dexmethasone prevent the release of lysosomal enzymes and, as a result, suppress the catabolism of acid mucopolysaccharides. Although corticosteroids are demonstrated to stabilize the lysosomal membrane and to inhibit a lysosomal enzyme (aryl sulfatase) activity, lysosomal hyaluronidase activity in rabbit iris was not affected directly by dexamethasone. It is necessary to determine whether corticosteroids suppress the release of lysosomal hyaluronidase from rabbit ocular lysosomes.

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Key words: lysosomal hyaluronidase, rabbit iris, indomethacin, acetylsalicylic acid, dexamethasone, epinephrine, cyclic AMP

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A method for preparing a viable corneal endothelial layer, completely denuded of overlying stroma. HUNSON SOONG, JORGE FISCHBARG, and TAKEO IWAMOTO.

A method for denuding Descemet’s membrane by dissection of all overlying stroma, while preserving the corneal endothelial layer, was developed and evaluated. The technique consists in treating the stroma with trypsin and surgically removing the softened stromal layers. With an automated thickness-measuring technique, the endothelium-Descemet preparation was found to range from 23 to 42 μm in thickness. Endothelial cell morphology was normal under specular and light-transmission microscopic examinations. Under electron microscopy, the endothelial cells appeared intact, except for an increase in the number of intracellular vacuoles. Occasionally, small portions of an intercellular space were found to be mildly dilated, but the over-all integrity of the functional complex was intact. In vitro, the viability of the preparation was comparable with that of a cornea with all layers intact. Endothelial resting membrane potentials, measured with intracellular microelec-
trodes, were found to be within the normal (33 ± 2 mV) range. It is thus possible to obtain a viable endothelial layer, completely stripped of stroma.

Preparations of functionally intact corneal endothelium, completely denuded of stroma, are desirable in many instances. In in vitro experiments, such preparations provide data which are specific for the endothelium and are untainted by the presence of stroma and epithelium. In deep lamellar keratoplasty, where full-thickness stromal replacement is contemplated, there would be advantages to preserving the integrity of host endothelium and avoiding penetration into the anterior chamber. Sherrard (personal communication) maintains that Descemet’s membrane, with its smooth surface, may provide a good lamellar bed for grafting. Two major impediments to surg-

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cally denuding Descemet's membrane of all overlying stroma are (1) the inherent mechanical fragility of Descemet's membrane and (2) the firmness of stroma. This report describes and evaluates a reliable method of using trypsin to facilitate removal of all stroma under in vitro conditions.

