Aerobic Responses of the Cornea to Alkali Measured In Vivo

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With the use of a micropolarographic system, the effects of a series of unbuffered sodium hydroxide concentrations on oxygen uptake by the corneal epithelium of rabbit were measured in vivo. Based on an exposure model of 10 sec, followed by a saline rinse, concentrations of greater than 0.10 N were found to cause an abrupt and severe decline in oxygen uptake, with oxygen flux responses associated with 0.13 N and greater being indistinguishable 10 min later from those of a cornea completely denuded of epithelium. Initial flux variations, characteristic of exposure responses to concentrations of 0.10 N or less, gradually diminished, however, stabilizing within 1 hr to levels not significantly different ($P < 0.01$) from pre-exposure fluxes or those of saline controls. Invest Ophthal-mol Vis Sci 24:582-585, 1983

Most detailed information on alkali burns of the eye has been qualitative, based on clinical or laboratory observations of the initial and healing phases of surviving tissue, or on histologic assessment of tissues following clinical loss or laboratory collection.1-12

Described here is an in vivo means of quantifying epithelial damage to the alkali exposed cornea, both at the outset, and throughout the later stages of healing.

A splash model of 10-sec exposure to unbuffered sodium hydroxide was used here to determine: (1) if a concentration threshold for corneal damage could be reliably identified using changes of oxygen uptake across the tear-epithelial interface, and (2) what the quantitative characteristics of progressive supra-threshold concentrations would be on oxygen dependent activity over the initial phases of such trauma.

Materials and Methods

A micropolarographic (25-μm cathode diameter, Clark-type electrode) system, described earlier for the measurement of oxygen flux across the tear-epithelial interface of the cornea, was used to quantify the differences of oxygen uptake before and after test solution exposure.13 A 12-μm thick polyethylene membrane over the end of the probe served both as an oxygen reservoir and as protection for the probe’s anode and cathode elements from contact with any residual test solution possibly left in the tissue. No biasing effect of the test solutions on probe performance could be demonstrated.

To explore the sensitivity of this method relative to other techniques in the literature, it was applied here to rabbit corneas, allowing long sequences of measurements to be made under uniform conditions. The corneas of each young adult animal were inspected carefully at the outset and monitored throughout. Ethyl carbamate general anesthesia was used, and the corneas were kept moist with misted saline.

A pre-exposure oxygen uptake average was established for each cornea, based on a series of eight measurements made at 3-min intervals over the 24-min period just preceding alkali, or saline control, exposure. On a population basis, the flux rates at none of those pre-exposure times differed significantly ($P < 0.01$) from any of the others. However, because of the slight differences in absolute rates found normally among corneas, the predosage baseline average for each was ratioed with the test (saline or NaOH) solution response values for that same cornea. The dashed horizontal reference lines in the figures indicate a ratio equal to 1.00; ie, no change. Points above the line indicate an increased rate of oxygen uptake by the cornea relative to pre-exposure values, and points below, a depression.

The primary concern of this study was with differential responses of the cornea to graded concentrations of alkali, in particular, following direct exposures of the epithelial surface to selected concentrations of sodium hydroxide for a standard period of
Fig. 1. The effects of various concentrations of sodium hydroxide on oxygen flux across the anterior surface of the cornea relative to pre-exposure (normal) flux levels, shown at eight times following a 10-sec exposure period to each concentration, then a saline rinse.

10 sec. Normal saline control series were included to determine the reliability of measurement of the technique. As the corresponding (ie, zero) concentration points in the successive frames of Figure 1 indicate, the associated flux measurements varied little (an average of 3%) from the predosage baseline level (dashed line), not differing significantly ($P < 0.01$) from it. In the course of those measurements as well, the smooth, flat end of the electrode was found by fluorescein examination, to have caused no significant damage to the corneal surface.

As in the saline control (zero concentration) series, each of the sodium hydroxide concentrations was maintained in contact with the corneal surface for 10 sec, being contained within a 8-mm diameter cylinder holding just enough liquid to assure confluent coverage of the entire enclosed area. Immediately following that 10-sec exposure period, the cylinder was lifted from the cornea, and the agent was washed away with a normal saline rinse. Each saline and sodium hydroxide concentration condition was measured as many as six times, each trial being made on a fresh, healthy cornea. Following each exposure period, oxygen flux measurements were repeated at times +1, +3, +5, +10, +15, +20, +30 and +60 min to monitor any oxygen flux changes induced.

Results

Findings for each of the series of sodium hydroxide concentrations studied here are summarized in Figure 1. The oxygen response fluxes associated with each sodium hydroxide concentration, measured at eight times following the 10-sec agent exposure and saline rinse, appear in the successive frames of Figure 1. Figure 2 shows the markedly different responses associated with concentrations of 0.10 N and 0.13 N NaOH. Figure 3 shows the histologic appearance of corneas following exposure to those concentrations, in addition to a cornea exposed only to normal saline. Such marked differences in tissue response to those two NaOH solution concentrations could be verified readily by fluorescein examination at the time of the experiment as well.

As indicated by the negligible changes of the saline control (0 time) responses from the predosage levels in Figure 1, the measuring probe itself, produced no detectable artifact due to the gentle physical contacts.
made with the corneal surface. These results confirm similar control observations described earlier.\textsuperscript{14}

**Discussion**

This micropolarographic method was first applied as a quantitative method for measuring corneal damage due to the ophthalmic anesthetics, and later, preservatives.\textsuperscript{14-15} At no postexposure time in either that study or this one were the oxygen uptake rates associated with the normal saline control dosages found to be significantly different from the pre-exposure level or the saline controls ($P < 0.01$). Initial flux variations did occur with concentrations of $0.10 \text{ N}$, or less, but those responses commonly stabilized to levels not significantly different from pre-exposure or saline control values within one hour.

Concentrations greater than $0.10 \text{ N}$ produced quite defined and enduring effects, however. The oxygen flux response associated with $0.13 \text{ N}$ exposure was sufficiently severe not to be distinguishable from that of an epithelially denuded cornea. Entire physical loss of the epithelium did occur occasionally at that concentration, but nearly always at the $0.60 \text{ N}$ concentration level, as was indeed confirmed histologically.

The oxygen flux decreased further, although in small steps, as the concentration was increased beyond $0.13 \text{ N}$. This further decrease suggests possible damage to stromal connective tissue and cells. Stromal
clouding was observed grossly with these higher concentrations and was most pronounced following the 0.60 N NaOH exposure.

It should be noted, however, that while corneal responses over the first hour of postexposure as examined here maintained a fairly consistent quantitative course, beyond that time, preliminary observations have shown recovery to a normal baseline to be wide ranging and individualized, from a few hours to several days, depending on the particular combination and degree of edema and cell loss finally sustained.

The in vivo polarographic method described here is a “splash” model for the evaluation of corneal responses to particular sodium hydroxide concentrations which appears then to be at least as sensitive as common methods of clinical assessment, but provides an alternative approach that is objective and quantifiable. It may not, however, be as sensitive as certain invasive techniques of histologic or electrical nature, which may reveal cellular changes sufficiently early that aerobic demands have not yet been overtly compromised.8-11

The in vivo applicability of this technique may prove useful for the quantitative monitoring of a range of corneal conditions including the natural healing courses of accidental and experimental burns, as compared with the ongoing effectiveness of particular therapeutic strategies.

Key words: cornea, alkali, oxygen, polarographic, rabbit

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References