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PRIMING OF THE FLUID PUMP BY OSMOTIC GRADIENTS ACROSS RABBIT CORNEAL ENDOTHELIUM

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Summary

The present study shows that the inclusion of 5% Dextran (average mol. wt. 40 000) in solutions to preserve in vitro rabbit corneal endothelium induces a sizable osmotic flow across the preparation which is superimposed on the existing fluid transport. Furthermore, even after fluid transport ceases due to in vitro deterioration, the Dextran-induced flow remains for some addition time. The osmotic permeability was 162 ± 17 μm/s in the presence of glucose and 451 ± 84 μm/s in its absence. The latter, comparatively high value suggests that such osmotic flow traverses the intercellular junctions. In addition, temporary (10—15 min) imposition of an osmotic gradient has a separate stimulatory 'priming' effect on the rate of fluid transport. Thus, the rate of fluid pumping increased by about 40% after challenge with Dextran. It was further noted that, after addition of Dextran, preparations in the absence of glucose escape gross deterioration for a time longer than those in the presence of glucose. On the other hand, mere addition of Dextran to a glucose-containing solution does not appear to prolong the estimated 'survival time' of the pumping mechanism. The sizable osmotic flows and the priming effect described here may provide a physiological context with which previously described Dextran effects on cornea preservation can now be compared.

Introduction

The question of the time-transient behavior of the fluid pumps in transporting epithelia kept in vitro has received comparatively little attention in the past. The matter has relevance to hypothetical future attempts to grow such epithelia in culture. It has also practical implications in areas such as corneal preserva-

tion, where it has been empirically found [15] that inclusion of Dextran in solutions employed in eye banks improves the transparency of corneal specimens. We have therefore studied the behavior of the transendothelial fluid pump and the superimposed osmotic flows when the tissue is maintained in vitro under various conditions. Aside from giving novel information on matters of endothelial osmotic permeability and tissue preservation, the results reveal an interesting effect by osmotic gradients on the rate of fluid transport, denoted here as 'priming' of the fluid pump.

Methods

Most of the details of the methods chosen for dissection and mounting of the endothelial preparation, as well as the chambers and the automatic technique for rate of fluid measurement employed have all been described elsewhere [2,6,7]. The solutions employed are detailed in Table I. The Dextran utilized was 40 000 in molecular weight, clinical grade, range 37 000—43 000, and came from Pharmaceuticals Inc., Cleveland, OH 44128 (catalog No. 101510). In order to determine the difference in osmotic pressure generated by it, Dextran from the batch above was added in varying proportions to an isotonic saline (NaCl, 9.5 g/100 ml), and the osmolality of each resulting solution was measured with an Advanced Instruments osmometer. From a plot of the results, it was determined that the addition of 5% Dextran results in an increase of 3.3 mosmol/l in osmotic pressure. The osmotic pressure of the standard solutions employed was (in mosmol/l ± S.E.): adenosine (SA solution), 292.6 ± 0.1; basal salts (S solution), 294.4 ± 0.2, and glucose (SG solution) 291.9 ± 1.0.

After enucleation of the eyes, the corneal epithelium was removed by scraping the cornea with a razor blade. The eye was subsequently dissected until only the endothelium plus stroma were left. This preparation was then mounted in a chamber, and the rate of fluid transport was monitored continuously; the temperature was 36.8°C. The standard procedure employed was to mount the two corneas of a given rabbit in two identical chamber set-ups and to use one preparation to conduct the tests while the other one serves as a control. In all cases, the same given solution was initially placed in both compartments of the chamber; the volume of these compartments was approx. 4 ml.

TABLE I

COMPOSITION OF BATHING SOLUTIONS EMPLOYED

Basal salts (mM): NaCl, 110.4; $NaHCO_3$, 39.2; $KHCO_3$, 3.8; KH_2PO_4, 1.0; $MgSO_4 \cdot (7H_2O)$, 0.78; $CaCl_2 \cdot (2H_2O)$, 1.7; sucrose, 5.0.

