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The instilled fluid dynamics and surface chemistry of polymers in the preocular tear film

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Using slit lamp fluorophotometry it was demonstrated that the rate of drainage of a vehicle placed in the eye increased with increasing volume and that polymer solutions increased the thickness of the precorneal tear film (PTF). By increasing the viscosity of the delivery vehicle, (e.g., a hydroxypropylmethylcellulose polymer solution), the PTF retention of fluorescein could be increased. The increased retention was shown to be due to an increase in the tear reservoir volume provided by the more viscous solutions. The PTF retention of fluorescein in a polyvinyl alcohol (PVA) vehicle was not as viscosity dependent, although PVA did seem to produce greater initial PTF fluorescence. This suggested that PVA initially produced a thicker PTF. The PTF retention of fluorescein by five commercial solutions did not have any relation to their wetting properties. The only good correlation with fluorescein retention in the PTF measured, seemed to be the ability of different polymer solutions to stabilize a thick layer of water as measured by the spontaneous spreading of polymer molecules at the air/liquid interface on wet glass surfaces. This model was designed to simulate tear film spreading in vivo. The results suggest that different polymer solutions may produce thicker PTF's than normal by virtue of their ability to drag water with them as they spread over the ocular surface with each blink. Mechanisms by which polymer solutions may increase the thickness of the PTF are discussed.

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For the past 20 to 30 years, synthetic polymers in aqueous solutions have had a wide use in ophthalmology: for prolonging the precorneal tear film (PTF) retention of drugs instilled in liquid ophthalmic vehicles, as tear substitutes for dry eye syndromes, and for the wetting and "cushioning" of contact lenses. At present there is little understanding of the importance of the various physicochemical properties of polymer solutions which enable these solutions to achieve the "optimum"

result in each of the uses cited. It is probable that in each case different properties assume major importance. This communication is concerned primarily with the retention of fluorescein in the PTF when administered in polymer vehicles and the characteristics of polymers which enable them to influence the thickness of the PTF.

There are a number of studies which demonstrate prolonged retention of various markers in the PTF[1-6] and increased intraocular concentration of drugs[1, 2, 7-13] when delivered in viscous polymer solutions. It has been shown in rabbits[1, 4, 11] that the greater the viscosity of the vehicle, the greater is the preocular retention and intraocular penetration of drugs. The only similar study in humans[2] showed little if any effect of increasing viscosity on the PTF retention and intraocular penetration of fluorescein.

In some of the studies mentioned above,[3-5, 7, 8] different polymers at the same concentration were compared for their ability to increase preocular retention and intraocular penetration of markers. However, the viscosity of the vehicles was never measured. As a result, it was implied that cellulose polymers were more efficacious than polyvinyl alcohol. However, we believe that this conclusion is misleading since at the same concentration, cellulose polymers produce a considerably higher viscosity (20- to 30-fold) than that of polyvinyl alcohol. For a proper comparison between various polymers, we believe their viscosity should be kept the same. In this study, using slit lamp fluorophotometry and human volunteer subjects, we examined the effect of varying the viscosity of two widely used polymers, hydroxypropylmethylcellulose (HMPC) and polyvinyl alcohol (PVA) on their ability to retain fluorescein in the PTF. In addition, five commercial ophthalmic solutions were similarly evaluated.

Whether or not polymer solutions increase the thickness of the PTF is subject to question.[2] The present study was designed to re-examine the thickness of the PTF in the presence of polymers in an attempt to elucidate the properties of polymer solutions which might influence tear film thickness.

Materials and methods

Polymer solutions. Hydroxypropylmethylcellulose (Methocel 65 HG, 4,000 cp.) was obtained from Barnes-Hind Pharmaceuticals, Inc., and polyvinyl alcohol (acetate content 12 per cent, 4 per cent solution—25 cp.) (Cat. No. 3396) was obtained from Polysciences, Inc. All commercial solutions were obtained from their respective manufacturers: Barnes and Hind wetting solution (Barnes-Hind Pharmaceuticals, Inc.), Adapt (Burton-Parson and Co., Inc.), Lacril and Presert (Allergan Pharmaceuticals), Visculose (Professional Pharmacal Co.).

All dry polymers were dissolved in a 0.9 per cent NaCl solution made with double-distilled water from an all glass still. Fluorescein was added to the polymer solutions to a final concentration of 5×10^{-4} Gm. per milliliter. The viscosity of all solutions was measured with a Brookfield Synchrolectric viscometer. A Rame-Hart contact angle goniometer was used to measure the advancing contact angle of solutions at equilibrium (approximately three to five minutes after drop deposition) and a Honeywell pressure transducer was used to measure surface tension by the Wilhelmy plate method. By using a pressure transducer to measure surface tension, instead of a torsion balance, errors due to the viscosity of the solution were avoided.

Application of the solutions. In order to avoid the differential effect that dilution by the normal tear volume might have on the viscosity of different polymer solutions, solutions were placed in the eyes of volunteer subjects by the following method. Twenty-five microliters of polymer solution was placed in the eye every three minutes for three applications (75 μl total). (Twenty-five microliters in our experience was the maximum volume which could be applied to the PTF without overflow and at three minutes after instillation the marginal strip visually assumed its normal dimensions such that an added 25 μl would not cause volume build-up and hence overflow.) Utilizing this method of instillation, we were assured that we were measuring the effect of the same solution taken from the vial without having significant alteration in its viscosity due to dilution by the existing tear volume (8 μl). Following the last instillation, subjects were asked to blink on cue every five seconds. This reduced large variations in the PTF fluorescence from one blink interval to another. Fluorescence from the center of the cornea was continuously recorded with a

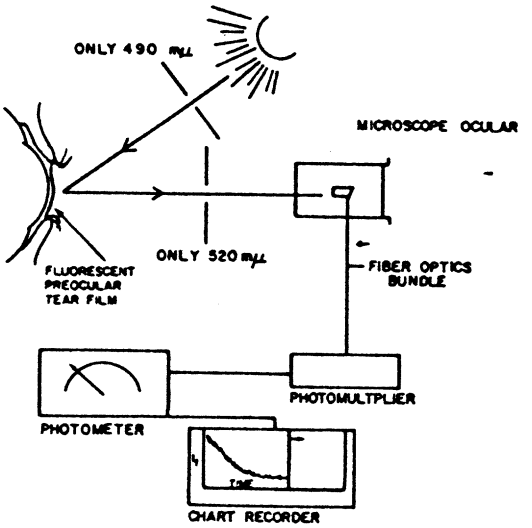

Fig. 1. The slit lamp fluorophotometric apparatus.

slit lamp fluorophotometer using a circle of illumination 2 mm. in diameter. If a subject sneezed, coughed, or felt ocular irritation during a trial, the trial was discarded and repeated on another day since fluorescence dropped precipitously following such an event. In each result reported for experimental subjects, eight determinations were made, two in each of four individuals. The mean values are reported.

