Studies on the Electrical Potential Profile across Rabbit Ileum

Effects of sugars and amino acids on transmural and transmucosal electrical potential differences

RICHARD C. ROSE and STANLEY G. SCHULTZ

From the Department of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213. Dr. Rose's present address is the Departments of Physiology and Surgery, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033. Please address reprint requests to the Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213.

ABSTRACT When isolated strips of mucosal rabbit ileum are bathed by physiological electrolyte solution the electrical potential difference (PD) across the brush border (ψ_{mc}) averages 36 mv, cell interior negative. Rapid replacement of Na in the mucosal solution with less permeant cations, Tris or choline, results in an immediate hyperpolarization of ψ_{mc}. Conversely, replacement of choline in the mucosal solution with Na results in an abrupt depolarization of ψ_{mc}. These findings indicate that Na contributes to the conductance across the brush border. The presence of actively transported sugars or amino acids in the mucosal solution brings about a marked depolarization of ψ_{mc} and a smaller increase in the transmural PD ($\Delta\psi_{ms}$). It appears that the Na influx that is coupled to the influxes of amino acids and sugars is electrogenic and responsible for the depolarization of ψ_{mc}. Under control conditions $\Delta\psi_{ms}$ can be attributed to the depolarization of ψ_{mc} together with the presence of a low resistance transepithelial shunt, possibly the lateral intercellular spaces. However, quantitatively similar effects of amino acids on ψ_{mc} are also seen in tissues poisoned with metabolic inhibitors or ouabain. Under these conditions $\Delta\psi_{ms}$ is much smaller than under control conditions. Thus, the depolarization of ψ_{mc} might not account for the entire $\Delta\psi_{ms}$ observed in nonpoisoned tissue. An additional electromotive force which is directly coupled to metabolic processes might contribute to the normal $\Delta\psi_{ms}$.

Although there is an abundant literature describing the transmural movements of solutes across small intestine and solute accumulation by intestinal

639

epithelium, it has become increasingly clear that insight into underlying mechanisms requires a detailed understanding of the individual properties of the mucosal and serosal boundaries of the absorptive cells. In recent years, methods have been described that permit the direct determination of unidirectional solute influx from the mucosal solution across the mucosal border into the intestinal epithelium (1). The present communication is concerned with a description of the electrical potential difference across the brush border and its response to actively transported sugars and amino acids. This information is essential for the interpretation of transmural electrical events and the analysis of movements of charged solutes across the brush border. Some of these data have been published in a preliminary report (2).

METHODS

New Zealand white rabbits (2.5–4 kg) on normal food intake were killed by intravenous injection of pentobarbital. The terminal ileum was excised, opened along the mesenteric border, and rinsed in buffered electrolyte solution until free of intestinal contents. In most instances the serosal musculature was stripped off, using glass microscope slides, to prevent spontaneous muscle contraction which would render prolonged micropuncture difficult. The stripping procedure has previously been demonstrated to remove the muscularis mucosa, submucosa, muscle layers, and serosa, leaving the epithelial layer and lamina propria apparently intact and undamaged (3).

Apparatus

The tissue is held in place between the halves of a Lucite chamber illustrated in Fig. 1. Each half contains holes for delivery and withdrawal of bathing solution and holes for insertion of agar bridges. The tissue (M) rested, mucosal surface up, on a disc of wire mesh (L) which was supported by the lower half of the chamber. The halves of the chamber were securely bolted together exposing 1.13 cm^2 of tissue surface to the bathing solutions.

A continuous supply of bathing solution at 37–$38°C$ was delivered by gravity from a reservoir to each half of the chamber. A nonpulsatile Holter pump (Model RL-175) was used to return fluid from the chamber to the reservoir. The fluid volume in each chamber was maintained at 1.0 ml and the flow rate through each chamber was 1.5 ml/min. The temperature of the reservoir was regulated by means of a water jacket which was connected to a constant temperature circulating pump. The contents of the reservoir were bubbled with humidified 100% O_2 when bicarbonate-free bathing solutions were employed or with O_2–CO_2 gas mixture ($95:5$, v/v) when the bathing solutions contained bicarbonate. The final pH of all bathing solutions was 7.0–7.2. The control medium contained (mM) NaCl, 142; KH_2PO_4, 1.5; K_2HPO_4, 4.2; $CaCl_2$, 1.2; and $MgCl_2$, 1.2. Na-free media were obtained by replacement of Na with either K, choline, or Tris. Sulfate medium was prepared by replacement of NaCl with 71 mM Na_2SO_4 and 90 mM mannitol and had the same measured osmolarity as the control medium.

Electrical Measurements

The transmural (mucosal solution to serosal solution) electrical potential difference, designated ψ_{ms}, was monitored continuously using Ringer–agar bridges connected through matched calomel half-cells to a Keithley model 602 electrometer, and was recorded on one channel of a Texas Instruments dual-channel recorder. These bridges were positioned approximately 2 mm from each surface of the tissue. Tissue resistance was measured by passing current from an external source through silver–silver chloride electrodes which made contact through Ringer–agar bridges with the bathing solution at the extreme end of each chamber.
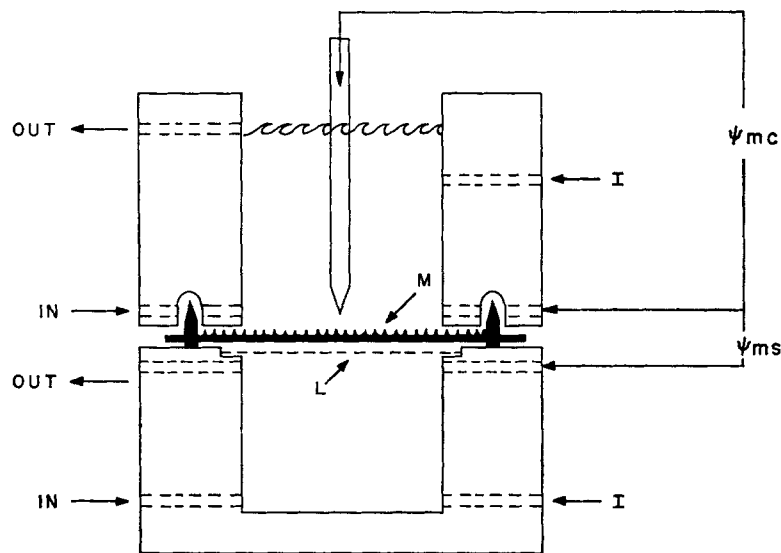

Figure 1. Schematic view of bathing chamber and electrode arrangement. Tissue (M) rests, mucosal surface up, on wire mesh (L). Ports for application of external current (I), measurement of ψ_{mc} and ψ_{ms}, and circulation of bathing solutions are shown.

Microelectrodes were prepared from 1.5–2 mm o.d. borosilicate glass tubing (A. H. Thomas Co., Philadelphia, Pa.) using a model M-1 micropipette puller (Industrial Science Associates, Inc., Ridgewood, N. Y.). The pipettes were immediately filled by boiling in methanol under reduced pressure for 10–20 min (4). They were then placed in distilled water for 15–20 min and allowed to exchange with 3 M KCl for at least 12 hr. Electrodes were used within 36 hr after filling.

Electrodes were selected for a resistance of 5–20 MΩ and a tip potential of less than 5 mv. A KCl-agar bridge connected the microelectrode to a calomel half-cell which, in turn, was connected to the high-impedance probe of a negative capacitance Medistor A-35 amplifier. The Ringer–agar bridge in contact with the mucosal bathing solution was used as the reference electrode and, therefore, in all further discussion the electrical potential of the mucosal bathing solution is taken as zero. The transmucosal PD (mucosal solution to cell interior), designated ψ_{mc}, was recorded on the

second channel of the dual-channel recorder. The transserosal PD, ψ_{cs} (cell interior to serosal solution, is defined by the relation $\psi_{cs} = \psi_{ms} - \psi_{mc}$.

The microelectrode was held by a Brinkman micromanipulator which was driven by hand with a minimum advance of 1–2 μ. The micromanipulator and bathing chambers were both secured to a shock-mounted plate and enclosed within a grounded, copper wire cage.

All errors are expressed as standard errors of the mean.

RESULTS

The criteria for an acceptable impalement were (a) an abrupt negative deflection of the microelectrode potential on advancing; (b) the maintenance of a stable PD for at least 15 sec; (c) an abrupt return to the base line upon withdrawal of the microelectrode; and (d) no change in electrode tip potential or resistance. Successful cell impalement by these criteria was difficult, perhaps due to movement of villi, and only 20–40% of the attempts were considered acceptable. A frequent observation was a rapid decline of the intracellular potential during the first few seconds after the electrode tip pierced the cell membrane, perhaps due to incomplete sealing between tissue and electrode. If the intracellular potential declined by more than 10–15% of its initial value, the observation was discarded. When the microelectrode tip resistance was large, the intracellular potential occasionally declined slightly immediately after impalement and then slowly increased by as much as 5–10 mv (for example see Fig. 6); this behavior is similar to that observed during the impalement of renal tubule (5) and rat colon (6) epithelial cells. These recordings were accepted if the intracellular potential reached a steady level.

