Corneal Hydration Control in Normal and Alloxan-Induced Diabetic Rabbits

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Reports of increased corneal thickness and altered endothelial morphology suggest that there is abnormal corneal hydration control in diabetic patients. To study the possible influence of hyperglycemia on corneal hydration control, experiments were done on normal and alloxan-induced diabetic rabbits to assess: (1) stromal dry weight, hydration, and swelling pressure; (2) corneal thickness and contact lens-induced edema recovery responses; and (3) endothelial homogenate sodium/potassium adenosinetriphosphatase (Na+/K+ ATPase) activity. The data show that 10 weeks of uncontrolled hyperglycemia in the rabbit results in abnormal corneal hydration control indicated by increased corneal thickness, increased stromal hydration, and a decreased ability to recover from contact lens-induced corneal edema. The stroma appears to be minimally involved in these changes; swelling pressures and dry weights of the normal and diabetic stroma were not significantly different. The measured decrease in diabetic rabbit endothelial homogenate Na+/K+ ATPase activity strongly suggests that endothelial fluid pump dysfunction is a major component in the abnormal corneal hydration control found in the uncontrolled diabetic rabbit. Invest Ophthalmol Vis Sci 31:2205-2213, 1990

Although retinopathy and cataract are the most dramatic ocular signs of diabetes mellitus, diabetes is a systemic disease influencing the entire eye. A wide range of corneal signs have been reported in diabetes including: increases in corneal thickness,1 decreased overall corneal oxygen consumption,2 abnormal wound repair,3,4 decreased corneal sensitivity,5-7 variations in endothelial permeability and fluid pump activity,8 alterations in endothelial morphology,9-11 and alterations in endothelial ratios of reduced to oxidized nicotinamide-adenine dinucleotide.12 Thus, the cornea of the poorly controlled diabetic is abnormal in many ways.

The primary function of the cornea is to provide a transparent refracting window for the eye. Corneal transparency is dependent on the maintenance of a relative degree of dehydration in the strongly hydrophilic stroma, mainly through the action of fluid pumping mechanisms in the corneal endothelium.13-15 Sodium/potassium adenosinetriphosphatase (Na+/K+ ATPase) is a major component of this mechanism; corneal administration of ouabain (a specific inhibitor of Na+/K+ ATPase) results in a marked increase in stromal hydration and corneal thickness.16-19 Reductions in Na+/K+ ATPase activity were reported in a number of diabetic tissues.20-27 These reports suggest that decreased enzyme activity, and fluid pump function, may also be found in the diabetic corneal endothelium. A reduction in fluid pump activity could also be associated with a number of changes, e.g., an increase in stromal hydration and corneal thickness and a decrease in the ability of the cornea to recover from stromal edema. Abnormal stromal hydration may also be due to changes in the mass or hydrophilic nature of the stroma. Changes in the hydrophilic nature of the stroma are possible since alterations in glycosaminoglycan metabolism have been reported in diabetic animals.28 To clarify the influence of hyperglycemia on corneal hydration control, experiments were done on normal and alloxan-induced diabetic rabbits to assess: (1) stromal dry weight, hydration, and swelling pressure; (2) corneal thickness and contact lens-induced edema recovery responses; and (3) endothelial Na+/K+ ATPase activity.

Materials and Methods

Establishing the Diabetic Rabbit Model

Fifteen 9-week-old male New Zealand White rabbits were obtained from a local supplier (Ray Nichols Rabbitry, Lumberton, TX). They injected in the marginal ear vein with a saline solution of 110 mg/kg alloxan.29 The diabetic state was verified by determining the blood glucose level before and 2 days after injection using an Ames Blood Glucometer II (Miles Inc., Elkhart, IN). Only animals with blood glucose
levels over 200 mg/dl were used in the study. Blood glucose levels were assayed weekly to assure that the diabetic condition was maintained during the 10-week period before measurements were begun. The blood glucose data are shown in Figure 1. Five of the alloxanized group of 15 were eliminated from the study because their blood glucose levels dropped below the diabetic criterion level during the 10-week adaptation period. The diabetic study group thus included ten rabbits. Diabetic signs in the alloxan-induced diabetic rabbits included hyperglycemia, increased thirst, increased urination, and sudden-onset cataracts of varying degree.