Solution	Basal salts	Nutrients	5% Dextran
Basal salts (S)	yes	—	—
Salts + glucose (SG)	yes	6.9 mM glucose	—
Salts + adenosine (SA)	yes *	5 mM adenosine	—
		0.1 mM oxidized glutathione	—
Salts, glucose and Dextran (SGD)	yes	6.9 mM glucose	yes
Salts + Dextran (SD)	yes	—	yes

* For SA solution, 5 mM adenosine substituted the 5.0 mM sucrose.

For most of the experiments, the rates of fluid movement were recorded for time intervals of 1—5 min. In addition, some experiments were left unattended overnight, in which case the recording time intervals were increased to 30 min. In those experiments which required a change in ambient solutions, the substitution was performed only on the endothelial, i.e., the aqueous side of the preparations. While the substituting solution was injected into the chamber with a syringe, another syringe simultaneously withdrew fluid in order to maintain its level inside the chamber as steady as possible. The solution that had been placed on the stromal side of the chamber at the beginning of the experiments was left undisturbed. During all experiments a hydrostatic pressure head of 10 cmH_2O was maintained on the endothelium. The results summarize the information obtained from 59 experiments. Wherever pertinent, the number of experiments for a given subgroup is given; all deviations reported are S.E.

Results

Control experiments

All of the main results reported here were obtained using paired rabbit eyes. It was therefore important to establish that, under each of the present conditions, the rate of fluid transport as a function of time for both paired preparations would be similar. The fact that this was indeed the case is exemplified in the control experiments with solutions S (basal salts), SA (adenosine) and SG (glucose) shown in Figs. 1—3. Both paired corneas were bathed with the same solution, and their rates of fluid transport were monitored as a function of time; their behavior was nearly the same. Control (maximal) rates of fluid pumping were (in $\mu l \cdot h^{-1} \cdot cm^{-2}$): 1.7 ± 0.5 ($n$ = 5) with S solution, and 2.8 ± 0.2 (n = 26) with SG solution. Test rates were 6.2 ± 0.3 (n = 16) with salts, glucose and Dextran (SGD) solution, and 9.7 ± 1.8 (n = 7) with SD solution. Other characteristics can also be seen in Figs. 2 and 3; survival time or in vitro 'life' is taken here to be the time during which the preparations pumped fluid in the normal forward or 'physiological' direction, from stroma to aqueous. As can be seen in those figures, the preparations bathed with adenosine (SA) solution developed a rate which was higher than that in those bathed with glucose (SG) solution, but 'lived' a much shorter time. At the end of this time, the deterioration of the preparations presumably resulted in decreased pump activity or increased hydraulic permeability or both. The hydrostatic pressure differences across the endothelium then induced water flow in the reverse direction.

Duration of 'forward' flow

The water flows before and after the addition of Dextran to the basal salts and glucose solutions are exemplified in Figs. 4 and 5, respectively. As can be seen in the figures, in the absence of glucose the osmotic flow resulting from Dextran addition (SD solution) lasted for a time much longer than that registered in its presence (SGD solution). This curious apparent 'adverse' effect due to the preparations, i.e., the times during which the preparations were able to sustain fluid movement from stroma to aqueous. These were as follows (in min): for S solution, 242 ± 32 (n = 11); for SD solution, 606 ± 319 (n = 7); for SG solution, 624 ± 59 (n = 8), and for SGD solution, 899 ± 83 (n = 16).

