Evaporation rate of water from the precorneal tear film and cornea in the rabbit

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A technique for the study of evaporation from the precorneal and corneal surfaces has been developed. Evaporation from the superficial lipid layer (SLL) has been measured to be \(10.1 \times 10^{-7} \text{ Gm.cm.}^{-2} \text{sec.}^{-1}\). When this lipid layer is removed, evaporation increases around fourfold. Evaporation from the dry epithelial surface occurs at a rate of \(1.8 \times 10^{-7} \text{ Gm.cm.}^{-2} \text{sec.}^{-1}\). After the epithelium is removed, a twentyfold increase in evaporation rate occurs. The specific resistances \((\omega)\) to evaporation of these layers have been calculated: epithelium, \(= 82.5 \text{ sec.} / \text{cm.}\); SLL, \(= 12.9 \text{ sec.} / \text{cm.}\); aqueous tears, \(\leq 1 \text{ sec.} / \text{cm.}\); stroma, \(\leq 0 \text{ sec.} / \text{cm.}\). Thus, the superficial lipid layer is effective in retarding evaporation of the precorneal tear film. Its effectiveness compares favorably to that of condensed monolayers of long-chain fatty alcohols on pure water, known to be efficient in retarding evaporation. The epithelium functions as a barrier to water flow and is highly effective in retarding evaporation in the absence of a tear film.

Key words: Corneal evaporation, tears, lipids, corneal epithelium, corneal stroma, corneal measurements, time factors, mathematical analysis, rabbits.

The precorneal tear film is thought to be a three-layered structure. This was first noted by Wolff, although he did not mention any experimental evidence to support this theory. Subsequently the structure of the tear film has been studied by Maurice and Mishima and is the subject of an extensive monograph by Ehlers.6 These studies showed that the film is about 6 to 7 \(\mu\) thick between blinks. A thin mucoid layer has been demonstrated on the surface of the corneal epithelium; a thick aqueous layer, which is seen to cover the epithelium, is covered by an extremely thin superficial lipid layer produced by the Meibomian glands. This layer has been shown to retard evaporation in the rabbit eye. Removal of the layer results in corneal thinning in the exposed eye.

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The first direct measurement of evaporation from the corneal surface was that of von Bahr. Subsequently, Mishima and Maurice measured corneal thickness changes in the exposed eye with and without the lipid layer and deduced the evaporation rate. Since the evaporation rate from the various surfaces of the precorneal film and cornea has not been directly measured, this study was undertaken to define the role played by each layer in the regulation of evaporation.

**Methods and materials**

New Zealand albino rabbits of both sexes weighing between 3 and 4 kilograms were anesthetized with intravenous pentobarbital and given a retrobulbar injection of 1 c.c. of 2 per cent lidocaine. No topical anesthetic was used because this has been reported to destroy the superficial lipid layer.

A special poly(methyl methacrylate) chamber, 8.0 mm. in diameter and 7.0 mm. high (Fig. 1), was constructed with a base ring curved to approximate the curvature of the anterior corneal surface. A small peripheral groove was ground into the base ring, and into the groove a small amount of methyl 2-cyanoacrylate was placed to serve as an adhesive, sealing the chamber to the corneal surface. Meticulous care was taken during the procedure not to disturb the encircled surface containing sequestered precorneal tear film. Desiccated air of relative humidity, $r < 0.01$, warmed to the temperature of the corneal surface (32.0 ± 0.2°C.) was passed into the chamber through a No. 20 gauge needle shaft and drawn out through a No. 23 gauge needle shaft through polyethylene tubing. The water vapor contained in the air was removed in a collecting chamber containing anhydrous CaCl$_2$, and was determined at the termination of the experiment by immediately weighing the chamber at an adjacent balance. The system was checked to assure that the inflowing dry air did not contain any detectable humidity, that the collecting chamber effectively removed the water from the outflow air, that no condensation of water occurred in the tubing, and that no detectable weight increase occurred during weighing (less than a minute).

