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ELECTRICAL CHANGES IN ISOLATED RAT JEJUNUM INDUCED BY HYPERTONICITY

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Summary

Mucosal hypertonicity produces a drop of transepithelial potential difference in isolated rat jejunum with a half time of about 15 s. The same effect is obtained when the osmolarity of both bathing solutions is raised simultaneously. Serosal hypertonicity produces an increase of transepithelial potential difference an order of magnitude lower than the drop produced by mucosal hypertonicity. The change in the short circuit current parallels the one in potential difference.

When the transepithelial potential difference is varied by adding different concentrations of glucose to the bathing media, the potential drop induced by mucosal hypertonicity is linearly related to the magnitude of the transepithelial potential difference just before the increase in osmolarity.

The drop of potential can be explained by a decrease of the electrical resistance of the extracellular shunt pathway due to opening of the tight junctions. The results can be accounted for in terms of an equivalent electrical circuit proposed for small intestine. Using this equivalent circuit model it is possible to obtain estimates of the values of the diffusion potential and the salt gradient across the tight junction.

Introduction

Many reports on transepithelial transport published in the last years support the view that a passive shunt pathway is an important factor in the regulation of solute and water movement across a wide variety of epithelial tissues [1—3]. Furthermore, this passive shunt pathway is located extracellularly, in the tight junctions between neighbouring cells and the lateral intercellular spaces and

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seems to account for the differences reported between "tight" tissues (high transmural electric resistance, high electric potential difference) and "leaky" tissues (low resistance and low potential difference) [1,3]. These differences between "tight" and "leaky" epithelia may be related to a different tight junction ultrastructure [4].

One method for studying the characteristics of the shunt pathway is to record the response of electric parameters and permeability when the osmolarity of the bathing media is altered. In this way, different responses of the tissue resistance to the osmolarity of the bathing medium have been observed among some epithelia and these differences seem to be concerned with the relative contributions of the junctional complexes and the intercellular spaces to the total shunt resistance [5,6].

However, these effects have been little studied in mammalian small intestine. A drop of transmural electric resistance has been reported when the mucosal bathing solution of rat small intestine was made hypertonic [7] and this drop has been ascribed to streaming potentials. Recently, the response of the electrical characteristics of bullfrog small intestine to changes in the osmolarity of the bathing medium has been reported [8]. These results were accounted for by means of an equivalent electric circuit proposed for small intestine [9,10], assuming that the electric resistance of the shunt pathway is inversely related to the osmolarity of the external medium.

This report is concerned with the electrical responses of rat small intestine to changes in the osmolarity of the mucosal and serosal bathing media, and was undertaken for the purpose of determining whether such changes are best accounted for in terms of streaming potentials, boundary diffusion potentials, or changes of shunt pathway electric characteristics.

Our results are accounted mainly for an effect of osmolarity changes on shunt electric resistance. This agrees with the current hypothesis for bullfrog small intestine [8].

Materials and Methods

Male and female Wistar rats which had been maintained on normal food and water intake, and which weighed between 200 and 230 g were used in all experiments. Rats were anesthetized with pentobarbital. After opening the abdomen, a 3 to 4 cm segment of distal jejunum was excised, cut open along the mesenteric border and rinsed clean of luminal contents. The rinsing solution had the same composition as the bathing medium and was bubbled with a mixture of 95% O_2 and 5% CO_2.

The segment of intestine was mounted as a flat sheet between two perspex (3 mm width) half chambers having an exposed area of 1.03 cm^2. The volume of each half chamber was 50 ml. The experimental set-up allowed the simultaneous incubation and recording of two adjacent segments in those experiments where a control was necessary. The elapsed time between the severance of the blood supply to the intestinal segment and the clamping of the flat sheet of the tissue on the half chambers did not exceed 1 min.

In all experiments, both sides of the tissue were initially bathed with identical solutions maintained at 38° C by means of a thermostatic bath and bubbled

continuously with a mixture of 95% O_2 and 5% CO_2. The bathing solutions were vigorously stirred by means of magnetic stirrers. Their composition in mM was: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0. The pH was adjusted to 7.4. D-Glucose and mannitol were present in every experiment at the concentrations stated.

