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# Dynamics of ocular surface pH

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SUMMARY We studied ocular surface pH in 161 subjects. The mean pH for 133 normal volunteers was 7·11, SD 1·5. We found that older women had a more alkaline pH than other subjects, that the pH shifted from acid to alkaline during the day, that one hour of eyelid closure caused an acid shift in pH, and that pH recovered to baseline values within 40 minutes after acid drop instillation. We explored the mechanism of pH regulation, and we believe that pH changes could affect contact lens toleration, drug effectiveness, and clinical signs in disease processes.

Using the direct contact microelectrode we have studied ocular surface pH in the inferior lateral fornix. Shifts in pH could have therapeutic and diagnostic significance. Both antibiotic effectiveness[1 2] and cell viability[3] are affected by pH.

pH changes may also help in understanding mechanisms of disease processes, since rosacea,[4] allergy, and bacterial infection[5] alter pH. Even toleration of soft contact lenses might be related to pH, because lens hydration decreases in acid pH.[6]

We present pH findings on 322 eyes (161 subjects) and explore mechanisms for the maintenance of, as well as deviations from, the steady state pH.

## Materials and methods

Subjects' age, sex, race, topical and systemic medication, and time of day were recorded. Patients taking substances known to be excreted in tears (methotrexate, aspirin, ampicillin, bacampicillin[7-9]) were excluded from the study. Informed consent was signed by all subjects.

Both eyes were tested. The eye to be measured first was selected at random by coin toss. A digital readout (Corning 125) pH meter was standardised with pH 7 and pH 10 solutions and adjusted to read samples at 32°C[10] before each measurement. A microelectrode pH probe (Microelectrodes, Inc. 410) was placed in the lateral inferior fornix (Fig. 1), and after a 5–10-second stabilisation period for the digital readout the pH was measured. Three consecutive readings were taken in each eye. The probe was cleaned with alcohol and rinsed with distilled water after use on each subject.

The ocular surface pH was tested in 133 health volunteers. One hundred and seventeen were selected from Grady Memorial Hospital and 16 were subjects not seeking eye care. Patients with inflammation due to allergy, conjunctivitis, lid abnormalities, or prolonged eyelid closure were excluded from this group. The subjects were divided into eight groups by age and sex. Effects on pH of age, sex, order or readings, first eye measured, and time of day were studied.

Six patients without ocular disease, who had been admitted to an orthopaedic ward, were tested eight times throughout one day. The measurements were taken every hour in both eyes from 0900 to 1700. Effects on pH of time of day were studied.

Fourteen healthy volunteers were tested to determine the effects of eyelid closure on ocular

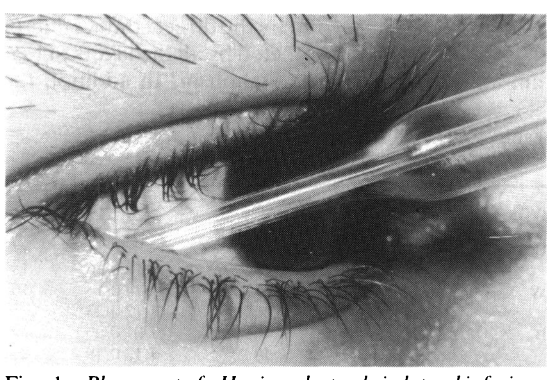

Fig. 1 *Placement of pH microelectrode in lateral inferior fornix.*

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surface pH. The eye to be patched was selected at random by coin toss. A cotton ball placed on the lid in combination with an eye patch assured lid closure. After one hour the patch was removed and the pH was immediately measured, followed by measurements in the unpatched eye.

Eight subjects were measured before drop instillation (phenylephrine 10%, pH 4·80, and Mydriacyl (tropicamide) 1%, pH 6·00), and subsequently until two measurements were found at preinstillation values. Effects on ocular surface pH after instillation of dilating drops with regard to pH recovery were studied.

## Results

For each of the normal subjects three separate pH readings were taken. As the three readings were not significantly different from one another, the mean of the three readings was obtained. Table 1 presents the means, standard deviation, and sample sizes for each of the eight groups. The mean pH values for the two age groups differed for males and females (analysis of variance $F=5·52$, $p<0·03$). The pH increased significantly with age in women.

The pH shifted from acidic to alkaline values when six individuals were tested throughout one day ($p<0·05$) (Fig. 2). There was a significant acidic shift in pH following one hour of lid closure. The patched eyes had a mean pH 7·20 before patching and pH 7·06 after one hour of eyelid closure (SE=0·16). The control eyes had a mean pH 7·19 before and pH 7·19 after one hour (SE=0·16).

