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MEMBRANE POTENTIALS OF EPITHELIAL CELLS IN RAT SMALL INTESTINE

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SUMMARY

1. Stripped sacs of rat jejunum in which the outer muscle layers had been removed were found to maintain substantial transport and electrical activities.

2. Mucosal and serosal membrane potentials of epithelial cells of normal and stripped everted sacs of rat jejunum were recorded *in vitro* together with the transmural potential difference.

3. The cell interior was negative relative to both serosal and mucosal fluids, the transmural potential being the sum of the two membrane potentials.

4. Changes in the transmural potentials in the presence of actively transferred hexoses and amino acids were entirely due to variations in the serosal potential, the mucosal potential being unchanged.

5. Serosal and transmural potential increases on the addition of galactose were consistent with Michaelis–Menten kinetics, giving apparent K_m values of 14·9 and 14·1 mM respectively.

6. Phlorrhizin, ouabain, 2,4-dinitrophenol and sodium fluoroacetate inhibited serosal potential changes in the presence of galactose.

7. Osmotic potentials resulting from transmural osmotic gradients originated from the serosal layers of the tissue.

8. The results are consistent with the concept of a serosally located, electrogenic sodium pump which is stimulated by actively transferred hexoses and amino acids. The sodium-dependent entry mechanism at the mucosal membrane is non-electrogenic.

INTRODUCTION

A potential difference is found across the wall of the small intestine and this is generated by the mucosal epithelial cells. The transmural potential difference consists of at least two potential steps – one across the luminal membrane and the other across the serosal or lateral membrane of the epithelial cell. These membrane potentials together with the transmural

potential difference can be measured using a micro-electrode technique. A number of previous workers have made such investigations in tortoise (Gilles-Baillien & Schoffeniels, 1965; Wright, 1966), bullfrog (White & Armstrong, 1970, 1971), hamster (Wright, 1966), rabbit (Rose & Schultz, 1970, 1971*a*, *b*) and most recently rat (Lyon & Sheerin, 1971). The changes in transmural potential difference obtained under different experimental conditions are reflected in changes in membrane potentials. Major differences between the contributions of the two membranes have been reported and have led workers to different interpretations of the generation of the transmural potential. To extend our knowledge of membrane potentials under different conditions and as a contribution to a better understanding of these potentials, the potential profile of the rat intestinal epithelium has been further studied.

METHODS

Three different potentials were measured:

The mucosal potential which is the potential difference across the mucosal membrane of the intestinal epithelial cell and was taken to be the potential of the micro-electrode with respect to the electrode in the mucosal fluid.

The serosal potential which is the potential difference across the serosal membrane of the epithelial cell and was taken to be the potential of the micro-electrode with respect to the electrode in the serosal fluid.

The transmural potential which is the potential difference of the electrode in the serosal fluid with respect to that in the mucosal fluid.

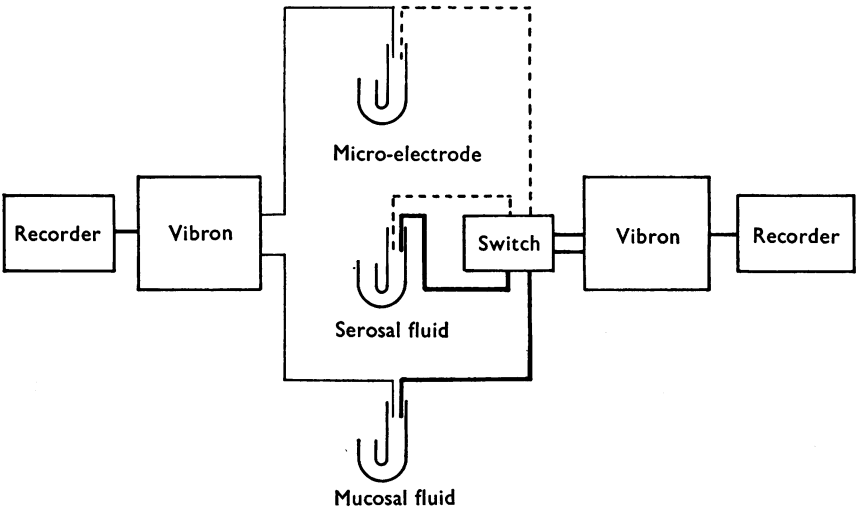
Electrodes. Micro-electrodes were drawn from Pyrex tubing (internal diameter 2 mm) using a vertical puller (Winsbury, 1956). All micro-electrodes were examined under the microscope and those not having a fine tip were discarded. The micro-electrodes were then filled with 3 M-KCl using a method based on that of Tasaki, Polley & Orrego (1954). Before use each micro-electrode was tested and only those with a resistance of 10–50 MΩ and a tip potential of 5 mV or less were selected for use. The micro-electrode was connected by means of a salt bridge (a polyethylene tube filled with M-KCl/3% agar) to a calomel half-cell.

