

# Reports

## Topical ascorbate decreases the incidence of corneal ulceration after experimental alkali burns. ROSWELL R. PFISTER, CHRISTOPHER A. PATERSON,\* AND SONIA ANDERSON HAYES.

*A double-masked study of the effects of topical ascorbate on alkali burns utilized 37 rabbit eyes subjected to 20 sec, 12 mm, 1N sodium hydroxide burns. Preliminary studies established the ocular acceptability, penetrability, and stability of the solutions used. Eighteen eyes in the experimental group were given two drops of 10% ascorbate in Adsorbotear vehicle hourly for 14 hr of each day. Nineteen eyes in the control group received Adsorbotear alone administered in the same manner. Over the course of 6 weeks one superficial ulcer developed in the ascorbate-treated group (5.6%), and nine ulcers formed in the control group (47.4%). The difference in the incidence of ulceration is significant statistically ( $p < 0.01$ ). The mean aqueous humor ascorbate concentration at the end of the experiment was 7 mg/dl in the control group and 37.2 mg/dl in the ascorbate-treated group; this difference is very significant ( $p < 0.001$ ). The study shows that topical 10% ascorbate administered 14 times per day to alkali-burned eyes significantly reduces the incidence of corneal ulceration in the rabbit eye. It confirms prior studies demonstrating that subcutaneous administration of ascorbate decreases corneal ulcerations after alkali burns.*

Ascorbate levels in the aqueous humor of rabbits remain persistently depressed to one-third normal for 4 weeks after a 12 mm, 1N sodium hydroxide burn for 20 sec.<sup>1</sup> The mechanism of this decrease was thought to be injury to the transport mechanism of the ciliary epithelium. Increasing the level of aqueous humor ascorbate in the alkali-burned eyes by subcutaneous injection effectively eliminated perforations in the experimental group, compared to a 60% incidence of perforation in the control group.<sup>1</sup>

When this experiment was repeated, 22% of the ascorbate-treated animals perforated or ulcerated, compared to 60% of the controls.<sup>2</sup> In the same study, ultrastructural investigation of the central, nonvascular, ulcerated, or perforated corneal tissues from both groups showed small numbers of fibroblasts with poorly developed or saccular endoplasmic reticulum, polyribosomal disarray, and pericellular fibrillary collagen. Fibroblasts from the central nonulcerated corneas of ascorbate-treated animals showed abundant endoplasmic reticulum, normal polysomal array, and pericellular mature collagen. Control corneas which did not

ulcerate showed mixed populations of cells with widely varying development of endoplasmic reticulum and polysomal disruption. Proline autoradiography showed grain localization over plump fibroblasts only in the ulcerated or perforated corneas, whereas ascorbate-treated animals showed grains over thin, active fibroblasts and pericellular mature collagen. Fibroblasts from ascorbate-treated nonulcerating corneas appeared to be actively engaged in collagen synthesis; this did not appear to be the case in ulcerating corneas. The results were construed as being highly suggestive of localized corneal tissue scorbustus in the face of extensive corneal collagen destruction in the alkali-burned control corneas.

The object of this study was to answer the question: Can ascorbate, delivered topically, influence the development of corneal ulcerations or perforations after alkali burns? To fully understand this problem, studies were also done to determine the ocular reaction of topically applied ascorbate, stability of ascorbate preparations, and corneal penetration of ascorbate.

**Materials and methods.** New Zealand Dutch strain female albino rabbits, weighing 3 to 4 kg, were used throughout the study.

**Ocular reaction study.** Solutions of ascorbate in concentrations of 0.5%, 1%, 2%, 5%, and 10% were prepared in distilled water and adjusted to pH 7.2 with sodium hydroxide. In addition, 10% and 20% ascorbate solutions in Adsorbotear (Burton-Parsons & Co.) were prepared and also adjusted to pH 7.2. Two drops of each solution were placed in the lower cul-de-sac of different rabbit eyes according to the technique of Fraunfelder.<sup>3</sup> The eyes were examined for duration of blepharospasm and the degree and duration of conjunctival injection.