Slit lamp fluorophotometer (SLFP). The SLFP used in these experiments was a modified version of the instrument described by Maurice.[14] It consisted of a light source provided by a Haag-Streit slit lamp, an interference filter placed in front of the light source emitting only the excitation wavelength of fluorescein (490 mμ), and another filter allowing only the emission wavelength of fluorescein (520 mμ) to pass. This light was then transmitted by a fiberoptics bundle to a photomultiplier which amplified the signal. The signal was then passed to a photometer and printed out on a chart recorder (Fig. 1). The more detailed specifications of this instrument may be found in an article by Waltman and Kaufman.[15] With the concentration of fluorescein used throughout this study, 5×10^{-4} Gm. per milliliter, and utilizing a 0.5 log neutral density filter in the photomultiplier housing, low sensitivity settings could be utilized on the photometer to minimize background noise. By comparing the fluorescence of the PTF after the application of a fluorescent solution with the fluorescence produced by known thicknesses of the same solution, estimates of the PTF thickness could be made. However, thicknesses of the PTF can only be accurately determined initially, since fluorescein is diluted by the basal tear flow with time. Basal tear flow has been calculated to be on

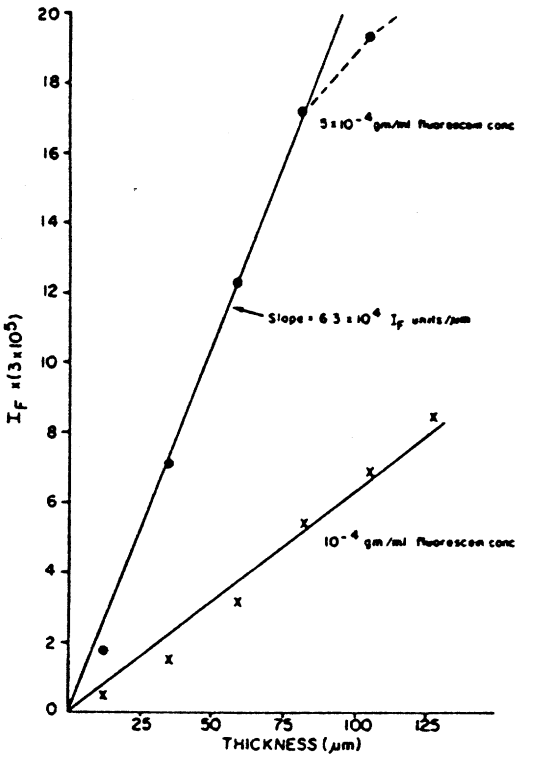

Fig. 2. Calibration of fluorescence versus thickness of the solution measured (0.9 per cent saline 5×10^{-4} Gm. per milliliter and 1×10^{-4} Gm. per milliliter of fluorescein).

the average 1.2 μl per minute and upon instillation of a vehicle can increase as much as 80 per cent.[16]

In order to equate fluorescence and thickness, a fluorescein solution of the same concentration as that used in the experiments described below (5×10^{-4} Gm. per milliliter) was placed between two glass slides separated at one end by a cover slip 150 μm thick such that a wedge of solution was formed. Measurements of fluorescence were then made at varying distances (varying thicknesses) from the cover slip.

In order to rule out the possibility of fluorescence quenching by polymers, the fluorescence of all solutions was measured at the same thickness (150 μm) and compared to the same concentration of fluorescein in saline. All solutions gave the same fluorescence ± 5 per cent as that of saline. For further information on fluorescence assay, see Udenfriend.[17]

Results

Calibration of fluorescence intensity and film thickness. Fig. 2 relates fluorescence and thickness as obtained from the fluo-

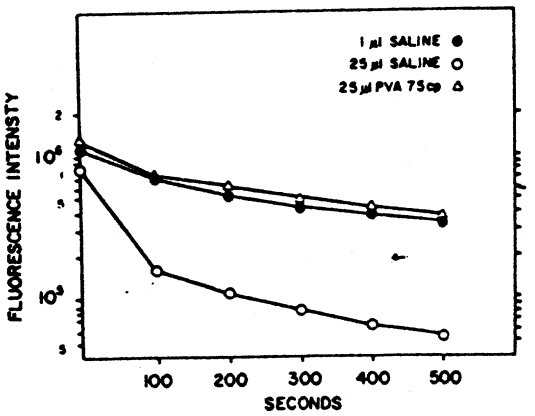

Fig. 3. The effect of varying the volume of the vehicle on the retention of fluorescein in the PTF and the prolonged retention of fluorescein by a PVA (75 cp.) solution.

rescence of varying thicknesses of the same solution of fluorescein (5×10^{-4} Gm. per milliliter and 1×10^{-4} Gm. per milliliter). From this result it can be seen that the relationship between intensity of fluorescence and solution thickness is linear up to approximately 80 μm thickness for the solution of 5×10^{-4} Gm. per milliliter used throughout these experiments. The assumption that fluorescence is linear at thicknesses below 20 μm might be questioned because of the possible adsorption of fluorescein molecules at interfaces. However, based on a simple calculation of the contribution to fluorescence of the number of molecules in a tightly packed fluorescein monolayer adsorbed at an interface and the contribution to fluorescence of the number of molecules in a 10 μ thick layer of fluorescein solution (5×10^{-4} Gm. per milliliter), the error would be 10 per cent. Also, since the surface tension of water is not changed by this concentration of fluorescein, we would not expect a significant contribution by adsorbed fluorescein molecules. Although we admit that this does not completely rule out fluorescein trapping at interfaces by adsorbed polymer networks.

