tion thus generated suppressed one half of the frequency-doubled response. The strength of the suppressive surround appeared to vary among the ON-OFF cells, since they exhibited a variable amount of frequency-doubled response at eccentric border positions.

Our experiments have shown that the X/Y classification based on a spatial linearity test works well for the Necturus retina. Cells which were found to be of the same type by independent response properties were also consistently and reliably found to respond similarly to the spatial linearity test. In addition, our results suggest that Necturus sustained ganglion cells (sustained-ON and sustained-OFF) are analogous to cat X ganglion cells and that Necturus transient-ON ganglion cells are analogous to cat Y ganglion cells. Although the ON-OFF cells could be classified as Y according to their linearity of spatial summation, they seem to be more analogous to the ON-OFF W cells of the cat retina due to their on and off responses, their large suppressive surround, and their other receptive field properties. Since linearity or nonlinearity of spatial summation appears to be an important feature of ganglion cells in the vertebrate retina, our finding of similar results in the Necturus may prove to be especially fruitful for generating a model of this phenomenon, since so much is already known of the synaptic interactions within this retina.

The E-I-E-I-O neurophysiological experiment management system was primarily written by Peter J. Goodeve (supported by NIH grant EY-00276 to H. B. Barlow) and generously supplied to our laboratory by A. B. Bonds. From the Center for Visual Science, University of Rochester, Rochester, N. Y. This study was supported in part by Contract N-14-76-C0189 between the University of Rochester and the Office of Naval Research and in part by National Institutes of Health grants 2P01 EY-01319 and 3701 EY-00086. Submitted for publication Nov. 8, 1978. Reprint requests: J. B. Tuttle, Center for Visual Science, University of Rochester, Rochester, N. Y. 14627.

Key words: Necturus, ganglion cell, spatial summation, X-like, Y-like, sinusoidal modulation, linearity

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The structure and function of basement membranes have been the subject of extensive investigation. The present study takes advantage of a new experimental procedure to yield ultrastructurally pure basement membranes and applies this methodology to Descemet’s membrane, a highly specialized ocular basement membrane. Rabbit Descemet’s membranes and associated endothelial cells were mechanically isolated without contaminating stromal elements. The endothelial cells were then solubilized and removed by treatment with detergents as verified by light microscopy and scanning and transmission electron microscopy. The Descemet’s membrane remained intact and retained its fibrillar fine structure. Therefore purity of starting material for ongoing morphological and biochemical studies of isolated Descemet’s membranes is demonstrated. These investigations will provide a valuable data base for comparison with disease-altered Descemet’s membranes.
Descemet’s membrane is an unusually thick and highly specialized basement membrane (BM) which lies subjacent to corneal endothelial cells in the vertebrate eye. Since this BM and its associated extracellular matrix are altered in numerous disease states and are relatively accessible for study, they have been the subject of numerous morphological and biochemical investigations.

Historically, most investigators have isolated Descemet’s membrane by mechanical methods. However, with this procedure stromal and/or endothelial cell contamination must be actively avoided. Ultrasound has also been employed in an effort to reduce cellular and other non-BM impurities, but this procedure is difficult to control and often results in fragmentation and morphological disruption of the membrane.

In the present study, we apply a standardized, reproducible procedure which utilizes detergents to solubilize cell membranes and remove other debris. This yields an isolated Descemet’s membrane which is structurally pure and provides an excellent starting material for experimental morphological and biochemical analyses.

Materials and methods. Eyes from 8-week-old New Zealand white rabbits were enucleated. Corneas were excised and placed in Spinner’s Minimal Essential Medium with 28 mM HEPES buffer, pH 7.2. Descemet’s membranes and associated endothelial cells were teased from the underlying stroma. Endothelial cells and other non-BM debris were removed following treatment with detergents. Briefly, tissues were osmotically shocked with distilled water (1 hr) prior to treatment with 3% Triton X-100 (2 to 4 hr) and a distilled water rinse. This was followed by exposure to DNAse (2000 Kunitz units) in 1M NaCl for 2 hr and 4% sodium deoxycholate for 2 to 4 hr at room temperature. Following extensive rinsing with distilled water, isolated Descemet’s membranes were prepared for morphological observation.

Control and detergent-treated Descemet’s membranes were fixed 1 hr at 4°C in glutaraldehyde-paraformaldehyde fixative followed by postfixation in 2% OsO4 in 0.144M sodium cacodylate buffer. Following ethanol dehydration some tissues were embedded in Epon-Araldite for light and transmission electron microscopy (TEM), and others were critical point–dried in CO2, placed on aluminum specimen stubs, and coated with carbon and gold palladium prior to observation by scanning electron microscopy (SEM).

