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Electrical Properties and Active Solute Transport in Rat Small Intestine

I. Potential Profile Changes Associated with Sugar and Amino Acid Transports

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Summary. Addition of D-glucose to the mucosal fluid resulted in a significant depolarization of the mucosal membrane potential (V_m) in rat duodenum, jejunum, and ileum accompanied by an increase in the transepithelial potential difference (PD_t). On the other hand, L-glucose did not induce PD_t and V_m changes. Glycine applied from the mucosal side also induced V_m-depolarization and PD_t-increment in the ileum. Phlorizin added to the mucosal fluid or ouabain added to the serosal fluid inhibited the sugar-dependent changes in PD_t and V_m.

According to the analysis with an equivalent circuit model for the epithelium, it was concluded that an actively transported solute induced not only a depolarization of the mucosal (brush border) membrane but also a hyperpolarization of the serosal (baso-lateral) membrane of an epithelial cell, so that the origin of solute-induced PD_t changes should be attributed to changes in emf's at both membranes. The hyperpolarization of the serosal membrane in the presence of an actively transported solute was attributed to a mechanism of serosal electrogenic sodium pump stimulated by the increase in the extrusion rate of Na^+ co-transported into the cell with sugar or amino acid.

It is a well-known fact that an active solute transport is associated with an increase in transepithelial potential difference (PD_t) of the small intestine (Barry, Dickstein, Matthews, Smyth & Wright, 1964; Schultz & Zalusky, 1964*b*; Lyon & Crane, 1966; Hoshi & Komatsu, 1968). However, the problem remains regarding interpretation of the origin of this solute-evoked PD_t change. Failing to observe a change in the mucosal membrane potential (V_m) in the presence of an actively transported solute, some investigators ascribed it to an increase in the emf of serosal (baso-lateral) membrane (Gilles-Baillien & Schoffeniels, 1965; Wright, 1966; Lyon & Sheerin, 1971; Barry & Eggenton, 1972), whereas others reported a significant depolarization of V_m due to an active solute transport, interpreting such a solute-evoked increase in PD_t

chiefly by a decrease in the emf of mucosal (brush border) membrane (Rose & Schultz, 1971; White & Armstrong, 1971).

Recently we reported that the membrane potential of the epithelial cell in rat small intestine under normal conditions was considerably greater than that hitherto reported (Okada, Sato & Inouye, 1975; Okada, Irimajiri & Inouye, 1976*a*). Experimental evidence for the existence of an electrogenic sodium pump on the serosal membrane was obtained in the course of these experiments (Okada & Inouye, 1976*b*; Okada, Irimajiri, Tsuchiya & Inouye, *in preparation*). In the present study, effects of glucose and glycine on the electrical potential profiles were studied to elucidate the origin of a solute-evoked PD_t change, and particular attention was given to the possible contribution of an electrogenic sodium pump to this process.

Materials and Methods

Essentially the same methods as those used in our previous work (Okada *et al.*, 1975) were employed. Briefly, a sheet of small intestine was prepared from adult rats of either sex fasted for 24–48 hr except for water provided *ad libitum*. The transepithelial potential difference (PD_t) and the mucosal membrane potential (V_m) were measured with respect to the reference electrode in the mucosal fluid. Recording microelectrodes filled with 3 M KCl solution were prepared by the glass fiber method (Tasaki, Tsukahara, Ito, Wayner & Yu, 1968). Glass fibers and tubings were pretreated with a strong acid in order to obtain microelectrodes with low tip potential (Okada & Inouye, 1976*a*). Resistances and tip potentials ranged from 10 to 28 MΩ (mean: 15 MΩ) and from 0 to −4.6 mV (mean: −1.6 mV), respectively. Criteria for an acceptable impalement were the same as described previously (Okada *et al.*, 1975).

Water jacketing was used to maintain the temperature of the tissue constant with the accuracy of 1 °C during each experiment. Bath temperatures were 35–37 °C (as control) and 2 °C (cooling).

Anoxia of the tissue was achieved by bubbling the bathing solutions on both sides with pure N_2.

