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AMPHOTERICIN B-INDUCED SODIUM TRANSPORT AND WATER FLOW ACROSS RABBIT CORNEAL EPITHELIUM

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Summary

We have determined fluid translocation across the cellular layers lining the cornea by measuring changes in corneal transparency. The loss of 1.3 $\mu l/cm^2$ fluid from the stroma causes an increase of +1% in transparency. Amphotericin B ($2 \cdot 10^{-6}$ M) when added to the tear side (= mucosal side) of the epithelium causes a rapid increase in potential difference of 12.3 ± 0.7 mV (mean ± S.E., $n = 6$) followed by a slower increase of 18.6 ± 1.5 mV. The electrical resistance is reduced from 3.2 ± 0.3 $k\Omega \cdot cm^2$ to 0.6 ± 0.1 $k\Omega \cdot cm^2$. The resulting increase in calculated short circuit current is accompanied by a decrease in transparency at a rate of 3.6 ± 1.0% per h, corresponding to an uptake of fluid by the cornea of 4.7 $\mu l \cdot cm^{-2} \cdot h^{-1}$. Replacement of the fluid bathing the endothelial side of the cornea, in order to prevent water movement from the aqueous compartment into the stroma, did not significantly alter this uptake of fluid. Thus the epithelial fluid transport which is reported to be normally slightly secretory, becomes absorptive in the presence of amphotericin B. Serosal hypertonicity (20 mM mannitol) increases the water influx into the cornea induced by amphotericin B. These results indicate that amphotericin B induces sodium-selective channels in the epithelium leading to an accumulation of NaCl and water in the stromal layer of the cornea. Ouabain reduces the potential and calculated short circuit current in epithelia pretreated with amphotericin B. Following addition of ouabain, the NaCl and water accumulated in the stroma leak away resulting in a transient increase in transparency. Finally, a model is proposed that includes a stromal compartment involved in fluid transport and that agrees with the results presented here.

Introduction

The cornea consists of a stroma lined by two cellular layers, the epithelium and the endothelium. The epithelium faces the external environment and is bathed by the tears. The endothelium faces internally and is bathed by the aqueous humor. Due to its high content of mucopolysaccharides, the stroma tends to take up water and swell. This passive influx of water is opposed by the active transport of water out of the stroma, principally by the endothelium [1]. Endothelial fluid transport has been proposed to be coupled to the transport of HCO_3^- [2]. The role of the epithelium in deturgescence of the stroma remains uncertain. Riley [4] has reported that the epithelium's major function is as a barrier to the passive movement of water into the stroma, however, following stimulation of epithelial Cl^- transport, the epithelium is able to transport fluid out of the stroma into the tears [3]. In the present study we have investigated whether the epithelium is capable of higher rates of fluid transport then hitherto reported.

The present experiments show that transmittance changes are linearly related to changes in water content and thus reflect fluid translocation across the cell layers lining the stroma. Amphotericin B has been reported in both the rabbit cornea and that of the amphibian to stimulate short circuit current, presumably due to an increase in sodium transport [5,6]. Following exposure of the mucosal surface of the epithelium to amphotericin B, there is a decrease in transmittance. From this transmittance decrease we have calculated a rate of fluid transport comparable to the rate of endothelial fluid transport reported by Mishima and Kudo [1].

Materials and Methods

New Zealand white rabbits weighing 2—2.5 kg were killed by a blow on the neck and exsanguination. The eyes were enucleated and kept in a moist atmosphere at 4°C. Corneal isolation and storage was performed as previously described [7]. The cornea was transferred to a modified Ussing chamber (cf. Fig. 1) adapted to fit the curvature of the cornea. The edges of the chamber were greased with silicon grease to improve sealing and to minimize edge dam-

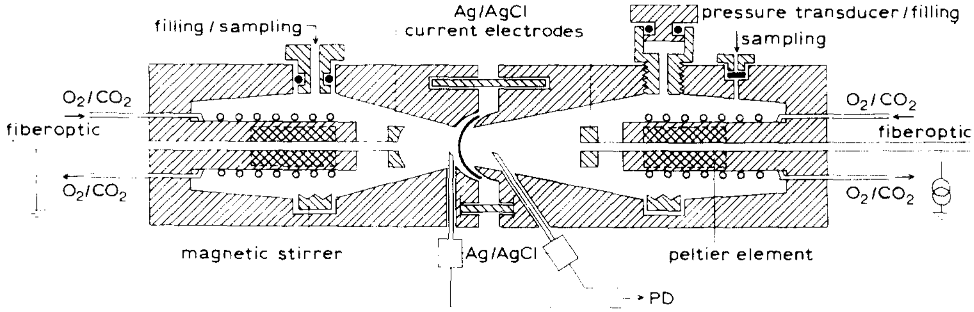


Fig. 1. A schematic diagram of the experimental set-up.

