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OSMOTICALLY INDUCED ELECTRICAL CHANGES IN ISOLATED BULLFROG SMALL INTESTINE

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SUMMARY

1. Steady state values of cell water, intracellular Na^+ and K^+ concentrations, and the electrical parameters E_{Tr}, E_m, and I_{sc} in the mucosa of isolated bullfrog small intestine were determined following immersion in sodium sulfate Ringer solutions with identical ionic composition but different osmolalities.

2. Cell water and intracellular K^+ concentration were inversely related to the osmolality of the bathing medium. During 1 h immersion, intracellular Na^+ concentration was not significantly affected by an increase or decrease in external osmolality.

3. Replacement of a hypotonic or an approximately isotonic (normal) medium by a medium of greater osmolality caused statistically significant decreases in E_{Tr}, I_{sc} and the (inside negative) magnitude of E_m. Conversely, when a hypertonic or a normal medium was replaced by one of lower osmolality, significant increases in the magnitude of these parameters were observed.

4. An equivalent circuit model for the epithelial cell layer, in which the resistance of a relatively highly conducting extracellular shunt pathway is assumed to be the major determinant of the electrical responses of the small intestine to external osmolality, has been shown to account satisfactorily for the observed changes in E_{Tr} and E_m. In terms of this model, the experimentally observed dependence of I_{sc} on external osmolality requires that, even when both the mucosal and the serosal sides of the tissue are bathed by identical media, isolated bullfrog small intestine maintains a finite diffusion potential across the shunt pathway. This is consistent with current views concerning transepithelial ionic transfer mechanisms.

INTRODUCTION

A large and rapidly increasing body of evidence strongly supports the concept that extracellular "shunt" pathways are an important factor in the regulation of trans-

Abbreviations: E_{Tr}, transmural potential difference; E_m and E_s, observed potential differences between an intracellular electrode and a reference electrode located in the mucosal and serosal bathing solution respectively; I_{sc}, short circuit current, m → s, mucosal to serosal; s → m, serosal to mucosal.

epithelial water and solute flows and the electrical characteristics of epithelial tissues [1]. In low resistance or "leaky" epithelia such as the small intestine and the gall bladder [2, 3] the shunt pathway (which has been identified as the tight junction-lateral/serosal space complex) appears to be the major route for passive ion permeation across the tissue. For example, in rabbit ileum [4] and in rat jejunum [5] the ionic conductance of the shunt pathway accounts for some 80–85 per cent of total tissue conductance.

As pointed out by Frömter and Diamond [2], the transepithelial potential difference (E_{Tr}) recorded between two identical bathing solutions is normally much smaller with "leaky" than with "tight" epitelia, and the low potentials recorded with the former could arise from a greater electrical shunting through the junctional pathways of the intrinsic potential differences (V_m and V_s) generated across the mucosal and lateral/serosal epthelial cell membranes by transmembrane ionic transfer mechanisms. Evidence for such shunting was obtained in studies on sugar and amino acid induced changes in E_{Tr} and in the measured mucosal and serosal membrane potentials (E_m and E_s) of isolated bullfrog small intestine [6] and rabbit ileum [7]. It was found that these changes could be accounted for by an equivalent electrical circuit for the epithelial cell layer in which V_m and V_s are electrically coupled through a relatively low resistance extracellular shunt pathway. According to this view, the magnitude of the observed potentials, E_{Tr}, E_m and E_s in the small intestine will, for a given set of conditions, depend on the effective resistance of the shunt pathway relative to the other resistive elements in the intestinal mucosa. Further, if V_m and V_s, together with their associated resistances, remain unchanged, the magnitudes of these measured potentials should respond in a predictable fashion to changes in the shunt resistance. For example, an increase in shunt resistance should result in an increased E_{Tr} and, conversely, a decrease in shunt resistance should be reflected in a decreased E_{Tr} [2]. On this basis it might be anticipated that hypertonicity of the extracellular medium, which causes cell shrinking and opening of the junctional spaces in certain epithelia [8, 9] could, by decreasing shunt resistance, cause a decrease in E_{Tr} whereas extracellular hypotonicity would have the opposite effect, and indeed, osmotically induced changes in the transmural electrical characteristics of isolated frog skin and renal tubular epithelium which are in qualitative agreement with these predictions have been described [10–12].

Although it has been found [13] that addition of mannitol to the mucosal bathing medium induces a sharp decrease in E_{Tr} across the isolated small intestine of the rat, no systematic investigation of the electrical responses of isolated small intestine to changes in the osmolality of the mucosal and serosal bathing media has, as far as we know, been reported. This paper describes a series of experiments in which the effects of extracellular osmolality on E_{Tr}, E_m and the short circuit current (I_{sc}) in isolated bullfrog small intestine were studied. The extent to which these changes can be interpreted in terms of a simple equivalent electrical circuit similar to those previously proposed for the small intestine [6, 7] is examined. For this purpose it is assumed as a working hypothesis that the effects of extracellular hypotonicity on E_{Tr}, E_m and I_{sc} are mainly due to an increase in the resistance of the shunt pathway and that the opposite is true for extracellular hypertonicity.

