both cornea and conjunctiva occurred as early as 15 min after bacterial inoculation. By 1 to 3 hr after bacterial inoculation, shrinkage of corneal epithelial cells was pronounced (Fig. 5, a and b). Fewer microvilli were seen on the shrunken regions of these affected cells, and the cell surfaces appeared roughened and irregular. Additionally, intercellular junctions were dilated to 1.0 μm in diameter (Fig. 5, b).

Discussion. Normally, Pseudomonas cannot infect the eyes of adult mice without prior corneal wounding.5 However, infant mice are particularly susceptible to inoculation of the organism beneath the unwounded fused eyelid and quickly die of Pseudomonas bacteremia.1 Pseudomonas eye infection in human infants, particularly those that are premature, has been shown to be the cause of bacteremia and death, despite antibiotic therapy.6 Thus, the infant mouse model provides an excellent correlation with clinical cases of Pseudomonas eye infection in newborns.

The present experimental study has established the mechanism by which Pseudomonas invade the unwounded ocular surface. The organism attached to and lysed the corneal and conjunctival epithelium and penetrated and lysed the respective stromas as early as 15 min after bacterial inoculation beneath the fused eyelid. Previously, other investigators using light and electron microscopy have reported phagocytosis of various organisms by conjunctival epithelial cells.7-9 Additionally, the enteric mucosal surface is active in phagocytosis of various microbes.9,10 However, in this study, we found no evidence to suggest that either conjunctival or corneal epithelial cells phagocytized Pseudomonas.

Further work is needed to determine why unwounded infant mouse eyes are particularly susceptible to invasion by Pseudomonas whereas adult mouse eyes are not. Whether this is due solely to microbial virulence factors or is also dependent upon host factors such as amounts of lysozyme, S-IgA, other immunoglobulins, complement, and/or additional factors remains to be resolved.

From the Departments of Anatomy and *Immunology/Microbiology, Wayne State University School of Medicine, Detroit, Mich. This study was supported by Public Health Service grants EY02986 and EY01935 from the National Eye Institute, Bethesda, Md. Submitted for publication Aug. 6, 1979. Reprint requests: L. D. Hazlett, Ph.D., Department of Anatomy, Wayne State University School of Medicine, 540 East Canfield Ave., Detroit, Mich. 48201.

Key words: unwounded immature cornea and conjunctiva, Pseudomonas aeruginosa, lysis, penetration, scanning and transmission microscopy

REFERENCES

Corneal swelling at low atmospheric oxygen pressures. ROBERT B. MANDELL AND ROBERT FARRELL.

Corneas of 25 human subjects were exposed to oxygen partial pressures, in a gas goggle, of either 6.9, 17.1, or 22.2 mm Hg, and the corneal swelling was measured by pachometry for 4 hr. Following an initial rise, the swelling stabilized after about 3 hr, in many subjects it was reversed, indicating an adaptation process. The average corneal swelling after 4 hr for the three oxygen partial pressures was 5.07%, 2.13%, and 1.66%, respectively. The average minimum oxygen requirement to prevent corneal swelling was at least 23 mm Hg and varied significantly among individuals. The variations in swelling responses were large enough to explain clinical differences in edema of patients wearing oxygen-permeable contact lenses, in the absence of adequate tear pumping.

Key words: oxygen, corneal swelling, gas goggle.
It is well known that the external cornea draws upon the atmosphere for its normal oxygen supply. However, the exact oxygen requirement of the cornea is still open to question. Polse and Mandell found the minimum partial pressure of oxygen, below which corneal edema was produced in humans, to be somewhere between 11.4 and 19 mm Hg. A range of uncertainty was given because the study was limited to only three subjects and there were no measures of repeatability.

The introduction of oxygen-permeable polymers for contact lenses has heightened interest in obtaining a more exact knowledge of the minimum corneal oxygen requirement. In particular, it is important to determine individual differences in minimum oxygen need. This has at least two applications. First, we need to know whether or not differences in the amount of edema found in patients wearing contact lenses may be attributed to individual differences in oxygen requirement or to the fit of the lenses. This would guide us in selecting the type of lens replacement or modification to be made. Second, we need to know that individual oxygen requirement as a basis for developing contact lenses with appropriate permeabilities.