A fairly constant intracellular potential could occasionally be measured for periods of 2–10 min. This provided an opportunity to change mucosal solutions with the microelectrode in place so that the immediate effects on ψ_{mc} of a single cell could be observed. Almost all the data reported in this paper were obtained in this manner.

A histogram of 185 impalements on 12 randomly selected tissues is given in Fig. 2. The values are distributed normally about a mean ψ_{mc} of -36 ± 0.5 mv. The mean ψ_{ms} of these tissues was 4.5 ± 0.3 mv. There was a slow decline in ψ_{ms} during the course of the experiment but the value of ψ_{mc} was constant for at least 90 min.

Tissue Resistance

Tissue resistance was measured by passing a 200 μa pulse of direct current across the tissue *during* a successful impalement. The change in transmural electrical potential difference, $\Delta\psi_{ms}$, averaged 11.1 ± 0.4 mv and the simultaneously recorded $\Delta\psi_{mc}$ averaged 4.2 ± 0.1 mv. The paired $\Delta\psi_{mc}:\Delta\psi_{ms}$ averaged 0.38 ± 0.01. These data are uncorrected for the electrical potential

difference contributed by the resistance of the bathing solution between the surfaces of the tissue and the agar bridges. In order to obtain corrected values, the tissue was removed and the electrical potential difference in response to 200 μa was measured; this value was subtracted from $\Delta\psi_{ms}$. The electrical potential difference between the mucosal agar bridge and the tip of the microelectrode was similarly measured after withdrawing the microelectrode a few microns from the cell; this value was subtracted from $\Delta\psi_{mc}$. The corrected $\Delta\psi_{mc}:\Delta\psi_{ms}$ averaged 0.29 $\pm$ 0.03. The agreement between the corrected and uncorrected ratios is not unexpected in view of the fact that the mucosal and serosal bridges are almost equidistant from the tissue surfaces.
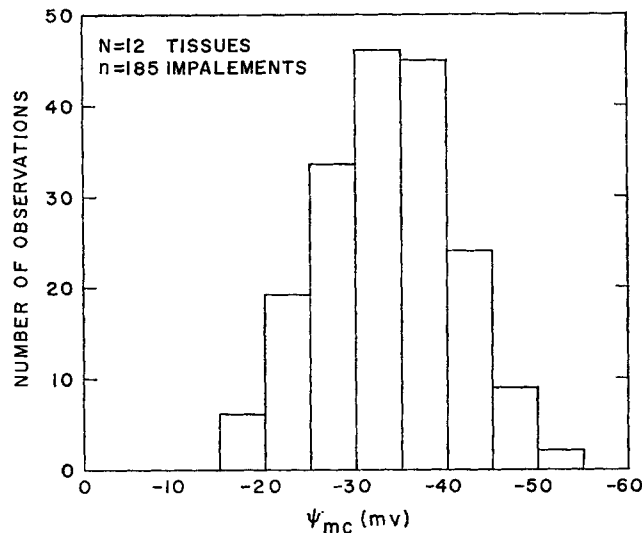

FIGURE 2. A histogram of ψ_{mc} on tissues bathed in control medium.

The corrected total transmural resistance averaged 21 $\pm$ 2 ohm-cm^2, a value that is considerably smaller than that observed using unstripped rabbit ileum (58 ohm-cm^2) (7).

In seven experiments, tissue resistance was measured before and after poisoning the tissue with KCN plus iodoacetate (10^{-3} M). In all but one instance there was a significant increase in transepithelial resistance which commenced within 3–8 min after introduction of the inhibitors and persisted for the remainder of the experiment (at least 60 min). The average control transepithelial resistance in these tissues was 21 $\pm$ 2 ohm-cm^2 and the average transepithelial resistance 20 min after the introduction of the metabolic inhibitors was 34 $\pm$ 5 ohm-cm^2. Thus, metabolic inhibitors bring about a highly significant increase in total transepithelial resistance.

Effects of Cations and Anions on ψ_{mc}

As illustrated in Fig. 3, the abrupt replacement of Na in the mucosal solution with the less permeant cation, Tris, resulted in a significant hyperpolarization of ψ_{mc}. Similar results were obtained when Na was replaced with choline. Conversely, when the tissue is initially bathed on both surfaces with Na-free, choline medium, the abrupt replacement of choline in the mucosal solution with Na results in a prompt depolarization of ψ_{mc} and an increase in ψ_{ms}, as
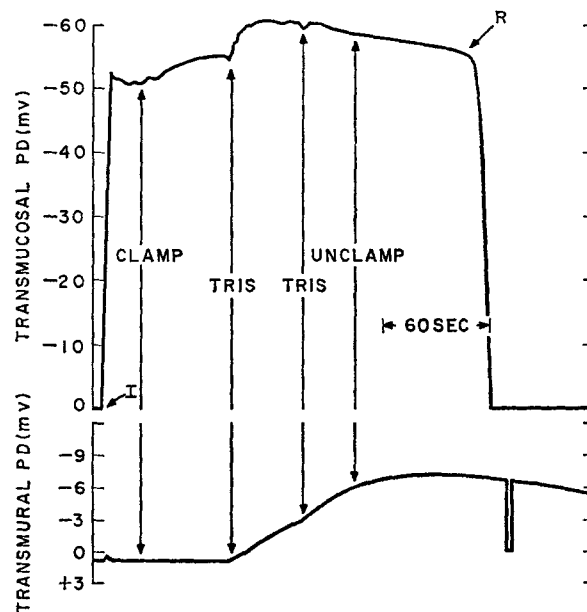

FIGURE 3. Changes in ψ_{mc} (top) and ψ_{ms} (bottom) when Na in the mucosal solution is replaced by Tris. *I* indicates time of impalement of cell; *R*, time of microelectrode retraction. The tube which delivers control medium to the mucosal surface was clamped at the time indicated. Tris medium (37°C) was injected into the mucosal chamber twice, and the flow of control medium was resumed. Figure traced from the original recording.

illustrated in Fig. 4. These results indicate that Na conductance contributes to ψ_{mc}. Hyperpolarization of the PD across the luminal membrane in response to replacement of Na in the luminal fluid with a less permeant cation has been reported for the proximal renal tubule of *Necturus* (8) and distal tubule of rat (9) and *Amphiuma* (10).

In contrast, complete replacement of Na in the mucosal solution with K did not have an immediate effect on ψ_{mc}, although, in many instances, a very slow decline was observed. These findings suggest that the brush border may be equally permeable to Na and K, a notion that is supported by direct measurement of [22]Na and [42]K influxes across this boundary (Rose and Schultz,

unpublished observations). Similar conclusions have been reported for the rat (9) and *Amphiuma* (10) distal renal tubule.

The effect of HCO_3 on ψ_{mc} was evaluated by comparing the mean value of ψ_{mc} when tissues were first bathed on both surfaces with control medium and then with medium containing 10 mM HCO_3.[1] As shown in Table I, there is no significant change in ψ_{mc} when this change in bathing solutions is made. Further, the presence of 5×10^{-3} M acetazolamide had no effect on the ψ_{mc} of tissues bathed in bicarbonate or bicarbonate-free media.
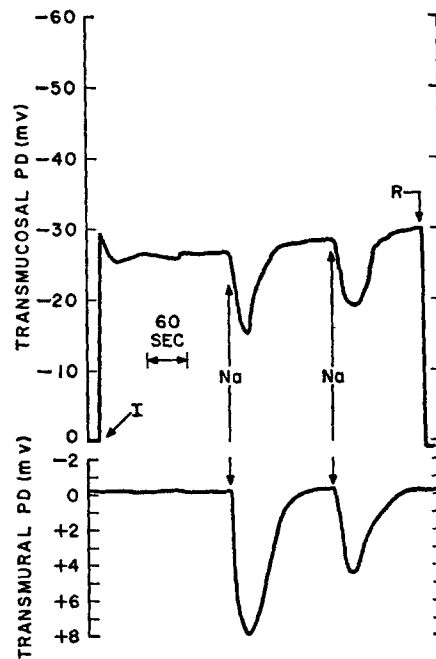

FIGURE 4. Effects of mucosal Na on ψ_{mc} and ψ_{ms} of tissue initially bathed in Na-free, choline medium. Rapid return to the control values is due to uninterrupted continuous circulation of the choline medium. Redrawn from original recording.

Finally, the abrupt replacement of Cl in the mucosal solution with SO_4 (maintaining constant osmolarity with mannitol) resulted in little or no change in ψ_{mc}; the $\Delta\psi_{mc}$ in nine such experiments averaged -2.5 ± 1.2 mv (the cell interior became slightly more negative).

Effect of Sugars and Amino Acids on the Potential Profile

The presence of actively transported amino acids and sugars in the mucosal solution increases the transmural PD and short-circuit current of small intestine from rabbit (11, 12) and several other species (13, 14).

---

[1] Because different gas mixtures were employed for bicarbonate and bicarbonate-free media, abrupt replacement of the mucosal solution was not feasible.

