namely the number or fraction of progeny which remained normal, 3% EDTA applied topically to the eye six times a day has a significant embryopathic effect, with only 30% of the progeny remaining normal. No teratogenic or embryopathic effects have been observed. Nevertheless, the implication of this study merits careful consideration if this compound (EDTA) is to be used in pregnancy, especially during the first trimester.

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The disulfide form but not the sulhydryl form of a boron hydride compound was found to be cataractogenic. Apparently this compound attaches to the sulhydryl group of Na-K ATPase in the lens epithelium inactivating this crucial enzyme. The consequence is that a defect in the cation pump activity arises, leading to a rapid influx of Na ions and loss of K ions and marked increase in hydration. These changes are thought to lead to opacification.

Boron hydride compounds have been investigated because of their possible usefulness in neutron therapy of brain tumors. To increase the uptake of the boron hydride anions into tumor cells, a sulhydryl moiety was incorporated into the structure. These compounds were taken up by tumor cells, but their toxicity was increased. Two interesting compounds were the sulhydryl (monomer) and disulfide (dimer) forms of a boron hydride compound (Fig. 1). The disulfide form caused an acute form of cataract in mice, but the sulhydryl form did not. This report deals with studies attempting to determine the cataractogenic effect of the boron hydride compound.

Material and methods. Cesium salt of the monomer (Cs₂B₅H₅S) and of the dimer, or the disulfide form (Cs₂B₅H₅S)₂, as well as their tritiated forms were furnished by Dr. Hideo Terao, Neurosurgical Laboratories, Massachusetts General Hospital, Boston.

A description of the incubation and chemical procedures used in these experiments has been reported previously. The medium employed was the TC-199, bicarbonate and glucose mixture as described previously. The incubations were for an overnight period unless otherwise specified.

Na-K ATPase was prepared from the capsule epithelium of 100 calf lenses. The tissue was homogenized in 10 volumes of 0.01M Tris buffer, pH 7.2. The homogenate was centrifuged, and the precipitate recovered was resuspended in Tris buffer and centrifuged. The washed precipitate was suspended in one third of the original volume of the buffer, and this suspension served as the enzyme source. Protein was determined by the Lowry method on the suspension. Na-K ATPase activity was determined as previously described.
Fig. 2. Electrolyte changes induced by boron hydride compound. The effects of the disulfide form of boron hydride compound at 0.1 and 0.5 mM concentrations on incubated rabbit lens. The contralateral lens served as control, and the results are given as the difference between the control and boron hydride-exposed lenses. In the control lenses the cation contents per lens were Na 2.3 ± 0.3 μEq and K 15.0 ± 0.8 μEq. The results are averages of quadruplicate runs.

In the tritium-labeled boron hydride experiments the rabbit lenses were incubated for an overnight period. The lenses were dissected into the capsule-epithelium, cortex, and nucleus fractions. Each fraction was treated with trichloroacetic acid (TCA) and centrifuged; the precipitate was washed 3 times with TCA, dried with alcohol, weighed, and counted.

Results. The oxidized or disulfide form was found to be toxic to mice. In addition to the general toxicity, an acute type of cataract was observed 3 to 4 hr. when an isotonic saline solution containing 2 mg. of the disulfide form was injected intraperitoneally into a mouse (20 gm.). The dozen mice receiving the injection all developed opacities that involved the entire lens. The sulfhydryl form, or monomer, on the other hand, did not produce cataracts.

To determine what the possible sequence of events was involved in this cataract, a study in vitro on the effects of this boron hydride compound was undertaken. We first determined that 0.5 mM of the disulfide form but not the sulfhydryl form did produce opacities in mouse, rat, and rabbit lenses in culture. In rabbit lens 0.5 mM of the disulfide form caused a marked increase in lens water and also major changes in cation levels. Sodium ion content increased markedly, and the potassium ion level dropped considerably. The results are shown in Fig. 2. Even at 0.1 mM concentration of the disulfide form the effect was obvious. The glutathione level was decreased by 25%. In contrast the deleterious effect was not evident when rabbit lenses were exposed to the sulfhydryl form of the boron hydride compound.