Ocular surface pH recovered to predrop instillation levels within 40 minutes in six subjects (Fig. 3).

## Discussion

As regards pH on the eye, the ocular surface is more than the interface between eye and air or eye and conjunctiva. At any point on the conjunctiva or cornea the pH, measured by the direct contact microelectrode, is probably affected by all the layers of tear film and epithelial cell metabolism. In addition the

Table 1 *Ocular surface pH measurements on subjects <40 and ≥40*

| *Race* | *Sex* | *Age* | *Mean SD* | *n* |
|---|---|---|---|---|
| B | M | <40 | 7·07 0·11 | 17 |
| | | ≥40 | 7·15 0·16 | 14 |
| W | M | <40 | 7·14 0·13 | 16 |
| | | ≥40 | 7·12 0·23 | 5 |
| B | F | <40 | 7·03 0·09 | 24 |
| | | ≥40 | 7·13 0·19 | 27 |
| W | F | <40 | 7·06 0·07 | 18 |
| | | ≥40 | 7·28 0·14 | 12 |

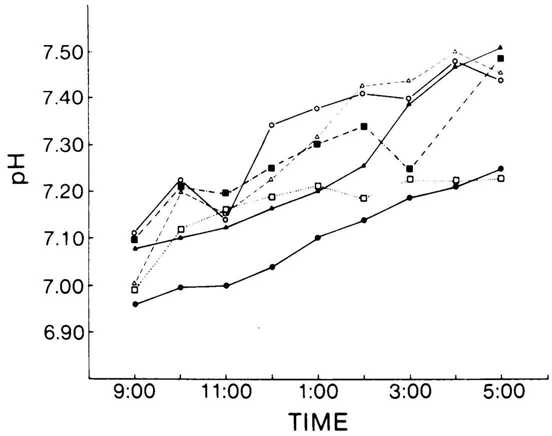


Fig. 2 *Changes of pH to alkaline as measured from 09 00 to 17 00 h. Six subjects.*

opposing surface, air or lid conjunctiva, also varies conditions on the ocular surface. Although we might define the ocular surface as tear film layers and epithelium, the many mechanisms that contribute to alterations in surface pH may have little correlation with exact structural definition.

Previous studies on pH have been limited by an anatomical approach to measurements. Those studies looked at variation in tear film pH by collecting samples at the meniscus,[11 12] a technique known to induce an alkaline error.[10 13] The only studies which have used the direct contact microelectrode have not investigated the variability of the readings at a specific point on the surface. Our data show that the pH on the conjunctival surface varies in ways that probably

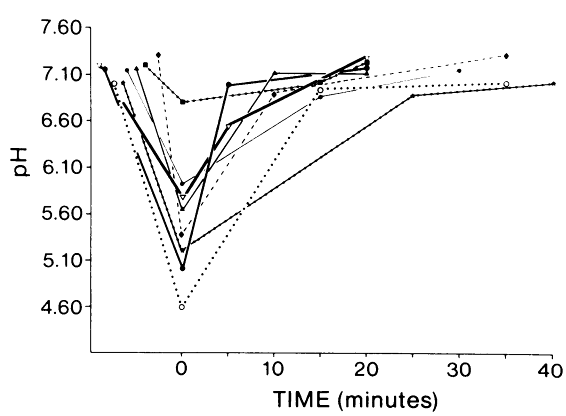


Fig. 3 *pH values before and after administration of drops. A pH reading was taken as baseline. One drop of tropicamide (pH 6·0) and one drop of phenylephrine (pH 4·8) were instilled in the lower fornix. 0 represents time of drop instillation. pH was measured until recovery to baseline values. Six subjects.*

contribute to contact lens intolerance and could influence both drug effectiveness and inflammatory responses.

We are not sure that ocular precorneal tear film and the pH means at various locations on the surface might not vary from the area we studied. Differences have been measured between upper and lower fornices.[14] We chose to measure ocular surface pH in the inferior lateral fornix because it is easily accessible to the tip of the probe, and the lateral fornix provides consistent coverage of the probe with lid conjunctiva where variation in temperature and atmospheric exposure are minimal. Our aim was to decrease stimulation and tearing; measurements in the fornix avoid the sensitive corneal surface. We found that no change occurs among three consecutive pH readings and that there is no difference between eyes. This indicates that no stimulation occurs that affects the readings with the technique as described.

pH as measured on the ocular surface is not simply a reflexive regulation of the tears but an important physical measurement influenced by the interaction of many factors. To categorise the complexities, our data suggest a steady state (with predictable fluctuations around a mean) which is maintained by (1) the influences of tear buffer capacity; (2) cellular products and secretions on the ocular surface; and (3) factors such as age and sex, which affect the mean pH.