We have repeated the experiments of Polse and Mandell with some modifications. The recording system of the pachometer was improved, and the gas goggles were redesigned for better comfort and improved optical quality. We increased the number of subjects and repeated measurements for several subjects for a reliability study.

Materials and methods. The corneal thickness measuring apparatus used in this experiment was described previously. It consisted of a Haag-Streit slit lamp and pachometer to which was added an electronic digital recorder. The gas goggles were modified swimming goggles with good optical quality which allowed pachometry measurements to be made while they were worn. The goggles were equipped with inlet and outlet tubes through which humidified gas was circulated. The gas was obtained from storage cylinders and humidified to 65% by passing it through a gas wash bottle placed in a constant-temperature water bath at 34°C.
Fig. 2. Corneal swelling after 4 hr exposure to low oxygen levels. The linear regression line and confidence limits at 2 S.D. are shown, together with the exponential regression line. The base line at 0.44% denotes average swelling in the control eyes. X one of two measurements at that concentration for the same subject.

Three mixtures of oxygen and nitrogen were used. The percent volumes of oxygen were 0.95%, 2.34%, and 2.77% (equivalent to 6.9, 17.1, and 20.2 mm Hg, assuming a vapor pressure of 30 mm Hg). The gas in each tank was analyzed to an accuracy of 0.01% by gas chromatography.

The subjects were 28 university students, 15 females and 13 males, between 18 and 35 years of age, who were applicants to the contact lens study. None was on medication, and all appeared to have normal tear characteristics by biomicroscopic examination. Several other candidates were eliminated because of poor fixation during pachometry or a decision on their part not to continue with the experiment due to discomfort.

Each subject received a general eye examination and a pachometry training session prior to the test day. On the test day the subject awakened at least 2 hr prior to the experiment, to avoid the effects of sleeping on corneal thickness. The goggles were adjusted in place, and baseline pachometry measurements were made. The gas was turned on, and pachometry was repeated each hour for a 4 hr test period (every half hour for the 2.77% oxygen mixture). The gas mixture was always passed before the left eye of each subject, and the right eye was exposed to room air as a control. Gas from the outlet tube was exhausted in a beaker of water and monitored to ensure that a positive pressure was maintained in the goggle throughout the experiment. Ten pachometry measurements were made of both the experimental and control eyes at each period of the testing session. (Some of the early runs did not include measurements of the control eye.)

Data are included for only those subjects and experimental runs in which measurements were possible for 4 hr. Several trials were aborted because the subjects were too uncomfortable to continue or because gas leakage from the goggles was detected.

Most subjects were available for measurements
with only a single gas mixture. The total number of subjects measured with each gas mixture was 13 at 2.77% $O_2$, 13 at 2.34% $O_2$, and 7 at 0.95% $O_2$. Five subjects had measurements at both the 0.95% and 2.34% oxygen levels. Measurements at the same oxygen concentration were repeated for 10 subjects for a reliability study.

**Results.** Corneal swelling was found in all subjects at the 0.95% and 2.34% oxygen levels and for 11 of 13 subjects at the 2.77% level. The average swelling curves are shown in Fig. 1. The data presented for the test eyes are absolute values. Low amounts of corneal swelling (mean 0.44%) were found in the control eyes, and this was attributed to the osmotic effects of tearing or experimental error. Since the swelling in the control eyes was very low and because data were not taken on all control eyes, their swelling values were not subtracted from the test eye data for the analysis. However, when swelling values for the control eyes were subtracted from the concurrent values for the test eyes in 19 subjects, no changes were found in the swelling trends.

Corneal swelling was evident in the first half hour of exposure to low oxygen levels. Thereafter, it increased to a maximum at 2 to 3 hr and was then reduced. This general trend was evident in the data for most individuals. When corneal swelling at the 3 and 4 hr test periods was compared, more swelling was found at 3 hr in 33 of the 43 total experimental runs (significant $\chi^2$ at 0.01% level). The average corneal swelling after 4 hr was 5.07%, 2.13%, and 1.66% for the 0.95%, 2.34%, and 2.77% oxygen levels, respectively. There was no significant difference in the swelling at the 2.34% and 2.77% oxygen levels at any time, but a significant difference was found between these and the 0.95% oxygen level at the 3 and 4 hr periods (Student's $t$ test at 0.05% significance level).