Membrane potentials were measured with reference to *mucosal and serosal electrodes*. These consisted of salt bridges which were in contact with the mucosal and serosal fluids respectively. The transmural potential was measured between the serosal and mucosal electrodes. Both mucosal and serosal electrodes were connected to calomel half-cells.

Electrical circuit. The potentials were measured by two Vibron electrometers (Electronic Instruments Ltd) which were linked to the electrodes via the three calomel half-cells, and were recorded on two Beckman recorders. The arrangement of the electrical circuit is shown in Text-fig. 1. One electrometer was used to record the mucosal potential while a switching device enabled the second electrometer to be used to measure both the serosal and transmural potentials. A correction, which took into account the tip potential of the micro-electrode and the imbalance between the calomel half-cells, was applied to the potentials recorded.

Animals. White female rats bred in the Sheffield Field Laboratories and weighing between 230 and 250 g were used. Before experiment the animals were maintained on an unrestricted diet (diet 86, Oxoid, London) with free access to water.

Experimental procedure. The rats were anaesthetized with I.P. Nembutal and the small intestine was removed and everted (Wilson & Wiseman, 1954). The experimental arrangement used to record membrane potentials is shown in Text-fig. 2. A 3·5 cm segment from the mid-region of the intestine was tied over a glass cannula designed to give firm support to the tissue. That part of the cannula which was covered by intestine was perforated in order to allow good electrical contact between the tissue and the serosal fluid (0·5 ml.). The preparation was set up in a glass vessel containing 50 ml. mucosal fluid which was gassed throughout the experiment with 95% O_2/5% CO_2. Both mucosal and serosal fluids were Krebs bicarbonate saline (Krebs & Henseleit, 1932) maintained at 37° C. The micro-electrode was held in a Leitz micromanipulator and before use was positioned with its tip immediately above the upper surface of the intestine.



Text-fig. 1. The arrangement of the electrical circuit used during the measurement of membrane potentials in epithelial cells of rat jejunum. —— Transmural potential, - - - - Serosal potential, —— Mucosal potential.

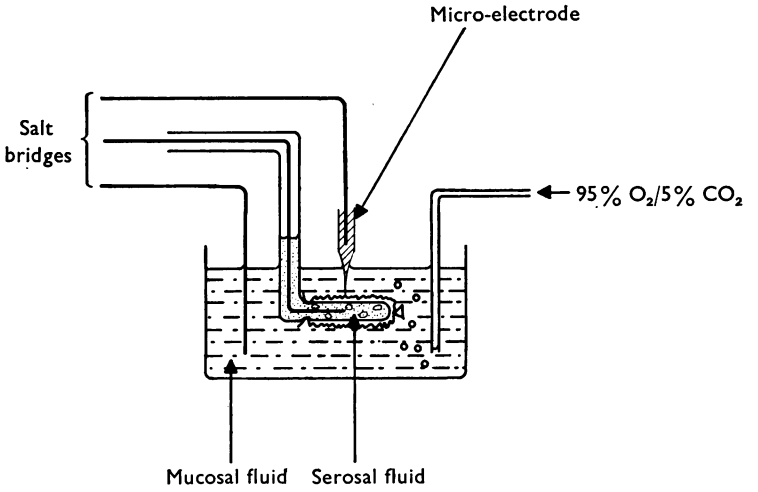
In all cases the preparation was left after setting up for at least 5 min, during which the transmural potential was monitored continuously, and no membrane potentials were measured until this potential was steady. To record membrane potentials the micro-electrode was slowly brought towards the gut surface by the micromanipulator. It was considered that the micro-electrode had entered a cell when there was a sharp increase in the negativity of the recording. In many cases this potential was not maintained after the initial impalement of the cell. Only those potentials that remained stable for more than 15 sec were included in the results.

Stripped sacs. In order to assess whether the muscle layers affected the potentials recorded, some experiments were carried out on preparations in which the outer muscle layer had been removed, using a method similar to that employed in the colon by Parsons & Paterson (1960). The ability of stripped sacs to generate a transmural potential was compared with that of normal everted sacs using the method of Barry, Dikstein, Matthews, Smyth & Wright (1964). Their transfer capacity was

measured as described by Barry, Matthews & Smyth (1961) and the terminology used to express the results is that defined in their paper.

Glucose and galactose were estimated by the total reducing power using the method of Nelson (1944) as modified by Somogyi (1952).

Results are expressed as mean values ± S.E. of the mean with the number of observations in brackets.



Text-fig. 2. The apparatus used to record membrane potentials. The micro-electrode was positioned immediately above the gut and mucosal and serosal electrodes were in contact with the mucosal and serosal fluids respectively. The mucosal fluid was gassed continuously with 95% O_2/5% CO_2.