Ten normal age- and sex-matched rabbits were also obtained and concurrently reared under the same conditions to provide the control group. All animals were housed in the Animal Care Unit of the University of Houston (AALAC approved) under a 12-hr/12-hr light-dark cycle (lights on at 7 AM). Animals were allowed free access to food (Purina Rabbit Chow, St. Louis, MO) and water and were handled in accordance with the ARVO Resolution on the Use of Animals in Research.

Statistical Analysis

Statistical analysis was done by analysis of variance using the SAS Language (Cary, NC) for Personal Computers. Tukey's test $(P = 0.05)$ was done when multiple comparisons were necessary.

Experiment 1: Corneal Thickness

Central corneal thickness measurements were made by lightly touching the water-filled probe of an ultrasound pachometer (Storz Corneo-Scan 2000, St. Louis, MO) to the tear film of the rabbit cornea and obtaining six readings whose mean was used as the recorded measurement. Since the rabbits did not appear to be uncomfortable during this procedure, no anesthetic was used. The probe was hand held and the position of the central cornea judged visually. This procedure has been shown to be valid due to the relative constancy of rabbit central corneal thickness.30

The corneal thicknesses of the right eyes of the experimental rabbits (19 weeks old) were measured at 2-hr intervals for 24 hr using an ultrasound pachometer set at a tissue velocity of 1580 m/sec.30 The mean of these readings was used to establish the baseline corneal thickness for each rabbit.

Experiment 2: Corneal Edema Recovery

Edema production: Corneal edema was produced with a thick soft hydrogel contact lens (+11.00D 3; Bausch & Lomb, Rochester, NY) worn behind the closed right eye for 2 hr.31-33 At the end of this time, the eye was briefly opened, the lens removed, and the corneal thickness quickly measured. This procedure was done on all rabbits. A control experiment, in which the eyes were closed for 2-5 hr with no contact lens worn, was done to establish the baseline edema due to eye closure alone.

Edema recovery: Both open-eye and closed-eye recovery were examined. First after inducing corneal edema, both eyes were opened, and the corneal thickness of the right eye was measured every 30 min for 3 hr. Then after inducing corneal edema, both eyes remained closed and were opened briefly only during the measurement process. Corneal thickness measurements were taken hourly on the right eye for 4 hr. These procedures were done on all rabbits.

Experiment 3: Stromal Hydration

One week after completing the edema-recovery trials, the animals were killed with an intravenous injection of T-61 Euthanasia Solution (Hoescht, New York, NY). The cornea of the right eye was immediately removed, the endothelium and epithelium gently scraped away, and an 8-mm button trephined out of the stromal tissue. The wet weight of the button was quickly assessed on an analytical balance (Mettler H311, Zurich, Switzerland). The button was then placed in a desiccating oven (105°C) for 24 hr and the dry weight obtained. Stromal hydration (mg water/mg dry weight) was calculated as the difference between the wet weight and the dry weight, divided by the dry weight. The method is essentially that of Freidman and Green.34

Experiment 4: Stromal Swelling Pressure

The left corneas from the rabbits of the previous experiment were used in this procedure. Immediately
after death the cornea was removed, the epithelium and endothelium gently scraped away, an 8-mm button trephined out of the central stromal tissue and stored at -10°C until use.

Stromal swelling pressures were measured with the method of Olsen and Sperling. An apparatus was constructed from an electronic balance (Mettler PC 4400, Zurich, Switzerland). Any force acting on the balance was countered by an equal and opposite force generated by the balance that maintained the balance in its zero position. The force generated was displayed by the instrument as a weight reading (g). A small plastic water bath was placed on the balance pan. A disc of porous steel was glued to the end of a micrometer screw gauge, which in turn was solidly attached to an external frame attached to the balance. When the stromal button was placed on the water bath under the porous steel, its thickness could be measured by the micrometer gauge and the swelling force recorded as a weight (g). This force can be converted to mm Hg by multiplying the swelling force by 1.456 (g/mm² to mm Hg). At the time of the experiment, a stromal button was removed from the refrigerator and allowed to thaw and partially dry at room temperature for 30 min. The zero position of the screw was established, and the micrometer gauge turned down to zero plus 50 μm thickness. Saline was added and the balance quickly zeroed. Swelling forces were recorded each 5 min for 30 min at each thickness setting (50, 100, 200, 300, and 400 μm). Twenty to 30 min were needed for the stromal swelling force to reach a steady equilibrium with the countering force from the balance. Thus each experiment required approximately 3 hr to complete.