Instilled fluid dynamics. When a drop of solution is applied to the eye, two processes occur simultaneously: the added volume in excess of the normal tear volume drains from the eye, and the solution is diluted by basal tearing and whatever reflex tearing elicited by the instillation of the drop. In Fig. 3, the decline in fluorescence produced by 1 μl of a 1.25 per cent fluorescein solution, 25 μl of saline, and 25 μl of PVA (75 cps.) (both containing the same amount of fluorescein as the 1 μl of 1.25 per cent fluorescein solution) are compared. Since 1 μl, the smallest volume we could accurately instill in the eye, means only a 13 per cent increase in the total tear volume, this small amount of solution probably did not induce significant additional drainage due to increased tear volume. So we interpreted the decline in fluorescence in this case to represent only the diluting effect of the basal tear flow. By the addition of 25 μl of saline, the PTF appears to be initially increased in thickness since the fluorescence intensity (I_F) produced by this concentration of fluorescein corresponds to a thickness of 16 μm from the calibration graph. The rapid initial decline in I_F, we believe, is due to a rapid decrease in PTF thickness as a result of a decreasing marginal tear strip volume due to drainage through the lacrimal apparatus. (The marginal tear strip volume was visibly enlarged upon instillation of 25 μl of saline and decreased to its normal dimensions at about 100 seconds by visual inspection.) The remainder of the I_F curve should represent dilution of the remaining fluorescein molecules in a tear film of normal dimensions (volume). If the remaining points of this curve are extrapolated to the ordinate, the fluorescence value obtained should be an approximation of the normal PTF thickness without added volume. This I_F corresponds to 4 μm which is less than the PTF thickness reported by Ehlers[15] of 4.5 to 8.5 μm using small paper sponges to soak up the PTF, and the value of 6 μm reported by Maurice, Mishima, and Wright[19, 20] using a SLFP to measure PTF fluorescence in rabbit eyes.

The fact that 25 μl of PVA solution (25

times more dilute than 1 μl of saline containing 1.25 per cent fluorescein) and 1 μl of saline containing 1.25 per cent fluorescein give approximately the same I_F indicates that PVA increases the thickness of the PTF since fluorescence intensity approximates thickness X concentration. Also, the increased thickness of the PTF produced by PVA seems to be maintained throughout the measurement period (500 seconds). If the I_F of the 1 μl solution of fluorescein in saline and the I_F of 25 μl of a polymer solution 25 times more dilute produce approximately the same initial fluorescence, then on the basis of a rough estimate of a normal tear volume of 7.7 μl (7 μl in marginal tear strips plus the volume under the palpebral conjunctiva and fornices,[16] 0.7 μl in the PTF if the film is 4 μm thick and 172 mm.^{2} in surface area[16]), the polymer solution increased the thickness of the PTF by a factor of 4 (assuming I_F = thickness × concentration, and complete mixing). Support for the above calculation comes from the experimental observation that the I_F produced by PVA corresponds to a thickness of ≃ 13 to 14 μm, which is 3.5 times the normal PTF thickness estimated to be 4 μm from our studies.

Effect of viscosity on fluorescein retention. In order to study the effect of viscosity on fluorescein retention, HPMC was prepared at viscosities of 20, 40, 80, and 120 cp. by varying polymer concentration. Fig. 4 compares the fluorescein retention in the PTF produced by each solution. It is evident that at 500 seconds the more viscous solutions give a higher I_F. (HMPC 120 cp. $I_F = 4 \times 10^5$ S.D. ± 0.37×10^5, HMPC 75 cp. $I_F = 2.4 \times 10^5$ S.D. ± 1.4×10^5, HMPC 40 cp. $I_F = 1.4 \times 10^5$ S.D. ± 0.5×10^5, HMPC 20 cp. $I_F = 1.1 \times 10^5$ S.D. ± 0.8×10^5). However, at 100 seconds all solutions have more nearly the same I_F. It was also noted at 100 seconds after instillation that the marginal tear strip in an eye which had received HPMC 120 cp. viscosity was visually larger than an eye which had received HPMC 20 cp. In order

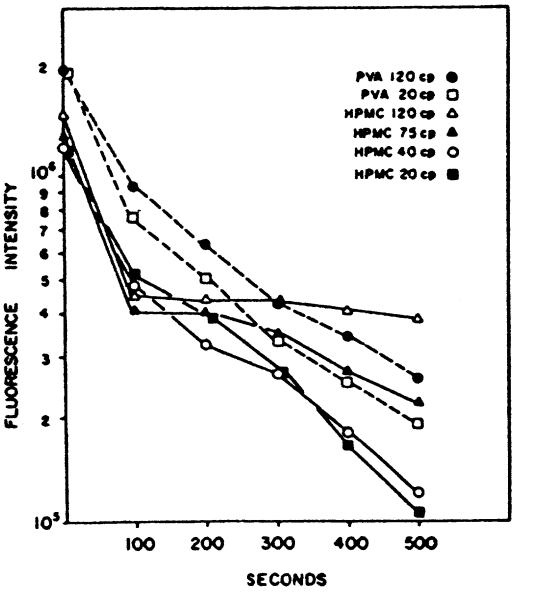

Fig. 4. The effect of varying viscosity of HPMC or PVA on fluorescein retention in the PTF.

Table I. The relative total PTF volume produced by polymer solutions using dilution technique (S.D.—standard deviation)

Polymer solutions	*Viscosity (centipoise)*	*Fluorescence of PTF sample after dilution*		
HPMC	120	82	S.D.	3.6
HPMC	20	45	S.D.	19.0
PVA	120	56	S.D.	5.1
PVA	20	45	S.D.	12.0

to demonstrate this enlarged volume, in a separate experiment, at 100 seconds after instillation of the vehicle, 10 μl of saline was added to an eye that had received a 120 cp. solution and one that received a 20 cp. solution. The resulting contents were allowed to mix for 5 blinks and by rotating the eye with the lids closed. A 5 μl aliquot was then removed from each eye with a micropipette, diluted in 2 ml. of saline, and the I_F measured in a spectrofluorophotometer. Table I contains the fluorescent values for the resulting samples. Larger values indicate a larger total marginal tear strip and conjunctival sac tear volume. The I_F for the sample taken from the eye receiving a 120 cp. solution was 82

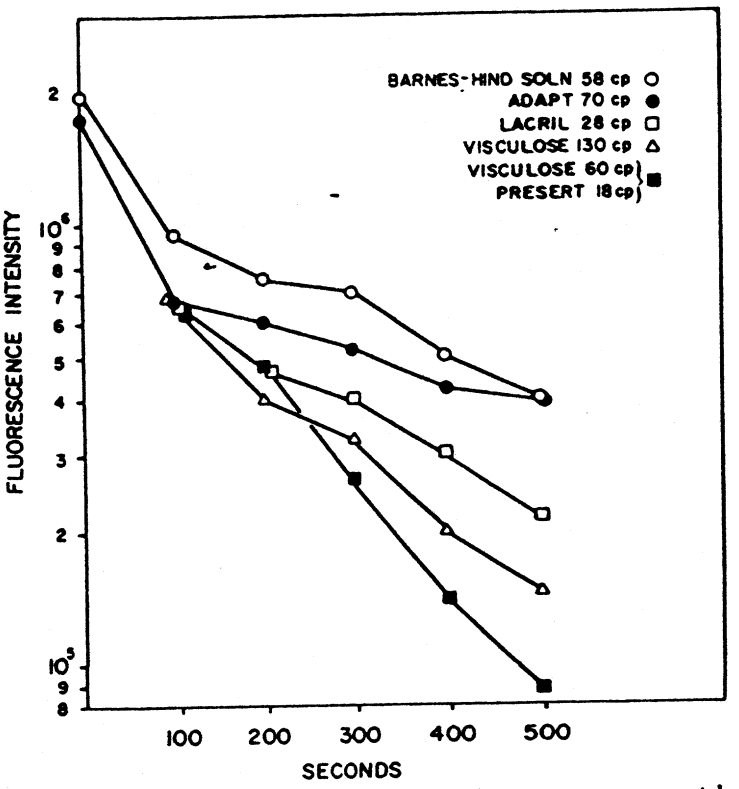

Fig. 5. The retention of fluorescein in the PTF produced by various commercial solutions.

