The Effects of Osmotic Shock on the Organ Cultured Mammalian Ocular Lens

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Freshly isolated rabbit lenses were incubated in anosmolar culture media to study the effects of osmotic shock on lens transparency and protein synthesis. The rate of protein synthesis was measured using three markers, namely α-crystallin, a water soluble protein, vimentin, a cytoskeletal protein and MP-26, an intrinsic membrane protein. It was found that only when lenses were cultured in a hyperosmolar medium of 450 mOsM, was there a significant decrease in the rate of lens protein synthesis. The hyperosmolar medium inhibited the synthesis of all three marker proteins. Lens clarity also depended on the solute used to prepare the hyperosmolar medium. Our experimental data showed that there is no relation between decreased protein synthesis and the appearance of lens opacity in the organ cultured rabbit lens. Invest Ophthalmol Vis Sci 25:586-593, 1984

It has been reported that the physiologic osmolarity of the lens and its surrounding fluids are essential for the maintenance of its normal metabolism and clarity. The inability of the ocular lens to maintain proper hydration is implicated as a causative factor in several types of hereditary, disease-related, and experimental cataracts. Kinoshita, et al reported that when mammalian lenses were incubated in a high glucose medium, they showed lenticular opacities, due to osmotic imbalance caused by sugar alcohols. The sugar alcohols are impermeable to the lens membranes, and when excessive amounts of sugar alcohols accumulate in the epithelial and fiber cells, intracellular molarity increases in relation to the extracellular fluid, and a net movement of water into the lens then causes swelling and eventual opacity. It has been demonstrated that diabetic cataracts also are due to an increased amount of sugar alcohols in the lens. In hereditary cataracts, like those found in the Philly and the Nakano mouse lens, lens opacity is preceded by lens swelling. It was reported further that these mouse lenses have impaired Na+, K+ ATPase activity, an enzyme intimately related with membrane permeability. It is evident from the literature that in the above-mentioned types of cataracts, the impairment of membrane permeability and decreased protein synthesis are the fundamental causes of the eventual appearance of lens opacity. It is known that in the case of hereditary mouse (Nakano) cataracts, the Na+, K+ pump is inhibited by a protein factor and in both diabetic and experimental glucose-induced cataracts, lens opacity is probably due to the breakdown of membrane permeability by the overloading of the cation pump. Even though the appearance of lens opacity and the concomitant diminished protein synthesis are well recorded, the relationship of these two pathologic events is not yet clear.

We studied the effects of different osmolar media on lens clarity and protein synthesis to determine whether there is a relationship between decreased protein synthesis and the appearance of lens opacity. Experiments were performed to establish the lowest effective osmolar medium that could induce lens opacity and inhibit protein synthesis. The use of different osmolar media enabled us to investigate the effects of lens swelling (hydration) as well as lens shrinkage (dehydration) on lens morphology and protein synthesis. It was found that the hyposmolar media used had no appreciable effect on either lens clarity or protein synthesis. Similar results were obtained with hyperosmolar media, until the molarity of the medium was 1.5 times the isosmolar medium. This medium inhibited lens protein synthesis, but lens clarity depended on the solute used to enhance the molarity of the medium.

Materials and Methods

Lenses were obtained from freshly killed New Zealand white rabbits of approximately 2 kg body weight and incubated in various osmolar TC-199. The pH of all culture media was adjusted to 7.4 with either 1 N NaOH or 1 N HCl and the osmolarity of the control medium was 310 mOsM. The pH of the medium also
was measured at the end of the incubation period to assure the maintenance of proper pH. Two hyperosmolar media were prepared by the addition of distilled water to the TC-199 medium, the molarity of these media were 230 and 260 mOsM. The hyperosmolar media were 360, 400, and 450 mOsM and were prepared by the addition of either sodium chloride or sucrose to TC-199. The osmolarity of all media was measured by a Precision osmometer (Precision System, Inc.; Sydbury, MA).9

To separate the effect of hyperosmolarity on the lens from possible effects of either sodium chloride or sucrose, media were prepared that had the same mM amount of sucrose or sodium chloride as that of the hyperosmolar media, but the final osmolarity of the medium was readjusted to that of isosmolar medium by the addition of distilled water. It was realized that the amount of intracellular ions and amino acids were diminished in these media and may have had some adverse effects on the morphology and protein synthesis. However, careful analysis of the experimental data revealed no such effect on the lens.

