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ELECTRICAL POTENTIALS ASSOCIATED WITH INTESTINAL SUGAR TRANSFER

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The rat small intestine has the capacity to transfer a number of substances, including hexoses, inorganic salts and fluid. Riklis & Quastel (1958) showed that movement of hexose was dependent on the presence of sodium, and Barry, Matthews & Smyth (1961) showed that a part of the fluid transfer was dependent on the presence of glucose, and part was independent of it. It is generally assumed that fluid movement in living tissues is related to movement of inorganic salts, and there must therefore be a complex inter-relation between transfer of sodium, glucose and fluid. Since electrical changes in other tissues are related to ion pumps, an investigation was undertaken into the relation between the electrical potential across the wall of the intestine and the transfer of fluid, various ions, hexoses and their derivatives. The present paper deals with transfer of fluid and sugars in relation to electrical changes, and preliminary communications of the results have been given by Barry, Dikstein, Matthews & Smyth (1961), and Barry, Matthews, Smyth & Wright (1962).

METHODS

White male rats of the Sheffield strain were used. Before experiments the rats were maintained on an unrestricted diet of rat cubes No. 86 (Oxoid, London). The work included three different kinds of experiments: (1) the effect of hexoses on the electrical potential difference across the gut wall, and this was studied both *in vitro* and *in vivo*; (2) determination of the rate of fluid transfer maintained by different sugars or their derivatives; and (3) the extent of metabolism of these sugars by the intestine: these last two were studied by *in vitro* methods. In the *in vitro* experiments the intestine was suspended in bicarbonate saline (Krebs & Henseleit, 1932), which was in equilibrium with a gas mixture of 5 % CO_2 and 95 % O_2. When anoxic conditions were required a gas mixture of 5 % CO_2 and 95 % N_2 was used.

Measurement of electrical potentials across intestine

Electrical recording. The potentials were led off from the solutions on each side of the gut wall to calomel half-cells by means of polythene tubes filled with M-KCl-3 %-agar, and these tubes are referred to subsequently as salt bridges. The pairs of calomel half-cells were selected to balance each other to within 1 mV. The half-cells were connected with a Hewlett–Packard vacuum tube voltmeter, with a minimum input impedance of 10 MΩ.

In vitro preparations. The preparation most frequently used was the everted sac of intestine (Wilson & Wiseman, 1954), in which there is only a small volume of fluid (the serosal fluid) in contact with the peritoneal layer of the gut. In some experiments a larger volume of serosal fluid was required, and in these cases a technique was used which had

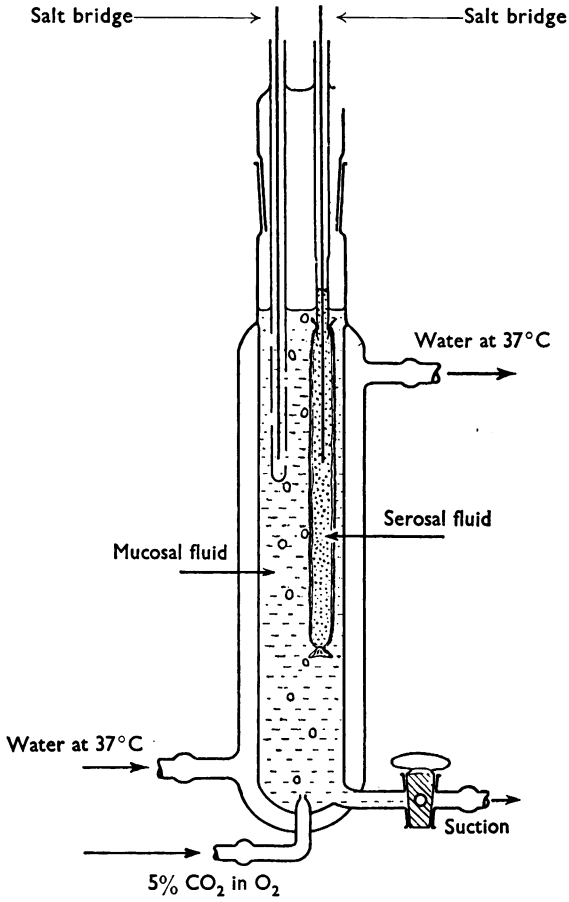

Fig. 1. Apparatus for recording the potential across the wall of everted sacs of small intestine. The sac, tied on to a cannula, contains the serosal fluid and is suspended in the mucosal fluid. Polythene tubes filled with M-KCl agar act as salt bridges for leading off the potential to calomel half-cells connected with a Hewlett–Packard voltmeter. The preparation is maintained at 37° C by means of warm water circulated through an outer jacket. The glass tube leading the salt bridge into the mucosal fluid has a number of holes which allow electrical contact with the mucosal solution and prevent trapping of bubbles around the end of the salt bridge.

been modified from that described by Wiseman (1953), by means of which saline can be circulated through segments of intestine. The modifications were (*a*) only one segment of intestine was used, and (*b*) the segment of intestine was everted. These *in vitro* preparations will be referred to subsequently as the everted sac and the everted segment respectively.