METHODS

Segments of proximal small intestine from bullfrogs (*Rana catesbeiana*) were used in these experiments. The isolation and preparation of these segments has been described elsewhere [14]. Three bathing media were employed. All three were sodium sulfate Ringer solutions. They had the same ionic composition and differed only in the amount of mannitol they contained. The medium which will be referred to as "normal" herein was identical to that described initially by Quay and Armstrong [14] and used subsequently in a number of investigations in this laboratory. It contained 102.4 mequiv. Na^+, 5 mequiv. K^+, 1.8 mequiv. Ca^{2+}, 51.2 mequiv. SO_4^{2-}, 2.1 mequiv. HPO_4^{2-}, 0.8 mequiv. $H_2PO_4^-$, 3.6 mequiv. gluconate$^-$ and 67.5 m.mol. mannitol per l. Its osmolality, measured with an Advanced Instruments, Inc. model 64-31 osmometer, was 208 ± 1 (S.E.) mosM per kg water. This is about 10 per cent less than the measured osmolality of frog plasma [15]. The media designated hypotonic and hypertonic in this paper contained respectively 17.5 and 117.5 mM mannitol. Their measured osmolalities were 151 ± 1 and 259 ± 2 mosM per kg water. The pH of all three media was 7.2. When necessary, small amounts of Tris buffer were used to adjust the pH to this value. On-line distilled water which had been further purified by two passes through a mixed ion exchanger and Analytical Reagent grade reagents were used in preparing these solutions. It was established in preliminary experiments that isolated bullfrog small intestine maintained relatively stable E_{Tr} and I_{sc} values in all three media. In addition, some experiments were performed in which the difference in osmolality between the normal medium and each of the other experimental media was increased to about 100 mosM per kg water by reducing the sodium sulfate content and adjusting the amounts of mannitol in all three solutions. Under these conditions it was found that E_{Tr} and I_{sc} in the hypotonic member of the set decreased fairly rapidly with time and that stable values of these parameters were not restored when the tissue was returned to a normal medium. All experiments were performed at room temperature (25 ± 0.5 °C).

Mucosal cell water, Na^+, and K^+ were measured following immersion of strips of intestine for various times ranging from 30 min to 3 h in the media described above. One percent (w/v) inulin, labelled with inulin-[^{14}C] carboxyl (New England Nuclear) was incorporated as an extracellular marker [15]. Following immersion the mucosa were scraped off, weighed while wet and dried to constant weight at 105 °C. The dried tissue was extracted for at least 48 h at room temperature in 0.1 M HNO_3 and its ^{14}C activity (relative to that of the bathing medium), Na^+ and K^+ content were determined as described in detail elsewhere [15, 16]. Mucosal cell water was taken as the difference between the total water content of the mucosal scrapings and their inulin "space" following immersion [15].

Two series of electrical measurements were made. In the first of these E_{Tr} and I_{sc} were measured in a conventional divided Ussing chamber fitted with a bubble lift for circulating and oxygenating the fluid in its mucosal and serosal halves. 100 percent oxygen was used for this purpose. This chamber (which had a window area of 0.33 cm^2 between the two halves), together with the methods used to monitor E_{Tr} and I_{sc} and the automatic voltage clamp device used to achieve short circuit conditions are fully described elsewhere [14, 17]. In the present experiments the tissue preparation was maintained in the open circuit condition and E_{Tr} was monitored continuously ex-

cept for periodic interruptions to measure I_{sc}. The bubble lift was fitted with drainage ports which permitted rapid emptying and refilling of both halves of the chamber. When the bathing medium was changed it was done simultaneously in both halves to avoid possible complications due to induced streaming potentials across the intestinal wall [13]. Also, during a change, the chamber was rinsed twice with the new medium and 2–3 minutes with bubbling were allowed between each rinse before the chamber was finally refilled and electrical measurements were resumed.

The divided perfusion chamber described by White and Armstrong [6] was used for the second series of electrical measurements. This chamber has a window area of 0.24 cm^2, permits rapid perfusion of both the mucosal and serosal sides of the tissue with a pre-oxygenated solution and allows the mucosal, the serosal, or both media to be changed quickly without interrupting perfusion. In these experiments E_{Tr} was monitored continuously under open circuit conditions and E_m in the epithelial cells was measured with high resistance microelectrodes. Cells were impaled from the mucosal side of the tissue. Following impalement of a cell and the establishment of a steady state E_m, the mucosal and serosal media were changed simultaneously with the microelectrode still in place and the resultant changes in E_{Tr} and E_m were followed. These parameters were recorded continuously on two channels of a Brush Mark 240 pen recorder. E_{Tr} was simultaneously displayed on one trace of a Hewlett-Packard model 132A dual beam oscilloscope and E_m was displayed on a Fairchild model 7050 multimeter. Numerical values for E_{Tr} and E_m were taken from the latter displays. Further details of the technique used to measure E_m and of the criteria used to assess the acceptability of cell impalments are given elsewhere [6]. In both series of electrical experiments a grounded electrode in the mucosal solution was the reference for potential measurements.

Student's t test was used to make statistical comparisons. Statements of significance are given at the 0.05 confidence level unless otherwise specified.

RESULTS

Water, K^+, and Na^+ content of mucosal cells

Table I summarizes the results obtained for the intracellular water, K^+ and Na^+ content of mucosal cells following immersion under various conditions. These results show that, as expected, the cells lost water in the hypertonic medium and gained water in the hypotonic medium. Following 3 h immersion the cell water content in the normal medium was significantly greater than that in the hypertonic medium and significantly less than that in the hypotonic medium. At all immersion times shown in Table I, the cell water content in the hypertonic medium was significantly less than the corresponding value in the hypotonic medium. In both these media, cell water remained relatively constant during immersion. In the hypotonic medium cell water did not change significantly between 0.5 and 3 h. Similarly, in the hypertonic medium, there was no significant change in cell water between 0.5 and 2 h. At the end of 3 h. immersion in this medium the cell water content was significantly greater than the corresponding value following 0.5 h immersion. This may reflect a slow entry of mannitol from this medium (in which its concentration was relatively high) into the cells [15].