**Reliability.** The data for all subjects are plotted in Fig. 2 in terms of corneal swelling after 4 hr of each oxygen concentration. It is of interest whether the swelling differences for the individual subjects represent true individual differences or simply experimental error. This was evaluated by comparing the variance of swelling among individuals to the variance of swelling when pachometry was repeated.

Ten subjects had their measurements repeated at the same oxygen concentrations: four at 0.95% $O_2$, four at 2.34% $O_2$, and two at 2.77% $O_2$. The variance from the mean of the two experimental trial means for the 10 replications was ±0.45%. This is significantly different from the variance of swelling for all the individuals at all oxygen levels which was ±1.31% (F ratio at 0.01 level of significance).

Five subjects had pachometry measurements taken at both the 0.95% and the 2.34% oxygen levels (Fig. 3). A correlation was found (0.77) for individual corneal swellings at the two oxygen levels, but the number of subjects was too low for statistical significance.

Unfortunately, there are insufficient data to firmly establish individual thresholds to the minimum oxygen requirement. However, possible values may be determined from an examination of the data of Fig. 2. An assumption that the relationship between corneal swelling and oxygen partial pressure is linear allows fitting a straight line which intercepts the zero axis at the 3.4% position. If an allowance is made for corneal swelling of 0.44% (average swelling in the control eyes) due to excess lacrimation or to dilution of the tears from
water vapor condensing in the goggles, the regression line intercept occurs at an oxygen level of 3.2%. Confidence limits at 2 S.D. from this would establish a range of thresholds of 2.0% to 4.4%. These values would necessarily be minimum for the oxygen threshold. It is unlikely that the regression line for corneal swelling and oxygen partial pressure is truly linear, but rather it is probably more nearly an exponential function. Such an assumption would shift the oxygen thresholds toward higher values. Unfortunately, here the error produced by corneal swelling due to factors other than oxygen deprivation becomes of greater significance. If an exponential relationship is postulated and if the assumption is made that 0.44% of the corneal swelling is due to nonoxygen factors, the average threshold shifts to 4.4%, with a predicted range of 3.3% to 5.5%.

**Discussion.** We found that the typical corneal swelling response to low oxygen levels was an increase in thickness for 2 to 3 hr, followed by stabilization or a thickness decrease, indicating adaptation. The mechanism of adaptation is subject to conjecture, since control of hydration in the cornea is not completely understood. Polse and Mandell estimated that the average threshold oxygen level which does not cause corneal swelling was between 11.4 and 19 mm Hg. Our results have revealed higher thresholds and significant differences in the minimum oxygen requirement for individuals. The average threshold was between 23 and 37 mm Hg, depending upon the allowance made for contaminating factors which could not be controlled. The individual thresholds for 95% of the subjects varied ±8.7 mm Hg from the mean value. These results are in close agreement with those found previously in animal studies using various histochemical measures of oxygen deprivation.

Although the threshold oxygen requirement is higher than previous estimates, the amount of corneal swelling at low oxygen levels is less than might be expected. Corneal swelling at the 0.95% O₂ level (6.9 mm Hg) varied among individual subjects between 2.4% and 7.3%. These limits are within the range commonly found clinically for both hard and soft contact lens patients. and suggest that many contact lens wearers may be exposed to lower oxygen levels at the precorneal surface than has been assumed. It explains, however, why soft contact lenses, which have permeabilities previously considered to be low and inconsistent with clinical results, are indeed sufficient to allow a clinically acceptable level of corneal edema.

Mandell et al. described the corneal swelling response to hard contact lenses as having essentially the same characteristics as were found with the low oxygen levels of our present study. They postulated that the stabilization or reversal of the swelling response was due to the cessation of tearing as adaptation occurred. It now appears that this response could also be explained as due to corneal adaptation to low oxygen levels.

The question remains as to what level of oxygen tension is needed at the precorneal surface of a contact lens wearer to avoid interference with the normal corneal physiology. Our results indicate that an oxygen tension as low as 7 mm Hg may be tolerable for short time periods, even though a level of about 35 mm Hg might be necessary for longer periods. Consequently, significant differences may exist in the oxygen needs of an extended-wear contact lens patient as contrasted to a daily-wear patient. It must also be considered that the corneal oxygen requirement of the sleeping patient may be less than during the diurnal period.