Experiment 5: Endothelial Na+/K+-Stimulated ATPase Activity

The corneal endothelial scrapings obtained from the previous experiments were assayed for Na+/K+ ATPase activity using the method of Whikehart and Soppet. In this procedure the two endothelial scrapings from both eyes of an individual rabbit were pooled into 500 μl of ice-cold distilled water and homogenized. An aliquot of this homogenate was then incubated in a water bath at 37°C for 1 hr in a medium of 2 mM adenosine triphosphate, 2 mM MgSO₄, 60 mM NaCl, 0.1 mM Ethylene Glycol-Bis (Beta Amino Ethyl Eter) Tetra Acetic Acid (EGTA), and 50 mM Tris HCl (pH 7.5) to determine Mg++-stimulated ATPase activity. Total ATPase activity was assessed in a similar manner except that 5 mM KCl was added to the medium. The Na+/K+ ATPase activity was defined as the difference between the total and Mg++-stimulated activities. A control experiment in which 1 mM of ouabain was added to both incubations confirmed this assumption. At the end of the incubation period, the reaction was stopped with 30% trichloroacetic acid and a dye marker added. The inorganic phosphate (P₄) released was then spectrophotometrically determined against a standard series. The activity was expressed as μmol of P₄ released/mg protein/30 min. Protein was determined using the standard Pierce Bicinchoninic Acid (BCA) (Rockford, IL) assay.

Results

Corneal Thickness

The baseline corneal thickness of the diabetic rabbit was found to be 373 ± 14 μm which is significantly thicker (P < 0.005) than that of the normal rabbit at 350 ± 14 μm. All estimates of error mentioned in this paper, unless otherwise specified, will be ± one standard deviation.

Edema Recovery

No significant difference (P > 0.25) was found between the edema induced in either the normal (29 ± 9 μm) or the diabetic (30 ± 11 μm) groups after 2 hr of eye closure when no contact lens was worn. The edema induced from eye closure remained constant with little variation over an additional 3 hr in both normal (35 ± 13 μm) and diabetic groups (32 ± 11 μm). Two hours of eye closure while wearing the contact lens resulted in significantly greater corneal edema in the normal rabbit (79 ± 10 μm) than in the diabetic rabbit (59 ± 8 μm) (P < 0.0001).

The results of the open-eye and closed-eye edema recovery experiments for normal and diabetic rabbits are shown in Figures 2 and 3. In the open-eye condi-
Fig. 3. Closed-eye recoveries from contact lens-induced corneal edema for normal and diabetic rabbits. Error bars represent ±1 SEM. The curve drawn through the normal data represents the best-fitting second-order polynomial regression.

It was found that corneal thickness recovered to baseline thickness in a nonlinear manner within 3 hr of opening the eyes and removing the contact lens. In the closed-eye condition, it was found that corneal thickness recovered to near closed-eye baseline thickness in an almost linear manner after about 4 hr of removal of the contact lens.

Linear-regression analysis was used previously to determine the approximate initial edema recovery rate in normal and aged human cornea. This analysis method was applied to the data from the normal and diabetic rabbits.

Because the rate of edema recovery may depend on the level of initial edema and differing levels of initial edema were produced by wearing the thick soft contact lens in the two groups, it was first considered necessary to compare edema-recovery rates from similar levels of initial edema. When calculated from data for 2 hr of edema recovery from an edema level of near 60 μm, the normal rabbit closed-eye corneal edema recovery rate (14.3 ± 1.7 μm/hr) was significantly faster (P < 0.005) than that of the diabetic rabbit (4.3 ± 1.4 μm/hr). Similarly, when calculated from data for 1.5 hr of edema recovery from an edema level of near 50 μm, the normal rabbit open-eye corneal edema recovery rate (27.3 ± 6.7 μm/hr) was also significantly faster (P < 0.05) than that of the diabetic rabbit (19.2 ± 9.8 μm/hr).