Bearing in mind that virtually all the intracellular K^+ in mucosal cells of bull-

TABLE I

Water, K^+, and Na^+ content of mucosal cells of isolated bullfrog small intestine following immersion at 25 °C under the conditions specified. Average values ±S.E. are given for the number of estimates shown.

Immersion medium	Time of immersion (h)	*n*	Cell water g/g dry wt	Cell K^+ μequiv./g dry wt	Cell K^+ μequiv./g cell water	Cell Na^+ μequiv./g dry wt	Cell Na^+ μequiv./g cell water
Hypertonic	0.5	7	2.57±0.13	357±11	127± 9	111±13	43±6
	1	7	2.69±0.09	337±20	126± 8	111±15	41±5
	2	7	2.74±0.13	365±12	136± 9	90±9	33±3
	3	9	2.84±0.08	356±18	126± 7	125±14	44±4
Hypotonic	0.5	6	3.87±0.14	354± 8	92± 3	142±25	36±6
	1	6	4.11±0.36	356±13	92±10	152±27	37±5
	2	6	4.15±0.14	320±28	78± 8	177±31	42±6
	3	8	3.90±0.14	317±17	82± 4	165±19	42±4
Normal	3	6	3.21±0.11	363±18	114± 5	118±11	37±2

frog small intestine appears to be in an osmotically active state [18], the intracellular K^+ concentrations shown in Table I are consistent with the data for cell water already discussed. Following 3 h immersion, the intracellular K^+ concentration was significantly greater in the hypertonic than in the normal medium. The opposite was true for cells immersed for 3 h in the hypotonic medium. A comparison of the cell water content and intracellular K^+ concentration of cells immersed for 0.5 h in hypertonic and hypotonic media respectively indicates that, under these conditions, the observed differences in intracellular K^+ concentration are satisfactorily accounted for by cellular shrinking or swelling and that there was no significant net movement of K^+ between the cells and the bathing medium. This is indicated by the fact that the total K^+ content of the cells (357±30 and 354±20 μequiv./g tissue dry weight) did not differ significantly under these two sets of conditions. Although there was a marginally significant difference ($0.05 > P > 0.025$) between the total K^+ content of cells following 1 h immersion in hypertonic and hypotonic Ringer solutions respectively (337±20 and 356±13 μequiv./kg tissue dry weight) there is reason to believe that this may be artifactual. The total K^+ content of cells immersed in hypertonic Ringer solution did not change significantly between 0.5 and 3 h and, with the exception of the value obtained after 1 h immersion, did not differ significantly from the K^+ content of cells immersed for 3 h in normal Ringer solution (363±18 μequiv./g tissue dry weight). Similarly, the total K content of cells immersed for 0.5 and 1 h, respectively, in hypotonic Ringer solution did not differ significantly from each other, from the value obtained following 3 h immersion in normal Ringer solution, or from the K content of of cells immersed for 0.5, 2 or 3 h in hypertonic Ringer solution. Thus, it seems reasonable to infer that, for immersion periods up to 1 h, intracellular K^+ concentration, under the conditions of our experiments, was regulated by net water movement between the cells and the bathing medium and that alterations in the osmolality of the medium had no marked effect on the K^+ permeability of the cell membranes.

On the contrary, the decrease shown in Table I for the K^+ concentration of cells which were immersed for 2 or 3 h in hypotonic Ringer solution appears to reflect

a real decrease in the total K^+ content of these cells. Following 3 h immersion under these conditions the total cell K^+ content (317 ± 17 μequiv./g tissue dry weight) was significantly less than that of cells immersed for the same length of time in normal or in hypertonic media.

During immersion in hypertonic Ringer solution, the intracellular Na^+ content and concentration did not change significantly between 0.5 and 3 h (Table I). Following 0.5 or 1 h immersion in a hypotonic medium, the intracellular Na^+ concentration did not differ significantly from the steady state value observed in normal media. This, together with the somewhat increased average intracellular Na^+ content under these conditions (Table I), suggests that there may have been some net entry of Na^+ into the cells during this time. However, the mean intracellular Na^+ content following 0.5 and 1 h immersion in a hypotonic medium (Table I) did not differ significantly from that observed in the normal medium ($P > 0.2$ in both cases). Following 3 h immersion in a hypotonic medium, the intracellular Na^+ concentration was significantly higher than the corresponding values after 0.5 and 1 h immersion. It was also significantly greater than the value found after 3 h immersion in a normal medium. In addition, the total intracellular Na^+ content following 3 h immersion in a hypotonic medium was considerably larger than the corresponding value following immersion in a normal medium, though, for the data shown in Table I, the difference between the two fell just short of statistical significance ($0.1 > P > 0.05$). These findings, together with the decrease in cell K^+ following 2 or 3 h immersion in a hypotonic medium, indicate a progressive deterioration of the mucosal cells during prolonged exposure to a hypotonic environment.