Everted sac. The rats were anaesthetized with pentobarbitone Nembutal (Abbott Laboratories), the small intestine was removed below the duodeno-jejunal flexure and divided into five segments, as described by Barry, B. A. *et al.* (1961). The division into five was to locate the position of the part of the intestine used, and for this purpose the sacs made from these segments are numbered I to V, starting from the jejunal end. It was, however, convenient not to use the whole of each segment, and sacs of about 10 cm were made from these. Although they are not complete fifths of the intestine they are nevertheless denoted by the terms sac I, sac II, etc., corresponding to the part of the gut from which they are made. It is essential to indicate the part of the gut from which the sac is made, as Barry, B. A. *et al.* (1961) have shown important differences in the transfer properties of different segments of the rat intestine. The experimental arrangement is shown in Fig. 1. At its upper end the everted sac was tied over a cannula; and the sac suspended from the cannula was immersed in 125 ml. of bicarbonate saline with a small glass weight attached

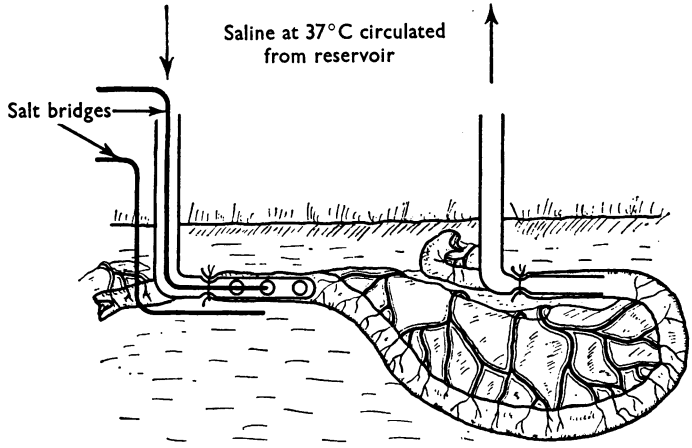

Fig. 2. Apparatus for recording the electrical potential across the gut wall *in vivo*. Two L-shaped cannulae enable saline to be circulated through the lumen of a segment of gut. One of these with perforated walls carries one of the salt bridges; the other salt bridge is held close to the peritoneal surface of the gut.

to the lower end to maintain the sac in a vertical position. The fluid surrounding the sac is referred to as the mucosal fluid, and is continuously gassed and maintained at 37° C by means of an outer water-jacket. The mucosal fluid could be changed within 30 sec by withdrawing under suction, and pouring in warmed gassed saline. A small volume (1–1·5 ml.) of bicarbonate saline was injected through the cannula into the sac of intestine, and this is referred to as the serosal fluid. A glass tube projected into the mucosal fluid, and through this and through the cannula supporting the sac the salt bridges were led off to the calomel half-cells.

Everted segment. After eversion of the intestine a segment about 10 cm in length was selected from the middle region and tied on to the apparatus in a manner similar to that described by Wiseman (1953). The volume of the mucosal solution, i.e. solution in the outer chamber in contact with the mucosal surface of the gut, was 80 ml., and the volume of the serosal solution, i.e. that circulated from the reservoir through the tube of everted intestine and in contact with its serosal surface, was 20 ml. Salt bridges dipped into the mucosal and serosal solutions for leading off the potentials.

In vivo. A modification of the technique of Sheff & Smyth (1955) was used. A rat was anaesthetized with pentobarbitone the abdominal cavity opened by a mid-line incision, and

the small intestine washed out with 0·9 % NaCl at 37° C. A short length (about 5 cm) of the intestine was selected and cannulated. The proximal cannula was L-shaped and contained a salt bridge forming contact with the mucosal fluid but not in contact with the mucosal epithelium (Fig. 2). A second bridge was introduced into the abdominal cavity close to the peritoneal surface of the segment of intestine selected for study. Electrical contact was made by running warm 0·9 % NaCl into the peritoneal cavity. Fluid (50 ml. of 0·9 % NaCl) was recirculated through the segment of intestine, and this fluid could be changed during the course of an experiment.

The position of the segment used was determined at the end of the experiment by removing and then weighing the small intestine proximal and distal to the segment. The position of the segment is described as proximal jejunum, mid intestine and distal ileum, these parts of the intestine *in vivo* corresponding roughly to sacs I, III and V *in vitro*.

Measurement of fluid transfer

Fluid transfer was measured in sacs made from fifths of everted intestine as described by Barry, B. A. *et al.* (1961), and the parameter used was the mucosal fluid transfer. This is the decrease in volume of the mucosal fluid during the period of incubation, but is calculated from the sum of the increase in fluid in the gut wall and the increase in volume inside the sac.

Measurement of sugar metabolized

Metabolism was measured by the technique described by Parsons, Smyth & Taylor (1958), in which an everted sac preparation is used with sugar initially present in known amount in both the serosal and mucosal solutions. After 1 hr incubation, the flask was plunged into boiling water for 3 min. The gut was ground with sand, pooled with the mucosal and serosal solutions, and an aliquot of this was taken for determination of sugar. The amount metabolized is the difference between the amount initially present and the amount recovered at the end of the experiment.

Chemical. The sugars and related substances used were all of A.R. quality; D-glucose, D-fructose and mannitol were obtained from British Drug Houses Ltd., D-galactose from Eastman Organic Chemicals, α-methyl-D-glucoside from Mann Research Laboratories, 3-*o*-methyl-D-glucopyranose from Light & Co. Ltd., and 2-deoxyglucose from Boots Pure Drug Co. Ltd. (The source is specified on account of the possibility that very small amounts of impurities could affect the results.) In referring collectively to these substances it will occasionally be found convenient (although inaccurate) to use the general term 'sugar', e.g. sugar-dependent potential, and to use the term 'transferable' to apply to those substances listed by Crane (1960) as capable of movement by the intestine against a concentration gradient.

