significant difference between its use with that of normal saline, plasma, or TC-199 (p < 0.005 in all cases) or albumin (t = 2.11; p < 0.05).

Two samples from each of the five tested substances were studied by SEM. Areas of membrane destruction were found in all the corneas. The type and pattern of damage was the same with all substances tested, the difference being the number of affected areas. This correlated well with the results of TB-stained corneas (Figs. 2 and 3).

Discussion. This study confirms the findings of Kaufman and Katz* that extensive damage results from contact between a methylmethacrylate intraocular lens and the corneal endothelium. Placing the intraocular lens in salt-poor human albumin or serum prior to endothelial contact resulted in a significant reduction in endothelial damage when compared to placing it in normal saline. The increased viscosity and protein composition of these solutions may account for the difference. Serum is more efficacious than plasma, suggesting that the fibrinogen or heparin in plasma negates some of the protective effect.

During surgery, contact between the intraocular lens and the corneal endothelium is often unavoidable, especially in cases of shallow anterior chambers. This study suggests that the use of human albumin or the patient's own serum to coat the intraocular lens prior to insertion may protect the corneal endothelium. Since both of these substances are easily obtainable in a sterile fashion, a clinical trial may be indicated. Prevention of damage to the corneal endothelium during surgery may reduce corneal edema and eliminate some of the problems of intraocular lens placement.

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Key words: intraocular implants, corneal endothelium.

REFERENCES

Enzyme-synthetic approach to demonstration of phosphorylase activity in the living rabbit cornea. Tsugio Amemiya and Hidehiko Yoshida.

An enzyme-synthetic method of demonstrating phosphorylase was applied to the living rabbit cornea, and polyglucose particles synthesized from glucose-1-phosphate in vivo were studied electron microscopically. In the corneas in which the medium for phosphorylase was applied from the anterior chamber or the bulbar subconjunctiva, synthesized polyglucose particles were found in the cytoplasmic matrices of the epithelium. When the medium was deposited in the conjunctival sac, a few synthesized polyglucose particles were found in the cytoplasmic matrices of only the superficial layer of the corneal epithelium. These findings suggest that metabolites for glycogen metabolism come mainly from the aqueous humor in the anterior chamber. The polyglucose particles synthesized by the enzyme-synthetic method in vivo resemble native glycogen particles. In addition, these particles were not overproduced because the synthesis of polyglucose is probably regulated in vivo.

There are three pathways by which metabolites reach the cornea from the surrounding media: (1) across the corneal limbus with the blood capillaries in the peripheral conjunctiva and sclera; (2) across the endothelium with the aqueous humor in the anterior chamber; (3) across the epithelium with the tear film. Phosphorylase is related to the following reaction: glucose-1-phosphate + (glucose), = (glucose),(1 + inorganic phosphate. It has been believed for some time that phosphorylase is a glycolytic and glycogenetic enzyme in vivo. However, at the present time, this enzyme is assumed to play a glycolytic role in vivo in glycogen metabolism. The enzyme-synthetic method applied to the living chick retina could demonstrate that polyglucose particles were synthesized in vivo from glucose-1-phosphate by the action of phosphorylase and branching glycosyltransferase. The findings obtained with this method are considered to be very similar to the condition in the living cell. When a large amount of glucose-1-phosphate is placed in any of the above-mentioned three pathways of metabolites in the cornea, polyglucose may be expected to be synthesized from glucose-1-phosphate by the action of phosphorylase and branching enzyme if these enzymes exist in the corneal epithelium and stromal kerocytes. The experiments described here were therefore undertaken to apply the media for histochemical demonstration to these three routes separately. The present paper clarifies the metabolic path-
Fig. 1. Electron micrograph of the epithelium of rabbit corneas in contact with the medium for phosphorylase by the enzyme-synthetic method in vivo. The medium was injected into the anterior chamber. Synthesized polyglucose particles, which stain well with lead citrate, are found in the cytoplasmic matrices. No synthesized polyglucose particles are seen in the intercellular space. (×50,000.)

Table I. Distribution and number of synthesized polyglucose particles in 1μ2 in the corneal epithelium in each experiment

<table>
<thead>
<tr>
<th>Incubation in medium</th>
<th>In vitro</th>
<th>Anterior chamber</th>
<th>Subconjunctiva</th>
<th>Conjunctival sac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial layer</td>
<td>60</td>
<td>50</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Deep layer</td>
<td>90</td>
<td>85</td>
<td>70</td>
<td>65</td>
</tr>
</tbody>
</table>

way as well as the location of the product by the enzyme-synthetic method of demonstrating phosphorylase.

