provides a new experimental model for the study of angiogenesis. Our test system has numerous advantages: it permits assessment and comparison of the angiogenic activity of substances isolated from non-neoplastic rat tissue and rat tumors such as the Walker 256 carcinosarcoma in an allogeneic in vivo system. This is important because a significant inflammatory response is induced by xenogeneic donor tissue in the rabbit corneal micropocket assay. The paucity of histologic inflammation in our experiments is probably related to the fact that test substances are of Sprague-Dawley origin. However, the scope of substances testable in our assay can be expanded to include xenogeneic material, since the rat can safely be rendered immune deficient by x-irradiation. The rat corneal micropocket assay has the additional advantages of being facile and economical.

These experiments demonstrate that both the crude ethanol extract and partially purified endothelial growth stimulatory factor from Walker 256 tumor homogenate have angiogenic activity in vivo. In addition, the partially purified factor is more potent than the crude ethanol extract since it effected a significantly higher capillary growth rate (0.21 vs. 0.08 mm/day, respectively, for the initial 4-day interval) at slightly lower dosages (0.11 O.D. 260/pellet vs. 0.16 O.D. 260/pellet). The fact that our low molecular-weight substance derived from Walker 256 tumor is angiogenic agrees with the findings of Weiss et al. However, we have demonstrated this in the animal of origin for this tumor-derived substance.

The absence of capillary regression before day 16 in corneas implanted with partially purified factor may mean that the capillaries have matured faster than those in corneas containing crude ethanol extract. However, since a continued stimulus is required for the maintenance of blood vessels in this system, this finding may simply indicate that the partially purified substance is more slowly released from the polymer than the ethanol extracted material.

In addition to being implicated in tumor growth, angiogenic substances play a prominent pathophysiological role in numerous ocular diseases, including corneal graft rejection, diabetic retinopathy, and retrolental fibroplasia. Our rat corneal micropocket assay could quicken the purification and characterization of these angiogenic substances as well as the naturally occurring inhibitors of angiogenesis. With increased understanding of neovascularization and its control, effective modalities for treating cancer and the neovascular ophthalmopathies can be formulated.

We are grateful to David Thompson, David Schroeder, and John Gallup for technical assistance.


Key words: angiogenesis, rat corneal micropocket assay, corneal neovascularization, tumor angiogenic factor

REFERENCES


Chemical effects of alkali on polymethylmethacrylate intraocular lenses. MILES A. GALIN, JOSEPH C. SALAMONE, ALFRED P. OLSON, AND AUDREY W. TUBERVILLE.

Polymethylmethacrylate intraocular lenses were left in 10% NaOH or 10% KOH for various periods of time. Contact angles were unaltered and electron microscopy

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for chemical analysis was unchanged. This concentration of alkali does not appear to chemically change the lens surface during the study period.

For nearly 30 years intraocular lenses of polymethylmethacrylate (PMMA) have been sterilized by the Ridley method.\textsuperscript{1,2} This technique is still employed in Europe but has been abandoned in the United States both because it is not a terminal form of sterilization and because the 0.1% NaOH concentration in which the lens is stored prior to use is an unsatisfactory sterilizing solution if seeded with a large inoculum such as $10^6$ organisms.\textsuperscript{3} In addition, the NaHCO\textsubscript{3} buffering solution used in the Ridley method has been a source of contamination in more than one case of intraocular infection.\textsuperscript{4}

Ethylene oxide (ETO) is the mandated terminal form of sterilization in this country for intraocular lenses.\textsuperscript{5} There are questions of carcinogenicity for this agent, and there is little known concerning its effects and the effects of minute concentrations of its breakdown products, ethylene chlorhydrin and ethylene glycol, on intraocular tissue. However, ETO has proved to be a safe and effective sterilizing agent according to the preliminary data from the Food and Drug Administration on the ongoing implant clinical investigation.\textsuperscript{6} The potential still exists, however, for sterilizing unclean lenses with ETO and introducing foreign but sterile material into the eye.\textsuperscript{7} In addition, in some quarters a clinical impression exists, which suggests that the incidence of sterile hypopyon is higher in ETO-sterilized lenses. Consequently, a search persists for a sterilization method that combines the best attributes of the cleaning and sterilization potential of NaOH and the terminal sterilization benefits of ETO.