All the sugars and related substances were estimated by total reducing power, by the method of Nelson (1944) as modified by Somogyi (1945). In the case of α-methyl-D-glucoside previous hydrolysis was carried out. To 5 ml. of the deproteinized filtrate 1·5 ml. of 30 % HCl was added and this was incubated at 100° C in a stoppered tube for 4 hr. It was then neutralized and made up to 10 ml. before estimation as glucose.

RESULTS

Electrical potential measurements

Effect of glucose

In vitro. When a sac of intestine from any part of the jejunum or ileum was set up in glucose-free saline, a small potential was recorded of 3–7 mV; the serosal side being positive to the mucosal side. This potential rapidly

fell off, but within 5–10 min became stabilized at 1–2 mV, and remained at this level for periods up to 1 hr. The time course of the changes in sac III is included in Fig. 3. Sac III usually showed a slightly higher potential

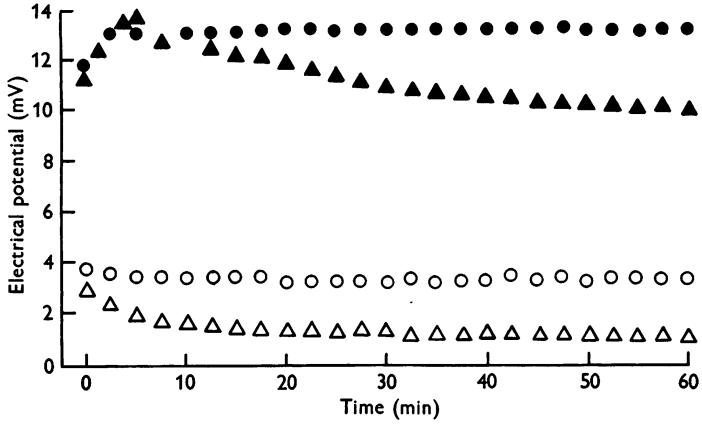

Fig. 3. Time course of the electrical changes across the intestine *in vivo* in the presence of 56 mM glucose (●), *in vitro* in the presence of 28 mM glucose (▲), *in vivo* in the absence of glucose (○), and *in vitro* in the absence of glucose (△). The concentrations of glucose were chosen to give maximum effects. In all figures, unless otherwise stated, the ordinate shows electrical potential, positive values indicating that the serosal side is positive to the mucosal side, and the abscissa shows time.

TABLE 1. Potential difference across the wall of the rat small intestine *in vitro* and *in vivo* in the absence and presence of glucose. The potential differences are expressed as the mean value with the standard error of the mean, the number of experiments being given in brackets. Positive values indicate that the serosal side is positive to the mucosal side

	Potential difference		
	Upper jejunum (mV)	Mid intestine (mV)	Lower ileum (mV)
In vitro			
No glucose	0·8±0·2 (5)	1·5±0·2 (14)	1·5±0·2 (24)
Glucose (28 mM)	5·6±0·3 (10)	12·0±0·2 (28)	5·8±0·5 (13)
In vivo			
No glucose	2·9±0·4 (5)	4·7±0·2 (16)	3·8±0·3 (10)
Glucose (56 mM)	7·3±0·4 (5)	11·1±0·3 (16)	7·4±0·7 (5)

than sacs I and V and the mean values of the potential in sacs I, III and V, 15 min after setting up the preparation, are given in Table 1.

On setting up the preparation in the presence of 28 mM glucose in both the mucosal and serosal fluids, a considerably higher potential was obtained, and this is illustrated for sac III in Fig. 3. The initial potential was 10–12 mV, and during the first few minutes could increase slightly.

This was followed by a slow but continuous decrease, but at the end of 1 hr there was frequently a potential as high as 10 mV.

When the preparation was set up in glucose-free saline, addition of glucose to the mucosal fluid gave an immediate rapid rise in potential, to the level obtained when glucose was initially present in both mucosal and serosal fluids. This is illustrated in Fig. 4, which also shows the fall in potential on replacing the mucosal solution containing 28 mM glucose

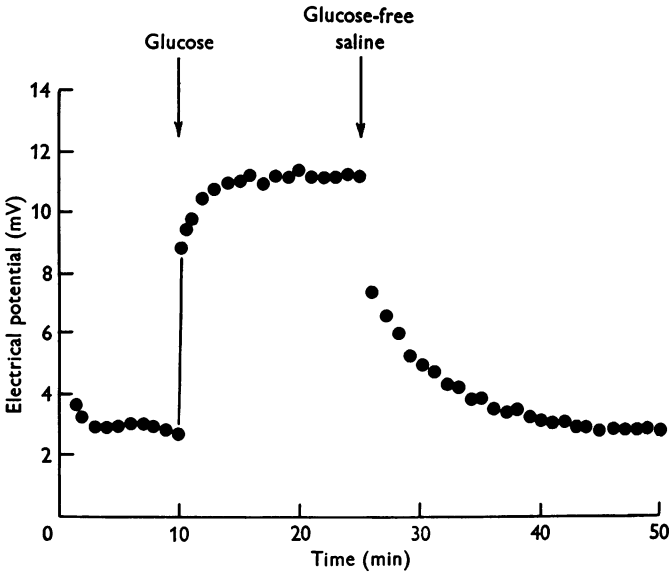

Fig. 4. Effect of glucose on the potential across the gut wall (sac III) *in vitro*. Glucose 28 mM was added to the mucosal fluid at the first arrow, and at the second was replaced by glucose-free saline.

with a glucose-free saline. The increase and fall of potential on addition or removal of glucose was extremely rapid, the change on addition beginning within 2 sec, and this included the time for mixing.