(S.D. ± 3.6), approximately twice that of the eye receiving the 20 cp. solution which was 45 (S.D. ± 19). Since there was a 15 per cent difference in the I_F, (i.e., PTF thickness) of the same solutions over the corneal surface at 100 seconds (Fig. 4), but the volume greatly different as shown by the above-mentioned dilution technique, this seemed to indicate that although two solutions of different viscosities may produce approximately the same I_F and hence about the same PTF thickness over the cornea, the concomitant volume in the marginal reservoir may be quite different.

When comparing the effect of PVA and HPMC on the PTF thickness or I_F (Fig. 4), it can be seen that initially and for the first 200 seconds PVA, regardless of its viscosity, produces a greater PTF fluorescence than HPMC of any viscosity. Also, for PVA, there is not as significant an effect of viscosity on I_F at 500 seconds as in the case of HPMC when comparing the same viscosities. (PVA 120 cp. $I_F = 2.7 \times 10^5$ S.D. ± 1.8×10^5, PVA 20 cp. $I_F = 1.8 \times 10^5$ S.D. ± 1.2×10^5). Furthermore, visually there was little difference in the marginal strip volume at 100 seconds. When samples from the marginal strip were taken at 100 seconds, as in the case of HPMC, the units of fluorescence of PVA 120 cp. and 20 cp. viscosity were, respectively, 56 (S.D. ± 5.1) and 45 (S.D. ± 12) (see Table I), which are not as significantly different as in the case of HPMC comparing the same viscosities. When compared to the fluorescence value of HPMC (20 cp.) of 45 (S.D. ± 19), there is not much difference between PVA (120 cp. or 20 cp. viscosity) and HPMC (20 cp. viscosity) in terms of PTF volume. These observations suggest that the higher I_F of HPMC of 120 cp. viscosity at 500 seconds than that of PVA of 120 cp. or 20 cp. viscosity is presumably due to a greater total marginal reservoir volume of HPMC after 500 seconds. A greater reservoir vol-

Table II. The wetting properties of polymer solutions

Polymer solutions	*Surface tension (dynes/cm.)*	*Viscosity (centipoise)*	*Contact angle (degrees)*	
			Glass	*Plexiglass*
HPMC	46	120	17	55
HPMC	46	75	16	56
HPMC	46	40	18	55
HPMC	46	20	18	54
PVA	43	120	18	52
PVA	46	20	19	52
Barnes and Hind	33	58	15	45
Adapt	50	70	11	56
Lacril	44	28	10	52
Visculose	23	130	20	50
Presert	34	18	16	48
H_2O	74	1	0	—

ume might be expected to produce greater PTF fluorescence for a longer period of time since dilution of this larger volume of fluorescent solution by basal secretion per unit time would be less than in the case of a smaller reservoir volume. PVA in contrast to HPMC does not seem to produce as significant an increase in the total reservoir tear volume with increasing viscosity and therefore not as high an I_F at 500 seconds after instillation as HPMC of 120 cp. viscosity.

Commercial polymer solutions. If one now compares the I_F produced by five commercial solutions (Fig. 5), the more viscous solutions generally give a higher I_F with the exception of Visculose of either 130 cp. or 60 cp. viscosity. In addition, it was observed that Visculose did not form a homogeneous film over the corneal surface. The solution appeared as if it had been smeared in lumps over the ocular surface and contained islands of fluorescence. However, when Visculose was diluted with 0.9 per cent saline to 60 cp. its film-forming properties improved. Similar poor film-forming properties were also observed with HPMC at a high viscosity (120 cp.), but not at viscosities of 75 cp. and below. However, in contrast, PVA at any viscosity as well as the other commercial solutions did not produce such non-homogenous films in the eye.

Surface chemical properties of ophthalmic polymer solutions. Table II contains the viscosity, surface tension, and advancing contact angles on glass, a completely hydrophilic surface, and plexiglass (polymethylmethacrylate), a hydrophobic surface (more analogous to corneal epithelium), for the solutions evaluated above. There is little difference in the surface chemical properties of these solutions and we do not feel that these properties of polymer solutions can sufficiently explain the differences in fluorescein retention produced by these solutions in vivo. The polymer solutions tested would not be expected to completely wet (i.e., contact angle of 0°) the glass used in these experiments since it was not pure silica glass. Based on its surface tension (23 dynes per centimeter), one might expect Visculose to have the best wetting properties. However, Visculose demonstrates little fluorescein retaining ability when compared to the other solutions in the eye and also forms a non-homogenous film over the ocular surface. We admit that the surface tension of Visculose seems unusually low. However, we have taken every precaution to eliminate the possible sources of error in the measurement of the surface tension of highly viscous solutions (e.g., utilization of a pressure transducer with very little displacement of the Wilhelmy blade, use of pre-wetted platinum blade etc.). Its poor film-forming properties may be due to a high surface viscosity or bulk viscosity.

Tear film thickness between blinks. When

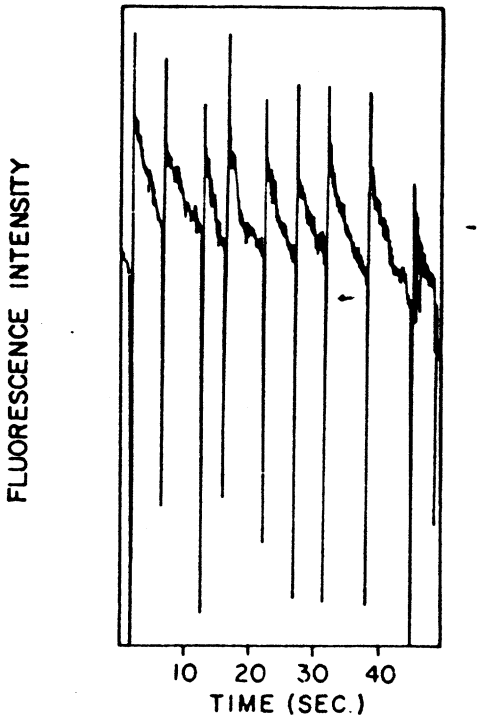

Fig. 6. The tear film stability between blinks. *A*, normal PTF. *B*, PTF in the presence of PVA solution (75 cp.). (The vertical lines represent blinks.)