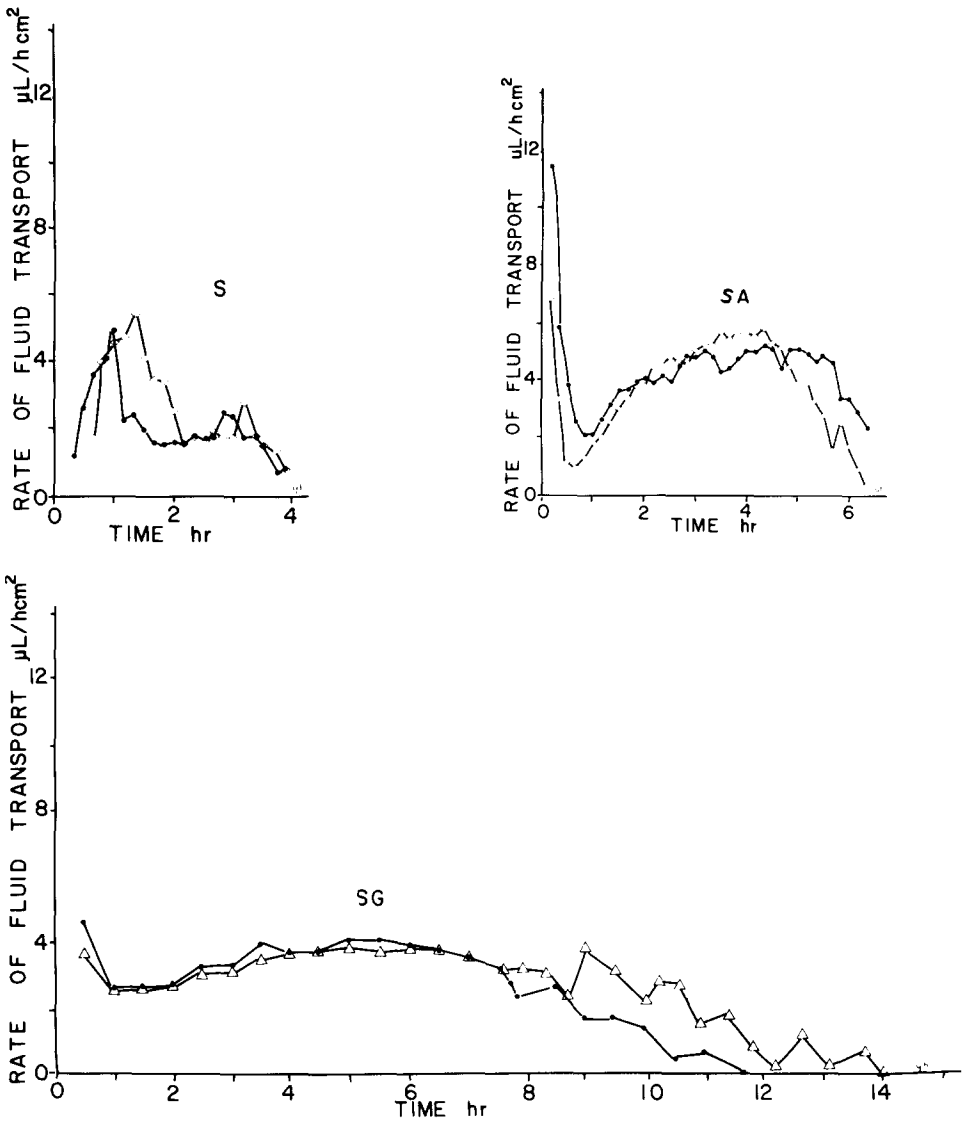


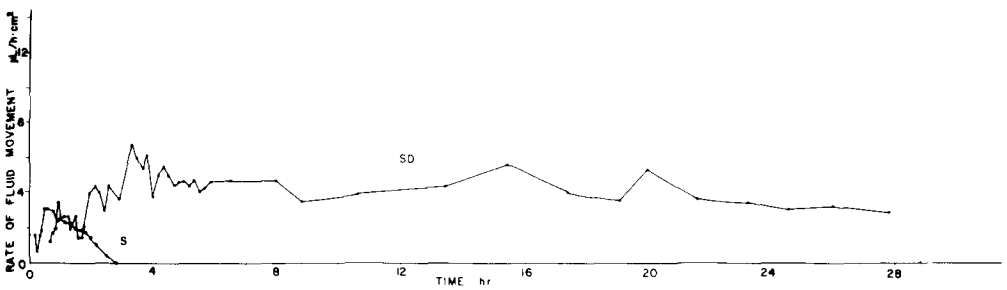
Fig. 1. The two curves represent two preparations obtained from the same animal (paired corneas). They were bathed in basal salts (S solution) on both sides. Zero time: time at which preparations were mounted.