Effect of medium osmolality on E_{Tr} and I_{sc}

Fig. 1 shows an experiment illustrating the response of E_{Tr} and I_{sc} to the osmolality of the bathing medium. In this experiment the tissue was first allowed to attain an electrical steady state in a normal Ringer solution. When the mucosal and serosal bathing media were replaced by a hypertonic Ringer solution both E_{Tr} and I_{sc} decreased markedly. When normal conditions were restored (arrow 2 of Fig. 1) both these parameters returned to values close to those previously recorded under these conditions indicating that the effects of the hypertonic medium were completely reversible. Fig. 1 also shows that replacement of the normal Ringer solution by a hypotonic medium elicited marked increases in E_{Tr} and I_{sc} although, in this experiment, E_{Tr} and I_{sc} under these conditions were less stable than they appeared to be in a normal or a hypertonic medium. Further, replacement of the hypotonic medium by a hypertonic solution induced a drastic decrease in E_{Tr} and I_{sc}. The polarity of both these parameters was temporarily reversed but this reversal was followed by a slow increase towards a low positive level. Finally, to check whether the tissue at this stage was capable of a normal electrical response to a known stimulus, glucose (final concentration 11 mM) was added to both the mucosal and the serosal halves of the chamber (arrow 5 of Fig. 1). The expected stable increases in E_{Tr} and I_{sc} [15] were recorded. However, the experiment shown in Fig. 1, and a number of similar experiments, indicated that, while the initial changes in E_{Tr} and I_{sc} following replacement of the normal medium with a hypertonic or hypotonic solution were completely reversible, the epithelial cells, though still functional, suffered progressive deterioration during exposure to a prolonged series of osmotic shocks. Therefore, for quantitative purpose, only the steady

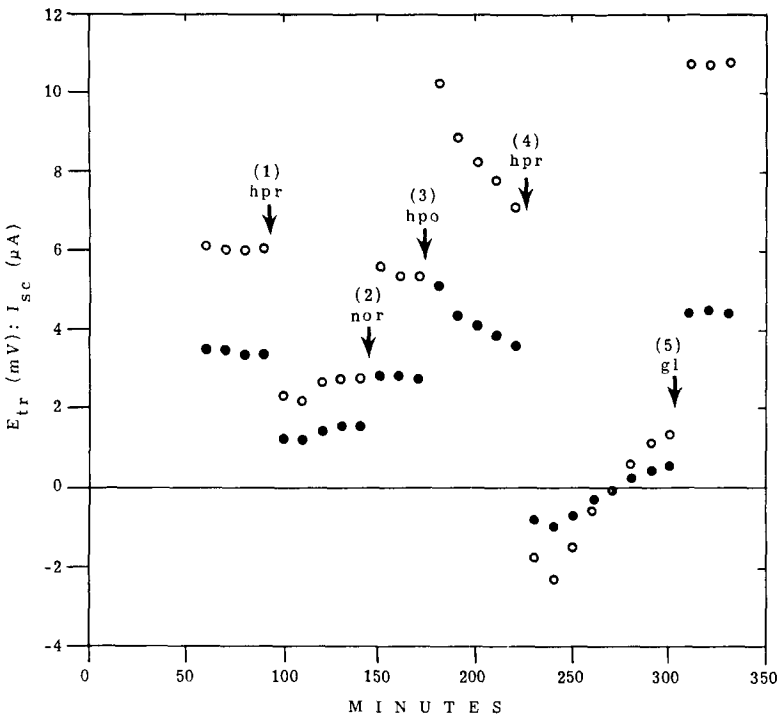

Fig. 1. Response of E_{Tr} (●) and I_{sc} (○) across isolated bullfrog small intestine to changes in the osmolality of the bathing medium. The tissue was first allowed to attain a steady state E_{Tr} and I_{sc} in a normal (nor) sodium sulfate Ringer solution (see text). Both the mucosal and serosal solutions were then replaced with a hypertonic (hpr) medium (arrow 1). At the times indicated by arrows 2, 3 and 4 the mucosal and serosal bathing solutions were replaced by normal, hypotonic (hpo) and hypertonic Ringer solutions respectively. Finally, glucose (gl) was added to both bathing media (arrow 5).

state values of E_{Tr} and I_{sc} obtained following the first change from a normal medium to a hypertonic or hypotonic medium were considered. In three experiments in which the tissue was first equilibrated with a normal Ringer solution and then exposed to a hypertonic medium, significant decreases in both E_{Tr} and I_{sc} were observed. E_{Tr} decreased from an average value of 2.6 ± 0.4 mV to 1.3 ± 0.2 mV. I_{sc} decreased from 12.0 ± 3.4 $\mu A/cm^2$ to 6.1 ± 1.4 $\mu A/cm^2$. Similarly, in five experiments in which a normal medium was replaced by a hypotonic solution, significant increases in E_{Tr} and I_{sc} were recorded. E_{Tr} increased from 2.8 ± 0.3 to 4.0 ± 0.3 mV and I_{sc} increased from 13.1 ± 1.4 to 18.3 ± 2.5 $\mu A/cm^2$.

It should be noted that in the experiment shown in Fig. 1 and similar experiments, it was not possible to record the time dependence of the early stages of the response of E_{Tr} and I_{sc} to changes in the osmolality of the medium. This is because of the time required for rinsing while the medium was being changed.

