To the Editor:

I would like to comment on a pair of papers by Huang, Tseng, and Kenyon that present some experimental data concerning the permeability of the rabbit corneal epithelium and endothelium to mannitol.

First, the permeability values they obtain for each layer are two orders of magnitude less than those found previously for mannitol or substances of similar MW by numerous other workers. Their value for Millipore is three orders of magnitude less than that which would be expected if the solution on either side was well stirred, which does not accord with their claim that it offers little or no resistance.

Second, their technique of measuring short-term tracer transfer across the intact cornea is not favored by workers who are experienced in the field because the results are difficult to interpret. The tracer after crossing one cellular barrier has to accumulate in and diffuse across the stroma before it crosses the other. This leads to low values for the transfer in the first hours. The tracer flux is, in fact, lowered by a factor of \((1-e^{-kt})\), where \(k\), the transfer coefficient across the endothelium, is probably about 1 hour for mannitol. The permeability could be underestimated by almost \(1/2\) if only readings over 2 hours were used. This could account, in part, for the points shown in their Figure 2 which show the transfer of mannitol across the entire cornea to be at least four times less than that across the cornea without its endothelium. Strangely, this is not reflected in the permeability values that are listed in their Table 1, where the difference is reported as only 30%. These discrepancies cast doubt on the validity of several conclusions of the paper.

Third, their citation practice is questionable; I will mention four instances.

1. Many textbooks describe how the permeability of the epithelium is much lower than that of the endothelium and that the hydration of the stroma is principally controlled by the endothelium. However, the authors prefer to cite several excellent papers that hardly mention these issues, if at all, and they rely particularly on one article, which shows the diffusion resistance of the layers to water to be little different from each other.

To the Editor:

The authors greatly appreciate the comments made by Dr. David Maurice on our papers, published in April 1989 and in March 1990. For in vitro studies,

2. Although they refer to four papers from the group of Thoft and Friend, they single out for omission the one that directly anticipates their own work and, in fact, gives similar results.

3. They believe that their paper is the first to compare the permeabilities of conjunctiva and cornea, but they are wrong; there are at least two predecessors.

4. The results of Tonjum on the penetration of horseradish peroxidase are misrepresented as referring to the corneal endothelium. On the contrary, he showed that in the corneal epithelium the tracer penetrated the intercellular spaces of much as found by Huang et al for the conjunctival epithelium.

Finally, it is difficult to accept some assertions that are advanced without any evidence. For example, that it is possible to rub the endothelium off an isolated rabbit cornea held in a petri dish by two pairs of forceps without affecting the epithelium. Again, that the conjunctival epithelium is permeable to 20K yet totally impermeable to 40K Dalton dextran, a molecule only 30% greater in diameter; cellular barriers do not have such sharp cut-offs.

It is a pity that the carelessness of the presentation mars what could be original in these papers, eg, in the first, the measuring of the permeability of the conjunctiva in the isolated tissue rather than in the intact eye.

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References

on ion transport and electric potential, i.e., transcellular pathway, have been long established by Maurice, Mishima, Klyce and others. However, such transcellular permeability is not the subject of our study, and should not be confused with the paracellular permeability, a point emphasized in our first paper.1 Because transcellular and paracellular pathways operate via entirely different mechanisms (to be detailed below), other reported data on transcellular permeability cannot be directly compared to ours.

We reported paracellular permeability of cornea as $0.12 \times 10^{-8}$ cm/sec to mannitol, one to two orders of magnitude lower than those previously reported for transcellular ion permeabilities $3-9 \times 10^{-8}$ cm/hr for Na$^+$ and Cl$^-$ as reported by Maurice3 and others. These different values however cannot be compared because the latter is driven by active ionic transport and electrical potential through the cellular membrane, whereas the former is mediated via the concentration-dependent passive diffusion through intercellular junctions. Interestingly, the paracellular permeability to mannitol of conjunctival tissue reported by us was $6.78 \times 10^{-8}$ cm/sec, which is also two orders of magnitude lower than $5 \times 10^{-3}$ cm/hr for the Na$^+$ (ionic) permeability reported by Maurice.4 These data suggest that there is an intrinsic difference between these two types of permeability with the transcellular one being consistently two orders of magnitude lower than the paracellular one. The Millipore filter (HAWP) used in our studies has a 0.45-$\mu$m pore size and a specific molecular cut-off. For the same reason, this filter is certainly expected to be more permeable to small MW ions that were tested in Dr. Maurice's study, than to such nonionic solute as mannitol.

As stated in the paper by Mishima and Hedbys,5 “three independent coefficients, i.e., permeability of the solute, hydraulic conductivity and reflection coefficient, are necessary to describe the permeability properties of a membrane.” The latter two factors were not pertinent in our studies, because these authors were dealing with ionic solutes. Dr. Maurice might be right that the transfer coefficient across the endothelium may require 1 hour for mannitol. This factor was not taken into consideration in our studies, nor in Maurice's original formula5 nor in that used by Thoft and Friend.7 By eliminating such a factor, all works including ours, have relied on the first-order approximation to calculate the permeability. To consider this factor, we extrapolate our curves of Figure 2 of the first paper1 for 1 additional hour and we arrive at the same conclusion but different from that suggested by Dr. Maurice that “the transfer of mannitol across the intact cornea to be at least four times less than that across the cornea without its endothelium.”

