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STUDIES ON TRANSMURAL POTENTIALS *IN VITRO* IN RELATION TO INTESTINAL ABSORPTION

VI. THE EFFECT OF SUGARS ON ELECTRICAL POTENTIAL PROFILES IN JEJUNUM AND ILEUM*

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SUMMARY

1. These studies were carried out to develop a routine procedure for microelectrode recording of transmembrane potential differences (PD's) across the mucosal and serosal membranes of the intestinal epithelial cell of small mammals.

2. The transmembrane PD's obtained using rat gut preparations *in vitro* were stable, reproducible and responded rapidly to changes in the composition of the bathing media.

3. Cell impalement in normal buffer ($[Na^+] = 145$ mM) always resulted in a potential change with the cell interior registering negative relative to both the mucosal and serosal bathing solutions.

4. Transmural PD values, representing the algebraic difference of mucosal and serosal transmembrane PD's, were similar to such values measured directly and reported earlier.

5. The addition of actively transported sugars to the mucosal bathing medium increased the transserosal PD in both jejunum and ileum.

6. The addition of ouabain to the serosal bathing medium caused a depression in transmural PD and in the glucose-induced increase in transmural PD (ΔPD). Both effects appear to be due primarily to a decrease in the transserosal PD.

7. The addition of phlorizin to the mucosal bathing medium produced a depression in the glucose-dependent ΔPD. This was not observed with serosal phlorizin.

8. All the results are consistent with the concept of an electrogenic ion extrusion mechanism acting at the serosal border of the rat intestinal absorbing cell.

INTRODUCTION

Sugar active transport across the mammalian small intestine has been shown to be associated with an increase in the transmural potential difference, while addition

Abbreviations: PD, potential difference; PD_m, transmucosal PD; PD_s, transserosal PD; PD_t, transmural PD ($PD_m - PD_s$); ΔPD_t, change (increment or decrement) in PD_t induced by addition of any non-electrolyte into the medium; O_m, mucosal ouabain; O_s, serosal ouabain; Phl_m, mucosal phlorizin; Phl_s, serosal phlorizin.

* Some of the principal findings of this study were presented at the 25th International Congres of Physiological Sciences, Munich, 1971.

of non-actively transported sugars causes a decrease in this potential difference[1–7]. Microelectrode studies using tortoise small intestine[7–9] have demonstrated that the cell interior is electrically negative with respect to the surrounding medium. Furthermore, it seems that the transmural potential (PD_t) may be adequately defined as the algebraic difference between transmucosal and transserosal PD's, PD_m and PD_s, respectively, each of different magnitude and apparently acting in series. Moreover, increases in PD_t in tortoise intestine, observed in the presence of actively transported sugars, are primarily the result of increases in PD_s alone. These findings suggest that the change in transmural potential difference, ΔPD_t, associated with active transfer of sugars, may be due to the presence of an electrogenic ion extrusion mechanism at the serosal face of the epithelial cell[8]. Recent experiments using bullfrog small intestine[10] and rabbit ileum[11] suggest that the increase in PD_t due to glucose transport may be attributed chiefly to a decrease in PD_m. The investigators using rabbit ileum have noted the differences between their results and those reported previously for tortoise small intestine. They suppose these differences may relate to a mucosal or serosal site for the species-specific mechanisms responsible for active sugar transport.

Microelectrode penetration of toad bladder epithelium from the mucosal surface has indicated two different electrical potential profiles: one of these appears as a voltage change in two steps[12]; the other, as a smooth, monotonic change in electrical potential[13]. It has been suggested that the voltage steps may be associated with the outer limiting cell membranes acting as ionic barriers. The smooth profile would seem to be a reflection of a transcellular ionic gradient.

Until now, excepting the unpublished data of FIELD AND CURRAN[14] and the recent work of ROSE AND SCHULTZ[11], most absorption studies with microelectrodes have been done with amphibian or reptilian epithelial cells. The studies presented in this paper concern the development of a microelectrode recording technique which may be easily used to investigate intestinal active transport in small mammals. The data herein present the electrical potential profiles obtained with rat mucosal epithelial cells, the effects upon these profiles of the addition of various sugars to the mucosal bathing medium, and the influence upon these profiles of ouabain and phlorizin. Our findings do indeed suggest that (1) PD_t may be adequately described as the algebraic difference between PD_m and PD_s and (2) the primary effect of actively transported sugars appears to be upon PD_s. Thus, these findings are consistent with and lend support to the concept of a serosal ion extrusion mechanism coupled with sugar active transport in the mucosal epithelial cell of rat gut.

METHODS

Tissue preparation

After a fast of 24–48 h during which water was available *ad libitum*, male Sprague–Dawley rats, approx. 130 ± 20 g, were killed by cervical fracture. An excised section of small intestine, either mid-jejunum or ileum, was suspended in fresh medium in a Petri dish. The piece of gut was opened to expose the mucosal surface, rinsed clean with fresh buffer solution, and mounted in the chamber described below. The interval between time of sacrifice and time of tissue mounting never exceeded 3 min; the first impalement occurred within 5 min of sacrifice.