continuous recordings of I were made after instillation of 1 μl of 1.25 per cent fluorescein, in some individuals, the PTF consistently decreased in fluorescence (thickness) between blinks (Fig. 6, *A*). However, under the same conditions, the PTF in other individuals either maintained its thickness or decreased in thickness depending upon the day of observation. However, with the exception of Visculose 130 cp. and HPMC 120 cp. viscosity, all of the polymer solutions tested as well as all of the commercial solutions allowed the PTF to increase in thickness between blinks (Fig. 6, *B*). After a complete blink, as the upper lid was raised, a film was seen to move vertically over an already fluorescent tear film. The duration of this phenomenon after instillation has not been studied extensively. However, when specifically looked for, it has been observed for as long as 20 minutes after instillation of polymer. The magnitude of the change

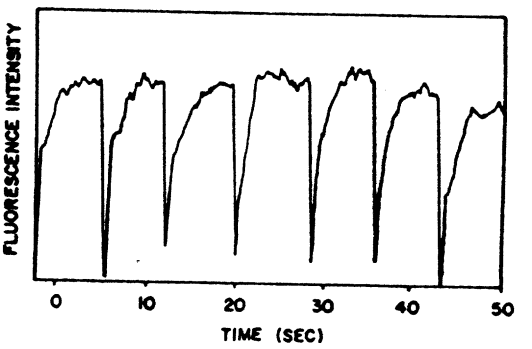

Fig. 6, *B*. For legend, see Fig. 6, *A*.

in thickness is difficult to measure since it begins instantaneously after a blink. Since the PTF is already fluorescent at time zero, it is conceivable that the increase in fluorescence we are observing is the continuation of spreading of a superficial polymer layer at the air/tear interface.

The water dragging capacity of moving polymer films. Since all polymers used in our studies exhibited surface activity (Table II), it is expected that they will adsorb at the air/tear interface in the same manner as does meibomian oil. The superficial layer of the PTF in the presence of surface active polymers probably exists as a mixed film of the two components. During a blink such as adsorbed mixed film will be compressed. After a blink, it will re-expand due to the surface pressure generated by the film's high state of compression. As the re-expanding film moves over the ocular surface, it carries with it a finite amount of water due to the viscous drag of its hydrophilic groups on the water beneath them.[21] In order to try to explain the differences in preocular fluorescence produced by the various polymer solutions and the increase in thickness of the PTF between blinks, we devised a method to measure the thickness of the water layer carried by moving surface adsorbed polymer molecules. If different polymers drag different amounts of water as they spread, then perhaps, in part, this could also explain the differences in preocular fluorescence observed with

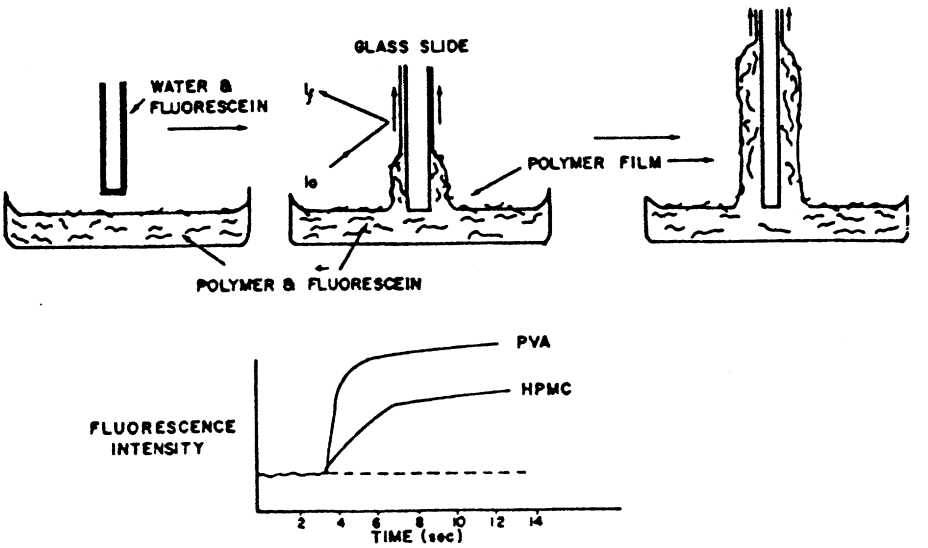

Fig. 7. Slide technique used to measure the thickness of moving polymer films.

polymer solutions of the same viscosity. The following experiment was performed to simulate the spreading of polymers at the air/tear interface. A clean glass slide was dipped into a beaker of 0.9 per cent saline (containing fluorescein at a concentration of 5×10^{-4} Gm. per milliliter) such that the total surface area of the wet portion of the slide was approximately 8 cm.^{2} (4 cm.^{2} × 2 sides). The polymer solution in question was prepared at the same fluorescein concentration and placed in a Petri dish with a surface area of 64 cm.^{2}. When the wet glass slide was touched to the surface of the polymer, the polymer solution was observed to climb the vertical slide. Polymer solutions will not spread on a dry glass slide because of the unfavorable energetics of wetting a dry surface. If at this time the change in fluorescence of the slide was measured as the moving film passed the SLFP sensing device (Fig. 7), the thickness of the film could be calculated using the calibration curve (Fig. 2). Table II shows that the thicknesses produced by the moving films differ considerably with Visculose giving the greatest thickness, an apparent contradiction to the in vivo result with Visculose. However, this system may not be analogous to the in vivo situation since

Table III. The thicknesses of moving polymer films

Polymer	*Viscosity (centipoise)*	*Thickness (μm) of water layer dragged by polymers*	
		64 cm.^{2}	*0.52 cm.^{2*}*
Barnes-Hind wetting solution	58	11	22
Adapt	70	16	17
Presert	18	—	16
Lacril	28	16	14
Visculose	130	30	11
PVA	120	19	18
PVA	20	19	12
HPMC	120	11	12
HPMC	20	9	9
Monomolecular film of PVA		13	

*Surface area of trough.

the surface area of the Petri dish is much larger than the surface area of a compressed film in the eye. Because of the large surface area of the Petri dish, the amount of surface film available for spreading is greater than that in vivo. We redesigned the system so that the area of the surface film available for spreading was 52 mm.^{2} which, although large for practical purposes, was the closest we could come to approximating the in vivo dimensions of the marginal tear strip. Forty microliters of solution was placed in a trough of 26 mm. by 2 mm. by 1.5 mm. deep, with a