**Materials and methods.** Male New Zealand albino rabbits weighing 2 to 3 kg were killed with an overdose of pentobarbital, and the eyes were enucleated. One eye was used for immediate dissection, and the second eye was stored temporarily in a moist, cold environment (7° to 8° C). The first eye was mounted in a special holder, and cornea trephination (8 mm diameter) down to mid-stromal level was performed. The epithelium within the trephined area was removed by blunt dissection, and the de-epithelialized surface was immersed for 15 min at 20° to 22° C in 47 mg/dl trypsin (bovine type III; Sigma Chemical Co., St. Louis, Mo.) dissolved in Ringers solution (Fig. 1). Following trypsin treatment, the stroma had almost a gel-like consistency. To arrest further digestion of the cornea, it was washed for 5 min in normal Ringer's solution that was stirred by a magnetic stirrer. It was then immersed for 15 min under gentle agitation at 20° to 22° C in 47 mg/dl soybean trypsin inhibitor (lyophilized type 1-S; Sigma) also dissolved in Ringer's solution; this inhibits trypsin on a mole-to-mole basis. After a final 5 to 10 min wash in normal Ringer's solution that was stirred by a magnetic stirrer. It was then immersed for 15 min under gentle agitation at 20° to 22° C in 47 mg/dl soybean trypsin inhibitor (lyophilized type 1-S; Sigma) also dissolved in Ringer's solution; this inhibits trypsin on a mole-to-mole basis. After a final 5 to 10 min wash in normal Ringer's solution, the cornea was ready to be dissected free of stroma. The stroma, softened by trypsin, was easy to dissect away, layer by layer, with the aid of a dissecting microscope, fine jeweler's forceps, and microsurgical scissors. As the last layer of stroma was carefully lifted with the forceps, a well-defined virtual space was realized between the stroma and Descemet's membrane. This deepest stromal layer was easily separated from Descemet's membrane, and the latter was identified by its distinct pearly sheen. Moreover, Descemet's membrane may be confirmed by applying Ringer's solution onto it—Descemet's membrane remains transparent, whereas stroma clouds up secondary to fluid imbibition. A small round section of about 1 mm in diameter was then cut out of the last stromal layer, consequently exposing Descemet's membrane. This hole was gradually enlarged by cutting around its edges with microsurgical scissors. Extreme care must be exercised to avoid contact of sharp points and edges of instruments with the membrane. Not only may Descemet's membrane tear easily, but the underlying endothelium may also be damaged by kinking, pressing, or drying of Descemet's membrane (Sherrard, personal communication). Following removal of all stroma within the perimeter of trephination, the cornea was mounted under a specular microscope fitted with an automatic attachment that allowed measurement of thickness without operator reading error. Specimen preparation time from enucleation to final mounting averaged 2 to 2½ hr. After mounting, the endothelial surface of the cornea was perfused with normal Ringer’s solution (37° C) at a rate of 0.38 ml/min with a pressure head of 15 cm H2O. Descemet's membrane was covered with silicone oil (Dow-Corning 200). Lacking structural support, the cornea totally denuded of stroma was prone to move in and out of focus in response to even minor fluctuations in perfusion pressure. Accurate thickness measurements and assessment of endothelial cell morphology were possible only if the perfusion pump was turned off during these critical periods which lasted 5 to 10 min. The Ringer's solution consisted of (mM): NaCl 110, KHCO3 3.8, NaHCO3 39, MgSO4 0.78, KH2PO4 1.0, CaCl2 1.7, glucose 6.9, adenosine 5.0, and oxidized glutathione 0.05.

At the conclusion of some of the experiments, after some 4 to 8 hr in vitro, only corneas which
Fig. 2. Light micrographs of denuded cornea. A, Cross-section of Descemet's membrane (D) and endothelium (E) after removal of full-thickness stroma. (H&E; 400×.) B, Cross-section of cornea at the perimeter of trephination. Epithelial layer (EP), partially digested stroma (ST), and layers of Descemet's membrane and endothelium (D&E) are seen in this view. (H&E; 60×.)

appeared to retain normal specular microscopic morphology were fixed for microscopic observation. This variation in incubation time did not have any significant effect on the structure of the fixed specimens as long as the fixation occurred before specular microscopic morphology showed any distortion. Eight corneas were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) for light-transmission microscopy. Additionally three corneas were fixed in 3.5% glutaraldehyde in 0.05M phosphate buffer and post-fixed in 1.0% osmium tetroxide in Caulfield buffer for electron microscopy.

Intracellular microelectrodes were used to record endothelial cell potentials from two trypsin-treated, de-epithelialized corneas. Glass-tipped micropipettes (20 to 150 MΩ) filled with 3M KCl were advanced into endothelial cells by a hydraulic driver, and cell potentials were measured. Only cells that were totally devoid of overlying stroma were impaled.

**Results.** The stromas of 20 corneas were treated with trypsin, and complete dissection down to Descemet's membrane was performed. In 17 of these, the procedure was successful, and perforation of Descemet's membrane was avoided. Of the 17, five corneas were washed only in Ringer's solution following the trypsin treatment, and 12 were treated additionally with soybean trypsin inhibitor. Endothelial cell morphology under
specular microscopy appeared normal in all 17 cases during the first 1 to 2 hr following trypsin treatment. After this initial period, however, the cells in the five preparations that were not post-treated with inhibitor evidenced morphologic changes indicative of suffering, such as loss of normal light reflectivity and loss or distortion of the regular hexagonal pattern. It follows that the trypsin dose needed to soften the stroma does cause cell damage if the enzyme is not inactivated with inhibitor immediately after the procedure. The 12 corneas that were treated additionally with inhibitor retained normal specular microscopic morphology up to 7 more hours under perfusion. However, after this period, progressive cellular distortion was evident in all cases. Thicknesses of healthy preparations ranged from 23 to 42 μm. The three failures, all occurring in the earliest attempts at dissection, were results of either trypsin overdose, with Descemet’s membrane eaten away (two cases), or accidental puncturing of the cornea (one case). In contrast, eight attempts to dissect down to Descemet’s membrane without the aid of trypsin were made; only one such case was successful.