Media

The basic incubation medium used in these experiments was Krebs–Henseleit bicarbonate buffer[15] with the following composition: Na^+, 145 mM; K^+, 6 mM; Ca^{2+}, 1.3 mM; Mg^{2+}, 1.2 mM; Cl^-, 127 mM; SO_4^{2-}, 1.2 mM; HPO_4^{2-}, 1.2 mM; HCO_3^-, 25 mM; and pH 7.4 ± 0.2.

The agar–buffer mixture, for partial filling of the barrel of the microelectrode and for filling the central support in contact with the serosal side of the tissue, consisted of 2 g of special agar-Noble (Difco Laboratories) per 100 ml of Krebs–Henseleit bicarbonate buffer. In all experiments, the mucosal and serosal sides of the gut preparation were exposed to media of the same ionic composition and concentration. Sugars, all reagent grade, were added only to the mucosal medium. Ouabain and phlorizin solutions were prepared as previously described[4,6].

Chamber

The excised piece of gut was mounted in a simple chamber consisting of two circular lucite compartments, an outer serosal one and an inner mucosal one (Fig. 1).

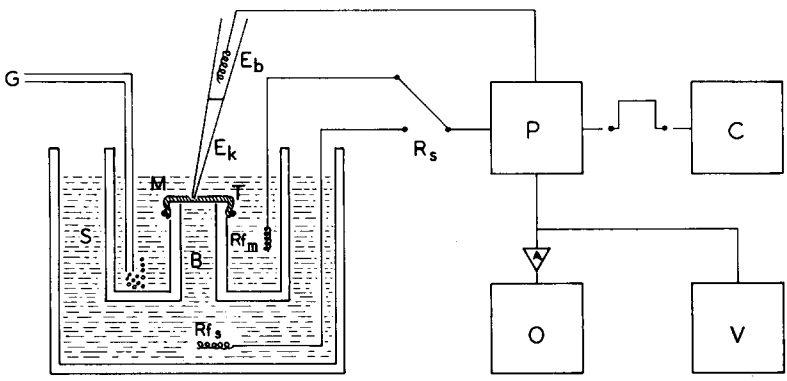

Fig. 1. Diagram of the incubation chamber and instrumentation used for the recording of transmembrane PD values. T, rat intestinal tissue, mucosa uppermost, mounted upon the central support filled with B, agar–buffer, and held in place by an O-ring; M and S, mucosal and serosal bathing media; G, gas inlet for O_2–CO_2 (95:5, v/v); Rf_m and Rf_s, mucosal and serosal reference electrodes; E_k, microelectrode tip filled with 3 M KCl; E_b, microelectrode barrel filled with agar–buffer; R_s, reference electrode reversing switch; P, preamplifier; C, square-wave generator (voltage calibrator); A, differential amplifier; O, storage oscilloscope; V, digital voltmeter.

The tissue was mounted, mucosa uppermost, upon the central support filled with the agar–buffer mixture. The serosal solution was brought into direct contact with the undersurface of the gut (serosa) through this mixture. The tissue was held in place over the open end of the central support by an O-ring. Thus, the tissue was stretched, without fluid leakage or damage, over the support. The inner (mucosal) compartment is open so that bathing media can readily be gassed and changed during the course of an experiment. The mucosal solution was gassed with O_2–CO_2 (95:5, v/v) at all times except during cell impalement when agitation due to gassing, on occasion, caused dislodgement of the microelectrode tip.

The entire chamber was placed in an outer container maintained at 37 ± 1°.

Microelectrodes

Glass micropipettes were pulled from 0.9–1.1 mm ID borosilicate capillary tubing, with a Narishige micropipette puller, to a tip diameter of < 0.3 μm. Initially, tip diameter was checked visually with a light microscope. After the relationship between tip diameter and tip resistance was established, resistance measurements alone were used to estimate tip diameter. The micropipettes were filled with freshly prepared and filtered 3 M KCl by boiling under reduced pressure. They were stored in 3 M KCl and used within 24 h. Tips were observed under a microscope to insure complete filling of the micropipettes. Microelectrodes prepared in this way had tip potentials of $< \pm 5$ mV and tip resistances usually between 40 and 55 MΩ (range, 25–75 MΩ). Tip resistance (the sum of resistances of tip and buffer prior to or after impalement; the sum of these plus intracellular resistance during impalement) was intermittently monitored during the course of the experiments, prior to, during and after impalement. Tip potential was checked before and after impalement. These checks gave assurance of stability of the electrical characteristics of the microelectrode and provided confidence in the measurements obtained. A sudden change in tip resistance to a value below 25 MΩ almost always indicated a break in the tip; these microelectrodes were immediately discarded. Tip potentials were always nulled to zero with the preamplifier balance adjustment prior to impalement.