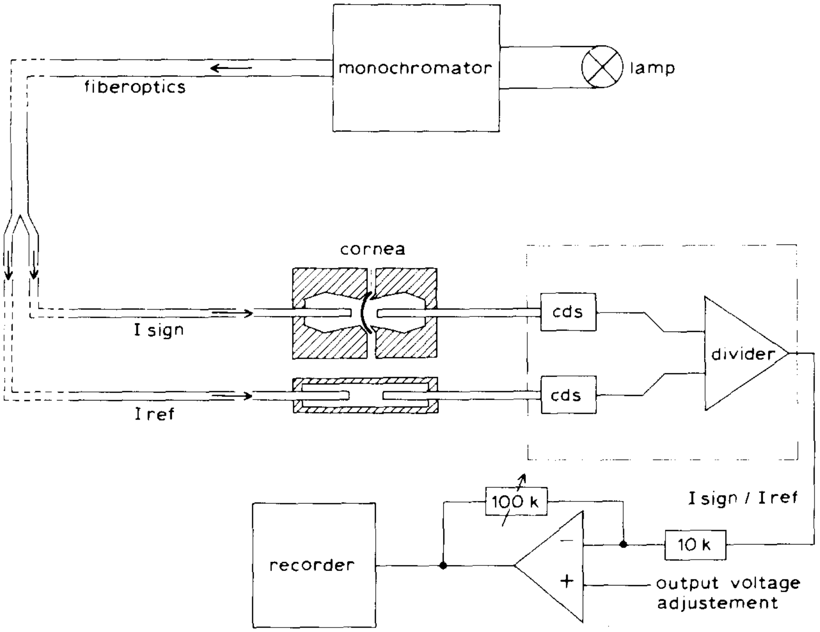


Fig. 2. A scheme of the light pathway.

age. The chamber permitted simultaneous measurement of potential difference, resistance and light transmittance. The light pathway is shown in Fig. 2. Light emitted by a tungsten lamp was passed through a monochromator (Beckman G 2400 Spektral photometer) to obtain a beam of wavelength 520 nm. The lightbeam was split by fiber optics and used in a dual-beam transmittance measuring device. Cadmium sulfide cells (RPY 58 A, Philips) were used to measure light intensity and a divider (434 B, Analog Devices) was used to calculate transmittance. The transmittance was set to 100% when there was no cornea in the Ussing chamber. Temperature at the tear side and the aqueous side could be controlled independently by means of peltier elements regulated by electrical control units essentially the same as those described by Augustus and Cuperus [8]. Unless otherwise stated the chamber temperature was kept at 35°C. Oxygenation was established by passing 100% O_2 in the case of solution 1 and 95% O_2 and 5% CO_2 in the case of solution 2 through silastic tubing coiled in the bathing solution. The diffusion of oxygen through the wall of the tubing was sufficient to oxygenate the solutions.

The bathing solutions contained the following substances (concentrations in mM). Solution 1: NaCl, 110; $CaCl_2$, 1.5; KCl, 3.8; KH_2PO_4, 1.0; Na_2HPO_4, 4.2; Na_2SO_4, 17; $MgSO_4 \cdot 7H_2O$, 1.0; glucose, 25. Solution 2: NaCl, 110; $NaHCO_3$, 21.2; $KHCO_3$, 3.8; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 1.0; $CaCl_2$, 1.5; Na_2SO_4, 9; glucose, 25. Adenosine, 5.0; reduced glutathione, 1.0 and L-ascorbic acid, 1.5 were added to bathing solution 2 to improve endothelial preservation (see also Ref. 7). In order to minimize interference of air bubbles in the measurement of transmittance, the surface tension was lowered by the addition of pluronic F 108 (BASF), 0.25 g/l. Colloid osmotic pressure was measured to be 0.07 kPa

(0.5 mmHg) and had no effect on measured parameters. Ouabain ($6 \cdot 10^{-4}$ M), amphotericin B ($2 \cdot 10^{-6}$ M) (fungizone, Squibb $6 \cdot 10^{-4}$ M) adenosine, reduced glutathione and L-ascorbic acid were added just prior to the experiment. Osmolality was measured with an Advanced Osmometer and adjusted to 310 mosM by adding mannitol. Resistance and potential difference were measured as described before [7].

