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Macromolecular drug absorption in the albino rabbit eye

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Summary

Proteins as well as polymers used in topical ophthalmic preparations are generally assumed to be excluded from the interior of the eye. Nonetheless, the corneal penetration of such high molecular weight compounds is of interest from both the drug toxicity and drug delivery points of view. The objective of this study was to determine, in a qualitative way, the mechanisms by which inulin permeated the cornea following topical dosing in the albino rabbit eye. Despite its molecular size (M.W. 5,000) and low *n*-octanol/buffer partition coefficient (1.27×10^{-3}), inulin was absorbed across the cornea. At 30 min post-dosing the amount of inulin recovered in the aqueous humor and anterior segment tissues of the eye was 0.12–0.37% of the topically applied dose. Results in this study suggest that the mechanism of corneal absorption of inulin is different from glucose and epinephrine. However, there is no evidence that a carrier or endocytosis is involved. It is suggested that further experiments are necessary to investigate the contribution of the paracellular pathway to the corneal absorption of inulin.

Introduction

It is generally accepted that the vast majority of ophthalmic drugs permeate the cornea via a passive diffusion mechanism. The other known mechanisms for drug transport: facilitated diffusion, active transport and endocytosis, are generally not considered. While admittedly these mechanisms may be unimportant for small molecules, this may not be the case for the larger ones such as polypeptides and proteins and particulate matters like liposomes and viruses. For such substances passive diffusion across the cellular membranes may in fact be secondary to facilitated diffusion, active transport or endocytosis. These mechanisms should be more carefully studied in the future for two reasons. First, peptides and proteins — notably epidermal growth factor, interferons, plasminogen activators and substance P antagonists — may become important therapeutic agents for certain ocular diseases or conditions. Second, polymers are common ingredients in ophthalmic preparations and from the toxicity standpoint it is important to evaluate their intraocular distribution.

There are resports in the literature indicating that inulin (M.W. = 5,000), a linear polymer of D-glucose and D-fructose in a molar ratio of 1 : 20 (McDonald, 1946), can permeate the cornea by an as yet unknown mechanism (Keller et al., 1980; Stratford et al., 1983). In 1968, Ryser (1968) stated

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that ^{14}C-labeled inulin was absorbed into sarcoma cells in culture via endocytosis. The objective of this study was to provide preliminary mechanistic information on the absorption of water-soluble large molecules into the eye using inulin as a model compound. This was studied both in the presence and absence of cetrimide, phlorizin and cytochalasin B, compounds known to interfere with carrier transport and endocytosis (Alvarado and Crane, 1962; Lucas and Duncan, 1983; Mizel and Wilson, 1972; Newey et al., 1959; Parsons et al., 1958; Wessells et al., 1971; Yahara et al., 1982). To rule out the non-specific effects these compounds may exert on the integrity of the corneal epithelium thereby altering solute transport, control experiments were conducted with D-glucose and DL-epinephrine.

Experimental

Materials

[^{3}H]Inulin (spec. act. 294.8 mCi/g), [^{14}C]inulin (spec. act. 41.9 mCi/g), [^{14}C]D-glucose (spec. act. 5 mCi/mmol), and [^{3}H]DL-epinephrine-HCl (spec. act. 7 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Both radiolabeled forms of inulin were found to be homogeneous in molecular weight by gel filtration chromatography on Sephadex G-25 column with 0.3% NaCl solution as the mobile phase and were used without further purification. Prior to the preparation of dosing solutions, flash evaporation was used to remove the solvents in which the radiolabeled glucose and epinephrine-HCl were dissolved.

Unlabeled inulin was purchased from Pfaltz and Bauer (Stamford, CT). D-Glucose, cetrimide, phlorizin, cytochalasin B and DL-epinephrine-HCl were purchased from Sigma Chemicals (St. Louis, MO). All compounds were used as received.

Male, albino, New Zealand rabbits weighing 2.5–3 kg were purchased from ABC Rabbitry (Pomona, CA).