Air flow was maintained at a constant rate for a given experiment by means of a Harvard withdrawal pump. Air flow rate was adjusted for the varying conditions of the experiment (1.91 to 7.64 c.c. per minute) to maintain an average relative humidity within the chamber below 50 per cent at 32°C, so that even at room temperature the air would not become saturated. Evaporation rates were then calculated at a relative humidity of 48.2 per cent. At a flow rate of 1.9 c.c. per minute, pressure differential across the chamber wall was measured to be negligible, < 0.2 torr.

The following method was used to estimate the average relative humidity in the chamber during a certain time interval providing a useful upper limit: the weight increase of the desiccant, $\Delta w$, during time $\Delta t$, was divided by the total volume of air, $F_a \cdot \Delta t$ (the contribution of the water vapor to the total volume can be neglected at
such low temperatures), that flowed through the chamber. The resulting average concentration of water vapor, $c$, was then divided by the concentration of saturated water vapor (Smithsonian tables) $c_{0}$ (e.g., $c_{0} = 3.383 \times 10^{-5}$ Gm.cm.$^{-2}$ at 32° C.). Thus,$$
abla = c/c_{0} = \Delta \omega/(c_{0} \cdot F_{A} \cdot \Delta t)$$

In all experiments data were collected at short intervals up to a total time of 20 minutes. At least four observations were made for each point recorded on the graph (Fig. 2), and the average standard deviation for measurements was 0.017 $\times 10^{-3}$ Gm.cm.$^{-2}$

Experiment A. Measurement of evaporation from the intact precorneal tear film. The lids were separated from the precorneal surface with a blepharostat. The chamber was applied directly to the corneal surface and held in place for 30 seconds to permit the polymerization of the adhesive to occur.

Experiment B. Measurement of evaporation from the precorneal tear film with the superficial lipid layer removed. The eye was prepared as above except that, after the blepharostat was applied, the corneal surface was rinsed copiously with a physiological artificial tear solution* to remove the lipid layer. Excess fluid was removed from the cul-de-sac with absorbent degreased tissue and the eye was left untouched for 2 minutes in an attempt to achieve a similar thickness of the tear film.

Experiment C. Measurement of evaporation from the corneal epithelial surface. The eye was prepared as above except that the entire tear film was gently removed by the application of absorbent filter paper which had been previously degreased with an organic solvent mixture of chloroform and methanol (2:1, v/v)$^{12, 13}$ to the corneal surface until the dry epithelial surface could be seen.

Experiment D. Measurement of evaporation from the stromal surface. After the above had been performed, the epithelium was removed with the use of a small electric motor and soft bur (Sears Manicure Kit) to loosen the epithelium, which was then removed with a No. 15 Bard-Parker blade.

Results

Experiment A. Measurement of evaporation from the intact precorneal film. Line A in Fig. 2 shows the weight of water evaporated from the surface as measured at intervals during a 20 minute period. These values can be divided into two phases: the first 10 minutes during which the weight of evaporated water increases at a constant rate ($10.1 \times 10^{-7}$ Gm. cm.$^{-2}$ sec.$^{-1}$) and the following 10 minutes in which a distinctly different and lower, approximately constant rate ($1.4 \times 10^{-7}$ Gm. cm.$^{-2}$ sec.$^{-1}$) is noted.

Experiment B. Measurement of evaporation from the tear film after the superficial lipid layer was removed. Again, two discrete phases with differing rates are noted. Line B shows the results from the tear film after the superficial lipid layer was removed. The rate of the second phase in Line B parallels that of the second phase in Line A. Line C shows the results measured from the epithelial surface. Only one phase with a rate similar to the second phases of A and B is noted. Line D shows the evaporation from the stromal surface which occurs at a rate similar to that from a pure water surface (Line E).

*Osmotic pressure (366 mOs), pH (7.3), and ionic constituents (K:Cl:Na ratio = 1.0:5.3:6.0)$^{10}$ approximately those of normal tears.