Sacs I and V also maintained a higher potential in the presence of glucose than in the absence of glucose, but in both I and V this was significantly smaller than the potential maintained by sac III (Table 1). Sac V showed considerable variation, both in the size of the potential maintained and in the variation in the potential with time, and variations in size are indicated by the values of the S.E. in Table 1. Like sac III, sac I showed a continuous slight fall in the potential during the period of incubation. In contrast the fall of potential in sac V was more rapid.

In vivo. The results obtained *in vivo* were closely parallel to those obtained *in vitro*. They differed mainly in that (*a*) the potentials in the

absence of glucose in the luminal fluid were higher than *in vitro* in the absence of glucose, and (*b*) the potential *in vivo* continued at a steady value throughout the experimental period. As *in vitro*, the potential was greater in the mid intestine than the proximal jejunum and distal ileum, and the variability was greatest in the distal ileum. Some of these points are illustrated in Table 1 and Fig. 3.

Effect of glucose concentration

The effects of glucose on the potential shown in Table 1 and Fig. 3 were obtained by using high concentrations of glucose, 28 mM *in vitro* and

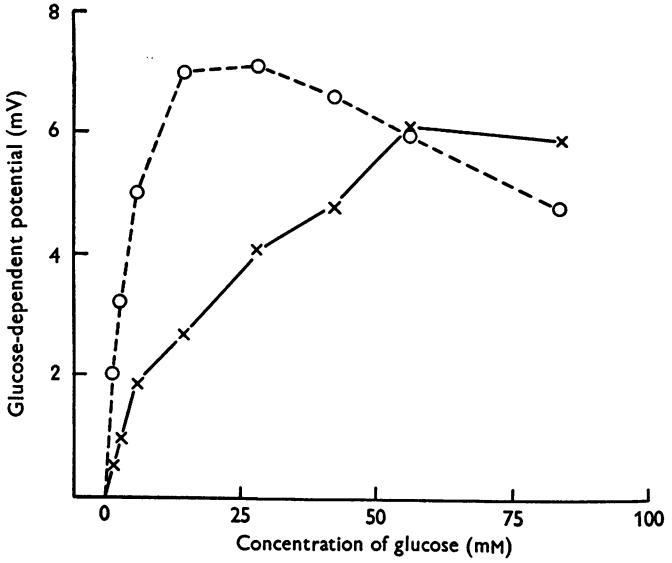

Fig. 5. Effect of glucose concentration on the potential across the wall of the gut both *in vitro* (O---O) and *in vivo* (X——X).

56 mM *in vivo*. The variation in the potential with glucose concentration was studied by varying the concentration of glucose, and Fig. 5 shows the relation between glucose concentration and glucose-dependent potential both *in vivo* and *in vitro* in the mid region of the small intestine. The glucose-dependent potential is regarded as the difference between the potential in the presence of glucose and in its absence. The points for the *in vitro* and *in vivo* conditions were each obtained from one experiment in which the glucose concentration in the mucosal fluid (*in vitro*) and luminal fluid (*in vivo*) was increased in a step-wise manner by successive additions of glucose. Figure 5 shows in both cases an increase in sugar-dependent potential with glucose concentration. The rise is more rapid

in vitro than *in vivo*, and shows a falling off at higher glucose concentrations. While the maximum glucose-dependent potential was greater *in vitro* than *in vivo* the total potential in both cases was about the same, since the potential in the absence of glucose was smaller *in vitro* than *in vivo*.

Effect of various sugars

The effects of a number of other sugars and related substances on the potential were tested both *in vitro* and *in vivo*. The sugars were galactose and fructose, and the other compounds were α-methyl-glucoside, 3-methyl-

TABLE 2. Potential difference across the wall of the mid intestine *in vivo* in the absence and presence of various substances. The initial concentration was in all cases 56 mM. The values are mean potentials with the standard error of the mean. The potential obtained before addition of the substance is given in the third column (control) and after addition in the fourth column. The difference between these is given in the last column as the 'sugar-dependent potential'. Negative values in the last column mean that the potential in the presence of the substance tested is smaller than the control potential

Test substance	No. of expts.	Control potential (mV)	Potential after addition of test substance (mV)	'Sugar-dependent potential' (mV)
Glucose	16	4·7 ± 0·16	11·1 ± 0·31	6·4 ± 0·34
Galactose	7	4·6 ± 0·24	11·6 ± 0·36	7·0 ± 0·38
3-*o*-Methyl-glucose	7	4·5 ± 0·35	6·8 ± 0·27	2·3 ± 0·23
α-Methyl-glucoside	5	4·4 ± 0·49	9·4 ± 0·49	5·0 ± 0·14
Fructose	4	3·8 ± 0·14	2·4 ± 0·12	−1·4 ± 0·16
2-Deoxy-glucose	4	4·0 ± 0·32	2·6 ± 0·43	−1·4 ± 0·20
Mannitol	8	4·3 ± 0·29	2·8 ± 0·29	−1·5 ± 0·07