The protocol for each experiment was as follows. Both sides of the tissue were bathed with 40 ml of solution for 30 min to check the stability of the electric parameters. At minute 30, 10 ml of the initial solution, containing the amount of mannitol necessary to give the final concentration indicated, were added to each compartment by means of a syringe.

The transepithelial potential difference (PD) was measured by means of two reversible calomel electrodes connected to the bathing solutions via saturated KCl-agar bridges. The tips of these agar bridges were placed as near as possible to the tissue. The voltage offset between these electrodes was compensated by means of an electrode compensating circuit [11]. The calomel electrodes were connected to a digital milivoltmeter (Fluke 8000A Digital Multimeter) with an input impedance of 10 MΩ. The PD was recorded continuously except for the brief period (not longer than one minute) necessary for the measurement of the short circuit current (I_{sc}) and the transepithelial electric resistance (R_M).

I_{sc} and R_M were measured at intervals following the method developed by Clarkson and Toole [12]. Two reversible calomel electrodes were used to pass an external current across the tissue. These electrodes were connected to the bathing solutions via KCl (2 M)-agar bridges. The tips of these agar bridges were placed as far as possible from the tissue so that there would be a current of uniform density across the tissue. These electrodes were equilibrated and compensated in a similar way to those used for measuring the PD. The current supplied by an external battery was regulated by means of a precision potentiometer (Helipot, 10 turns, Beckmann Inst. Ltd.) and measured on a microammeter (Fluke 8000A Digital Multimeter).

All the results are shown as means ± S.E.M. Student's *t*-test was used for statistical comparisons.

Results

Fig. 1 shows the response of PD when the osmolarity of the bathing media was changed in different ways. When the bathing solution contained 20 mM D-glucose, the PD (serosal solution positive) 30 min after mounting the tissue averaged 8.6 ± 0.3 mV (29 experiments). After mannitol was added to the mucosal solution, a drop of PD was recorded with a half-time of about 15 s (Fig. 1a). The change of PD (ΔPD) taken as the difference between the PD value at 35 min, when the PD reached a new stable value, and the initial PD at 30 min, was −7.4 ± 0.4 mV (21 experiments) when 170 mM mannitol was added, and −4.4 ± 0.4 mV (8 experiments) following addition of 85 mM mannitol.

If both the mucosal and the serosal solutions were made hypertonic simultaneously by adding mannitol, the PD changed in the same way as it did when only the mucosal solution was made hypertonic (Fig. 1b). Under these conditions, in 6 experiments ΔPD averaged −7.0 ± 0.5 mV with 170 mM mannitol

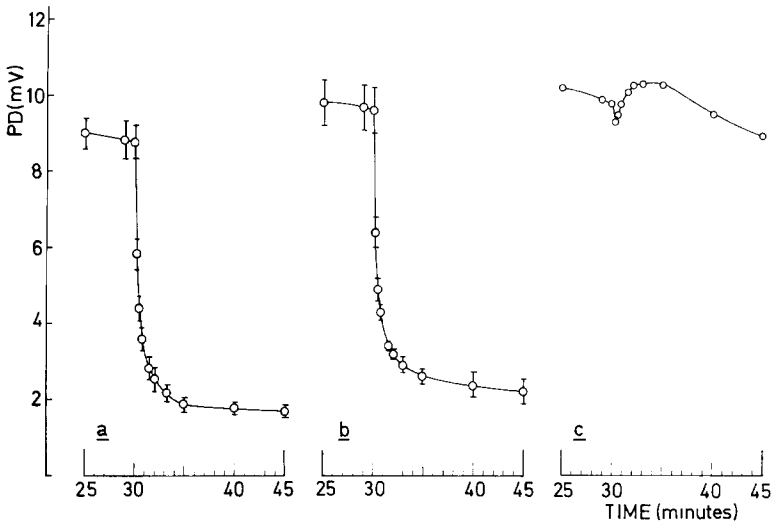

Fig. 1. PD change when the osmolarity of the bathing medium is raised with adding 170 mM mannitol, (a) to the mucosal solution only (11 experiments), (b) to both mucosal and serosal solutions simultaneously (6 experiments), (c) to the serosal solution only (5 experiments). The S.E.M. in (c) has been omitted for the sake of clarity.

and −3.9 ± 0.2 mV with 85 mM mannitol. When both solutions were made hypertonic, ΔPD did not differ significantly ($P > 0.4$) from its value when the osmolarity of the mucosal solution alone was increased.