Each lens was incubated in 25 ml of the culture medium in a closed system maintained in a water bath at 33.8°C ± 1°C.9 Contralateral lenses were incubated in control and experimental media for 6, 24, or 48 hr. Six hours before the end of the incubation period, lenses were exposed to 3H-leucine (Amersham; Chicago IL) at a concentration of 50 μCi/ml of medium (specific activity 130 Ci/mM). It should be noted that the lenses were pulsed with the isotopic medium only for the 6 hr prior to the termination of the experiment and were not maintained in the medium containing 3H-leucine throughout the incubation period. Thus, the data obtained in these experiments reflects the rate of protein synthesis at 0–6 hr, 18–24 hr, and 42–48 hr—not the cumulative incorporation of radioactive precursors over the 48-hr culture period. Lenses were isolated in a cold buffer consisting of Tris-HCl, MgCl2, β-mercaptoethanol (TM buffer).10 The capsule and adhering epithelium were removed, the superficial cortex was dissected, and its protein content was obtained.10 Three specific protein markers were used to study protein synthesis. Alpha-crystallin was chosen to represent the water soluble proteins, vimentin for the cytoskeletal proteins, and MP-26 for the membrane proteins. Alpha-crystallin was isolated by gel chromatography,10 vimentin was extracted with 8 M urea,11 and MP-26 was extracted as described previously.12 All three proteins were purified further by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The gels were stained with amido-black and scanned by a soft-laser scanning densitometer (LKB Instruments; Gaithersburg, MD). The desired bands were excised and dissolved in a 30% hydrogen peroxide solution. The amount of radioactivity in each band was measured by liquid scintillation spectrometry. The amount of 3H-leucine pool in 6-, 24-, and 48-hr lenses was determined by monitoring the amount of 3H-leucine in the 50 μl TCA soluble lens homogenate by liquid scintillation spectrometry.9

The lens dry weight was obtained by dehydrating lenses in a 60°C incubator for 72 hr. A CCRG camera (courtesy of Dr. C.V. Harding, Kresge Eye Institute; Detroit, MI) was employed to photograph control and experimental lenses.13

Results

Initial experiments were performed to determine the lowest effective osmolarity of the experimental culture medium that could inhibit lens protein synthesis and have an effect on lens clarity.

Figure 1 shows the data from such an experiment. Five experimental media were used with osmolarities ranging from 230 mOsM (hyposmolar) to 450 mOsM (hyperosmolar) and one control medium with an osmolarity of 310 mOsM (isosmolar). Only sucrose was used to increase the osmolarity of the media. The lenses were incubated in the different media for a period of 48 hr and α-crystallin synthesis was used as a marker for protein synthesis. Only the 450 mOsM medium significantly depressed α-crystallin synthesis, and the lenses incubated in this medium displayed prominent posterior cortical opacity. All other experimental media neither inhibited α-crystallin synthesis nor caused lenticonular opacity. On the basis of these findings, experiments were designed to study the effect of the 450 mOsM medium prepared with either sodium chloride or sucrose on the synthesis of cortical α-crystallin, vimentin, and MP-26 at 6, 24, and 48 hr of culture. Sucrose and sodium chloride were used in these experiments to examine any possible effect of the solute on the observed inhibition of α-crystallin synthesis. It was found that 180 mM sucrose or 90 mM sodium chloride is needed in the medium to obtain an osmolarity of 450 mOsM. Two other culture media also were prepared as mentioned in Materials and Methods, which contained 180 mM sucrose or 90 mM sodium chloride, but had a final osmolarity of 310 mOsM (isosmolar).