RESULTS

Stripped sacs

The stripping of the intestine resulted in a weight loss of 22·8 ± 1·2 (12) % and examination under the microscope revealed that most of the outer muscle layer had been removed (Pl. 1). The ability of stripped sacs to generate transmural potential differences and to transport sugars actively was compared with that of normal sacs (Tables 1 and 2). The potential differences were slightly lower in the stripped preparations but the increases in the potential difference in the presence of actively transferred sugars (transfer potentials) were similar in the two preparations. Stripped sacs have retained their ability to transfer sugars actively since they are able to establish a significant concentration gradient for both glucose and galactose. Their transfer capacity for both fluid and hexose is lower than that of normal sacs but it is still substantial. That the stripped preparation is an active tissue is confirmed by the observation that it metabolizes a significant amount of glucose. It can therefore be considered

that this is a viable preparation and can be used to measure electrical and transfer parameters of the rat small intestine.

TABLE 1. Transmural potential differences in stripped sacs compared with those in normal sacs of rat jejunum. Hexoses, when present, were at a concentration of 28 mM, in both mucosal and serosal fluids. The potential difference is given in mV

	Stripped	Normal
No added solute	1·0 ± 0·1 (6)	1·9 ± 0·1 (6)
Glucose	11·6 ± 0·7 (6)	13·5 ± 0·4 (6)
Galactose	8·6 ± 0·5 (6)	9·4 ± 0·4 (6)

TABLE 2. Transfers of fluid and hexose by stripped and normal sacs of rat jejunum after 30 min incubation in the presence of 28 mM glucose or 28 mM galactose in both mucosal and serosal fluids. Results are expressed per g initial wet weight

	Stripped	Normal
No. expts	6	5
Mucosal glucose transfer (μmole)	131·9 ± 4·4	224·4 ± 15·9
Final glucose concn. gradient (mM)	22·5 ± 2·3	36·6 ± 2·2
Glucose metabolized (μmole)	54·1 ± 6·7	66·9 ± 5·6
Mucosal galactose transfer (μmole)	71·9 ± 12·5	88·3 ± 9·2
Final galactose concn. gradient (mM)	20·9 ± 1·6	22·5 ± 3·5

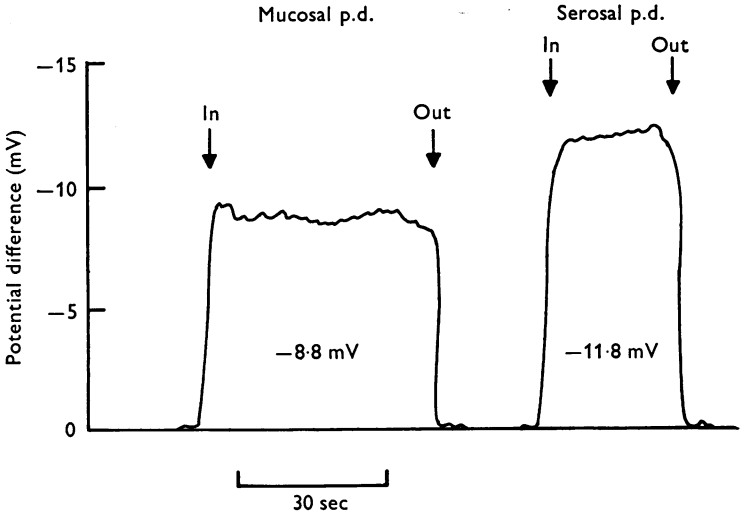
Measurement of membrane potentials

When the micro-electrode entered an epithelial cell of a normal sac the potential became negative relative to both mucosal and serosal electrodes. This change was reversed when the micro-electrode was withdrawn. A typical recording is shown in Text-fig. 3. Mean values for mucosal, serosal and transmural potentials are shown in Table 3. Whilst the intracellular electrode is always negative with respect to both serosal and mucosal electrodes, the mucosal potential is shown in most Text-figures and Tables as a negative step and the serosal potential as a positive step. In the absence of sugar, the serosal potential step of 11·9 mV is slightly greater than the mucosal potential step of 9·3 mV. The sum of the mean potentials gives a calculated value for the transmural potential of 2·6 mV which agrees well with the measured transmural potential of 2·7 mV. A similar pattern of results is obtained with stripped sacs as with normal sacs.

Effect of actively transferred hexoses and amino acids

On addition of 28 mM glucose the mucosal potential was unchanged but the serosal potential increased sharply (Table 4). This occurred in both normal and stripped sacs (Table 3). Again there is close agreement between calculated and measured transmural potentials. However, in stripped sacs

the serosal and transmural potentials were somewhat lower than in normal sacs. The removal of the external muscle layer will have reduced the diffusion barrier behind the epithelial cell, allowing much closer equilibration between the serosal fluid and the fluid in contact with the serosal pole of the cell. Changes in the ionic composition of the fluid adjacent to the serosal membrane will be dissipated more rapidly in stripped sacs so that the ionic gradients across this membrane may differ in stripped and normal sacs. This could account for the slight differences in potential. Other sugars which are actively transferred but not metabolized by rat



Text-fig. 3. A recording of membrane potentials. The intestinal preparation was bathed on both sides with Krebs bicarbonate saline with no added solute. The arrows labelled in and out show the entry and withdrawal of the micro-electrode. The measured transmural potential was 2·9 mV.