Methods

Preparation of dosing solutions

Solutions of inulin were prepared in a phosphate buffer at pH 7.4, spiked with 0.25–1 mCi of [^{3}H]inulin/ml of solution to yield a final inulin concentration of 1–20 mM, and rendered isotonic with the addition of NaCl. To verify that the tritium label on an inulin molecule remained intact over the time course of an experiment, a control experiment was conducted using a 1 mM inulin solution prepared with [^{14}C]inulin as the tracer. The ocular inulin concentrations at 30 min were found not to be statistically different ($P = 0.10$) from those using inulin solutions containing [^{3}H]inulin as the tracer. Based on this result, the tritiated compound was used as the tracer in all inulin solutions.

Solutions of glucose, 1–25 mM in concentration, were prepared as described for inulin solutions. A 1 mM solution of epinephrine was prepared similarly except that the buffer contained 0.2% sodium bisulfite, which was included to ensure chemical stability of epinephrine. About 0.25 mCi of labeled material was added to each milliliter of each dosing solution. All dosing solutions were prepared fresh immediately prior to each experiment.

Effect of instilled solution concentration on the ocular absorption of inulin

Twenty-five microliters of an inulin solution, 1, 5, 10, 15, 17.5 or 20 mM in concentration, were instilled onto the cornea of both eyes of each rabbit, collecting in the cul-de-sac. At 30 min post-dosing, the rabbit was killed with an overdose of Eutha-6 (Western Medical Supply, Arcadia, CA). The whole conjunctiva, aqueous humor, corneal epithelium, corneal stroma-endothelium, and iris-ciliary body were obtained in that order and processed for radioactivity determination as previously described (Stratford et al., 1983). To demonstrate the barrier effect of the corneal epithelium in limiting the ocular absorption of inulin, this experiment was repeated with 3 inulin solutions, 1, 10 and 20 mM in concentration, using rabbits whose corneal epithelia were removed by scraping with a no. 11 blade 60 min prior to solution instillation (Stratford et al., 1983).

To contrast the ocular absorption of inulin with other water-soluble molecules, another experiment was conducted using D-glucose, which is the termi-

nal sugar residue in an inulin molecule. Five solutions of glucose, 1, 3, 10, 15 and 25 mM in concentration, were used. The experimental procedure was as described for inulin.

Effect of cetrimide, phlorizin and cytochalasin B on the ocular absorption of inulin

To test the hypothesis that the corneal transport of topically applied inulin involved a sugar carrier or endocytosis, experiments were conducted whereby 25 μl of a 1 mM inulin solution containing 1 mg/ml cetrimide, 1 mM phlorizin, or 10 μg/ml cytochalasin B, was instilled to the rabbit eye. Both cetrimide and phlorizin are sugar-carrier poisons (Alvarado and Crane, 1962; Lucas and Duncan, 1983) whereas cytochalasin B is an inhibitor of endocytosis (Mizel and Wilson, 1972; Wessells et al., 1971; Yahara et al., 1982). Where necessary, the above solutions were rendered isotonic by adding NaCl. As before, rabbits were killed at 30 min post-instillation and processed for tissue sampling.

Control experiments were conducted whereby cetrimide, phlorizin and cytochalasin B were incorporated at the concentrations specified above into separate 1 mM solutions of glucose, whose corneal transport may involve a carrier, and epinephrine-HCl, whose corneal transport is by simple diffusion.

Results

Ocular inulin concentration at 30 min post-dosing

Despite its molecular size (M.W. 5,000) and low *n*-octanol/buffer partition coefficient [1] (1.27×10^{-3}), inulin was absorbed across the cornea. At 30 min post-dosing, the amount of inulin recovered in the aqueous humor and anterior segment tissues of the albino rabbit eye was 0.12–0.37% of the topically applied dose (Table 1). Fig. 1 shows the inulin concentration in the aqueous humor and anterior segment tissues at 30 min post-instillation of inulin solutions ranging from 1 to 20 mM in concentration. The profiles do not suggest saturation of ocular uptake of inulin in any tissue or fluid. Rather, as the instilled solution

TABLE 1

PERCENT OF INSTILLED INULIN DOSE RECOVERED IN THE AQUEOUS HUMOR AND ANTERIOR SEGMENT TISSUES OF THE ALBINO RABBIT AS A FUNCTION OF INSTILLED SOLUTION CONCENTRATION

Solution concentration (mM)	Percent of dose recovered [a]
1	0.12 ± 0.017 (16)
5	0.19 ± 0.029 (10)
10	0.16 ± 0.019 (12)
15	0.17 ± 0.025 (10)
17.5	0.29 ± 0.048 (12)
20	0.37 ± 0.041 (20)

[a] Mean ± standard error of the mean; figure in parentheses denotes number of eyes.