Figure 2 shows the effect of hyperosmolar medium on the α-crystallin synthesis. The solute used to prepare the experimental medium is shown on the top of the bar graph. In all experiments medium A represents control medium (isosmolar), medium B is hyperosmolar (450 mOsM) with either sucrose or sodium chloride, and medium C (isosmolar) has either 180 mM sucrose or 90 mM sodium chloride, but a final osmolarity of 310 mOsM. It is evident from the histogram that medium B (hyperosmolar), causes a significant decrease of α-crystallin synthesis at 24 and 48
Fig. 1. Alpha-crystallin synthesis by the organ cultured rabbit lens fiber cells incubated in various osmolar medium (mOsM) for 48 hr. The data represent the average of five experiments, and each experiment used six lenses. In each experiment, one whole lens was incubated in experimental medium (hyper or hyposmolar) and the contralateral whole lens was cultured in control (isosmolar) medium. At the end of the experiment lenses were isolated in cold TM buffer, the capsule along with the adhering epithelium were removed, the superficial cortical fibers were dissected, and their protein content was isolated. Alpha-crystallin was purified by gel chromatography and SDS-PAGE. T-bars represent the standard error of the mean.*

hr of culture when compared with the medium A (isosmolar). At 6 hr of incubation, only medium B (hyperosmolar), prepared with sucrose, produced a statistically significant decrease of α-crystallin synthesis but not medium B (hyperosmolar) prepared with sodium chloride. When these data were compared with those obtained from the lenses incubated in medium C (isosmolar) (containing either 180 mM sucrose or 90 mM sodium chloride) for 6 and 48 hr, it was found that at 6 hr of culture, only the sucrose containing medium C (isosmolar) resulted in a decrease of α-crystallin synthesis. The lenses cultured for 48 hr in medium C had no effect on the α-crystallin synthesis, when compared with lenses cultured in control medium.

Figure 3 shows the effects of the different culture media on vimentin synthesis. The data presented in the bar graph clearly show that the rate of vimentin synthesis is considerably decreased by medium B (hyperosmolar, sucrose) at 6, 24, and 48 hr of culture. The effect of medium C (isosmolar, sucrose) on vimentin synthesis is comparable with that obtained from the lenses cultured in medium B (hyperosmolar, sucrose). On the other hand, vimentin synthesis is only significantly decreased at 6 hr of culture in medium B (hyperosmolar, sodium chloride). At 24 and 48 hr of cultivation, the rate of vimentin synthesis by the lenses incubated in all media was similar. The lenses cultured in medium C (isosmolar, sodium chloride) for 6 hr showed a slight decrease in the rate of vimentin synthesis compared with that of the control lenses.

Figure 4 shows the rate of MP-26 synthesis by lenses when cultured in either medium A, B, or C. The lenses cultured in medium B (hyperosmolar, sucrose) showed a significant inhibition of MP-26 synthesis at 6, 24, and 48 hr. When the lenses were incubated in medium C (isosmolar, sucrose), there was an inhibition of MP-26 only at 6 hr but not at 48 hr. The lenses cultured in medium B (hyperosmolar, sodium chloride) for 24 or 48 hr, showed a considerable depression of MP-26 synthesis, whereas those incubated for 6 hr showed only a slight decrease in MP-26 synthesis. When the

* Ordinate—CPM = counts per minute per gel band. Density = amount of protein per gel band. Abscissa = osmolarity (mOsM) of the incubation medium.
lenses were cultured in medium C (isosmolar, sodium chloride) for 6 hr, there was a minor decrease in the MP-26 synthesis, but no such depression was found in the MP-26 synthesis by the lenses cultured for 48 hr.

Table 1 shows the amount of $^3$H-leucine from the lenses cultured for 6, 24, or 48 hr, in the various experimental media. It should be noted that the lenses were exposed to the medium containing $^3$H-leucine only for the last 6 hr of the culture period, so the data actually reflects the $^3$H-leucine pool size for 0-6, 18-24, and 42-48 hr of culture. It is obvious from the results that, the lenses cultured in the different osmolar medium showed similar or increased amounts of radioactivity compared with the control lenses. This data indicates that the observed decrease in the protein synthetic activity is not due to a lack of radioactive precursors.

The dry weights of the control and experimental lenses also were measured (data not presented). The data suggests that the lenses cultured in the experimental media did not show any appreciable loss of weight. There also was no detectable amount of lens proteins found in the culture medium.

Figure 5 shows the effect of control and experimental media on lens clarity at 0 (a), 24 (b), and 48 (c) hours of incubation. There were five different media used for these experiments. Medium A was the control medium, and the lenses cultured in this medium at 0, 24, or 48 hr remained clear.