When 170 mM mannitol was added to the serosal solution only, the PD fell slowly during the first 15 s and then rose to a stable value at 35 min (Fig. 1c). In 5 experiments ΔPD was 0.5 ± 0.1 mV. This was significantly different from zero ($P < 0.01$).

I_{sc} was measured just before adding mannitol and at 32 min. The I_{sc} change paralleled the change in PD in all experiments. Thus, when the mucosal solution was made hypertonic the relative decrease of PD, (PD at 32 min − PD at 30 min)/PD at 30 min, was −0.76 ± 0.02 and the relative decrease of I_{sc}, (I_{sc} at 32 min − I_{sc} at 30 min)/I_{sc} at 30 min, was −0.77 ± 0.02, in 21 experiments with 170 mM mannitol ($P > 0.2$). In 8 experiments with 85 mM mannitol the corresponding relative changes in PD and I_{sc} were −0.42 ± 0.03 and −0.43 ± 0.03 respectively ($P > 0.6$).

The fact that there is no significant difference between the relative changes of PD and I_{sc}, suggests that there should not be any change in the transmural electric resistance values due to osmotic effects. Fig. 2 shows the time course of R_M in the control experiments (no change of osmolarity) and in each of the three conditions of hypertonicity examined (mucosal hypertonicity only, serosal only, or both serosal and mucosal). It is seen that there are no differences between the four sets of experiments before or just after the increase of osmolarity. There is a decrease 10 min after the mucosal solution alone or both the mucosal and serosal solutions were made hypertonic. However, the differences from the control values were never significant ($P > 0.1$). This small change in transmural resistance will be further considered in relation to the equivalent electrical circuit model shown in Fig. 5.

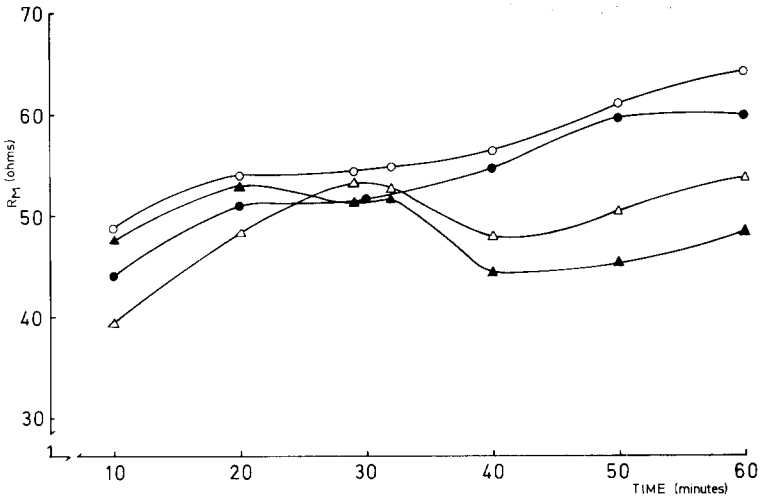

Fig. 2. R_M course during the first hour. The values at 60 min remained constant till the end of the experiments at 80 min. ●———●, control experiments with no change in the osmolarity of external solutions (10 experiments); △———△, the osmolarity of both external solutions was increased simultaneously at 30 min by adding 170 mM mannitol (6 experiments); ○———○, as above but raising the osmolarity of the serosal solution only (6 experiments); ▲———▲, as above but raising the osmolarity of mucosal solution only (12 experiments).

Relation between ΔPD and the initial PD

The changes of PD with an increase of osmolarity reported above may have several causes. A drop of PD produced by mucosal hypertonicity may be due to a build up of streaming potentials by osmotic flow of water from the serosal to the mucosal compartment [7]. It is also possible that boundary diffusion potentials account for the drop of PD produced by serosa-to-mucosa water flow. This could give rise, by sieving effect, to an increase of salt concentration in the unstirred layer of the serosal border. This gradient of salt concentration could induce an ionic diffusion toward the mucosal solution across the tissue and, because of the cationic selectivity of the tissue [13], build up a mucosal-positive diffusion potential.