Materials and methods. Rabbits were anesthetized with Nembutal or urethan. In one group, the aqueous humor in the anterior chamber was replaced by the medium for phosphorylase. Immediately after the aqueous humor had been suctioned out at the corneal limbus by a syringe with a 22-gauge needle, 0.5 ml. of the medium was injected into the anterior chamber at the opposite corneal limbus. In a second group, the same medium was injected into the subconjunctiva of the bulbus. In a third group, the conjunctival sacs were filled with the same medium so as to soak the corneal surface. After 20 min. with the medium in each experiment, the corneas were totally resected at the limbus by Graefe's knife and immediately fixed in 6 percent glutaraldehyde in cacodylate buffer at pH 7.4 for 30 min.; then after being washed for 10 min. with cacodylate buffer at pH 7.4, they were divided into peripheral and central parts and cut into small pieces individually. The specimens were post-fixed in 1 percent osmium tetroxide in sodium veronal buffer (pH 7.4), dehydrated in a series of ethanols, and embedded in Epon 812.

The conventional histochemical method for
phosphorylase was also applied to the cornea. Under Nembutal or urethan anesthesia, the cornea was totally resected at the limbus and incubated in the same medium for 20 min. Then the cornea was prepared for electron microscopy as described above.

Some corneas from each experimental group were tested with α-amylase digestion after fixation with glutaraldehyde following incubation with the medium.

As a control experiment, the aqueous humor in the anterior chamber was replaced with cacodylate buffer at pH 7.4 by the above-mentioned techniques, and after 20 min, the cornea was totally resected at the limbus and prepared for routine electron microscopy as described above.

Before the ultrathin sections were made, 1 to 2 μm slices were cut and stained with periodic acid–Schiff stain (PAS). Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined in a Hitachi HU 11A or 11D electron microscope.

Results. In the corneas incubated in the medium for phosphorylase by the conventional histochemical method or permeated with the medium in vivo, the epithelium stained weakly with PAS. The specimens tested with α-amylase digestion and those in contact with cacodylate buffer injected into the anterior chamber showed negative staining with PAS.

In the corneas permeated with the medium for phosphorylase injected into the anterior chamber or the subconjunctiva, synthesized polyglucose particles were found in the cytoplasmic matrices of the epithelium (Fig. 1) and the stromal keratocytes. These particles were 290 to 360 Å in diameter and stained fairly well with lead citrate in spite of being unstainable with uranyl acetate. Sometimes these particles were located as a mass among the tonofibrils. The distribution of the synthesized polyglucose particles is shown in Table I. In the corneas permeated with the medium from the surface in vivo, a small number of synthesized polyglucose particles were located in the cytoplasmic matrices of the epithelium and found mainly in the superficial layer. These particles were 290 to 360 Å in diameter and stained fairly well with lead citrate but were unstainable with uranyl acetate.

In the corneas in contact with cacodylate buffer injected into the anterior chamber, no synthesized polyglucose particles were found.

In the corneas incubated in the medium by the conventional histochemical method, synthesized polyglucose particles were located in the cytoplasmic matrices of the epithelium (Fig. 2).
Fig. 3. Electron micrograph of the epithelium of a rabbit cornea treated with \( \alpha \)-amylase after contact with the medium for phosphorylase injected into the anterior chamber. Empty spaces in the cytoplasmic matrices may represent digested polyglucose particles. (x50,000.)

and the stromal keratocytes. Sometimes polyglucose particles were found in the intercellular space. These particles were 220 to 290 A in diameter, and they stained less well with lead citrate but were stainable with uranyl acetate. The distribution of the synthesized polyglucose particles is shown in Table I.

In the corneas tested with \( \alpha \)-amylase digestion in all experimental groups, no synthesized polyglucose particles were seen in the cytoplasmic matrices of the epithelium (Fig. 3) or the stromal keratocytes.

