Biochimica et Biophysica Acta, 443 (1976) 181–189

BBA 77397

IONIC PERMEABILITY OF EPITHELIAL TISSUES

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(Received January 16th, 1976)

SUMMARY

The overall permeability of epithelial tissues to solutes is generally determined by analyzing net or unidirectional transepithelial fluxes in response to transepithelial differences of concentration and/or electrical potential using relations that describe diffusional movements across a single membrane. If the solute is uncharged and diffusional movements are transcellular, the overall transepithelial permeability coefficient is determined by the permeabilities of the two limiting cell membranes combined in series. However, if the solute is charged and the pathway for transepithelial movement involves diffusional flows across at least two membranes arranged in series (i.e. transcellular transport), the value of the overall transepithelial permeability coefficient determined using relations that describe ionic diffusion across a single membrane is not an accurate measure of the permeabilities of the two limiting membranes combined in series. Further, if ionic diffusion is transcellular, permeability coefficients determined from studies of transepithelial fluxes are not only quantitatively incorrect but can also result in grossly erroneous interpretations of changes in transepithelial permeabilities and faulty inferences regarding the route of transepithelial ionic diffusion.

INTRODUCTION

An important characteristic of epithelia is the resistance offered to transepithelial diffusion of ions or, conversely, the passive ionic permeability of the tissue. Two approaches have been employed to determine, directly, the ionic permeability coefficients of epithelia. The first involves studies of net transepithelial movements in response to transepithelial concentration and/or electrical potential differences [1, 2]. The second, more frequent approach, involves examining the effect of an applied transepithelial electrical potential difference on the uni-directional (tracer) flux of an ion when both external bathing solutions have identical compositions [3–9]. Both approaches assume that chemical and electrical driving forces are equivalent. More important, both approaches infer that physically meaningful information about the permeabilities of the two limiting cell membranes can be derived directly from studies of passive trans-epithelial movements.

The purpose of this communication is to demonstrate that these approaches are applicable only if diffusional ionic movements are, largely if not entirely, restricted to extracellular pathways and thus closely conform to transport across a single barrier. If the pathway for transepithelial ionic diffusion is transcellular, involving movements across at least two membranes arranged in series, the overall permeability coefficient derived from such studies is not an accurate measure of the combined permeability coefficients of the two limiting membranes. We will also show that a uni-directional transepithelial flux may conform with equations describing flow across a single barrier, within experimental error, in spite of the fact that two membranes are involved; under these circumstances the investigator(s) may be misled into assigning an incorrect route for ionic diffusion and incorrect physical significance to the derived permeability coefficient.

DEFINITIONS

The symbols we will employ are illustrated in Fig. 1. m, c, and s designate the mucosal, intracellular and serosal compartments respectively. J_{jk} designates the unidirectional (tracer) flux from j to k. ψ_{mc} is the electrical potential difference across the mucosal membrane with reference to the mucosal solution; ψ_{cs} is the potential difference across the serosal membrane with reference to the cell interior; ψ_{ms} is the potential difference across the tissue with reference to the mucosal solution; hence, $\psi_{ms} = \psi_{mc} + \psi_{cs}$. P_m and P_s are the permeability coefficients of the mucosal and serosal membranes, respectively, to an ion which, for the sake of simplicity, we will assume to be a monovalent cation. P is the "overall" permeability coefficient of the epithelium for that ion; c_j is the concentration of the ion in compartment j; and R, T and F have their usual meanings.

The permeability coefficient of a monovalent cation across a homogeneous barrier may be defined in several equivalent ways [4, 10]. Thus, if diffusion is driven only by a concentration difference (i.e. when the potential difference is zero)

$$P = J/\Delta c \tag{1}$$

where J is the net flux and Δc is the concentration difference across the barrier. For diffusion of a monovalent cation driven only by an electrical potential difference,

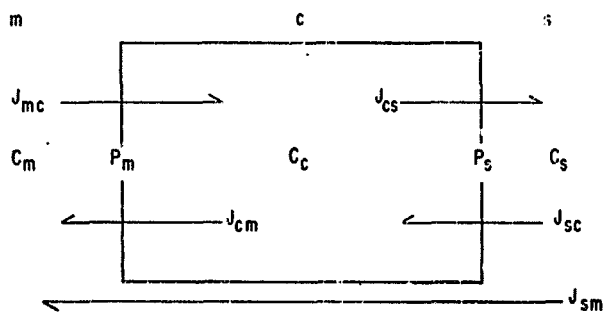

Fig. 1. An illustration of the notations that are employed in this communication.

$$P = RTJ/cF\Delta\psi \tag{2}$$

where c is the concentration in the two surrounding solutions and $\Delta\psi$ is the PD across the barrier. The signs of Δc and $\Delta\psi$ in Eqns. 1 and 2 are chosen so that a positive net flux takes place from higher to lower concentration or from a higher to lower electrical potential.

