ties 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 subconjunctiva 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 subconjunctival 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, Sakyu-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 corneas 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...
The purpose of these experiments is to determine chick corneal $O_2$ consumption before, during, and after the critical period of transparency development and to see what effect thyroxine or thiouracil has on $Q_O$.

**Materials.** Fertile eggs of white Leghorn domestic fowl were used and staged according to Hamburger and Hamilton. Two series of animals received 0.1 ml. injections onto the chorioallantois: either 10 mg. of thiouracil in 0.9 percent saline or thyroxine (1 or 5 $\mu$g) in 0.1N NaOH and 0.9 percent saline, adjusted to a pH of 8.2 to 8.5 with 1N HCl. Controls were similarly injected with their respective vehicles. The $O_2$ consumptions from corneas between stages 38 and 45 and 2-7 weeks hatched were measured with an oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio). A corneal pair was incubated in 1 ml. of Ringer's solution containing (millimolar) NaCl, 118.4; KCl, 4.7; CaCl$_2$, 3.3; MgSO$_4$, 2.5; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 25.0; and glucose, 11.1. The solution was gassed with 5 percent CO$_2$ and 95 percent air to bring the final pH to 7.4. Specially modified chambers were used to incubate the corneas in the oxygen-monitoring system. Corneal $O_2$ consumptions were measured at 38°, 24°, and 14° C, and the $Q_{10}$ calculated. The $O_2$ content of the solutions at these temperatures was calculated based on oxygen solubility in Krebs-Ringer's.

**Results and discussion.** Measured as microliters of oxygen consumed per hour per corneal pair, $O_2$ consumption rises during development from 3.2 at stage 38 to 4.0 at stage 40. It plateaus between stages 40 and 45 and rises again after hatching to 6.2 in the 2-week-old chick (Fig. 1). This increase is probably related to changes in corneal cellularity. The rise in consumption between stages 38 and 40 can be correlated with the rise in total potassium concentration [$K^+$] seen during this time. This rise in [$K^+$] is concomitant with the increasing cellularity of the cornea as epithelial cells and fibroblasts proliferate. Cell proliferation is over by stage 40, and this is reflected as a plateau in $O_2$ consumption. After hatching, the corneas continues to grow, and presumably the cell number increases since a rise in $O_2$ consumption is observed. A similar rise in $O_2$ consumption with increasing age has been observed in rabbits.

The $Q_{10}$ (microliters of $O_2$ consumed per hour per milligram dry weight) decreases throughout the developmental period studied (Table I). In fact, the $Q_{10}$ is highest at the earliest stage studied (stage 38, 3.60; not shown). This indicates that the extracellular mass is increasing within the cornea relative to the cell number. After stage 40, when the cell number in the stroma stabilizes, there is continued laying down of collagen and ground substance, which increase the corneal mass without a similar increase in cells.

Thyroxine treatment at stage 38 elevated mean $O_2$ consumption at stage 40 (Fig. 1) but not significantly. The elevation is consistent with the increased [$K^+$] of thyroxine-treated corneas at this stage and may reflect increased corneal cellularity. Thiouracil treatment at stage 36 significantly decreased $O_2$ consumption at stages 42 and 45 compared to untreated controls (Fig. 1). This is

**Table I. Oxygen consumption and $Q_{10}$ values in developing chick cornea**

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>Temperature (°C)</th>
<th>$O_2$</th>
<th>$Q_{10}$+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 40</td>
<td>7</td>
<td>24</td>
<td>2.5±0.07</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>24</td>
<td>1.3±0.05</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14</td>
<td>0.8±0.03</td>
<td>1.56</td>
</tr>
<tr>
<td>Stage 42</td>
<td>8</td>
<td>38</td>
<td>2.2±0.11</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>1.4±0.11</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.6±0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 45</td>
<td>10</td>
<td>38</td>
<td>1.7±0.08</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24</td>
<td>0.8±0.02</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>14</td>
<td>0.4±0.02</td>
<td>1.80</td>
</tr>
<tr>
<td>Two weeks</td>
<td>3</td>
<td>38</td>
<td>1.6±0.09</td>
<td>2.03</td>
</tr>
<tr>
<td>after</td>
<td>3</td>
<td>24</td>
<td>0.7±0.03</td>
<td>2.03</td>
</tr>
<tr>
<td>hatching</td>
<td>2</td>
<td>14</td>
<td>0.3±0.03</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S.E.