Medium B was the hyperosmolar medium prepared with sucrose. Lenses incubated in this medium immediately displayed an anterior opacity and faint nuclear opacity along with large vacuoles in the polar region. After 24 hr in this medium, the large vacuoles in the polar region still persisted, but there was no anterior or nuclear opacity; instead, lenses displayed a distinct posterior cortical opacity near the suture lines. This posterior cortical opacity and the polar vacuoles remained visible in the 48-hr cultured lenses.

The lenses cultured in medium Bn, a hyperosmolar medium prepared with sodium chloride, also showed immediate anterior opacity and slight nuclear opacity, but no vacuoles could be detected until 24 hr of culture. But by 24 hr of incubation lenses appeared clear, and
they remained clear for the remainder of the experiment.

The lenses cultured in medium Cs, an isosmolar medium with 180 mM sucrose, showed an immediate anterior opacity and slight nuclear opacity, but no vacuoles in the polar region. At 24 hr of culture, the anterior and nuclear opacity cleared up, but there was a distinct posterior capsular opacity and numerous small vacuoles in the polar region. By 48 hr of incubation, posterior capsular opacity became even more pronounced; but there were no detectable vacuoles in the polar region. It also is interesting to note that in all cases of posterior cortical opacity, the area of the suture lines remained clear.

Medium Cn also is an isosmolar medium, with 90 mM sodium chloride, and the lenses cultured in this medium for 0, 24, and 48 hr showed neither opacity nor vacuolation. These lenses remained similar in appearance to the lenses incubated in the control medium.

Discussion

The results obtained in this study show that lenses can function normally with a moderate perturbation of the osmolarity of the surrounding medium and only when the osmolarity of the bathing medium was as high as 1.5 × isosmolar, was protein synthesis and clarity of the lens affected. Lenses cultured in the hyperosmolar medium B (sucrose or sodium chloride) for 24 or 48 hr showed a significant decrease in α-crystallin and MP-26 synthesis; whereas only those lenses cultured for 6 hr in the medium B (sucrose) showed diminished α-crystallin and MP-26 synthesis. It has been reported by several investigators using tissue culture systems that the effect of a hyperosmolar medium on the protein synthesis is independent of the solutes used to prepare it. It is possible that the observed differences found in our experiments could be due to stimulatory effects of the increased levels of Na⁺, Cl⁻ in the culture medium on the lens cation pump, which can temporarily counteract the effects of hyperosmotic medium on the lens membrane permeability. This possible effect on the membrane permeability could sustain a normal cellular ionic balance thus maintaining a steady rate of protein synthesis. It should be noted that this suggestion excludes any direct effects of high Na⁺, Cl⁻ levels in the culture medium on protein synthesis.
The results for lenses cultured in medium C (isosmotic, sucrose, or sodium chloride) for 48 hr showed that these solutes had no direct effect on either α-crystallin or MP-26 synthesis. Only the lenses cultured for 6 hr in medium C (sucrose) but not in medium C (sodium chloride) showed decreased protein synthetic activity, and these results are probably due to the direct or indirect effects of sucrose. How sucrose effects protein synthesis in the lens is not yet clear.

Sucrose has been shown to have a lipogenic effect in the intestinal cells. This could possibly alter membrane permeability, thus causing inhibition of protein synthesis. It also has been shown that in intestinal cells, sucrose is hydrolyzed by enzymes, like sucrase or other saccharidases, to form glucose and fructose. In the lens cells this process could result in the formation of sugar alcohols. It should be noted, however, that the presence of any saccharidases in the lens cells

Table 1. TCA soluble \(^3\)H-leucine pool (CPM/50 \(\mu\)l)

<table>
<thead>
<tr>
<th>Time</th>
<th>Isomolar A (control) 310 mOsM</th>
<th>Hyperosmolar B (sucrose) 450 mOsM</th>
<th>Hyperosmolar B (sodium chloride) 450 mOsM</th>
<th>Isomolar C (sucrose) 310 mOsM</th>
<th>Isomolar C (sodium chloride) 310 mOsM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 hr</td>
<td>26,966</td>
<td>44,780</td>
<td>29,741</td>
<td>73,875</td>
<td>54,829</td>
</tr>
<tr>
<td>18-24 hr</td>
<td>58,829</td>
<td>79,435</td>
<td>90,522</td>
<td>58,977</td>
<td>50,125</td>
</tr>
<tr>
<td>42-48 hr</td>
<td>46,907</td>
<td>48,328</td>
<td>97,345</td>
<td>58,977</td>
<td>50,125</td>
</tr>
</tbody>
</table>

\(A = \) isomolar medium. \(B = \) hyperosmolar medium. These media are prepared by addition of the appropriate amount of either sucrose or sodium chloride to the isomolar medium in order to obtain a medium having 180 mM sucrose or 90 mM sodium chloride and a final osmolality of 450 mOsM.