Fig. 2. Two paired preparations bathed in adenosine (SA) solution on both sides.

Fig. 3. Two paired preparations bathed in basal salts solution plus glucose (SG solution) on both sides.

Osmotic permeability

From the maximal osmotic flows measured in the presence of Dextran, the osmotic permeability (P_{os}) of the endothelium can be calculated ($P_{os} = \sigma \cdot Lp$). As mentioned above, the osmotic pressure difference due to Dextran was 3.3 mosmol/l. It was assumed that in the preparations bathed with glucose plus



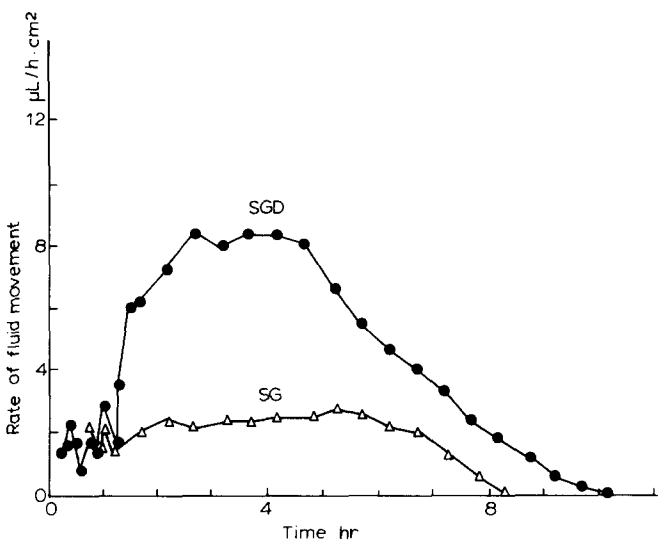


Fig. 4. Two paired preparations. Lower curve: control preparation, bathed with S solution on both sides. Top curve: test preparation, with stromal side bathed with S solution and aqueous side bathed with SD solution.

Fig. 5. Two paired preparations. △———△, control preparation bathed on both sides with SG solution. ●———●, test preparation with SG solution on the stromal side and SGD solution on the aqueous side.

Dextran (SD solution), the net fluid flow measured was due to two independent processes, namely (1) the intrinsic fluid pump, and (2) the osmotic flow due to Dextran. Therefore, in order to compute the osmotic permeability, the maximal rate of fluid movement in the test preparation (SGD solution) and the time at which it took place were both noted, and the rate of fluid transport observed at that same time in the control preparation (SG solution) was subtracted. For the case of the SD solution, at the time of maximal flow in the test preparations (4—6 h) the rate of flow in the control preparations (S solution) was nearly zero, so no correction was employed. The osmotic permeability values obtained in this fashion were: (a) 162 ± 17 µm/s in the presence of glucose (SGD solution), and (b) 451 ± 84 µm/s in its absence (SD solution). A comparison of these with values previously measured in this and other laboratories is given in Table II.

TABLE II

COMPARISON OF CORNEAL ENDOTHELIUM OSMOTIC PERMEABILITY MEASUREMENTS

Reference	Method and condition	Osmotic agent	Osmotic permeability value in μm/s ($P_{os} = \sigma LpRT/Vw$)
16	time transient	sucrose	184 ± 47
18	time transient	sucrose	215 ± 55
10	steady state	sucrose	20 ± 1
9	steady state	sucrose	38 ± 2
8	steady state (high conductance)	sucrose	115 ± 6
8	steady state (low conductance)	sucrose	26 ± 3
This paper	steady state (glucose present)	Dextran (M_r 40 000)	162 ± 17
This paper	steady state (glucose absent)	Dextran (M_r 40 000)	451 ± 84
13	time transient, theoretical extrapolation	sucrose	588 ± 84