glucose, 2-deoxyglucose and mannitol. Mannitol was included as a substance not transferred by the intestine, and hence one which might give information about osmotic effects. The results of a series of experiments *in vivo* are given in Table 2 for the mid intestine. In this table, the last column gives the difference in potential caused by addition of the substances tested, and this is denoted as the 'sugar-dependent potential'. From this table it is seen that galactose has an effect very similar to glucose. In many experiments the potential caused by galactose was slightly higher than that caused by glucose, but the difference in a series of experiments was not significant. A smaller sugar-dependent potential was caused by 3-methyl-glucose and α-methyl-glucoside. The sugar-dependent potential was negative with fructose, 2-deoxyglucose and mannitol. The effect of all the substances was reversible, and this is illustrated in Fig. 6. This shows the results of experiments in which the same preparation was used for testing out a series of substances added as shown by the arrows. About 5 min after the addition of each, the luminal fluid was replaced by fresh 0·9% NaCl, and the next substance was

then tested. At the end of the experiment glucose was added again, and this showed that the response of the gut to glucose had not altered during the course of the experiment. Figure 7 shows the results of testing some

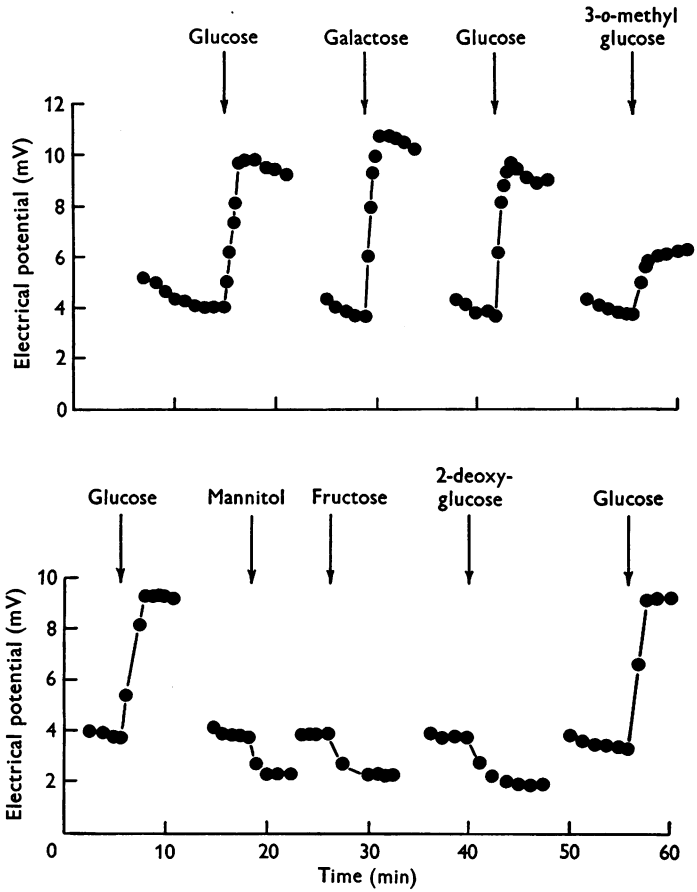

Fig. 6. Effect of a number of substances on the potential across the intestinal wall *in vivo*. The same preparation was used for all these experiments, and at the arrows the substance indicated was added to the mucosal fluid to make a concentration of 56 mM. The mucosal fluid was subsequently replaced by 0·9 % NaCl, and the next substance added. At the end of the experiment glucose was used to show that the preparation still produced its original response.

of these substances *in vitro*, in order to indicate the variation in the potential with time. The rate of fall of potential with galactose and α-methyl-glucoside was similar to that with glucose, but the 3-methyl-glucose potential rapidly fell away to the no-glucose level.

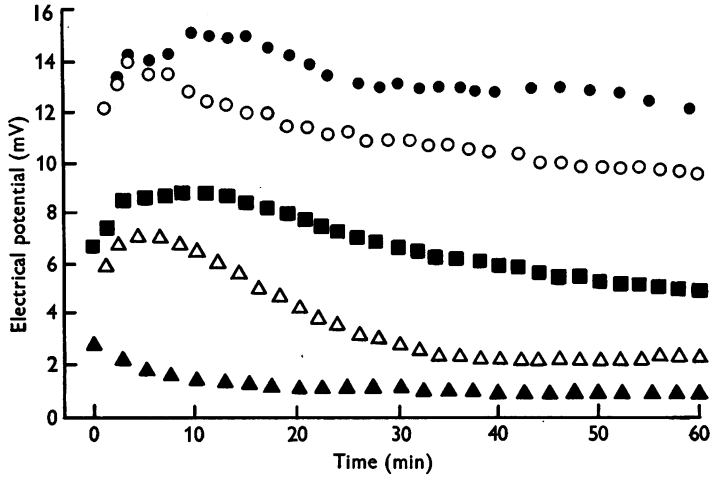

Fig. 7. Time course of potential difference across the mid intestinal region of the gut *in vitro* in the presence of galactose (●), glucose (○), α-methyl-glucoside (■), 3-methyl-glucose (△), and no added sugar (▲). It is seen that the effects differ not only in the size of the potential but also in its rate of decay.