A major stabiliser to the pH on the surface of the eye is the bicarbonate buffer system in the aqueous portion of tears.[15] Our data and other data[14,15] strongly support this concept. The slow rise in pH during the day can best be attributed to the diffusion of $CO_2$ to the atmosphere. With loss of $CO_2$ the concentration of $H^+$ decreases and an alkaline shift in pH results.

$$\text{Equilibrium: } CO_2+H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$\text{Eye open: } \downarrow CO_2+H_2O \leftarrow H_2CO_3 \leftarrow \downarrow H^+ + HCO_3^- \quad (\text{pH} \uparrow)$$

After an hour of eyelid closure, $CO_2$ is retained in the aqueous tears.

$$\text{Eyes closed: } \uparrow CO_2+H_2O \rightarrow H_2CO_3 \rightarrow \uparrow H^+ + HCO_3^- \quad (\text{pH} \downarrow)$$

A rapid drop in pH occurs as shown by our results. The changes in pH associated with lid closure and atmospheric exposure are almost surely related to $CO_2$ concentration primarily involving this buffer system.[15]

The bicarbonate buffer system is most important in the maintenance of a consistent pH. But there are protein buffers and cellular secretions that contribute to changes and fluctuations in pH.

There are two tear proteins that exert a buffer capacity in the aqueous portion of tears. Lysozyme is a basic protein and specific tear albumin is acidic. A decrease in lysozyme, which is known in keratoconjunctivitis sicca and smog eye irritation, may decrease tear buffering capacity.[16]

Meibomian glands secrete lipids as a stable composition of cholesterol and cholesteryl esters on to the surface of the tear film. A low-grade infection, however, changes these Meibomian lipids.[17,18] Bacteria generate enzymes that lyse long-chain components of these lipid secretions, forming free fatty acids that are irritating to the tear film and corneal surface, creating an unstable tear film[17] and probably altering pH on the ocular surface.

Mucin, which is alkaline and secreted by goblet cells, is dissolved within the overlying aqueous layer and interacts with the outer lipid layer of the tear film.[17] Changes in the quality and quantity of mucin, as seen with dry eye or acid burns, could influence pH on the ocular surface.

Sex and age affect the mean pH. Our studies show that females over 40 have an alkaline shift in the mean. We also find a steady increase in the mean surface pH with age in all subjects.

Tear fluid changes that occur with aging include a higher incidence of dry eye. This is especially true of elderly females. Perhaps mechanisms involved in dry eye, such as an increase in mucin or other non-specific aging changes, could participate in pH alterations on the ocular surface.

Ocular surface pH means are also affected by time of day. We have shown on the ocular surface, as others have in tears,[12] that pH becomes more alkaline as the day progresses. The bicarbonate buffer system probably has the most important influence on this alkaline shift in pH. But, since the tear constituents of aqueous, mucin, and oils are secreted, other factors may also be important. The bodily secretions of saliva[19] and urine[20] as well as blood[21,22] show daily changes in pH. Known influences include hormonal in some cases, metabolic in others. One may speculate that the secretions comprising tears could be similarly affected.

If the ocular surface pH is made acid, the return to the steady-state levels is probably by reflex tearing. Our data showed that lowering the pH with 2 drops (Mydriacyl, pH 4·80, and phenylephrine, pH 6·00) results in a rapid return within 20–40 minutes to initial pH, probably secondary to the tearing induced by irritation from the acid substances. The dilution effect of the reflex tears is probably most important to the return of the pH to its level before the drops were given.

Deviations from the steady state pH can also occur from disease processes. pH is acid in rosacea patients, as measured by direct contact microelectrode.[4] An allergic reaction in the eye is seen to cause an alkaline shift in pH[5] when studied with a pH sensitive dye, bromthymol blue. Further confirmation is needed in allergy and other conditions which might alter ocular surface pH, such as dry eye and bacterial infection, by direct-contact microelectrode measurement.

The hydration of soft contact lenses can be decreased by acid changes in pH. Permalens water content decreases 8% when the pH goes from 7·4 to 6.[6] This has been thought to be a reason for contact lens intolerance and possibly cause of the tight lens syndrome.[6] Previous reports[4] have shown acid changes in rosacea on the conjunctival surfaces that could be in ranges that would make rosacea patients poor contact lens candidates.