In vivo the intraocular pressure amounts to 2.4 kPa (18 mmHg). However, we had observed in preliminary experiments that high hydrostatic pressure (1.5 kPa, 11.3 mmHg) causes potential resistance and transmittance to decline rapidly after 3 to 4 h incubation. For rabbit cornea these phenomena have not been reported previously. One explanation for this may be that the decline in potential and resistance cannot be detected by measuring only the short circuit current. Furthermore, transmittance measurements in rabbit cornea, especially with longer incubation periods, have rarely been described. Zadunaisky et al. [9] have reported in frog cornea a similar rapid fall in potential and resistance when using a high hydrostatic pressure. As reported for frog cornea, these adverse effects could be avoided in our preparation by using a hydrostatic pressure just sufficient to prevent wrinkling of the tissues, i.e. a pressure of 0.13 kPa (1 mmHg). An additional advantage of such a low hydrostatic pressure is that any signifcant effect on water translocation can be ruled out.

Hydration of the cornea was calculated as the ratio of water content to dry weight of a punched-out plug of the cornea. Wet weight was determined after gently blotting the corneal plug on filter paper. Dry weight was obtained after baking for 24 h at 95°C.

In soma experiments, silicon oil MS 200 (Merck-Schuchard) was used at the aqueous side to prevent water movement from the aqueous compartment to the stroma across the endothelium.

The results are given as the mean ± S.E.

Results

The relationship between corneal hydration and light transmittance

The relationship between hydration and transmittance was found to be linear in the range tested, i.e. transmittances as low as 50%. Variation in water content was established by prolonged incubation of the cornea with or without ouabain and sometimes after removal of the epithelium and endothelium by brushing. The slope of the line relating hydration and transmittance is —0.13 g H_2O/g dry weight per Δ% transmittance ($r = 0.93$, 21 observations). Since the dry weight of corneal tissue was found to be 10.2 ± 0.4 mg $\cdot$ cm^{-2} ($n = 10$) an increase in hydration of 1.3 μl H_2O per cm^2 corresponds to a 1% reduction in transmittance.

To check that our indirect method of measuring water transport gives valid results we incubated corneas in bathing solution 2 which contained ouabain. Trenberth and Mishima [10] using corneal thickness as a measure of corneal hydration and using also an HCO_3^--containing bathing solution (cf. our solution 2) have reported that ouabain causes fluid accumulation at a rate of 4.3 μl $\cdot$ h^{-1} $\cdot$ cm^{-2}. In this study we have found a rate of transmittance decrease of 2.0 ± 0.3% per h ($n = 7$) corresponding to a water accumulation rate of 2.7 μl $\cdot$

$h^{-1} \cdot cm^{-2}$. The agreement of this figure with that reported by Trenberth and Mishima [10] who used an independent method supports the validity of our method of measuring net fluid translocation across the lining cellular layers of the cornea. Because we wished to study water transport initiated by the corneal epithelium we have attempted to minimize active transport by the endothelium. Hodson and Miller [2] found that Ringer solutions free of CO_2 and HCO_3^- inhibited the active contribution of the endothelial layer to corneal deturgescence. So we used bathing solution 1 which did not contain CO_2 or HCO_3^- to inhibit endothelial water transport. Because transmittance is not significantly lowered during the first 80 min of incubation in bathing solutoon 1, we can conclude that bathing solution 1 has not altered the water content of the cornea. This indicates that either endogenous CO_2 and HCO_3^- activate the fluid pump and/or the epithelium contributes to deturgescence.

