work and outflow apparatus. They do, however, expand prior limits, suggesting a richness and complexity of innervation not previously envisioned.

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Key words: substance P, trabecular meshwork, innervation, monkey, aqueous humor, outflow

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Sodium citrate reduces the incidence of corneal ulcerations and perforations in extreme alkali-burned eyes — acetylecysteine and ascorbate have no favorable effect.

Roswell R. Fister, Mary Lou Nicolabo, and Christopher A. Paterson.

Alkali-burned eyes (45 sec, 12 mm, 4N NaOH) were subjected to topical treatment with 10% ascorbate, 20% acetylecysteine, 10% ascorbate together with 20% acetylecysteine, 10% citrate, or Adsortebor vehicle. Only citrate-treated eyes showed a significant decrease in corneal ulcerations and perforations (17%) compared with ascorbate (58%), acetylecysteine (81%), ascorbate/acetylecysteine (100%), or Adsortebor (75%). In the citrate-treated eyes there was a significantly reduced incidence of band keratopathy (17%) but an increased incidence of hyphema (100%). Both groups receiving acetylecysteine developed acellular corneal caps, the result of peripheral ulceration undermining the central cornea. Polymorphonuclear neutrophils (PMN) were substantially increased at the base of the cap in the acetylecysteine- and acetylecysteine/ascorbate-treated eyes at day 56. At the end of the experiment, citrate-treated eyes showed substantially fewer stromal PMN than any other group. These results show that topical citrate has a most favorable effect on the incidence of corneal ulceration and perforation after alkali burning.

Ascorbate levels are depressed in the aqueous humor of rabbit eyes burned with alkali. When the concentration of ascorbate in aqueous humor is raised by subcutaneous injections or by topical applications in burns (12 mm, 1N NaOH, 20 sec), the incidence of corneal ulceration is greatly reduced. When the burn is prolonged to 35 sec ascorbate reaches adequate concentrations in the eye and corneal ulcerations are reduced only after topical applications. In the 35 sec burn, damage to the ciliary epithelium and/or the ciliary body vascular supply may be the reason for failure of systemic ascorbate to reduce the incidence of corneal ulcerations.

The present study was designed to determine whether there are limits to the favorable effects of topical ascorbate when used on a more severe burn (12 mm, 4N NaOH, 45 sec). For comparative purposes, acetylecysteine (a collagenase inhibitor) and acetylecysteine together with ascorbate groups were also studied. Citrate, a food compound with-
Table I. Incidence of corneal ulceration, perforation, and vascularization in alkali burn model

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Ulceration and perforation (%)</th>
<th>Perforations (%)</th>
<th>Total vascularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorbotear (control)</td>
<td>12/16 (75)</td>
<td>8/16 (50)</td>
<td>2/16 (13)</td>
</tr>
<tr>
<td>Citrate</td>
<td>2/12 (17)*</td>
<td>0/12 (0)*</td>
<td>12/12 (100)*</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>14/16 (88)</td>
<td>10/16 (63)</td>
<td>6/16 (38)</td>
</tr>
<tr>
<td>Acetylcysteine</td>
<td>13/16 (81)</td>
<td>10/16 (63)</td>
<td>4/16 (25)</td>
</tr>
<tr>
<td>Acetylcysteine/ascorbate</td>
<td>14/14 (100)c</td>
<td>5/14 (36)</td>
<td>3/14 (21)</td>
</tr>
</tbody>
</table>

*p < 0.001 vs. control.
*p = vs. control.
*a 0.01 > p > 0.001 vs. control.

Table II. Associated findings in alkali burned eyes

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hyphema (%)</th>
<th>Band keratopathy (%)</th>
<th>Corneal cap (%)</th>
<th>Translucent corneal areas (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorbotear (control)</td>
<td>2/16 (13)</td>
<td>14/16 (88)</td>
<td>0/16 (0)</td>
<td>3/16 (19)</td>
</tr>
<tr>
<td>Citrate</td>
<td>12/12 (100)*</td>
<td>2/16 (17)*</td>
<td>0/16 (0)</td>
<td>12/12 (100)*</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>4/16 (25)</td>
<td>6/16 (38)*</td>
<td>0/16 (0)</td>
<td>7/16 (44)</td>
</tr>
<tr>
<td>Acetylcysteine</td>
<td>8/16 (50)</td>
<td>8/16 (50)*</td>
<td>10/16 (63)*</td>
<td>2/16 (13)</td>
</tr>
<tr>
<td>Acetylcysteine/ascorbate</td>
<td>6/14 (42)</td>
<td>7/14 (50)c</td>
<td>10/14 (71)*</td>
<td>6/14 (42)</td>
</tr>
</tbody>
</table>