To eliminate most of the "battery" effect, due to liquid junction potentials, each micropipette was modified so that about one-half of the 3 M KCl solution within the barrel was replaced with the agar–buffer mixture. A syringe, fitted with a blunt, smooth-tip needle was used to inject the fluid mixture into the pipette. After the agar set, the pipette was inspected for the presence of any air bubbles. These, of course, would produce an electrical resistance due to a break in the conductivity pathway through the agar–KCl mixture or between the agar–electrolyte and the Ag–AgCl wire which is inserted into it.

The microelectrode was placed in a micropipette holder and supported in a Prior micromanipulator. Between impalements, the microelectrode tip was immersed in buffer solution.

Recording of electrical potentials

The Ag–AgCl wire, inserted into the micropipette, was connected to the input terminal of a preamplifier. Ag–AgCl reference electrodes were suspended in each compartment of the chamber. The latter were connected to the bath input of the preamplifier by way of a switching device permitting measurement of intracellular potentials against either the mucosal or serosal reference set at zero. The preamplifier output was connected by shielded cable to the + (d.c.) input of a Tektronix Type 3A3 differential amplifier and to the input of a Hewlett–Packard 3430A digital voltmeter. Voltage changes were observed with a Tektronix Type 564 storage oscilloscope and actual voltage readings were obtained from the digital voltmeter. An oscilloscope calibration of 10 mV, brought into the preamplifier test terminal and observed as a square wave, provided a means for determination of tip resistance. Electrical integrity of the microelectrode, as indicated by tip potential and tip resistance, was checked against the square wave and compensation, when required, was provided by the preamplifier.

All instrumentation, except the oscilloscope and the digital voltmeter, was

housed in a Faraday cage. The cage and the individual instruments were tied together with a single shielded cable ending in an outside earth ground, completely separated from third-wire grounding in the building.

PD_m—PD_s differences were determined before the tissue was mounted in the chamber to detect and correct for any small deviations from zero inherent in the system. With the mucosal reference set at zero, these PD_t values, rarely more than ± 1 mV, were always accounted for in the recording of PD_s values.

After mounting the tissue, the microelectrode tip was inserted into an epithelial cell with the micromanipulator. The PD across the mucosal face of the cell, PD_m, was recorded against the mucosal reference. When the reference was switched to the serosal side, the PD across the serosal face of the cell, PD_s, was recorded. The calculated transmural potential, PD_t, was expressed as the algebraic difference between PD_m and PD_s. PD_t values calculated in this way compare very favorably with transmural PD's determined directly and reported previously[1-6].

Statistical variation of the data

The data presented in this report represent mean values: the standard errors in percent for all values ranged from ± 1 to ± 7 %.

General procedure

The following procedure was adopted for the experiments described herein. The microelectrodes were prepared as noted above. Krebs–Henseleit bicarbonate buffer at $37 \pm 1°$ was added to both the mucosal and serosal compartments. Preliminary measurements of tip potential, tip resistance and mucosal-serosal PD's were recorded. The animal was then sacrificed, the gut prepared and mounted and the PD's were rerecorded. Before impalement, the mounted tissue imposed an electrical asymmetry upon the system whose measured value invariably was identical with the calculated PD_t or resting transmural PD. Upon cell impalement, PD_m and PD_s values were measured and recorded. Following four impalements the microelectrode tip was withdrawn and the buffer was removed by aspiration and replaced with fresh buffer containing the substance which was to be tested. After eight impalements in the test medium, the microelectrode tip was again withdrawn and the mucosal solution removed and replaced with normal buffer. A final set of four PD_m and PD_s impalement values was then recorded. Thus, a total of eight readings with the test substance and eight readings without it, four before and four after contact with the test substance, were obtained for each tissue.

In some instances, the mucosal fluid was evacuated and replaced while holding the microelectrode tip within the cell. The values obtained in these instances did not differ from those obtained as described above.

After final impalement, the tissue was removed and the system was checked to determine whether or not any electrical changes had occurred during the time of the experiment. Any such changes noted in the mucosal–serosal PD or in the microelectrode tip potential or resistance, the latter indicating a corresponding change in the physical characteristics of the tip due to cell penetration, was cause for discarding the recorded data. It should be noted, however, that this happened very rarely during experiments which, according to the described sequence, were usually completed within 30 min from the time of sacrifice.

Visual control of impalements was provided by observing methylene blue-stained tissue with a Vickers binocular microscope mounted on a pillar stand.

RESULTS AND DISCUSSION

Profiles of electrical potentials in the absence of glucose

Our initial studies were carried out to determine the general nature of the electrical responses recorded as the microelectrode advanced through the mucosal surface of the gut. It readily became apparent that the first response originated in the absorptive epithelial cell layer and usually was characterized by a sudden decrease in potential. A deeper response, typical of that seen with impaled smooth muscle cells[16], was distinguished from the first response by slow repetitive action potentials of -25 to -50 mV amplitude. That the first response originated in the epithelial layer was confirmed by light microscopy of methylene blue-stained tissue. Simultaneous observation of the oscilloscope tracing and the spatial relationship of the microelectrode tip to the tissue surface made it clear that penetration of the mucosal membrane of the absorptive cell was responsible for the sudden decrease in electrical potential. These observations also demonstrated that the electrical changes did not originate in goblet cells nor in cells of the crypts of Lieberkühn. Thereafter, our efforts were concentrated upon the first, epithelial cell, response.