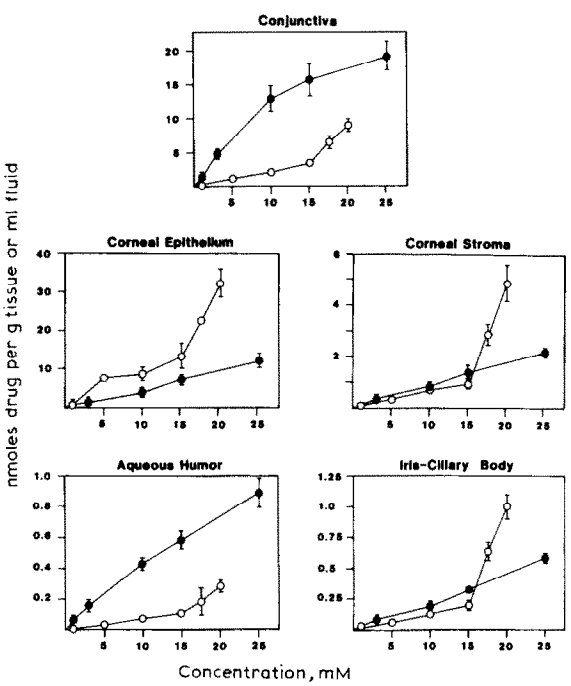

Fig. 1. Inulin concentration in the conjunctiva, corneal epithelium, corneal stroma, iris-ciliary body and aqueous humor at 30 min following the instillation of 25 μl of inulin and glucose solutions of varying concentrations. An average of 12 eyes were used for each instilled concentration. Error bars represent standard error of the mean. Where not shown, they were smaller than the size of the symbol. Key: ○, inulin; ●, glucose.

[1] This was determined by measuring the ratio of equilibrium concentration of inulin in 10 ml of *n*-octanol to 1 ml of a pH 7.4 buffer containing 1×10^7 cpm of ^{14}C-labeled inulin.

concentration exceeded 15 mM, there was a disproportionate increase in ocular inulin concentration with instilled solution concentration. This pattern was not observed for glucose, a molecule 30 times lower in molecular weight than inulin, which showed a linear increase in ocular concentration over essentially the same concentration range of 1–25 mM. Interestingly, as shown in Table 2, the presence of glucose (75 mM) in the same solution as inulin (1 mM) enhanced the ocular uptake of inulin by a factor of 5–13 but had no effect on the ocular uptake of epinephrine. This latter finding suggests that the absorption enhancement effect with respect to inulin was not due to changes in the integrity of the ocular surfaces.

Pretreating the rabbit eye with 6 consecutive doses of a 20 mM inulin solution instilled 5 min apart 30 min prior to dosing also enhanced the absorption of inulin from a 1 mM solution into the conjunctiva and the corneal epithelium. However, the extent of enhancement was less than from co-administration with glucose (Table 2). Since this treatment has a positive effect on the absorption of epinephrine into the conjunctiva and corneal epithelium also, it is conceivable that a 20 mM inulin solution caused changes at these epithelial surfaces to enhance transcellular solute transport. In a control experiment, the same regimen of multiple instillation of buffer onto the rabbit eye did not affect the ocular absorption of inulin or epinephrine (data not shown).

Fig. 1 also shows that, whereas the glucose concentration in the conjunctiva and the aqueous humor was expectedly higher than inulin concentration, the glucose concencentration in the corneal stroma and iris-ciliary body was not statistically different ($P = 0.10$) from the inulin concentration until the instilled solution concentration exceeded 15 mM. Beyond this concentration, the inulin concentration in these ocular tissues as well as the corneal epithelium was actually higher than the glucose concentration.