The next series of experiments involved the rubidium ion uptake and runout studies. We found that the rubidium uptake was depressed when the lens was exposed to the boron disulfide compound but not to the monomer. The decrease in the rubidium uptake ranged from 23% to 28% with 0.1 mM of the boron compound and 38% to 45% at 0.2 mM under the conditions previously described. Furthermore the rubidium runout was not affected, indicating that the membrane permeability to cations was not compromised. These results suggested that the disulfide boron hydride form may have an adverse effect on the cation pump mechanism. For this reason the effects of the monomer and dimer forms of the boron compound on the Na-K ATPase were studied. With a preparation of Na-K ATPase from calf lens it was found that the disulfide

### Table I. Boron hydride disulfide effect on lens Na-K ATPase

<table>
<thead>
<tr>
<th>Boron compound (mM)</th>
<th>Na-K ATPase activity*</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>155</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>78</td>
</tr>
<tr>
<td>0.1</td>
<td>79</td>
<td>49</td>
</tr>
<tr>
<td>0.01</td>
<td>113</td>
<td>27</td>
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</table>

*Activity of Na-K ATPase is expressed as nanomole of phosphate released per hour per milligram of protein. The results are averages of triplicates. The sulfhydryl form gave no inhibition.
Fig. 3. Incorporation of tritium-labeled boron hydride compound. Rabbit lenses were incubated in either the monosulfide or disulfide forms of boron hydride compound. The initial specific activities of the two forms used were essentially equal. The open bar represents result with the dimer compound.

form but not the monomer markedly inhibited the ATPase. The results are shown in Table I. The inhibition was 50% at 10⁻⁵M and dropped to 27% at 10⁻⁶M. The boron hydride compound also inhibited Na-K ATPase prepared from calf retina. These results suggested that the boron disulfide group was probably reacting with the —SH group of the Na-K ATPase, which then led to enzyme inactivation.

In addition to the effect of boron hydride compound directly on the Na-K ATPase enzyme, a study was made on the incubated lens. Incubation of the rabbit lens at 0.5 mM disulfide boron hydride compound caused 60% inhibition of the Na-K ATPase in 3 hr.

Another series of experiments involved the use of either monomer or dimer forms of the boron hydride compound which were labeled with tritium. In Fig. 3 is shown a comparison of the distribution of radioactivity when either the monomer or dimer forms were incubated with rabbit lens. A much greater degree of incorporation of tritium was observed with labeled disulfide form than with the sulhydryl compound. The capsule-epithelium fraction was found to incorporate the highest level of radioactivity. Only small amounts of isotope penetrated to the cortex and nucleus. Apparently the radioactivity was principally incorporated into the epithelium and not the capsule, for in other experiments, scraping the inner layers of the capsule led to a substantial decrease in the counts recovered.

These results suggest that the effect is primarily on the epithelium, and although the boron hydride disulfide can react with any —SH group, the finding is consistent that Na-K ATPase is involved. This —SH-containing enzyme is membrane bound, and the highest activity is found in the epithelium.

Discussion. In previous studies we found that membrane sulfhydryl groups play a crucial role in osmotic regulation of the lens. Electrolyte and water balance in the lens are determined by permeability characteristics of the membrane and the cation pump activity. Studies have revealed that there are two types of sulfhydryl groups involved in osmotic regulation. There are —SH groups that regulate membrane permeability to cations. There is also a —SH group of the membrane-bound Na-K ATPase, an enzyme that is a principal component of the cation pump mechanism. The study with diamide indicated that loss in glutathione (GSH) leads to an increase in cation permeability and also to a decrease in cation pump activity. Azoester is another oxidant which affects both the runout of cations and cation pump activity. In contrast iodosobenzoate and the boron hydride disulfide appear to affect only the cation pump activity. However, with iodosobenzoate the decrease in cation pump activity could be demonstrated only in the lens incubated in the absence of glucose. If glucose is present in the medium, no change in cation pump activity could be shown by iodosobenzoate or with other —SH oxidants. The explanation for these findings is that the oxidant oxidizes the membrane —SH along with GSH. GSH usually maintains the membrane —SH, but when excessive oxidant is present, the GSH level itself is decreased. In the presence of glucose, however, the GSH level is maintained by the continual regeneration of NADPH through the hexose monophosphate shunt mechanism. Thus the inactivation of ATPase by these oxidants is reversible. However, in the case of the boron hydride compound the decrease in cation pump activity occurs even in the presence of glucose. This suggests that the inactivation of Na-K ATPase appears irreversible. Apparently the mixed disulfide formed by the reaction of the disulfide of the boron hydride compound and the —SH of the Na-K ATPase is difficult to reverse.