Fig. 2 shows simultaneous recordings of the response of E_{Tr} and E_m, during impalement of a single cell, to changes in the osmolality of the bathing medium. Following impalement of a cell in normal Ringer solution and the establishment of a steady E_m, the preparation was perfused with a hypotonic solution (Fig. 2, arrow 1). During

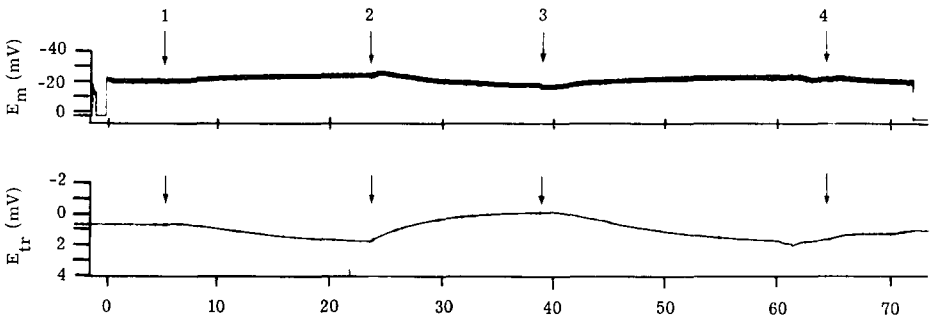

Fig. 2. Response of E_{Tr} (lower tracing) and E_m (upper tracing) to changes in medium osmolality. Arrow 1, normal Ringer solution replaced by hypotonic solution. Arrow 2, hypotonic solution replaced by hypertonic solution. Arrow 3, hypotonic solution re-introduced. Arrow 4, preparation perfused with normal medium. Numerical values of E_{Tr} and E_m were recorded as described under Methods.

this time E_{Tr} increased from 1.2 to 2.3 mV and E_m increased from −24 to −27 mV. When the hypotonic perfusate was replaced by a hypertonic solution (Fig. 2, arrow 2) E_{Tr} decreased to 0.2 mV and E_m fell to −21 mV. During a second perfusion with a hypotonic medium (Fig. 2, arrow 3), E_{Tr} and E_m again increased, E_{Tr} to 2.0 mV and E_m to −28 mV. Finally (Fig. 2, arrow 4) when the preparation was again perfused with a normal medium, E_{Tr} decreased to 1.3 mV and E_m fell to −25 mV.

It is apparent from Fig. 2 that, even if one allows for a delay in the attainment of uniform composition in the chamber fluid following a change of perfusate, osmotically induced responses in E_{Tr} and E_m are relatively slow. Following a change in the osmolality of the perfusate, at least 10–15 min are required for E_{Tr} and E_m to reach a new steady state. Thus, experiments of the kind shown in Fig. 2 require that, following impalement, the microelectrode be kept in the cell interior for a considerable time. This severely limts the number of successful recordings which can be achieved with a single preparation. Table II summarizes the results obtained in experiments of this kind. In these experiments the tissue was first allowed to develop a steady E_{Tr} in normal Ringer solution. A mucosal cell was then impaled. When E_m had stabilized the preparation was perfused with a hypertonic or a hypotonic medium. E_{Tr} and E_m were allowed to reach a steady state and their values were recorded. At this point the per-

TABLE II

Response of E_{Tr} and E_m in isolated bullfrog small intestine to changes in the osmolality of the bathing medium (mean values ±S.E.). A positive value for ΔE_{Tr} means that E_{Tr} became more positive with respect to the grounded mucosal solution and vice versa. A positive ΔE_m means that the cell interior became less negative with respect to the mucosal solution and vice versa. Numbers in parentheses are number of animals and number of successful impalements, respectively.

Change in perfusate	ΔE_{Tr}	ΔE_m
Isotonic → hypertonic (9 : 15)	−1.4±0.2	2.7±1.2
Isotonic → hypotonic (19 : 30)	1.4±0.2	−2.8±0.5
Hypertonic → hypotonic (12 : 15)	1.7±0.3	−4.2±1.2
Hypotonic → hypertonic (15 : 21)	1.8±0.2	4.7±0.9

fusion medium was again changed. If it was hypertonic, it was replaced by a hypotonic solution and vice versa. E_{Tr} and E_m were again allowed to reach a steady state and their values were again recorded. In the context of these experiments, a "steady state" E_m means that this parameter remained constant within 1 mV for at least 2 min before the perfusate was changed. Following withdrawal of the microelectrode the tissue was once more perfused with normal Ringer solution and E_{Tr} was permitted to return to its baseline value. At this point a second impalement was made and a second sequence of measurements was begun. Table II includes the results obtained on first changing from a normal medium to a hypertonic or hypotonic perfusate and following a second change from a hypertonic to a hypotonic medium or vice versa. Results from all recordings in which the microelectrode remained in the cell throughout the sequence of changes described above, and in which the electrode tip potential did not change by more than $\pm$ 1 mV between impalement and withdrawal, are included in Table II. It is clear from this table that, when the osmolality of the bathing medium was increased, both E_{Tr} and E_m decreased in absolute magnitude. On the other hand, when the osmolality of the bathing medium was decreased, E_{Tr} and E_m increased in magnitude. These changes in E_{Tr} and E_m were statistically significant ($P < 0.5$). Further, the changes in potential recorded for a given increase in osmolality were of similar magnitude to those observed for a corresponding decrease in osmolality and, in every instance, ΔE_m was greater than the corresponding ΔE_{Tr}.