The effects of amphotericin B addition on transmittance, potential and resistance

The lowest concentration of amphotericin B giving a maximal response in the potential difference was found to be $2 \cdot 10^{-6}$ M. Addition of $2 \cdot 10^{-6}$ M amphotericin B to the tear side caused changes in potential, resistance and transmittance that are summarized in Table I and Fig. 3. In particular there is a reduction in transmittance (indicating water accumulation in the stroma) beginning after 25 min of exposure to amphotericin B. The water permeability of the epithelium is very low and this low permeability may impede the action of amphotericin B in increasing water movement across the epithelium. Because Hardy et al. [11] have reported that serosal hypotonicity increases the water permeability of the toad bladder, we have tried to enhance the action of amphotericin B on corneal water transport by using a hypertonic bathing solution on the aqueous side of the cornea (i.e. an additional 20 mM mannitol). Mannitol gradients across the endothelium are dissipated within a few minutes [12] and thus in our experiments the entire osmotic gradient is acting across the epithelium. In control experiments we have found that serosal hypertonicity does not influence the potential, resistance or transmittance of the cornea in the absence of amphotericin B.

The potential change consists of two separate phases. The first phase is a hyperpolarization of 12.3 ± 0.7 mV ($n = 6$) which is completed within a few

TABLE I

THE EFFECT OF AMPHOTERICIN B ON POTENTIAL DIFFERENCE AND RESISTANCE

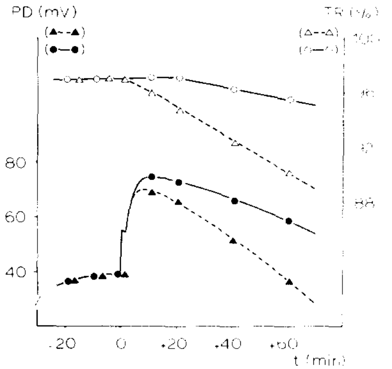
Mean values ± S.E. ($n = 6$) of potential difference (p.d., in mV) and resistance (R, in $k\Omega \cdot cm^2$) before and after tear side (TS) amphotericin B ($2 \cdot 10^{-6}$ M) addition. R_{30} denotes R 30 min after amphotericin B addition. AS, aqueous side.

20 mM mannitol AS	Before amphotericin B		After amphotericin B			
	p.d.	R	p.d.		p.d.$_{max}$	R_{30}
			first step	second step		
—	36.7 ± 1.7	3.2 ± 0.3	12.3 ± 0.7	18.6 ± 1.5	65.1 ± 28	0.6 ± 0.1
+	41.4 ± 2.5	3.0 ± 0.4	10.0 ± 1.5	13.2 ± 1.6	63.7 ± 1.9	0.7 ± 0.1

seconds and is not significantly changed by serosal hypertonicity. When the solution bathing the tear side contained no Na^+ (tetraetylammonium and choline substitution) the addition of amphotericin B resulted in a small depolarization of a few mV indicating the Na^+ selectivity of the amphotericin B-induced potential response. The second slower rise in potential of 18.6 ± 1.5 mV is significantly reduced by serosal hypertonicity to 13.2 ± 1.6 mV ($n = 6, P < 0.05$).

30 min after addition of amphotericin B the resistance has stabilized at a level of 0.6 ± 0.1 $k\Omega \cdot cm^2$ from the initial value of 3.2 ± 0.3 $k\Omega \cdot cm^2$ and this response is not significantly altered by serosal hypertonicity. After at most 25 min of exposure to amphotericin B the potential began to fall linearly (see Fig. 3). This potential fall was accompanied by a fall in transmittance. Serosal hypertonicity increased both the fall in potential and the fall in transmittance. Corneal resistance remains stable and is unchanged by serosal hypertonicity. In the absence of serosal hypertonicity amphotericin B causes transmittance to fall at a rate of 3.6 ± 1.0% per h ($n = 6$) which corresponds to a water flow into the cornea of 4.8 $\mu l \cdot h^{-1} \cdot cm^{-2}$. When mannitol is applied to the aqueous side the rate of transmittance fall after amphotericin B is 9.5 ± 0.8% per h ($n = 8$), corresponding to a water movement of 12.4 $\mu l \cdot h^{-1} \cdot cm^{-2}$ which is significantly higher than in the absence of serosal hypertonicity ($P < 0.001$). The effect of serosal hypertonicity starts just after its application to the amphotericin B-treated corneas as can be seen in Fig. 4. The parallel fall of potential and transmittance (see Figs. 3 and 4) suggests a relationship between these two parameters. We have plotted the rate of fall of transmittance against the rate of fall of the potential during the period when their falls were linear. The resulting graph is shown in Fig. 5 and reveals a linear relationship with a correlation coefficient of $r = 0.76$.