Lastly, using the electric model proposed for small intestine [8—10] a decrease of PD may be produced (see Eqn. 1) by either a decrease of shunt pathway electric resistance or an increase of the diffusion potential across the tight junctions between the lateral intercellular spaces and the mucosal solution, according to the standing-gradient osmotic hypothesis [14,15].

As is shown in the Discussion, it is possible to rule out some of these possibilities if we know the dependence of ΔPD, produced by a change of osmolarity, on the initial PD value, when this initial PD is brought to different values by changes in the glucose concentration of the medium.

For this purpose, three sets of experiments were carried out. The medium used in each set had the ionic composition cited above (Materials and Methods) but the glucose concentration was changed, using mannitol to maintain the isotonicity of the initial bathing solution. Each set showed a different PD value at 30 min. When the bathing solution contained 20 mM glucose, the PD value

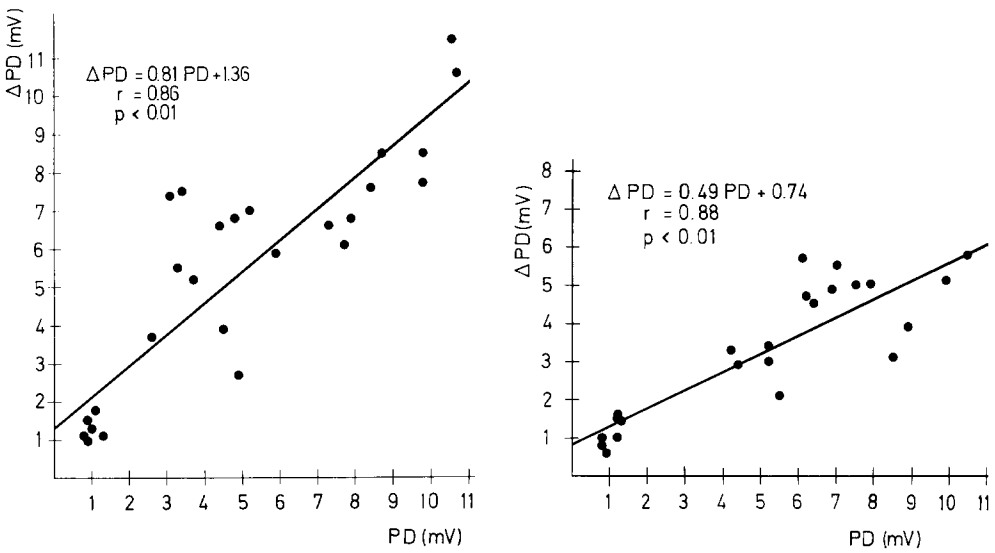

Fig. 3. Relation between initial PD and (—ΔPD) in response to mucosal hypertonicity (170 mM mannitol).

Fig. 4. Same as Fig. 3 but adding only 85 mM mannitol.

at 30 min averaged 8.6 ± 0.5 mV (10 experiments). With 5 mM glucose plus 15 mM mannitol the PD averaged 4.0 ± 0.3 mV (10 experiments) and when glucose was completely replaced by mannitol (20 mM), the PD was 1.0 ± 0.1 mV (7 experiments). The ΔPD values produced by mucosal hypertonicity were also proportionally lower. Fig. 3 shows the relation between (—ΔPD) and the initial PD when the mucosal solution was made hypertonic by adding 170 mM mannitol. The data can be fitted by a regression line having a slope of 0.81 and an intercept of 1.36 mV. A similar linear relation was obtained with a mucosal hypertonicity of 85 mM. The slope and intercept were 0.49 and 0.74 mV, respectively (Fig. 4). All regression lines reported are significant at the 0.01 confidence level.