Priming effect

It was observed quite often during these experiments that the rate of fluid transport determined in the presence of standard glucose (SG) solution could be made to increase by brief exposure of the preparation to an osmotic gradient. The rate remained at a higher level even after the gradient was subsequently abolished and in spite of repeated washings with fresh solution. This interesting phenomenon, which can be described as a priming of the fluid pump, was typically induced by challenging the preparations for short periods

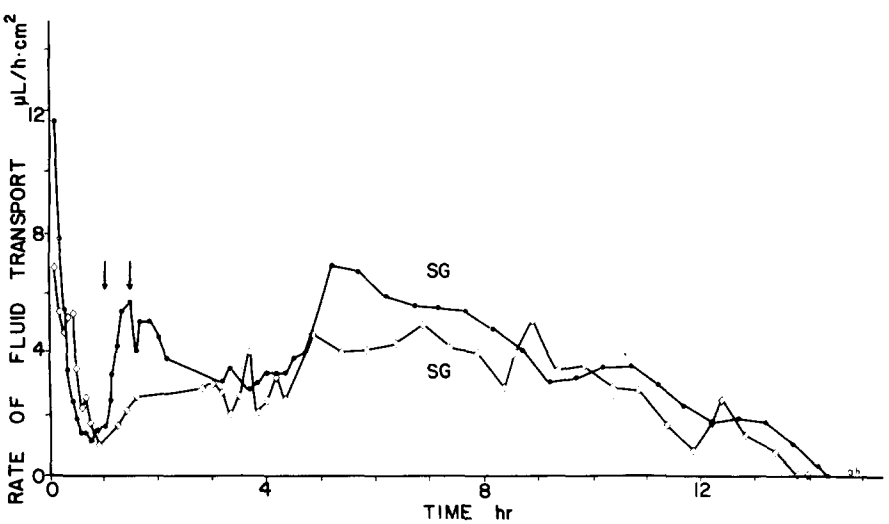


Fig. 6. Comparison of transport rates showing the enhancing or priming effect of an osmotic gradient. The test preparation (●———●) was initially bathed with SG solution, then challenged with 5% Dextran for 5 min (SGD solution) on the aqueous side (first arrow), then returned to SG solution (second arrow). The control preparation (◇———◇) was simply bathed with SG solution and allowed to run its course.

of time (10—60 min) with SGD solution; washings with fresh SG solution instead had no effect. The maximal rates of fluid transport in primed preparations were 40—50% higher than those in control preparations, as exemplified in Fig. 6. This effect was investigated in detail in eight experiments performed, as usual, with paired eyes. One of the preparations was bathed with SG solution throughout the experiment, and served as a control. After the challenge, the test preparations transported with average maximal rates of 4.5 ± 0.7 $\mu l \cdot h^{-1} \cdot cm^{-2}$. At the times when maximal rates showed in the test preparations, the average rate in the control preparations was 2.8 ± 0.4 $\mu l \cdot h^{-1} \cdot cm^{-2}$. The paired differences (test − control rates) were highly significant (Student's t-test, $0.01 > P > 0.001$). A similar effect was also observed if the gradient was due to sucrose addition.

Discussion

The present results demonstrate obvious Dextran-induced increases on both the rate of flow and the time for which flow ensues in the forward (or physiological) direction for the preparations. The results are especially striking when the addition of Dextran to basal salt solution is considered, since in fact the average flow seen in the presence of SD solution is larger than that seen with SGD solution. Similarly, interesting observations can be noted when the survival times are examined. Preparations bathed with SD solution exhibit forward flow for a period about 7-times longer than those with S solution. In contrast, preparations bathed with SGD solution survive only some 3—4-times longer than those with S solution.

The observation that treatment with SD solution increases both the osmotic flows and the longevity of the preparations as compared to what takes place with glucose-containing SGD solution would seem at first paradoxical. However, contrary to intuitive expectations, glucose addition might affect adversely the 'in vitro' preservation of the tissue. For instance, it could stimulate metabolic activity by the endothelium so that a critical component(s) would be exhausted comparatively sooner. A similar pattern emerges when adenosine is used (cf. Figs. 2 and 3). The preparations bathed in SA solution pump at a higher rate but live less than those bathed in SG solution; adenosine would play here the role mentioned above for glucose. On the other hand, interesting as these observations may be, their possible application to the area of corneal preservation is not straightforward. In the absence of information on the metabolic condition of the endothelial cells deprived of their normal glucose substrate, one cannot obviously advise usage of Dextran and omission of glucose. Still, as would be expected, the present findings agree with the notion that global lowering of the metabolic activity of the tissue results in better preservation. This principle has been employed for a long time by eye banks which keep their samples refrigerated.