Preventing the transport of water across the endothelium from the aqueous side into the stroma by bathing the endothelial side with silicon oil (MS 200)



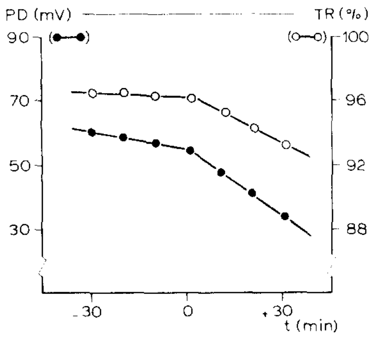
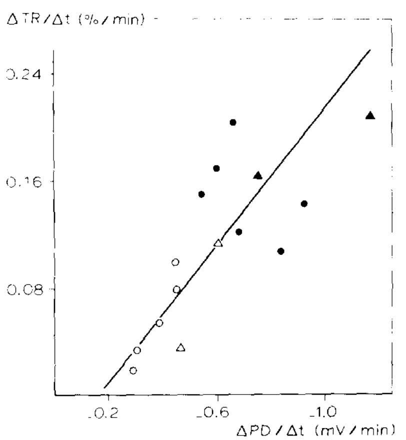


Fig. 3. The effect of $2 \cdot 10^{-6}$ M amphotericin B addition to the tear side bathing solution upon potential difference (p.d.) and transmittance (TR) with or without 20 mM mannitol aqueous side. Amphotericin B is added at time $t = 0$. Without mannitol: p.d. (●———●), TR (○———○); with mannitol: p.d. (▲- - - - -▲) and TR (△- - - - -△).

Fig. 4. The effect of serosal hypertonicity (20 mM mannitol, aqueous side) upon potential difference (p.d.) (●———●) and transmittance (TR) (○———○) in amphotericin B conditions. Mannitol is added at time $t = 0$.



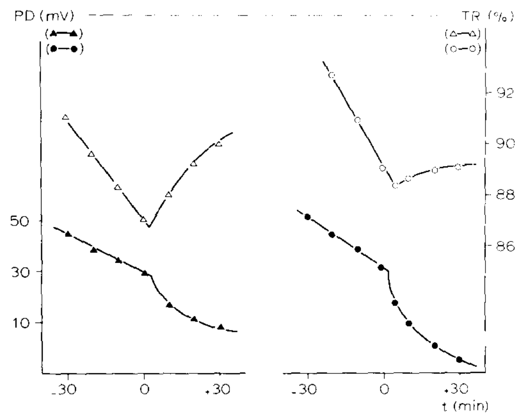


Fig. 5. The relationship between the rate of decrease of transmittance (TR) and potential difference (p.d.) after amphotericin addition. The rates were determined when TR and p.d. fell linearly. AS, aqueous side; TS, tear side. (○) bathing solution 1; (●) bathing solution 1 + AS 20 mM mannitol; (△) bathing solution 2 TS and bathing solution 1 AS; (▲) bathing solution 2 TS and bathing solution 1 AS + 20 mM mannitol.

Fig. 6. The effect of ouabain ($6 \cdot 10^{-4}$ M) on potential difference (p.d.) and transmittance (TR) in amphotericin conditions + 20 mM mannitol. (△——△) and (○——○) TR; (▲——▲) and (●——●), p.d. TS, tear side; AS, aqueous side. The triangles denote bathing solution 1 TS and AS. Circles denote bathing solution 1, TS and bathing solution 1 Cl^- substituted by SO_4^{2-}, AS.

did not significantly alter the effects of amphotericin B on potential or resistance. The transmittance fall due to amphotericin B amounted to 2.4 ± 0.2% per h ($n = 3$) which is not significantly lower than that observed without blocking the endothelial side, $P < 0.35$, the two situations being different. From this we conclude that the water transport following exposure to amphotericin B is mainly across the epithelium rather than across the endothelium.