TABLE 3. Membrane potentials in stripped and normal everted sacs of rat jejunum in the absence of added solute and in the presence of 28 mM glucose in both mucosal and serosal fluids. Three measurements of membrane potentials were made from each intestinal preparation

	Mucosal p.d. (mV)	Serosal p.d. (mV)	Transmural p.d. (mV)
No added solute			
Stripped	−9·2 ± 0·1 (12)	11·3 ± 0·1 (12)	2·1 ± 0·1 (12)
Normal	−9·3 ± 0·2 (12)	11·9 ± 0·3 (12)	2·7 ± 0·3 (12)
28 mM glucose			
Stripped	−9·5 ± 0·2 (12)	18·3 ± 0·2 (12)	8·8 ± 0·2 (12)
Normal	−9·4 ± 0·1 (12)	19·8 ± 0·3 (12)	10·6 ± 0·3 (12)

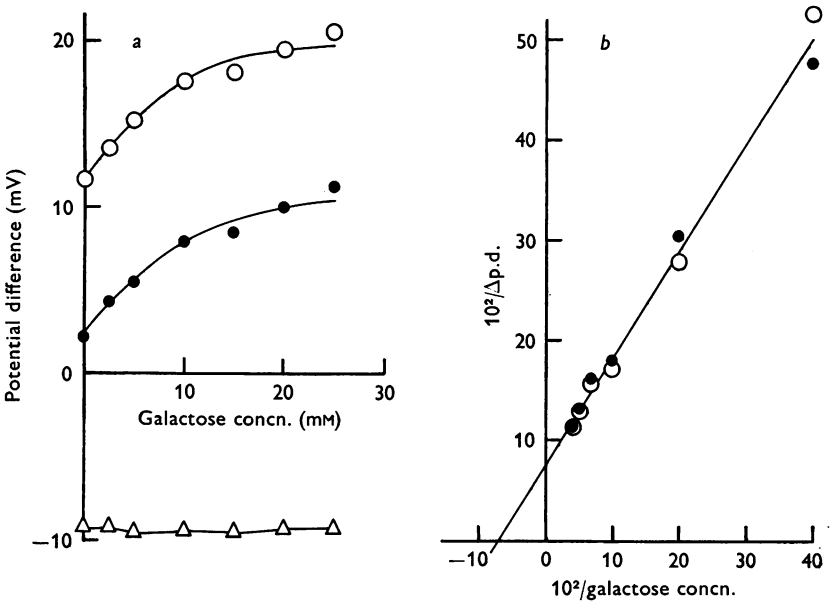
small intestine also increase the transmural potential (Table 4) and again this increase is totally due to changes in the serosal potential. Similar findings in the presence of actively transferred amino acids demonstrate that in the development of a transfer potential the mucosal potential is unchanged and the increased transmural potential results from an increase in the serosal potential.

TABLE 4. Effect of actively transported hexoses and amino acids in both mucosal and serosal fluids on membrane potentials in rat jejunal epithelial cells. Three intracellular recordings were made from each preparation

	Mucosal p.d. (mV)	Serosal p.d. (mV)	Transmural p.d. (mV)
No added solute	$-9{\cdot}3 \pm 0{\cdot}2$ (12)	$11{\cdot}9 \pm 0{\cdot}3$ (12)	$2{\cdot}7 \pm 0{\cdot}3$ (12)
28 mM glucose	$-9{\cdot}4 \pm 0{\cdot}1$ (12)	$19{\cdot}8 \pm 0{\cdot}3$ (12)	$10{\cdot}6 \pm 0{\cdot}3$ (12)
28 mM galactose	$-9{\cdot}7 \pm 0{\cdot}2$ (12)	$17{\cdot}5 \pm 0{\cdot}5$ (12)	$7{\cdot}9 \pm 0{\cdot}5$ (12)
28 mM 3-*O*-methyl glucopyranose	$-9{\cdot}5 \pm 0{\cdot}2$ (12)	$15{\cdot}6 \pm 0{\cdot}4$ (12)	$6{\cdot}1 \pm 0{\cdot}2$ (12)
20 mM L-alanine	$-9{\cdot}2 \pm 0{\cdot}1$ (9)	$16{\cdot}9 \pm 0{\cdot}2$ (9)	$7{\cdot}7 \pm 0{\cdot}2$ (9)
20 mM L-glycine	$-9{\cdot}3 \pm 0{\cdot}1$ (9)	$15{\cdot}3 \pm 0{\cdot}4$ (9)	$6{\cdot}0 \pm 0{\cdot}3$ (9)

Effect of hexose concentration

The magnitude of the hexose transfer potential increases with hexose concentration (Barry *et al.* 1964) and the relationship between them is consistentwith Michaelis–Menten kinetics (Lyon & Crane, 1966). If transfer potentials result entirely from increases in the serosal potential it would be expected that changes in the serosal potential with hexose concentration would also follow Michaelis–Menten kinetics. This relationship was tested using galactose (Text-fig. 4*a*). The serosal potential increased with the galactose concentration and saturation appeared to occur. Lineweaver–Burke plots were made using the reciprocals of the transfer potentials and of the changes in serosal potential against the reciprocal of the galactose concentration (Text-fig. 4*b*). From these plots two values for the apparent K_m for the galactose transfer potential were calculated. Using the galactose-dependent increase in the serosal potential a value of 14·9 mM was obtained, while using the rise in the transmural potential a figure of 14·1 mM was obtained. The close agreement between these two figures emphasizes the relationship between the transfer potential and the rise in the serosal potential.