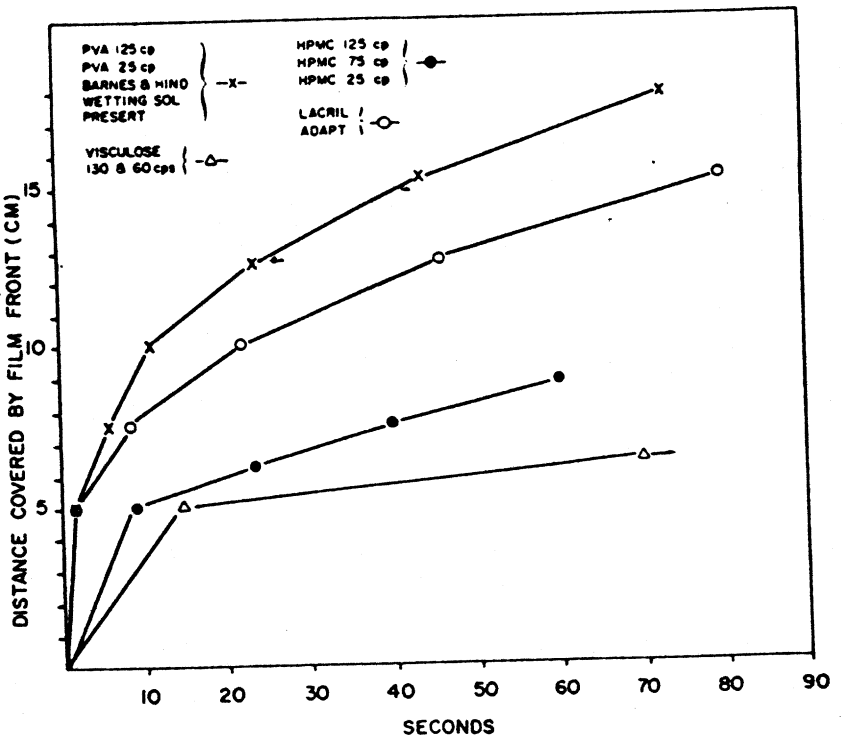

Fig. 8. The surface spreading capacity of polymer solutions.

surface area of 52 mm.^{2}. When films of polymers were allowed to spread from this small trough, the thicknesses they produced (Table III) corresponded more closely to the preocular fluorescence order observed in the eye by the same solutions. Barnes-Hind wetting solution and Adapt produced thicknesses of 22 μm and 17 μm, respectively, and Visculose the smallest, 11 μm. PVA of 120 cp. viscosity gave a greater thickness than HPMC 120 cp., 18 μm and 12 μm, respectively, also as observed in vivo. Presert produced a thickness of 16 μm on the glass slide but in the eye it had the least fluorescein retaining ability along with Visculose 60 cp. The poor ability to retain fluorescein in the eye might be partially explained by the fact that Presert had the lowest viscosity of all of the solutions tested and retention of fluorescein may be partially viscosity dependent. From these experiments it was apparent that the magnitude and geometry of the surface area of the trough used for polymer spreading could affect the thickness of moving polymer films. Since the surface pressure would be the same in both systems, it cannot account for the observed differences. However, in a large dish, the transfer of a surface film to the wet glass surface would occur rapidly because of the availability of an already adsorbed polymer film. In a trough of smaller surface area, the same process would require migration of polymer molecules from the bulk solution to the surface and their subsequent surface transport to the wet slide. In this case, factors such as energy of adsorption, entanglement, and aggregation between polymer molecules in the bulk solution may influence the kinetics of the process and subsequently the thickness of the dragged water layer. Some of the factors mentioned might be operative in vivo accounting for the good correlation observed with the small trough and the PTF fluorescence. Since it appears that the kinetics of spreading of polymer films

may influence the thickness of the dragged sublayer of solution. The height to which polymer films rise on a wet glass slide was measured as a function of time (Fig. 8). A film will continue to move vertically as long as molecules from the bulk solution diffuse to the air/water interface thereby increasing the surface pressure. It is apparent that Barnes-Hind wetting solution, Adapt, Presert, and PVA spread much faster than HPMC or Visculose. We believe that the ability of a compressed film to spread spontaneously has some relevance to the fluorescence and hence the thickness of the PTF observed in the eye with the same solutions since such polymer films have the ability to move more water as they spread.

In an attempt to see if the thickness of a moving film could be explained by a moving monomolecular film of polymer, a monomolecular film of PVA was formed on the surface of a large trough by touching a glass rod, previously dipped into PVA solution, to the surface of a trough containing saline and fluorescein (5×10^{-5} Gm. per milliliter). Polymer molecules were allowed to spread on the saline surface until no more molecules could spread on the surface of the trough. A clean wet glass slide was touched to the surface of the trough allowing the monolayer to spread on the glass surface. The thickness of water layer dragged by the monomolecular film was 13 μm (Table III), similar to that produced by the bulk solution of PVA 20 cp. (12 μm) when spread from the small reservoir. This suggests that the thickness of the water layer dragged on a glass surface by PVA can be accounted for by a monomolecular film of the same polymer, indicating that the polymer molecules at the surface are mainly responsible for the dragged water layer and that those PVA molecules in the bulk solution do not significantly influence the thickness of the dragged water layer. However, as mentioned previously, factors such as entanglement and aggregation of polymer molecules could conceivably influence this process with other polymer types and under different spreading conditions (e.g., HPMC spreading from the small 40 μl trough).

Discussion

The role of viscosity and instilled volume of ophthalmic vehicles. Chrai and coworkers[22] have shown that the rate of drainage of an instilled drug from the eye of rabbits is related to the volume of the drug solution instilled and increases with increasing volume. In Fig. 3, we have shown that the retention of fluorescein is much greater if the same amount of fluorescein is instilled in 1 μl versus 25 μl. The same authors[1] have also shown that the rate of solution drainage is related to viscosity and decreases with increasing viscosity of methylcellulose vehicles. Using HPMC solution as a vehicle, we have also demonstrated that increasing viscosity prolongs the retention of fluorescein in the PTF. However, this conclusion does not appear to apply to PVA solutions on the basis that there did not appear to be a significant difference in the fluorescein retention produced by PVA 120 cp. or PVA 20 cp. at 500 seconds after instillation. Also by the dilution technique, PVA 120 cp. and PVA 20 cp. had almost the same reservoir volume at 100 seconds when compared to HPMC 20 cp., in contrast to HPMC 120 cp., Table I. It is possible that the molecular conformation as well as entanglement of HPMC molecules might retard its drainage. Adler, Maurice, and Patterson,[2] using SLFP, observed a slightly higher PTF fluorescence with viscous drops when compared to saline, but did not feel that the increased fluorescence observed with viscous drops was due to a decreased drainage rate (prolonged contact time) of fluorescent polymer solution from the PTF. These investigators contended that the increased fluorescence observed with the viscous solution was due to a greater initial saturation of the PTF by the viscous drop, since a saline drop would drain more quickly. Actually, one would expect that a more viscous drop

would tend to mix more slowly and less completely within the same period of time as a less viscous drop. These authors have suggested that the time of mixing is important, but this has not been our experience.