**Ascorbate stability studies.** Ascorbate solutions of 10% concentration were prepared in distilled water and in Adsorbotear, as described above. The vials were capped and refrigerated. The ascorbate concentration in each vial was determined at 1, 2, 3, 4, and 10 days, by the techniques of Maickel<sup>4</sup> as modified by Zannoni et al.<sup>5</sup>

**Corneal penetration of topical ascorbate.** The penetration study was designed to provide information to assist in the selection of an appropriate topical treatment schedule for the experimental group of rabbits subjected to ocular alkali burns. It is known that alkali burning rapidly destroys the

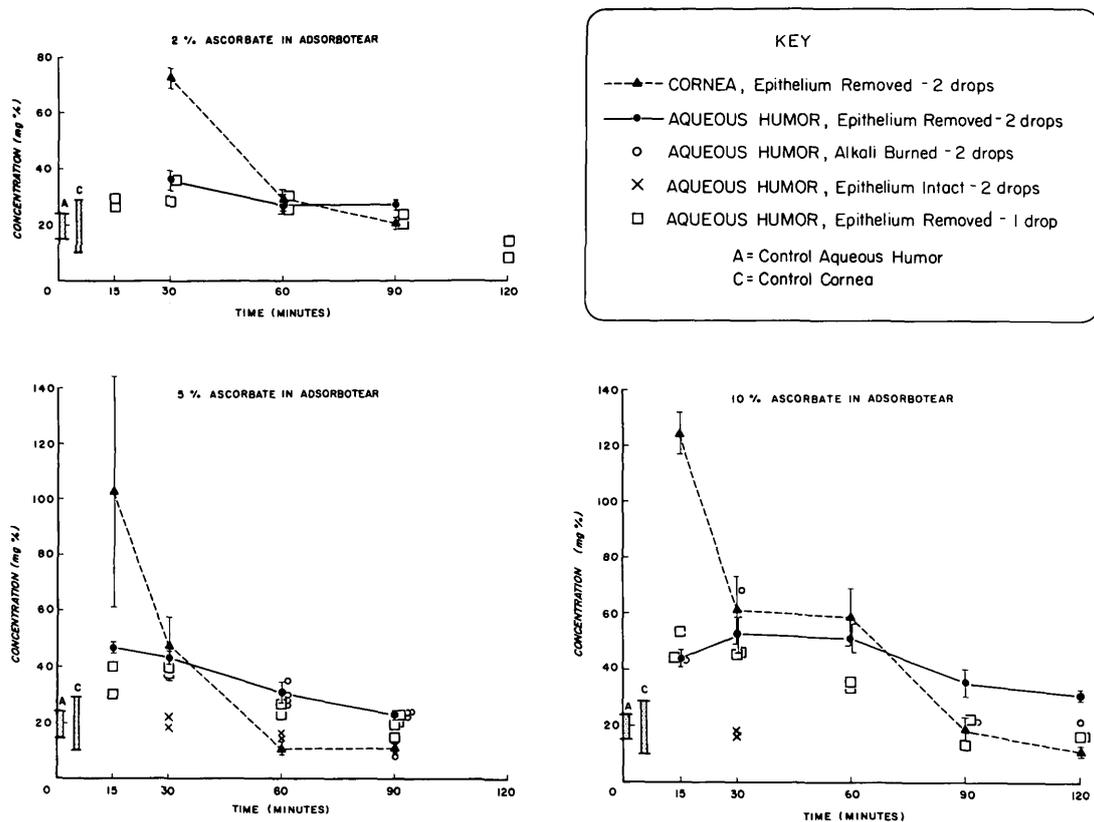


Fig. 1. Level of ascorbic acid in the cornea and aqueous humor is depicted at various times following topical application of 2%, 5%, and 10% ascorbate in Adsorbotear. Penetration was determined under a number of different experimental conditions. Aqueous humor levels are expressed as mg/dl, and corneal levels as mg/dl wet weight. Each point associated with a vertical bar (standard error) is the mean of at least four determinations. Points without a vertical bar represent single determinations.

corneal epithelium, thereby removing a significant permeability barrier to the penetration of topical medications. Rather than use alkali-burned eyes in the penetration study, we believed that an eye with the epithelium scraped off would provide a more convenient and consistent model.