The effect of adding L-alanine (20 mM, final concentration) to the solution bathing the mucosal surface of the ileum is illustrated in Fig. 5. There was an immediate 10 mv decrease in the absolute magnitude of ψ_{mc} (i.e., the cell interior became less negative) and an increase of 3 mv in ψ_{ms}. The continuous supply of control solution flowing into the mucosal chamber washes away the amino acid in approximately 20 sec and ψ_{mc} returns to its initial value. Similar results were obtained with L-phenylalanine which also is transported across the ileum by a Na-dependent mechanism (15). The introduction of D-valine which is not subject to Na-dependent carrier-mediated transport by this tissue (Schultz and Yu-Tu, unpublished observations) did not significantly affect ψ_{mc} or ψ_{ms}.

Alanine influx across the brush border is enhanced by Na and is associated with a concomitant increase in Na influx (16). In order to test whether the

TABLE I

EFFECT OF BICARBONATE ON ψ_{mc}

Bathing solution	ψ_{mc}
	mv
Control	35.6±1.8 (11)
Control with acetazolamide	35.8±1.3 (41)
Bicarbonate	32.6±1.4 (11)
Bicarbonate with acetazolamide	31.6±1.7 (28)

The composition of the bicarbonate medium was (mM): NaCl, 142; KH$_2$PO$_4$, 0.2; K$_2$HPO$_4$, 1.2; CaCl$_2$, 0.9; MgCl$_2$, 1.2, and KHCO$_3$, 10. Number of impalements given in parentheses.

depolarization of ψ_{mc} following the addition of amino acids is dependent upon the presence of Na in the bathing medium the membrane was bathed for 15 min by solutions in which Na was replaced by Tris. Fig. 6 shows a typical example of the complete absence of an effect of alanine on ψ_{mc} and ψ_{ms} when Na is not present in the mucosal solution.

The addition of D-glucose (20 mM, final concentration) to the mucosal solution results in a depolarization of ψ_{mc} and an increase in ψ_{ms} (Fig. 7) similar to that elicited by alanine. Also, illustrated is the lack of a marked effect on ψ_{mc} or ψ_{ms} following the addition of 20 mM mannitol, which is neither actively transported nor metabolized by the tissue. The actively transported but poorly metabolized D-galactose has an effect on ψ_{mc} similar to that of glucose. Fructose, which crosses the mucosal border by a carrier-mediated process that is not dependent on the presence of Na in the mucosal solution (17), did not elicit a significant electrical effect. The presence of phlorizin (10^{-3} M) in the mucosal solution prevented the effect of glucose on
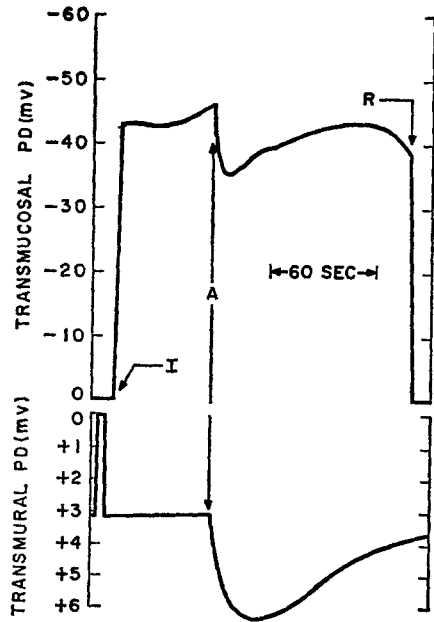
Figure 5

Figure 6

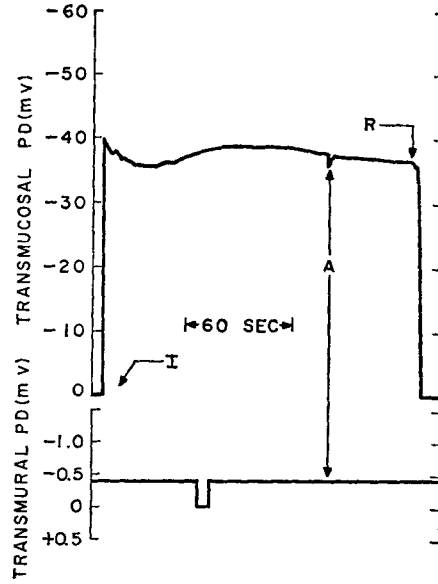FIGURE 5. Effects of L-alanine (*A*) on ψ_{mc} and ψ_{ms}.

FIGURE 6. Effects of L-alanine (*A*) on ψ_{mc} and ψ_{ms} of tissue bathed in Na-free, Tris medium.
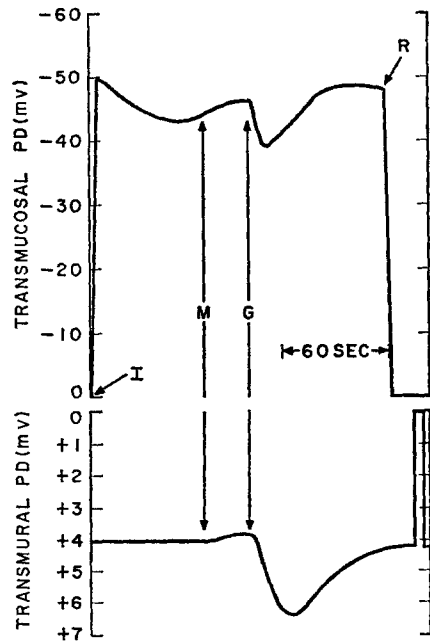
FIGURE 7. Effects of D-mannitol (*M*) and D-glucose (*G*) on ψ_{mc} and ψ_{ms}.

ψ_{mc} but did not affect the response to alanine. Thus, it appears that the effect on ψ_{mc} is restricted to sugars and amino acids that exhibit Na-coupled entry across the brush border.

In 39 experiments similar to those illustrated in Figs. 5 and 7, the initial ψ_{ms} and ψ_{mc} averaged 2.5 ± 0.1 mv and −32 ± 2 mv, respectively. Following the addition of alanine or glucose, $\Delta\psi_{ms}$ averaged +1.9 ± 0.1 mv and $\Delta\psi_{mc}$ averaged +6.0 ± 0.5 mv[2]. The average paired value of $\Delta\psi_{ms}:\Delta\psi_{mc}$ was 0.41 ± 0.06 mv. The relation between the initial ψ_{mc} and $\Delta\psi_{mc}$ is illustrated in Fig. 8. Clearly, the lower[3] the initial ψ_{mc} the smaller the response to the addition of glucose or alanine and the relation between these two parameters appears to be linear. A plot of $\Delta\psi_{ms}:\Delta\psi_{mc}$ vs. initial ψ_{mc} is given in Fig. 9. In only 5 out of 39 experiments, was $\Delta\psi_{ms}:\Delta\psi_{mc}$ greater than or equal to 1 and in 4 out of these 5 instances the initial ψ_{mc} was lower than −16 mv. However, $\Delta\psi_{ms}:\Delta\psi_{mc}$ is independent of the initial ψ_{mc} when the latter exceeds −20 mv.

It should be noted that in these experiments the amino acid or sugar was introduced directly into the mucosal chamber in amounts calculated to give a final concentration of 20 mM. Since the continuous circulation of control medium was not interrupted, the actual final concentration is not known accurately. However, the continuous circulation provided a means of demonstrating, within a short duration, that ψ_{mc} returns to its original value when the amino acid or sugar is washed out of the mucosal chamber. In view of the fact that spontaneous changes in ψ_{mc} upon prolonged impalement are not uncommon, it seemed more important to demonstrate the return of ψ_{mc} to the original value than to define accurately the concentrations of sugars or amino acids. Several experiments were performed to determine whether the washout significantly affects $\Delta\psi_{mc}$ or $\Delta\psi_{ms}$. In these experiments, circulation was interrupted so that the effects of sugars or amino acids were sustained at peak values; however, $\Delta\psi_{mc}$, $\Delta\psi_{ms}$, and $\Delta\psi_{ms}:\Delta\psi_{mc}$ did not differ significantly from the values observed during continuous flow.

Previous studies have shown that Na-dependent alanine influx across the brush border is not affected by metabolic inhibitors or ouabain (18). However, these agents abolish active transmural alanine transport and the ψ_{ms}. In order to examine the effect of inhibitors on the ability of alanine to depolarize ψ_{mc}, the following experiments were performed: Each piece of tissue was bathed initially in control medium and shown to respond to alanine as il-

[2] It should be noted that although changes in ψ_{mc} and ψ_{ms} in response to sugars or amino acids commence simultaneously, the peak $\Delta\psi_{ms}$ is reached later than the peak $\Delta\psi_{mc}$. This was a consistent finding. The most probable explanation for this observation is that micropuncture was generally carried out close to the center of the exposed tissue area and the sugar or amino acid was introduced by syringe close to the microelectrode. Since ψ_{ms} is the cumulative result of responses on the part of the total cell population the lag may be due to the fact that cells in the center of the tissue are exposed to the sugar or amino acid earlier than cells situated in more peripheral areas of the tissue.