Table 3 shows that removing the corneal epithelium prior to instilling 1, 10 and 20 mM inulin solutions increased the uptake of inulin into the corneal stroma 30–150 times, into the aqueous humor 70–140 times, and into the iris-ciliary body 9–24 times. In the case of the 1 mM inulin solution, the extent of increase was higher than from co-administration of glucose or pretreatment with 20 mM inulin solutions (Table 2).

Effect of cetrimide, phlorizin, and cytochalasin B on the ocular absorption of inulin

Table 4 shows the effect of cetrimide, phlorizin and cytochalasin B on inulin concentration in the aqueous humor and various anterior segment tissues at 30 min post-instillation of 1 mM inulin

TABLE 2

EFFECT OF PRETREATMENT WITH MULTIPLE DOSES OF 20 mM INULIN SOLUTION AND OF CO-ADMINISTRATION WITH GLUCOSE ON OCULAR INULIN AND EPINEPHRINE CONCENTRATIONS AT 30 min FOLLOWING THE TOPICAL INSTILLATION OF 25 μl OF A 1 mM DRUG SOLUTION

Tissue/fluid	nmoles drug/g tissue or ml fluid [a]					
	Inulin			Epinephrine		
	Control	Inulin [b]	Glucose [c]	Control	Inulin [b]	Glucose [c]
Conjunctiva	0.13 (0.012; 16)	0.34 [d] (0.067; 10)	0.93 [d] (0.16; 8)	0.57 (0.04; 8)	1.47 [d] (0.26; 11)	0.57 (0.002; 10)
Corneal epithelium	0.74 (0.13; 17)	3.33 [d] (0.59; 11)	9.57 [d] (2.93; 10)	1.51 (0.33; 9)	2.78 [d] (0.37; 8)	1.92 (0.37; 8)
Corneal stroma	0.061 (0.009; 15)	0.26 [d] (0.04; 11)	0.28 [d] (0.15; 9)	0.29 (0.04; 10)	0.43 [d] (0.05; 11)	0.31 (0.04; 11)
Iris-ciliary body	0.014 (0.002; 13)	0.016 (0.003; 10)	0.154 [d] (0.03; 10)	0.047 (0.004; 12)	0.048 (0.005; 11)	0.059 (0.009; 11)
Aqueous humor	0.009 (0.001; 21)	0.007 (0.001; 9)	0.045 [d] (0.007; 10)	0.052 (0.008; 12)	0.052 (0.004; 11)	0.051 (0.006; 12)

[a] The figures within parentheses represent standard error of the mean and number of eyes, respectively.

[b] The rabbit eyes were treated with 6 doses of 20 mM inulin solution prior to the instillation of a 1 mM inulin or epinephrine solution. See text for details.

[c] The 1 mM inulin (or epinephrine) solution was prepared in a 75 mM glucose solution.

[d] Significantly different from control at $P < 0.01$.

TABLE 3

EFFECT OF ABRADING THE CORNEA ON THE OCULAR CONCENTRATION OF INULIN AT 30 min FOLLOWING TOPICAL INSTILLATION OF 25 μl OF 1, 10 AND 20 mM INULIN SOLUTIONS [a]

Tissue/fluid	nmoles inulin/g tissue or ml fluid [b,c]					
	1 mM		10 mM		20 mM	
	Intact	Abraded	Intact	Abraded	Intact	Abraded
Conjunctiva	0.13 (0.012; 16)	0.12 (0.016; 17)	2.05 (0.37; 11)	2.10 (0.23; 16)	8.82 (0.98; 19)	8.70 (1.82; 8)
Corneal stroma	0.061 (0.009; 15)	9.14 (0.985; 17)	0.71 (0.08; 12)	88.7 (14.0; 9)	4.91 (0.71; 18)	145.4 (33.0; 8)
Iris-ciliary body	0.014 (0.002; 13)	0.33 (0.04; 21)	0.12 (0.01; 10)	4.79 (0.76; 17)	1.00 (0.10; 19)	8.90 (1.31; 9)
Aqueous humor	0.009 (0.001; 21)	0.58 (0.16; 8)	0.073 (0.005; 12)	10.3 (0.76; 16)	0.29 (0.03; 22)	22.9 (3.2; 9)

[a] The corneal epithelia of the rabbits in the 'abraded cornea' group were removed by scraping with a no. 11 blade 60 min prior to solution instillation. See text for details.
[b] The figures within parentheses represent standard error of the mean and number of eyes, respectively.
[c] All three treatment groups are significantly different from the control at $P < 0.01$. The exception is the conjunctiva.