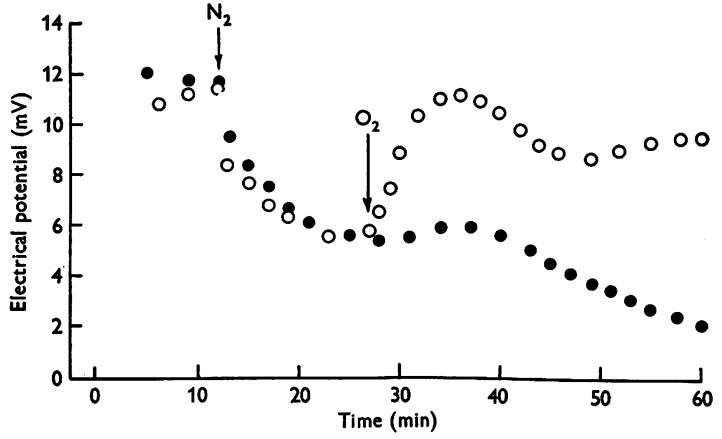

Fig. 8. Time course of the potential across the wall of everted sac of mid intestinal region of rat gut. Both experiments started aerobically, and anoxic conditions were introduced at the first arrow. In one (○) aerobic conditions were restored at the second arrow, but in the other anoxia continued throughout the experiment.

Effect of anoxia

The effects of anoxia were studied only *in vitro*, and typical effects are shown in Figs. 8, 9 and 10. Figure 8 includes two experiments, in each of which the intestine was set up in aerobic conditions with glucose present

and after about 10 min was exposed to anoxia. In one, anoxia was continued until the end of the experiment, in the other aerobic conditions were restored after nearly 20 min of anoxia, as indicated by the second arrow in Fig. 8.

Under anaerobic conditions the potential falls rapidly, but this fall is followed by a phase lasting up to 20 min when the potential remains at about 6 mV and may even show a slight rise. A further fall in potential then takes place, but even after 1 hr from the beginning of anoxia the potential has not completely disappeared. If oxygen is readmitted during the stable phase (i.e. when the potential remains at about 6 mV), a con-

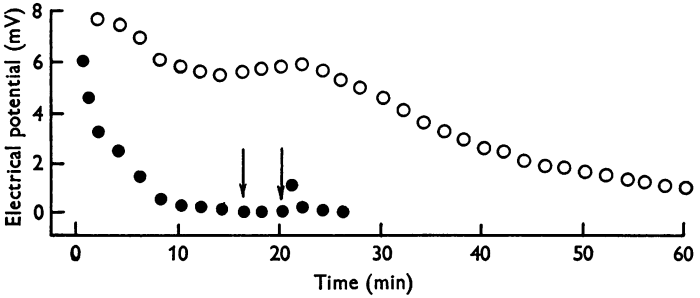

Fig. 9. Time course of the potential across the wall of everted sacs of mid intestinal region of rat gut. Both experiments were started in anoxic conditions. In one experiment (○) 28 mM glucose was present throughout. In the other experiment (●) glucose was absent initially, but was added to the mucosal fluid at the first arrow, and aerobic conditions were restored at the second arrow.

siderable degree of recovery occurs, as is shown in Fig. 8. The amount of recovery depends on the duration of anoxia and if this lasts until the final fall in potential has set in, very little recovery is possible. The effect of anoxia depends largely on the presence or absence of glucose, and this is illustrated in Fig. 9, which shows the results obtained from two preparations in anoxic conditions from the beginning of the experiment (one in the presence of 28 mM glucose and one in the absence of glucose). The potential falls much more rapidly in the absence of glucose, and neither addition of glucose nor restoration of aerobic conditions causes any significant recovery.

It has already been seen that galactose is capable of maintaining as large a potential as that maintained by glucose. This, however, applies only to aerobic conditions and in anoxia galactose is not an effective substitute for glucose (Fig. 10). With galactose the rapid fall in potential resembles that obtained in the absence of glucose, and furthermore on readmission of oxygen no recovery takes place.

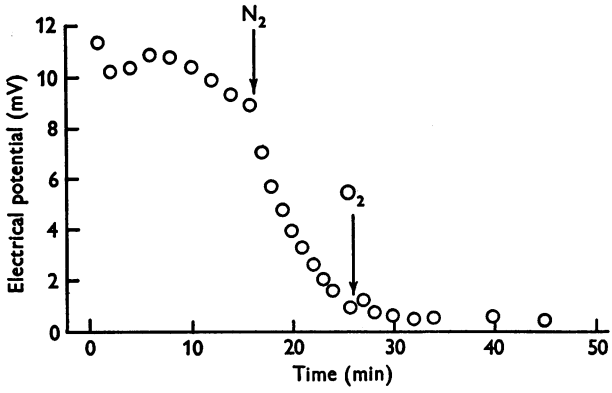

Fig. 10. Time course of potential across the wall of everted sac of mid intestinal region of rat gut. Galactose (28 mM) was present in the mucosal fluid throughout, and the experiment was started in aerobic conditions. At the first arrow anoxia commenced, and at the second arrow aerobic conditions were restored.