Control mucosal and serosal fluids were phosphate-buffered saline containing (in mM) NaCl, 127.0; KCl, 2.7; $CaCl_2$, 0.9; $MgCl_2$, 0.5; Na_2HPO_4, 8.0; KH_2PO_4, 1.5; and mannitol, 20.0 (pH 7.3 ± 0.1). The K^+-free saline was prepared by replacing of KCl and KH_2PO_4 with NaCl and NaH_2PO_4 on an equimolar basis. When glucose or glycine was applied to tissue preparations, mannitol in the mucosal fluid was replaced by 20 mM D-glucose or 20 mM glycine to maintain the osmolarity of the bathing fluids constant. Ouabain (Merck) and phlorizin (Wako) were added cumulatively to the test solutions.

All data are the means ± SE.

Results

Effect of Glucose on the Transmural Potential Difference

When the mucosal fluid was replaced with a phosphate-buffered saline containing 20 mM D-glucose, increases in the transepithelial potential

Table 1. Effect of 20 mM glucose on the transepithelial potential difference in rat small intestine

Solute	Tissue	n[a]	PD_t		ΔPD_t (mV)
			Control (mV)	Glucose (mV)	
D-Glucose	Duodenum	9	2.3±0.4	4.9±0.3	+2.6
D-Glucose	Jejunum	5	2.0±0.3	5.7±0.4	+3.7
D-Glucose	Ileum	7	1.3±0.4	5.4±0.2	+4.1
L-Glucose	Duodenum	3	2.2±0.3	2.2±0.3	0

[a] n: the number of individual experiments.

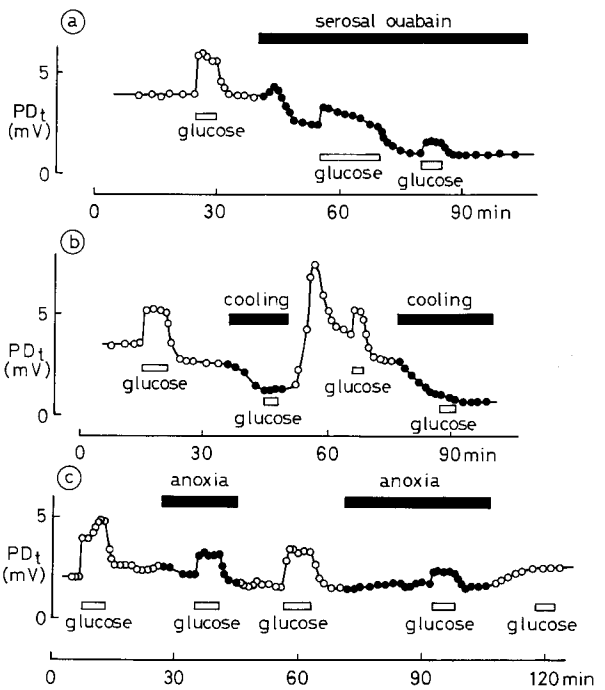

Fig. 1. Sugar-evoked PD_t changes in rat duodenum, and effects of serosal ouabain (*a*), cooling (*b*) and anoxia (*c*) thereon

differences (PD_t) were always observed irrespective of whether the small intestine, duodenum, upper jejunum or lower ileum was used as the test preparation, as shown in Table 1 and Fig. 1. Such a glucose-dependent increment in PD_t was slightly larger in the lower small intestine than in the upper small intestine. On the other hand, L-glucose brought

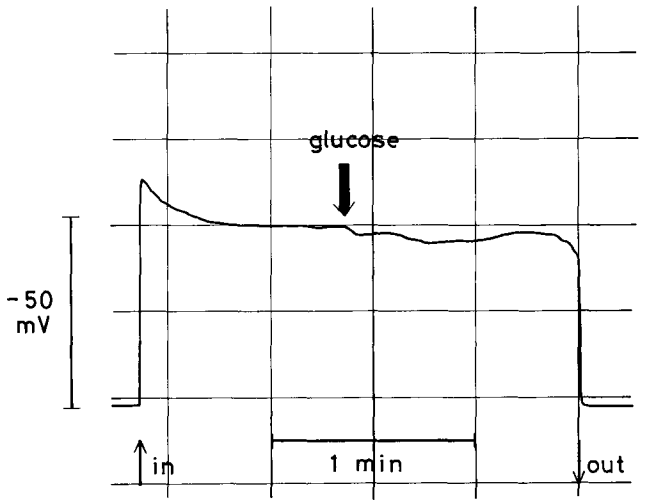

Fig. 2. Recording of the membrane potential in rat duodenum exemplifying transient membrane depolarization by adding D-glucose into the mucosal fluid