[3] Terms such as "lower than" or "greater than" refer to the absolute values of the PD.
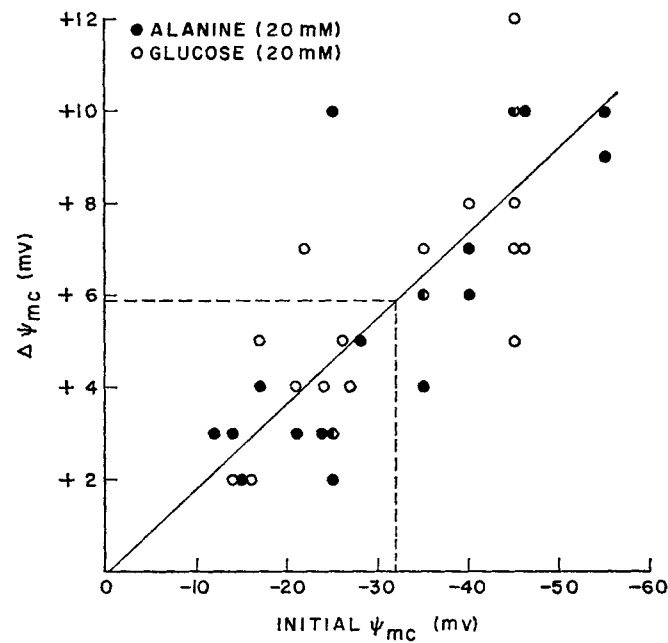
FIGURE 8.   Relation between initial ψ_{mc} and $\Delta\psi_{mc}$ in response to alanine (solid circles) or glucose (open circles). Dashed lines indicate average values. The line was obtained by least-squares regression analysis.
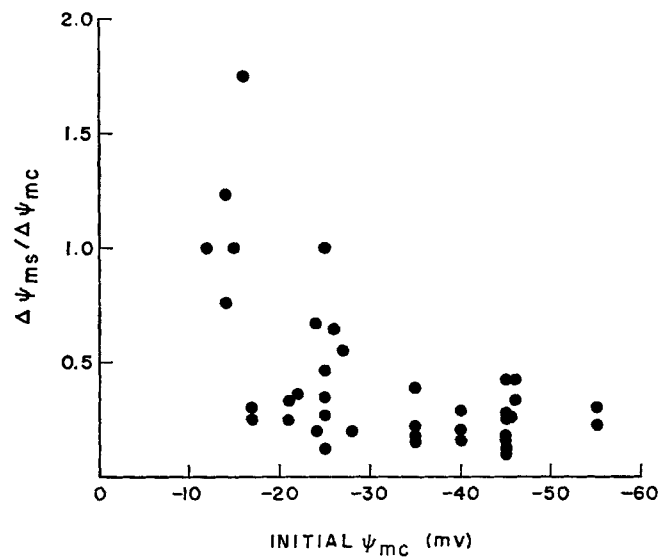

FIGURE 9.   Relations between $\Delta\psi_{ms}$:$\Delta\psi_{mc}$ and initial ψ_{mc}.

lustrated in Fig. 5. The tissue was then *rapidly* poisoned by introducing KCN, ouabain, and iodoacetate (10^{-3} M, final concentration) into the bathing solutions. A decline in ψ_{ms} commenced *immediately* after introduction of the poisons and values close to zero were reached within 10 min. L-Alanine was added to the mucosal solution after 15–30 min when ψ_{ms} was close to zero and ψ_{mc} had already declined significantly from its original value. Fig. 10 shows the depolarizing effect of L-alanine on ψ_{mc} when cell metabolism has been inhibited. However, under these conditions the effect on ψ_{ms} is minimal. Thus, in the poisoned tissue changes in ψ_{mc} are not accompanied by marked
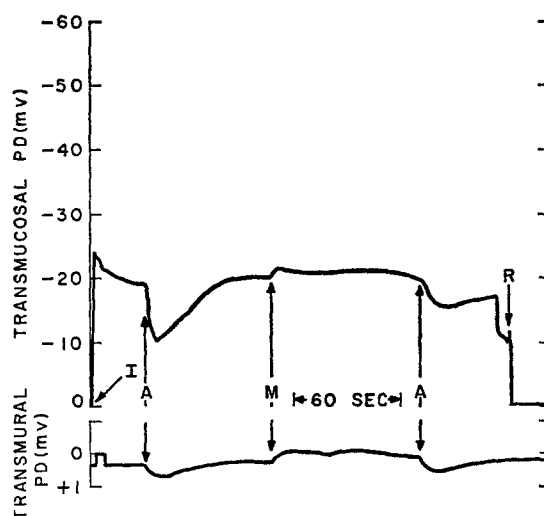

FIGURE 10.   Effect of L-alanine (A) and D-mannitol (M) on ψ_{mc} and ψ_{ms} of tissue bathed in control medium containing KCN, ouabain, and iodoacetate (10^{-3} M). Figure traced from the original recording.

changes in ψ_{ms}. In six experiments similar to that illustrated in Fig. 10, the initial ψ_{ms} and ψ_{mc} were $+0.3 \pm 0.02$ mv and -17 ± 2 mv, respectively. In response to alanine $\Delta\psi_{mc}$ averaged $+6.7 \pm 1.3$ mv and $\Delta\psi_{ms}$ averaged only $+0.2 \pm 0.1$ mv; paired $\Delta\psi_{ms}:\Delta\psi_{mc}$ averaged 0.04. As is also shown in Fig. 10, the addition of mannitol to the mucosal solution brings about a small decrease in ψ_{ms} and a slight hyperpolarization of ψ_{mc}. This effect on ψ_{ms} has been reported previously and has been attributed to "streaming potentials" in response to water flow induced by the osmotic effect of the added solute (19). In 13 experiments, the effect of 20 mM mannitol, fructose, or D-valine on ψ_{ms} averaged -0.2 mv. Under control conditions the contribution of this "osmotic effect" to $\Delta\psi_{ms}$ in response to alanine or glucose is negligible. However, in poisoned tissues the average "osmotic effect" is equal to the average $\Delta\psi_{ms}$ observed in response to glucose or alanine and, if ignored, would lead

to a significant underestimate of $\Delta\psi_{ms}$. The average $\Delta\psi_{ms}:\Delta\psi_{mc}$ corrected for the osmotic effect is 0.06.

Thus, there are two important differences between control and poisoned tissues with respect to the response to alanine or glucose. First, in spite of the fact that the initial ψ_{mc} in poisoned tissue is significantly lower than that in control tissues, $\Delta\psi_{mc}$ is essentially the same under these conditions. The relation shown in Fig. 8 does not apply when the initial ψ_{mc} is reduced by poisoning; $\Delta\psi_{mc}$ in the poisoned tissues is more than twice that which would be predicted by this relation. Second, $\Delta\psi_{ms}:\Delta\psi_{mc}$ in poisoned tissue is significantly lower than that observed in control tissues.

DISCUSSION

Previous data on the electrical potential difference across the brush border of mammalian small intestine appear to be limited to those reported by Wright in a paper dealing primarily with the electrical potential profile across the tortoise small intestine (20). He reports that ψ_{mc} in hamster midjejunum averaged approximately -8 mv in the presence of glucose and increased to approximately -10 mv upon the addition of phlorizin. The values of ψ_{mc} observed in the present study on rabbit ileum are considerably greater than those reported by Wright and are in closer accord with those reported for bullfrog small intestine (21) and rat colon (6). Thus, although the transmural electrical potential difference in stripped rabbit ileum is rather small (2–5 mv in the absence of sugars and amino acids) the intracellular electrical potential is quite significant and cannot be ignored in studies dealing with the accumulation of charged solutes. The average ψ_{mc} of 36 mv, interior negative, can account for a fourfold accumulation of a monovalent cation or an eightfold accumulation of a divalent cation (e.g. Ca) without the necessity of invoking active transport processes. Further, short-circuiting the tissue with an external current sufficient to abolish the spontaneous ψ_{ms} does not markedly affect ψ_{mc}.

Effects of Sodium-Coupled Amino Acid and Sugar Transport

The addition of actively transported amino acids or sugars to the mucosal solution results in a significant depolarization of ψ_{mc} (i.e. the cell interior becomes less negative with respect to the mucosal solution). This is not observed with D-valine, mannitol, or fructose whose influxes across the brush border are independent of Na. Further, the effect on ψ_{mc} is not observed in a Na-free medium, so that it cannot be attributed to a nonspecific change in brush border permeability; for example, an increase in permeability to all ions brought about by the presence of these actively transported nonelectrolytes. Previous studies have shown that sugar (22) and amino acid influxes (16, 23, 24) across the brush border of rabbit ileum are dependent upon Na and are associated with a concomitant, stoichiometric increase in Na influx.

In the light of these observations, the effect on ψ_{mc} is almost certainly the result of the electrogenic[4] entry of Na coupled to the entry mechanisms for sugars and amino acids.