Green and Downs<sup>6</sup> showed that topically applied pilocarpine penetrated the rabbit cornea better when Adsorbotear, rather than saline or a number of other preparations, was used as the vehicle. Since Adsorbotear presumably increased corneal contact time of a drug, it seemed appropriate to use this product in the relatively unwettable alkali-burned rabbit cornea.

Rabbits were lightly anesthetized with sodium pentobarbital administered via a marginal ear vein. Two drops of proparacaine were instilled into each eye. Each eye was then gently proptosed and the corneal epithelium carefully scraped from

virtually the entire corneal surface using a scalpel blade. Within 15 min of removing the epithelium, 2 drops of 2%, 5%, or 10% ascorbic acid in Adsorbotear (pH 7.2) were instilled into each eye. At predetermined times, aqueous humor samples were collected by paracentesis. The animal was then killed with an overdose of sodium pentobarbital, and the cornea was excised. The aqueous humor and corneal tissue were immediately processed for the ascorbic acid assay.

In a limited number of separate experiments, ascorbate penetration was determined after administration, in varied conditions: (1) in eyes with intact corneal epithelium, (2) at 1 hr after a standard alkali burn (see below), and (3) following instillation of only 1 drop of the preparation.

*Topical ascorbate studies.* Twenty rabbits were anesthetized with intravenous sodium pentobarbital via a marginal ear vein. Topical proparacaine

was instilled into each eye, the eyes were proptosed, and as described previously, a 12 mm plastic well was centered on the cornea.<sup>1</sup> Sodium hydroxide (1N 0.4 ml) was pipetted into the well, allowed to remain for 20 sec, and then pipetted out. Five milliliter of saline were used to irrigate the interior of the well and the cornea. Alternate rabbits were then placed in the experimental group (10 rabbits) and the control group (10 rabbits).

On the basis of the results of the above studies, the 10 experimental rabbits received 2 drops of 10% ascorbate (0.073 ml containing 7.3 mg of ascorbic acid) dissolved in Adsorbotear vehicle (pH 7.2) in both eyes at hourly intervals. The ascorbate drops were made up fresh every 7 days. The remaining solution was analyzed on the seventh day to ensure consistency of the ascorbate solution.

The control rabbits received 2 drops of vehicle alone at hourly intervals. All drops were begun 2 hr after burning and continued daily from 8:00 A.M. to 9:00 P.M. inclusive, for 6 weeks. Each animal was kept in a separate restrainer during the day. At the conclusion of the day's drops, ¼ inch of 0.5% erythromycin ointment was instilled into all eyes, and the animals were returned to cages for a 10 hr period with food and water. From day 28 to day 36, erythromycin ointment was applied to all animals three times daily for antibiotic prophylaxis. During the course of the experiment, seven experimental and 11 control eyes were treated with erythromycin four times a day for a mucoid discharge. Two eyes in the experimental group and one eye in the control group were eliminated from the study because of corneal infection with large infiltrates and hypopyon.

The study was conducted in a double-masked manner with the examiner (R. R. P.) unaware of the rabbit groupings until the conclusion of the experiment. Each eye was examined three times per week. Detailed notes were made on conjunctival injection, presence of external or corneal infection, presence and size of epithelial defects, ulcers, descemetocelles, or perforations, and presence of amount of corneal vascularization.

So that accurate levels of ascorbate in the aqueous humor could be determined on the last day of the experiment, drops begun at 8:00 A.M. were continued in the usual fashion to 2:00 P.M. Between 2:15 P.M. and 2:45 P.M., aqueous humor samples were obtained from five rabbits in each group by paracentesis. The usual drops were administered to the remaining 10 rabbits until 3:00 P.M., when aqueous humor samples were col-