Light-transmission microscopic evaluation of the successfully dissected specimens showed both the endothelium and Descemet’s membrane to be intact, with the latter being completely free of stroma (Fig. 2, A). Stroma exposed to trypsin (Fig. 2, B) showed disruption of the fibers.

Under electron microscopy (Fig. 3), Descemet’s membrane, endothelial cell ultrastructure, and junctional complexes appeared virtually normal. There was, however, an increased number of cytoplasmic vacuoles, mostly located in the basal portion of the endothelial cells and containing a finely filamentous substance whose identity remains yet to be determined. There was also an occasional dilatation of an intercellular space that contained this filamentous material, but the junctional complex was apparently undisturbed. It is possible that the filamentous substance is a secretory product for elaborating new Descemet’s membrane or a digestive product of phagocytosis, both of which may occur in reaction to chemical stimul-
lation. The variations were typical of the average specimen.

In order to assess survival of cell membrane potential following dissection, two corneas were used for microelectrode studies. A mean cell potential of 33 ± 2 mV (range 12 to 52 mV) was obtained up to 5 hr after trypsin treatment. This value is well within normal limits of undenuded corneal endothelium. It follows that the presence of stroma has little or no effect on endothelial resting membrane potential.

**Discussion.** Success in achieving survival of the endothelial layer was documented by normal cellular morphology under the specular microscope up to 8 hr at 37° C and by normal resting membrane potentials of up to 5 hr duration. This may be attributed to at least two factors: (1) an effective barrier provided by Descemet’s membrane to enzymatic degradation and (2) the immediate inactivation of trypsin by its inhibitor. The resistance of Descemet’s membrane to chemical reagents and likewise to pathologic processes is well documented. Descemet’s membrane has often been seen to offer resistance and remain intact even when the entire cornea has become purulent.

In vitro thickness measurements of the endothelial-Descemet preparation by specular microscopy were greatly facilitated by the total removal of stroma—the absence of stroma eliminated unwanted stromal planes of reflection. This preparation would be useful for experiments relating to corneal endothelial fluid transport or metabolism where the presence of stroma may introduce an additional factor. The use of the preparation in laboratory investigations is promising. Clinical use of this technique is only a conjecture and is by no means to be construed as practical in its present stage.

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**Key words:** Descemet’s membrane, stroma, endothelium, trypsin, soybean trypsin inhibitor, specular microscope

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**Cornea endothelial bicarbonate fluxes following preservation in solutions of varying composition. Keith Green, James G. Buyer, and David S. Hull.**

Numerous modifications have been made in MK solution, primarily concerned with alterations in both bicarbonate concentration and PCO2. Variations of the solutions from high bicarbonate to low bicarbonate and either high or low PCO2 were made which resulted in a decreased net bicarbonate flux across the endothelium after a 3-day storage period. Even with drastic changes in the storage solution there was a relatively small change in the net bicarbonate flux. Higher passive fluxes were found at a pH above 8, and a higher net flux with a bicarbonate concentration of 17.5 mM or higher. Variation in PCO2 made little difference to the net endothelial fluxes; when a high PCO2 was used, there was a concurrent elevation of PO2 in the storage solution, but no beneficial effect was found on the endothelial transport system. It was apparent that a good storage solution contained a buffer (bicarbonate was better than phosphate) and 5% dextran and was at a neutral pH (between 7 and 8).

Recent identification of a net movement of bicarbonate across the corneal endothelium, which is correlated with endothelial fluid transport, has lead to speculations regarding the enhancement of corneal preservation in media of varying HCO3− concentration. Hodson compared corneas stored in moist chamber, Ringer solution, or HCO3− and CO2-free solution for up to 8 days. The HCO3− and CO2-free solution maintained corneas near physiological thickness. When compared to MK solution, the two solutions were comparable in terms of the maintenance of corneal thickness.

Since we have recently identified changes in the