Discussion

Streaming potentials and boundary diffusion potentials

There are few reports on the effects of hypertonicity in mammalian small intestine. Smith and Wright [7] reported that an increase of mucosal osmolarity in in vitro rat small intestine caused a decrease of the PD value. They related this PD drop to streaming potentials, which were noted earlier in rabbit gallbladder [16]. Therefore, they concluded that these supposed streaming potentials were associated with water flows induced by osmotic gradients between the external solutions but not with isotonic water flow coupled to active solute transport, and interpreted this as indicating that these two flows went through different pathways across the epithelium. However, Wedner and Diamond [17] showed that most, if not all, of the water flow induced by an electric current across the gallbladder, could be accounted for by a transport number effect. This effect

builds up a salt concentration gradient between the unstirred layers adjacent to the membrane and induced a water flow in the same direction as the electric current. According to this interpretation, little water flow is due to a true electrosmotic flow. Thus, Wedner and Diamond [17] inferred that these potentials were not streaming potentials but diffusion potentials similar in origin to electroosmotic flow. We will consider below these diffusion potentials in small intestine.

Our results do not support streaming potentials as the origin of the recorded ΔPD. First, it is difficult to see how the same streaming potentials would be produced when either the mucosal bathing solution only is made hypertonic or when both the mucosal and serosal solutions are made hypertonic with no external osmotic gradients. Second, as Figs. 3 and 4 show, ΔPD is proportional to the initial PD. Since the different initial PD values were achieved by changing the glucose concentration of the bathing medium, this result is difficult to reconcile with a change in PD due to streaming potentials. This point is considered further in the discussion of the electrical equivalent circuit given below.

Another possible explanation of the reported ΔPD is to suppose that it is due to boundary diffusion potentials [17]. An osmotically induced water flow in the serosal to mucosal direction could, by a sieving effect, produce a salt accumulation in the unstirred subserosal layer and deplete the salt concentration in the unstirred layer adjacent to the mucosal border. Since the unstirred subserosal layer is larger than the mucosal one (the subserosal layer includes the remaining tissue below the lamina propia) the main effect is a salt accumulation in the serosal unstirred layer. Such a salt gradient across the tissue would produce a mucosal positive diffusion potential, because of the cationic selectivity of the tissue. Conversely, a mucosal to serosal water flow would produce salt depletion in the unstirred serosal layer and a serosal positive diffusion potential. However, these boundary diffusion potentials would be proportional to concentration ratios, so that depletion of a given amount of salt at the serosal border (serosal hypertonicity) should cause a larger ΔPD than accumulation of the same amount of salt (mucosa hypertonic). This is not consistent with our results. Moreover, it is difficult to explain, in terms of boundary diffusion potentials, the ΔPD found when both solutions are made hypertonic and the linear relationship between ΔPD and PD.

Equivalent electrical circuit for small intestine

An equivalent electrical circuit for the small intestine has been proposed [9,10]. This model has been extended recently [8] by inserting a diffusion potential into the shunt pathway even when there are no ionic gradients between the external solutions and assigning to it a direction opposite to that of the measured transepithelial PD. This equivalent electric circuit is shown in Fig. 5.

When this equivalent circuit is analyzed the potential difference between the serosal and mucosal solution is given by:

$$PD = [(V_s - V_m)R_j - V_j(R_m + R_s)]/R_t \quad (1)$$

where $R_t = R_m + R_s + R_j$.

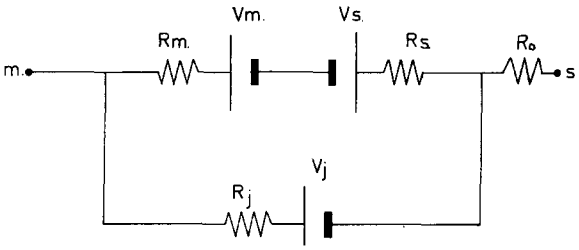

Fig. 5. Equivalent electric circuit for small intestine. V_m is the electromotive force due to ionic diffusion across the mucosal membrane and R_m is the input resistance of this membrane. V_s and R_s are the corresponding parameters for the baso-lateral membrane. V_j is a diffusion potential across the tight junction and R_j is the resistance of this shunt pathway. m and s designate the mucosal and serosal solutions, respectively. R_0 is the resistance of the subserosal layer.