Results. When Descemet’s membrane and associated endothelial cells were isolated mechanically, the tissue curled inwardly (Fig. 1, a), and although keratocytes and other stromal contaminants were not present (as verified by light microscopy of random samples) corneal endothelial cells closely adhered to the posterior surface of the membrane where they maintained their epithelial characteristics.

Following treatment with detergents, cells were lysed and plasmalemmae solubilized. The corneal endothelium was completely removed, although Descemet’s membrane remained morphologically intact (Fig. 1, b). These observations were confirmed by SEM, which showed that the membrane was not fragmented or morphologically disrupted (Fig. 2).

Although light microscopic observations (Fig. 1, b) suggested that both the stromal and endothelial surfaces of Descemet’s membrane were smooth and uniform, TEM of cross-sections through the membrane showed that opposite surfaces were morphologically disparate (Fig. 3). One of the surfaces was sharply demarcated, the immediately subjacent portion of the membrane was finely granular, and the opposing surface was rough and fibrillar. This is not surprising, since the anterior and posterior portions of the in vivo Descemet’s membrane differ morphologically. Most importantly, however, the membrane was completely free of cellular contamination and other non-BM debris. Therefore we were confident that isolation by detergent solubilization yields a nonfragmented morphologically pure Descemet’s membrane which provides as excellent starting material for biochemical analyses. In fact, approximately 65% of the collagen in these isolated Descemet’s membranes can be extracted following a brief pepsinization and collagen purification scheme (unpublished data).

Discussion. BMs (including Descemet’s membrane) are extracellular structures which function in support, filtration, and compartmentalization. It has been shown that these electron-dense extracellular matrices are composed of noncollagenous and collaginous components. Controversy exists regarding the number of collagen species and their chemical interactions in BM, and it seems possible that some of the discrepancies may be related to the methods employed to isolate the BM for analyses. The importance of obtaining noncontaminated BM for chemical analyses therefore is evident and is corroborated by studies which indicate that reported differences in BM composition may be attributed to impurities in the starting material.
Fig. 1a. Light micrograph of mechanically isolated Descemet’s membrane. Corneal endothelial cells adhere closely to the membrane (arrows), whereas stromal contaminants are not seen. (Toluidine blue; ×500.)

Fig. 1b. Light micrograph of mechanically isolated Descemet’s membrane treated with sodium deoxycholate. Note absence of cellular material. (Toluidine blue; ×375.)
Fig. 2. Scanning electron micrograph of the turned up edges (arrows) of deoxycholate-treated Descemet's membrane. The membrane has a sheetlike appearance. Both stromal and endothelial surfaces of the isolated Descemet's membrane are visible (a and b). (×9300.)

Fig. 3. Transmission electron micrograph of adjacent surfaces of curled portion of Descemet's membrane. Note the smoother endothelial cell surface as compared to the more fibrillar nature of the stromal surface. (×18,900.)
It is generally accepted that morphology is the best criterion for determining the purity of BM preparations.\textsuperscript{16-11} In the present investigation Descemet's membrane and associated endothelial cells were mechanically removed from the posterior surface of the cornea. Preservation of the morphological integrity of the membrane was effected by avoiding the usual isolation methods of sonication and centrifugation and employing a reproducible detergent solubilization procedure.\textsuperscript{5, 6, 14} to yield morphologically pure Descemet's membrane.

These detergent-treated membranes are free of corneal endothelial cell contamination, are not fragmented, and appear as rigid sheets of extracellular matrices which are ultrastructurally indistinguishable from their in vivo counterparts. Furthermore, our results are consistent with studies of other BM obtained by detergent solubilization, which show amino acid and carbohydrate profiles virtually identical to those isolated by conventional sonication.\textsuperscript{5, 6}

In 1974, Perlman and coworkers\textsuperscript{12, 13} used trypsin to separate Descemet's membrane from corneal endothelial cells following 2 to 3 weeks in vitro incubation of Descemet's membrane-endothelium explants. Although this isolation procedure offers a potential alternative to the method described in the present study, caution is required because the effect of trypsinization on the integrity of Descemet's membrane has not been studied in depth. Preliminary ultrastructural studies suggest that at least some BMs are markedly digested by trypsin.\textsuperscript{14} This is consistent with the analyses by Kefalides\textsuperscript{15} of ovine and canine Descemet's membrane, which showed that approximately 6.4% of the amino acids in these structures are lysine or arginine, both of which are known to be involved in trypsin-sensitive peptide bonds.\textsuperscript{16} Moreover, 75% of the carboxylic portion and 71% of the peptide portion of Descemet's membrane are solubilized following treatment with trypsin.\textsuperscript{15} Therefore it seems reasonable to suggest that the use of trypsin for isolation of Descemet's membrane carries risks of altering significantly its morphological and chemical properties.