DISCUSSION

There is now abundant evidence that the total transmural resistance (R_t) of epithelial tissues is sensitive to changes in the osmolality of the bathing medium [1, 3, 10, 25–27]. In addition, direct measurements of the passive resistive properties of isolated frog skin [10] and toad urinary bladder [27] have shown that, in these tissues, the decrease in E_{Tr} induced by mucosal hypertonicity is due mainly to a decrease in shunt resistance, the apical and lateral/serosal cell membrane resistances being relatively little affected. Conversely, with the isolated toad bladder, a reduction in the osmolality of the mucosal bathing medium has been found to cause a marked increase in shunt resistance [27]. Although comparable studies on the passive electrical properties of low resistance epithelia, such as the small intestine, have not yet been reported and there appears to be some variation, between different types of epithelia, in the tissue response to m $\rightarrow$ s and s $\rightarrow$ m osmotic gradients respectively [25–30], the available evidence is consistent with the supposition that, when the isolated small intestine is exposed to symmetrical bathing media, an inverse relationship exists between the shunt resistance and the osmolality of the external medium [7]. The data shown in Table I for the water content of epithelial cells of isolated bullfrog small intestine indicate that the volume of these cells increased significantly when the tissue was immersed in a hypotonic medium and decreased significantly during exposure of the tissue to a hypertonic bathing solution. Such swelling and shrinking of the epithelial cell layer could, by decreasing or increasing the volume of the extracellular spaces [8, 9] cause concomitant increases or decreases in transepithelial shunt resistance. Thus, it may be proposed as a working hypothesis that an osmotically induced increase or decrease in the extracellular shunt resistance is a major determinant of the electrical responses of isolated bullfrog small intestine to hypotonic and hypertonic media respectively.

Equivalent electrical circuit for the small intestine

The equivalent electrical circuit shown in Fig. 3 permits the analysis, in terms of a decrease or an increase in shunt resistance, of the electrical responses of isolated bullfrog small intestine to hypertonic and hypotonic bathing solutions. In this figure, V_m and V_s represent the intrinsic electromotive forces across the mucosal and lateral/serosal membranes of an epithelial cell. R_m and R_s are the corresponding membrane resistances*. V_j is a diffusion potential across the tight junction. V_j may be considered to arise, as a result of active solute transfer, between the fluid in the closed ends of the lateral/serosal spaces and the mucosal bathing solution [19]. R_j is the resistance of this extracellular shunt pathway. Points 1, 2 and 3 in Fig. 3 represent potential measuring electrodes in the mucosal medium, the cell interior, and the serosal medium, respectively. In this discussion it is assumed that point 1 in Fig. 3 is maintained at zero (ground) potential at all times.

In Fig. 3, V_m and V_s are given the orientations which are normally observed for the measured membrane potentials (E_m and E_s) in the small intestine [6, 7, 20–23]. On the basis of the studies of Machen and Diamond [24] with isolated rabbit gall bladder, V_j is assigned an orientation opposite to that of the spontaneous transmural potential difference (E_{Tr}). With the exception that V_j is considered to have a finite value when the mucosal and serosal sides of the tissue are bathed by symmetrical solutions, the circuit illustrated in Fig. 3 is identical to those previously proposed for the small intestine by White and Armstrong [6] and by Rose and Schultz [7].

Effect of external osmolality on E_{Tr} and E_m

Analysis of the circuit shown in Fig. 3 leads to the following expression for E_{Tr}:

$$E_{Tr} = [R_j(V_s - V_m) - V_j(R_m + R_s)]/R_t \qquad (1)$$

where $R_t = (R_m + R_s + R_j)$. It is at once apparent from eqn 1 that both the magnitude and orientation (with respect to a grounded mucosal solution) of E_{Tr} under any given set of circumstances will depend upon the difference between the terms $R_j(V_s - V_m)$ and $V_j(R_m + R_s)$. This difference will depend on the magnitude of R_j if all other param-

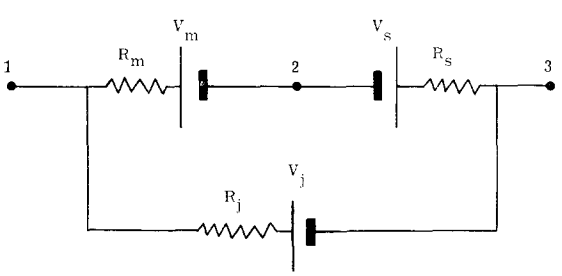

Fig. 3. Equivalent electrical circuit for epithelial cell of small intestine (see text for detailed description).

* In addition to the internal resistances of the batteries represented by V_m and V_s, R_m and R_s may include parallel shunt resistances across the mucosal and baso-lateral cell membranes, respectively [7].

eters on the right hand side of Eqn 1 remain constant. If $R_j(V_s-V_m) > V_j(R_m+R_s)$, E_{Tr} will be serosal positive and vice versa. If R_j is very large, i.e. $R_j \simeq R_t$, E_{Tr}, will approach the limiting (serosal positive) value (V_s-V_m). As R_j decreases, E_{Tr} will also decrease because of the increased magnitude of the negative term $-[V_j(R_m+R_s)/R_t]$ and the concomitant decrease in the ratio R_j/R_t. Thus, if one assumes that, under the conditions of the present study, R_j is inversely related to external osmolality, the results shown in Fig. 1 and in Table II for the effect of hypertonic and hypotonic media on E_{Tr} are consistent with the predictions of Eqn 1. Eqn 1 is also consistent with the conclusion [2] that the relatively low spontaneous E_{Tr} values, compared to those found with "tight" epithelia, which are normally recorded when "leaky" epithelia are exposed to symmetrical bathing solutions are due to a greater degree of shunting, in the leakier epithelia, of the membrane potential differences, V_m and V_s, across the junctional pathway.

