J. Membrane Biol. 31, 221–232 (1977)

Electrical Properties and Active Solute Transport in Rat Small Intestine

II. Conductive Properties of Transepithelial Routes

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Received 26 April 1976

Summary. The transepithelial resistance, the cell membrane resistance and the ratio of resistances of the serosal (baso-lateral) to the mucosal (brush border) cell membrane were measured in rat duodenum, jejunum and ileum by means of microelectrode techniques. These measured values were not affected in the presence of actively transported solutes in the mucosal bathing fluid.

Contribution of an electrical conductance through the extracellular shunt pathway to the total transepithelial conductance was quantitatively estimated using an electrically equivalent circuit analysis. These values estimated in respective tissues of small intestine were approx. 95% of the total transepithelial conductance, remaining unaffected by an active solute transport.

From these data, the changes in emf's of the mucosal and serosal membrane induced by D-glucose or glycine were separately evaluated.

Since the report of Ussing and Windhager (1964) it has become increasingly clear that the transepithelial, paracellular shunt pathways play a significant role in the transport of ion, water and solutes in a variety of epithelial tissues (Frömter & Diamond, 1972; Whittembury, Rawlins & Boulpaep, 1973; Schultz, Frizzell & Nellans, 1974; Ussing, Erlij & Lassen, 1974). Contribution of such a shunt pathway to electrical conductance would be greater in "leaky" tissues (e.g., small intestine, gallbladder and kidney proximal tubule) than that in "tight" tissues (e.g., frog skin, urinary bladder and gastric mucosa) (Frömter & Diamond, 1972; Claude & Goodenough, 1973).

In the small intestine, Clarkson (1967) first indicated the significance of the existence of an independent channel for passive ion transport across the intestinal epithelium. Rose and Schultz (1971) and White and Armstrong (1971) suggested that the extracellular shunt pathway is highly conductive, from their observations of effects of actively transported solutes on the potential profiles. We also adhered to this concept

from the observation of electrical interaction between the mucosal (brush border) and serosal (baso-lateral) membrane (Okada, Sato & Inouye, 1975) and from that of different values for ionic permeability coefficients across the cell membranes and across the whole epithelia (Okada *et al.*, 1975; Okada, Irimajiri & Inouye, 1976). It has been stressed, however, that a quantitative evaluation of the electrical conductance of this shunt pathway was imperative in order to decide precisely the origin of the electrical potential changes induced by active solute transport (Rose & Schultz, 1971; Schultz *et al.*, 1974; Okada, Tsuchiya, Irimajiri & Inouye, 1977). Direct evaluations of the partial ionic conductances of the extracellular pathway were carried out in rabbit ileum (Frizzell & Schultz, 1972) and in rat jejunum (Munck & Schultz, 1974) from measurements of voltage clamped ion fluxes. To procure a quantitative estimation of the electrical shunt conductance using an electrophysiological method, a cable analysis, as made by Frömter (1972) and Reuss and Finn (1974), is one of the most appropriate methods because of the existence of electrical cell-to-cell coupling in epithelial tissues (Loewenstein & Kanno, 1964; Loewenstein, Socolar, Higashino, Kanno & Davidson, 1965; Loewenstein, 1966). Due to the morphological complexity of the rat small intestine, application of a cable analysis is almost technically impossible; therefore, a different electrophysiological approach was used in our present work. The effects of D-glucose and glycine on the shunt conductance were also examined in an attempt to provide further information regarding the origin of a solute-evoked potential change in rat small intestine.

Materials and Methods

Tissue Preparation

Adult rats of either sex were fasted for 24–48 hr except for water provided *ad libitum*. Under ether anesthesia and maintenance of a normal blood supply, a segment of the small intestine was rinsed free of intestinal contents by a syringe, incised along the mesenteric border, and mounted on a lucite chamber as described previously (Okada *et al.*, 1975). The area of the window between the two halves of this chamber was 0.28 cm^2. We employed an O-ring similar to the one used by Frizzell and Schultz (1972) to prevent damage to the ring of tissue clamped between the chambers.