*p < 0.001 vs. control
*p = 0.01 > p > 0.001 vs. control.
*p 0.05 > p > 0.02 vs. control.

out antiscorbutic properties, was also evaluated as a possible placebo for the ascorbic acid studies. In actuality, sodium citrate proved to be an effective therapeutic agent.

Materials and methods

General considerations. Forty New Zealand Dutch strain albino rabbits weighing 2.0 to 2.5 kg were anesthetized by intramuscular injections of 9 mg/kg xylazine and 9 mg/kg ketamine HCl supplemented by topical tetracaine (0.5%). Each eye was then proposeted and a 12 mm lucite well was held firmly on the cornea. A solution of 4N NaOH (0.4 ml) was pipetted into the well and allowed to remain in contact with the cornea for 45 sec. The alkali was then aspirated from the well and the eye was flushed with 5 ml of normal saline. Erythromycin ointment (0.5%) was immediately instilled.

Rabbits were randomly assigned in groups of eight to (1) ascorbate treatment, (2) acetylcysteine treatment, (3) acetylcysteine together with acetylcysteine treatment, (4) citrate treatment, or (5) vehicle treatment (Adsorbotear).

The Student’s t test was used to statistically analyze the results (two-tailed test).

Solution preparation. Solutions of sodium ascorbate (10%) and sodium citrate (10%) were prepared by dissolution of ascorbic acid or citric acid crystals in Adsorbotear vehicle at room temperature. The final pH value of each solution was adjusted to 7.2 with 10N NaOH, converting the acidic solutions to the sodium salt. These solutions were prepared fresh every 7 days. Acetylcysteine (20%) solution was prepared fresh every 3 days by dissolving the powder in Adsorbotear solution. All solutions were kept refrigerated.

Treatment regimen. Animals treated with ascorbate, acetylcysteine, citrate, or Adsorbotear received 2 drops of the appropriate solution in each eye 14 times a day on the hour and 2 drops of the vehicle (Adsorbotear) alone on the half hour. Animals treated with ascorbate/acetylcysteine received 2 drops of ascorbate solution on the hour and two drops of acetylcysteine on the half hour 14 times daily. All animals received erythromycin ointment three times a day. The heads of the animals were restrained during the 14 hr dropping schedule. The animals were returned to their cages for food and water in the remaining hours of the day.

Clinical observations and tissue analysis. Each animal was examined twice a week with a binocular dissecting microscope and a slit lamp to determine the presence of corneal ulceration, perforation, vascularization, or infection. The examiner (R. R. P.) was unaware of the animal grouping until the end of the experiment. The dropping regimen was continued until the animals were killed on days 55 and 56. The anterior chambers were absent in all but six eyes, thereby precluding useful biochemical analyses of the aqueous humor.
Fig. 1. Translucent corneal area (arrowheads) in citrate-treated eyes permits better visualization of the hyphema.

All eyes were enucleated, cut in half, and fixed in 10% formalin in preparation for histologic examination.

Results. The extremely severe alkali burn employed in this study resulted in the development in all eyes of phthisis or severe hypotony. None of the eyes became infected.

The major clinical changes observed in the five study groups are summarized in Table I. There was little difference in the percentage incidence of corneal ulceration and perforation between those eyes receiving only Adsorbotear (75%) and those receiving ascorbate (88%) or acetylcysteine (81%) therapy. Eyes receiving acetylcysteine together with ascorbate treatment appeared, if anything, to have a higher incidence (100%) of ulceration and perforation than that in the control (p < 0.046). The most surprising observation was that after this severe burn, the citrate-treated groups gave only a 17% incidence of ulceration without any perforation, a significantly lower ulceration and perforation incidence than that in the control group (p < 0.01).