As for the priming effect observed, it may be speculated that the osmotic gradient would induce a shift in tissue properties. It might cause alterations in the geometry or the pumping rate of the tissue, which would persist even after the gradient is suppressed, and would in turn lead to more efficient fluid pumping. This interesting effect, which resembles a 'positive feedback', undoubtedly calls for further investigation.

The present results shed additional light on the area of the osmotic permeability of the endothelial layer. Table II shows that the osmotic permeability value derived from the experiments with SGD solution is in line with other values previously obtained. In the case of the previous measurements the osmotic agent was sucrose while here it was Dextran. However, a surprising feature among the present results is that the value derived from the experiments with SD solution is about 3-times larger than the earlier 'high-conductance' value for the steady state (see Table II) obtained under more physiological conditions. It may be speculated that under the non-physiological conditions created in a decaying preparation deprived of glucose, the osmotic pathway (i.e., the intercellular junctions) may dilate. It seems also conceivable that, under the conditions above, somehow the whole extent of this pathway (i.e., the entire cell perimeter) might become available for water permeation, while under physiological conditions some zones of the tissue might be excluded. The actual value of the osmotic permeability might be even larger, since an increase in diameter of the osmotic pathway may lead to a decrease in the reflection coefficient for Dextran. Some indication that a large permeability could indeed be evidenced under particular conditions can be found in the time-transient results given also in Table II. An extrapolation of previous data from this laboratory (Ref. 8, Fig. 4, range from 0 to 10 mosmol/l) suggests a permeability as large as 400—800 μm/s; and a calculation [13] recently made with some of the same data yielded a value near 600 μm/s.

Lastly, it is interesting to compare the order of magnitude of the basal or control rates of fluid transport (in the presence of SG solution) with the increase seen in SGD solution. The magnitude of the Dextran-induced osmotic flows is about the same as that of the normal rate of fluid transport (2.8 $\mu l \cdot h^{-1} \cdot cm^{-2}$ with SG solution as compared to 6.2 $\mu l \cdot h^{-1} \cdot cm^{-2}$ with SGD solution; maximal flows). Since the osmotic pressure difference created by the Dextran gradient is only 3.3 mosmol/l, it follows that such a small osmotic gradient suffices to induce a movement of fluid quite comparable to that normally induced by the physiological fluid pump. Evidence from this laboratory has been reported to support the idea that osmotic flows across corneal endothelium largely traverses its junctions [3,5,8]. If we choose to assume that the route for osmotic flows and for the flow induced by the fluid pump is the same (cf. Ref. 5), it would seem that such a pump across the corneal endothelium could operate simply by creating an effective osmotic gradient of a few mosmol/l across some defined barrier within the tissue, such as the intercellular junctions. This possibility has been already suggested in recent publications concerning this preparation [4,5], and the present evidence is once more consistent with it. It has also been advanced as an explanation for fluid transport across other leaky epithelia. Thus, on the basis of theoretical arguments [17], it was concluded that isotonic flow across *Necturus* proximal tubule was best explained by a small osmotic pressure difference which would induce such flow across the junctions. Is has also been argued [11] that most of the fluid transported across *Necturus* gall bladder must be passing across the junctions, although that evidence did not allow the conclusion that such flow is necessarily osmotic. And it has been maintained [1] that a difference of only 0.65 mosmol/l between lumen and bathing solution osmolarities would account for

the observed rate of fluid absorption by rabbit kidney proximal tubule. It is therefore apparent that further experimental and theoretical studies of these mechanisms are both desirable and promising.

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