Electrical Measurements

The arrangement of apparatus used for the electrical measurements was almost identical with that described elsewhere (Okada *et al.*, 1975), except for two swirled Ag-AgCl wires freshly prepared for each experiment. Setting these wires as close as possible to the tissue

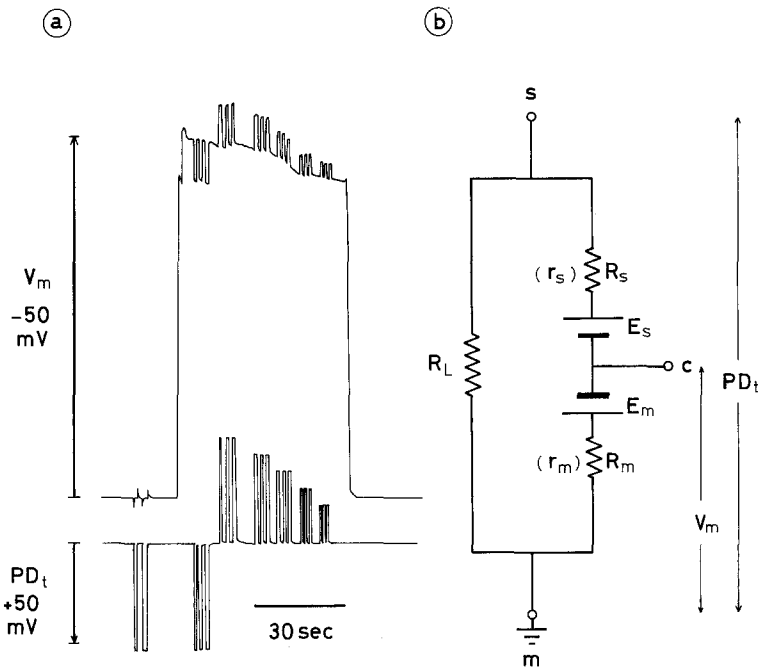

Fig. 1. (*a*) Recording of changes in the mucosal membrane potential (V_m) and in the transepithelial potential difference (PD_t) produced by transepithelial current pulses of -155, $+155$, $+130$, $+105$, $+80$ and $+55$ μA, applied successively from the serosal to the mucosal side. (*b*) An equivalent 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. r_m and r_s are the resistances of the mucosal and serosal membranes, respectively, of an epithelial cell (or a single unit in its electrical behavior composed of a certain number of cells; *see* text). R_m and R_s are the lumped resistances of the mucosal and serosal membranes, respectively, in an epithelial layer. R_L represents a transepithelial shunt resistance. The diffusion potential through the transepithelial shunt pathway is neglected as both the mucosal and serosal fluids have identical electrolyte compositions. *m*, *s* and *c* designate the mucosal, serosal and cellular fluids, respectively

preparation, they were then connected to the electrical system consisting of a battery and a voltage divider, which served for passage of the current across the tissue.

The transepithelial resistance (R_t) of the epithelium was measured at 37 °C by pulses of 20–300 μA (around 1 sec). Because the tissue behaved as an ohmic resistor as seen in Fig. 1*a*, R_t was simply calculated from a linear current-voltage relation as ohm × cm^2 by referring to the unit area of the tissues. The Ag-AgCl wires had been set close enough to the tissue that the resistance of the fluid between these current-passing electrodes was usually negligible.

Using the system of applying the constant voltage pulse, which was successfully employed for the measurement of high resistance (up to 6,000 MΩ) across the thin glass wall near the tip of microelectrodes (Okada & Inouye, 1976), the effective cell membrane resistance (r_M) was determined. A pulse of 100 ~ 400 msec was delivered through the intracellular electrode after obtaining a stable membrane potential, and the short potential deflections thus induced were recorded by an electric recorder (Hitachi QPD-53) using a transient memory (Transidyne, Neurograph Model N-3). As the current-voltage relations was closely

linear as seen in Fig. 1 *a*, the r_M values were calculated using Ohm's law from these potential deflections after subtracting the electrode resistance.

Putting the serosal and mucosal cell membrane resistance per one cell (or per one unit of cells as discussed below) as r_s and r_m, respectively, the ratio of $r_s/r_m(=\mu)$ was determined from observations of the voltage divider ratio produced by a transepithelial pulse with the microelectrode in a cell. A typical result thus measured is shown in Fig. 1 *a*. Usually the serosal membrane potential deflection (ΔV_s) was calculated by subtracting the mucosal membrane potential deflection (ΔV_m) from the transepithelial potential deflection (ΔPD_t):

$$(\Delta PD_t - \Delta V_m)/\Delta V_m = r_s/r_m = \mu. \tag{1}$$

Estimation of Shunt Conductance ("Leakiness")

Because of the complicated structure of an intestinal epithelium (villous structure) and an epithelial cell (micro-villous structure), an equivalent circuit analysis had to be employed using the measured values of resistances in order to estimate the contribution of an electrical conductance through the extracellular shunt pathway to the total transepithelial conductance ("leakiness").