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Fig. 2. The weight of water evaporated and evaporation rates of water from the precorneal and corneal surfaces as measured over a 20 minute period. Line A shows the results from the intact tear film. Two discrete phases with differing rates are noted. Line B shows the results from the tear film after the superficial lipid layer was removed. Again, two discrete phases with differing rates are seen. The rate of the second phase in Line B parallels that of the second phase in Line A. Line C shows the results measured from the epithelial surface. Only one phase with a rate similar to the second phases of A and B is noted. Line D shows the evaporation from the stromal surface which occurs at a rate similar to that from a pure water surface (Line E).
Experiment B. Measurement of evaporation from the precorneal tear film without the superficial lipid layer. Line B in Fig. 2 illustrates the greatly increased evaporation rate (38.0 \times 10^{-7} \text{ Gm. cm.}^{-2} \text{ sec.}^{-1}) measured during the initial 5 minutes. A second phase with a considerably decreased evaporation rate (1.8 \times 10^{-7} \text{ Gm. cm.}^{-2} \text{ sec.}^{-1}) can be seen to commence at approximately 10 minutes. The interval between 5 and 10 minutes can be viewed as a transition period. In the initial phase a maximum increase in evaporation rate of fourfold is noted after the lipid layer is removed. This difference decreases rapidly after the first 5 minutes to approximate the rates for the intact tear film at 15 to 20 minutes (1.4 \times 10^{-7} \text{ Gm. cm.}^{-2} \text{ sec.}^{-1}).

The same method was used to measure evaporation from the precorneal surface of the Macacus rhesus monkey eye. Results with and without the lipid layer were in close agreement with those obtained for the rabbit eye.

Experiment C. Measurement of evaporation from the epithelial surface. Line C in Fig. 2 illustrates the weight of evaporated water collected from the epithelial surface over different lengths of time. The weight of evaporated water increases uniformly in time for the 20 minute period at a rate approximating that of the second phase of the previous two experiments (1.8 \times 10^{-7} \text{ Gm. cm.}^{-2} \text{ sec.}^{-1}).

Experiment D. Measurement of evaporation from the stromal surface. Line D in Fig. 2 illustrates that the increase in evaporated water is a linear one over the 20 minute period which approximates the initial phase of the evaporation from the tear film without the lipid layer (41.1 \times 10^{-7} \text{ Gm. cm.}^{-2} \text{ sec.}^{-1}).

Discussion

In the open eye there is a continual evaporation from the precorneal tear film resulting in a hypertonicity of the tear film which in turn effects a flow of water from the aqueous humor through the cornea and into the tear film.\textsuperscript{4, 6, 11} Earlier studies\textsuperscript{4} have demonstrated that this evaporation accounts for a small degree of corneal thinning when the eye is open; removal of the superficial lipid layer of the precorneal tear film results in a marked increase in evaporation and appreciable corneal thinning.\textsuperscript{3} Mishima and Maurice\textsuperscript{3} have reported an evaporation rate of 2.2 to 3.7 \mu l. cm.\textsuperscript{2} hr.\textsuperscript{-1} from the undisturbed surface of the rabbit cornea. Destruction of the superficial lipid layer of the tear film resulted in a 10- to 20-fold increase in evaporation. A much earlier study by von Bahr\textsuperscript{3} reported a normal evaporation rate similar to this latter figure. This has been attributed to his inadvertent destruction of the then unknown superficial lipid layer.\textsuperscript{3}

The nature, thickness, and mechanism of action of the superficial lipid layer are yet to be elucidated. It has been suggested that the superficial lipid layer is probably several molecules thick,\textsuperscript{15} but this is by no means proved. The fact that it can retard evaporation, however, is generally accepted.

In this study an attempt has been made to measure the effect on evaporation of various layers of the precorneal tear film and the cornea in vivo. The method used was artificial in the sense that the area studied was isolated from its normal milieu of tear flow and replacement, but this provided a method by which the constituents of evaporation could be separated and studied in a controlled environment.

During a 20 minute period, measurement of evaporation with the lipid layer intact reveals two discrete phases of evaporation (see Line A, Fig. 2). It is probable that the initial phase represents evaporation from the tear film itself. During the first 10 minutes, approximately 0.3 mg. of water was evaporated and this value agrees with the amount of tears sequestered within the area measured. After the tear volume had been exhausted, the second phase, which showed a distinctly lower evaporation rate, would be a measurement from the corneal surface.