Text-fig. 4. Effect of galactose concentration on membrane potentials. The sugar was initially present at the same concentration in both mucosal and serosal fluids. (*a*) the measured potentials; (*b*) the relationship between the reciprocal of galactose concentration and the reciprocals of the changes in serosal and transmural potentials caused by this sugar. Each point represents the mean of nine observations obtained from three preparations. The mucosal potential is denoted by the symbol △, the serosal potential by ○, and the transmural potential by ●.

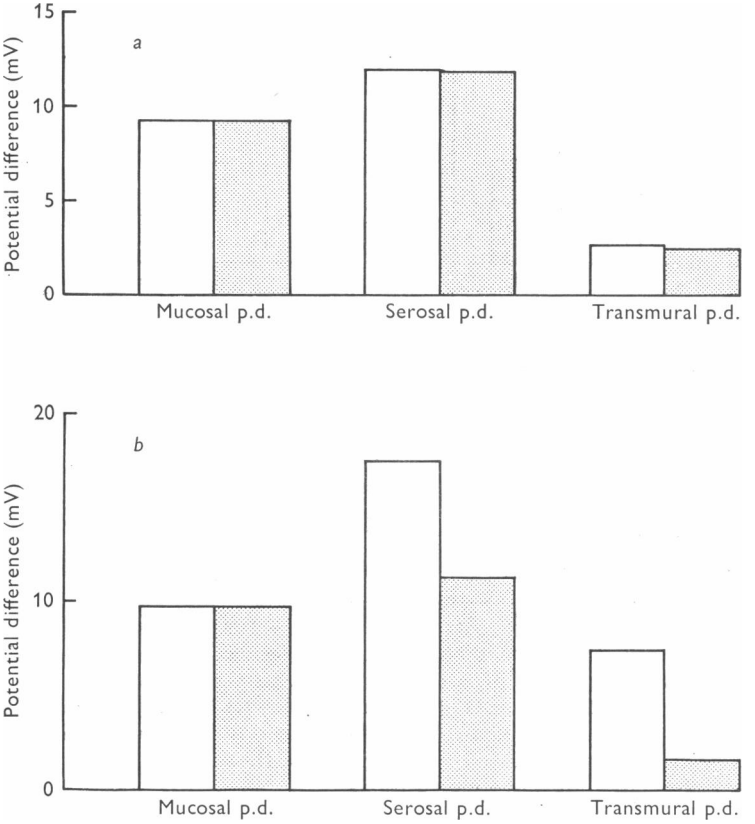
Effect of phlorrhizin

Phlorrhizin competitively inhibits active hexose transport (Newey, Parsons & Smyth, 1959) and also abolishes the transfer potential (Barry *et al.* 1964). Its effect on membrane potentials was therefore investigated, phlorrhizin being added to the mucosal fluid to give a final concentration of 5×10^{-4} M. Phlorrhizin had no effect on either the membrane potentials or the transmural potential in the absence of sugar (Text-fig. 5). However, when 28 mM galactose was present, phlorrhizin reduced the transmural potential by causing a decrease in the serosal potential, but was without effect on the mucosal potential.