Effect of inhibitors

Phlorrhizin. Phlorrhizin is known to have an effect on sugar movement in the intestine over a very wide range of concentration. In low concentrations, from 10^{-6} M upwards, it inhibits entry of glucose from the mucosal fluid, while in concentrations of 10^{-3} M it also inhibits metabolism. Its effect on the glucose-dependent potential *in vivo* is illustrated in Fig. 11. Phlorrhizin (10^{-5} M) produces a small but definite fall in potential, and this fall increased in a step-wise manner with increasing concentration. Phlorrhizin had no effect on the potential when no sugar was present. If glucose was added after phlorrhizin (10^{-3} M) the effect of glucose addition was to reduce the potential to that characteristic of fructose or mannitol. It is also evident from Fig. 11 that the effect of phlorrhizin on the potential was reversible. Phlorrhizin had a similar effect, not illustrated here, on the potential caused by galactose, 3-methyl-glucose and α-methyl-glucoside.

The effects of phlorrhizin were similar *in vitro* (Fig. 12) when added to the mucosal fluid, except that it was perhaps slightly more effective, and a concentration of 5×10^{-4} M was sufficient to abolish the glucose-dependent potential, whereas a concentration of 10^{-3} M was required to do this *in vivo*. As *in vivo*, the effects of phlorrhizin were reversible. These *in vitro* effects are illustrated in Fig. 12, which also shows that the effect of phlorrhizin can be seen in anoxic conditions in presence of glucose.

2,4-Dinitrophenol. The effect of 2×10^{-4} M 2,4-dinitrophenol in the presence of glucose aerobically was very similar to that of anoxia (Fig. 13).

Higher concentrations had no greater effect, so that it can be assumed that 2×10^{-4} M 2,4-dinitrophenol had a maximum effect. Anaerobically in the presence of glucose, 2,4-dinitrophenol did not cause further fall in potential, and if the experiment was started aerobically in presence of 2,4-dinitrophenol, changing over to anaerobic conditions did not affect the potential. In the presence of galactose the effect of 2,4-dinitrophenol was much greater than that in presence of glucose (Fig. 13), so that in this respect also the effect of 2,4-dinitrophenol resembled that of anoxia.

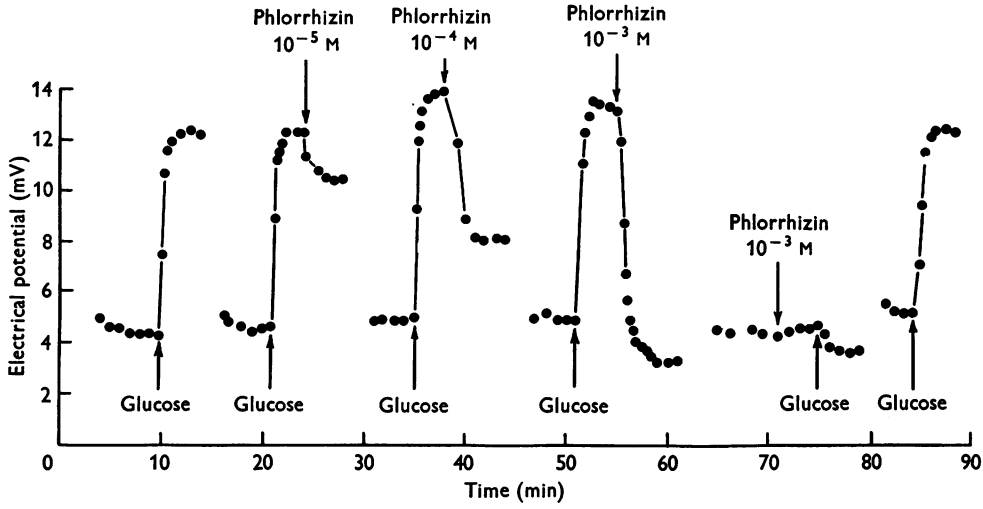

Fig. 11. Effect of phlorrhizin on the potential across the wall of the mid intestinal region of rat gut *in vivo*. The same preparation was used throughout. Solutions of 0·9 % NaCl were circulated through the intestine, and at the lower arrows glucose was added to make a final concentration of 56 mM. At the upper arrows phlorrhizin was added to produce the concentration shown. Where the record is broken the phlorrhizin and glucose-containing solution was washed out and replaced with 0·9 % NaCl.

Iodoacetate in a concentration of 5×10^{-5} M had little immediate effect on the potential, and in this respect differed strikingly from phlorrhizin. There was, however, a steady subsequent fall in potential, and this change was irreversible. These results are consistent with the view that iodoacetate is not a very specific inhibitor of glucose transfer by the intestine, but causes general irreversible damage to the gut.

Fluoroacetate. The effect of fluoroacetate (10^{-2} M) *in vitro* is shown in Fig. 14. In the presence of glucose the effect was very small, and comparison with Fig. 3 shows that the fall in potential was no greater than in the absence of fluoroacetate. In the presence of galactose the effect of

