
The corneas of 12 eye donors with maturity-onset diabetes were obtained, and the corneal epithelial basement membranes were studied by transmission electron microscopy. Similar tissue was obtained from 12 nondiabetic eye donors who were matched for age (within 2 years) and race. The mean thickness of the corneal epithelial basement membrane in nondiabetic patients was 0.33 μm (±0.11 S.D.), which gives a normal range of 0.11 to 0.55 μm. None of the nondiabetic basement membranes lay outside this range. The basement membranes of four of the 12 diabetics exceeded this thickness. No race or sex difference was seen in basement membrane thickness, nor was a clear trend seen with age. Multilaminated basement membranes were seen in eight diabetic patients and six nondiabetic patients. Multilamination was more clearly related to basement membrane thickness than to the presence or absence of diabetes.

It is well established that capillary endothelial basement membrane abnormalities are a feature of diabetes mellitus. Thickening of the endothelial basement membrane is the most easily recognized change. As yet, however, little attention has been directed to the basement membrane of surface ectoderm-derived epithelial cells elsewhere in the body.

Since the introduction of vitrectomy for treatment of diabetic vitreous hemorrhage, the corneas of patients with diabetes have received closer attention. During vitrectomy the corneal epithelium of diabetic patients often becomes edematous and cloudy; to restore clarity and allow the operation to proceed, the corneal epithelium in these patients has to be removed. Such patients subsequently have considerable difficulty with corneal re-epithelialization. The corneal epithelium heals poorly and is loosely attached to the underlying basement membrane, forming "mounds" or irregularities with a tendency to produce recurrent erosions. Key factors predisposing to these corneal epithelial complications include the presence of diabetes, preexisting decreased corneal sensation, the surgical debriement of the corneal epithelium, or cataract extraction performed at the time of vitrectomy. The basement membrane of corneal epithelium removed from diabetic patients during vitrectomy was found to be thicker than that from nondiabetic patients, and in some diabetics it was multilaminated.

The present study systematically examines post-mortem tissue obtained from diabetic and nondiabetic eye donors to see whether diabetes was associated with corneal epithelial basement membrane abnormalities.

### Methods

Eleven whole eyes and one pair of corneas from diabetic eye donors were obtained from the Medical Eye Bank of Maryland. These eyes were collected from 12 consecutive eye donors who were diabetic. Corneas were also obtained from 12 nondiabetic eye donors who were matched for age and race with the diabetic donors. The eyes from the nondiabetic donors were also collected consecutively. The age of the nondiabetic eye donors was matched within 2 years with the age of the diabetic donors. Because of the difficulty in obtaining the tissue, it was not possible to match for sex. Six eyes came from diabetic females, whereas only two control eyes were from females. All 12 of the diabetic eye donors had maturity-onset diabetes, and all had been treated with insulin at some time.

The whole eyes were initially fixed in 10% formaldehyde. A 4 mm segment of temporal cornea was removed by cutting vertically through the cornea and then around the limbus. This was done so that the globe was not destroyed for subsequent light microscopy. The cornea was then placed in McCarey-Kaufman medium. The corneal buttons were also sectioned 4 mm from the limbus to give a corneal segment. This was placed in the glutaraldehyde-formaldehyde fixative. After fixation, the blocks were washed in S0rensen's phosphate buffer, treated with buffered 2% osmium tetroxide, dehydrated through a graded series of acetones, and embedded in Mollenhauer's mixture of Epon epoxy resin and Araldite. After embedding, the blocks were cut into sections and stained with uranyl acetate and lead citrate for transmission electron microscopy.