The mean length of time for appearance of corneal ulceration was 23 days (range 17 to 35 days) in the control (Adsorbotear) group, 19 days (range 17 to 35 days) in the ascorbate-treated group, and 36 days in the citrate-treated group. There were no statistical differences between these groups, but a trend for later ulceration in the citrate-treated eyes is evident. It should be noted that the only two eyes ulcerating in the citrate-treated group occurred in one animal at 27 and 45 days. The presence of a corneal cap in the majority of eyes in the acetylcysteine-treated groups prevented the exact determination of the day of perforation. All perforations were verified histologically.

Fig. 2. Corneal caps were present in a large percentage of eyes from the two groups treated with acetylcysteine. A, Clinical appearance of cap (arrowheads); B, Histologic appearance of acellular cap, with sheets of PMN infiltrating the base.

A summary of additional observations is given in Table II. In the first 2 to 3 weeks hyphemas were noted in all the citrate-treated eyes compared with only 13% to 50% in the other groups. The chelation of calcium by citrate may have led to bleeding in the anterior segment of these eyes. On the other hand, this difference could be more apparent than real because the higher prevalence of translucent corneal areas (Fig. 1) present in all of the citrate-treated eyes allowed better visualization of the anterior chamber. Although the translucent areas in the citrate-treated corneas noted from the second to the fifth week appeared to be thinner than normal cornea, histological confirmation was not possible because all the corneas vascularized by the end of the experiment.

Band keratopathy occurred least frequently in the citrate-treated groups (p < 0.001). The reduction in the number of eyes with bands in all other groups compared with that in the Adsorbotear group shows that any form of treatment in the experiment seems to inhibit band formation.

In both acetylcysteine-treated groups (with and without ascorbate treatment) a significant number of acellular and non ulcerated portions of cornea, 6
to 8 mm in diameter (caps), were found with associated corneal perforation (p < 0.001). The caps were isolated from peripheral cornea by a deep corneal trench, heavily infiltrated by polymorphonuclear neutrophils (PMN), through three fourths of the corneal stroma (Fig. 2). The ulcer undercut the cap and perforated posteriorly and centrally. There were virtually no fibroblasts found in the caps or in the bases of eyes from either group treated with acetylcysteine.

A 1 mm fringe of neovascularization was noted in the peripheral corneas of all eyes at 17 days, increasing to 2 mm by 24 days. There was no difference in the amount of corneal vascularization between the different groups at the time when corneal ulcers develop. Only after day 24, when most of the corneal ulcers had already formed, did vascularization of the citrate-treated eyes accelerate ahead of the other groups. In an average period of 43 days (range 38 to 55 days) all citrate-treated eyes had totally vascularized. The high incidence of perforation or failure to totally vascularize the cornea in all the other groups by the end of the experiment (56 days) prevented any comparison.

The number of PMN present in the stroma of the citrate-treated eyes were substantially fewer than that in any other group.

**Discussion.** The great severity of the alkali burn employed in this study is indicated by the fact that almost all eyes became phthisical during the period of observation. In spite of this degree of injury the citrate-treated eyes showed significantly fewer ulcerations and no perforations when compared with any other group.

Ascorbate had no effect on the incidence of corneal ulceration or perforation in this animal model. In previous studies it was shown that less severely burned eyes responded well to ascorbate therapy. In these earlier studies ascorbate appeared to reverse scurbutic changes in corneal cells and collagen as shown by autoradiographic and electron microscopic techniques. In the more severe burn inflicted in this study, ascorbate apparently failed to accelerate the repair processes sufficiently to reduce corneal ulceration. Other unpublished studies (Pfister, Haddox) show significantly reduced numbers of fibroblasts in the burn area up to 2 weeks after the burn compared with fibroblast presence in eyes burned less severely. If the favorable effects of ascorbate are dependent on the presence of fibroblasts in the burned area, then the absence of any healing attributable to ascorbate could be anticipated with more severe burns. The fact that citrate treatment had a very beneficial effect in these more severely burned corneas suggests a mechanism of action for citrate that may be partially or completely independent of cellular repair processes.

In this animal burn model the use of acetylcysteine as a collagenase inhibitor failed to reduce corneal ulcerations or perforations with or without concurrent ascorbate therapy. In fact, when ascorbate and acetylcysteine treatment were combined, the incidence of corneal ulcers increased when compared with that in control eyes. This would appear to be the result of the heavy infiltration of PMN clustered around the base of the "cap" described in Results. The presence of a corneal cap with few or no fibroblasts was a distinctive finding in a large percentage of eyes from both acetylcysteine-treated groups.