As illustrated in Figs. 5, 7, and 9, the depolarizing effect of glucose and alanine on ψ_{mc} is larger than the increase in ψ_{ms}. This is schematized more clearly in Fig. 11 for the data shown in Fig. 7. Thus, the initial value of ψ_{mc} was -46 mv and the initial ψ_{ms} $+4$ mv, giving an initial ψ_{cs} of $+50$ mv. Following the addition of glucose, ψ_{mc} decreased to -39 mv and ψ_{ms} increased to $+6.5$ mv. Thus, ψ_{cs} actually decreased by 4 mv to a value of $+46$ mv. Because $\Delta\psi_{ms}$ reflects changes in the entire population of cells, a possible
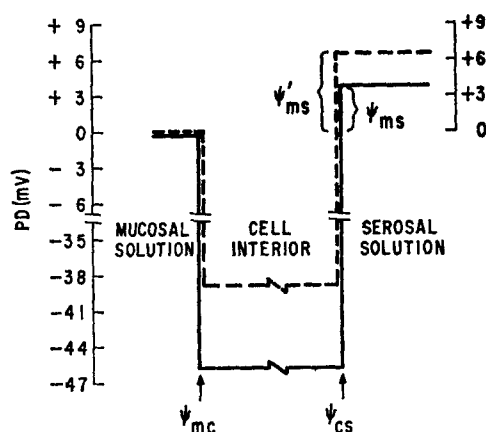

FIGURE 11. The electrical potential profile of an epithelial cell of isolated rabbit ileum before (solid line) and after (dashed line) the addition of glucose. ψ_{ms} designates the transmural PD in the absence of glucose and ψ'_{ms} designates the transmural PD with glucose (20 mM) present in the mucosal solution. Data taken from Fig. 7.

explanation for the observation that $\Delta\psi_{ms} < \Delta\psi_{mc}$ is that the population of cells that were successfully impaled is not representative of the total cell population. However, impalement was carried out in a random fashion and, as shown in Fig. 9, with the exception of five instances, the values of $\Delta\psi_{ms}$: $\Delta\psi_{mc}$ lie within a narrow range. If

$$\frac{\sum\limits^{n} \Delta\psi_{mc}}{n} = \Delta\psi_{ms}$$

[4] The term electrogenic implies that the electromotive force is generated directly by the operation of a carrier mechanism that brings about a net displacement of charge. Electroneutrality is maintained by the passive movements of other ions through pathways in parallel with the carrier mechanism. The depolarization of ψ_{mc} is too rapid to be ascribed to changes in ionic gradients across the mucosal membrane and, under steady-state conditions, glucose or alanine does not bring about significant changes in intracellular Na or K concentrations (3). However, the possibility that $\Delta\psi_{mc}$ is the result of changes in ion concentrations in the unstirred layers on either side of the mucosal membrane (e.g. a decrease in local extracellular Na and an increase in local intracellular Na) cannot be excluded.

where n represents the total number of responding cells, a random sampling should disclose more instances in which $\Delta\psi_{ms} > \Delta\psi_{mc}$. The possibility that only cells capable of responding with a large $\Delta\psi_{mc}$ were susceptible to successful impalement cannot be ruled out. However, as will be shown below, a more likely explanation for the observation that $\Delta\psi_{ms}:\Delta\psi_{mc} < 1$ emerges from an analysis of a simple equivalent circuit model.

These observations are in complete agreement with those reported by White and Armstrong (21) for bullfrog small intestine, a preparation that closely resembles rabbit ileum with respect to the interactions between sugar or amino acid transport and the transport of Na (25, 26). However, these results are diametrically opposite to those reported by Wright (20) and Gilles-Baillien and Schoffeniels (27) for tortoise small intestine. These investigators reported that the increase in transmural electrical potential difference following the addition of sugars (20, 27) or amino acids (27) to the solution bathing the mucosal surface of tortoise small intestine can be attributed entirely to an increase in ψ_{cs}; no change in ψ_{mc} was implicated. These differences may be attributable to apparent species differences with respect to the mechanism of amino acid transport. Gilles-Baillien and Schoffeniels (28) have concluded that L-alanine crosses the brush border of tortoise small intestine passively and that the active step for transmural transport of this amino acid is located at the serosal or lateral membranes. In rabbit ileum, Na-dependent mechanisms located in the brush border appear to be responsible for the transport of sugars and amino acids from lower concentrations in the mucosal solution to higher concentrations within the cell (1, 3). Thus the differences between our data and those obtained using tortoise small intestine may reflect different locations for the mechanisms responsible for active transmural amino acid and sugar transport. It is somewhat more difficult to explain the observations of Wright using hamster midjejunum, where the effect of glucose on ψ_{ms} was also attributed mostly to an increase in ψ_{cs}. A possible explanation is suggested by the very low intracellular electrical potentials observed by Wright. It should be recalled that ψ_{cs} is not measured directly, but is derived from the difference between ψ_{mc} and ψ_{ms}. Wright's conclusion is based on the observation that ψ_{ms} increased but ψ_{mc} *did not change* significantly. It is quite possible that, in Wright's study, the impaled cells were damaged (e.g. the membrane failed to seal around the microelectrode) resulting in a very leaky cell that was incapable of responding to glucose with a large depolarization of ψ_{mc} (see below). The absence of a response would then lead to the conclusion that a change in ψ_{cs} was responsible for the observed change in ψ_{ms}. This explanation is consistent with the relations shown in Figs. 8 and 9. Values of $\Delta\psi_{ms}:\Delta\psi_{mc}$ greater than unity were only observed when the initial ψ_{mc} was low (≤ -16 mv). Under these circumstances an increase in ψ_{cs} would have to be postulated to account for the entire $\Delta\psi_{ms}$. A more likely explanation is

that the *impaled* cell was damaged so that the observed $\Delta\psi_{mc}$ is not a reliable measure of the change in ψ_{mc} occurring in the remaining cell population.

An Equivalent Electrical Circuit

The relation between ψ_{mc} and ψ_{ms} can be analyzed by means of the electrical circuit illustrated in Fig. 12. In this circuit E_m designates an electromotive force operating across the mucosal membrane, R_1 represents the internal resistance of this battery, and R_2 represents a shunt resistance across the mucosal membrane. E_s, R_3, and R_4 are the respective parameters for the serosal (and/or lateral) membranes. R_6 is a transepithelial shunt resistance and E_L and R_5 are included to account for the possibility of transepithelial diffusion potentials arising from ionic gradients across the tissue. The remainder of the
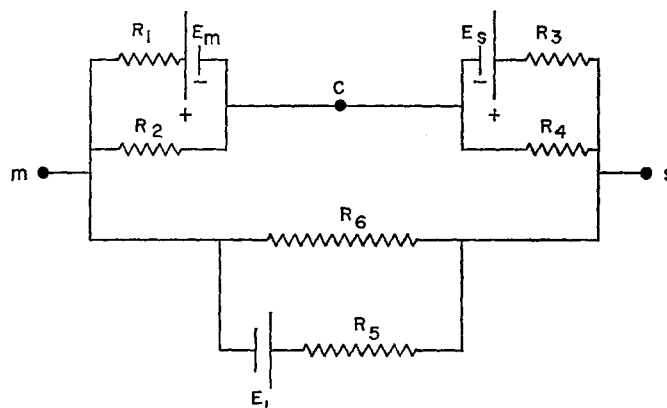

Figure 12. An equivalent electrical circuit. m, c, and s designate the mucosal, intracellular, and serosal electrodes, respectively.

discussion will deal with conditions in which the mucosal and serosal solutions have identical ionic compositions so that we can consider $E_L = 0$. Thus, the resistance of the shunt pathway is simply $R_5 R_L$ where $R_L = R_6/(R_5 + R_6)$.

The solutions of this circuit for ψ_{mc} and ψ_{ms} are:[5]

$$\psi_{mc} = - R_m [R_1 R_s E_s + E_m (R_3 R_s + R_5 R_L)] : R_T \qquad (1)$$

and

$$\psi_{ms} = R_5 R_L (E_s R_s - E_m R_m) : R_T \qquad (2)$$

where

$$R_s = R_4 : (R_3 + R_4)$$
$$R_m = R_2 : (R_1 + R_2)$$

[5] The orientations of the electromotive forces, as shown in Fig. 12, are included in the solution so that E_m and E_s designate absolute values.

and

$$R_T = R_1 R_m + R_3 R_s + R_5 R_L.$$

The relation between a change in ψ_{mc} and the accompanying change in ψ_{ms} resulting from a change in E_m alone is

$$\Delta\psi_{ms} : \Delta\psi_{mc} = 1/[1 + (R_3 R_s : R_5 R_L)]. \tag{3}$$

Thus, a change in E_m resulting from the addition of sugars or amino acids to the mucosal solution will bring about a change in ψ_{ms} that is linearly related to the change in ψ_{mc}. $\Delta\psi_{ms}$ will equal $\Delta\psi_{mc}$ only when $R_5 R_L$ is infinite. When $R_5 R_L = 0$, a change in ψ_{mc} will not be accompanied by a change in ψ_{ms}. Clearly, when $R_5 R_L = 0$, $\psi_{ms} = 0$, $\psi_{mc} = -\psi_{cs}$, and the system resembles a suspension of symmetrical cells under all conditions. However, when $0 < R_5 R_L < \infty$, $\Delta\psi_{ms} < \Delta\psi_{mc}$. Thus, the possibility that the impaled cells are not representative of the total population need not be invoked to explain our observation. However, this possibility cannot be excluded as a contributory factor.