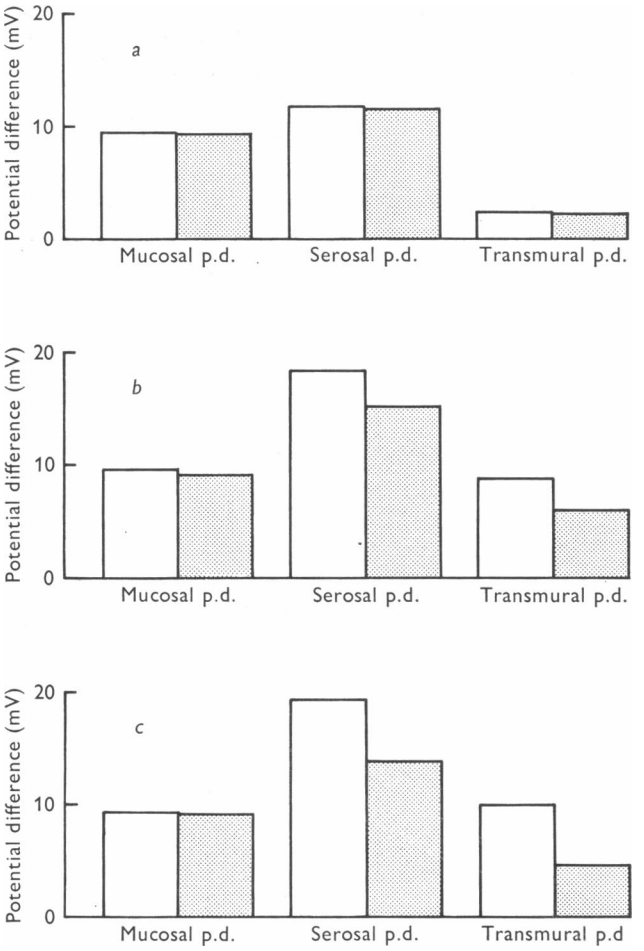
Effect of ouabain

The findings so far are consistent with the concept of a serosally located, electrogenic sodium pump whose activity is stimulated by the active transport of sugars and amino acids. This pump is thought to be inhibited by ouabain (Schultz & Zalusky, 1964*a*) and it was therefore of interest to

study the effect of this cardiac glycoside on intestinal membrane potentials (Text-fig. 6). As ouabain is only effective from the serosal side of the cell, stripped sacs were used to reduce the diffusion barrier between the cell and serosal fluid. Ouabain was added to the serosal fluid to give a final concentration of 10^{-3} M, and in the absence of hexose it had no effect on any of the three potentials measured. However, in the presence of glucose ouabain reduced the transmural potential by causing a fall in the serosal potential and this was even more marked in the case of galactose. No changes in the mucosal potential were observed.



Text-fig. 5. Effect of 5×10^{-4} M phlorrhizin in the mucosal fluid on potentials measured in the absence of added solute (*a*) and in the presence of 28 mM galactose (*b*). The light areas indicate control values while the dark areas indicate potentials measured in the presence of phlorrhizin. Each value represents the mean of twelve observations obtained from four preparations.



Text-fig. 6. Effect of 10^{-3} M ouabain in the serosal fluid on potentials measured in the absence of added solute (*a*), in the presence of 28 mM glucose (*b*) and in the presence of 28 mM galactose (*c*). The light areas indicate control values while the dark areas indicate potentials measured in the presence of ouabain. Each value represents the mean of twelve observations obtained from three preparations.

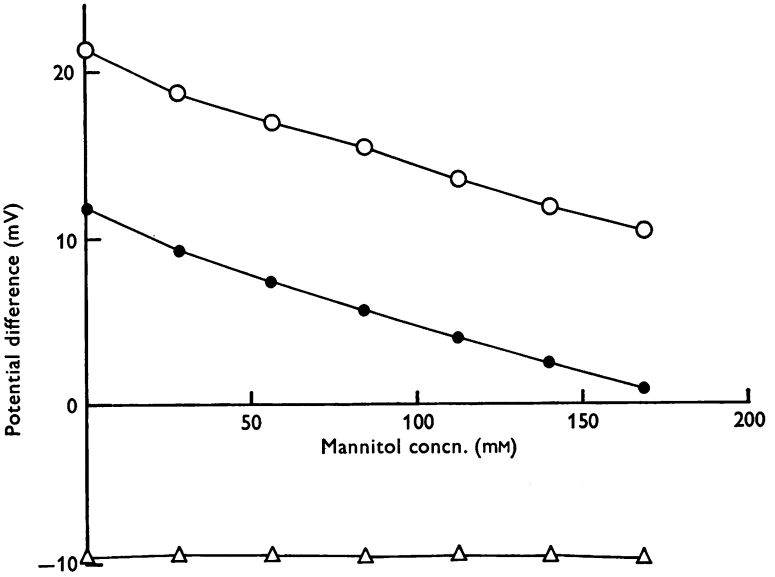
Effect of metabolic inhibitors

The effects of 2,4-dinitrophenol (2×10^{-4} M) and sodium fluoroacetate (10^{-2} M) were determined by adding these inhibitors to the mucosal fluid when 28 mM galactose was present (Table 5). Both inhibitors caused a fall in the transmural potential which resulted solely from a decrease in the serosal potential. Both these inhibitors block the production of high energy compounds from aerobic metabolism; 2,4-dinitrophenol by uncoupling

oxidative phosphorylation and sodium fluoroacetate by blocking the Krebs TCA cycle. Thus the maintenance of the serosal potential in the presence of galactose depends on a supply of energy derived from aerobic metabolism.

TABLE 5. Effect of metabolic inhibitors in the mucosal fluid on membrane potentials in the presence of 28 mM galactose in both mucosal and serosal fluids. Three recordings of the membrane potentials were made on each preparation and these were taken 10 min after addition of the inhibitor

	Mucosal p.d. (mV)	Serosal p.d. (mV)	Transmural p.d. (mV)
Control	$-9{\cdot}4 \pm 0{\cdot}1$ (9)	$19{\cdot}9 \pm 0{\cdot}5$ (9)	$10{\cdot}4 \pm 0{\cdot}6$ (9)
2×10^{-4} M DNP	$-9{\cdot}4 \pm 0{\cdot}1$ (9)	$11{\cdot}0 \pm 0{\cdot}2$ (9)	$1{\cdot}6 \pm 0{\cdot}2$ (9)
Control	$-9{\cdot}3 \pm 0{\cdot}1$ (9)	$19{\cdot}5 \pm 0{\cdot}9$ (9)	$10{\cdot}1 \pm 0{\cdot}9$ (9)
10^{-2} M sodium fluoroacetate	$-9{\cdot}2 \pm 0{\cdot}1$ (9)	$13{\cdot}5 \pm 0{\cdot}4$ (9)	$4{\cdot}3 \pm 0{\cdot}5$ (9)