Since the edema-recovery rate may independent of the initial level of edema, edema-recovery rates were also calculated for both closed-eye and open-eye edema recovery conditions for, respectively, the first 2 hr and 1.5 hr after lens removal. In the closed-eye condition, the normal rabbit (8.7 ± 5.6 μm/hr) recovered from lens-induced corneal edema at a significantly faster rate (P < 0.05) than the diabetic rabbit (3.9 ± 3.9 μm/hr). Similarly, in the open-eye condition, the normal rabbit cornea (31.1 ± 4.6 μm/hr) recovered from edema at a significantly faster rate (P < 0.05) than the diabetic cornea (20.9 ± 9.8 μm/hr).

These data indicate that the initial rate of edema recovery of the diabetic rabbit cornea, as found by linear-regression analysis, is significantly slower than that of the normal rabbit cornea irrespective of whether the initial edema recovery rate is or is not dependent on the initial level of corneal edema.

Nonlinear-regression analysis portrays a more realistic model of corneal edema-recovery dynamics. The model can be expressed as:

\[ T = B + S_i \exp\left(-Dt\right) + e \]

where T is the instantaneous corneal thickness (μm), B is the baseline corneal thickness (μm), \( S_i \) is the induced corneal swelling (μm), D is a calculated deswelling factor, t is the time after the start of the edema recovery (min), and e represents the error term of the mathematical model (ie, measurement error). This expression adequately predicts the open-eye edema recovery in normal human subjects of various ages and in elderly subjects with Fuchs' corneal endothelial dystrophy. The expression also provides clinical indices of corneal hydration control such as (1) a corneal deswelling rate in percent recovery per hr, and (2) a time to remove 95% of the initial edema (T95%). These edema-recovery constants can be calculated by entering the raw corneal thickness data from the edema-recovery experiments into a nonlinear regression procedure (SAS NLIN: Marquardt method). For a more detailed explanation of this protocol, see Mandell et al. and Polse et al.

This analysis can be applied to the corneal edema-recovery data for normal and diabetic rabbits. The results of this analysis are shown in Table 1. In the open-eye condition, the normal rabbit cornea (41.4 ± 11.3%/hr) recovered from lens-induced edema at a significantly faster rate (P < 0.01) than the diabetic cornea (26.0 ± 11.5%/hr).

Table 1. Corneal edema recovery constants calculated from the exponential model using nonlinear regression analysis

<table>
<thead>
<tr>
<th>Condition</th>
<th>PPRH (%/hr)</th>
<th>T95% (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-eye</td>
<td>Normal</td>
<td>41.4 ± 11.3</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>26.0 ± 11.5</td>
</tr>
<tr>
<td>Closed-eye</td>
<td>Normal</td>
<td>25.6 ± 10.7</td>
</tr>
<tr>
<td></td>
<td>Diabetic*</td>
<td>—</td>
</tr>
</tbody>
</table>

Error term represents ± 1 SD. Ten rabbits were used in each group.

* Data did not fit criteria for exponential model.
Table 2. Hydration and dry weights of corneal stroma buttons from normal and diabetic rabbit corneas

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromal hydration (mg water/mg dry weight)</td>
<td>3.43 ± 0.25</td>
<td>3.77 ± 0.28</td>
</tr>
<tr>
<td>Stromal dry weight (mg)</td>
<td>5.49 ± 0.51</td>
<td>5.27 ± 0.28</td>
</tr>
</tbody>
</table>

Estimates of measurement error are ± 1 SD. Ten stromal buttons were used in each group.

rabbit cornea (26.0 ± 11.5%/hr). The T95% was also significantly greater \( P < 0.01 \) in the diabetic rabbit cornea (12.1 ± 6.0 hrs) than in the normal rabbit cornea (6.2 ± 2.5 hrs). Similar comparisons were not done for the closed-eye condition because the diabetic rabbit data could not be modeled using the exponential model. The ability of the model to predict the normal rabbit closed-eye edema-recovery data, but not the diabetic rabbit closed-eye edema-recovery data, is interpreted as demonstrating that the closed-eye edema-recovery response is abnormal in the diabetic rabbit cornea.