Amphotericin B incubation and subsequent ouabain addition

In order to examine whether the decrease in transmittance following exposure to amphotericin B was really a consequence of active Na^+ transport by the epithelium, ouabain ($6 \cdot 10^{-4}$ M) was added to the aqueous side of the cornea. Fig. 6 shows that with a lag time of 2 min ouabain causes an additional fall in potential which is accompanied by a transient increase in transmittance. Simultaneous incubation with amphotericin B and ouabain causes the potential to decline to zero. The total increase in transmittance following the addition of ouabain is dependent on the actual transmittance at the moment of ouabain addition. Fig. 7 is a plot of the total transmittance increase due to ouabain against the transmittance in amphotericin B conditions at the moment of ouabain addition. All experiments, both with and without serosal hypertonicity, were used for the construction of this figure. The correlation coefficient $r = 0.91$. The line crosses the abscissa at 96% transmittance, i.e. a cornea with 96% transmittance following amphotericin B will not become more transparant after ouabain addition. A similar phenomenon, that is an increase in transmittance following inhibition of the (Na^+-K^+)-ATPase in corneas treated with amphotericin B is seen when the cornea is cooled to 0°C. The closed triangles in

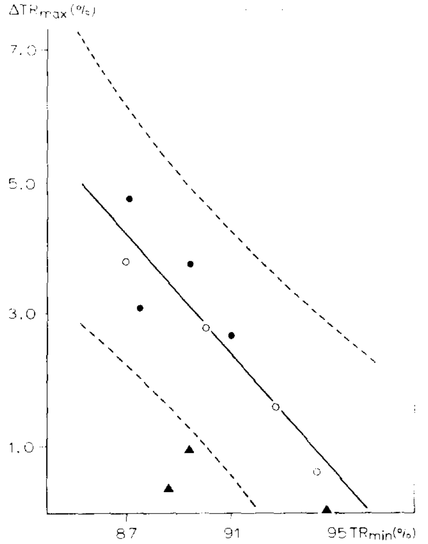


Fig. 7. Maximum increase in transmittance (TR) is plotted to the actual TR reached at the moment $6 \cdot 10^{-4}$ M ouabain is added to the amphotericin B ($2 \cdot 10^{-6}$ M) pretreated cornea. AS, aqueous side. (○) bathing solution 1; (●) bathing solution 1 + 20 mannitol AS; (▲) bathing solution 1 + 20 mM mannitol AS + AS Cl^- substituted by SO_4^{2-}.

Fig. 7 denote corneas which were incubated in Cl^--free solutions at the aqueous side (SO_4^{2-} replacing Cl^-). These corneas did not recover as well as the control corneas. For the two corneas with transmittance lower than 91% the increase in transmittance was significantly different from the calculated linear regression line (the broken lines in Fig. 7 indicate the 97.5% confidence limits).

Discussion

In this study we have used an indirect method for measuring net fluid transport across the lining cellular layers of the rabbit cornea. We have found that there is a linear relationship between corneal hydration and transmittance. The existence of this linear relationship is supported by the findings of Farrell et al. [13] who reported for the rabbit cornea a linear relationship between thickness and transmittance. However, Zadunaisky and Lande [14], using frog cornea, found a more complex relationship between water content and transmittance. The frog cornea also differs from the rabbit cornea in that the frog cornea swells considerably in the first hours of incubation [14]. This phenomenon is not observed in the rabbit cornea. The ouabain incubation experiments give rise to two comments. First, the water uptake of $2.7\ \mu l \cdot cm^{-2} \cdot h^{-1}$ we have calculated from the transmittance decrease agrees with the value of $4.3\ \mu l \cdot cm^{-2} \cdot h^{-1}$ reported by Trenberth and Mishima for the rabbit cornea [10]. This finding confirms that transmittance is suitable for measuring net fluid translocation across the lining cell layers. Secondly, since Trenberth and Mishima [10] used changes in stromal thickness (which are large in comparison to those of the lining cellular layers) the similarity of our results to theirs suggests that swelling of the lining cellular layers does not contribute to the transmittance changes that can be observed.