When unidirectional tracer fluxes are determined,

$$P = {}_0J_{jk}/c_j \tag{3}$$

where ${}_0J_{jk}$ is the unidirectional flux from j to k when $\psi_{jk} = 0$. As discussed in detail by Essig and Li [10], Eqn. 3 assumes the absence of isotope interactions or coupling between the flow of the species under study and the flows of other solutes or solvent.

Referring to Fig. 1, when an uncharged solute diffuses from s to m,

$$\begin{aligned} J_s &= P_s(c_s - c_c) \\ J_m &= P_m(c_c - c_m) \quad \text{and} \\ J &= P(c_s - c_m) \end{aligned} \tag{4}$$

where J_s is the net flux across the serosal membrane; J_m is the net flux across the mucosal membrane; J is the net flux across the epithelium, and all fluxes are positive in the s-to-m direction. From Eqns. 4 it is a trivial exercise to show that when a steady-state is achieved, so that

$$\begin{aligned} J &= J_m = J_s, \\ P &= P_m P_s/(P_m + P_s) \end{aligned} \tag{5}$$

Thus, under these circumstances, P is a valid measure of the permeabilities of the two limiting membranes combined in series. That is, if P is determined from measurements of the net flux of an uncharged solute across the tissue driven by a transepithelial difference in concentration, the result is a physically meaningful measure of the permeabilities of the two limiting membranes to that solute combined in series. Stated otherwise, if we could determine P_m and P_s independently, P would be given by Eqn. 5.

We now enquire whether the same is true for charged solutes whose movements are entirely diffusional, and three approaches will be explored (as mentioned above, for simplicity we will use the example of a monovalent cation).

Determination of net fluxes

One approach to the evaluation of P from the measurement of net transepithelial movements of an ion in response to differences in concentration and/or electrical potential is based on Eqn. 6 that can readily be derived from the theory of non-equilibrium or irreversible thermodynamics [11]

$$P = J/(\Delta c + \bar{c}F\psi_{jk}/RT) \tag{6}$$

where $\Delta c = c_k - c_j$, $\bar{c} = (c_j + c_k)/2$, and $\psi_{jk} = \psi_k - \psi_j$; J is positive in the direction k-to-j. It should be noted that the derivation of Eqn. 6 assumes that Δc is small so that $\bar{c}$, the "average" concentration in the membrane, can be approximated by the arithme-

tic mean of the two surrounding concentrations [11]. If Eqn. 6 is written for J_m, J_s and J (using the present notation) when $c_m = c_s$, it can be readily shown that

$$P_m = J_m/[(c_c - c_m) + (c_c + c_m)F(\psi_m - \psi_c)/2RT]$$

and

$$P_s = J_s/[(c_m - c_c) + (c_c + c_{in})F(\psi_c - \psi_s)/2RT]$$

Therefore, when $J_m = J_s = J$ (i.e. the steady-state) (Recall that J is defined as positive in the s-to-m direction; i.e. $J = J_{sm} - J_{ms}$)

$$P = (RTJ/Fc_m\psi_{ms})$$

but

$$P_m P_s/(P_m + P_s) = RTJ/[F\psi_{ms}(c_m + c_c)/2]$$

Thus, P will equal $P_m P_s/(P_m + P_s)$ only when $c_c = c_m$; this in general is not true for ions whose movements across epithelial tissues are entirely diffusional.