about no changes in PD_t, a finding which is in accord with the observation made by Lyon & Sheerin (1971). Since the sugar-evoked potential is closely related to the active sugar transport in the small intestine of various animals (Barry *et al.*, 1964; Schultz & Zalusky, 1964*b*; Lyon & Crane, 1966; Wright, 1966; Hoshi & Komatsu, 1968), the activity of sugar active transport appears to be slightly higher in the lower small intestine of rat than in the upper one, as the extracellular shunt conductance was not so different in both tissues (Okada, Irimajiri & Inouye, 1977).

Depolarizing Effect of Glucose on the Membrane Potential

In order to observe the effect of D-glucose on the mucosal membrane potential (V_m), droplets of high concentrated D-glucose (300 mM) were added to the perfused mucosal saline after the measured V_m value had reached a stable state. On addition of glucose, a transient small depolarization of V_m occurred as shown in Fig. 2. To observe such a depolarizing effect of glucose at a steady state, a control phosphate-buffered saline on the mucosal side was replaced by turns with a phosphate-buffered saline containing 20 mM D-glucose after five successful impalements of

Table 2. Effect of 20 mM glucose on the mucosal membrane potential in rat small intestine

Solute	Tissue	V_m		ΔV_m (mV)	Significance of difference from controls
		Control (mV)	Glucose (mV)		
D-Glucose	Duodenum	-56.6 ± 0.9 (48)	-53.1 ± 0.9 (49)	+3.5	$P<0.01$
D-Glucose	Jejunum	-54.9 ± 0.9 (34)	-51.4 ± 1.1 (26)	+3.5	$P<0.05$
D-Glucose	Ileum	-54.3 ± 0.1 (30)	-49.9 ± 1.3 (14)	+4.4	$P<0.05$
L-Glucose	Duodenum	-52.5 ± 1.3 (10)	-52.5 ± 1.2 (8)	0	$P \simeq 1$

Numbers in parentheses indicate number of observations.

the microelectrode into the epithelial cells. One example of the results thus obtained is as follows:

$$-55.5 \pm 3.3 \text{ mV (control)} \rightarrow -51.1 \pm 3.0 \text{ mV (glucose)}$$
$$\rightarrow -55.0 \pm 3.2 \text{ mV (control)} \rightarrow -53.8 \pm 2.3 \text{ mV (glucose)}$$
$$\rightarrow -57.7 \pm 2.0 \text{ mV (control)} \rightarrow -60.0 \pm 1.5 \text{ mV (glucose)}$$
$$\rightarrow -60.9 \pm 1.9 \text{ mV (control)}$$

These results suggest that exposure of the cells to glucose brings about depolarization (decrease in V_m), and the V_m values tended to increase (hyperpolarize) gradually after repeated exposure to D-glucose. Averages of all data obtained by similar measurements are presented in Table 2 which shows that V_m was depolarized significantly in the presence of D-glucose ($P<0.05$). This finding is consistent with the observations made by Rose and Schultz (1971) and White and Armstrong (1971). Such a sugar-dependent depolarization of V_m in rat small intestine is greater in the lower part of the small intestine than in the upper one. L-glucose did not affect the V_m values, nor the PD_t values.