TABLE 4

EFFECT OF CETRIMIDE (1 mg/ml), PHLORIZIN (1 mM), AND CYTOCHALASIN B (10 μg/ml) ON OCULAR INULIN CONCENTRATION AT 30 min FOLLOWING INSTILLATION OF A 1 mM INULIN SOLUTION [a]

Tissue/fluid	nmoles inulin/g tissue or ml fluid [b]			
	Control	Cetrimide	Phlorizin	Cytochalasin B
Conjunctiva	0.13 (0.012; 16)	2.08 (0.18; 12)	0.87 (0.15; 9)	0.60 (0.12; 10)
Corneal epithelium	0.74 (0.13; 17)	13.4 (2.32; 12)	3.60 (0.46; 9)	1.96 (0.37; 10)
Corneal stroma	0.061 (0.009; 15)	1.37 (0.15; 12)	0.38 (0.08; 9)	0.27 (0.03; 10)
Iris-ciliary body	0.014 (0.002; 13)	0.26 (0.02; 11)	0.31 (0.11; 9)	0.11 (0.02; 10)
Aqueous humor	0.009 (0.001; 21)	0.11 (0.012; 12)	0.37 (0.067; 9)	0.015 (0.001; 12)

[a] All three treatment groups are significantly different from the control at $P < 0.01$.
[b] The figures within parentheses represent standard error of the mean and number of eyes, respectively.

solutions. Clearly, all 3 compounds enhanced inulin absorption ($P < 0.01$). Cetrimide was the most effective of the three, increasing inulin absorption about 18 times. But it was far less effective than removing the corneal epithelial barrier in terms of enhancement in ocular absorption of inulin (Table

TABLE 5

EFFECT OF CETRIMIDE (1 mg/ml), PHLORIZIN (1 mM), AND CYTOCHALASIN B (10 μg/ml) ON OCULAR GLUCOSE CONCENTRATION AT 30 min FOLLOWING INSTILLATION OF A 1 mM GLUCOSE SOLUTION

Tissue/fluid	nmoles glucose/g tissue or ml fluid [a]			
	Control	Cetrimide	Phlorizin	Cytochalasin B
Conjunctiva	1.41 (0.20; 10)	9.29 [b] (1.49; 10)	1.35 (0.21; 10)	1.69 (0.17; 9)
Corneal epithelium	0.55 (0.07; 10)	6.59 [b] (0.85; 9)	0.57 (0.074; 8)	0.61 (0.045; 10)
Corneal stroma	0.11 (0.021; 10)	0.59 [b] (0.089; 10)	0.12 (0.02; 11)	0.13 (0.009; 11)
Iris-ciliary body	0.030 (0.004; 10)	0.14 [b] (0.02; 10)	0.024 (0.003; 11)	0.036 (0.004; 11)
Aqueous humor	0.065 (0.012; 12)	0.40 [b] (0.051; 10)	0.063 (0.007; 12)	0.077 (0.007; 12)

[a] The figures within parentheses represent standard error of the mean and number of eyes, respectively.
[b] Significantly different from control at $P < 0.01$.

TABLE 6

EFFECT OF CETRIMIDE (1 mg/ml), PHLORIZIN (1 mM), AND CYTOCHALASIN B (10 μg/ml) ON OCULAR EPINEPHRINE CONCENTRATION AT 30 min FOLLOWING INSTILLATION OF A 1 mM EPINEPHRINE SOLUTION

Tissue/fluid	nmoles epinephrine/g tissue or ml fluid [a]			
	Control	Cetrimide	Phlorizin	Cytochalasin B
Conjunctiva	0.57 (0.04; 8)	1.68 [b] (0.18; 11)	0.66 (0.08; 10)	0.56 (0.11; 12)
Corneal epithelium	1.51 (0.33; 9)	4.28 [b] (0.40; 9)	2.15 (0.36; 9)	1.63 (0.33; 10)
Corneal stroma	0.29 (0.043; 10)	0.86 [b] (0.10; 10)	0.30 (0.029; 12)	0.36 (0.056; 12)
Iris-ciliary body	0.047 (0.004; 12)	0.29 [b] (0.038; 11)	0.048 (0.006; 12)	0.040 (0.005; 12)
Aqueous humor	0.052 (0.008; 12)	0.16 [b] (0.022; 10)	0.46 (0.004; 11)	0.046 (0.006; 11)

[a] The figures within parentheses represent standard error of the mean and number of eyes, respectively.
[b] Significantly different from control at $P < 0.01$.