After removal of the lipid layer, evap-
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oration from the tear film occurred at a much higher rate, approximately four times greater than with the lipid layer intact* (see Line B, Fig. 2). After the initial or tear phase in which the entire thickness of the tear film had been evaporated (this film of artificial tears might have been thicker, totalling 0.6 mg. in volume, than that occurring naturally, and was possibly due to the rinsing technique), a second, corneal phase, similar to that recorded with the lipid layer, was observed.

After the tear film had evaporated, an evaporation rate from the epithelial surface, identical to the corneal phase above, was observed (1.8 x 10⁻⁷ Gm. cm.⁻² sec.⁻¹) (see Line C, Fig. 2). This value is presumed to be a measure of evaporation from the cornea without a tear film. After the epithelium had been removed, a rate similar to that obtained from the tear film without a lipid layer was obtained from the stromal surface (see Line D, Fig. 2). It is probable that this represents a maximum evaporation rate obtainable under these conditions, since a similar value was obtained from a pure water surface!* (see Line E, Fig. 2).

This study demonstrates only a fourfold increase in evaporation rate upon removal of the lipid layer compared to a 10- to 20-fold increase reported by Mishima and Maurice. Any given evaporation rate however, is mainly determined by the experimental conditions which alter the resistance of the overlying air layer, a major factor retarding evaporation. This means that rates cannot be directly compared between different studies and that the retardation of evaporation has to be expressed in more meaningful terms, such as specific resistance to evaporation. The concept of specific resistance was first introduced by Langmuir and Schaefler, and was later analyzed in more detail by Archer and

| Table I |
|-----------------|----------------|
|                | Specific resistance (sec/cm.) |
| Superficial lipid layer | ≥ 12.9 |
| Aqueous tear film | ≤ 1.0 |
| Epithelium | ≥ 82.5 |
| Stroma | ≤ 0 |

La Mer. The specific resistance, $\omega$, is defined as:

$$\omega = \frac{(c_0 - c)}{(f_m - f_s)} \cdot \frac{1}{1 - r}$$

where $f_m$ and $f_s$ are the specific (i.e., per unit area) evaporation rates of water surface covered with lipid layer and of a pure water surface, respectively, and $(c_0 - c)$ is the driving force of evaporation across the lipid layer and the adjacent stagnant air layer. The specific resistance thus defined has the dimensions of reciprocal velocity (sec./cm.) and is characteristic of the lipid layers only, provided the experimental conditions satisfy certain requirements. For our purposes, the equation was modified to:

$$\omega = c_0 \cdot \frac{(1 - r)}{(f_m - f_s)}$$

where $c_0$ is the saturation concentration of water vapor in air at the temperature of this experiment and $r$ is the relative humidity in the chamber at a steady state. In calculating specific resistances we assumed that both the natural and artificial tear films have an equilibrium vapor pressure equal to that of pure water. The validity of the steady state assumption was checked by plotting the reciprocal humidity versus the air flow rate which yielded a straight line in agreement with the theory (see Appendix).

The specific resistances* to evaporation (or apparent specific resistance) for each of the layers studied are listed in Table I. Epithelium seems to offer the greatest resistance to evaporation in the absence of a tear film, followed by the lipid layer.

*The evaporation rates were calculated at the same relative humidity, $r = 0.482$.

†A slightly greater evaporation rate measured from the stromal surface as compared to a pure water surface can be explained by the greater surface area exposed on the stromal surface. This has been calculated to be 1.4 times that of pure water.

*The term “apparent specific resistance” is more appropriate in referring to the epithelium, since its complex structure may not be analogous to a homogeneous lipid layer and different mechanisms may be operative.
This is in contrast to the findings of Mishima and Maurice, who have stated that evaporation from the cornea is not hindered by the epithelium. Their conclusion is based on the fact that they did not observe any appreciable difference in corneal thinning with and without the epithelial surface. In this study we have found that the epithelial resistance to evaporation is five to six times greater than that of the lipid layer.