**Table I.** Summary of findings in alkali-burned eyes treated with topical Adsorbotear (controls)

| Rabbit No.                   | Clinical observations                               | Ascorbate level* (mg/dl) |
|------------------------------|---|--------------------------|
| No. 11 OD                    | No ulcer (totally vascularized at day 38)           | 5.59                     |
| OS                           | No ulcer (totally vascularized at day 38)           | 6.87                     |
| No. 12 OD                    | Deep ulcer (day 12 to day 26)                       |                          |
| OS                           | Superficial ulcer (day 15 to day 22)                | Died day 26              |
| No. 13 OD                    | Deep ulcer (day 21 to day 38)                       | -                        |
| OS                           | Superficial ulcer (day 29 to day 31)                | 12.46                    |
| No. 15 OD                    | No ulcer (totally vascularized at day 38)           | -                        |
| OS                           | No ulcer (totally vascularized at day 38)           | 11.40                    |
| No. 16 OD                    | No ulcer (totally vascularized at day 38)           | 10.69                    |
| OS                           | No ulcer (totally vascularized at day 38)           | 11.82                    |
| No. 17 OD                    | Ulcer-descemetocoele (day 17 to day 38)             | -                        |
| OS                           | Ulcer-descemetocoele-perforation (day 17 to day 38) | -                        |
| No. 18 OD                    | No ulcer  | 2.13                     |
| OS                           | Ulcer-descemetocoele (day 12 to day 38)             | 6.37                     |
| No. 19 OD                    | No ulcer  | 5.74                     |
| OS                           | No ulcer (totally vascularized at day 38)           | 7.57                     |
| No. 20 OD                    | Superficial ulcer (day 24 to day 38)                | 4.89                     |
| No. 21 OD                    | No ulcer  | 5.52                     |
| OS                           | Superficial ulcer (day 36 to day 38)                | 0.29                     |
| Mean ascorbate concentration |   | 7.03                     |
| S.D.                         |   | 3.72                     |

\*At end of experiment.

lected from 3:15 P.M. to 3:45 P.M. The protein content of all aqueous humor samples was determined by the Lowry method.<sup>7</sup>

The results were treated statistically by the chi-square test and significance of difference in proportion method.<sup>8</sup>

#### Results

**Ocular reaction study.** All ascorbate solutions of 10% or less, in distilled water or Adsorbotear, instilled topically into rabbit eyes caused mild conjunctival injection and mild blepharospasm for less than 2 min. The 20% ascorbate solutions caused moderate conjunctival reaction and moderate-to-marked blepharospasm for about 5 min. On the

**Table II.** Summary of findings in alkali-burned eyes treated with topical 10% ascorbate in Adsorbotear (experimentals)

| Rabbit No.                   | Clinical observations                        | Ascorbate level* (mg/dl) |
|------------------------------|--|--------------------------|
| No. 1 OD                     | No ulcer                                     | Not available            |
| No. 2 OD                     | No ulcer                                     | 45.85                    |
|                              | OS No ulcer                                  | 21.37                    |
| No. 3 OD                     | No ulcer                                     | 51.58                    |
|                              | OS No ulcer                                  | 26.11                    |
| No. 4 OD                     | No ulcer                                     | 25.97                    |
|                              | OS No ulcer                                  | 39.83                    |
| No. 5 OS                     | Superficial ulcer (day 15 to day 38)         | 14.37                    |
| No. 6 OD                     | No ulcer                                     | 25.76                    |
|                              | OS No ulcer                                  | 41.53                    |
| No. 7 OD                     | No ulcer                                     | 67.36                    |
|                              | OS No ulcer                                  | 57.17                    |
| No. 8 OD                     | No ulcer                                     | 34.53                    |
|                              | OS No ulcer                                  | 35.80                    |
| No. 9 OD                     | No ulcer                                     | 32.62                    |
|                              | OS No ulcer                                  | 36.79                    |
| No. 10 OD                    | No ulcer                                     | 35.52                    |
|                              | OS No ulcer (totally vascularized at day 38) | 39.83                    |
| Mean ascorbate concentration |  | 37.18                    |
| S.D.                         |  | 13.25                    |

\*At end of experiment.

