Osmotic Effects on the Amino Acid-concentrating Mechanism in the Rabbit Lens*

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A characteristic feature of the development of sugar cataracts in the rabbit is the occurrence of a depression in the level of amino acids in the lens a few days after the animals are either placed on a high galactose diet or injected with alloxan. Kinsey and Reddy (1, 2) have shown by means of experiments in vitro and in vivo that a nonmetabolizable amino acid is not accumulated as rapidly in the lenses of diabetic rabbits as in those of normal animals. Thus, the drop in the amino acids that is observed in the development of sugar cataracts probably reflects a decrease in the efficiency of the amino acid-concentrating mechanism.

The finding of van Heyningen (3) that there is an accumulation of polyols in sugar cataracts has led to the proposal of an osmotic theory to explain the development of sugar cataracts (4-7). According to this hypothesis, the increased concentration of sugar in the aqueous humor leads to an enhanced sugar uptake by the lens. The resultant high concentration of sugar accelerates its reduction to an alcohol. Since the lens membranes are relatively impermeable to sugar alcohols, these compounds tend to accumulate as they are formed so that a hypertonic condition is created. Osmotic equilibrium is restored by the movement of water from the aqueous humor into the lens fibers, which are relatively impermeable to sugar alcohols or to the osmotic swelling of the lens. This report deals with a study that indicates that the ability of the lens to concentrate amino acids, α-aminoisobutyric acid and l-aminoctylpentane-1-carboxylic acid, is markedly influenced by osmotic changes in the lens.

EXPERIMENTAL PROCEDURE

Rabbit lenses weighing 175 ± 10 mg were incubated in 10 ml of medium, 9 ml of which were a salt solution. The salt solution consisted of 130.7 mM NaCl, 4.2 mM KCl, 0.6 mM MgSO4, 0.3 mM NaH2PO4, 0.25 mM KH2PO4, 29 mM NaHCO3, and 1 mM KHCO3. The other 1 ml of the incubating medium was a sugar solution, 300 mM in either fructose (control medium), galactose, glucose, or mannose. Since glucose appears to be the only sugar to provide sufficient energy to maintain the lens (4) a concentration of 5 mM was always present in the final medium in addition to the 30 mM sugars. The concentration of calcium ion was adjusted to 1.25 mM in the final mixture. The incubation medium was equilibrated with 95% air and 5% CO2. Except when otherwise indicated, the final concentration of α-aminoisobutyric acid-3-14C was 0.1 mM and that of 14C-cycloleucine was 0.5 mM. Other incubation procedures and chemical analyses were carried out in the manner previously described (5-8). At the end of the incubation period, the lens was removed from the culture tube, rolled on filter paper to remove adherent fluid, and weighed in a tared test tube. It was then homogenized in 3 ml of 10% trichloroacetic acid. After centrifugation, the precipitate was washed with alcohol and acetone, dried, and weighed. The average water content of 30 lenses incubated in the control medium was 68.9% ± 0.3 (s.d.). Thereafter this value was used to calculate the water content of lenses incubated in the control medium from their wet weights. Changes in the water content of lenses because of incubation in media of different tonicities or of high aldose concentration were estimated from the differences in weights of paired lenses, one of which was incubated in the control medium and the other in a medium of a different composition. This procedure seemed reliable since the two lenses of a rabbit agreed in weight within 1 mg when incubated separately in media of identical toxicity. The toxicities of the media were determined with an osmometer purchased from the Advanced Instrument Company.

Aliquots of the medium before and after incubation and of the trichloroacetic acid filtrate of the lens were taken for counting of radioactivity. The activity of the medium after incubation was used for the calculation of the ratio of lens to medium radioactivity. The radioactivity determinations were made in 20 ml of phosphor solution and counted in a scintillation counter (9).

RESULTS

Effect of Aldoses on Amino Acid Transport—Lenses were incubated for 21 hours in media containing AIB and high levels of either galactose, glucose, mannose, or fructose so that the effect of these sugars on AIB uptake could be ascertained. In these experiments one lens was placed in an experimental sugar

*The abbreviation used is: AIB, α-aminoisobutyric acid.
medium while the paired control lens was incubated in the fructose control medium. Results with lenses from different animals were sufficiently consistent that the values could be expressed as the means of a number of experiments (Table I). Since the distribution ratio of AIB (counts per minute per ml of lens water divided by counts per minute per ml of incubation medium) was 24.6 after incubation in the fructose medium, it is apparent that AIB was accumulated against a concentration gradient. The AIB ratio was lower in the lenses incubated in the other sugars. In these lenses not only was less AIB taken up but an increase in water also diluted the concentration of AIB. The drop in the ratio appeared to be correlated with increases in sugar alcohol and water. The lowest ratio was observed in the lenses incubated in the galactose medium where the accumulation of sugar alcohol and increase in water were the highest. Similarly, the distribution ratio was only slightly affected by the small increases in water and sugar alcohol that occurred in high mannose medium and was moderately lowered by the intermediate levels of water and sugar alcohol in the high glucose medium.