As already pointed out, if $V_j\ (R_m+R_s) > R_j\ (V_s-V_m)$, Eqn 1 predicts that E_{Tr} will be mucosal positive even though (V_s-V_m) may be positive with respect to a mucosal reference point and active m→s transfer of a positively charged ion such as Na^+ may be occurring. In the limit, i.e. when $(R_m+R_s) \simeq R_t$, E_{Tr} will approach the limiting value $-V_j$ and, for very low values of R_j, E_{Tr} will be largely determined by V_j and will be relatively insensitive to the value of (V_s-V_m) [4]. This appears to be the situation for isolated rabbit gallbladder both in the presence and in the absence of an osmotic gradient [24, 31] and could also account, in part at least, for the finding [14] that, when isolated bullfrog small intestine is immersed in an isotonic sodium chloride medium (in which E_{Tr} is normally serosal positive and the mean I_{sc} is consistent with a net m→s transfer of positive charge), a mucosal positive spontaneous E_{Tr} is occasionally observed.

Fig. 3 leads to the following expression for E_m:

$$E_m = -[V_m\ (R_s+R_j)+R_m\ (V_s+V_j)]/R_t \tag{2}$$

The negative sign on the right hand side of Eqn 2 indicates that the cell interior is negative with respect to a grounded mucosal reference electrode. If $R_j \ll R_t$ then, for a small change, ΔR_j, in the former parameter, R_t can be assumed to remain relatively constant*. Under these conditions one obtains

$$\Delta E_m = -V_m\ (\Delta\ R_j)/R_t \tag{3}$$

Eqn 3 predicts that, for the conditions specified, an increase in R_j will result in hyperpolarization of the mucosal membrane, i.e. the cell interior will become more negative with respect to the mucosal solution. Conversely, a decrease in R_j will result in depolarization of the mucosal membrane.

The results obtained in the present investigation (Fig. 2 and Table II) are consistent with this prediction. It is apparent from Table II that replacement of either a normal or a hypertonic medium by a hypotonic medium of identical ionic composition resulted in a statistically significant ($P < 0.05$) increase in the negativity of the cell interior with respect to the mucosal solution (i.e. E_m increased). Similarly, when

* At present the numerical value of the ratio R_j/R_t is not known for isolated bullfrog small intestine under the conditions of our experiments. For isolated rabbit [4] and rat [5] small intestine it has been estimated to lie in the range 0.1–0.2 so that this assumption does not seem unreasonable.

either a normal or a hypotonic medium was replaced by an ionically equivalent hypertonic medium, E_m decreased significantly. Thus, it would appear that, under the conditions of the present experiments, the electrical responses of isolated bullfrog small intestine to changes in external osmolality are in fact dominated by osmotically induced increases or decreases in the transepithelial shunt resistance. Table I shows that the changes in epithelial cell water content which resulted from the imposition of an external osmotic gradient were accompanied by parallel changes in intracellular K^+ concentration, and by inference [18] in intracellular K^+ activity. As has already been pointed out, the increase or decrease in intracellular K^+ concentration which occurred during exposure of the cells to a hypertonic or a hypotonic bathing medium were, for immersion periods up to 1 h, satisfactorily accounted for by the concomitant changes in cell water, i.e. there was no net K^+ uptake by or K^+ loss from the cells, indicating that, over the range of osmolalities employed, there was no dramatic change in the K^+ permeability of the cells. Nevertheless, since the external K^+ activity was the same in all three immersion media used in the present study, there were significant differences between the steady state inside/outside K^+ activity ratio (a_{ki}/a_{ko}) attained in each of them. The observed changes in this ratio (see Table I) would, on the basis of the constant field equation [32], be expected to result in hyperpolarization of the mucosal membrane by hypertonic media and depolarization of this membrane by hypotonic media. The quantitative relationship between E_m and a_{ki}/a_{ki} has not yet been systematically studied in the small intestine. Hence the magnitude of the changes in E_m which would be expected for the changes in a_{ki}/a_{ko} which are suggested by the data of Table I cannot be accurately estimated. There are indications [33] that the dependence of E_m on a_{ki}/a_{ko} is numerically small in epithelial cells of small intestine, but, in any event, it seems clear that, in the present experiments, any change in E_m that resulted from an alteration in this ratio was outweighed by the opposing change in E_m induced by an increase or decrease in R_j (Table II).

Effect of external osmolality on I_{sc}

A consequence of electrical shunting, through conducting junctional pathways, of the intrinsic electromotive forces generated across epithelial tissues is that in the so-called open circuit condition (i.e. when no current from any external source is being driven across the tissue) a finite "inner current" [10] will flow around a loop such as that diagrammed in Fig. 3. In other words, for a finite shunt resistance, the open circuit condition is in reality a partially short circuited state, and, for any given set of conditions, the degree of short circuiting in the absence of an external current supply is inversely related to the magnitude of the shunt resistance. Taking the circuit shown in Fig. 3 as a basis for analysis, it is instructive to examine the influence of shunt resistance on the short circuit current as it is conventionally defined [34]. This is the external current which must be supplied, when the tissue is immersed in symmetrical bathing media, to maintain E_{Tr} at zero. For this purpose points 1 and 3 in Fig. 3 are considered to be connected to an external source of current.