Hyperpolarizations Due to Repeated Exposure to Glucose

The V_m values listed in Table 2 consist of two groups, one which already had been exposed twice or more to D-glucose and the other which had never been exposed. As summarized in Table 3, the above-suggested hyperpolarization of V_m induced by repeated exposure to glucose was statistically significant at a 5% level both in the absence and presence of glucose. The measured V_m values depend, as described later (Eq. 2), not only upon the emf of mucosal membrane (E_m) but also

Table 3. Comparison of V_m values between exposure and nonexposure of the duodenum to D-glucose

	V_m		ΔV_m (mV)
	Control (mV)	Glucose (mV)	
(1)[a]	-53.9 ± 1.6 (15)[c]	-51.1 ± 1.1 (25)[c]	+2.8
(2)[b]	-57.8 ± 1.1 (33)[c]	-55.3 ± 1.1 (24)[c]	+2.5
Significance of difference between (1) and (2)	$P<0.05$	$P<0.05$	–

[a] V_m obtained in the epithelial cells which had not been previously exposed to D-glucose.
[b] V_m obtained in the epithelial cells which had been previously exposed to D-glucose.
[c] Numbers in parentheses indicate number of observations.

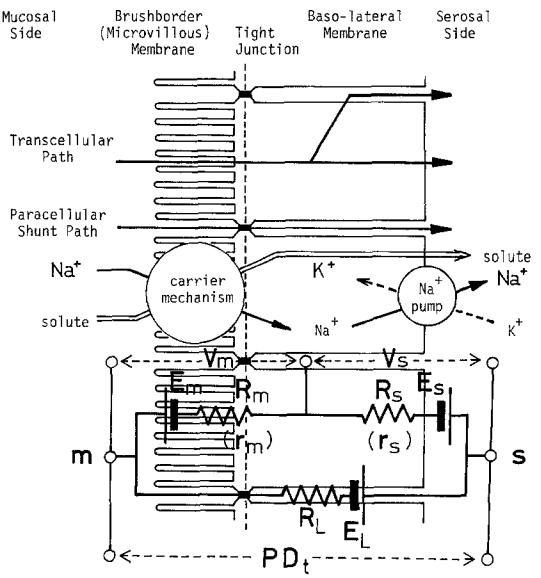

Fig. 3. A schematic view of transepithelial pathways and an equivalent circuit model for an intestinal epithelium. E_m and E_s are the emf's for the mucosal (brush border) and serosal (baso-lateral) membranes, respectively. E_L is the diffusion potential through the transepithelial shunt pathway. r_m and r_s are the resistances of the mucosal and serosal membranes of an epithelial cell, respectively. R_m and R_s are the lumped resistances of the mucosal and serosal membranes in an epithelial layer, respectively. R_L represents a transepithelial shunt resistance. *m* and *s* designate the mucosal and serosal fluids, respectively

upon the emf of serosal membrane (E_s) because of the presence of a relatively low resistance extracellular shunt pathway (Frizzell & Schultz, 1972). Many investigators have claimed the existence in intestinal epithelia of an electrogenic sodium pump located on the serosal membrane (Schultz & Zalusky, 1964*b*; Crane, 1965; Gilles-Baillien & Schoffeniels,

Table 4. Effect of 20 mM glycine on the electrical potentials in rat small intestine

Tissue	PD_t		ΔPD_t (mV)	V_m		ΔV_m (mV)
	Control (mV)	Glycine (mV)		Control (mV)	Glycine (mV)	
Duodenum	1.7±0.2(7)	2.1±0.2(7)	+0.4	−56.0±0.6(69)	−55.8±0.9(38)	+0.2
Jejunum	3.3±0.6(6)	3.8±0.6(6)	+0.5	−54.7±0.8(27)	−54.2±0.8(25)	+0.5
Ileum	1.8±0.4(4)	4.9±0.4(4)	+3.1	−55.3±0.9(47)	−50.0±1.1(43)	+5.3

Numbers in parentheses indicate number of observations.

1965; Wright, 1966; Lyon & Sheerin, 1971; Barry & Eggenton, 1972; Okada & Inouye, 1976*b*; Okada *et al., in preparation*). The results shown in Table 3 therefore suggest the possibility that the electrogenic sodium pump remains stimulated to a certain degree after withdrawal of glucose from the mucosal fluid.