Thus, it is seen that hypertonicity may produce a decrease of PD if it changes the extracellular shunt pathway characteristics, either by decreasing R_j or increasing V_j (the latter being similar to the effect of boundary diffusion potentials). However, the change in the PD due to a change of V_j only, in the absence of other changes in the parameters of the circuit, will be:

$$\Delta PD = -\Delta V_j(R_m + R_s)/R_t \quad (2)$$

that is, ΔPD would be directly related to $(R_m + R_s)/R_t$ and to the possible change induced in V_j by hypertonicity. On the other hand, it has been shown [9,10] that the increase of PD produced by Na^+-dependent actively transported sugars is mainly due to an increase of the difference $(V_s - V_m)$, possibly by a decrease of V_m only. Then, if the recorded ΔPD when the bathing medium osmolarity is changed is due only to a change of V_j, Eqn. 2 predicts that this ΔPD should be independent of the $(V_s - V_m)$ value, i.e. the glucose concentration. This is not in agreement with the results shown in Figs. 3 and 4. However, Rose and Schultz [9] pointed at the possibility of a contribution of a change in V_j in the increase of PD produced by actively transported sugars. If it is so, it is possible that the ΔV_j induced by hypertonicity depends on the glucose concentration, and so ΔPD as it can be seen from Eqn. 2. The present work does not allow an insight in this problem, although an increase of the extrussion of Na^+ into the lateral spaces produced in response to the presence of glucose, would increase V_j to which an orientation opposite to that of the PD is assigned [8,15], and therefore the change in PD by glucose would not be in agreement to the observations of Rose and Schultz [9].

Under given experimental conditions, the magnitude of the streaming potential will depend upon the characteristics of the shunt pathway. Thus, streaming potentials should depend upon the R_j/R_t ratio and one might think that they will be related to the initial PD value, which is also dependent upon R_j/R_t [1]. However, when the initial PD value is changed by changing the glucose concentration of the bathing medium, $(V_s - V_m)$ is altered without any change of the R_j/R_t ratio. Thus, under these conditions, R_j/R_t should not change and, if ΔPD is a streaming potential, it should not exhibit the dependence on initial PD found in our experiments (Figs. 3 and 4).

The simplest explanation of our results is that a change of medium osmolarity produces a change of shunt pathway resistance. If ΔPD is due to a change in

R_j only, without any other change in the parameters of the circuit, then from Eqn. 1:

$$\Delta PD = (V_s - V_m + V_j)(R_m + R_s)\Delta R_j / R_t(R_t + \Delta R_j) \quad (3)$$

that is, a decrease of R_j produces a concomitant decrease of PD which is directly related to $(V_s - V_m + V_j)$. As the $(V_s - V_m + V_j)$ value is increased by the bathing medium glucose [9,10], it seems that ΔPD produced by hypertonicity will increase with the glucose concentration, as it is seen in the results shown in Figs. 3 and 4.

Armstrong et al. [8] considered as a working hypothesis that the change of PD found in bullfrog small intestine when the medium osmolarity was changed, was due to a change of R_j. They reported a drop of PD when both external solutions were made hypertonic and an increase of PD when both solutions were made hypotonic. However, the I_{sc} parallels the change of PD, R_M remaining constant as found in our experiments (Fig. 2). This constancy of the transepithelial electric resistance R_M, may seem to be in disagreement with the hypothesis of a change in shunt resistance, but it should be noted that R_M is given by:

$$R_M = [(R_m + R_s)R_j/(R_m + R_s + R_j)] + R_0 \quad (4)$$

where R_0 is the electric resistance of the subserosal layer, which acts as a barrier to diffusion [9]. If R_j changes, the others parameters of Eqn. 4 remaining constant, the change of R_M will be:

$$\Delta R_M = \Delta R_j(R_m + R_s)^2 / R_t(R_t + \Delta R_j) \quad (5)$$

i.e., $\Delta R_M < \Delta R_j$. As will be shown below, the decrease of R_j is almost 80% of the initial value when the mucosal solution is made hypertonic by adding 170 mM mannitol and close to 50% when 85 mM mannitol is added. Rose and Schulz [9] estimated R_j in rabbit ileum to be approximately 28 $\Omega \cdot cm^2$. If the same value is accepted for rat small intestine, a decrease of R_M less than 22 $\Omega \cdot cm^2$ or 14 $\Omega \cdot cm^2$ will be expected when 170 mM or 85 mM mannitol respectively is added.