One logical product that might fulfill these requirements is NaOH at a concentration that has the appropriate $d$ number, does not lose efficacy in storage, does not alter the lens with time, and does not require dilution or buffering. Ten percent NaOH has been shown to sterilize a slurry of $10^6$ organisms and a looped implant in approximately 3 hr.\textsuperscript{8} Sterilization is maintained for at least 2 years of shelf life (personal observation, M. A. G.). Furthermore, since NaOH is not absorbed by PMMA, simple rinsing (as done even for ETO-sterilized lenses) is adequate in the operating room. We do not know, however, what such a high concentration of NaOH might do to the chemistry of the lens. In particular, a strong base such as NaOH could hydrolyze the surface methyl ester groups according to the following reaction\textsuperscript{9\textendash}12.

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_2-\text{C} & \quad \text{CH}_2-\text{C} \\
+ \text{NaOH} & \quad + \text{CH}_3\text{OH} \\
\text{C}=\text{O} & \quad \text{C}=\text{O} \\
\text{O} & \quad \text{O}^- \\
\text{Na}^+ & \quad (\text{II}) \\
\text{CH}_3 & \quad \text{(I)}
\end{align*}
\]

where I represents one subunit of the PMMA molecule at the lens surface. If this surface hydrolysis occurs, the lens surface would change from neutral to anionic (negative). Also, since sodium polymethacrylate, the homopolymer of complete structure II, is highly water soluble whereas PMMA is water insoluble, the surface would be expected to become more hydrophilic as surface hydrolysis occurs. Because of these possibilities, a study was undertaken to ascertain the effect of strong base on the surface of PMMA intraocular lenses.

**Materials, methods, and results.** Perspex CQ was obtained from the same sheets used to make iris clip and anterior chamber lenses\textsuperscript{*} and was provided as a clear sheet of material sandwiched between two sheets of "waxed" paper. Sixteen 1 by 1 cm samples were used for contact angle measurements, and eight 1 by 2 cm samples were used for electron spectroscopy for chemical analysis (ESCA) studies. No pieces with gross defects such as scratches, chips, or cracks caused by cutting were chosen as test specimens. Each sample was cleaned with an anionic sulfonate detergent typically used for hard contact lenses and was then exhaustively rinsed in deionized water. The detergent was completely removed by rinsing, as evidenced by the lack of any sulfur peaks in the ESCA scan.

Contact angles were measured at 20° C on each sample with a drop image projection technique whereby the shadow of a drop is projected onto a screen calibrated for angle measurements to the nearest 1 deg. Contact angles were 60 ± 2 deg in all cases. Eight 1 cm pieces were then submerged in 10% NaOH at 20° C for periods of 5 min to 4 days. The samples were removed and rinsed, and their contact angles were remeasured. No change from the original contact angle was observed. The other eight 1 cm samples were placed in 10% NaOH at 50° C for up to 1160 hr (approximately

\*\textsuperscript{Kindly provided by the Intraocular Lens Manufacturers Association.}

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equivalent to 9000 hr at room temperature). Contact angles (60 ± 2 deg after 12 hr and 59 ± 2 deg after 1160 hr) were essentially unchanged.

ESCA analysis, which is capable of detecting specific atoms in concentrations as low as 1% (atomic), was performed on the remaining eight samples. The specimens were submerged in a 10% KOH solution at 50° C for up to 4 days. Controls were treated with deionized water only. KOH was used instead of NaOH because of possible contamination by environmental sodium. Broadband spectra and shortscans of the carbon 1s/potassium 2p binding energy region in ESCA were taken for each sample. From these measurements it is possible to determine the elemental composition of the surface (20 to 50 Å deep), including the percentage of carbon atoms that are carbonyl carbon (C==O) or hydrocarbon carbon (—C—), as well as the percent of surface K⁺.

Elemental compositions of control and experimental samples were identical (±1 atom/100 atoms), whereas slightly higher values of hydrocarbon carbon atoms (approximately 61% as compared with a theoretical value of 57%) and ester carbonyl carbon atoms (approximately 17% as compared with a theoretical value of 14%), and slightly lower values of oxygen atoms (20% as compared to a theoretical value of 28%) were found, compared with a PMMA surface that has equivalent amounts of—CH₃ and —C—OCH₃ groups exposed. Approximately a 1% silicon impurity was also noted. Significantly, there was no indication of potassium or any other cationic atom that might have undergone ionic exchange with potassium.