Effect of Glycine on Electrical Potentials

Table 4 summarizes the effect of 20 mM glycine on the measured potentials. The changes in PD_t suggest that the activity of glycine transport is remarkably low in rat duodenum or jejunum, but fairly high in the ileum. The V_m changes induced by mucosal glycine were quite small in the duodenum and jejunum and were not statistically significant ($P>0.25$) as shown in Table 4. On the contrary, V_m in the ileum was depolarized remarkably in the presence of glycine ($P<0.005$), being consistent with the previous observations made in rabbit ileum (Rose & Schultz, 1971) and in bullfrog small intestine (White & Armstrong, 1971) when other amino acids were used.

Effect of Phlorizin

The effect of 10^{-4} M phlorizin added to the mucosal fluid on the potential changes induced by D-glucose was investigated in the duodenum. In the presence of phlorizin, differences between the potentials (PD_t and V_m) obtained under normal conditions and those obtained on exposure to D-glucose were not significant with $P>0.25$, as shown in Table 5. Thus the mucosal phlorizin inhibited the glucose-dependent changes in electrical potentials. This observation is related to the well-

Table 5. Effect of mucosal phlorizin and serosal ouabain on the potentials obtained in a control saline and in a saline containing 20 mM D-glucose

Condition	Potential	Control (mV)	Glucose (mV)	Significance of difference from controls
Phlorizin[a]	PD_t	3.2 ± 0.2 (3)[c]	3.2 ± 0.3 (3)[c]	$P \simeq 1$
	V_m	-52.4 ± 1.4(10)[c]	-51.8 ± 1.6(7)[c]	$P > 0.25$
Ouabain[b]	PD_t	1.7 ± 0.2 (8)[c]	2.6 ± 0.3 (8)[c]	$P > 0.05$
	V_m	-50.3 ± 1.2(20)[c]	-49.2 ± 1.3(12)[c]	$P > 0.50$

[a] 10^{-4} M phlorizin added to the mucosal saline.
[b] 3×10^{-4} M ouabain added to the serosal K^+-free saline. (15–60 min after addition).
[c] Numbers in parentheses indicate number of observations.

known fact that phlorizin inhibits competitively the active entry of glucose into the cell from the mucosal side at this concentration, without any effect on cellular metabolism (Newey, Parsons & Smyth, 1959). It seems obvious, therefore, that an active entry mechanism of glucose at the mucosal membrane is a prerequisite for the glucose-dependent V_m depolarization. Phlorizin never affected the glycine-induced changes in PD_t and V_m.

Effect of Mucosal Ouabain

A variety of doses of ouabain (up to 3×10^{-4} M) added to the mucosal control saline had no effect on the magnitude of PD_t and V_m, for about 50 min. In the presence of 3×10^{-4} M ouabain in the mucosal fluid, the PD_t and V_m values in duodenum were 2.0 ± 0.1 mV (n=3) and -52.8 ± 1.2 mV (n=15), respectively. These values were not significantly different from those in the absence of ouabain ($P > 0.50$). Replacement of the mucosal fluid containing ouabain with a saline containing both D-glucose (20 mM) and ouabain, resulted in an increase in PD_t to 4.1 ± 0.1 mV (n=3) and a decrease in V_m to -49.9 ± 1.2 mV (n=10). A higher dose of ouabain (10^{-3} M) did not affect the potentials up to 10 min. However, after a longer application the potentials decreased gradually. Since swelling of the epithelial cell resulting in cell desquamation was detected in the histological sections, these potential decreases could hardly be assigned to a specific drug action. Our results obtained with mucosal ouabain of 3×10^{-4} M or less support the view that the mucosal membrane does not have any ouabain-sensitive electrogenic

pump activities (Okada & Inouye, 1976*b*; Okada *et al., in preparation*), and moreover that the entry mechanism of glucose at the mucosal membrane was not affected by the mucosal ouabain (Csáky & Hara, 1965).