The question raised is, how does increasing the viscosity of a polymer solution increase the retention of a substance in the PTF? The following conclusions from this study might answer part of the question. By increasing the viscosity of a vehicle, one in effect increases the duration of the vehicle in the eye probably by decreasing the drainage rate thereby increasing the marginal tear strip volume for a longer period of time compared to nonviscous solutions. The increased marginal tear strip volume would then act as a reservoir for fluorescein such that with each blink fluorescein is respread in the PTF over the ocular surface. Although this mechanism seems applicable in the case of HPMC, it does not appear to be as applicable to PVA, since PVA does not appear to increase the marginal strip volume as significantly with increasing viscosity. Even though HPMC 120 cp. at 100 seconds after instillation has a larger reservoir volume than PVA of 120 or 20 cp. viscosity, PVA still gives a greater PTF fluorescence, indicating that this polymer is capable of forming a thicker PTF. This also implies that a larger reservoir volume is not necessarily required for the formation of a thicker PTF. This may indicate that only a certain minimum quantity or volume of polymer solution is needed to create the film thickness measured over the cornea with the SLFP and beyond this critical amount the excess remains in the reservoir. This is based on the observation that HPMC 120 cp. and HPMC 20 cp. produce nearly the same fluorescence at 100 seconds after instillation, yet have greatly different reservoir volumes by the dilution technique at the same time.

Theoretical considerations[20-23] indicate that a thicker PTF might prolong PTF breakup. The following pieces of data from this study indicate that the PTF is increased in thickness by polymer solutions:

1. The initial fluorescence of the PTF after instillation of a polymer solution corresponds to a 15 to 20 μm thickness from our calibration graph, which is considerably greater than the thickness of 4 to 10 μm reported by Ehlers,[15] and 6 μm reported by Mishima[19] and Maurice and Wright,[20] for the normal PTF.
2. One microliter of saline and 25 μl of PVA, both containing the same amount of fluorescein, produce the same PTF fluorescence, suggesting that PVA has increased the PTF thickness.
3. Certain polymer solutions allow the PTF to increase in fluorescence (thickness) between blinks.

When comparing different commercial polymer solutions, fluorescein retention does not seem to be strictly dependent upon viscosity (Fig. 5). Barnes-Hind wetting solution and Adapt, 60 and 70 cp. viscosity, respectively, produced greater fluorescein retention than Visculose 130 cp. or 60 cp. In order to try to explain these results we decided to investigate the water dragging capacity of spreading polymer films from these solutions. We succeeded in demonstrating that spreading films of Barnes-Hind wetting solution, Adapt and Presert were approximately twice the thickness of films of Visculose. We would like to emphasize that we could not demonstrate any correlation between the in vitro wetting properties of these polymer solutions such as surface tension and advancing contact angle and their fluorescein retention in the eye. The only correlation we could find with fluorescein retention in the PTF was the thickness of the dragged aqueous layer produced by these polymers and their capacity for surface spreading on wet glass surfaces, indicating that these two parameters of polymer solutions may be important in fluorescein retention and in providing a thick PTF. This reasoning is supported

by the experimental observation that polymer solutions in the eye increase the PTF thickness between blinks.

There appear to be a number of rational uses for polymer solutions in ophthalmology. Their use as wetting agents for contact lenses has been discussed.[20, 24, 25] Holly and Lemp[24, 26] have examined the effects of polymers as ocular wetting agents and feel that certain polymers demonstrate mucomimetic properties, advocating their use as tear substitutes for certain types of tear deficiencies. In the absence of mucin, which they feel to be the natural corneal wetting agent,[27] polymers may affect corneal wetting by adsorption at the corneal/tear interface and by decreasing the surface tension of the tear through interaction with the superficial lipid layer.

Mechanisms of stabilization of precorneal tear film by polymers. A thick PTF might be expected to be of benefit in aqueous deficient dry eyes and may even prevent the abnormal PTF breakup observed in mucin-deficient dry eyes.[20] One may envision at least three mechanisms by which polymer solutions may increase the PTF thickness. (1) By increasing the volume of fluid available in the marginal tear strip, (2) by absorption of polymers at either the corneal/aqueous or mucin-aqueous interface, or (3) by a film of polymer solution at the air/tear interface supporting a layer of water beneath it or by dragging a layer of water with it as it spreads over the ocular surface with each blink.

The first mechanism of increasing film thickness is alluded to by Ehlers.[18] From this study, it seems that the PTF can be transiently increased in thickness by the increase in volume provided by 25 μl of saline and for a more substantial time by increasing the viscosity of the vehicle (25 μl PVA 75 cp. vs. 25 μl saline 1 cp.) (Fig. 3). At present, it is difficult to say how long just an increased marginal tear strip volume alone can increase the thickness of the PTF without quantitative measurements of fluorescein dilution and tear flow. Our general impression is that with the most viscous solutions this phenomenon may persist for as long as 20 minutes.

In the second mechanism, the adsorbed polymer by virtue of its hydrophilic-lipophilic character, would be capable of adsorbing by multiple points of attachment at the corneal/tear or mucin/aqueous interface extending into the adjacent aqueous phase, thereby stabilizing a thick layer of water adjacent to the adsorbing surface. In a broad sense, this adsorbed polymer would be acting like a sponge of loose structure.

The adsorption of polymers to the corneal surface has been studied by Lemp and Szymanski[25] who measured changes in the contact angle of a drop of saline on the corneal surface of isolated rabbit eyes before and after polymer adsorption. This study essentially demonstrated that polymers have strong adsorptive properties which in some cases are more significant than those of mucin and that some adsorbed polymers resist considerable washing by saline.