A second approach is to evaluate net fluxes assuming that movements across the entire tissue and each of the two limiting membranes conform to the Goldman "constant field" equation [12], viz.

$$J = \frac{PF\psi_{jk}}{RT} \cdot \left\{ \frac{c_k - c_j \exp(-F\psi_{jk}/RT)}{1 - \exp(-F\psi_{jk}/RT)} \right\} \tag{7}$$

When $c_m = c_s$, Eqn. 7 indicates that

$$P = (RTJ/Fc_m\psi_{ms}) \tag{8}$$

However when Eqn. 7 is written for J_m and J_s and these equations are solved for P_m and P_s we find that

$$P_m P_s/(P_m + P_s) = \left[\frac{RTJ/F}{\psi_{mc}X + \psi_{cs}Y} \right]$$

where

$$X = \left[\frac{c_c - c_m \exp(-F\psi_{mc}/RT)}{1 - \exp(-F\psi_{mc}/RT)} \right]$$

and

$$Y = \left[\frac{c_m - c_c \exp(-F\psi_{cs}/RT)}{1 - \exp(-F\psi_{cs}/RT)} \right]$$

It follows that, $P = P_m P_s/(P_m + P_s)$ only when $c_c = c_m$; this condition is identical to that derived above. Thus, in general, the value of P determined from an analysis of net fluxes across an epithelium in response to an imposed potential difference using the "constant field" flux equation does not accurately reflect the combined P_m and P_s.

Determination of unidirectional fluxes

In the absence of isotope interactions, or coupling to flows of other solutes or solvent, the unidirectional diffusional flux of a monovalent cation from k-to-j is given by [3, 4, 10, 13, 14]

$$J_{kj} = {}_0J_{kj} \cdot \left\{ \frac{F\psi_{jk}/RT}{\exp\,(F\psi_{jk}/RT)-1} \right\} \tag{9}$$

where, according to eqn. 3, ${}_0J_{kj}/c_k = P$. As shown by Schultz and Zalusky [3], for small values of ψ_{jk} Eqn. (9) can be approximated by

$$J_{kj} = {}_0J_{kj} \exp\,(F\psi_{jk}/2RT) \tag{10}$$

(when $\psi_{jk} = \pm 50$ mV, this approximation results in an error of less than 15%, which is reasonably acceptable given the usual experimental errors involved in unidirectional flux determinations).

Thus, for a monovalent cation when $c_m = c_s$ we may write (see Fig. 1)

$$\begin{aligned} J_{mc} &= P_m c_s \exp\,(-F\psi_{mc}/2RT) \\ J_{cm} &= P_m c_c \exp\,(F\psi_{mc}/2RT) \\ J_{cs} &= P_s c_c \exp\,(-F\psi_{cs}/2RT) \\ J_{sc} &= P_s c_s \exp\,(F\psi_{cs}/2RT) \end{aligned} \tag{11}$$

and

$$J_{sm} = Pc_s \exp\,(F\psi_{ms}/2RT) \tag{12}$$

Under steady-state conditions [15]

$$J_{sm} = J_{sc}J_{cm}/(J_{cm}+J_{cs}) \tag{13}$$

so that, from Eqn. 11

$$J_{sm}/c_s = \left[\frac{P_s \exp\,(F\psi_{cs}/2RT) \cdot P_m \exp\,(F\psi_{mc}/2RT)}{P_s \exp\,(-F\psi_{cs}/2RT)+P_m \exp\,(F\psi_{mc}/2RT)} \right]$$

or, since $\psi_{ms} = \psi_{mc}+\psi_{cs}$,

$$J_{sm}/c_s = \left[\frac{P_s P_m \exp\,(F\psi_{ms}/2RT)}{P_s \exp\,(-F\psi_{cs}/2RT)+P_m \exp\,(F\psi_{mc}/2RT)} \right] \tag{14}$$

Thus when $\psi_{ms} = 0$ (see Eqn. 3), ${}_0J_{sm}/c_s$ (or P), in general, will not equal $P_sP_m/(P_s+P_m)$ unless $\psi_{mc} = \psi_{cs} = 0$*. For those epithelial tissues studied to date, when $\psi_{ms} = 0$ (i.e., short-circuit condition) $\psi_{mc} = -\psi_{cs} \neq 0$ (cf. ref. 16). It follows that the value of P determined from unidirectional flux measurements across epithelial tissues is not an accurate measure of the "lumped" series array of P_m and P_s.

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* It should be noted that if $\psi_{mc} = \psi_{cs} = 0$ when $\psi_{ms} = 0$, then $c_m = c_c = c_s$ which is the condition for P to be equal to $P_mP_s/(P_m+P_s)$ when P is determined from studies of net transepithelial transport (Eqns. 6 and 7).

