sodium and potassium ions. Although we have no evidence to date whether this particular system operates in an intact retina, nevertheless the results of the present study show that there must be some mechanism to hold the intracellular concentration of free calcium at a low level in order to prevent inhibition of the Na\(^+\)-K\(^+\)-ATPase.

We thank Jennifer Benner and Ellen Yates for excellent technical assistance.

From the Institute of Biological Sciences, Oakland University, Rochester, Mich. This work was supported in part by grants EY-01219 and EY-00541 from the National Eye Institute, United States Public Health Service. Submitted Nov. 9, 1979. Reprint requests: Dr. Barry S. Winkler, Institute of Biological Sciences, Oakland University, Rochester, Mich. 48063.

Key words: retina, Na\(^+\)-K\(^+\) ATPase, Ca\(^2\)+-activated Mg\(^2\)+ ATPase, calcium

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The effect of ascorbic acid on experimental acid burns of the rabbit cornea. Patricia Wishard and Christopher A. Paterson.

The corneas of albino rabbits were subjected to 45 sec, 12 mm, 2.3N hydrochloric acid burns. Of 18 eyes in nine rabbits receiving no treatment (controls), 11 (61%) developed ulceration sometimes progressing to desemet-
Material and methods. Twenty male and female New Zealand strain albino rabbits, weighing approximately 2.8 kg were used. The animals were anesthetized with intramuscular ketamine hydrochloride (15 mg/kg) and xylazine (7.5 mg/kg). Immediately prior to burning, 2 drops of proparacaine hydrochloride were applied topically. A circular plastic well, 12 mm in diameter, was placed on the cornea of each proptosed eye and filled with 2.3N hydrochloric acid. After 45 sec the acid was aspirated from the well, and the interior of the well was rinsed for approximately 5 sec with saline. The well was then removed, and the saline irrigation was continued for an additional 5 sec. Erythromycin ophthalmic ointment was instilled in each eye immediately after the injury and daily thereafter for the duration of the experiment.

Following the burn procedure the animals were placed alternately into either the control or experimental group. The 10 rabbits in the experimental group received a daily subcutaneous injection of 1.5 gm of ascorbic acid as 10 ml of a freshly prepared, neutralized aqueous solution. In each case, the first injection was given within 2 hr of the burn. The 10 rabbits in the control group received no further treatment other than the daily erythromycin ointment.

All eyes were examined daily by penlight, noticeable changes were further examined under a hand-held slit lamp. Particular attention was paid to the presence and degree of ulceration, descemetocoele formation, perforation, and neovascularization. After 30 days, aqueous humor and blood samples were obtained from all rabbits, under anesthesia. These samples were taken 24 hr after the last injection of ascorbic acid. Ascorbic acid levels in aqueous humor and plasma were determined by the method of Maickel and Zannoni et al.11

Results. One animal in both the control and experimental groups died during the course of the experiment. One eye of one animal in the experimental group became infected. Thus the data are drawn from 18 control eyes and 17 experimental eyes.

The ascorbic acid levels in aqueous humor and plasma at the end of the experiment are given in Table I. The animals receiving subcutaneous ascorbic acid manifested plasma and aqueous humor levels significantly above those in the control group. The ascorbic acid levels in the aqueous humor of the treated rabbits were, except for one value of 12 mg/dl, all in excess of 20 mg/dl.

The clinical observations made on the two groups are summarized in Table II. The acid-burned corneas began to show neovascularization during the second week after the injury; no significant difference was noted between the treated and control groups. There was, however, a very significant difference in the incidence of ulceration and perforation in the two groups. Although 61% of the eyes in the untreated animals developed ulcers of varying severity, only one eye (5.9%) in the ascorbate-treated group developed an ulcer, and this involved only the anterior corneal stroma.

Discussion. The results of this study demonstrate that 45 sec, 12 mm, 2.3N hydrochloric acid burns to rabbit corneas induce corneal ulceration and perforation in a fashion similar to that following alkali burns.12 Thirty days after the acid burn, the ascorbic acid in the aqueous humor of untreated rabbits is severely reduced to a level similar to that found after alkali burns. These observations are in keeping with the report that hydrochloric acid applied to the rabbit cornea results in a rapid decrease in the pH of aqueous humor.12 This acidic environment must inflict significant damage to the ciliary processes, thus reducing the active transfer of ascorbic acid14 from plasma into the aqueous humor.

Subcutaneous administration of ascorbic acid to rabbits subjected to ocular acid burns restores the level of ascorbic acid in the aqueous humor to concentrations usually in excess of normal concentrations. These elevated levels are found 24 hr following a subcutaneous injection of ascorbic acid. We may then presume that the aqueous humor level of ascorbic acid has certainly been much higher during the prior 24 hr.

The clinical observations leave little doubt that raising the level of ascorbic acid in the aqueous humor correlates with a highly significant reduction in the incidence of acid burn–induced corneal ulceration and perforation. The dramatic difference between the incidence of ulceration in control and ascorbic acid–treated rabbits was not the

Table I. Plasma and aqueous humor ascorbic acid levels (mg/dl) in control and experimental (ascorbate-treated) rabbits at end of experiment*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td>6.0 ± 0.6 (17)</td>
<td>33.0 ± 2.7 (17)</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.8 ± 0.03 (9)</td>
<td>1.8 ± 0.2 (19)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., with number of determinations in parentheses. Difference between control and experimental levels of ascorbic acid in aqueous humor and plasma is statistically significant (p < 0.01 by t test).

*30 days after the burn.

565
Table II. Clinical observations at end of experiment (day 30)*

<table>
<thead>
<tr>
<th></th>
<th>Control group (untreated, 18 eyes)</th>
<th>% incidence</th>
<th>Experimental group (ascorbate-treated, 17 eyes)</th>
<th>% incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcers†</td>
<td>8</td>
<td>61%</td>
<td>1</td>
<td>5.9%</td>
</tr>
<tr>
<td>Descemetoceles</td>
<td>2</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Perforations</td>
<td>1</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No ulcers</td>
<td>7</td>
<td>39%</td>
<td>16</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

*The difference in the number of ulcers, descemetoceles and perforations (combined) between the control and treated groups is statistically significant (p < 0.001; normal approximation for a test between two binomial frequencies).
†The category of ulcers extends from superficial to less than full-thickness ulcers.

result of altered corneal neovascularization patterns, since these were similar in both groups. It must be presumed that the mechanism of action of ascorbic acid in reducing corneal ulceration in acid-burned eyes is the same as that described in the Introduction for alkali-burned eyes.

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Key words: cornea, acid burn, ulceration, perforation, ascorbic acid

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