced by cells of different sizes and shapes. Elongated or fusiform shapes predominated with a tendency to produce large pseudorosettes. Amorphous cell debris and, occasionally, white cells were present on the endothelial surface. Most of the endothelial cells showed a profuse number of microvilli, occasional cilia and cytoplasmic expansions suggesting that some cell overlapping was present.

Two day stage. Nitroblue tetrazolium preparations of clear corneas at this stage showed isolated spots of increased cellular staining and occasional areas of dense staining where the cells had an elongated appearance. Corneas with mild to moderate degree of stromal edema showed areas of endothelial repair (Fig. 10). This area stained deeply with NBT as well as the surrounding regenerating cells.

Scanning electron microphotographs of clear corneas showed that most of the endothelial surface was acquiring the normal hexagonal arrangement, however, there were many areas with fusiform and giant cells. A similar pattern of regeneration was observed in cat corneas. Elongated and giant cells were observed near areas of focal regeneration. Macrophages and dispersed pigment granules were frequently observed on the endothelial surface of cat corneas.

Three to 7 day stage. NBT stained preparations of endothelium of clear cor-
Fig. 7. Damage caused by tip overheating. Rabbit corneal stroma and Descemet's membrane has puckered around the limbal opening. (SEM ×200) (En = endothelium.)

Fig. 8. Large areas of rabbit endothelial destruction (Des). These lesions are possibly caused by mechanical trauma and overheating. Small lesions (arrow) involve individual or multiple cells and might be caused by projected particles or bubbling. (SEM ×200) (En = endothelium.)
neulas at this stage were normal except for occasional areas of increased cellular staining which were always related to cells of abnormal shape and size. The endothelial surface had regenerated in most instances as could also be observed in SEM photographs. These cells, however, still showed a diffuse number of microvilli with enhanced delineation of intercellular spaces.

**Seven to ten day stage.** Clear corneas at this stage showed normal endothelial pattern and staining with NBT. Scanning EM
photographs showed normal appearance of the endothelial surface with recovery of the normal hexagonal shape of endothelial cells except for isolated areas where larger or smaller cells were present (Fig. 11). The number of microvilli on the cell surface was decreased and for the most part the cell junctions were tight and prominent.

Control corneas removed and processed for NBT staining or S.E.M. after no intraocular procedure showed no endothelial changes. In addition to Experiment 2 which actually was a control for Experiment 3, we found that luxation of soft lens material (young animals) into the anterior chamber caused areas of increased NBT staining in broad streaks or irregular patches in 8/10 eyes. Prolapse of hard lens material in larger rabbits caused similar patterns in 7/10 eyes but with actual loss of endothelium. These changes were confirmed in S.E.M. preparations (Table I).

Discussion

The potential corneal endothelial damage caused at various stages of the phacoemulsification procedure were analyzed in four separate experiments. The first step was to analyze the effect of the calcium-free balanced salt solution supplied with the phacoemulsifier and induced fluid turbulence, since the infusion-aspiration flow with this instrument is relatively rapid (15 to 25 c.c. per minute). It appeared, that neither the fluid volume or speed of infusion caused severe cell alterations for periods of five minutes in this study and up to 20 minutes in the previous one. Swelling appeared to be related mostly to the lack of calcium in the solution. The large amount of fluid that circulates through the anterior chamber with similar irrigating systems has been of concern to other investigators, leading them to advise a manually controlled aspiration by an assistant; however, with the Cavitron instrument a controlled flow is necessary for its proper functioning and needle cooling. Fast flow of the irrigating solution for periods up to five minutes caused minimal degrees of endothelial edema as demonstrated by NBT-stained flat endothelial
Table I

<table>
<thead>
<tr>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4 Control A</th>
<th>4 Control B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure</td>
<td>Plasma-Lyte irrigation, 5 min.</td>
<td>Ultrasound irrigation, 5 min.</td>
<td>Phacoemulsification, 10 min.</td>
<td>Lens Lux into anterior chamber</td>
<td>No procedure</td>
</tr>
<tr>
<td>NBT</td>
<td>No increased staining</td>
<td>Diffuse increased staining, cytoplasmic vacuolation</td>
<td>Increased staining, cell edema, vacuolation, cell loss</td>
<td>Focal staining, cell loss</td>
<td>No staining</td>
</tr>
<tr>
<td>TM</td>
<td>Not done</td>
<td>Normal or swollen cells</td>
<td>Cytoplasmic vacuolation, cell destruction</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>SEM</td>
<td>Endothelial cell edema, stretched cell junctions</td>
<td>Endothelial cell edema, stretched cell junctions, focal cell destruction</td>
<td>Cell edema, vacuolation and death, focal and diffuse cell destruction</td>
<td>Normal, with focal damage</td>
<td>Normal</td>
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Amount of cell trauma

0 ++ +++ + 0

Code: NBT = nitroblue tetrazolium stain; TM = transmission electron microscopy; SEM = scanning electron microscopy.

preparations and scanning electron microphotographs. We felt that five minutes of fast and continuous flow could be compared with a ten-minute procedure where a similar amount of fluid was used while the lens was emulsified. An irrigation of twenty minutes or longer with a calcium-free solution would produce changes which may not be present in most eyes in which this procedure was done.