1. Calculated from the following formula:

$$Q_{10} = \left[ \frac{K_{T_2}}{K_{T_1}} \right]^{\frac{T_1 - T_2}{10}}$$

where $T_1 = 14° C$, $T_2 = 38° C$, and $K_{T_1}$ and $K_{T_2}$ are measured $O_2$'s at temperatures $T_1$ and $T_2$, respectively.
also consistent with the previously reported reduced $[K^+]$ of these corneas.11

Measurement of $Q_O$ at various temperatures permitted calculation of corneal $Q_a$. The $Q_a$ progressively increases from 1.56 at stage 40 to 2.03 in the 2-week-old hatched chick (Table I). This last value compares favorably with the $Q_a$ value obtained in adult rabbit cornea (1.96)9 and indicates that the metabolism of the hatched chick cornea, but not the embryonic cornea, is similar to that of other biological systems.

From the Departments of Physiology and Ophthalmology, The Medical College of Wisconsin, Milwaukee. This investigation was supported in part by a Milwaukee Children's Hospital Research Fellowship in Ophthalmology to Eileen Masterson, Children's Hospital, Milwaukee, Wisconsin and a research grant from the National Eye Institute, EY-00933. Submitted for publication April 27, 1977. Reprint requests: Dr. H. F. Masterson, Department of Physiology, National Eye Institute, National Institutes of Health, Bethesda, Md.

Key words: oxygen consumption, cornea, chick embryo, thyroxine.

REFERENCES


Choroid tension and facility of aqueous outflow. ROBERT A. MOSES AND WALTER J. GRODZKI, JR.

Tension of the choroid of enucleated eyes was adjusted by incising the sclera and lengthening the eye. The facility of outflow increased in direct proportion to choroid stretch. The present results are compared with reported results of anterior chamber deepening and lens depression in vitro and with accommodation and goniospasm in vivo. Choroid stretch in the enucleated eye is less efficient in increasing outflow facility than is equivalent choroid movement in accommodation. Anterior chamber deepening and lens depression also appear to be relatively inefficient means of enhancing outflow facility, although goniospasm appears to be relatively efficient. The suggestion is made that traction of the ciliary body on the trabecular mesh in an axipetal direction is more efficient in enhancing outflow facility than is traction in the meridional direction.

Considerable evidence exists that ciliary muscle contraction increases the facility of aqueous outflow. Thus Armaly and Jepson found an increase of facility with accommodation, and the facility-increasing effect of miotic drugs is well known. Increase of facility of outflow is also known to occur during perfusion of the anterior chamber when the anterior chamber deepens, presumably in part due to traction on the ciliary body through the zonules by the retroplaced lens. The effect of lens depression was quantitated by Van Buskirk and Grant who showed that facility is directly related to retrodisplacement of the lens. Traction on the ciliary body by the zonules during lens depression is backward and inward toward the axis of the eye, simulating to some degree ciliary muscle tension on the trabecular mesh. The present report describes an attempt to simulate ciliary muscle contraction by tensing the choroid.

Materials and methods. Nineteen enucleated human eyes 1 to 5 days post-mortem were used. The steps in preparation of the eye for perfusion (Fig. 1) are as follows:

1. Mark a circle 17 mm. in diameter on the anterior sclera parallel to the limbus.
2. Place a 5 mm. trephine hole in the cornea,