Effect of Serosal Ouabain

Ouabain of 3×10^{-4} M added to the serosal control medium induced no significant changes in PD_t and sugar-evoked potentials up to 60 min after application. Since the intestinal preparation employed here had an intact subepithelial layer, a relatively long latency and a high dose would be required to achieve a full effect of the drug applied on the serosal side. A higher dose of serosal ouabain (10^{-3} M) affected remarkably the profile of electrical potentials without histological deterioration of tissues. An example of the experiments is shown in Fig. 1*a*. On exposure to ouabain, PD_t and the sugar-evoked potential declined gradually and decreased to 1.0 and 1.6 mV, respectively, at about 40 min after addition of ouabain. When a K^+-free saline was used for the serosal fluid, such ouabain effects on electrical potentials were observed at a lower concentration (3×10^{-4} M) with a shorter latency (around 15 min), as summarized in Table 5. The addition of ouabain to the serosal bathing medium caused a spontaneous time-dependent decay in PD_t and the sugar-evoked potential change, a fact in good agreement with many previous observations (Schultz & Zalusky, 1963; 1964*a*; Csáky & Hara, 1965; Lyon & Sheerin, 1971). The average V_m value measured in the duodenum between 15 and 60 min after addition of 3×10^{-4} M ouabain to the serosal K^+-free fluid was slightly depolarized (around -50 mV) compared with those measured in the absence of ouabain (Tables 2, 3 and 4). As is well-known (Kerkut & York, 1971; Koketsu, 1971), ouabain applied to the serosal side is expected to inhibit, to a certain degree at least, the elctrogenic sodium pump located on the serosal membrane (Okada & Inouye, 1976*b*; Okada *et al., in preparation*). Consequently, decreases in the measured V_m values could be explained by the depolarization of E_s resulting from the depressed activity of the ouabain-sensitive electrogenic pump as a result of electrical coupling through a relatively high conducting extracellular shunt pathway [see Eq. (2) and Fig. 3]. During a long exposure to ouabain on the serosal side, the effects of glucose on the potentials were studied in the duodenum (Table 5 and Fig. 1*a*) and the results indicated that the serosal ouabain inhibited to a considerable extent the glucose-dependent changes in PD_t and V_m,

Table 6. Effect of cooling and anoxia on the transepithelial potential difference in rat duodenum

	Condition	n[a]	PD_t		ΔPD_t
			Control (mV)	Glucose (mV)	
Cooling	35 °C	6	2.8±0.1	5.1±0.2	+2.3
	2 °C[b]	6	1.0±0.1	1.1±0.1	+0.1
Anoxia	O_2	8	2.1±0.2	4.3±0.3	+2.2
	N_2[c]	8	1.5±0.3	2.3±0.3	+0.8

[a] The number of individual experiments (=the number of animals used).
[b] 10–20 min after cooling at 2 °C.
[c] 10–20 min after bubbling of both media with pure N_2.

and that the inhibition of an active extrusion of Na^+ at the serosal membrane brought by the serosal ouabain also inhibits the sugar entry coupled with Na^+ entry through the mucosal membrane (Schultz & Curran, 1970). Although we did not make systematic observations regarding the effect of ouabain on the glycine-induced potential changes, a few observations on rat ileum revealed that the circumstances were quite similar to those seen with glucose.

Effect of Cooling on the Solute-Evoked Potential

Solute-evoked changes in PD_t obviously have a relation to an activity of the electrogenic pump located on the serosal membrane. Since cooling is expected to inhibit such an electrogenic activity (Kerkut & York, 1971; Koketsu, 1971), the effect of low temperature of the tissue (2 °C) was examined in rat duodenum.

As shown in Fig. 1*b*, PD_t decreased gradually down to a lower steady value (approximately +1 mV, Table 6) within 10 min. At a steady state of PD_t values under the cooled condition, 20 mM D-glucose applied to the mucosal control saline evoked no significant changes in PD_t (Fig. 1*b*, Table 6). Under such conditions, glucose never affected the V_m values, which were depolarized by cooling (around 10 mV) (Okada & Inouye, 1976*b*; Okada *et al., in preparation*). On rewarming the tissue, a rapid PD_t increase occurred and was always accompanied by a transient "overshoot" (6.0±0.4 mV at the peak, n=6), and then returned to the original level within 10–20 min. Measurement of the V_m during the short period

of an "overshoot" could not accurately be made. Though a tendency of hyperpolarization of V_m was observed, the explanation for the origin of this "overshoot" is still open to question. A sugar-evoked potential was also recovered after re-warming as shown in Fig. 1*b*.