The circuit illustrated in Fig. 12 is also consistent with the relation shown in Fig. 8. When $E_L = 0$ and $\Delta\psi_{mc}$ is due to a change in E_m alone

$$\Delta\psi_{mc} = (\Delta E_m) R_m (R_3 R_s + R_5 R_L) : R_T$$

Thus, both ψ_{mc} and $\Delta\psi_{mc}$ decrease with decreasing R_m (i.e. an increase in shunt conductance across the mucosal membrane).[6] The relation between $\Delta\psi_{mc}$ and the initial ψ_{mc} is simply

$$\frac{\Delta\psi_{mc}}{\psi_{mc}} = \frac{(E_m)_f - (E_m)_i}{(E_m)_i + [R_1 R_s E_s : (R_3 R_s + R_5 R_L)]}$$

where the subscripts i and f designate initial and final values. Therefore, when all other parameters remain constant, $\Delta\psi_{mc}$ will be a linear function of the initial ψ_{mc} and both will approach zero with decreasing R_m.

Thus, the response of ψ_{ms} to the addition of sugars or amino acids to the mucosal solution may be attributed, at least in nonpoisoned tissue, to the effect on ψ_{mc} together with the presence of a transepithelial shunt pathway; the size of $R_5 R_L$ compared to that of $R_3 R_4 : (R_3 + R_4)$ determines the extent to which the change in ψ_{mc} is attenuated. On the basis of these data alone there appears to be no need to implicate an electrogenic mechanism at the serosal membrane (i.e. a mechanism that would be manifest by an increase in E_s).

[6] Thus, if R_m of *the impaled cell* is decreased by incomplete sealing around the microelectrode, $\Delta\psi_{mc}$ will be spuriously reduced. This, as discussed above, is offered as the formal explanation for Wright's findings (20).

This analysis supports the previous suggestions of Barry et al. (29, 30) and of Hoshi and Komatsu (31) that the increase in ψ_{ms} is directly attributable to the transfer mechanisms for sugars and amino acids. However, as will be discussed below, other factors might contribute to the over-all $\Delta\psi_{ms}$.

This analysis also provides an explanation for the parallelism between results obtained from studies of transmural electrical potential difference and short-circuit current, and results obtained from studies of sugar influx across the brush border of rabbit ileum. Schultz and Zalusky (11) demonstrated that the increase in short-circuit current (ΔI_{sc}) across rabbit ileum is a saturable function of the glucose concentration in the mucosal solution, and that lowering the Na concentration in the bathing media reduced the maximal increase in current but did not affect the concentration of glucose needed to elicit a half-maximal increase. Goldner et al. (22) demonstrated that the coupling coefficient between sugar and Na influxes across the brush border is unity and that lowering the Na concentration in the mucosal solution did not affect the concentration of sugar needed to elicit a half-maximal influx (K_t) but decreased the maximal influx (J^{imax}). The observations that (a) sugar influx is accompanied by an equivalent increase in Na influx; (b) when all other parameters are constant, $\Delta\psi_{ms}$ is a linear function of $\Delta\psi_{mc}$; and (c) ΔI_{sc} is a linear function of $\Delta\psi_{ms}$ (7, 11) provide a rigorous explanation for these findings.

It is instructive to inquire whether these observations could be attributed to a change in $either$ E_s or E_L $alone$. Clearly, an increase in ψ_{ms} could result from an increase in E_s alone (e.g. an electrogenic pump that extrudes Na from the cell). However, if $only$ E_s changes then

$$\Delta\psi_{ms}:\Delta\psi_{mc} = - (R_5 R_L : R_1 R_m).$$

Thus, an increase in E_s will result in an increase in ψ_{ms} and a $hyperpolarization$ of ψ_{mc} (the cell interior would become more negative). Fordtran et al. have demonstrated that the presence of glucose or galactose in the lumen increases Na absorption by the human jejunum and brings about an increase in the transmural PD (32). These investigators have suggested that the increased Na absorption is the result of solvent-drag secondary to glucose-induced water absorption. According to their model, the increased Na absorption in response to actively absorbed sugar flows through extracellular channels (see Fig. 10, reference 32) and the increased transmural PD could then be attributed to electrokinetic phenomena such as streaming potentials. According to the circuit illustrated in Fig. 12 the model proposed by Fordtran et al. is equivalent to saying that $\Delta\psi_{ms}$ is the result of an increase in E_L alone. However, under these conditions

$$\Delta\psi_{ms}:\Delta\psi_{mc} = 1 + (R_3 R_s : R_1 R_m).$$

Thus, if $\Delta\psi_{ms}$ is due entirely to a change in E_L, the increase in ψ_{ms} would be *greater* than the depolarization of ψ_{mc}. Indeed, the resistance measurements reported above suggest that $R_sR_s \gtreqqless 2 R_1R_m$ so that $\Delta\psi_{ms}:\Delta\psi_{mc}$ would be approximately 3, a value that is 10 times greater than that observed.

In summary, although a change in E_m *alone* can satisfy these observations, changes in E_s *or* E_L *alone* lead to predictions that are irreconcilable with our data or those of White and Armstrong.

Finally, it should be stressed that the relation between $\Delta\psi_{mc}$ and $\Delta\psi_{ms}$ given in equation (3) is based on the assumption that E_s and all the resistance pathways remain constant when E_m is changed. Ignoring for the moment possible changes in E_s, let us focus on factors that could influence R_sR_L and, hence, the relation between $\Delta\psi_{mc}$ and $\Delta\psi_{ms}$. Clearly, increasing R_sR_L by replacement of Na and/or Cl in the bathing solutions with either an uncharged solute (e.g. mannitol [33, 34]) or less permeant electrolytes (e.g. Tris [34, 35]) will increase $\Delta\psi_{ms}:\Delta\psi_{mc}$. Further, the transepithelial shunt resistances in renal tubular epithelium (36) and frog skin (37) are sensitive to alterations in extra-cellular osmolarity. Hypertonicity generally reduces the resistance whereas hypotonicity increases this resistance; these changes may be attributable to the effects of cell swelling or shrinking on the width of the intercellular spaces. Studies in progress in this laboratory indicate that rabbit ileum behaves similarly. Thus, if the bathing solutions are rendered hypertonic by the addition of a high concentration of an actively transported sugar or amino acid, the effect of $\Delta\psi_{mc}$ on $\Delta\psi_{ms}$ will be diminished.

The Effect of Metabolic Inhibitors on the Electrical Potential Profile

The above analysis suggests that the effect of sugars and amino acids on ψ_{ms} can be attributed to (*a*) a primary effect of electrogenic Na influx on ψ_{mc}; and (*b*) electrical coupling between transmucosal and transmural events influenced by a low resistance transepithelial shunt. However, observations on poisoned tissues suggest that this explanation *may not* be sufficient.

As shown in Fig. 10, the addition of alanine to the mucosal solution results in a significant depolarization of ψ_{mc} even in tissues poisoned with metabolic inhibitors and ouabain. These observations are consistent with previous findings that Na-dependent alanine influx across the brush border is not affected by metabolic inhibitors or ouabain (18). Further, Curran et al. (38), using mucosal strips of ileum that had been treated with ouabain, have demonstrated that the coupled Na–alanine influx mechanism can bring about movement of Na into the epithelium against a Na concentration difference. Under these conditions, at least part of the driving force for Na movement is the chemical potential difference of alanine across the membrane. Eddy (39) has demonstrated the same phenomenon for the case of coupled Na and glycine transport by poisoned ascites tumor cells. The observations illustrated

in Fig. 10 may represent the electrical consequences of the observations by Curran et al.

However, for the purposes of the present argument, the important observation illustrated in Fig. 10 is that in spite of the marked depolarization of ψ_{mc}, the associated increase in ψ_{ms} is very small, though not negligible. Under control conditions, $\Delta\psi_{ms}$ in response to the addition of alanine or glucose averages $0.4 \Delta\psi_{mc}$; exclusion of the six high values shown in Fig. 9 yields an average $\Delta\psi_{ms}:\Delta\psi_{mc}$ of 0.3. However, in the poisoned tissues, the corrected $\Delta\psi_{ms}:\Delta\psi_{mc}$ is only 0.06. According to equation (3), this change in $\Delta\psi_{ms}:\Delta\psi_{mc}$ could result from a marked decrease in R_sR_L and/or an increase in R_3R_s. Currently there is no evidence for either of these changes. In fact, the total transepithelial resistance of poisoned tissue is significantly greater than that of the control; a decrease in the resistance of the parallel shunt must result in a decrease in transepithelial resistance.