### Table I. Occurrence of multilaminated corneal epithelial basement membrane in 12 diabetic/nondiabetic pairs (odds ratio, 1.67)

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<th>Diabetic</th>
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<tr>
<td>Multilaminated</td>
<td>3</td>
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<tr>
<td>Not multilaminated</td>
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Fig. 1. Light photomicrograph showing the generalized distribution and uniformity of thickening of the corneal epithelial basement membrane in diabetic patients. A, Basement membrane of normal thickness. B, Thickened and multilaminated basement membrane. Arrows delineate basement membrane width. (PAS stain.)

were aligned and trimmed so that vertical sections could be cut through the midpoint of the straight side of the corneal segment, that is, through the central cornea 4 mm from the limbus. Vertical sections 500 Å thick were stained first with uranyl acetate and then counterstained with lead citrate and were examined on a JEOL 100-B transmission electron microscope.

The corneal epithelial basement membrane was measured in transmission electron micrographs in a masked fashion by two observers, with neither observer knowing whether a particular micrograph belonged to a diabetic or a nondiabetic patient. For each cornea 12 measurements equally spaced along the basement membrane were made on a representative photograph (×35,000), and the mean and standard deviation were calculated. If the two observers differed in their measurements, their results were averaged. This procedure was repeated on photographs (×10,000) of adjacent but nonoverlapping areas of basement membrane.

Results. Thin sections were always examined by light microscopy before electron microscopy; when whole eyes were obtained, light microscopy was routinely performed. Corneal epithelial basement membrane thickening could be detected by light microscopy and was best seen with periodic acid–Schiff staining. When basement membrane thickening was observed, it was seen as a fairly uniform increase in thickness along the length of the membrane in the central cornea (Fig. 1).

The measurements of corneal epithelial base-
Fig. 2. Graph showing the mean corneal epithelial basement membrane thickness by age for 12 paired diabetic and nondiabetic eye donors. Measurements made on photomicrographs taken at 35,000×. Error bars, 1 S.D.

The mean thickness of the epithelial basement membrane in the nondiabetic corneas was 0.33 μm (S.D. 0.11 μm). This gave a "normal" range of 0.11 to 0.55 μm (mean ± 2 S.D.). None of the nondiabetic basement membranes lay outside this range (Figs. 2 and 3). In four of the 12 diabetic corneas the epithelial basement membrane thickness exceeded 0.55 μm, the upper limit of the "normal" range. This difference is significant (chi-square test, 6.13; p = 0.01).

Eleven corneas had basement membranes 0.33 μm thick or less (the mean for nondiabetic donors), and two of these were multilaminated, whereas of the 13 membranes that were thicker than 0.33 μm, 12 were multilaminated. The one that was not was 0.34 μm. Eight of the 12 basement membranes from diabetic eye donors were multilaminated, whereas only six of 12 from nondiabetic eye donors were. However, an analysis of pairs showed that there were five pairs in which the diabetic partner had a multilaminar basement membrane and the nondiabetic partner did not, and three pairs in which the basement membrane of the nondiabetic partner was multilaminated and that of the diabetic partner was not (Table I). This gave an odds ratio for the occurrence of a multilaminar basement membrane in a diabetic of only 1.67, which is not statistically significant.

No clear association could be found between age, race, sex, or the duration of diabetes and corneal epithelial basement membrane thickness. Although it was not possible to accurately determine the time of onset of diabetes in all the diabetic donors, the basement membrane measured only 0.25 and 0.37 μm in thickness in the two who had had diabetes the longest (18 and 20 years). A survey of the severity of diabetes, past medical history, cause of death, autopsy findings, and specimen handling did not reveal any factor or factors that correlated with basement membrane thickness.

Whole eyes from 11 of the 12 diabetic eye donors were studied by light microscopy. The
diabetes-related pathology of these eyes did not seem to correlate with the corneal epithelial basement membrane thickness. There were two patients with "background diabetic retinopathy" who also had neovascularization, but their corneal epithelial basement membrane was only moderately thickened (0.74 and 0.42 μm). Two of the three patients with the thickest basement membranes had no other ocular diabetes-related changes identified by light microscopy, and the only change seen in the third was hyalinization of the basement membrane of the ciliary body.