Yasuda and Stone have calculated water flow through stroma under normal conditions to be $1.3 \times 10^{-7}$ Gm. cm.$^{-2}$ sec.$^{-1}$, a value in close agreement with our measurement of evaporation from the epithelial surface. The resistance of the epithelium to water flow, however, has been measured and was found to be low enough to allow evaporation at a rate greater than that measured from the "dry" (blotted) epithelial surface in our study. Since the evaporation rate measured at times longer than 10 minutes in our studies from the tear film (with and without a lipid layer) and that from the "dry" epithelial surface itself are remarkably close, it is highly probable that we are dealing with a common phenomenon. It is possible that initially there is evaporation of freely available water from the tear film resulting in concentration of hydrated mucoid with a subsequent decrease in the equilibrium vapor pressure and hence the evaporation rate. At the same time the tear film residue will also become more hypertonic, increasing the water flow through the epithelium. The decreasing evaporation rate and the increasing rate of supply will stabilize at a steady state mucoid and electrolyte concentration, resulting in an approximately constant evaporation rate considerably lower than the initial rate. In the absence of epithelium, the rate of flow of water supply is greater and the spreading and orientation of mucus glycoproteins would also be considerably altered.

It is also possible that in the previous studies utilizing corneal thickness as an indicator of evaporation the method was not sensitive enough to record this difference which occurs in a matter of minutes. The hypothesis of hydrated mucoid covering the corneal surface is an attractive one which is compatible with the known physical properties of mucus.

Under normal conditions, with a tear film covering the intact epithelium, the lipid layer nevertheless plays the essential role in the regulation of evaporation of the tear film. A specific resistance of 12.9 sec. per centimeter compares favorably with those reported for long-chain lipid monolayers which have a considerable retarding effect on evaporation of bodies of water. The nature and mechanism of the action of the lipid layer will be discussed in a future communication.

The role played by each of these layers may be important in defining the pathogenesis of corneal changes in various disease states which affect either the Meibomian glands, and therefore the lipid layer, and/or the epithelium or the tear fluid. Moreover, this method of measuring evaporation affords a model for the study of tear replacements in the control of evaporation.

REFERENCES

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Appendix

We assumed only one rate-controlling process: the passage of water vapor across the phase boundary which includes the stagnant air layer adjacent to the surface. The thickness of the air layer is assumed to be independent of the airflow rate between the experimental limits. Actually, the thickness of the air layer above a pure water surface decreases with increasing airflow rates but the magnitude of the change within the experimental limits is small, amounting to only a fraction of a second per centimeter. Constant temperature and pressure and steady state conditions are assumed to exist throughout the system.

Under such conditions, the concentration of the water vapor in the chamber will be independent of time and as a first approximation can be expressed as the average humidity in the chamber. This steady state concentration of water vapor will then be given by the ratio of the evaporation rate, \( F_e \), and the airflow rate, \( F_a \):

\[
\frac{F_e}{F_a} = c. \tag{1}
\]

If the total resistance to evaporation across the phase boundary is \( \omega \), the evaporation rate will be given:

\[
F_e = A (c_0 - c)/\omega = A (1-c/\omega), \tag{2}
\]

where \( c = c_0 \) is the average relative humidity in the chamber and \( A \) is the surface area of the evaporating liquid phase. Between these two equations, \( F_e \) can be eliminated, yielding the relative humidity in terms of the resistance and airflow rate:

\[
1/\omega = F_a/\omega A + 1 = f_s \omega + 1, \tag{3}
\]

where \( f_s \) is the specific airflow rate, i.e., the airflow rate per unit area of the evaporating surface.

A more refined analysis of the system kinetics has also been made, where laminar airflow and increasing water vapor concentration in the direction of flow were assumed. However, measurements made on pure water at various airflow rates as a function of time showed that: (1) the amount of water evaporated increases linearly with time at all the airflow rates utilized, and (2) the reciprocal of the relative humidity increases linearly with the airflow rate according to Equation 3.

Hence, the assumptions made in this calculation are realistic and justified for our experimental conditions.