Addition of amphotericin B to the tear side bathing solution gave a biphasic potential and resistance response consisting of a fast and a slow phase. The first potential step is most probably due to a depolarization or the mucosal membrane resulting from a fast increase in P_{Na^+}/P_{Cl^-}. Holz [15] has reported that application of amphotericin B on one side of thin lipid membranes causes a cation-selective permeability increase. The hyperpolarization that we have observed following amphotericin B addition to the mucosal bathing solution suggests that at least initially, the amphotericin B-induced pores are more selective for Na^+ than for K^+. Furthermore, reversing the Na^+ gradient across the mucosal membrane reversed the polarity of this response. Finally, microelectrode experiments in progress in our laboratory have shown the first potential step to take place across the mucosal membrane of the epithelium. This increase in Na^+ permeability of the tear side membranes of the epithelium is reflected in the decrease in resistance (Table I).

The second potential step may be the result of an increased sodium transport rate [16]. Candia and Neufield [6] have shown that amphotericin B stimulates short circuit current in rabbit cornea, which they ascribed to an increase in active sodium transport. Stimulation of short circuit current by amphotericin B explicable by an increase in active sodium transport has also been reported in amphibian cornea [5] and toad bladder [17]. Two distinct phases in the initial rise in short circuit current or potential have not been described in these tissues. These findings strongly suggest that amphotericin B stimulates active Na^+-transport in rabbit corneal epithelium also, by making more Na^+ available to the pump.

It is most striking that the fluid transport as given by the transmittance fall starts at the moment potential is beginning to decrease, while the resistance is not falling significantly at that time. This transmittance change shows that amphotericin B alters the normal direction of transport of water across the epithelium (which is believed to be slightly secreotyr [3]) to that characteristic of a reabsorptive epithelium such as that of the proximal tubule.

Preliminary histological and electron-microscopic experiments indicate that the lateral intercellular spaces dilate after the addition of amphotericin B. Such wide lateral intercellular spaces are described in several epithelia which transport fluid from the mucosal to the serosal side, e.g. rabbit gallbladder [8], renal collecting tubule [19] and toad bladder [20]. Serosal hypertonicity makes the potential and transmittance fall, after amphotericin B addition, larger. This additional transmittance fall indicates an increase in net volume flow into the stroma. The increased volume flow, however, can be caused either by an increase in osmotic gradient (sic) or by an increase in water permeability such as that suggested by Hardy et al. [11] for the toad urinary bladder. The increased transmittance fall due to serosal hypertonicity in the presence of amphotericin B suggests that the stimulated Na^+ transport itself creates an osmotic gradient across the epithelial layer. Bindslev et al. [21] have pointed out the possibility that permeability changes in toad urinary bladder at the tight junctions are induced by osmotic gradients. In the rabbit cornea, osmotic gradients across the epithelium do indeed cause transmittance changes after application of amphotericin B that suggest a greater hydraulic conductivity (Festen, C.M.A.W., unpublished results). An increase in water permeability following application of amphotericin B has been reported also in the toad cornea by Parisi et al. [22]. The results we have discussed so far can be summarized by saying that amphotericin B leads to a stimulation of active sodium transport accompanied by a net flow of water into the stroma. The magnitude of this accompanying water flow is enhanced by an increased water permeability, resulting both from the direct action of amphotericin B and from an osmotic gradient across the epithelium. The osmotic gradient may be secondary to the increased Na^+-transport or due to imposed serosal hypertonicity.

In rabbit ileum [24] and in toad urinary bladder [21] the opening of the lateral intercellular spaces and the resultant increase in water permeability are accompanied by an increase in the conductance of the paracellular pathway. However, in rabbit corneal epithelium, following exposure to amphotericin B a decrease in shunt resistance resulting from the opening of the lateral intercellular spaces may be hard to detect, since the cellular route has a low resistance. The depolarization seen following amphotericin B can be explained by a lowering of the shunt resistance acting in concert with an increase in the sodium gradient across the epithelium. These lead to intra-epithelial loop currents and hence to the observed depolarization of the transcorneal potential.

The major part of the water flow induced by amphotericin B is across the epithelium, since replacement of the aqueous bathing solution by silicon oil (which prevents the water flow from the aqueous to the stromal side of the endothelium) does not significantly reduce the accumulation of water in the stroma.

Green [24] has reported that there is a close coupling between passive swelling of the stroma and the loss of Na^+ bound to mucopolysaccharides in the stroma. In particular, Green was only able to demonstrate stromal swelling when the amount of sodium bound was reduced. We have found that amphotericin B can cause stromal swelling without lowering the amount of Na^+ bound.