**Table III.** Analysis of clinical observations in control and experimental groups

|                                   | Controls* | Experimentals† | p value |
|-----------------------------------|-----------|----------------|---------|
| Total ulcers                      | 9 (47.4%) | 1 (5.6%)       | 0.002   |
| Ulcers or descemetocoeles, day 38 | 6 (35.3%) | 1 (5.6%)       | 0.014   |
| Total descemetocoeles             | 3 (15.8%) | 0 (0.0%)       | 0.039   |
| Descemetocoeles, day 38           | 2 (11.8%) | 0 (0.0%)       | 0.018   |
| Perforations                      | 1 (5.9%)  | 0 (0.0%)       | 0.134   |
| Total corneal vascularization     | 7 (41.2%) | 1 (5.6%)       | 0.006   |

\*Seventeen eyes on day 38; nineteen eyes during experiment.  
†Eighteen eyes throughout.

basis of these findings, it was concluded that the maximum concentration that can be employed is 10% ascorbate.

*Ascorbate stability studies.* The concentration of ascorbic acid remained about the same in refrigerated, capped vials for a period of 10 days. On the basis of this experiment, each solution was prepared fresh every 7 days and kept under refrigeration or on ice at all other times.

*Corneal penetration studies.* The results of the penetration experiments are shown in Fig. 1. It is clear that topical administration of all preparations

raised the level of ascorbic acid in the cornea and aqueous humor in the eyes from which the epithelium had been scraped. For both 2% and 5% ascorbic acid, the level in the aqueous humor and cornea peaked at 15 to 30 min and then declined rapidly toward control levels at 60 min. Only with the 10% preparation did the levels of ascorbic acid in the aqueous humor and cornea remain markedly above control levels at 60 min after instillation.

With 2% and 5% preparations, there was little difference between the degree of penetration of ascorbic acid into aqueous humor following 1 or 2 drops. For the 10% solution, however, 2 drops appeared to be somewhat more effective in achieving lasting elevation of ascorbic acid levels in the aqueous humor. At the time periods studied, penetration of ascorbic acid into the aqueous humor of eyes 1 hr after an alkali burn seemed very similar to that in the scraped eye.

Finally, the intact corneal epithelium appeared to act as a formidable barrier to the penetration of ascorbic acid. The levels of ascorbic acid in the aqueous humor of non-scraped eyes was well within the range of the control levels.

*Topical ascorbate treatment of alkali-burned eyes.* A summary of the results of this experiment is shown in Tables I and II. In the control group, nine of 19 eyes ulcerated or perforated (47.4%), compared to one of 18 eyes (5.6%) in the ascorbate-treated group (Tables I and II). The relatively large difference in the incidence of ulceration between the two groups during the experiment (47.4% vs. 5.6%) diminished to 35.3% vs. 5.6% at the end of the experiment because one control animal with bilateral ulcers died at 26 days and one ulcer in a control animal vascularized before the end of the experiment. The one ulcer in the ascorbate-treated group was very superficial, whereas the control group showed four superficial ulcers, two deep ulcers, two descemetocoeles, and one perforation. Seven control corneas fully vascularized, as opposed to one in the ascorbate group. These clinical data are summarized in Table III.

Conjunctivitis, treated with erythromycin, occurred in seven of the ascorbate-treated eyes and 11 of the control eyes. There was a single non-infectious hypopyon in each group. Two animals in the control group developed band keratopathy, but none did in the ascorbate group.

The mean aqueous humor ascorbate level at the end of the experiment in the control group was 7.03 mg/dl (n = 13) and 37.18 mg/dl (n = 17) in the ascorbate group; this difference is significant (p < 0.001). The one ascorbate-treated animal

that ulcerated showed an aqueous humor ascorbate level of 14.37 mg/dl. This value is significantly less than those in the rest of the ascorbate-treated group ( $p < 0.02$ ). The aqueous humor protein content in the experimental group was  $175 \pm 77$  mg/dl and was  $177 \pm 73$  mg/dl in the control group. There is no significant difference between those values.