Although it is hazardous to draw firm conclusions from experiments on poisoned tissues, these findings suggest that under control conditions an additional electromotive force that is dependent upon metabolic energy may contribute to the total $\Delta\psi_{ms}$ in response to amino acids or sugars. For example, if the Na that enters the cell across the brush border coupled to the entry of sugars or amino acids is extruded from the cell across the lateral or serosal membranes by means of an energy-dependent, electrogenic active transport mechanism, the total $\Delta\psi_{ms}$ would reflect changes in both E_m (less negative) and E_s (more positive). Inhibition of the electrogenic Na extrusion mechanism with either metabolic inhibitors or ouabain would make $\Delta\psi_{ms}$ entirely dependent on ΔE_m. Alternatively, Na could be extruded into the lateral spaces by means of an electrically neutral active transport mechanism but diffusion potentials and/or streaming potentials resulting from the movements of Na and water through these channels could bring about a contribution from E_L to the $\Delta\psi_{ms}$. Inhibition of this *neutral* pump would abolish the contribution from E_L and decrease $\Delta\psi_{ms}:\Delta\psi_{mc}$. Machen and Diamond have recently suggested that the small PD across rabbit gallbladder may be attributed to diffusion potentials generated in the lateral intercellular spaces (40).

The rapidity of the response of ψ_{ms} to metabolic inhibitors also supports the notion that an energy-dependent transport process contributes directly to the total ψ_{ms}. As noted in these studies as well as in previous studies on rabbit ileum (7), rat small intestine (41), and rat colon (42), metabolic inhibitors bring about an immediate decline in ψ_{ms} (see for example Fig. 13 of reference 41); the onset of this effect is much too rapid to be reasonably attributed to significant changes in ionic gradients. Although changes in membrane permeabilities cannot be excluded a direct effect of metabolic inhibitors on an energy-dependent transport process that contributes directly to ψ_{ms} seems a more likely explanation.

Further speculation on this point is certainly unwarranted. Suffice it to say, that a contribution to $\Delta\psi_{ms}$ in response to sugars or amino acids from a metabolically dependent electromotive force other than ΔE_m cannot be excluded and that further study of the factors that determine and influence E_s and E_L is clearly necessary. (See *Note Added in Proof*.)

Transepithelial Shunts and the Electrical Potential Profile

In recent years considerable attention has been focused on the possible role of extracellular transepithelial pathways in the transport of water and solutes

TABLE II

TRANSEPITHELIAL RESISTANCES

Tissue	Species	R	Reference
		ohm-cm²	
Ileum (S)	Rabbit	21	Present study
Ileum (U)	Rabbit	58	(7)
Ileum (U)*	Rat	60–80	(44)
Jejunum (U)*	Rat	28	(29)
Jejunum (U)	Rat	61	(45)
Small intestine (U)†	Bullfrog	120–200	(46)
Colon (S)†	Rat	140	(42)
Gallbladder (U)*, †	Fish	113	(47)
Renal proximal tubule	Rat	5–6	(48)
Skin	Frog	2500–5000	(49)
Urinary bladder	Toad	2400	(50)
Urinary bladder	Turtle	1750	(51)

(U) designates unstripped preparations.

(S) designates preparations in which part or all the outer muscle layers were removed.

* Values are approximate since cylindrical or spherical preparations were employed and the total areas are not well-defined.

† Values are overestimates since corrections for fluid resistance were not made.

by a variety of epithelial tissues (e.g. reference 43). There is also considerable evidence that these shunt pathways may be the predominant factors in determining total transepithelial electrical resistances. Relatively low transepithelial resistances are characteristic of tissues such as small and large intestine, renal tubules, and gallbladder compared to frog skin and isolated toad urinary bladder (Table II). These low resistance tissues bring about absorption of large quantities of solutes and water, have relatively high hydraulic conductivities, and possess characteristically wide and conspicuous lateral intercellular spaces. Further, wherever studied, the membranes surrounding these epithelial cells are capable of sustaining relatively large transmembrane electrical potential differences (35–80 mv). For example, Frömter

and his associates have demonstrated that the transtubular electrical potential difference in rat proximal renal tubule is close to zero and that the intracellular electrical potential may be as high as 80 mv, interior negative (52). The transtubular resistance reported by these workers is only 5–7 Ωcm^2 (48). Thus, it seems inescapable that the extremely low transepithelial resistance is due to extracellular shunts and that this may be responsible for the absence of a significant transtubular PD. Hoshi and Saki (53) have arrived at a similar conclusion with respect to the proximal tubule of the newt kidney. Further, it seems highly likely that the lateral intracellular spaces are the anatomic counterparts of this parallel low resistance shunt and that their total ionic conductance sets the upper limit for the total transepithelial resistance. This notion is consistent with the observations that factors that bring about cell swelling, such as perfusion with hypotonic solutions (36, 37) or metabolic inhibition (present studies and reference 54), increase transepithelial resistance whereas shrinking of cells by exposure to hypertonic solutions has the opposite effect (36, 37). As pointed out by Clarkson (44), in the case of the small intestine areas of denudation due to spontaneous exfoliation of cells could also contribute to low resistance transepithelial shunts.[7]

As discussed by Boulpaep (55) and Giebisch (8) low resistance transepithelial shunts severely complicate the interpretation of transmembrane and transmural electrical potential differences and their responses to changes in the surrounding media. These shunts result in coupling among electrical events at the mucosal and serosal membranes as well as electromotive forces that may arise in the shunt pathway itself. Thus, ψ_{mc} is not a measure of the electromotive force across the mucosal membrane alone unless $R_5 R_L$ is infinite. Indeed, E_m could be zero and the entire ψ_{mc} and ψ_{ms} could be attributed to E_s. Further, if the composition of the solution on the mucosal side of the tissue is changed, the resulting change in ψ_{mc} could reflect changes in E_m and/or E_L. If, on the other hand, the solutions bathing both surfaces of the tissue are changed in the same manner, E_L may be zero but ψ_{mc} could reflect changes in E_m and/or E_s. Finally, the absence of a significant transepithelial PD (or the absence of a change in PD in response to an increase in ion transport) does not rule out the possibility of electrogenic

---

The present data permit estimates of $R_1 R_m$, $R_3 R_s$, and $R_5 R_L$ from the observation that $R_3 R_s \cong 2 R_1 R_m$, equation (3), and measurements of total transepithelial resistance. According to the circuit illustrated in Fig. 12 the total transepithelial resistance is given by:

$$R_E = R_5 R_L \; (R_1 R_m + R_3 R_s) : (R_5 R_L + R_1 R_m + R_3 R_s)$$

For nonpoisoned tissue, $R_E = 21$ ohm-cm^2 and $\Delta\psi_{ms} : \Delta\psi_{mc} = 0.3$, so that $R_5 R_L \cong 28$ ohm-cm^2 $R_3 R_s \cong 56$ ohm-cm^2, and $R_1 R_m \cong 28$ ohm-cm^2. Using the data obtained on poisoned tissue, $R_5 R_L \cong 35$ ohm-cm^2; $R_3 R_s \cong 600$ ohm-cm^2; and $R_1 R_m \cong 300$ ohm-cm^2. The latter values for $R_1 R_m$ and $R_3 R_s$ more closely resemble the specific resistances of epithelial surface membranes than do the former (53, 56).

transport processes or the presence of markedly asymmetric electromotive forces across the mucosal and serosal membranes.

Note Added in Proof   Results obtained in this laboratory (Frizzell and Schultz, unpublished observations) indicate that at least 85 % of the total tissue conductance can be attributed to ionic diffusion through a transepithelial extracellular shunt pathway. These direct measurements support the contention that the relative resistance of the shunt is too low to account for the relation between $\Delta\psi_{ms}$ and $\Delta\psi_{mc}$ observed in nonpoisoned tissue assuming a change in E_m alone.

BIBLIOGRAPHY

1. Schultz, S. G., P. F. Curran, R. A. Chez, and R. E. Fuisz. 1967. Alanine and sodium fluxes across mucosal border of rabbit ileum. *J. Gen. Physiol.* **50**:1241.

2. Rose, R. C., and S. G. Schultz. 1970. Alanine and glucose effects on the intracellular electrical potential of rabbit ileum. *Biochim. Biophys. Acta.* **211**:376.

3. Schultz, S. G., R. E. Fuisz, and P. F. Curran. 1966. Amino acid and sugar transport in rabbit ileum. *J. Gen. Physiol.* **49**:849.

4. Frank, K., and M. C. Becker. 1964. Microelectrodes for recording and stimulation. *In* Physical Techniques in Biological Research. Volume V. Electrophysiological Methods. W. L. Nastuk, editor. Academic Press, Inc., New York. 22.

5. Giebisch, G. 1958. Electrical potential measurements on single nephrons of *Necturus*. *J. Cell. Comp. Physiol.* **51**:221.

6. Edmonds, C. J., and O. E. Nielsen. 1968. Transmembrane electrical potential differences and ionic composition of mucosal cells of rat colon. *Acta Physiol. Scand.* **72**:338.

7. Schultz, S. G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567.

8. Giebisch, G. 1968. Some electrical properties of single renal tubule cells. *J. Gen. Physiol.* **51**(5, Pt. 2): 315s.