The active sodium transport by rabbit corneas exposed to amphotericin B is diminished following the addition of ouabain, as has been reported by Candia et al. [5] for the amphibian cornea.

If the active transport of Na^+ is inhibited, the accumulated Na^+ and water may diffuse out of the stroma. Inhibition by ouabain of the active Na^+ and water accumulation in the stroma may thus lead to a transient increase in transmittance, as observed, before the normal swelling process reasserts itself.

In corneas swollen by pretreatment with amphotericin B, replacement of Cl^- by SO_4^{2-} in the aqueous bathing solution reduces the transmittance increase observed following ouabain. There are two possible (but not mutually exclusive) explanations for this. Firstly, that the movement of Na_2SO_4 across the lining cell layers of the cornea is restricted in comparison to that of NaCl. Secondly, that the activity of the Cl^- transporting mechanism which has been reported to be able to dehydrate the stroma [3] is inhibited by the Cl^--free bathing solution, thus reducing the water efflux seen following ouabain administration.

The linear relationship between the transmittance increase after ouabain and the actual transmittance reached at the moment of ouabain addition implies

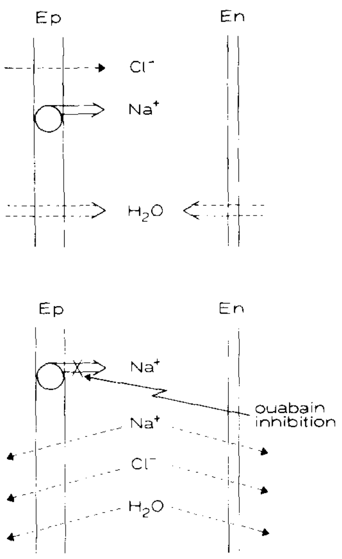


Fig. 8. A model of rabbit cornea with the amphotericin B-induced ion and water movements (upper panel) and the result of subsequent ouabain addition (lower panel).

that a constant fraction of the accumulated water is released. That fraction of accumulated water not released is probably bound to the stromal components.

Our findings agree with the model of the rabbit cornea shown in Fig. 8. It consists of a stromal compartment separated from the tears by the epithelium and from the aqueous by the endothelium. Transport processes across these layers can load or deplete the stromal compartment of ions. The loading or depletion of the stromal compartment is accompanied by fluid translocation.

Amphotericin B sharply stimulates the Na^+ transport across the corneal epithelium, loading the stromal compartment with NaCl. In response to the resulting NaCl gradient, water is translocated across the epithelium and probably also across the endothelium. When water transport across the endothelium from the aqueous to the stroma is blocked with silicon oil the epithelium is able to transport up to 3.1 $\mu l \cdot cm^{-2} \cdot h^{-1}$ following stimulation of the Na^+ transport by amphotericin B. Ouabain stops the active flux of Na^+ into the stromal compartment and hence the active water translocation across the epithelium (see lower panel of Fig. 8). Subsequently, the accumulated salt and water leak away. In this model, the stroma as a whole is the primary site of the hypertonicity driving water translocation, rather than the lateral intercellular spaces.

The same model may be applied to the endothelium as well, so that in the rabbit cornea a mechanism operates similar to that proposed for fluid movement across the proximal tubule by Andreoli and Schafer [25]. Andreoli and Schafer argue that small osmotic gradients across an epithelial layer can be the driving force for fluid translocation. The $NaHCO_3$ gradient necessary for a fluid translocation of 5 $\mu l \cdot cm^{-2} \cdot h^{-1}$ across the endothelium into the aqueous (as for example seen following the rewarming of corneas swollen by cooling) can be estimated. From the L_p ($42 \cdot 10^{-12}$ $cm^3 \cdot dyne^{-1} \cdot s^{-1}$) and the σ_{NaCl} (0.45) reported by Klyce and Russell [12] together with the assumption that $\sigma_{NaHCO_3} \simeq \sigma_{NaCl}$ [2], we have calculated that an osmotic gradient of 2.8 mosM suffices for the amount of fluid translocation across the endothelium as seen following rewarming of the cornea. Suggestions that the stroma is a compartment involved in the fluid transport have been made by Klyce and Russell [12]. Further experiments are necessary to check the proposed model. The experimental set-up as described in this report can be used to measure the relevant parameters potential, resistance and via the transmittance, the fluid translocation across the epithelium or the endothelium.

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