Concerning the literature citation, we would like to reply as follows:

1. The paper by Maurice5 that Dr. Maurice4 believed we failed to cite is not pertinent to our work.2 That paper focused on the electrical potential and ion transport of conjunctiva in vivo and did not study the paracellular permeability of nonionic solutes, which was the subject of our two studies. Furthermore, our studies focused on the in vitro aspect of the paracellular permeability.

2. We do agree with Dr. Maurice that it was authors' oversight to omit the citation of Thoft and Friend's8 earlier work on the permeability of regenerated corneal epithelium in vivo. However, there are several differences between their and our works, which are noteworthy. First, their study was performed in vivo and the tracers, including mannitol, were applied on the epithelial surface, whereas our in vitro study applied the tracer to the endothelial side. Secondly, it was not clear how and to what extent their epithelial wounds were performed. Even if one assumed that their epithelial wounding was similar to part of ours, i.e., up to limbus, the permeability to mannitol of the newly regenerated epithelium is approximately two-fold higher than the normal intact epithelium. This two-fold difference is also noted in our experiment (normal versus regenerated was $0.0015 \pm 0.0021$ cm/hr in Thoft and Friend's7 and 0.12 vs 0.23 $\times 10^{-8}$ cm/sec in our.2 Thirdly, their use of glue to adhere a polycarbonate tube to the epithelium could have caused epithelial damage. This point was mentioned in their study and might account for the high permeabilities they observed. Fourthly, it is also well known that in vivo bathing of the ocular surface for 90 min, as employed by Maurice4 and Thoft and Friend7 using a tear solution, can lead to significant conjunctival chemosis and epithelial edema unless a physiological bathing fluid has been used.8,9 This in vivo approach could have created an artifact and the derived permeability for ionic or nonionic solutes in vivo may not necessarily reflect their in vivo condition. This might further contribute to additional difference between their7 and our data.2 Lastly, the paracellular permeability in vascularized and nonvascularized corneas with conjunctiva-derived epithelium was not studied by Thoft and Friend. We do feel that the above points should have been included in the discussion of our second paper.

3. The study of Tonjum10 on the penetration of horseradish peroxidase (HRP) was conducted in vitro and HRP was found in the intercellular space of superficial corneal epithelia after 2 hr perfusion. At 30 min perfusion, Tonjum did not observe HRP in the superficial interepithelial spaces, a finding consistent with ours in which in vivo perfusion was performed for up to 45 min. If our perfusion were to be extended up to 2 hr, we might expect to see more HRP in the corneal stroma and/or epithelium. We observed a to-
pographic difference in tracer distribution such that HRP was readily detected in the intercellular spaces of conjunctival epithelium, to a much less extent in the cornea. This difference could be attributed to the lack of a vascular supply in the cornea and to the differential resistance of tissue perfusion between corneal and conjunctival tissues. This further illustrated the limitation of the in vivo approach to compare these two epithelial tissues, which have an intrinsic difference in blood supply. That was also why out studies have focused on in vitro approach.

Finally, the handling of the corneal tissue to avoid unnecessary damage to the cellular layers has been a concern for permeability studies. The technique employed in our studies was physically demonstrated to Dr. Maurice during his visit to Eye Research Institute in the summer of 1984. While the cornea is suspended over a petri dish containing perfusion media without touching the epithelium, the endothelium can be gently rubbed off without difficulty. No criticism was made by Dr. Maurice at that time. Since only the central 5 mm of cornea was used for our perfusion study, incomplete removal of the peripheral endothelium, if any, would not matter. Moreover, if there were incomplete removal of central corneal endothelium, we should not have observed the augmented effects of Figure 2D, as compared with Figure 2C. The low permeability noted in the remaining epithelial barrier further indicates that there is negligible, if any, epithelial damage.

As far as the molecular cut-off is concerned, the corneal tissue is well known for its low permeability to such nonionic tracers as fluorescein and mannitol, whereas that of the conjunctival tissue has not been characterized. Solute permeation may be hindered by the presence of subepithelial tissues, such as corneal stroma, conjunctival substantial propa, and Tenon's capsule. The discrepancy of sharp distinction of cellular barriers to molecular size between 20,000 and 40,000 dextran may be due to a nonlinear relationship between molecular weights and sizes of the tracers. For example, the diameters of molecules of fluorescein (MW 300), HRP (MW 40,000), and albumin (MW 67,000) were 1.1, 3, 7.4 nm, respectively. Tracers with different molecular sizes may face different resistance while permeating through the subepithelial tissue. As indicated by Dr. Maurice in his book (p. 58), "The largest molecule that can diffuse in the normal stroma is around 12 nm in diameter. Since the spaces between the fibril are probably as great as 30 nm. It is likely that the ground substance also plays a part in restricting solute movement." The above statement was also confirmed by our studies showing that stromal edema-induced alteration of interlamellar space was accompanied by a significant increase of permeability, exceeding that of the deturgessed stroma.

In summary, we feel strongly that there is an intrinsic difference in our permeability studies of paracellular nonionic solutes versus by most others of transcellular (ionic solutes) cited by Dr. Maurice. For those works in which nonionic solutes were used, none has characterized and compared the permeability between corneal and conjunctival tissues, as we did in a defined in vitro system.

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