As shown in Fig. 1, the time course of the AIB uptake by the lens in the control medium appears almost linear over the 21-hour period studied. When lenses were incubated in either a high galactose or high glucose medium, the AIB ratio leveled off by the 8th hour of incubation. Considerable increases in both sugar alcohol and water occurred as shown in Fig. 2. During the 21 hours of incubation the increase in hydration paralleled the accumulation of dulcitol. In a previous study we found that this relationship was maintained for a period as long as 72 hours (6). As revealed in Fig. 2 the increases in water and sugar alcohol were more pronounced in the lenses incubated in the galactose than in the glucose medium.

Effect of Varying Tonicity of Incubating Medium—The effect of incubation in the glucose or galactose media on the AIB uptake seems to be related either to the accumulation of sugar alcohol or to changes in hydration of the lens. Conceivably, the accumulation of high levels of sugar alcohol may be inhibitory to the amino acid concentrating mechanism. Another possibility is that the osmotic swelling of the lens which results from the increase in sugar alcohol content could alter the AIB-concentrating mechanism. The osmotic effect was evaluated by a determination of the changes in the distribution ratio of AIB that occurred when lenses were incubated in media of different tonicities. For this purpose, one lens was incubated in the control medium while the paired control lens was incubated in a medium which had an osmolality of 292 and the other lens from the same rabbit in a medium of a different tonicity. The results, shown graphically in Fig. 3, indicate that the lens responds to changes in the tonicity of the medium, taking up water when incubated in a hypotonic medium and losing water in a hypertonic medium. The lens is sufficiently sensitive to detect a 10 milliosmolar difference in tonicity, a change of approximately 5% in the tonicity of the medium. Through a range of tonicities differing from the control by +32 to -64 milliosmolar, the changes in water content of the lens appear to depend linearly upon the tonicity differences. Departure from linearity is observed when the lens was incubated in a medium which is 90

<table>
<thead>
<tr>
<th>Medium</th>
<th>Increase in Polyl</th>
<th>Water</th>
<th>AIB Distribution ratio*</th>
<th>Cycloleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles/lens</td>
<td>mg/lens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>6.9 ± 0.5</td>
<td>19.5 ± 1.5</td>
<td>24.6 ± 3.5 (128)</td>
<td>25.8 ± 3.6 (14)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.9 ± 0.7</td>
<td>12.5 ± 1.2</td>
<td>10.5 ± 2.0 (14)</td>
<td>12.9 ± 1.6 (7)</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.2 ± 0.2</td>
<td>4.2 ± 0.9</td>
<td>17.2 ± 1.6 (7)</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as counts per minute per ml of lens water divided by counts per minute per ml of incubation medium.
milliosmolal hypotonic to the control medium. In this hypotonic solution, apparently, secondary changes occurred in the lens which caused it to take up more water than can be accounted for by the difference in tonicity. At the other extreme, when the lens was exposed to a solution which was 60 milliosmolal hypertonic to the control medium, the resultant decrease in water is less than would be expected. When the 21-hour incubation was replaced by a much shorter period of incubation, such as 4 hours, the water loss at this tonicity is linear with the other values. Thus, the lens does respond as an osmometer through a limited range of tonicities. However, it seems incapable of maintaining its normal permeability characteristics upon protracted exposure to tonicities differing by more than 60 milliosmolal from the control medium.

As is shown in Fig. 4, the tonicity of the medium markedly affects the ability of the lens to concentrate AIB. The more the lens swells, the less effectively can it concentrate AIB. Thus, the osmotic response of the lens to change in tonicity of the medium seems to be reflected in the effectiveness of the AIB-concentrating mechanism.