Stromal Swelling Pressure

The stromal swelling pressure results for normal and diabetic rabbits are shown in Figure 3. A simple linear relationship was found between log swelling pressure (mm Hg) and stromal thickness (μm) in both normal and diabetic rabbits. No significant difference was found between normal and diabetic rabbit stromal swelling pressures over the range of 50-400-μm stromal thickness \( P > 0.25 \).

Stromal Hydration

The stromal hydration results for normal and diabetic rabbits are given in Table 2. The diabetic rabbit stroma (3.77 ± 0.25 mg water/mg dry weight) was found to be significantly more hydrated \( P < 0.01 \) than that of the normal (3.43 ± 0.25 mg water/mg dry weight). The stromal button dry weights for the normal (5.49 ± 0.51 mg) and the diabetic (5.27 ± 0.28 mg) groups were not significantly different \( P > 0.25 \).

Endothelial Na+/K+-Stimulated ATPase Activity

The results of the endothelial homogenate ATPase assays for both normal and diabetic rabbits are given in Table 3. The diabetic Na+/K+ ATPase activity was significantly reduced compared with the normal Na+/K+ ATPase activity \( P < 0.05 \). No statistical difference was found between the diabetic total ATPase and Mg++ ATPase activities \( P > 0.05 \), between the Mg++ ATPase activity of the normal and diabetic groups \( P > 0.05 \), or between the protein determinations for normal and diabetic rabbit endothelial homogenates \( P > 0.25 \).

Discussion

Corneal Thickness

The average central corneal thickness of the normal adult (19-week-old) rabbit was found to be 350 ± 14 μm which agrees with previous reports by Prince and Walkenbach. Ten weeks of hyperglycemia resulted in a significant increase (23 μm) in corneal thickness for age- and sex-matched alloxan-induced diabetic rabbits. These results agree with a similar finding of increased corneal thickness in a study of type I human diabetic patients.

Corneal Edema Recovery

Two hours of contact lens wear induced less corneal edema in the diabetic rabbit (59 μm) than in the normal rabbit (79 μm). Wearing a thick soft contact lens causes a hypoxic environment in the corneal epithelium. Lactic acid produced by hypoxic epithelial metabolism is thought to diffuse into the stroma and draw water into this tissue by raising its osmotic pressure. If epithelial metabolism is reduced in the diabetic state, then it is possible that lactic acid production may also be decreased, reducing the edema produced by epithelial hypoxia. Some support for this possibility is found in a study by Graham et al who reported a reduction in corneal epithelial oxygen consumption of 42% and 35%, in diabetic rat and human subjects, respectively. Thus, the decreased levels of edema seen in the diabetic rabbit may be a sign of what they term the “generalized metabolic malaise” of diabetes.

Table 3. Corneal endothelial homogenate ATPase activities for normal and diabetic rabbits

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total ATPase</th>
<th>Mg++ ATPase</th>
<th>Na+-K+ ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.036 ± 0.003</td>
<td>1.01 ± 0.05</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.035 ± 0.003</td>
<td>0.80 ± 0.06*</td>
<td>0.69 ± 0.07</td>
</tr>
</tbody>
</table>

All ATPase activities are expressed as μmol P i released/mg protein/30 min. Protein concentrations are given as mg protein/50 μl homogenate. Estimates of errors of measurement are ± 1 SEM.

* Difference between the normal and diabetic group means is statistically significant (Tukey Test, \( P = 0.05 \).
The measurement of the recovery from contact lens-induced corneal edema has been proposed as a clinical test to allow assessment of normal corneal hydration control.\textsuperscript{31-33,37,38,44,45} Prolonged recovery times have been reported in elderly patients\textsuperscript{33,38} and in cases of Fuchs' endothelial dystrophy,\textsuperscript{37,45} with decreased endothelial fluid pump activity being proposed as a possible cause. Diabetes has been associated with altered endothelial morphology\textsuperscript{9-12} and biochemistry.\textsuperscript{12} When combined with the previous reports of prolonged corneal edema-recovery times in cases of assumed endothelial dysfunction, these findings suggest that diabetic subjects could also have prolonged edema-recovery times. Our results confirm this in the diabetic rabbit, with the diabetic animals having a significant reduction in their ability to recover from contact lens-induced corneal edema.