Epithelial cell impalement in Krebs–Henseleit bicarbonate buffer always resulted in a potential change with the cell interior being negative relative to both the mucosal and serosal media. With most impalements, the initial immediate fall in PD_m, to an average of about -15 mV, was followed by a slow positive drift with the potential reaching an average value between -9 and -10 mV within a minute which was stable for many minutes. With this type of profile the potential did not undergo further change when the microelectrode was advanced into the cell. In contrast, a very much smaller number of impalements was distinguished by a smooth, continuous voltage drop. It is important to note that no hysteresis effect was observed with either type of profile upon withdrawal of the microelectrode tip from the cell.

The smooth profile, similar to that observed by CHOWDHURY AND SNELL[13] in toad bladder, may be due to the microelectrode tip moving through an intracellular ionic gradient. Presumably this gradient may be maintained by specific ionic conductances at the mucosal and serosal borders of the impaled cell.

It seems reasonable to suppose that the decrease in PD_m is the consequence of microelectrode penetration of the mucosal barrier of the cell. Similarly, the change in PD_s at the opposite side of the cell may be ascribed to the serosal barrier. In instances when more than two steps in potential are observed, it is possible that these steps may represent penetration of discreet intracellular ionic compartments, perhaps associated with particular cell organelles. Alternatively, they may be due to exit of the microelectrode tip into the lateral spaces between adjacent cells and subsequent penetration into the neighboring cell. This is more likely, as has been observed under the microscope, in those instances when the microelectrode tip enters the mucosal surface at an angle.

Variations in the measured transmembrane PD's of rat jejunal epithelial cells, in the absence of added substrates (A) and in the presence of glucose (B), with the tissue preparation bathed on both sides in Krebs–Henseleit bicarbonate buffer, are

shown in the distribution curves of Fig. 2. More than two-thirds of 790 successful impalements in buffer alone gave values between −6.18 and −12.70 mV (mean ± S.E., −9.44 ± 0.12 mV; range, −2.5 to −22.5 mV). These PD_m values are quite similar to those observed by WRIGHT[9] in tortoise small intestine and those referred to by FIELD AND CURRAN[14] in unpublished experiments with rabbit ileum. However, our values differ somewhat from those obtained by GILLES-BAILLIEN AND SCHOFFENIELS[8] in tortoise small intestine and from the data of ROSE AND SCHULTZ[11] in rabbit ileum. Comparisons of all these data suggest that the differences in recorded PD_m values may be due to microelectrode differences rather than species differences alone.

Variations in PD_s recordings obtained in each of the impalements also are shown in Fig. 2. Again, more than two-thirds of these values lie between −9.06 and −15.78 mV (mean ± S.E., −12.42 ±0.12 mV; range, −5.0 to −25.5 mV). The algebraic difference between PD_m and PD_s values gives a mean PD_t value of +2.98 mV which is similar to previously reported transmural PD values[3–6].

The effect of D-*mannitol on transmembrane PD's*

Assessment of the influence of graded concentrations of D-glucose, and of other sugars and substrates, upon transmembrane PD's requires correction for osmotic disequilibria. Experiments with isosmotic concentrations of D-mannitol in place of

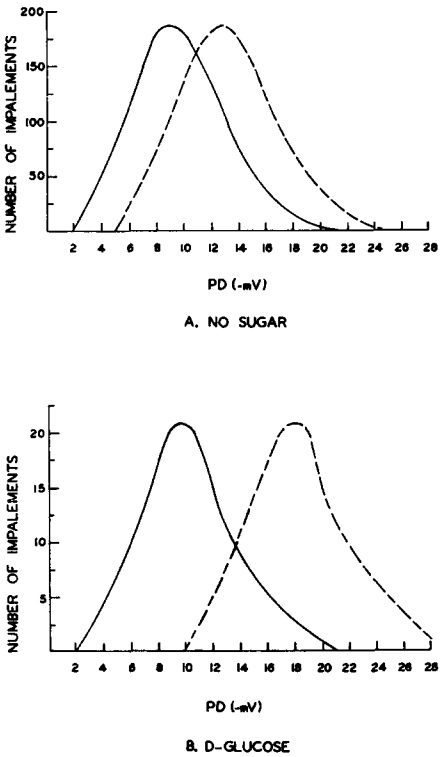

Fig. 2. Distribution curves showing the variation in the measured transmembrane PD's of rat jejunal epithelial cells. All measurements were recorded following stabilization which occurred within 1 min of impalement. A. In the absence of added substrates. B. In the presence of 20 mM D-glucose. ———, PD_m; — — —, PD_s.