For a PMMA repeat unit characterized by structure I (which has seven scattering centers, four of which are hydrocarbon carbon scattering centers, one carbonyl carbon scattering center, and two oxygen scattering centers), the fact that the theoretical values of each group were not obtained would indicate a slightly greater preference for carbon on its surface than for oxygen. However, since surface analysis is dependent on the history and treatment of the sample, additional studies will have to be performed before an understanding of the behavior of the groups on the PMMA can be obtained.

Discussion. The Ridley technique for intraocular lens sterilization was applied originally to biconvex, solid lenses. Ten percent NaOH for 1 hr is effective against 10⁶ organisms in such a system. Storage in 0.1% NaOH, part of the original Ridley technique, was clinically adequate despite the fact that such a solution does not sterilize 10⁶ organisms. This implies that transfer to storage solution was aseptically performed or that contamination was of such low order that prolonged storage was self-sterilizing.

The introduction of looped lenses, with accompanying tunnels and irregular surfaces, required a reevaluation of the Ridley technique. Indeed, it was found that 3 hr in 10% NaOH was now required⁸ The fact that no positive cultures have ever been obtained from lenses stored in 0.1% NaOH solutions after only 1 hr in 10% NaOH again indicates that no contamination occurred or potential inocula were of low concentration.

There are clinical advantages to NaOH sterilization. The lens is cleaned by alkali most effectively so that any residual materials such as oils or polishes are removed. Some studies have incriminated such residues in pseudophakic inflammatory responses.¹⁴ Although these studies have employed 10% NaOH, we have data indicating that lenses left in 5% NaOH for 24 hr are sterile even when contaminated with a slurry of 10⁶ organisms, including Bacillus subtilis.¹⁵ This concentration of alkali, then, provides a terminal form of sterilization for all types of PMMA lenses, does not require dilution, and does not require buffering. Furthermore, our present study implies that at least for one year at room temperature and at the concentrations of base studied, this concentration would not alter the lens surface.


Key words: intraocular lens, NaOH sterilization, Ridley method, contact angle, electron spectroscopy for chemical analysis

REFERENCES

The efficacy of brief periods of reverse occlusion in promoting recovery from the physiological effects of monocular deprivation in kittens. David P. Crewther, Sheila G. Crewther, and Donald E. Mitchell.*

The relative effects of short daily periods of reverse occlusion in promoting recovery from the physiological effect of monocular deprivation in kittens were examined with a view to identifying a neurophysiological basis for the visual improvement observed with minimum occlusion therapy in amblyopia. Kittens were monocularly deprived from near birth until 5 weeks of age, at which time they were reverse-sutured and housed in total darkness. Each kitten received a short period of visual exposure through its initially deprived eye each day for either a fixed number of days or for a constant total visual exposure spread over a different number of exposure sessions. Electrophysiological recordings from single cells in the visual cortex were made the day after the last visual exposure. Kittens that received daily periods of reverse occlusion as brief as 30 min for 20 days showed a substantial degree of reversal of cortical ocular dominance. Other experiments indicated that 20 hr of reverse occlusion distributed over a number of brief daily sessions was far more effective in promoting physiological recovery than the same total period of exposure imposed in only two sessions. In general these results suggest that a given period of reverse occlusion may be more effective in promoting recovery with distributed than with massed periods of occlusion.

For over two centuries the accepted treatment for amblyopia has included occlusion of the better eye for a time in order to force the child to use the amblyopic eye. Although it is generally agreed that greater improvement in the vision of the amblyopic eye is achieved with full-time occlusion, a growing body of evidence indicates that even brief daily periods of patching can be quite effective. Since brief periods of patching are far more acceptable to a child than full-time occlusion, it is important to explore this approach in greater detail including identification of its neurophysiological basis through animal models.

Kittens monocularly deprived by eyelid suture from near birth to 5 weeks of age or more appear behaviorally blind when first forced to employ their deprived eye. This functional blindness is accompanied by striking physiological changes in the visual cortex, where the vast majority of cells can be excited only by visual stimuli presented to the formerly open eye. However, substantial recovery can be observed both behaviorally and physiologically after termination of the period of monocular occlusion, particularly if the animal is forced to employ its formerly deprived eye by occlusion of the other eye, a procedure generally referred to as reverse occlusion. In this situation the distribution of ocular dominance of cortical cells can be completely reversed, so that all cells are now excited only by visual stimuli delivered to the formerly deprived eye. (Ocular dominance refers to the relative excitatory influence of each eye on a cortical cell.) The dramatic shift in the ocular dominance of cortical cells is also accompanied by equally rapid improvements in visual behavior and acuity. Most studies of the physio-