Our study also shows the various problems associated with the use of linear-regression analysis for the calculation of initial edema-recovery rates. When using this method number of assumptions are required. For example, it is assumed that the edema-recovery rate is linear over the first 1.5 hr after lens removal in the open-eye experiment and that the rate of edema recovery is or is not dependent on the initial level of edema. These assumptions are limiting and still need to be verified. A further difficulty with the linear-regression method is that a valid baseline corneal thickness must be established. This is difficult in the rabbit due to the known diurnal variation of its corneal thickness.\textsuperscript{46,47}

The nonlinear exponential model, on the other hand, is free of these assumptions and offers a more powerful and realistic clinical model. It is interesting to consider the diabetic rabbit closed-eye edema-recovery data. These data were not able to be modeled using the nonlinear regression method. Does this indicate a flaw in the model? Since the model adequately predicts the normal rabbit closed-eye edema-recovery data, it would seem not. This result suggests that the diabetic process results in a major alteration in the rabbit closed-eye corneal edema-recovery response in such a way as to make it functionally dissimilar to normal. Further study is needed to confirm this suggestion.

**Stromal Hydration**

The normal rabbit stromal hydration data from this study (3.43 mg water/mg dry weight) agree well with literature values (3.0-3.5 mg water/mg dry weight) reported by Freidman and Green.\textsuperscript{34} The increased diabetic rabbit stromal hydration values of 3.77 mg water/mg dry weight, combined with the finding of no significant difference between the dry weights of the normal and diabetic stromal buttons, suggest that the increase in corneal thickness in the diabetic rabbit was primarily due to an increase in the amount of water in the stroma rather than an increase in actual stromal mass.

**Stromal Swelling Pressures**

A recent study\textsuperscript{48} argued that the increased corneal thickness in patients with localized scleroderma (morphea) may be due to alterations in the hydrophilic character of the stroma from changes in the various components of the glycosaminoglycan matrix. Alterations in glycosaminoglycan metabolism have been reported in the vascular system of the diabetic rat,\textsuperscript{28} after transient corneal edema,\textsuperscript{49} and in certain corneal disorders such as lattice degeneration and keratitis.\textsuperscript{50} The increase in corneal thickness seen in the diabetic animal may be due to an increase in the hydrophilic nature of the stroma. Our finding of no significant difference (Fig. 4) between the swelling pressures of the normal and diabetic rabbit stromal buttons argues against this proposal. The primary site of corneal alterations occurring in the diabetic rabbit may lie outside the stroma.

**Endothelial Na+/K+ ATPase Activity**

The normal rabbit endothelial homogenate Na+/K+ ATPase activity measured in this study of 0.36 \( \mu \)M P\textsubscript{i}/mg protein/30 min agreed well with the values reported in the literature (0.28\textsuperscript{51} and 0.53\textsuperscript{52} \( \mu \)M P\textsubscript{i}/mg protein/30 min). A 69% reduction in the Na+/K+ ATPase activity was found in the diabetic rabbit endothelial homogenate. Reductions in Na+/K+ ATPase activity or pump site concentra-
tions have also been reported in glycosylated bovine lens epithelium, during the development of galactosemic cataract, in the retina of the alloxan-treated rabbit, in the heart and skeletal muscle, and the renal glomeruli of the streptozotocin-treated rat, in the sciatic nerve of the alloxan-treated rat and in the peripheral nerves of the streptozotocin-treated rat, the BB Wistar rat, and the alloxan-treated rabbit. Thus, it would seem that the hyperglycemic condition of diabetes is associated with a reduced Na+/K+ ATPase activity across a wide range of tissues, including the corneal endothelium.

The interpretation of assay results which use crude endothelial homogenates in the assessment of endothelial Na+/K+ ATPase activity may be questioned since confounding effects from intracellular organelles may occur. However these effects are considered to be relatively small; studies showed that most of the endothelial enzyme is located in the lateral plasma membranes. There is a procedure to isolate purified endothelial plasma membranes, and a future study using purified plasma membranes is planned to allow a more accurate quantification of the reduction of Na+/K+ ATPase activity occurring in the diabetic state.