various sugars were carried out to evaluate the necessary corrections. Calculated PD_t values were depressed at all concentrations of D-mannitol up to 20 mM (Fig. 3). These values have been used to correct all sugar PD_t data for the osmotic effects of their graded concentrations. The PD_m at 20 mM D-mannitol was -9.39 ± 0.81 mV ($n = 16$), a value practically the same as that obtained in the presence of buffer alone. PD_s, however, fell to -10.68 ± 0.78 mV ($n = 16$), corresponding to a decrease in PD_t of 1.69 mV relative to the PD_t in normal buffer (see Table I). The addition of D-mannitol to the mucosal compartment during impalement did not alter PD_m while PD_s decreased by 1.65 ± 0.19 mV ($n = 10$). With ileal tissue PD_m increased

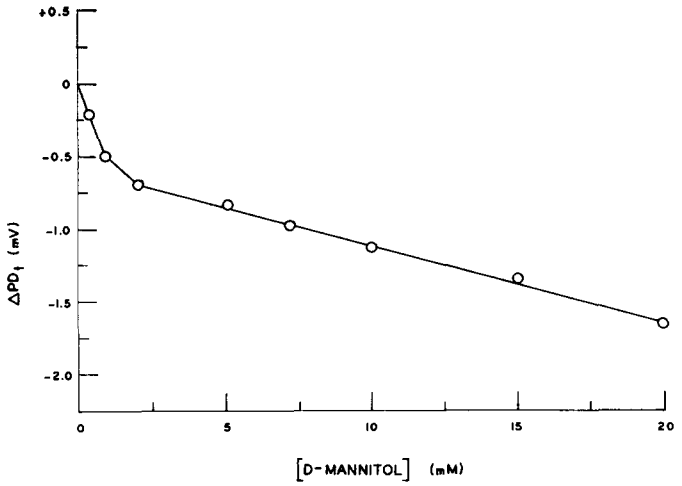

Fig. 3. Effect of graded concentrations of D-mannitol upon the PD_t in rat jejunum. Concentrations used were 0.5, 1.0, 2.0, 5.0, 7.0, 10.0, 15.0, and 20.0 mM. Each point represents the mean of at least 16 impalements.

TABLE I

COMPARISON OF THE EFFECTS OF 20 mM D-GLUCOSE UPON JEJUNAL AND ILEAL PD_m AND PD_s VALUES

Measurements of PD_m and PD_s are represented by the mean $\pm$ S.E. All values are expressed in mV. PD_t values are calculated as the algebraic difference between PD_m and PD_s data. The last two columns compare ΔPD_t values obtained in these studies with those measured directly as transmural PD's and reported elsewhere.

	PD_m	PD_s	PD_t	ΔPD_t	
				This study	*Previous studies (ref. No.)*
A. Jejunum					
1. Buffer alone ($n = 790$)	-9.44 ± 0.12	-12.42 ± 0.12	$+2.98$		
2. With D-glucose ($n = 53$)	-10.33 ± 0.68	-17.64 ± 0.69	$+7.31$	$+4.33$	$+4.27$ (1, 4)
B. Ileum					
1. Buffer alone ($n = 116$)	-10.14 ± 0.34	-13.14 ± 0.35	$+3.00$		
2. With D-glucose ($n = 12$)	-9.76 ± 0.32	-16.95 ± 0.33	$+7.19$	$+4.19$	$+5.50$ (1, 2, 4)

by 0.44 ± 0.05 mV ($n = 9$) and PD_s fell 1.11 ± 0.06 mV ($n = 9$). It is clear that the primary effect of D-mannitol is on the transserosal electrical barrier with the net effect resulting in a decrease in the calculated PD_t. The direction of these effects and their magnitude correspond closely to directly measured transmural data[1,5].

Profiles of electrical potentials in the presence of D-*glucose*

Distributions of PD_m and PD_s values obtained in the presence of 20 mM D-glucose are shown in Figure 2B. The mean PD_m value in the presence of D-glucose, −10.33 ± 0.68 mV ($n = 53$), did not differ significantly from the PD_m value, −9.44 mV, in its absence. However, the mean PD_s value with D-glucose, −17.64 ± 0.69 mV ($n = 53$), does differ from the value of PD_s, −12.42 mV, in its absence. Thus, it is clear that the primary transmembrane effect of glucose is upon the serosal barrier of the cell. The calculated PD_t in the presence of 20 mM D-glucose is +7.31 mV [−10.33 − (−17.64)], an increase of 4.33 mV over the value recorded in the absence of D-glucose (+2.98 mV). The calculated glucose-dependent ΔPD_t is essentially the same as glucose-dependent ΔPD values reported earlier[1–6].

In an attempt to verify the sugar effect upon PD_s, experiments were carried out in which the mucosal buffer was removed by aspiration and replaced by a sugar-containing medium while the microelectrode remained in place within the cell. Replacement of the buffer with 20 mM D-glucose caused no change in PD_m. However, PD_s showed a net increase of 4.09 ± 0.33 mV (Fig. 4). These findings confirm the observations described above in which ΔPD_t values due to the presence of D-glucose are essentially changes in PD_s, rather than in PD_m and PD_s.