Implications

We now examine the consequences if (a) J_{sm} is transcellular and is given by Eqn. 14 but, (b) the relation between J_{sm} and ψ_{ms} is incorrectly described by Eqns. 3 and 10, i.e.

$$J_m = Pc_s \exp(F\psi_{ms}/2RT) \tag{15}$$

Equating Eqns. 14 and 15 we see that

$$P = P_m P_s/\{P_m \exp(F\psi_{mc}/2RT) + P_s \exp(-F\psi_{cs}/2RT)\} \tag{16}$$

It follows that the P determined from the relation between J_{sm} and ψ_{ms} (Eqns. 3, 10 and 15) will overestimate the lumped permeability given by $P_m P_s/(P_m+P_s)$ (Eqn. 5) whenever $\psi_{mc} < 0$ and $\psi_{cs} > 0$. In epithelia such as mammalian small intestine [17], rabbit gallbladder [4], and renal proximal and distal tubule [18], ψ_{mc} is negative with respect to the mucosal or luminal solution; in all epithelia studied to date ψ_{cs} is positive (as defined) (c.f. ref. 16).

Further, since P is a function of ψ_{mc} and ψ_{cs} it could be affected by experimental conditions that do not affect P_m or P_s. For example, a given agent may alter the permeability of the mucosal membrane to ion i, thereby changing the electrical resistance of that membrane and thus the values of ψ_{mc} and ψ_{cs} at any (imposed) ψ_{ms}. This would result in a change in P for ion j, determined using Eqn. 15, in spite of the fact that P_m and P_s for ion j were not affected. Thus, the determination of P using Eqn. 15 will not only result in a quantitatively erroneous estimate of $P_m P_s/(P_m+P_s)$ but could also lead to qualitatively erroneous conclusions if diffusion is primarily transcellular.*

In addition, we see (Eqn. 14) that if P_m and P_s are constant, a plot of J_{sm} vs. $\exp(F\psi_{ms}/2RT)$ will, in general, not be linear if the route of passive ionic diffusion is transcellular (i.e. the slope, which is determined by P, is a function of ψ_{ms}). A nonlinear plot might lead one to conclude, incorrectly, that P_m or P_s are functions of ψ_{ms}.

Thus, the next questions are, how nonlinear would the relation between J_{sm} and $\exp(F\psi_{ms}/2RT)$ be if the diffusional flows are transcellular? Could nonlinearity vs. linearity serve to distinguish between transcellular vs. paracellular diffusion? And, what errors would be introduced by incorrectly deducing a paracellular route if one cannot distinguish between transcellular and paracellular diffusion?

These questions cannot be answered generally since the answers depend upon the relation between the electrical resistance of the mucosal membrane (R_m) and that of the serosal membrane (R_s) as well as the relation between P_m and P_s. Nevertheless we can illustrate the answers to these questions by using two examples.

Assume that under short-circuit conditions, $\psi_{mc} = -45$ mV and that $R_m = R_s$; these assumptions are not unreasonable for leaky epithelia [17]. Then, if $P_m = P_s$, the true P given by $P_m P_s/(P_m+P_s)$ should be $P_m/2$. However, if P is obtained from a plot of J_{sm} vs. $\exp(F\psi_{ms}/2RT)$ we see from Table I (example a) that P_m/P is less than 2 over the range ± 50 mV so that the value of P determined from such a plot would overestimate the true permeability by more than a factor of 2. At the same time we

* The same conclusion holds when P is determined from net transepithelial fluxes using Eqns. 6 or 7. Thus, any perturbation that affects c_o will affect P in spite of the fact that P_m and/or P_s need not be affected.

TABLE I

RELATIONS BETWEEN OVERALL AND ELEMENTAL PERMEABILITIES

ψ_{ms}, ψ_{mc} and ψ_{cs} are in mV. P_m/P is given by Eqn 16. $P/_0P$ is the calculated value of P_m/P normalized to the value when $\psi_{ms} = 0$ (i.e. the short-circuit condition). As discussed in the text, in both examples we assume that $R_m = R_s$. Example (a) is for the case when $P_m = P_s$ and example (b) is for the case when $P_m = 10\,P_s$.