Since the mucosal and serosal fluids had identical ion compositions, we can adopt the model shown in Fig. 1 *b* as an equivalent circuit for an epithelium by negelcting the diffusion potential through the paracellular shunt pathway. According to this model, the measured R_t and r_M values could be expressed by the following equations:

$$R_t = \frac{R_L}{R} \cdot (R_m + R_s), \qquad R = R_m + R_s + R_L \tag{2}$$

$$r_M = \frac{r_m \cdot r_s}{r_m + r_s} = \frac{n\mu}{(1+\mu)^2} \cdot (R_m + R_s), \tag{3}$$

where R_m $(=r_m/n)$ and $R_s(=r_s/n)$ are the lumped resistances of the mucosal and serosal membranes per unit area of the epithelium, R_L respresents a transepithelial shunt resistance per unit area, and n is the effective cell number per unit area. Using these equations, the contribution of shunt conductance (G_L) to the total transepithelial conductance (G_t), so called "leakiness", can be expressed as follows:

$$\frac{G_L}{G_t} = \frac{R_t}{R_L} = 1 - \frac{n\mu}{(1+\mu)^2} \cdot \frac{R_t}{r_M}. \tag{4}$$

The "leakiness" of the tissue, $L(\%) \equiv 100\, G_L/G_t$, can be calculated by using measured R_t, r_M and μ values, when the effective cell number (n) is correctly estimated.

Solutions

A phosphate-buffered saline of pH 7.3 ± 0.1 was employed as the control bathing fluid and contained (in mEquiv/liter) 143.0 Na^+, 4.2 K^+, 0.9 Ca^{++}, 0.5 Mg^{++}, 132.5 Cl^-, 1.5 $H_2PO_4^-$ and 8.0 HPO_4^{--} with 20.0 mM mannitol. Mannitol in the mucosal fluid was sometimes replaced by 20 mM D-glucose or glycine. All data in the tables are the means $\pm$ SE.

Results

Effects of Sugar and Amino Acid on Electrical Resistance

After the transepithelial resistances per unit area (R_t) were measured under normal conditions, the effect of D-glucose or glycine in the mucosal

Table 1. Effect of 20 mM solute on the transepithelial resistance per unit area (R_t)

Tissue	R_t (Ω-cm^2)			
	Control	Glucose	Glycine	Total
Duodenum	97.2±5.1(26)	104 ±10.2(13) [$P>0.50$]	89.6±5.9(9) [$P>0.25$]	97.7±3.6(48)
Jejunum	66.4±7.1(5)	64.7± 7.1(5) [$P>0.50$]	65.3±7.6(5) [$P>0.50$]	65.5±3.9(15)
Ileum	90.1±11.0(5)	86.2± 9.9(5) [$P>0.50$]	86.2±9.3(5) [$P>0.50$]	87.6±5.4(15)

Numbers in parentheses and square brackets indicate number of observations and the significant difference from the control, respectively.

fluid on the R_t values was observed. The data thus obtained are summarized in Table 1. The R_t values in jejunum are smaller than those in duodenum and ileum. The error due to conductance through the edge damage (Dobsen & Kidder, 1968; Walser, 1970; Helman & Miller, 1971; 1974) was practically negligible in these experiments, because almost the same value of R_t (variations within ±15%) was obtained irrespective of whether the chambers had an exposed area of 0.28, 0.50 or 0.64 cm^2. The R_t values obtained in the present study are slightly higher than those reported previously in various intestinal epithelia (Asano, 1964; Schultz & Zalusky, 1964; Barry, Smyth & Wright, 1965; Field, Fromm & McColl, 1971; Munck, 1972). Such a discrepancy may be due to different experimental conditions employed (e.g., preparation methods of tissue samples, an edge damage, etc.), as we confirmed the R_t values of the same magnitude as presented here by means of an ac bridge method (Irimajiri & Okada, *unpublished observation*). At any rate, the presence of 20 mM D-glucose or 20 mM glycine that can produce significant potential changes as reported in the preceding paper (Okada *et al.*, 1977) did not produce any significant changes in the R_t values as shown in Table 1 ($P>0.25$). This finding is in good agreement with reports by Schultz and Zalusky (1964) and Barry *et al.* (1965).