\(C = \) isomolar medium. This medium contains either 180 mM sucrose or 90 mM sodium chloride, but by the addition of distilled water, a final osmolality of 310 mOsM, was maintained.
has not yet been established. The lenses incubated in medium C (sucrose) for 48 hr must have at least partially regained their homeostasis, as there was no inhibition of lens protein synthesis, even though the lenses remained opaque. The effect of hyperosmotic shock on vimentin synthesis showed that hyperosmotic medium inhibits vimentin synthesis at 6, 24, and 48 hr of culture. Only the lenses cultured in medium B (sodium chloride) for 24 hr showed no decrease in their vimentin synthesis. The lenses cultured for 6 hr in medium C (sucrose or sodium chloride) showed decreased vimentin synthesis. The decreased vimentin synthesis in medium C (sucrose) is probably due to the presence of an increased amount of sucrose in the culture medium, but the reason for the minor depression in medium C (sodium chloride) is not clear, especially since the latter medium did not cause any change in the synthetic rates of a-crystallin or MP-26. The lenses cultured in medium C (sucrose) for 48 hr showed a slight decrease in vimentin synthesis, probably due to the increased amount of sucrose in the culture medium. How the increased amount of sucrose was able to inhibit the synthesis of vimentin while showing no effect on either a-crystallin or MP-26 synthesis is not clear.

The initial appearance of anterior cortical opacity of the organ cultured lenses in the hyperosmotic media could be due to dehydration of the superficial cortical fiber cells and this anterior opacity cleared up within hours as the lens cells were able to regain their normal water balance. Our data suggest that the inhibition of lens protein synthesis is not directly related to the formation of the lens opacity observed in these experimental lenses. In general, diminution of the protein synthesis is affected by the hyperosmolar medium; whereas lens opacity is produced by the increased amount of sucrose (180 mM) in the culture medium. It is not clear how the hyperosmolar medium inhibits or decreases protein synthesis. It has been proposed that the hyperosmotic medium could alter cellular Na⁺, K⁺ balance, and this ionic change could interfere with peptide chain initiation—thus inhibiting protein synthesis. In a cell-free system, sodium ions could inhibit cellular protein synthesis; whereas increased concentrations of potassium ions in the culture medium reverse the inhibitory effects of ionophore an...
tibiotic Nigericin on yeast cell protein synthesis. Re-
be reversed, there was a marked decrease in the rate 
dehydration, when sodium ion concentration should 
concentration of sodium ions is reported to be intimately 
related with the protein synthetic activity. In the lens 
cells it has been shown that in both hydration and dehydratation, when sodium ion concentration should be reversed, there was a marked decrease in the rate of protein synthesis. So, it is still not clear how the concentrations of sodium ions affect the rate of protein synthesis.

The posterior cortical opacities of the lenses are probably due to conformational changes or the aggregation of lens proteins, produced by either ionic or lens water imbalance. How the increased amount of sucrose in the culture medium could cause altered ionic balance or lens water homeostasis is not known. However, it is clear from our data that the presence of increased amounts of sucrose in the culture medium produces posterior opacity (Fig. 5). It also should be noted that the lens cultured in hyperosmolar medium B (sucrose) shows less posterior opacity (Fig. 5, Bsc) than that of the lens incubated in isosmolar medium C (sucrose) (Fig. 5, Csc). This data indicates that the hyperosmolar medium affects production of posterior opacity.

It is paradoxical that the hyperosmolar medium that significantly decreases lens protein synthesis could protect lenses from developing osmotic cataract. It should be noted, however, that the protective action of the hyperosmolar medium on the lens clarity cannot be effective for more than a short duration, as the sustained decrease of lens protein synthesis will definitely alter lens metabolism and homeostasis, which are essential for the maintenance of lens clarity.

Key words: hyperosmolar medium, dehydration, osmotic cataract, intracellular ions, protein synthesis, hydration, rabbit lens

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References