ψ_{ms}	ψ_{mc}	ψ_{cs}	Example (a)		Example (b)	
			P_m/P	$P/_0P$	P_m/P	$P/_0P$
50	−20	70	0.96	1.12	7.2	1.53
40	−25	65	0.93	1.08	6.6	1.40
30	−30	60	0.90	1.05	6.0	1.28
20	−35	55	0.88	1.02	5.5	1.17
10	−40	50	0.86	1.00	5.1	1.09
0	−45	45	0.86	1.00	4.7	1.00
−10	−50	40	0.86	1.00	4.4	0.94
−20	−55	35	0.88	1.02	4.1	0.87
−30	−60	30	0.90	1.05	3.9	0.83
−40	−65	25	0.93	1.08	3.6	0.77
−50	−70	20	0.96	1.12	3.4	0.72

see that the values of P normalized to that observed when $\psi_{ms} = 0$, $(P/_0P)$, differ by less than 12 % over the range ±50 mV. Thus, given a reasonable experimental error of ±10 % in the determination of J_{sm}, a plot of J_{sm} vs. exp $(F\psi_{ms}/2RT)$ would not deviate significantly from linearity. Hence, under these conditions one might conclude that the route for transepithelial diffusion is extracellular and overestimate the true lumped transcellular permeability by more than a factor of 2.

If $R_m = R_s$ and $P_m = 10\,P_s$ (example b), $P_mP_s/(P_m+P_s) = P_m/11$. The data given in Table I indicate that a plot of J_{sm} vs. exp $(F\psi_{ms}/2RT)$ should be discernably nonlinear when $|\psi_{ms}| > 20$ mV. However, if experiments are carried out within the range of ±20 mV, nonlinearity may not be apparent and the value of P_m/P of 4.1–5.5 is considerably lower than "true" value of 11. Obviously, if J_{sm} is determined at only two values of ψ_{ms} [8, 9] so that nonlinearity cannot be detected, the use of Eqn. 16 (or Eqn. 9) would result in a significant overestimate of $P_mP_s/(P_m+P_s)$.

CONCLUSIONS

In summary, the diffusion of solutes across any epithelial tissue may involve transcellular movements and/or paracellular movements; the former implies strictly diffusional movements across at least two membranes arranged in series. A fundamental question is: can studies of transepithelial movements in response to transepithelial conjugate driving forces (i.e. electrical and/or chemical potential differences) [11] provide direct information regarding the properties of either or both routes? More specifically, if diffusion is transcellular and if P_m and P_s are determined independently using Eqns. 6, 7 or 9 would the value of P determined from studies of transepithelial diffusion be equal to $P_mP_s/(P_m+P_s)$? We have shown that:

(a) for uncharged solutes, if P_m and P_s are independent of concentration, the value of P determined from studies of transepithelial diffusion is given by $P_mP_s/(P_m+P_s)$. However,

(b) permeability coefficients of ions determined from net transepithelial fluxes or unidirectional transepithelial tracer fluxes using Eqns. 6, 7 or 9, in general, are physically meaningful only if the movements are restricted to paracellular pathways and thus approximate flows across a single barrier. Thus, these approaches are, in general, likely to be most valid when applied to "leaky epithelia".

(c) If diffusional flows are transcellular, permeability coefficients derived from equations that treat the epithelium as a single barrier may significantly overestimate the "overall" permeability of the transcellular route given by $P_mP_s/(P_m+P_s)$. Further, the permeability coefficient determined using Eqn. 6, 7 or 15 may be influenced by conditions that do not affect P_m or P_s. The use of these equations to determine the permeability of the tissue to an ion may thus lead to grossly erroneous quantitative and qualitative conclusions.

(d) Under some circumstances the relation between a unidirectional tracer flux (i.e. J_{sm}) and the transepithelial potential difference (ψ_{ms}) may appear to conform with Eqns. 9 and 14, within experimental error, in spite of the fact that J_{sm} is transcellular. This apparent conformity can lead to an erroneous assignment of the route for diffusion and an incorrect assessment of the tissue permeability.

Finally, it should be apparent that:

(a) the relation between a unidirectional transepithelial flux and the potential difference must be explored over a wide range of potential differences; clearly, determinations of J_{sm} at only two potential differences cannot disclose a possible non-linear relation.

(b) A non-linear relation between J_{sm} and $\{(F\psi_{ms}/RT)/[\exp(F\psi_{ms}/RT)-1]\}$ or $\exp(F\psi_{ms}/2RT)$ need not imply interactions among tracer flow and the flow of the abundant species, other solutes or solvent; nonlinearities can result simply from the fact that passive transepithelial diffusion involves diffusional movements across two membranes arranged in series.

ACKNOWLEDGEMENTS

This work was supported by research grants from the National Institutes of Health (NIAMDD) (AM16275 and AM18199), the Western Pennsylvania Heart Association and the Richard Wechsler Research Foundation.

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