9. Giebisch, G., G. Malnic, R. M. Klose, and E. E. Windhager. 1966. Effect of ionic substitutions on distal potential differences in rat kidney. *Amer. J. Physiol.* **211**:560.

10. Sullivan, W. J. 1968. Electrical potential differences across distal renal tubules of *Amphiuma. Amer. J. Physiol.* **214**:1096.

11. Schultz, S. G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. *J. Gen. Physiol.* **47**:1043.

12. Schultz, S. G., and R. Zalusky. 1965. Interactions between active sodium transport and active amino-acid transport in isolated rabbit ileum. *Nature (London).* **205**:292.

13. Schultz, S. G., and P. F. Curran. 1968. Intestinal absorption of sodium chloride and water. *In* Handbook of Physiology, Section 6. Alimentary Canal. C. F. Code, editor. American Physiological Society, Washington, D.C. 3:1245.

14. Schultz, S. G., and P. F. Curran. 1970. Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**:637.

15. Hajjar, J. J., and P. F. Curran. 1971. Characteristics of the amino acid transport system in the mucosal border of rabbit ileum. *J. Gen. Physiol.* **56**:673.

16. CURRAN, P. F., S. G. SCHULTZ, R. A. CHEZ, and R. E. FUISZ. 1967. Kinetic relations of the Na–amino acid interaction at the mucosal border of intestine. *J. Gen. Physiol.* **50**:1261.

17. SCHULTZ, S. G., and C. K. STRECKER. 1970. Fructose influx across the brush border of rabbit ileum. *Biochim. Biophys. Acta.* **211**:586.

18. CHEZ, R. A., R. R. PALMER, S. G. SCHULTZ, and P. F. CURRAN. 1967. Effect of inhibitors on alanine transport in isolated rabbit ileum. *J. Gen. Physiol.* **50**:2357.

19. SMYTH, D. H., and E. M. WRIGHT. 1966. Streaming potentials in the rat small intestine. *J. Physiol. (London).* **182**:591.

20. WRIGHT, E. M. 1966. The origin of the glucose dependent increase in the potential difference across the tortoise small intestine. *J. Physiol. (London).* **185**:486.

21. WHITE, J. F., and W. McD. ARMSTRONG. 1970. Abstracts of the Biophysical Society 14th Annual Meeting. Baltimore, Maryland. 36a.

22. GOLDNER, A. M., S. G. SCHULTZ, and P. F. CURRAN. 1969. Sodium and sugar fluxes across the mucosal border of rabbit ileum. *J. Gen. Physiol.* **53**:362.

23. PETERSON, S. C., A. M. GOLDNER, and P. F. CURRAN. 1970. Glycine transport in rabbit ileum. *Amer. J. Physiol.* **219**:1027.

24. SCHULTZ, S. G., L. YU-TU, O. O. ALVAREZ, and P. F. CURRAN. 1970. Dicarboxylic amino acid influx across the brush border of rabbit ileum. Effects of amino acid charge on the sodium–amino acid interaction. *J. Gen. Physiol.* **56**:621.

25. QUAY, J. F., and W. McD. ARMSTRONG. 1969. Enhancement of net sodium transport in isolated bullfrog intestine by sugars and amino acids. *Proc. Soc. Exp. Biol. Med.* **131**:46.

26. ARMSTRONG, W. McD., D. L. MUSSELMAN, and H. C. REITZUG. 1970. Sodium, potassium and water content of isolated bullfrog small intestinal epithelium. *Amer. J. Physiol.* **219**:1023.

27. GILLES-BAILLIEN, M., and E. SCHOFFENIELS. 1965. Site of action of L-alanine and D-glucose on the potential difference across the intestine. *Arch. Int. Physiol. Biochim.* **73**:355.

28. GILLES-BAILLIEN, M., and E. SCHOFFENIELS. 1966. Metabolic fate of L-alanine actively transported across the tortoise intestine. *Life Sci.* **5**:2253.

29. BARRY, R. J. C., D. H. SMYTH, and E. M. WRIGHT. 1965. Short-circuit current and solute transfer by rat jejunum. *J. Physiol. (London).* **181**:410.

30. BARRY, R. J. C., J. EGGENTON, and D. H. SMYTH. 1969. Sodium pumps in the rat small intestine in relation to hexose transfer and metabolism. *J. Physiol. (London).* **204**:299.

31. HOSHI, T., and Y. KOMATSU. 1970. Effects of anoxia and metabolic inhibitors on the sugar-evoked potential and demonstration of sugar-outflow potential in toad intestine. *Tohoku J. Exp. Med.* **100**:47.

32. FORDTRAN, J. S., F. C. RECTOR, JR., and N. W. CARTER. 1968. The mechanisms of sodium absorption in the human small intestine. *J. Clin. Invest.* **47**:884.

33. BARRY, R. J. C., J. EGGENTON, D. H. SMYTH, and E. M. WRIGHT. 1965. Sodium concentration and hexose-dependent potentials in the small intestine. *J. Physiol. (London).* **182**:40P.

34. SCHULTZ, S. G., P. F. CURRAN, and E. M. WRIGHT. 1967. Interpretation of hexose-dependent electrical potential differences in small intestine. *Nature (London).* **214**:509.

35. LYON, I., and R. K. CRANE. 1966. Studies on transmural potentials in vitro in relation to intestinal absorption. I. Apparent Michaelis constants for Na-dependent sugar transport. *Biochim. Biophys. Acta.* **112**:278.

36. WINDHAGER, E. E., E. L. BOULPAEP, and G. GIEBISCH. 1966. Electrophysiological studies on single nephrons. Proceedings 3rd International Congress on Nephrology, Washington, D.C. **1**:35.

37. USSING, H. H., and E. E. WINDHAGER. 1964. Nature of shunt path and active transport path through frog skin epithelium. *Acta Physiol. Scand.* **61**:484.

38. CURRAN, P. F., J. J. HAJJAR, and I. M. GLYNN. 1970. The sodium-alanine interaction in rabbit ileum. Effect of alanine on sodium fluxes. *J. Gen. Physiol.* **55**:297.

39. EDDY, A. A. 1969. A sodium ion concentration gradient formed during the absorption of glycine by mouse ascites–tumour cells. *Biochem. J.* **115**:505.

40. Machen, T. E., and J. M. Diamond. 1969. An estimate of the salt concentration in the lateral intercellular spaces of rabbit gall bladder during maximal fluid transport. *J. Membrane Biol.* **1**:194.

41. Barry, R. J. C., S. Dikstein, J. Matthews, D. H. Smyth, and E. M. Wright. 1964. Electrical potentials associated with intestinal sugar transfer. *J. Physiol. (London).* **171**: 316.

42. Edmonds, C. J., and J. Marriott. 1968. Electrical potentials and short-circuit current of an in vitro preparation of rat colon mucosa. *J. Physiol. (London).* **194**:479.

43. Diamond, J. M., and W. H. Bossert. 1967. Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* **50**:2061.

44. Clarkson, T. W. 1967. The transport of salt and water across isolated rat ileum. Evidence for at least two distinct pathways. *J. Gen. Physiol.* **50**:695.

45. Asano, T. 1964. Metabolic disturbances and short-circuit current across intestinal wall of rat. *Amer. J. Physiol.* **207**:415.

46. Quay, J. F., and W. McD. Armstrong. 1969. Sodium and chloride transport by isolated bullfrog small intestine. *Amer. J. Physiol.* **217**:694.

47. Diamond, J. M. 1962. The mechanism of solute transport by the gall bladder. *J. Physiol. (London).* **161**:474.

48. Hegel, U., E. Frömter, and T. Wick. 1967. Der electrische Wandwiderstand des proximalen Konvolutes der Rattenniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **292**:274.

49. Cereijido, M., and P. F. Curran. 1965. Intracellular electrical potentials in frog skin. *J. Gen. Physiol.* **48**:543.

50. Civan, M. M. 1970. Effects of active sodium transport on current-voltage relationship of toad bladder. *Amer. J. Physiol.* **219**:234.

51. Klahr, S., and N. S. Bricker. 1964. Na transport by isolated turtle bladder during anaerobiosis and exposure to KCN. *Amer. J. Physiol.* **206**:1333.

52. Frömter, E., and U. Hegel. 1966. Transtubulare Potentialdifferenzen an proximalen und distalen Tubuli der Rattenniere *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **291**:107.

53. Hoshi, T., and F. Sakai. 1967. A comparison of the electrical resistances of the surface cell membrane and cellular wall in the proximal tubule of the newt kidney. *Jap. J. Physiol.* **17**:627.

54. Lew, V. L. 1970. Short-circuit current and ionic fluxes in the isolated colonic mucosa of *Bufo arenarum. J. Physiol. (London).* **206**:509.

55. Boulpaep, E. L. 1967. Ion permeability of the peritubular and luminal membrane of the renal tubular cell. *In* Symposium über Transport und Funktion intracellular Elektrolyte. F. Kruck, editor. Urban and Schwarzenberg, Munich. 98.

56. Loewenstein, W. R. 1966. Permeability of membrane junctions. *Ann. N.Y. Acad. Sci.* **137**:441.