There is strong evidence supporting the concept that shunt pathway resistance is sensitive to changes of medium osmolarity, either in the so-called tight epithelia [3,18—24] or in the leaky epithelia [5,25,26]. However, the specific response of the tissue to mucosal hypertonicity seems to differ between these epithelia. In leaky tissues an increase of R_M due to a collapse of lateral intercellular spaces is found [3,5,26—28], whereas in tight tissues, a decrease of R_M by opening of the tight junctions is found [3,18,20,22]. From an electrical point of view, the shunt pathway can be considered as a series array of two resistive elements: the tight junction and the lateral intercellular space, and it is possible that each of these resistances changes in a different way when the medium osmolarity is altered or when an external current is passed across the tissue [5]. Thus, any change in R_j will depend upon the relative change in each of these resistances. This, in turn, will depend on the tissue under consideration and the experimental procedures [6].

In rabbit ileum [29], the main resistance to diffusion in the shunt pathway

seems to lie in the tight junctions, not in the intercellular spaces. It has been shown that the permselectivity properties of rat jejunum are similar to those of rabbit ileum [13]. It seems possible, then, to assert that the main effect of mucosal hypertonicity in mammalian small intestine consist of an opening of the tight junctions with a concomitant increase in shunt pathway electric conductance. In the same way could be explained the increase of passive permeability to sugars observed in rat small intestine [30] and frog jejunum [25], when the external osmolarity is raised.

Asymmetrical characteristics under osmotic gradients have been reported in a wide variety of tissues [3,5,25–28]. The asymmetrical behaviour shown by rat jejunum in our experiments suggests that mucosal hypertonicity is the necessary condition to cause the opening of the tight junctions in this epithelium. However, further investigations and morphological studies of tight junctions are necessary to confirm this point.

Estimation of the diffusion potential V_j

Eqn. 3 may be rearranged, following substitution for $(V_s - V_m)$ from Eqn. 1, to give:

$$\Delta PD = (PD + V_j)(R_m + R_s)\Delta R_j / R_j(R_t + \Delta R_j) \quad (6)$$

that is, a linear equation in PD with a slope $(R_m + R_s)\Delta R_j / R_j(R_t + \Delta R_j)$ and an intercept $V_j(R_m + R_s)\Delta R_j / R_j(R_t + \Delta R_j)$. We may compare this Eqn. 6 with the regression lines in Figs. 3 and 4. Dividing the intercept by the slope in Eqn. 6, gives V_j. If our experimental regression equations are correct, the same V_j value should be obtained from the line in Fig. 3 (170 mM mannitol) as from the line in Fig. 4 (85 mM mannitol). The regression equation for Fig. 3 gives: $V_j = 1.36/0.81 = 1.7$ mV; that for Fig. 4 gives: $V_j = 0.74/0.49 = 1.5$ mV. These results are in good agreement, however, this value must be seen as an approximate estimate of V_j because of the error in determination of the intercept. Machen and Diamond [15] obtained a V_j value of 1.4 mV in rabbit gallbladder.

If we consider $R_j << (R_m + R_s)$ [13], the slope in Eqn. 6 is $\Delta R_j / R_j$ and when this is compared with the slopes of the experimental regression lines for Figs. 3 and 4, a relative decrease of 80% in shunt pathway resistance is obtained when mucosal hypertonicity is induced by adding 170 mM mannitol. Addition of 85 mM mannitol results in a decrease of 50% in shunt resistance.

It is possible to obtain from the averaged V_j value of 1.6 mV an estimation of the salt gradient in the lateral intercellular spaces if Machen and Diamond's [15] hypothesis for rabbit gallbladder is accepted, as a first approximation, for rat jejunum. Using the Goldman constant field equation for Na^+, K^+ and Cl^-, the values of relative permeabilities across rat jejunum shunt pathway [13] of $P_K/P_{Na} = 2.4$ and $P_{Cl}/P_{Na} = 0.2$ and the ionic concentrations in the mucosal solution, one finds that the salt concentration within the lateral spaces is about 15 mM higher than in mucosal solution. The assumptions underlying this estimate are discussed in detail by Machen and Diamond [15].

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