Counteracting Inhibitory Effect of Aldoses with Osmotically Compensated Media—Since the increase in hydration of the lens produced by hypotonicity seems to have an adverse effect on the accumulation of AIB, osmotic changes may be sufficient explanation for the observed inhibitory effect of aldoses on this transport system. Accumulation of sugar alcohol in the lens fibers should be similar in osmotic effect to incubation in a hypotonic medium. In both cases the movement of water into the lens is a response to the higher tonicity of the intralenticular fluid relative to the tonicity of the environment. If the depressed AIB uptake is due solely to an osmotic effect, prevention of the swelling of the lens despite the accumulation of sugar alcohols should preserve the normal capacity of the lens to concentrate amino acids.

To test this experimentally, lenses were incubated in high glucose or galactose media, the tonicity of which was progressively increased during the course of the incubation to compensate for the internal accumulation of sugar alcohol. In the galactose experiments the initial experimental medium was made 30 milliosmolal hypertonic to the control medium by the addition of sorbitol. Every 4 hours the incubation medium was replaced by one that was 30 milliosmolal higher in sorbitol than the previous one until, after 12 hours, a tonicity 120 milliosmolal above that of the control medium was attained. Incubation was continued for a total of 21 hours. The control medium was changed as frequently as the galactose medium, but the tonicity was held
constant throughout the experiment. As shown in Table II, the weight of lenses incubated in the compensated galactose medium was within 1.3 mg of the control, and the AIB distribution ratio was near the normal value.

The results with the high glucose-compensated medium are also listed in Table II. Because accumulation of sugar alcohol is less marked in the presence of high glucose levels, the compensation of tonicity by the addition of sorbitol was done in 20-milliosmolal increments, starting at a concentration 20 milliosmolal hypertonic to the control, until the final medium was 80 milliosmolal hypertonic to the control. Incubation in the high glucose-compensated medium caused the lenses to lose about 2 mg in weight while the distribution ratio of AIB was essentially normal.

The results of these experiments demonstrate that it is possible to attain normal AIB distribution ratios in lenses that have accumulated large amounts of sugar alcohols if the medium is so compensated that there is no significant influx of water in the lenses. The contention that the depressed AIB levels that occur when lenses are exposed to high levels of aldoses are due primarily to osmotic changes is therefore supported. Any inhibitory effect of the sugar alcohols or other substances that may have been accumulated appears to be minimal.

**Effect of Aldoses on Exit Process** The adverse effect on AIB accumulation of swelling the lens could result either from an increase in the rate of the exit process or from a decrease in the rate of uptake. Evidence has been obtained that the rate of exit of AIB from a lens exposed to galactose is higher than from a normal lens. Lenses were preloaded with tracer levels of AIB in a normal medium, and one lens of each pair was further incubated in a control medium while the other was incubated in a medium containing 30 mM galactose. The reentry of AIB was prevented both by the addition of 10 mM methionine to the media

**Table II**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Tonicity</th>
<th>Distribution ratio* of AIB</th>
<th>Change in water</th>
<th>Poliol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>293</td>
<td>24.3 ± 2.9</td>
<td>+19.5 ± 1.5</td>
<td>+6.9 ± 0.3</td>
</tr>
<tr>
<td>Galactose</td>
<td>293</td>
<td>7.6 ± 1.2</td>
<td>-1.3 ± 0.8</td>
<td>+7.9 ± 0.4</td>
</tr>
<tr>
<td>Galactose</td>
<td>323-413</td>
<td>22.7 ± 1.5</td>
<td>+12.5 ± 1.2</td>
<td>+4.9 ± 0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>293</td>
<td>25.0 ± 6.4</td>
<td>-2.1 ± 1.0</td>
<td>+5.6 ± 0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>312-373</td>
<td>26.2 ± 5.7</td>
<td>-2.1 ± 1.0</td>
<td>+5.6 ± 0.3</td>
</tr>
</tbody>
</table>

* See footnote to Table I.
and by the replacement of the media every 4 hours so that the concentration of AIB in the medium never exceeded 0.001 M. That the uptake of AIB is inhibited by methionine under these conditions is shown in Table III. The presence of 10 mM methionine in the external medium effectively blocks the uptake of AIB when the concentration of the amino acid is 0.001 M or 0.0025 M. As shown in Fig. 5 the rate of exit of AIB over 24 hours is more rapid from the lenses exposed to galactose than from those in the control medium. At the end of 24 hours the AIB level in the galactose lenses is about 50% of the level in the control lenses. It thus appears that one factor contributing to a depressed level of AIB in the galactose-exposed lens is an increase in the exodus of the amino acid from the lens.