3). Moreover, cetrimide also enhanced the ocular absorption of glucose about 7 times (Table 5) and epinephrine about 4 times (Table 6), suggesting that it affected solute transport by virtue of its non-specific effect on the integrity of the corneal epithelium. In contrast, phlorizin and cytochalasin B had no effect on the ocular absorption of glucose (Table 5) or epinephrine (Table 6).

Discussion

Biological membranes are generally assumed to be impermeable and inpenetrable barriers to the uptake and transport of macromolecules. Nonetheless, there have been occasional reports on the ability of macromolecules to traverse some biological membranes such as the mucosa of the small intestine (Taniguchi et al., 1980; Walker et al., 1972; Walker and Isselbacher, 1974). Loehry et al. (1970) demonstrated that water-soluble substances in the molecular weight range of 8000–80,000 permeated the small intestinal wall of the rabbit by an as yet unknown mechanism and at a rate inversely proportional to their molecular size. There is additional evidence that polyethylene glycol traverses the intestine via the paracellular pathway (Nellans, 1979) and that horseradish peroxidase, a water-soluble protein with a molecular weight of 40,000 and a molecular diameter of 3 nm, traverses the intestine via endocytosis (Cornell et al., 1971; Walker et al., 1972).

When compared with the intestine, much less is known about the mechanisms for transport of macromolecules into the eye. Thus far, endocytosis has been implicated as a mechanism by which horseradish peroxidase is absorbed into the conjunctiva of the guinea pig (Steuhl and Rohen, 1983), the corneal epithelium of the rat (Iwata et al., 1975), the corneal endothelium of the rabbit (Kaye et al., 1973). However, the paracellular pathway has been shown to be unavailable to the corneal transport of this protein (Iwata et al., 1975; Tonjum, 1974). Ahmed and Patton (1985) recently demonstrated that approximately 80% of topically applied inulin recovered in the iris-ciliary body at 20 min post-dosing was derived from the non-corneal route, although almost all of the inulin recovered in the aqueous humor was still derived from drug diffusion across the cornea. Our study was concerned primarily with the involvement of carriers and endocytosis relative to diffusion in the corneal transport of inulin. To a more limited extent this study was also concerned with the paracellular pathway. Thus, experiments were conducted to investigate the effect of instilled solution concentration, sugar-carrier poisons (cetrimide and phlorizin), and inhibitor of endocytosis (cytochalasin) on ocular uptake of inulin. It was assumed that precorneal fluid dynamics was unaffected by any of these treatments.

Over an instilled concentration range of 1–20 mM, which spans the range of typical affinity constants for carrier-mediated transport of monosaccharides (Caspary, 1977; Malathi et al., 1973), the inulin concentration in the corneal epithelium

and other anterior ocular tissues increased bi-linearly with instilled concentration (Fig. 1). This finding suggests that a carrier played a minimal role, if at all, in ocular inulin uptake. This lack of involvement of a carrier in the ocular absorption of inulin is supported by the finding that cetrimide and phlorizin, which are inhibitors of sugar transport carriers (Alvarado and Crane, 1962; Lucas and Duncan, 1983), enhanced rather than inhibited ocular inulin absorption (Table 4). The absorption enhancement effect of cetrimide is probably the consequence of its mild detergent effect on biological membranes, since it also enhanced the ocular absorption of two other hydrophilic substances, glucose (Table 5) and epinephrine (Table 6). On the other hand, the absorption enhancement effect of phlorizin with respect to inulin must occur at a site other than the plasma membrane of the corneal and conjunctival epithelia, since it did not affect the ocular absorption of glucose (Table 5) or epinephrine (Table 6). Judging from the disparity in the extent of enhancement in solute transport, as a comparison between Tables 3 and 4 would reveal, neither cetrimide nor phlorizin disrupted the integrity of the corneal epithelium to the same degree as mechanically breaching the corneal epithelial barrier.