As shown in Table 2, the effective resistance of intestinal epithelial cell membrane (r_M) is around 20 MΩ and these values obtained in each tissue were not so significantly affected by the presence of an actively transported solute (D-glucose or glycine) ($P>0.10$). Recently, we confirmed by intracellular current injection with the modified bridge circuit (W-P, M701) that the r_M values were around 20 MΩ and remained unaffected by these solutes (Okada & Irimajiri, *unpublished observation*). We may easily postulate, however, that electrical coupling through low

Table 2. Effect of 20 mM solute on the effective cell membrane resistance (r_M)

Tissue	r_M (MΩ)			
	Control	Glucose	Glycine	Total
Duodenum	24.3±2.2(47)[a]	19.9±2.6(25) [$P>0.10$][b]	21.2±2.1(27) [$P>0.50$]	22.3±1.5(99)
Jejunum	23.8±2.4(17)	19.3±1.9(19) [$P>0.10$]	21.6±2.7(10) [$P>0.50$]	21.5±1.2(46)
Ileum	21.5±2.4(20)	26.4±5.6(8) [$P>0.25$]	26.3±2.8(14) [$P>0.10$]	24.0±2.1(42)

[a] and [b], *see* Table 1.

Table 3. Effect of 20 mM solute on the ratio of the serosal to the mucosal cell membrane resistance (μ)

Tissue	$\mu=r_s/r_m$			
	Control	Glucose	Glycine	Total
Duodenum	16.3±0.7(89)[a]	14.8±0.9(24) [$P>0.10$][b]	15.0±1.0(40) [$P>0.25$]	15.7±0.4(153)
Jejunum	11.3±0.9(53)	9.5±0.7(28) [$P>0.05$]	10.2±0.6(33) [$P>0.25$]	10.5±0.4(114)
Ileum	4.3±0.3(100)	3.7±0.4(40) [$P>0.10$]	4.2±0.5(35) [$P>0.50$]	4.1±0.2(175)

[a] and [b], *see* Table 1.

resistance cell-to-cell junctions exists in the intestinal epithelial cells as in other epithelia studied by many investigators (Loewenstein & Kanno, 1964; Loewenstein *et al.*, 1965; Loewenstein, 1966; Silverblatt & Bulger, 1970; Blum, Hirschowitz, Helander & Sachs, 1971; Frömter, 1972; Reuss & Finn, 1974). In such a case, the measured r_M values cannot be regarded as the cell membrane resistance of a single cell but rather as the effective membrane resistance of a lump of a certain number of cells electrically coupled and assumed to behave as a single unit electrically. It follows that n in Eqs. (3) and (4) should be read as the average number of such units per unit area.

The ratio of the serosal to the mucosal cell membrane resistance (μ) was calculated by Eq. (1) using the data obtained from the measurements of ΔPD_t and ΔV_m as shown in Fig. 1*a*. The μ values thus obtained are tabulated in Table 3. These results indicate that the μ values in the upper small intestine are greater than those in the lower small intestine and are assumed to be due to morphological differences in epithelial

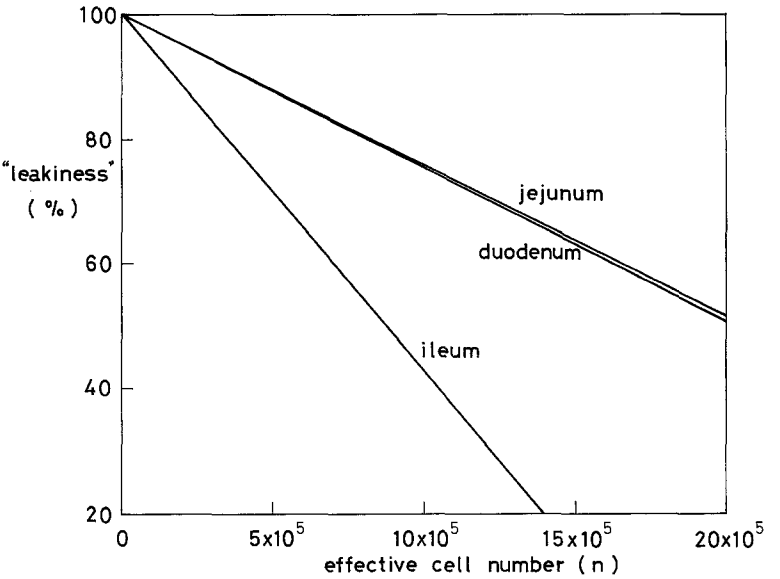

Fig. 2. Contribution of the shunt conductances to the total transepithelial conductances ("leakiness") (%) plotted against the effective cell number (n)

cells among the respective areas of small intestine. The μ values remained practically unaltered in the presence of an actively transported solute as shown in Table 3 ($p > 0.05$).