**Effect of Aldoses on Entry Process**—In order to study the effect of swelling the lens upon the active uptake of AIB, it was necessary to find some means of isolating this component from the passive components. For this purpose, lenses were exposed for brief periods to tracer levels of AIB so that nearly initial rates of uptake could be observed because the level of AIB in the lens never reached that of medium. One lens of a pair was incubated in the control medium and the other in the galactose medium for 21 hours, a tracer level of AIB was added, and the incubation was prolonged for an additional 30 minutes. According to the results listed in Table IV, lenses swollen in galactose took up roughly 30% as much AIB during the 30-minute period as did the control lenses. The conclusion must be drawn from this experiment that the active concentration of AIB is itself diminished when the lens is swollen.

### Table IV

**Uptake of AIB by rabbit lens incubated in presence of galactose**

<table>
<thead>
<tr>
<th></th>
<th>Total in lens</th>
<th>Amount in lens water</th>
<th>Amount in medium</th>
<th>Distribution ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m.</td>
<td>c.p.m./ml</td>
<td>c.p.m./ml</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>394 ± 44</td>
<td>3377 ± 420</td>
<td>5160 ± 100</td>
<td>0.65 ± 0.12</td>
</tr>
<tr>
<td>Galactose</td>
<td>136 ± 25</td>
<td>957 ± 180</td>
<td>5288 ± 00</td>
<td>0.18 ± 0.02</td>
</tr>
</tbody>
</table>

* See footnote to Table I.

**DISCUSSION**

The crucial effect that the hydration of the lens has on the ability of this organ to maintain normal concentrations of amino acids is clearly indicated by these studies in vitro. In order to isolate the active component from the passive efflux of AIB, it proved necessary to perform the experiments under entirely different conditions; consequently it is not possible to assess the relative importance of efflux and transport in the depression of the AIB distribution ratio in the swollen lens. The stretching of the fibers that results from increased hydration would probably lead to an enhanced porosity of the membranes with a consequently accelerated rate of escape of concentrated substances. Whether the decrease in the rate of accumulation is due to a dilution of the concentration of the AIB and carrier at the site of transport must await further investigation.

The sugar alcohols that accumulate in the lens on exposure to high levels of aldoses are not evenly distributed but are concentrated principally at the equator and at the anterior surface.4

4 J.H. Kinoshita, unpublished results.
This phenomenon is a reflection of the fact that transport into the lens occurs predominantly through the mediation of a sheet of epithelial cells that are found under the capsule on the anterior and not the posterior surface of the lens. Thus the effective concentration of sugar alcohols is especially high in the neighborhood of the epithelial cells. It may be for this reason that a higher level of sorbitol in the medium was required to compensate for dulcitol accumulation than would be expected on the basis of the actual dulcitol concentration in the lens. That is, after 21 hours of incubation in a galactose medium, 7 mmoles of dulcitol had accumulated, and this was equivalent to a 70 mM solution. However, an external sorbitol concentration of 120 milliosmolar was required to maintain hydration and AIB uptake near normal. Probably the high concentration of sorbitol was needed to compensate for the actual level of dulcitol in the epithelial cells.

From these experiments in vitro it becomes apparent that in sugar cataracts the depression in the amino acid level is probably caused by an increase in hydration of the lens. Furthermore, other substances such as potassium and sodium that are maintained in the lens against a concentration gradient are also similarly affected during the development of sugar cataracts (10). Thus, the increase in hydration of the lens produced by the intracellular accumulation of sugar alcohol alters the internal environment and contributes to the progressive loss in viability of the lens during cataract development.

**SUMMARY**

The lens is capable of concentrating α-aminoisobutyric acid against a concentration gradient, but this capacity is impaired by exposure of the lens to high levels of certain aldoses. The presence in the lens of high levels of these aldoses leads to an accumulation of sugar alcohols. The consequence of the increase in polyols in the lens is a hypertonicity which results in osmotic swelling. If the lens is prevented from swelling by appropriate increases in the tonicity of the medium, the α-aminoisobutyric acid uptake is restored to nearly normal levels. Thus, the increase in hydration of the lens is primarily responsible for the loss in efficiency of the amino acid-concentrating mechanism.

The independent study of the entry and exit processes of α-aminoisobutyric acid has revealed that both processes are affected by the lens swelling induced by sugar alcohol accumulation.

**REFERENCES**

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