Observations on rat ileum with glycine (20 mM) also showed that cooling had much the same inhibitory effect on the glycine-evoked potential.

All these data indicate that the uphill solute transport mechanism in intestinal epithelia is remarkably dependent on temperature.

Effect of Anoxia on the Solute-Evoked Potential

To observe the O_2-dependency of the sugar-evoked potential, both the mucosal and serosal bathing media were saturated with N_2 by bubbling. Since the chamber bathing the tissue preparation was open to the air, complete anoxia was not achieved, however, bubbling with N_2 in excess 30 min resulted in a considerable decrease in the sugar-evoked potential and these potentials were not restored after re-oxygenation as shown in Fig. 1*c*. This bubbling with N_2 was apparently effective to observe the anoxia effect on the preparation. A short-lasting anoxia (for 10–20 min) depressed to a certain extent the glucose-evoked potential and such potentials were recovered after re-oxygenation (Fig. 1*c*). The experimental results with 20 mM D-glucose obtained at this reversible stage of anoxia (10–20 min period are summarized in Table 6 and indicate that the electrogenic activity associated with the sugar transport in intestinal epithelia is O_2-sensitive, and may be consequently dependent on cellular metabolism. Moreover, such a reversible anoxia results in a significant depolarization in V_m and is explicable in terms of depressed pump activity, as described elsewhere (Okada & Inouye, 1976*b*; Okada *et al., in preparation*).

With 20 mM glycine, quite a similar inhibition due to anoxia of the evoked potential was observed in rat ileum.

Discussion

There is some dispute as to whether the PD_t changes evoked by sugars and amino acids should be attributed chiefly to a hyperpolarization of serosal membrane (Gilles-Baillien & Schoffeniels, 1965; Wright, 1966;

Lyon & Sheerin, 1971; Barry & Eggenton, 1972) or a depolarization of mucosal membrane (Rose & Schultz, 1971; White & Armstrong, 1971). According to our observations, the addition of actively transported solute to the mucosal fluid resulted in a significant depolarization of V_m accompanied by an increase in PD_t in rat small intestine, and these solute-dependent changes in V_m and PD_t were blocked by cooling to 2 °C, serosal ouabain and anoxia. Since Lyon & Sheerin (1971) and Barry & Eggenton (1972) did not observe such a solute-induced change in V_m in rat small intestine, the discrepancy cannot be attributed to species differences but rather to particular conditions of the intestinal tissues employed in the experiments.

The intestinal epithelium can be classified as a leaky tissue which has a low-resistance, transepithelial, extracellular pathway (Frömter & Diamond, 1972), so that the resulting potential changes cannot be straightforwardly interpreted in terms of either the emf of mucosal membrane (E_m) or that of serosal membrane (E_s) without making use of an electrical equivalent circuit analysis. Since both the mucosal and serosal sides of the tissue were bathed in identical electrolyte media in the present experiments, we can safely adopt the following equivalent circuit model (Fig. 3) by neglecting the diffusion potential through the shunt pathway ($E_L=0$ in Fig. 3). According to this model, PD_t and V_m can be expressed by the following equations,

$$PD_t=\frac{R_L}{R}\cdot(E_s-E_m) \tag{1}$$

$$V_m=-E_m-\frac{R_m}{R}\cdot(E_s-E_m) \tag{2}$$

where $R=R_m+R_s+R_L$. Here it should be noted that both E_m and E_s are referred to the cell interior (Fig. 3) and so the former is defined in the opposite direction to V_m [Eq. (2)]. Because the transepithelial resistance (Schultz & Zalusky, 1964*a*; Barry, Smyth & Wright, 1965; Okada *et al.*, 1967) and the membrane resistances (Okada *et al.*, 1977) are not affected by the presence of actively transported solutes, the solute-evoked changes in PD_t and V_m would be written as follows,