How is hyperglycemia linked with reduced Na+/K+ ATPase activity? There are two main hypotheses. Garner and Spector suggest a local inhibition where hyperglycemia may result in the formation of an intermediate Schiff base which then stabilizes by an Amadori rearrangement, resulting in the inactivation of the enzyme through restriction of conformational changes. They provide some experimental evidence to support this claim. Another proposal by Greene and Lattimer, summarized by Winegrad, suggests that increased blood glucose levels result in a cascade of effects across a wide range of physiologic systems. Increased glucose may inhibit the transport of myo-inositol into the cell. This could result in a decrease in phosphatidylinositol hydrolysis, a decrease in diacylglycerol formation, a reduction in protein kinase C activity, and finally a decrease in Na+/K+ ATPase activity. The decreased Na+ transport increases the negative feedback into the system, eventually leading to a marked overall decrease in Na+/K+ ATPase activity. Various evidence supports this model. Decreased amounts of myo-inositol have been measured in diabetic nerves, and reduced myo-inositol may limit metabolic turnover of membrane phosphoinositol which is associated with a possible protein kinase C-mediated decrease in Na+/K+ ATPase activity. This decrease can be reversed by myo-inositol supplementation or by administration of protein kinase C agonists or gangliosides. It would therefore seem possible that hyperglycemia may have both a local and systemic effect in reducing the activity of corneal endothelial Na+/K+ ATPase.

Epithelial Involvement

Although the involvement of the epithelium in corneal hydration control was not addressed by this study, it is useful to consider the possible influences of diabetes on the anterior cornea and how these changes may affect corneal hydration control. Diabetic corneal epithelial changes reported in literature include increased fragility, increased permeability, altered collagen synthesis, abnormal basement membrane formation, and increased glycogen and glucose storage. Of particular interest to this discussion is the finding of a fivefold increase in epithelial permeability to fluorescein in long-term type II diabetic patients, suggesting that loss of the epithelial barrier function may play a significant part in the corneal edema seen in the diabetic rabbit. It should be noted, however, that the influence of aging on epithelial permeability was ignored in this study.

The influence of changes in the precorneal tear film also depends on the integrity of the epithelial barrier. Reports of increases in the glucose concentration of the tears could either aid stromal dehydration by increasing the osmolarity of the tears or decrease in the epithelial barrier function is normal, or if the barrier function is compromised, increase stromal osmotic pressures and result in stromal edema. A fluid pump has been reported in rabbit and human corneal epithelium, and although the ability of this pump to deswell the stroma is small, it may be influenced by the hyperglycemic precorneal tear film. The epithelial transport mechanisms are thought to be primarily concerned with maintaining the normal hydration of the epithelium itself.

Thus, it can be seen that current knowledge concerning the impact of diabetic epithelial changes on corneal hydration is sparse. Further studies are needed to assess the contribution that diabetic epithelial changes play in the loss of normal corneal hydration control in the diabetic patient.

Conclusion

This study showed that 10 weeks of uncontrolled hyperglycemia in the rabbit results in increased corneal thickness, increased stromal hydration, and a decreased ability to recover from contact lens-induced corneal edema. The stroma does not appear to be greatly involved with these changes because the swelling pressures and dry weights of the normal and diabetic stroma were not significantly different. When combined with previous reports of altered dia-
abetic endothelial morphology, permeability, and biochemistry, the finding of decreased endothelial Na+/K+ ATPase activity strongly suggests that endothelial fluid-pump dysfunction is a major component of the abnormal corneal-hydration control seen in the diabetic rabbit. The interaction of blood-glucose control on corneal-hydration dynamics is an intriguing area for future research.

Our results also suggest that clinicians should remain aware of the variations of blood glucose in their diabetic patients and should consider carefully the possible negative impact of endothelial dysfunction in a poorly controlled diabetic patient before prescribing contact lenses or refractive surgery.

Key words: cornea, corneal thickness, diabetes, endothelial pump, stromal swelling pressure

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References