**Discussion.** The initial discovery that subcutaneously administered ascorbate could reduce or eliminate corneal ulceration and/or perforation<sup>1, 2</sup> prompted us to determine whether similar results could be obtained with topical administration. Studies reported in this paper have shown that topical 10% ascorbate in Adsorbotear is acceptable to the rabbit eye in terms of ocular irritation and reaction and remains stable for at least 10 days. Penetration studies indicate high corneal and aqueous humor ascorbate levels for at least 1 hr after topical instillation of 2 drops of 10% ascorbate in Adsorbotear. The use of drops hourly 14 times per day, therefore, appeared to be the most reasonable therapeutic regimen for the definitive experiment.

This study clearly shows that the topical application of 10% ascorbate in Adsorbotear administered 14 times per day significantly reduces the incidence of corneal ulceration and perforation after an alkali burn of the rabbit eye. The mechanism of this effect is presumed to be restitution of corneal levels of ascorbate to promote collagen synthesis by fibroblasts in the cornea. With adequate corneal ascorbate, the relative tissue scorbatus is overcome so that hydroxylation of proline and extrusion of mature collagen from fibroblasts can be accomplished. Integrity of the cornea may then be assured by replacing denatured collagen with that which is newly synthesized.

In prior experiments,<sup>1, 2</sup> 60% of the control corneas ulcerated or perforated, compared to 47.4% in the present study. Two possible explanations are that Adsorbotear used topically improved the prognosis of the alkali-burned eye or that biologic variation yielded a slightly different result under these testing circumstances. On the basis of our experience with this model system, we believe the latter is more likely.

During the course of the experiment, more ulcers were present in the control group than were found at its completion (6 weeks). One rabbit with bilateral ulcers died 10 days before the experiment ended, and the ulcer of one other eye became vascularized and was no longer classified as an ulcer. The difference between the control vs. ascorbate-treated groups compares favorably with

the two preceding experiments in which ascorbate was administered systemically.<sup>1, 2</sup>

This experiment reaffirms the finding that the level of aqueous humor ascorbate in control eyes does not correlate with the presence, time of appearance, or degree of ulceration.<sup>2</sup> The development of corneal ulcers after alkali burns seems to be influenced only when aqueous humor ascorbate levels are elevated to normal or above normal levels by topical or parenteral therapy. Examination of the present results leaves two areas of question. First, one eye which showed a superficial ulcer in the ascorbate-treated group showed the lowest aqueous humor ascorbate level (14 mg/dl). Whether ascorbate penetration into this cornea was deficient or other factors were operative is unknown. Second, total corneal vascularization occurred in seven control eyes but in only one ascorbate-treated eye. These differences may not be as great as they appear, since it must be remembered that any animals with a very small residual avascular zone would not be classified as totally vascularized. Previously we have noted no inhibition of corneal vascularization by ascorbate treatment.

At the end of the experiment the mean aqueous humor ascorbate was significantly elevated in the experimental group as compared to the control group. The aqueous humor levels of ascorbate in the experimental group did not attain a level as high as that noted after the penetrability studies using 10% ascorbate. This may be explained by the smaller size of the epithelial defects and the extensive corneal vascularization present in the experimental eyes. Presumably this aqueous humor ascorbate elevation persisted in the experimental group during the 14 hr dropping period. Despite the withdrawal of topical ascorbate over a 10 hr resting period each night, the 14 hr daytime schedule was most effective in reducing the incidence of ulceration in alkali-burned eyes.

It was not surprising that there was no difference in the aqueous humor content of protein in the control and experimental eyes at the end of the experiment. However, the observation does imply that both groups initially received similar degrees of trauma and that the ascorbate-treated group showed no improvement in the integrity of the blood-aqueous barrier, at least with respect to the permeability to plasma proteins.

These studies clearly demonstrate that topical administration of ascorbate hourly for 14 hr of the day significantly reduces the incidence of ulcer development after experimental alkali burns in rabbit eyes.