Concerning the third mechanism, we have shown that spreading polymer films, including a monomolecular film of PVA, have the capability of dragging with them an aqueous layer 10 to 20 μm thick, provided there is sufficient water available for them to move as well as sufficient polymer to produce a monomolecular film at the air/tear interface. In the eye, the amount of fluid available to move is strictly controlled by normal homeostatic mechanisms. By applying a drop of polymer solution to the eye, sufficient water becomes available for the polymer film to drag. As long as there are polymer molecules remaining at the air/tear interface, the amount of water in the PTF should be increased by the amount of water that is stabilized by the hydrophilic groups of the polymer as well as by its surface spreading characteristics, even though the basal tear flow input and output remains the same as normal. It is a common observation that in a soap

film hundreds of millimicra can be stabilized in a vertical frame by the adsorption of surfactant molecules at the air water interface. Also a vertical film of water, not more than 100 μm, is prevented from falling due to gravity by the presence of surface active molecules at the air/tear interface. A surface film having a high surface pressure gradient acts as a force in a vertical direction to counteract the downward force of gravity. The surface film is capable of supporting the water in between the corneal surface and the air/tear interface because of its hydrophilic groups. The hydrophilic groups of the surface film serve as "anchor points" to stabilize the water layer beneath them. When the surface molecule moves, the entire network of water molecules organized by the film moves also. We have measured the thickness of this water layer to be 10 to 20 μm which is less than but approximates the 25 to 30 μm thickness for oleic acid monolayers reported by Schulman and Teorell[21] using an entirely different method and is in agreement with the subjective observations made by Brown and Dervichian.[23] Interestingly, the thickness of the PTF in the presence of polymers which we have measured is of the same order of magnitude, 10 to 20 μm. Since the diameter of a water molecule is 3 Å, this thickness would be equivalent to 40,000 water molecules stacked end-on-end.

The capacity of polymer molecules to move water as they spread is by no means unique but probably a property of surface-active molecules in general. In our experience fatty acids, phospholipids, and cholesterol as well as a number of detergents have been shown to move similar thicknesses of water (unpublished data) but because of the toxic and irritating effects of some of these substances in the eye, their use is prohibited.

There is no doubt that the normal tear film components such as meibomian gland secretion, mucin, albumin, globulin, etc. are partially responsible for the stabilization of the normal PTF and it is quite probable that they demonstrate some of the phenomena described above. However, the fact remains that polymer solutions can increase the thickness of the normal tear film. Holly[29] has recently published a study on the surface chemistry of normal tear film component analogues, and has alluded to studies presently being undertaken to include the interactions of these components with polymers. It would be interesting to compare the spreading characteristics of polymers versus normal tear film components as well as their interactions and water dragging capacity.

One may envision the mechanism of polymer induced thickening of the PTF in the following way: two forces act to spread tears over the ocular surface. (1) The upper eyelid, by a mechanical action, pulls water with it as it is raised, and the amount of water moved is enhanced by (2) the vertical spreading of polymer molecules pushing and/or dragging additional water with them as they spontaneously spread at the tear/air interface (Fig. 9). Polymer molecules which are capable of rapid adsorption at the air/tear interface will move from the bulk solution to the surface and continue to provide a surface-pressure gradient which induces spreading until equilibrium is reached. The duration of polymer-induced tear film thickening would be a function of the retention time of the polymer in the eye. Meibomian secretion, because of its limited solubility in water, would be expected to have a long retention time since the normal tear flow would occur between the air/tear surface film of meibomian secretion and the ocular surface. Highly water-soluble polymers which are less surface active would have a short retention time in the PTF since they would be removed by the normal tear flow. Based on this reasoning, perhaps more surface-active polymers should be experimented with in the framework provided above.

This study has only examined the tear film for a brief time after the application

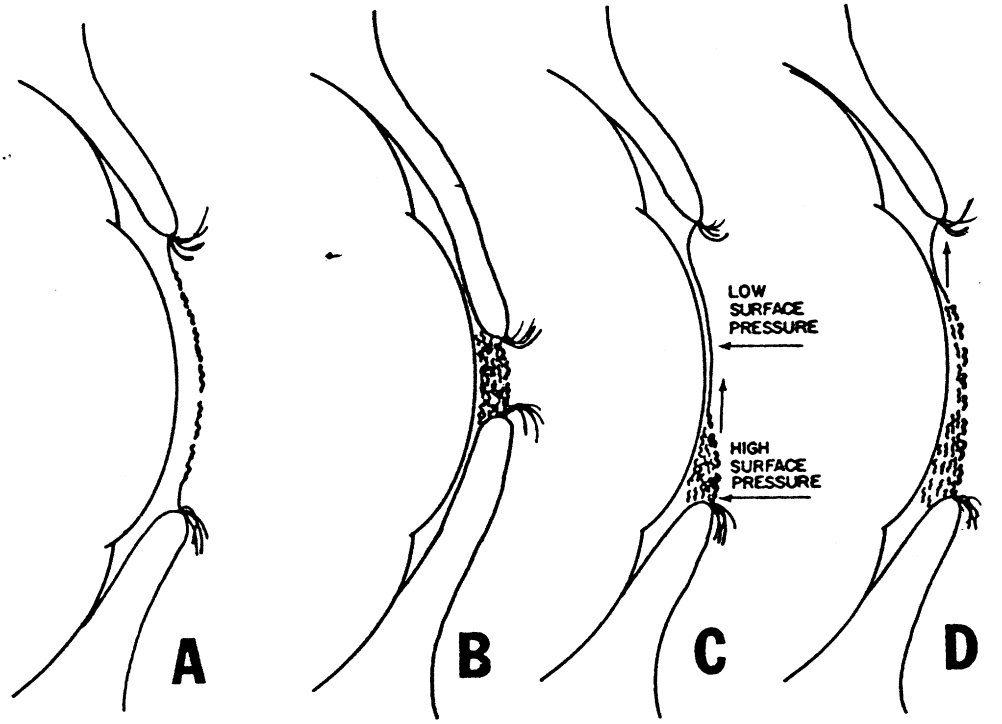

Fig. 9. Schematic representation of a moving polymer film during a blink.

of polymer solutions and, at this time, it is impossible to predict how long such effects as tear film thickening persist. It is entirely possible that the PTF thickening by polymers is strictly due to mechanical spreading of a viscous polymer solution by the upper eyelid during a blink. It is also possible that the long-term desirable effects of polymers are entirely different from those introduced in this text and that the different polymers utilized in this study may prove to have completely different desirable properties when examined from a different standpoint, such as adsorption to the corneal surface.

We have succeeded in demonstrating that polymer solutions increase the thickness of the PTF. When comparing different polymer solutions, the retention of fluorescein in the PTF is not strictly related to the viscosity of the vehicle or its surface wetting properties but there is a good correlation with its surface spreading characteristics and its ability to drag water. The increased thickness of the PTF observed with polymers may be attributed to a spreading surface film of polymer molecules. We believe that a polymer's capability to move water and stabilize a thick aqueous layer may play an important role in the use of polymer solutions in ophthalmology, especially as tear substitutes in people with dry eye syndromes. We hope that future studies will further characterize the properties of polymers involved in this phenomenon.

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