Temperature measurements at the ultrasonic tip have been performed by others who found no thermal hazard when irrigation-aspiration flow was maintained. On two occasions, however, we were able to study the effects of the tip overheating on the corneal endothelium. In one eye, it was due to the obstruction of the inflow line and in the other, exhaustion of the irrigating solution for a few seconds. In these accidents, the destruction of the endothelium is widespread and shrinkage of Descemet’s and corneal stroma occurs if the tip is in contact with these tissues. Even through the silicone jacket, the overheating can produce puckering and contraction of the lips of the wound at the limbal area.

The possibility of tissue destruction by ultrasonic energy has been one cause for concern about the safety of such instruments in the eye. With the Cavitron instrument, though, (as well as with two other instruments which one of us has studied), the ultrasonic energy at the tip is minimal. The maximum energy intensity of 0.003W/cm² calculated at the tip is well below the upper limit of tolerance of 0.5W/cm²/5 minutes suggested by Baum for clinical application. A Japanese study of damage produced by an ultrasound tip within the eye determined that stress on ocular structures was less with the tip perpendicular to the ocular axis than parallel to it. The surgical technique, however, requires that the tip be used in a position parallel to the iris plane. Even if held over the lens surface, its vibration causes an increased permeability to NBT, in addition to intracytoplasmic vacuolation and separation of intercellular junctions; the last two, seemed to correspond to the irrigation effect and were less pronounced in the cat than in the rabbit cornea.

Endothelial changes of varying degrees consistently occurred when the lens nucleus was luxated into the anterior chamber and manipulated with the cystotome or the phacoemulsification tip. These changes have been observed in control eyes (luxa-
tion alone) without previous irrigation or ultrasound effect and are, therefore, related and common to all extracapsular techniques in which the lens rubs the back of the cornea. Even with the most gentle maneuvering the lens nucleus will touch and produce changes in the endothelial surface upon which the irrigating solution flow and ultrasonic vibration will have additional effect. These studies showed, however, that young cat and rabbit corneas partially regenerated the endothelium within 48 hours after surgery and fully recovered in seven days, very much as it occurs in most clinical cases, but these changes are a potential risk in aged corneas. For this reason, Kuwashara, in a series of studies paralleling those of Kelman, felt that a retro-pupillary technique of lens aspiration was preferable and reduced corneal damage. It should be noted that in a recent study with the scanning electron microscope, Emery found no endothelial damage in cat corneas after phacoemulsification. Our short-term studies have not allowed us to study changes at the level of Descemet's membrane following endothelial cell regeneration, but it is known that retrocorneal membranes can develop in the endothelial layer following the destruction of these cells by localized corneal freezing.

It has been claimed by several authors that the incidence of serious corneal complications in patients undergoing this procedure is small if cases are properly selected. The incidence of reversible striate keratopathy has varied from 6 to 23 per cent in some initial series and is attributed to excessive manipulation. In intra-capsular extraction, though, the incidence may be as great or greater. Of more concern is the occurrence of permanent corneal edema or opacification. This is variously reported 0 per cent, 1 per cent, 2 per cent, 0.4 per cent, 0.5 per cent, and 10 per cent in a recent series of 50 operations by experienced surgeons. The rate of complication tends to increase in patients over 60 years of age due to a higher incidence of senile endothelial changes, cornea guttata, shallower anterior chamber and denser cataract nucleus. One of the conditions which is a high risk for this type of operation is that of a patient with Fuchs' dystrophy without guttata, since these changes in corneas of normal thickness are difficult to detect by slit-lamp biomicroscopy and tend to occur in the older patients. At the present time however phacoemulsification patients are twelve (12) years younger (56.2 years) than patients who have intracapsular extractions.

Although the endothelial changes described here were rapidly reversed by endothelial healing and regeneration in healthy animals such healing may have a different course in humans. A note of caution is warranted in patients with senile lens and corneal endothelial alterations and particular attention should be placed in eyes with possible Fuchs' dystrophy without guttata. Careful pachymetry may detect these corneas if they appear thicker than normal. Possibly those with measurements over 0.5 mm. should not be candidates for this procedure based on our studies.

REFERENCES