Discussion. The polyglucose shown in the in vivo cornea by the enzyme-synthetic method of demonstrating phosphorylase must be that synthesized from glucose-1-phosphate in the medium, since it was found that polyglucose was not synthesized in the control medium without glucose-1-phosphate and that the polyglucose synthesized from uridine diphosphate glucose (UDPG) by glycogen synthetase in the enzyme-synthetic method was quite different in size and electron density from that synthesized by phosphorylase.\(^4\) In the paraboloid of the accessory cone of the chick retina, the polyglucose particles synthesized from UDPG by glycogen synthetase were fine and less stainable with lead citrate.\(^4\) On the other hand, the polyglucose particles synthesized from glucose-1-phosphate by phosphorylase and branching glycosyltransferase were large and better stained with lead citrate.\(^8\) Therefore the difference between the polyglucose particles synthesized by glycogen synthetase and by phosphorylase with branching glycosyltransferase is clear. The synthesized polyglucose particles show a great tendency to aggregate in cluster, various sizes, an affinity for lead citrate, stainability with uranyl acetate, location in the cytoplasmic matrices, positive amylase digestion test, and void area around the particles. These characteristics of synthesized polyglucose are quite different from those of ribosomes. Since phosphorylase is located in the cytoplasmic matrices,\(^5\) the intercellular reaction product in the in vitro experiment is an artifact.

The synthesized polyglucose particles in the in vivo experiment were larger, better stained with lead citrate, and fewer than in the in vitro specimens. Thus the polyglucose particles demonstrated in vivo are very similar to physiological glycogen particles. Similarities in size and electron density are due to the conditions for incubation with the medium in vivo in spite of the fact that the concentration of glucose-1-phosphate in tissue is artificially higher than normal. The fact that fewer polyglucose par-
ticles are seen in the in vivo experiment than in the in vitro one suggests that synthesis of polyglucose is regulated in the cell even under in vivo histochemical conditions.

Another use of the enzyme-synthetic approach in the in vivo cornea has been shown in the present study. The number of synthesized polyglucose particles in the corneal epithelium indicates the amount of glucose-1-phosphate in the medium which permeates the corneal epithelium mainly from the anterior chamber. Such speculation cannot come from conventional histochemistry. Most of the glucose in the cornea has been found to be transported across the endothelium to the epithelium in the cornea from the aqueous humor in the anterior chamber. The present study also shows that polyglucose may be synthesized from glucose-1-phosphate supplied mainly from the anterior chamber. Glucose-1-phosphate in the medium injected into the bulbar conjunctiva probably reaches the corneal epithelium mainly through the anterior chamber and partly from the corneal limbus, because as far as the principal metabolites are concerned, the vascular system directly supplies only a very limited peripheral region of the cornea. Such a limited vascular supply of metabolites in the cornea may explain the difference in number of synthesized polyglucose particles between the central and peripheral cornea in the experiment with bulbar conjunctival injection of the medium. The transportation of metabolites from the corneal limbus may be limited to the superficial layer of the corneal epithelium.

From the Department of Ophthalmology, Faculty of Medicine, Kyoto University, Kyoto, Japan. Submitted for publication Feb. 25, 1977. Reprint requests: Tsugio Amemiya, M.D., Department of Ophthalmology, Faculty of Medicine, Kyoto University, Sakyou-ku, Kyoto-shi 606 Japan.

Key words: rabbit, cornea, phosphorylase, glycogen metabolism, electron microscopic histochemistry.

REFERENCES


Oxygen consumption in the developing chick cornea. EILEEN MASTERSON* AND HENRY F. EDELHAUSER.

Embryonic chick corneae at different stages of development were evaluated for O₂ consumption. Some embryos were treated with thyroxine or thiouracil. In untreated animals, corneal QO₂ (oxygen consumption/hr./mg. dry weight) decreased from 3.60 at stage 38 to 1.59 after hatching. The temperature coefficient Q₁₀ increased from 1.55 at stage 40 to 2.03 after hatching. If O₂ consumption is calculated as microliters of O₂ consumed per hour per corneal pair, it increases between stage 38 (3.20) and hatched chicks (6.20) with a plateau between stages 40 and 45. Thiouracil treatment reduced O₂ consumption by the cornea at stages 42 and 45, and thyroxine treatment elevated it at stage 40.

During the development of corneal transparency in the chick, i.e., stages 40 to 45, the cornea dehydrates, and there is an inverse relationship between corneal water content and degree of transparency. Thyroid hormone affects the rate of corneal dehydration and transparency development, with thyroxine accelerating these processes and thiouracil (a thyroid inhibitor) retarding them. The mechanism of corneal deturgescence has yet to be elucidated, although its dependence in the adult on an intact metabolism has been well established. Corneal metabolism requires oxygen, and there are several studies on the oxidative activities of adult rabbit and human...