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**Key words:** corneal wound healing, corneal burns, alkali (corneal ulcer) (corneal perforation), vitamin C

#### REFERENCES

1. Levinson, R. A., Paterson, C. A., and Pfister, R. R.: Ascorbic acid prevents corneal ulceration and perforation following experimental alkali burns, *INVEST. OPHTHALMOL.* **15**:986, 1976.
2. Pfister, R. R., and Paterson, C. A.: Additional clinical and morphological observations on the favorable effect of ascorbate in experimental ocular alkali burns, *INVEST. OPHTHALMOL. VISUAL SCI.* **16**:478, 1977.
3. Fraunfelder, F. T.: Extraocular fluid dynamics; how best to apply topical ocular medication, *Trans. Am. Ophthalmol. Soc.* **74**:457, 1976.
4. Maickel, R. P.: A rapid procedure for the determination of adrenal ascorbic acid. Application of the Sullivan and Clarke method to tissues, *Anal. Biochem.* **1**:498, 1960.
5. Zannoni, V., Lynch, M., Goldstein, S., and Sato, P.: A rapid micromethod for the determinations of ascorbic acid in plasma and tissues, *Biochem. Med.* **11**:41, 1974.
6. Green, K., and Downs, S. J.: Ocular penetration of pilocarpine in rabbits, *Arch. Ophthalmol.* **93**:1165, 1975.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* **193**:265, 1951.
8. Bancroft, H.: *Introduction to Biostatistics*, New York, 1957, Hoeber-Harper, pp. 115-182.

#### Sensitizing activity of 9,13-dicis retinal in bleached photoreceptors of the skate.

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*9,13-dicis Retinal was externally applied to photoreceptors of isolated skate retina that previously had been desensitized by bleaching irradiation. This treatment led to a significant lowering of photoreceptor threshold and to the intracellular formation of isorhodopsin II, an ar-*

*tificial visual pigment containing 9,13-dicis retinal as its chromophore. These results suggest that isorhodopsin II can function in situ to promote an increase in the visual sensitivity of skate photoreceptors.*

Although all naturally occurring visual pigments contain as their chromophore the 11-*cis* form of retinal (or 3-dehydroretinal), the 11-*cis* isomer is not unique in its ability to bind at the chromophoric site of opsin. Recently, it has been shown that a photosensitive pigment, "isorhodopsin II," is formed on the incubation of extracted opsin with 9,13-*dicis* retinal, a stereoisomer of the native chromophore.<sup>1</sup> In certain respects, the photochemistry of isorhodopsin II is similar to that of rhodopsin and "classic" isorhodopsin ("isorhodopsin I," containing 9-*cis* retinal)<sup>2</sup>; for example, the bleaching of all three pigments leads to the formation of opsin and all-*trans* retinal. In other ways, isorhodopsins I and II differ significantly from rhodopsin; the isopigments maximally absorb light at relatively shorter wavelengths, and their quantum efficiencies of bleaching are less than that of the naturally occurring pigment.<sup>1-3</sup> Furthermore, the formation of isorhodopsin II in vitro occurs much more slowly than does the formation of rhodopsin or isorhodopsin I.<sup>1</sup> The distinctive properties of these pigments clearly depend on the configuration of their retinal chromophore.

The fact that opsin can combine with synthetic isomers of retinal raises an interesting question: Do the resulting analogs of rhodopsin exhibit physiological activity if they are induced to form within the photoreceptors? Recent evidence has suggested that isorhodopsin I can indeed act in the visual process.<sup>4-6</sup> For example, treatment of the partially bleached, isolated skate retina with 9-*cis* retinal leads to both the formation of isorhodopsin I in the receptors and a substantial lowering of photoreceptor threshold. Here we present evidence that 9,13-*dicis* retinal, when applied to the skate retina under similar conditions, also promotes the formation of pigment (isorhodopsin II) and a sensitization of the receptors. Our findings suggest that isorhodopsin II can at least partially mimic the visual activity of rhodopsin.

**Methods.** The isolated skate retina, utilized in the present experiments, is a preparation in which the photoreceptors do not ordinarily regenerate visual pigment.<sup>4-6</sup> Sections of retina (approximately 3 by 5 mm) were isolated from the tapetal region of the dark-adapted eye; the techniques of dissection and electrophysiological recording, as well as the composition of the Ringer's solution, were as previously described.<sup>6</sup> Photoreceptor po-