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REFERENCES


Simplification of glutathione-bicarbonate-Ringer solution: its effect on corneal thickness. JAMES K. McENERNEY AND GHOLAM A. PEYMAN.

Glutathione and adenosine can safely be omitted from glutathione-bicarbonate-Ringer solution (GBR) which is used as an irrigating solution during vitrectomy, without significant reduction in the solution’s efficacy or increase in corneal toxicity. Bicarbonate appears to be an essential ingredient and should not be omitted from the Ringer solution.

With the advent of automated instruments to remove and replace vitreous, vitreous surgery has become more prevalent, and with it has come the need for extended intraocular irrigation and vitreous replacement. Currently used intraocular irrigating solutions (normal saline, balanced salt solution, and Ringer-lactate) have been shown to be damaging to the corneal endothelium and result in significant corneal swelling. McCarey et al. and others have proposed a glutathione-bicarbonate-Ringer solution containing adenosine (GBR; Table 1), which has demonstrated ability in maintaining the physiologic state of the cornea and lenses during perfusion and incubation studies. Currently GBR either must be entirely made up fresh or the unstable components (adenosine, glutathione, and bicarbonate) must be individually added to the basic salt solution at the time of ocular surgery, which is not only inconvenient but allows for the possibility of infectious contamination. It is the attempt of this investigation to analyze what role, if any, adenosine and glutathione play in the effectiveness of GBR to maintain corneal thickness during in vitro perfusion. In addition, the effect of phosphate buffer substitution for bicarbonate buffer is studied in a newly proposed glucose-phosphate-Ringer solution (GPR). No controlled studies have previously been reported which maintained constant pH, osmolarity, temperature, pressure, and electrolyte amounts during simultaneous perfusion with GBR and GBR with labile elements omitted.

Materials and methods. Corneas obtained from 5 to 10 lb. albino rabbits were prepared in the manner described by Dikstein and Maurice and mounted in a dual-chambered specular microscope. All infusions were carried out with paired corneas from the same albino rabbit being perfused under identical conditions of temperature (36° to 37° C) and pressure (15 to 20 mm. Hg) for the two solutions under analysis. The basic electrolyte and dextrose components (Nos. 1 to 7, Table 1) of the solutions under analysis were either prepared fresh or obtained from stock solutions. They contained 4 μg/ml gentamicin and were refrigerated at 4° C. for periods up to 4 days. The unstable components (Nos. 8 to 10, Table 1) were all added fresh at the time of infusion, the pH and osmolarity were also checked at this time, and adjustments made if indicated. Every 15 min. for a total of 180 min. corneal thickness was measured three times, and the average value recorded.

Results. Perfusion of the corneal endothelium with glutathione-deficient GBR (Fig. 1) resulted in good maintenance of corneal thickness throughout 180 min. with a mean net loss of 4 μm (n=4). After 60 min. of perfusion when the corneal preparations appeared to have equilibrated with the specific temperature and pressure conditions of the perfusion chamber, glutathione-deficient GBR induced a mean swelling of 2 μm/hr. The paired control corneas perfused with GBR containing glutathione underwent a mean net loss of 5 μm over 5 μm over 180 min. and hourly swelling rates of 5 μm/hr. after 60 min.

Corneas perfused with adenosine-deficient GBR (Fig. 2) also appeared to resist swelling, with a mean net loss of 5 μm (n=5) over the 180 min. perfusion. These corneas underwent mean hourly swelling rates of 3 μm/hr. after 60 min. of perfusion. Control corneas perfused simultaneously with adenosine-containing GBR decreased 8 μm in thickness over 180 min. and did not swell at all after 60 min. of perfusion.

GBR deficient in adenosine and glutathione (Fig. 3) demonstrated a mean net gain in corneal thickness of 11 μm (n=5) over the entire