Electrical profiles in rat ileum

Rat ileal preparations tested in the manner indicated above, yielded profiles which were very similar to those obtained with the jejunum. In 116 impalements in the absence of sugar, the PD_m value (mean ± S.E.) was −10.14 ± 0.34 mV while the PD_s value was −13.14 ± 0.35 mV. The calculated PD_t was 3.00 mV. The addition

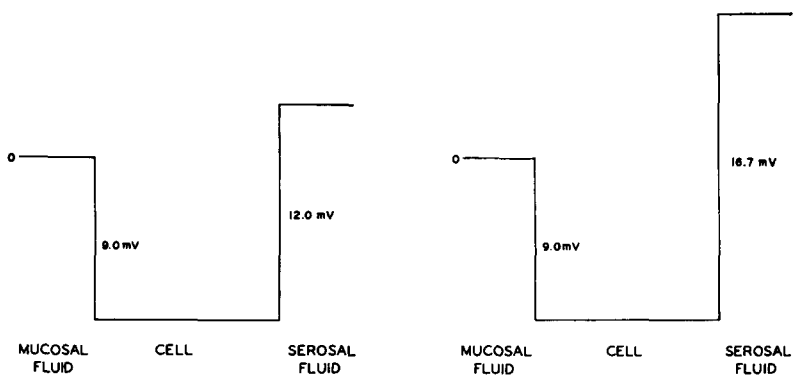

Fig. 4. Potential profiles across the intestinal epithelial cells of rat jejunum. A. In the absence of sugar. B. In the presence of 20 mM D-glucose. Profiles represent direct effects of solution changes during impalement. Diagrams depict the results of a typical impalement.

of 20 mM D-glucose caused a slight decrease in the PD_m value of 0.38 ± 0.33 mV ($n = 12$). This amounted to a glucose-dependent change in PD_t of 4.19 mV (compare with 4.33 mV for the jejunal ΔPD_t value). Transmembrane PD's recorded during ileal impalement, gave values which did not differ from the ileal values cited above.

Table I summarizes the effects of D-glucose in both jejunum and ileum. It is clear that ileal transmembrane electrical responses parallel those obtained with jejunal preparations both in the absence and presence of D-glucose.

The sugar-dependent increment of transmural PD, ΔPD, is associated with the active transport of D-glucose in the small intestine of many animals[1,3,9]. It has been suggested that the substrate-dependent (sugar or amino acid) increase in PD across the small intestine of the tortoise is due to the presence of an electrogenic ion pump located at the serosal or lateral membranes of the epithelial cell[8,9]. The data presented in this report for rat jejunum and ileum are certainly consistent with this hypothesis.

Effects of graded concentration of sugars upon jejunal PD_t values

The effects of graded sugar concentrations upon PD_m and PD_s measurements, and upon the calculated PD_t values, were studied with five different sugars. The results are shown as double-reciprocal plots in Fig. 5. It may be seen that these sugars appear to interact with the mucosal epithelial cell in a manner characteristic of that commonly observed with actively transported sugars. The vertical intercepts suggest that these sugars have approximately the same v_{max} value, about 4 mV (range, 3.6 to 4.5 mV). The K_m values for these sugars varied from 4 to 25 mM: D-glucose,

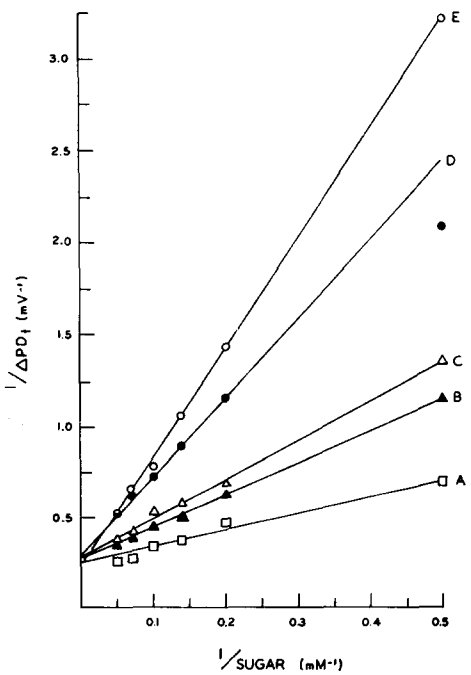

Fig. 5. Double-reciprocal plots showing the effects of graded concentrations of 5 different sugars upon the PD_t. Concentrations used were 2, 5, 7, 10, 15 and 20 mM. Each point represents the mean of 16 impalements. A, D-glucose; B, D-galactose; C, D-fucose; D, 3-*O*-methyl-D-glucose; E, D-xylose.