Now, if V_j is set equal to zero so that the shunt pathway becomes a simple resistive element of the circuit shown in Fig. 3, analysis of this circuit by conventional methods gives the following expression for I_{sc},

$$I_{sc} = (V_s - V_m)/(R_m + R_s) \tag{4}$$

Eqn 4 predicts that, as long as V_m, V_s, R_m and R_s remain unchanged, I_{sc} is independent of the value of R_j. On the other hand, if V_j is assigned a finite value one obtains

$$I_{sc} = [(V_s - V_m)/(R_m + R_s)] - V_j/R_j \tag{5}$$

and, in these circumstances I_{sc} depends directly on R_j if all other parameters of Eqn 5 remain constant since the negative term on the right hand side of the equation is an inverse function of R_j.

Three important consequences of Eqn 5 may be noted. These are as follows: first, a change in I_{sc} which results from an increase or decrease in R_j alone is indicative of the existence of a finite diffusion potential in the shunt pathway. The fact that, in the present experiments, I_{sc} across isolated bullfrog small intestine increased in a hypotonic medium and decreased in a hypertonic solution may therefore be interpreted as indicating that this tissue, like the isolated rabbit gallbladder [24, 31], can maintain a finite diffusion potential across the junctional pathway even when the mucosal and serosal bathing solutions are symmetrical. Second, in epithelial tissues which maintain such diffusion potentials, E_{Tr} and I_{sc} are, in a certain sense, coupled since an increase or decrease in R_j affects both these parameters in a similar fashion. Third, it is apparent from Eqn 5 that although the I_{sc} under these conditions corresponds to net transepithelial ion transport [34], it does not necessarily reflect the intrinsic rate of m→s ion pumping by the tissue. It is also evident that, for epithelial tissues which possess relatively low resistance shunt pathways, the ratio E_{Tr}/I_{sc} does not provide a valid measure of total transepithelial resistance, regardless of the value of V_j.

The orientation of V_m in the small intestine

A further point which emerges from the results described in this paper concerns the true orientation of the electromotive force V_m across the mucosal membrane of the small intestine. In high resistance epithelia such as frog skin [10, 34] and toad bladder [35], E_m under open circuit conditions usually has the opposite orientation to that normally found in low resistance epithelia such as the small intestine [6, 7, 20–23] and the proximal renal tubule [36], i.e. the cell interior is electrically positive with respect to the mucosal solution. Similarly, V_m in high resistance epithelia is normally assigned an orientation opposite to that shown in Fig. 3 [37]. In a theoretical analysis of electromotive forces in epithelial tissues, Schultz [38] pointed out that, under certain conditions, a mucosal positive value for the measured parameter E_m may be consistent with an opposite orientation for V_m. This conclusion is readily confirmed as follows. Inversion (with respect to the reference point 1) of the sign of V_m in Fig. 3 leads to the following expression:

$$E_m = [V_m (R_s + R_j) - R_m (V_s + V_j)]/R_t \tag{6}$$

It is apparent from this equation that $E_m > O$ when $[V_m (R_s + R_j)] > [R_m (V_s + V_j)]$ and $E_m < O$ when $[V_m (R_s + R_j)] < [R_m(V_s + V_j)]$, and that, in theory, a reduction in R_j without change in the other parameters on the right hand side of equa-

tion [6] could lead to an inversion of E_m*. In support of this conclusion Schultz [38] cites the observation of Ussing and Windhager [10] that the five to eight fold decrease in the shunt resistance of isolated frog skin induced by exposing the outer surface of the tissue to a hypertonic urea-sulfate Ringer solution resulted in a reversal of E_m under open circuit conditions and correctly infers that, in low resistance epithelia such as the small intestine, the observed orientation of E_m under open circuit conditions may not necessarily reflect the true orientation of V_m.

The results obtained in the present study lead to more definite conclusions concerning the orientation of V_m in the small intestine under open circuit conditions, if one assumes as before that the response of E_m to changes in the osmolality of the bathing medium is largely mediated by osmotically induced changes in R_j. If the orientation of V_m is the opposite of that shown in Fig. 3, then, by analogy with Eqn 3, one obtains for a small change, ΔR_j, in R_j

$$\Delta E_m = V_m (\Delta R_j)/R_t \tag{7}$$

In terms of Eqn 7, if V_m has the opposite orientation to the (inside negative) measured E_m, an increase in R_j should result in a decrease in the magnitude of E_m and vice versa. This is the converse of the results obtained in this investigation (Fig. 2 and Table II). These, as already noted, were consistent with the predictions of Eqn 3 which is based on the assumption that V_m and E_m have the same orientation in the small intestine. Thus, if the assumptions underlying the present discussion are valid, the results shown in Fig. 2 and Table II support the conclusion that the inside negative mucosal membrane potential normally observed in the small intestine under open circuit conditions accurately reflects the orientation, though not the magnitude, of the corresponding transmembrane electromotive force V_m. They also suggest a method (i.e. alteration of the shunt resistance) by which the true orientation of V_m in other leaky epithelia can be ascertained from measurements of E_m.

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* It should be noted that if the orientations assigned to V_m, V_s and V_j in Fig. 3 correspond to the real orientation of these parameters in the small intestine, Eqn 3 predicts that a change in R_j alone will affect the magnitude but not the orientation of E_m. Hence, in these circumstances, E_m and V_m should have the same orientation regardless of the value of R_j.

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