Text-fig. 7. Effect of an osmotic gradient on membrane potentials in the presence of 28 mM glucose in both mucosal and serosal fluids. Mannitol was added to the mucosal fluid at the concentrations shown. Each point represents the mean of six observations obtained from three preparations. The mucosal potential is denoted by the symbol △, the serosal potential by ○, and the transmural potential by ●.

Effect of osmotic gradients

The transmural potentials are modified by osmotic gradients across the wall of the small intestine (Smyth & Wright, 1964, 1966). In this study the membrane potential as well as transmural potential changes have been

recorded with increasing osmotic gradients produced by the addition of the impermeant solute mannitol to the mucosal fluid. In the presence of glucose (Text-fig. 7), both the transmural and serosal potentials fell with increasing osmotic gradient, whilst the mucosal potential was unchanged. Transmural and serosal potentials fell at the constant rate of 0·064 mV/mM mannitol.

DISCUSSION

The transmural potential across the small intestine is the sum of at least two potentials, those across the mucosal and serosal membranes of the epithelial cell. The present study has confirmed that the inside of the cell is negative with respect to both mucosal and serosal fluids, although the magnitude of the membrane potentials are low compared with those found in many other epithelial tissues. For example, in the rat colon mucosa (Edmonds & Nielsen, 1968), in the proximal and distal regions of the kidney tubule (Windhager & Giebisch, 1965) and in the toad bladder (Janacek, Morel & Bourguet, 1968) membrane potentials of up to 70 mV have been recorded.

It is well established that the active transfer of non-electrolytes causes a rise in the transmural potential difference. The application of intracellular recording techniques has enabled workers to draw conclusions concerning the site of origin of the transfer potential. In these experiments the transfer potential in the rat small intestine is seen to be entirely due to an increase in the serosal potential, the mucosal potential remaining unchanged. This is in agreement with the recent results of Lyon & Sheerin (1971) also in the rat, Gilles-Baillien & Schoffeniels (1965) in the tortoise and Wright (1966) in the hamster. These observations are consistent with the concept of a serosally located, electrogenic sodium pump, which is stimulated by the presence of actively transported sugars and amino acids (Schultz & Zalusky, 1964*b*, 1965; Crane, 1965). On the other hand, experiments with rabbit ileum (Rose & Schultz, 1970, 1971*a*, *b*) and bullfrog intestine (White & Armstrong, 1970, 1971) have suggested that the transfer potential results from a reduction in both mucosal and serosal potentials, a larger decrease occurring at the mucosal membrane. These workers consider that the entry of the actively transferred substance, together with sodium, into the cell is an electrogenic process which results in a depolarization of the mucosal membrane. They suggest that the observed fall in the serosal potential is due to the presence of an intercellular shunt pathway which transmits potential changes from the mucosal to the serosal membrane. Rose & Schultz have criticized Wright's (1966) experiments on the grounds that the impaled cells were damaged so that they were unable to respond to an actively transferred substance by a

depolarization of the mucosal membrane. If the serosal potential was calculated from measured transmural and mucosal potentials, the absence of a change in the mucosal potential of the impaled cell would lead to the conclusion that the increased transmural potential resulted from a rise in the serosal potential. However, Rose and Schultz's objection cannot be applied to Wright's experiments nor to those reported here, as both serosal and mucosal potentials were measured simultaneously, together with the transmural potential. Gilles-Baillien & Schoffeniels (1965) and Lyon & Sheerin (1971) also made direct measurements of both mucosal and serosal potentials. The absence of an effect of actively transferred substances on the mucosal potential in these experiments could be explained if the entry of sodium into the cell was balanced by an equivalent efflux of another monovalent cation. It has been suggested (Bosačková & Crane, 1965) that when the carrier loaded with sodium and the actively transferred sugar reaches the inner side of the mucosal membrane the sodium and sugar dissociate from the carrier and the sodium site on the carrier is then occupied by potassium. The sugar is unable to associate with the potassium carrier complex so that this crosses the membrane while the sugar remains within the cell. Such a mechanism would not be expected to generate a potential difference since every sodium ion entering the cell on the carrier is balanced by a potassium ion leaving the cell.