4.3 mM; D-galactose, 6.8 mM; D-fucose, 8.3 mM; 3-*O*-methyl-D-glucose, 15.4 mM; and D-xylose, 25.0 mM. These v_{max} and K_m values are similar to those found elsewhere[2–6,17]. For example, mean K_m values in the literature vary from an average of 6.0 mM for D-glucose, 9.5 mM for D-galactose, 13.5 mM for 3-*O*-methyl-D-glucose to 24.8 mM for D-xylose. The fact that D-xylose can be accumulated against a concentration difference has been reported previously[18].

Effects of various monosaccharides upon PD_t values

Table II shows the relative effects, at 10 mM, of a number of D- and L-sugars upon PD_t values calculated from PD_m and PD_s data measured in rat jejunal and ileal preparations. Sugars known to be actively transported appear at the top of the list and their effects are much the same in both sections of the gut. Among the L-sugars, tested only in the jejunum, L-xylose was most effective. The marked jejunal-ileal differences due to D-fructose, D-mannose and 2-deoxy-D-glucose are rather striking and will be studied further to more fully understand the nature of their interactions with the membranes of intestinal epithelial cells.

The data in Table II give a good indication of the importance and effects of the C-2 and C-6 hydroxyl groups, through their presence and their spatial orientation, upon active transportability. The sugars having a C-2 hydroxyl group with the D-

TABLE II

EFFECT OF VARIOUS SUGARS UPON PD_t VALUES

All sugars were tested at 10 mM; ΔPD_t values represent the mean ± S.E. and are expressed in mV.

Sugar	*Number of impalements*	ΔPD_t
A. Jejunum		
D-Glucose	16	+3.31 ± 0.19
D-Galactose	16	+2.29 ± 0.10
6-Deoxy-D-glucose	16	+1.94 ± 0.19
D-Fucose	16	+1.93 ± 0.11
D-Xylose	16	+1.32 ± 0.07
3-*O*-Methyl-D-glucose	16	+1.29 ± 0.04
L-Xylose	16	+1.10 ± 0.09
L-Fucose	16	+0.78 ± 0.28
L-Rhamnose	16	+0.51 ± 0.01
L-Glucose	8	+0.38 ± 0.02
L-Mannose	16	+0.22 ± 0.10
D-Fructose	16	+0.16 ± 0.03
D-Mannose	16	+0.13 ± 0.06
2-Deoxy-D-glucose	16	+0.11 ± 0.03
L-Sorbose	16	+0.04 ± 0.10
2-Deoxy-D-galactose	16	+0.01 ± 0.26
B. Ileum		
D-Glucose	16	+4.34 ± 0.01
D-Galactose	16	+3.81 ± 0.34
3-*O*-Methyl-D-glucose	16	+1.82 ± 0.04
D-Mannose	16	+1.75 ± 0.22
D-Xylose	8	+1.46 ± 0.02
D-Fructose	16	+0.65 ± 0.19
2-Deoxy-D-glucose	16	+0.60 ± 0.16

glucose configuration obviously have marked effects upon jejunal and ileal PD_t values. The importance of this hydroxyl group and its orientation relative to the affinity of sugars for the intestinal active transport mechanism are well known[6,19–20]. When this group is in the axial orientation as in D-mannose, or is absent as in 2-deoxy-D-glucose, active transport is greatly diminished or does not occur. The absence of the C-6 hydroxyl group, as in 6-deoxy-D-glucose and in D-fucose, diminishes somewhat the effect of the sugar upon the PD_t value and decreases the apparent affinity of the sugar for the membrane carrier, when compared with D-glucose (see Fig. 5). The absence of the C-6 carbon atom, as in D- and L-xylose, also decreases the affinity of the sugar for the carrier but does not entirely abolish it (see Fig. 5). These findings, relating the influence of the C-6 carbon atom and the presence or absence of its hydroxyl group to sugar transportability, confirms the suggestion that this position in the monosaccharide molecule has considerably less influence than does the C-2 group.

Effects of mucosal and serosal ouabain upon transmembrane PD's

In jejunum and ileum, mucosal ouabain (O_m) causes a small depression in PD_t

TABLE III

THE EFFECTS OF MUCOSAL AND SEROSAL OUABAIN UPON TRANSMEMBRANE PD'S IN RAT JEJUNUM AND ILEUM

Ouabain concentration, 0.1 mM; D-glucose concentration, 20 mM; PD_m and PD_s values are represented by the mean ± S.E.; PD's are expressed in mV.