These results provide supporting evidence that some contact lens patients are basically more prone than others to corneal edema from contact lens wear.

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**Key words:** cornea, oxygen tension, pachometry, edema, corneal thickness, contact lenses

**REFERENCES**

The effect of induced diabetes on the electroretinogram components of the pigmented rat. E. L. PAUTLER AND S. R. ENNIS.

The fast and slow components of the electroretinogram (ERG) of the pigmented rat were observed 2, 4, and 19 weeks after the induction of diabetes by streptozotocin. The diabetic state was confirmed by measures of serum and urine glucose and the appearance of polydipsia and polyuria. There was no apparent effect on the b-wave of the ERG. As shown in Table I, the diabetic state was further characterized by an increase in the osmolality of the serum.

As illustrated in Fig. 1, changes in the c-wave of the ERG were apparent as early as 2 weeks after the induction of diabetes. As the period of diabetes was extended, further reductions in the c-wave of the experimental animals was observed.

A number of recent reports have presented evidence that a change in permeability of the blood-retinal barrier is the earliest ocular abnormality of diabetes in both humans and experimental animals. Although the pathological involvement of the retinal capillaries has been well established, recent evidence suggests the barrier component represented by the pigment epithelium (PE) is also affected. It has been argued that the observed changes in the infoldings and effective surface area of the basal surface of the PE may reflect alterations in the transport and barrier functions of this tissue. Since the c-wave of the electroretinogram (ERG) is believed to be generated primarily by the PE, it is possible that the functional changes in the PE observed in the diabetic state may be reflected in the generation of this potential. The present study focuses on the changes in ERG components observed in the early stages of streptozotocin-induced diabetes in rats, with particular emphasis on the c-wave.

Methods and materials. Pigmented rats (Long-Evans strain) used in this study were from established colonies maintained by our laboratory. Rats were 60 to 70 days old and weighed 250 to 300 gm at the beginning of the study. Randomly assigned rats were fasted 16 hr before the injection of streptozotocin, 65 mg/kg body weight, via heart puncture. Streptozotocin (lot S-0130; Sigma Chemical Co., St. Louis, Mo.) was dissolved just prior to use in acidified 0.9% saline (pH 4.5) at a concentration of 50 mg/ml. Control animals were injected with acidified saline alone. Diabetes was established by the demonstration of polyuria, glycosuria, and impaired growth in the experimental animals. Serum and urine glucose were measured by the glucose oxidase method on a Beckman glucose analyzer. Serum osmolality was measured on a Wescor Model 5100B vapor pressure osmometer. Eyes were prepared for histological examination as previously described.

For ERG recordings, rats were dark-adapted for 24 hr and then anesthetized with an intraperitoneal injection (70 mg/kg) of sodium pentobarbital. For local anesthesia, 0.5% proparacaine HCl was applied around the eye, and the pupils were dilated with 1% tropicamide (Mydriacyl, Alcon Laboratories Inc., Ft. Worth, Texas). Differential recordings were made by cotton wicks threaded through a small polyethylene tube filled with saline, connecting to a Ag-AgCl wire. One of these electrodes was inserted behind the palpebral conjunctiva for a distance of 2 to 3 mm, and the other was placed on the cornea. A needle electrode under the scalp connected to ground. Responses were recorded on a Grass 70A polygraph after preamplification by a Grass P16 DC amplifier. A Grass PS 2 photostimulator placed 1 m from the eye was employed as light source. To obtain c-wave data, stimuli of relatively long (10 sec) duration were provided by high-frequency flicker at the rate of 50 flashes/sec. The duration was under manual control. Single-flash stimuli were used in determining the b-wave intensity-amplitude curves. The light intensity was controlled by neutral-density filters in conjunction with the intensity controls on the Grass photostimulator.

Results. As shown in Table I, the diabetic state was established by measures of urine glucose, serum glucose, serum osmolality, and body weight. The levels of glucose were greatly elevated in both the serum and urine of the experimental animals, who also lost weight during the early phases of the experiment. Polydipsia and polyuria were observed in the experimental animals. The diabetic state was further characterized by an increase in the osmolality of the serum.

As illustrated in Fig. 1, changes in the c-wave of the ERG were apparent as early as 2 weeks after the induction of diabetes. As the period of diabetes was extended, further reductions in the c-wave of the experimental animals was observed.