Despite the slight ambiguity in the nature of measured r_M values, it can be concluded that the active solute transport in the small intestine does not produce significant changes in electrical properties of the epithelial cell membrane.

Shunt Conductance Contribution

In view of our finding that the measured R_t, r_M and μ values did not significantly change in the presence of D-glucose or glycine, the shunt conductance contribution ("leakiness") as expressed by Eq. (4) should be nearly constant irrespective of whether or not the epithelium is exposed to an actively transported solute. Using the R_t, r_M and μ values averaged on the total measurements, the L value (%) of each tissue was calculated by Eq. (4) while varying the effective cell number (n). The results are illustrated in Fig. 2. Here it is clearly shown that if the n values are smaller than $2 \times 10^6/\text{cm}^2$ in the duodenum and jejunum, or $8 \times 10^5/\text{cm}^2$ in the ileum, these intestinal epithelial can be classified as a "leaky" tissue (Frömter & Diamond, 1972).

The dimension of the intestinal epithelial cells was around 15 μm $\times$ 15μm in the histological horizontal cross section as reported previously (Okada & Inouye, 1976*b*). Taking into account the villous structure on the mucosal surface of the intestine, the number of cells in unit area would be in the order of 10^6. It is a well-known fact, however, that most epithelial cells are electrically coupled, and Blum *et al.*, (1971) reported that such an electrical coupling was found within a radial distance of up to 100 μm (i.e., about 10 cells) in gastric mucosa. If such is also the case in the small intestine, the effective value of n assumed as above would be approximately the order of 10^5. Hence the intestinal epithelium of rat should be a "leaky" tissue as claimed by many investigators (Rose & Schultz, 1971; White & Armstrong, 1971; Frizzell & Schultz, 1972; Frömter & Diamond, 1972; Schultz *et al.*, 1974; Okada *et al.*, 1975; 1976).

Discussion

We concluded from data in this study that the rat intestinal epithelium is a "leaky" tissue which has a relatively high conductive extracellular shunt pathway, and the shunt conductance contribution in each tissue is not affected by exposure to an actively transported solute. The exact magnitude of the shunt conductance compared to the cellular conductance does, however, remain uncertain until the n values have been estimated.

From Eqs. (2) and (3), n can be expressed by the following equation:

$$n = \frac{(1+\mu)^2}{\mu} \cdot \frac{r_M}{R_t} \cdot \frac{R_L}{R}$$

$$= \frac{(1+\mu)^2}{\mu} \cdot \frac{r_M}{R_t} \frac{1}{(1+\mu)\dfrac{R_m}{R_L}+1}. \tag{5}$$

From an equivalent circuit analysis (*see* Eqs. (1) and (2) in the preceding paper: Okada *et al.*, 1977), R_m/R_L can be easily expressed by the emf value of mucosal membrane (E_m) and the measured PD_t and V_m values as follows:

$$\frac{R_m}{R_L} = -\frac{E_m + V_m}{PD_t}. \tag{6}$$

Here, it should be noted that E_m is defined in the opposite direction to V_m (Fig. 1*b*), as in the preceding paper (Okada *et al.*, 1977). If E_m

Table 4. Effect of 20 mM solute on the shunt conductance contribution ("leakiness")

Tissue	Effective cell number	Leakiness $= G_L/G_t$ (%)		
		Control	Glucose	Glycine
Duodenum	1.9×10^5	96	94	95
Jejunum	2.3×10^5	95	93	94
Ileum	1.1×10^5	93	94	94

be known, therefore, the effective cell number can be estimated by Eqs. (5) and (6) using the measured PD_t, V_m, R_t, r_M and μ values.