	PD_m	PD_s	PD_t	ΔPD_t	
				Caused by D-*glucose*	*Caused by ouabain*
A. Jejunum					
Buffer alone *			+2.98		
Mucosal glucose, mucosal ouabain					
Ouabain ($n = 24$)	−10.72 ± 0.57	−13.52 ± 0.57	+2.80		−0.18
Ouabain + glucose ($n = 24$)	− 8.42 ± 0.40	−15.62 ± 0.46	+7.20	+4.40	
Mucosal glucose, serosal ouabain					
Ouabain ($n = 28$)	− 9.63 ± 0.40	−11.29 ± 0.39	+1.66		−1.32
Ouabain + glucose ($n = 32$)	− 9.61 ± 0.48	−14.03 ± 0.47	+4.42	+2.76	
B. Ileum					
Buffer alone *			+3.00		
Mucosal glucose, mucosal ouabain					
Ouabain ($n = 24$)	−10.55 ± 0.58	−13.11 ± 0.58	+2.56		−0.44
Ouabain + glucose ($n = 24$)	− 8.22 ± 0.36	−15.88 ± 0.36	+7.66	+5.10	
Mucosal glucose, serosal ouabain					
Ouabain ($n = 24$)	− 9.42 ± 0.47	−11.51 ± 0.47	+2.09		−0.91
Ouabain + glucose ($n = 24$)	− 9.41 ± 0.49	−15.11 ± 0.49	+5.70	+3.61	

* See Table I.

values (Table III). The ΔPD_t, caused by the addition of D-glucose, is elevated when the sugar is added either before or after O_m, or together with the cardiotonic steroid. This enhancement effect varies from a slight one in the jejunum to a greater one in the ileum. The O_m enhancement of the glucose-dependent ΔPD_t seems to be due chiefly to a direct influence of ouabain upon the mucosal surface of the cell. This effect appears as a lowering of the PD_m. The slight fall in PD_s, noted with O_m, could be a reflection of the PD_m effect mediated through the intracellular Na^+ gradient.

Serosal ouabain (O_s) causes a 44 % reduction in PD_t, a much greater inhibition than that noted with O_m in both sections of the gut. With O_s, too, the sugar-dependent increase in PD_t was lower in the presence of the steroid, especially in the jejunum. It seems clear that O_s has its primary effect upon the serosal border of the cell, causing a decrease in PD_s, both in the presence and absence of mucosal glucose. These findings are fully in accord with data suggesting the existence of a basally located, ouabain-sensitive, electrogenic Na^+-extrusion mechanism in mucosal epithelial cells of the small intestine[7–9].

TABLE IV

THE EFFECTS OF MUCOSAL AND SEROSAL PHLORIZIN UPON TRANSMEMBRANE PD'S IN RAT JEJUNUM AND ILEUM

Phlorizin concentration, 10^{-6} M; D-glucose concentration 20 mM; PD_m, and PD_s values are represented by the mean ± S.E.; PD's are expressed in mV.

	PD_m	PD_s	PD_t	ΔPD_t	
				Caused by glucose	*Caused by phlorizin*
A. Jejunum					
Buffer alone *			+2.98		
Mucosal glucose, mucosal phlorizin					
Phlorizin ($n = 32$)	−9.63 ± 0.51	−12.63 ± 0.50	+3.00		+0.02
Phlorizin + glucose ($n = 24$)	−8.31 ± 0.48	−14.19 ± 0.48	+5.88	+2.88	
Mucosal glucose, serosal phlorizin					
Phlorizin ($n = 24$)	−9.77 ± 0.37	−12.62 ± 0.37	+2.85		−0.13
Phlorizin + glucose ($n = 24$)	−9.62 ± 0.40	−16.86 ± 0.43	+7.24	+4.39	
B. Ileum					
Buffer alone *			+3.00		
Mucosal glucose, mucosal phlorizin					
Phlorizin ($n = 24$)	−9.52 ± 0.43	−12.62 ± 0.41	+3.10		+0.10
Phlorizin + glucose ($n = 24$)	−9.05 ± 0.38	−15.61 ± 0.43	+6.56	+3.46	
Mucosal glucose, serosal phlorizin					
Phlorizin ($n = 24$)	−9.52 ± 0.37	−12.51 ± 0.37	+2.99		−0.01
Phlorizin + glucose ($n = 24$)	−9.52 ± 0.46	−16.72 ± 0.47	+7.20	+4.19	

* See Table I.

Effects of mucosal and serosal phlorizin upon transmembrane PD's

The addition of 10^{-6} M phlorizin to the mucosal medium (Phl_m), in the absence of D-glucose, caused a very small increase in PD_t as a result of minor effects upon PD_m and/or PD_s values (Table IV). The small increases in PD_t values are consistent with the phlorizin-dependent increment of PD values reported earlier[6]. This effect of phlorizin may be due to the release of glucose from the glucoside by a β-glucosidase (phlorizin hydrolase) present in the microvillar membrane of the intestinal brush border[21]. In the presence of 20 mM D-glucose, however, Phl_m inhibits the glucose-induced ΔPD_t about 33 % in the jejunum and about 18 % in the ileum. Manifestation of these inhibitory effects with mucosal phlorizin may be the result of a direct inhibition associated with the mucosal membrane and the subsequent decrease in transmucosal glucose entry. These effects, in turn, could be expected to be reflected in a depression in the transcellular glucose–Na^+ gradient with a diminution in active cation extrusion at the serosal border.

Serosal phlorizin, Phl_s, had no effect on the PD_m, PD_s, PD_t or the glucose-dependent ΔPD_t.

Detailed studies of the effects of both phlorizin and ouabain will be presented in future reports.

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