Antibiotic effectiveness in vivo changes with pH.[1] The aminoglycosides cannot penetrate tissues at pH above 7·4.[1] Gentamicin is the most commonly used aminoglycoside in ophthalmology, and it has been shown that conditions of reduced pH may decrease the antibacterial effect of this drug.[1] The effectiveness of gentamicin as well as other ophthalmic aminoglycosides, such as neomycin and tobramycin, needs to be investigated when the ocular surface is altered, since an alkaline pH, as shown by our studies, is common in older females.

Cell viability in vitro is best at pH 7·4–7·8; pH lower than 6·8 or higher than 7·6 slows growth.[3] Cells involved in ocular healing are also affected in vitro by pH changes. For example, the optimum growth of fibroblasts occurs when the pH is in the range of 7·4 to 7·8.[23]

We conclude that ocular surface pH varies significantly in both healthy and disease states. Understanding of the surface pH could have therapeutic and diagnostic significance and might help in the understanding of complexities of disease processes and intolerance of eyes to contact lenses.

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**References**

1 Vandaux P. Peripheral inactivation of gentamicin. *J Antimicrob Chemother* 1981; **8** (suppl A): 17–25.
2 Barza M. Principles of tissue penetration of antibiotics. *J Antimicrob Chemother* 1981; **8** (suppl C): 7–28.
3 Fischer A. Growth of fibroblasts and hydrogen ion concentration of the medium. *J Exp Med* 1921; **34:** 447–54.
4 Jaros PA, Coles WH. Ocular surface pH in rosacea. *Contact Lens Assoc J* 1983; **9:** 333–6.
5 Norn MS. Hydrogen ion concentration of tear fluid. *Acta Ophthalmol (Kbh)* 1968; **46:** 189–200.
6 McCarey BE, Wilson LA. pH, osmolarity and temperature effects on the water content of hydrogel contact lenses. *Contact Intraocul Lens Med J* 1982; **8:** 158–67.
7 Doroshow JH, Gershon YL, Gaasterland DE, Hubbard SP, Young RC, Myers EE. Ocular irritation from high-dose methotrexate therapy: Pharmokinetics of drug in tear film. *Cancer* 1981; **48:** 2158–62.
8 Valentic JP, Leopold IH, Dea FJ. Excretion of salicylic acid into tears following oral administration of aspirin. *Ophthalmology* 1980; **87:** 815–20.
9 Simon C, Malercyzyk V, Klaus M. Absorption of bacampicillin and ampicillin and penetration into body fluids (skin blister fluid, saliva, tears) in healthy volunteers. *Scand J Infect Dis* 1978; suppl 14: 228–32.
10 Abelson MB, Udell IJ, Weston JH. Normal human tear pH by direct measurement. *Arch Ophthalmol* 1981; **99:** 301.
11 Hill RM. Laboratory studies. *Int Ophthalmol Clin* 1981; **21:** 223–36.
12 Carney LG, Hill RM. Human tear pH. *Arch Ophthalmol* 1976; **94:** 821–4.
13 Norn MS. Human tear pH. *Arch Ophthalmol* 1977; **95:** 170.
14 Fischer FH, Wiederholt M. Human precorneal tear film pH measured by microelectrodes. *Albrecht von Graefes Arch Klin Ophthalmol* 1982; **218:** 168–70.
15 Hill RM, Carney LG. Human tear responses to alkali. *Invest Ophthalmol Visual Sci* 1980; **19:** 207–10.
16 Pretsch RC, Pearlman ME. Human tear lysozyme variables. *Arch Ophthalmol* 1973; **90:** 94–6.
17 Lemp MA. Precorneal fluid and blinking. *Int Ophthalmol Clin* 1981; **21:** 55–64.
18 Brown SI, Dervichian OG. The oils of the Meibomian glands. *Arch Ophthalmol* 1969; **82:** 537–40.
19 Dawes C. Circadian rhythms in the flow rate and composition of unstimulated and stimulated human submandibular saliva. *J Physiol (Lond)* 1975; **244:** 535–48.
20 Elliot JS, Sharp RF, Lewis L. Urinary pH. *J Urol* 1959; **81:** 339–43.
21 Shock NW, Hastings AB. Studies of the acid-base balance of the blood. *J Biol Chem* 1934; **104:** 585–601.
22 Hastings AB, Eisele CW. Diurnal variations in the acid-base balance of the blood. *Proc Soc Exp Biol Med* 1940; **43:** 308–12.
23 Hosford GN, Hicks AM. Hydrogen ion concentration of tears. *Arch Ophthalmol* 1935; **13:** 14–25.