The mechanism by which citrate protects the alkali-burned cornea from ulceration is unknown. Citrate was not found to have any capacity to inhibit collagenase, hence its property as a chelator of heavy metals has no apparent effect on this enzyme. Citrate-treated corneas did not appear to have a different pattern of vascularization while ulcers were developing. It therefore seems very unlikely that the decrease in incidence of ulcerations in the citrate-treated group is directly related to corneal vascularization. In view of these findings it seems more plausible to consider some other mechanism(s) responsible for the favorable citrate effects in this burn model.

Jeff Haddox and Robert Dodson assisted in data evaluation and manuscript preparation. Kathy Fleck typed the manuscript.


**Key words:** sodium citrate, sodium ascorbate, acetylcysteine, alkali burns, eye, rabbit

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The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway. Adriana Fiorentini, Lamberto Maffei, Mario Pirchio, Donatella Spinelli, and Vittorio Porciatti.

Electroretinogram (ERG) responses to alternating gratings have been recorded in patients with temporary occlusion of the retinal artery, retrobulbar optic neuritis, and other ganglion cell diseases. The ERG responses to alternating gratings were absent in the patients in whom the ganglion cell activity was depressed. The ERG responses to light flashes or to homogeneous flickering light were normal. These findings corroborate recent evidence from studies on the cat, showing that the ERG in response to alternating gratings is correlated with ganglion cell activity.

The electroretinogram (ERG) in response to flashes of light is currently used as a clinical test for the diagnosis of retinal diseases. Its validity, however, is limited to the disorders of the outer layers of the retina. Diseases of the optic nerve or of the ganglion cell layer do not significantly affect the flash-evoked ERG. This is because the various components of the ERG originate in the receptor layer, the inner nuclear layer, and the pigment epithelium. The ganglion cells do not contribute to the flash ERG.

An ERG can be obtained in response to patterned visual stimuli such as alternating gratings, in which contrast is reversed several times per second with no temporal change in mean luminance. An alternating grating of suitable spatial frequency may be expected to be a powerful stimulus for the visual neurons that, like the retinal ganglion cells, have their receptive fields organized in regions with antagonistic responses. The antagonistic organization of the receptive field makes these neurons relatively insensitive to flashes of diffused light. On the other hand an alternating grating, because of its constant mean luminance, is not an effective stimulus for the retinal sources of the flash ERG.

A recent experiment in the cat has shown that no pattern reversal ERG can be recorded after retrograde degeneration of the ganglion cells induced by chronic section of the optic nerve. In the same preparation, the flash-evoked ERG is normal.

This finding demonstrates that integrity of the ganglion cells is necessary for the generation of the pattern reversal ERG.

Prompted by these results in the cat, we have recorded the ERG in response to alternating gratings in man in the attempt to find evidence for an electrical retinal response correlated with ganglion cell activity. The ERG evoked by alternating gratings has been recorded in normal subjects and in a number of patients with retinal or optic nerve diseases causing either degeneration or dysfunction of the ganglion cells. We have found that the ERG in response to alternating gratings is dramatically affected in patients with diseases of the ganglion cells and is absent in cases in which the ganglion cells are expected to be degenerated. In all these patients the flash-evoked ERG was normal.

Sinusoidal vertical gratings of variable spatial frequency and contrast were electronically generated on a circular television screen (mean luminance 20 cd/m², 25 cm diameter). The gratings were alternated in phase at the rate of 8 Hz (16 reversals/sec) and were viewed monocularly from a distance of 57 or 114 cm with natural pupils. The same display was used to generate homogeneous sinusoidally modulated light (0 to 40 cd/m², 8 Hz). Flashes were generated by a strobe lamp.

The ERG was recorded with a variation of the electrode of Maffei and Campbell, consisting of a small silver plate introduced between the lower eyelid and the eyeball after instillation of Novocain. An equal electrode placed in the other eye was used as reference. This was done to avoid interference with cortical evoked potentials. The ground electrode was placed on the forehead. The cortical visual-evoked potentials (VEP) were simultaneously recorded with silver plate electrodes placed on the midline 2 cm above the inion and at the vertex. The ERG and VEP signals were amplified and filtered by band pass filters (12 db/ octave). Filtering was between 5 and 50 Hz for alternating gratings and flickering light and between 2 and 50 Hz for light flashes.

The filtered signals were fed into two analog