The observations that actively transported sugars and amino acids did not increase the potential across the serosal membrane of rabbit and bullfrog intestinal epithelial cells does not exclude the possible existence of an electrogenic sodium pump at this membrane, since its electrical effect might be obscured by the presence of a low resistance intercellular shunt pathway. It has been suggested (Frömter & Diamond, 1972) that the rat small intestine is a 'leaky' membrane and might therefore possess a significant shunt pathway. This suggestion is based on the low transmural resistance of this tissue which is in the order of $30\,\Omega/cm^2$ (Barry, Smyth & Wright, 1965). However, this figure is related to the serosal area and it may be more accurate to relate tissue resistance to the mucosal area, since it is the mucosal epithelial layer that is responsible for the transmural electrical activity of the gut. It has been estimated that the area of the epithelium is 30 times greater than the serosal area (Wilson, 1962) and therefore the resistance of the small intestine would be in the order of $900\,\Omega/cm^2$ mucosal area. Similar arguments apply to values for the osmotic water permeability which are also related to the serosal area. On the basis of these revised figures the rat small intestine would appear to be a particularly 'tight' membrane. This is supported by the observations that in the rat small intestine changes in the potential across one membrane were not reflected in an alteration of the potential across the other membrane.

Changes both in the serosal potential and in the transmural transfer potentials obtained at different galactose concentrations were consistent with saturation kinetics. Reciprocal Lineweaver-Burke plots gave apparent K_m values of 14·9 mM from changes in the serosal potential and 14·1 mM from the transmural transfer potentials. This close agreement emphasizes the dependence of the galactose transfer potential on changes in the serosal potential difference.

Phlorrhizin competitively inhibits intestinal hexose transfer and reduces the transmural hexose transfer potential. This reduction results entirely from a modification of the serosal potential response. Phlorrhizin effects are consistent with an electrogenic sodium pump situated at the serosal membrane. Experiments with ouabain provided additional support for such a hypothesis, since ouabain is thought to be a specific inhibitor of the sodium, potassium-dependent ATPase that produces energy for the sodium pump (Dunham & Glynn, 1961). In the presence of glucose and galactose ouabain decreased the transfer potential by an effect on the serosal potential. Again this suggests that a sodium pump, whose activity is stimulated by actively transferred hexoses, is located at this membrane. In the absence of hexoses ouabain had no effect on intestinal membrane potentials and it therefore appears that this sodium pump does not make a significant contribution to the endogenous potential. The sodium pump appears to depend on energy derived from aerobic metabolism since 2,4-dinitrophenol and sodium fluoroacetate reduced the serosal potential. The mucosal potential was unaffected by these metabolic inhibitors and so may either be due to passive processes or may use energy supplies that are independent of aerobic metabolism.

Osmotic gradients across the small intestine led to a reduction in the transmural potential. Again this resulted from a decrease in the serosal potential. Streaming potentials are thought to be due to the movement of fluid through the polar regions of membranes (Wright & Diamond, 1969), the direction of the potential change in this case indicating that the membranes must bear negative charges. The origin of the streaming potential appears to be to the serosal side of the epithelial cell, a similar situation being observed by Lyon & Sheerin (1971), although the range of osmotic gradients that they used was much smaller than those reported here.

The results obtained from this investigation support the view that the active transport of sugars and amino acids increases the transintestinal potential by causing a rise in the activity of an electrogenic sodium pump situated at the serosal membrane of the epithelial cells. It appears that the sodium-dependent entry of these substances into the cell across the mucosal membrane is a non-electrogenic process since no change in the potential across this membrane was observed.

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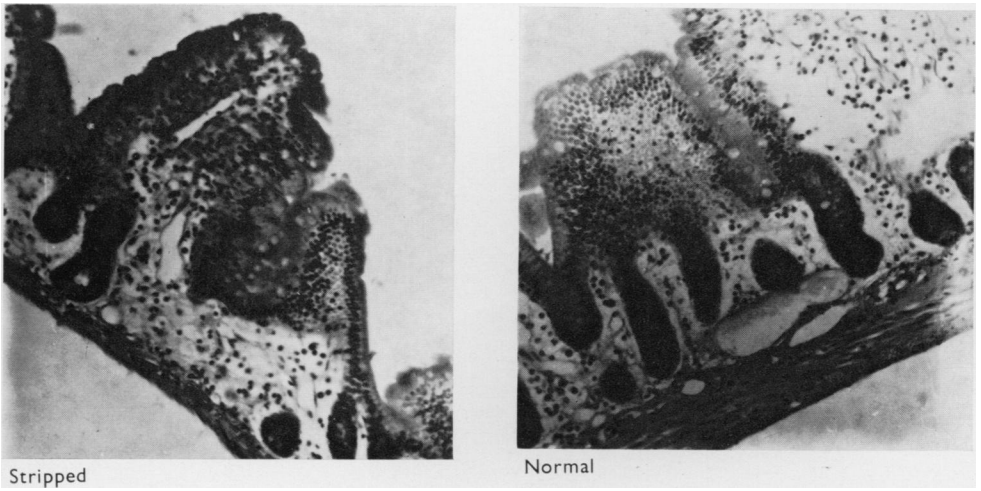
EXPLANATION OF PLATE

Comparison of stripped and normal sacs of rat jejunum. Stained with haematoxylin + eosin. Transverse section × 180.


Stripped
Normal