**Discussion.** Attention has been drawn to the difficulties of measuring the thickness of basement membranes. Previous reports have usually concentrated on the measurement of capillary basement membranes, for which the effect of oblique sections through vessels must be taken into consideration. Oblique sectioning is much less a problem with cornea, since it is possible to embed and align the cornea accurately. In addition, a section that was 15 degrees away from the vertical would increase the thickness by less than 5%.

In our study every effort was made to examine the same area of cornea in each case, that is, central cornea 4 mm from the limbus cut tangentially to the limbus. The blocks containing the corneal segments were semitransparent, and they could be accurately aligned. Unfortunately, the same fixative was not used for all specimens. As outlined in the Methods, whole eyes were initially fixed in formalin. Most of the diabetic corneas came from whole eyes, whereas only the minority of nondiabetic corneas did. Although fixation artifact could account for small differences in basement membrane thickness, it is unlikely that it could cause the sevenfold increase in thickness seen in some cases.

**Fig. 3.** Transmission electron micrographs of corneal epithelial basement membrane. **A,** Normal appearance in a diabetic donor. **B,** Moderately thickened and multilaminar in a nondiabetic donor. **C,** Grossly thickened and multilaminar in a diabetic donor. **Arrows,** Basement membrane width.
Conclusions based on electron microscopy are always subject to sampling errors caused by the very small piece of tissue contained in each section. The high magnification of electron microscopy, however, does add a degree of precision to measurement not attainable at lower magnification. In our study we confirmed by light microscopy the generalized distribution of corneal epithelial basement membrane thickening seen in some diabetic patients. We quantified this increase in thickness at high magnification (×35,000), where measurements were thought to be more accurate and reproducible. Finally, we verified these measurements by examining adjacent areas at a lower magnification (×10,000). We consider therefore that the measurements of the corneal epithelial basement membrane made at high magnification are truly representative of the central cornea.

Corneal epithelial basement membranes were examined by Kenyon et al. The material they examined was somewhat selective because it was obtained from diabetic patients who had advanced diabetic retinopathy with vitreous hemorrhage that was severe enough to warrant surgery. Further, the corneal epithelial status was such that the epithelium became opaque during surgery and had to be removed. The patient age and indication for surgery in the 15 nondiabetic patients they studied were not given. They reported that the epithelium obtained from diabetic patients tended to have an intact basement membrane with it. In the nondiabetic patients the epithelium separated through the basal cell layer. This was associated with a rupturing of the basal cells, and presumably little basement membrane remained for study.

Our study is the first to specifically examine the intact corneal epithelial basement membrane in diabetes and to include appropriate controls. The tissue was obtained from eye donors and was matched for age and race to reduce the effect that these variables may have had on corneal epithelial basement membrane thickness, since it is known that capillary basement membrane thickness increases with age. Further, the specimens were examined from intact corneas, with the basal epithelial cells and their basement membrane retaining a normal relation to Bowman’s membrane and the underlying corneal stroma. Although Descemet’s membrane was examined in many of the electron microscopy sections, no obvious abnormalities were found.

Our study showed that there was a considerable variation in the thickness of the corneal epithelial basement membrane, although in one third of the diabetic patients this membrane was significantly thicker than that of the nondiabetic controls and lay outside the “normal” range. Our study found no clear relation between corneal epithelial basement membrane thickness and age or sex. This may be because most patients studied were over the age of 50 and after this age there is little change in capillary endothelial basement membrane thickness and a sex difference is no longer apparent. Also, no clear association could be found between the duration of diabetes and the corneal epithelial basement membrane thickness. This lack of correlation may well be an effect of the small number of patients in this study, since other studies on capillary basement membrane thickness have shown a clear increase in thickness with increasing duration of the disease.