In summary, detergent treatment is a simple method for producing ultrastructurally pure, non-fragmented Descemet's membrane. This procedure has the obvious advantage of histarchitectural preservation for experimental morphological studies and also assures purity of the starting material for biochemical analyses presently being carried out in our laboratory. We are also applying this standardized isolation procedure to other ocular and nonocular BMs to allow morphological and biochemical comparisons of BM isolated by identical methodology.

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Key words: Descemet's membrane, cornea, corneal endothelium, basement membranes, rabbit

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12. Perlman M., and Baum, J. L.: Synthesis of a collage-
Activation of initial wound healing responses in rat corneas in organ culture by mesodermal growth factor. Virginia L. Weimar.

In organ culture, mesodermal growth factor (MGF) from mouse submaxillary glands stimulates fibroblast formation, mitosis, and migration of cells into the dead-cell zone adjacent to the wound edge of rat corneal buttons. A classic dose-response relationship between the width of the stromal fibroblast zone and the dose of MGF suggests that fibroblast zone width may be used as a measure for screening agents which influence the initial stages of corneal wound healing.

Normal corneal wound repair has been accepted as occurring in three stages: the lag, fibroblastic or cellular, and cicatricial phases.† The lag phase was so named because it was considered, until recently, to be a time when the wounded cornea waited passively for the invasion of healing cells.‡ Weimar has shown, however, that the lag phase is in fact a phase of explosive activity in which the events determining wound repair are initiated.§ These earliest stages of corneal wound healing have been reproduced in organ culture.¶ This provides a model system in vitro in which those processes which initiate and regulate the initial stages of corneal stromal wound repair may be studied in a controlled synthetic environment.

The initial stages of corneal wound healing include a gradual transition of quiescent fibrocytes adjacent to the wound edge to spindle-shaped fibroblasts within 24 hr of wounding. This transformation is characterized by cell hypertrophy, increased intensity of staining, and the development of numerous large nucleoli. These cellular changes have been quantified in cultured corneas by means of an image analysis computer§ and have served as guides in the isolation of two factors (F-2 and mesodermal growth factors (MGF)) with potent growth-stimulating activities for corneal stromal cells from rabbits.† One of these factors, MGF, has recently been shown to accelerate corneal wound healing in vivo in the rabbit. 19

In this report we show that MGF is also a potent growth-stimulating agent for corneal stromal cells of another species, the rat. In addition, MGF also stimulated endothelial cell enlargement and mitosis.

The stromal responses could be rapidly quantified by measuring the width of the zone of activated fibroblasts in coronal sections. This method is discussed with regard to the assay of potential stimulatory agents for wound healing.

Materials and methods. Organ cultures were prepared from the corneas of Sprague-Dawley rats, about 200 gm in weight and of either sex. The animals were sacrificed by decapitation, and the eyes were enucleated into Ringer’s solution. The cornea was dissected with a small rim of sclera retained for handling. The iris and lens were excised, and 3 mm corneal buttons were cut with a trephine. The buttons were soaked for 20 min in sterile organ culture medium prior to culturing. The composition of the organ culture medium was as follows: Eagle’s minimum essential medium (Earle’s base) containing phenol red and supplemented with glutamine, 2 mM/L; Eagle’s nonessential amino acids, each 0.1 mM/L; penicillin, 100 U/ml; streptomycin, 100 μg/ml; fungizone, 0.25 μg/ml; FeSO₄·7H₂O, 0.426 mg/L; CuSO₄·5H₂O, 0.238 mg/L; ZnSO₄·7H₂O, 0.143 mg/L; MnCl₂·4H₂O, 0.053 mg/L; CoCl₂·6H₂O, 0.105 mg/L; and (NH₄)₆Mo₇O₂₄·4H₂O, 0.114 mg/L. For culture, the buttons were transferred by loop to 2 ml of organ culture medium in Falcon cluster dishes. The buttons (3 buttons/well) were floated on the medium which contained 0 to 10 μg/ml MGF added in a volume of 10 μl of distilled water. All buttons were incubated for 48 hr at 39°C in a humidified air-CO₂ incubator.

At the end of the culture period, the buttons were fixed in Carnoy’s solution overnight, rehydrated by passage through successively lower concentrations of alcohols, water, and then frozen in liquid nitrogen where they may be stored indefinitely. Frozen buttons were serially sectioned tangentially to the surface of the cornea, starting from the anterior surface. Tangential sectioning permits the examination of cellular detail which is not visible in sagittal sections. The sections were stained simultaneously in Giemsa stain (to minimize slide-to-slide variations in stain intensity) and were mounted in Permount.

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