As demonstrated in our previous works (Okada & Inouye, 1976*c*; Okada *et al.*, 1977; Okada, Irimajiri, Tsuchiya & Inouye, *in preparation*), the electrogenic sodium pump located on the serosal membrane results in an electrical polarity of the intestinal epithelium and is inhibited by cooling to 2 °C, short-lasting anoxia or long-lasting exposure to serosal ouabain. As the first approximation, therefore, we may regard that the V_m value obtained under the condition of inhibiting the pump activity is equal to E_m. As we had obtained -50 mV for V_m in the duodenum under these inhibiting conditions (Okada & Inouye, 1976*c*; Okada *et al.*, 1977; Okada *et al.*, *in preparation*), it is reasonable to assume $E_m \simeq 50$ mV in epithelial cells in rat small intestine. Using the PD_t and V_m values measured previously (Okada *et al.*, 1977), and the R_t, r_M and μ values obtained here together with the E_m value thus estimated, the effective cell number was found to be around 2×10^5 for the duodenum and jejunum, and 1×10^5 for the ileum as shown in Table 4. These results suggest that about 5–10 cells are electrically coupled, a finding in good agreement with the report on gastric mucosa by Blum *et al.* (1971). We can thus evaluate the "leakiness" (L) in each tissue by incorporating these n values into Eq. (4). As seen in Table 4, the results indicate that a rat intestinal epithelium is remarkably "leaky" and the magnitude of L was not affected by an active solute transport. By means of cable analysis, several investigators had assessed the shunt conductance contribution in "leaky" epithelia: namely, 96% for *Necturus* gallbladder (Frömter, 1972), 99% for kidney proximal tubule (Whittembury *et al.*, 1973). In contrast, relatively low values (about 64% and 20%) had been reported in "tight" epithelia (toad urinary bladder and *Necturus* fundic gastric mucosa, respectively) (Reuss & Finn, 1974; Spenney, Shoemaker & Sacks, 1974). Our results are in full accord with the results obtained in "leaky" epithelia. From the data of flux measurements,

Table 5. Electrical potential changes induced by 20 mM solute

Solute	Tissue	ΔPD_t (mV)	ΔV_m (mV)	ΔE_m (mV)	ΔE_s (mV)
Glucose	Duodenum	+2.6	+3.4	−6	+12
	Jejunum	+3.7	+3.5	−8	+36
	Ileum	+4.1	+4.4	−19	+52
Glycine	Duodenum	+0.4	+0.2	−1	+3
	Jejunum	+0.5	+0.5	−1	+1
	Ileum	+3.1	+5.3	−17	+45

Schultz and his collaborators reported around 85% and 80% for the L values in rabbit ileum (Frizzell & Schultz, 1972) and in rat jejunum (Munck & Schultz, 1974), respectively. These values are also in fair accordance with our own evaluations herein.

As the contribution of shunt conductance to the total transepithelial conductance was not affected by an uphill transport of D-glucose or glycine in rat small intestine, the electrical potential changes (ΔPD_t, ΔV_m) associated with these transports as reported in the preceding paper (Okada *et al.*, 1977) should be ascribed solely to the membrane emf changes ΔE_m and/or ΔE_s). Since the values of $R_L/(R_m+R_s+R_L)$ and $R_m/(R_m+R_s+R_L)$ can be easily calculated by the L value estimated here, Eqs. (3) and (4) in the preceding paper (Okada *et al.*, 1977) enable evaluation of the ΔE_m and ΔE_s values. These values thus estimated are tabulated in Table 5 together with the ΔPD_t and ΔV_m values obtained previously (Okada *et al.*, 1977). The results show that a remarkable hyperpolarization of E_s as well as a depolarization of E_m were associated with sugar and amino acid transports. The magnitude of ΔE_m and ΔE_s values thus estimated are, of course, largely dependent on the magnitude of n value, estimation of which is necessarily an approximate one. The qualitative conclusions stated in the preceding paper (Okada *et al.*, 1977) as well as in this report would remain unaffected irrespective of the magnitude of n of less than 10^6. Thus our analysis based on the equivalent circuit analysis supports the view brought forward by Schultz *et al.* (1974) and by ourselves (Okada *et al.*, 1977) concerning the origin of the solute-evoked potential changes in small intestine; namely, both E_m-depolarization and E_s-hyperpolarization build up the solute-evoked potential changes.

Thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript. This work was supported in part by the grant from the Ministry of Education of Japan.

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