The results from topical application of cytochalasin B, an agent known to disrupt the microfilaments involved in endocytosis (Mizel and Wilson, 1972; Wessells et al., 1971; Yahara et al., 1982), were similar to those observed with cetrimide and phlorizin. This compound caused an approximate 4-fold increase in the concentration of inulin in the aqueous humor and the anterior segment tissues studied, suggesting that endocytosis played a minimal role, if at all, in the ocular absorption of inulin. Since cytochalasin B did not affect the ocular absorption of glucose (Table 5) or epinephrine (Table 6), its absorption enhancement effect with respect to inulin must occur at a site other than the plasma membrane proper of the corneal and conjunctival epithelia (Johnstone et al., 1980). As is the case with the corneal endothelium (Kaye et al., 1974) and the kidney epithelial lining (Cereijido et al., 1981), it is possible that cytochalasin B disrupts the tight junctions of the paracellular shunt pathway, thereby enhancing ocular absorption of inulin. Overall, the apparent lack of involvement of a carrier and endocytosis in the corneal transport of inulin seems to suggest that paracellular transport (Schultz, 1977) plays a rather important role. The implicit assumption is that inulin, 15 Å in molecular radius, is small enough to permeate the paracellular pathway.

The reason for the abrupt increase in ocular inulin concentration beyond an instilled concentration of 15 mM, as shown in Fig. 1, is as yet unavailable. For the time being, this abrupt increase may be interpreted as a switch in the dominant mechanism of inulin transport from, say, paracellular to transcellular transport due to undefined changes in the nature of the ocular surfaces including the rheological characteristics of the mucus layer and glycocalyx overlying these surfaces (Nichols et al., 1985). This speculation is supported by the finding that pretreating the rabbit eye with a 20 mM inulin solution increased the conjunctival and corneal uptake of not only inulin but also a compound which is absorbed by transcellular diffusion, namely, epinephrine (Table 2). Based on the much larger surface area of the plasma membrane than the paracellular space, it is reasonable to expect that a switch in the mechanism from paracellular to transcellular transport would markedly increase the corneal absorption of substances which would otherwise utilize the paracellular route.

A few comments on the ocular absorption of inulin relative to glucose at 30 min post-dosing are in order. We propose that the higher glucose than inulin concentration in the conjunctiva, as shown in Fig. 1, was partly the result of retention of glucose in this tissue following incorporation into the biosynthetic pathways in the conjunctival cells, including mucin biosynthesis in the goblet cells (Schachter, 1978). In contrast, the approximately equal glucose and inulin concentration in the corneal stroma and iris-ciliary body, at instilled solution concentrations below 15 mM, is probably fortuitous, for it may be the consequence of rapid diffusion of glucose into the anterior chamber, where it gives rise to a much higher glucose than inulin concentration in the aqueous humor. The higher inulin than glucose concentration in the corneal epithelium is postulated to be the com-

bined result of rapid diffusion of glucose away from this tissue and immobilization of inulin in the mucus layer or glycocalyx at the corneal surface. Probably immobilization of inulin occurred following chain entanglement with the glycoproteins comprising the mucus layer and glycocalyx. This rapid movement of glucose through ocular tissues was found to enhance the ocular absorption of inulin but not epinephrine from a 1 mM drug solution prepared in a 75 mM glucose solution (Table 2). Presumably this absorption enhancement effect of glucose is due to an osmotic effect it exerts on ocular tissues (Jacob and Duncan, 1982).

In summary, topically applied inulin can permeate the cornea and reach the aqueous humor, albeit to a much smaller extent than glucose and epinephrine. While there is no evidence to support the involvement of a carrier or endocytosis, results in this study suggest that the mechanism of corneal absorption of inulin is different from glucose or epinephrine. It is suggested that the paracellular pathway plays an important role in the corneal absorption of inulin. Future experiments are necessary to quantitate the contribution of this pathway, relative to transcellular diffusion, to the corneal absorption of inulin and other large molecules such as peptides and proteins.

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