Although multilaminated basement membrane changes were more commonly seen in the diabetic patients than in the nondiabetic, the presence of multilaminated basement membranes was more closely related to basement membrane thickness than to the presence or absence of diabetes. The increase in basement membrane thickness in diabetes has been attributed to both an increased synthesis of basement membrane and a decreased degradation, possibly associated with an excess of glycosylation. Sorbitol accumulates in the corneal epithelium of diabetic patients, and it has been suggested that it may contribute to the poor adhesion. It is possible that sorbitol may interfere with the normal glycosylation of the basement membrane. It is therefore of considerable interest that 50% of the basement membranes from nondiabetic eye donors were multilaminated. There is no clear explanation for the occurrence of multilaminated basement membranes, although it has been postulated that they are related to cell death and regeneration caused by focal injury to the basal cells.

In conclusion, this study of corneas obtained from age-matched and race-matched pairs of diabetic and nondiabetic eye donors showed that (1) the corneal epithelial basement membrane was sometimes, but not always, abnormally thickened in diabetics; (2) multilamination of basement membranes was present in corneas from some diabetics and some nondiabetics; and (3) multilamination of basement membrane correlated more closely with basement membrane thickness than with the presence or absence of diabetes.

We thank the technicians of the Medical Eye Bank of Maryland for their full cooperation in obtaining the eye
donor tissues used in this study and also Dr. W. Richard Green and Dr. Susie Humphreys for their help and advice.


Key words: diabetes mellitus, corneal epithelium, basement membrane, transmission electron microscopy, multilaminated basement membrane

REFERENCES


Effect of arachidonic acid on normal and dystrophic retinal pigment epithelium in tissue culture. BRENDA J. TRIPATHI AND RAMESH C. TRIPATHI.

With carmine particles used as markers, no significant difference was observed between the phagocytic activity of 1- to 3-week-old confluent monolayer cultures of normal and dystrophic retinal pigment epithelial (RPE) cells harvested from 1-day-old Royal College of Surgeons rats. The phagocytic activity of both normal and dystrophic RPE was markedly depressed in a medium containing 1 μg/ml arachidonic acid (AA), and the cells rapidly assumed a rounded profile. With 100 μg/ml AA, the phagocytic activity of dystrophic RPE was differentially reduced compared with that of the normal sample (p < 0.001); this effect was subsequently accompanied by a gradual change in the shape of the cells. Lower concentrations of AA (3 μg/ml and below) did not produce a significant effect in either group.

Arachidonic acid (AA) is the major polyunsaturated fatty acid (PUFA) constituent of retinal pigment epithelium (RPE), accounting for some 16.6% by weight of total phospholipids. AA is also present in phospholipids of the rod outer segments (5.6% by weight) together with the other PUFAs, docosahexaenoic acid (23% by weight), and trace amounts of linoleic and linolenic acids. Elevated levels of plasma AA have been reported in some patients with primary pigmentary degeneration of the retina. Fibroblasts cultured from one patient who had pigmentary degeneration of the retina and progressive spinocerebellar degeneration incorporated greater amounts of 14C-arachidonate into triglyceride and esterified cholesterol than did normal human fibroblasts. These studies led to the hypothesis that a defect in arachidonate metabolism may adversely affect the normal functioning of the RPE, at least in patients with some types of retinitis pigmentosa.

This report is concerned with the effect of varying concentrations of AA on the morphology and phagocytic activity of the RPE cultured from normal and dystrophic rats of the Royal College of Surgeons (RCS) strain.

Materials and methods. Cultures of RPE were initiated from 1-day-old dystrophic rats of the RCS pink-eye strain and from their congenic controls, RCS+dy* rats. The animals were descendants of breeder pairs kindly supplied by Dr. M. M. LaVail. The animals were anesthetized with sodium pentobarbital and were washed in three changes of sterile saline. In a sterile environment and with the aid of a dissecting microscope, the lids were opened and the eyes were enucleated. The globes were opened at the ora serrata, and the retina was dissected out from the posterior segment. The RPE cells were gently recovered from the posterior segment by microdissection and transferred to a cover glass of a Sterilin disposable tissue culture chamber containing TC 199 culture medium (Gibco) with 10% fetal calf serum and 10% penicillin/streptomycin antibiotic at pH 7.4.