$$\Delta PD_t=\frac{R_L}{R}\cdot(\Delta E_s-\Delta E_m) \tag{3}$$

$$\Delta V_m=-\Delta E_m-\frac{R_m}{R}\cdot(\Delta E_s-\Delta E_m). \tag{4}$$

Using these two equations, it can easily be shown that ΔE_m should be a negative value, as the measured ΔPD_t and ΔV_m were positive values as seen in Tables 1, 2 and 4. It is evident that the change in emf of the mucosal membrane induced by actively transported solutes should be "depolarization". Putting the ratio of ΔPD_t to ΔV_m as k ($\Delta PD_t/\Delta V_m = k$), ΔE_s can be written as

$$\Delta E_s = \frac{-\Delta E_m}{R_L + kR_m} \cdot \{kR_s - (1-k)R_L\}. \quad (5)$$

Because the term of $\{-\Delta E_m/(R_L+kR_m)\}$ is positive, the sign of ΔE_s depends on whether $\{kR_s-(1-k)R_L\}$ is positive or negative; namely, $\Delta E_s \gtrless 0$ for $R_s/R_L \gtrless (k^{-1}-1)$. As seen in Tables 1, 2 and 4, all the values of k obtained in our observations were larger than 0.5, so that $1 > (k^{-1}-1)$. ΔE_s should be, therefore, positive, if $R_s > R_L$, that is, $G_L/G_t > (\mu+1)/2\mu+1)$, where G_L and G_t is the conductance of the paracellular shunt pathway ($=1/R_L$), and the total transepithelial conductance [$=(1/R_L)+1/(R_m+R_s)=(1/R_L)+\mu/R_s(\mu+1)$], respectively, and $\mu = R_s/R_m$. As discussed in our previous reports (Okada *et al.*, 1975; 1977) and as measured in fact in rat small intestine (Okada *et al.*, 1976*c*), μ is far greater than 1. Hence a hyperpolarization of emf of the serosal membrane ($\Delta E_s > 0$) occurs for $G_L/G_t > 0.5$. As noted by other workers (Rose & Schultz, 1971; Frömter & Diamond, 1972), the small intestine is a very leaky tissue, G_L/G_t being around 0.85 in rabbit ileum (Frizzell & Schultz, 1972) and around 0.80 (Munck & Schultz, 1974) or 0.95 (Okada *et al.*, 1977) in rat small intestine. Thus it is concluded that the emf of serosal membrane could be hyperpolarized in the presence of actively transported solutes. Depending on the magnitudes of the values of $\Delta PD_t/\Delta V_m$ and G_L/G_t, a variety of cases could be expected to occur under different experimental conditions. Such a circumstance possibly provides an explanation for the widely discrepant views on the origin of the evoked potential of small intestine. The most plausible explanation for the results obtained in our work here is that a hyperpolarization of the serosal membrane as well as a depolarization of the mucosal membrane are brought about by the presence of actively transported solutes in the mucosal fluid. Schultz, Frizzell and Nellans (1974) also suggested this possibility from a reconsideration of the data obtained by Rose and Schultz (1971) in the light of the presence of a high conducting extracellular shunt pathway.

Why depolarization of mucosal membrane occurs remains unknown. It seems highly improbable that an electrogenic sodium pump takes

part in this depolarization at the mucosal membrane, as the mucosal ouabain had no effect on the potential profiles. It has been reported that intracellular ion concentrations change remarkably in the presence of actively transported solutes (Schultz, Fuisz & Curran, 1966; Koopman & Schultz, 1969; Csáky & Esposito, 1969; Armstrong, Musselman & Reitzug, 1970; Okada, Irimajiri & Inouye, 1976*b*). Such changes in intracellular ion concentrations (especially decreases in the K^+ concentration) might relate to the depolarization of the mucosal membrane. Moreover there is another possibility, as suggested by Schultz *et al.*, (1974), that a rheogenic carrier solute transport mechanism operates at the brush border membrane (Fig. 3). Hyperpolarization of serosal membrane induced by an active solute transport could be ascribed to stimulation of an electrogenic sodium pump located at the serosal membrane in the light of the effect of repeated exposure to D-glucose and the effect of serosal ouabain on the potential profiles.

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