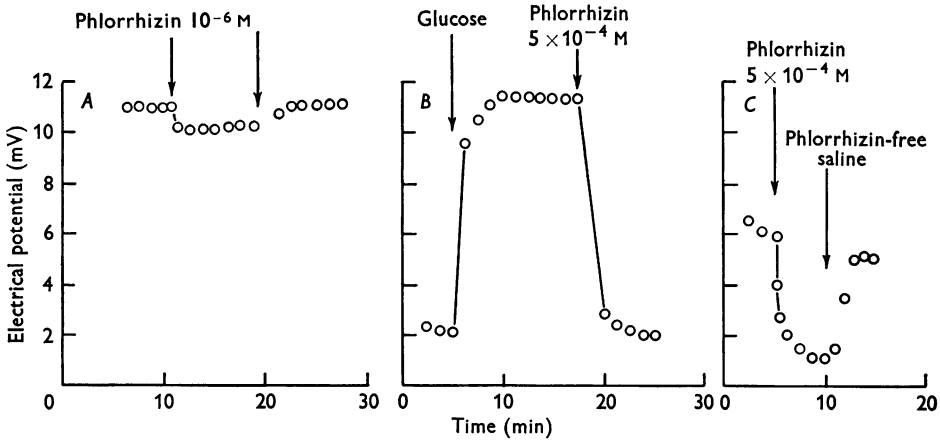

Fig. 12. Effect of phlorrhizin on the potential across the wall of a sac of rat everted gut made from the mid intestinal region. The three records were from separate experiments. In *A* glucose (28 mM) was initially present; at the first arrow phlorrhizin was added to the mucosal fluid to make a final concentration of 10^{-6}M and at the second arrow the mucosal solution was replaced by phlorrhizin free solution containing glucose 28 mM. *B* shows the effect of addition of glucose to make a concentration of 28 mM and the subsequent addition of 5×10^{-4} M phlorrhizin. In *C* the preparation has been set up in anoxic conditions with glucose 28 mM; at the first arrow phlorrhizin was added to the mucosal solution to give a final concentration of 5×10^{-4} M, and at the second arrow the mucosal fluid was replaced with phlorrhizin-free saline containing glucose (28 mM).

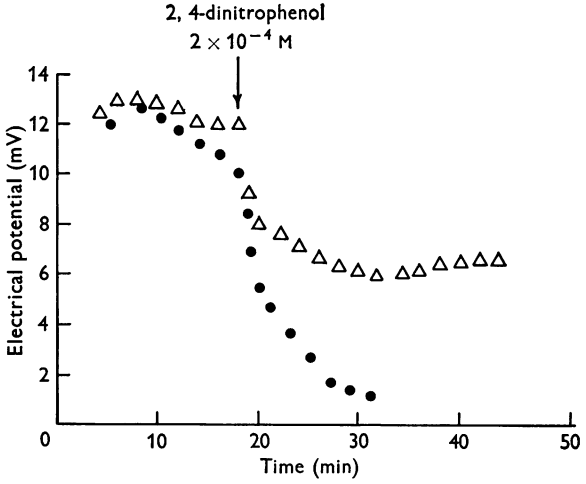

Fig. 13. Effect of 2,4-dinitrophenol on the potential across the wall of a sac of rat everted intestine made from the mid intestinal region in presence of glucose 28 mM (△) and galactose 28 mM (●). 2,4-dinitrophenol was added at the arrow to make a final concentration of 2×10^{-4} M.

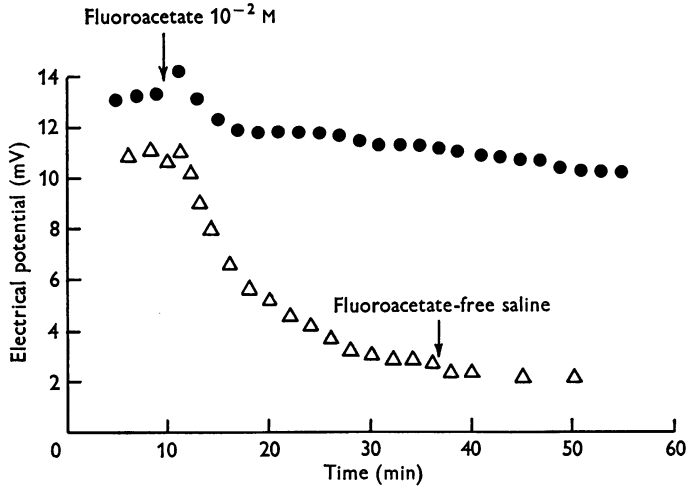

Fig. 14. Effect of fluoroacetate on the potential across the wall of a sac of rat everted intestine made from the mid intestine region in presence of 28 mM glucose (●) and 28 mM galactose (△). At the first arrow fluoroacetate was added to the mucosal fluid to give a final concentration of 10^{-2} M; at the second arrow in the galactose experiment the mucosal solution was replaced with a bicarbonate-saline solution with 28 mM galactose.

fluoroacetate was much greater and there was a profound fall in potential, which was irreversible.

Effect of hexoses in serosal fluid

These experiments were carried out with everted segments instead of the everted sac, as it was necessary to make rapid changes of the serosal fluid, a procedure not possible with the everted sac. The addition of 28 mM glucose and galactose to the serosal fluid had no appreciable effect on the potential. High concentrations (168 mM) caused increase in potential, but the rate of increase was much slower than when these sugars were added to the mucosal fluid (Fig. 15). As this concentration caused considerable change in the osmolarity of the serosal solution, experiments were also carried out with mannitol, which does not cause a potential when present in the mucosal fluid. It is seen in Fig. 15 that mannitol (168 mM) also caused a potential, but this was smaller than that caused by either glucose or galactose. In the case of glucose and galactose, phlorrhizin added to the mucosal fluid reduced the potential to the level obtained with mannitol. Addition of phlorrhizin in the presence of mannitol had no effect on the potential. It thus appears that there are two components in the potential caused by additions of glucose and galactose to the serosal fluid, (1) an osmotic effect and (2) a specific effect of the transferable hexose.

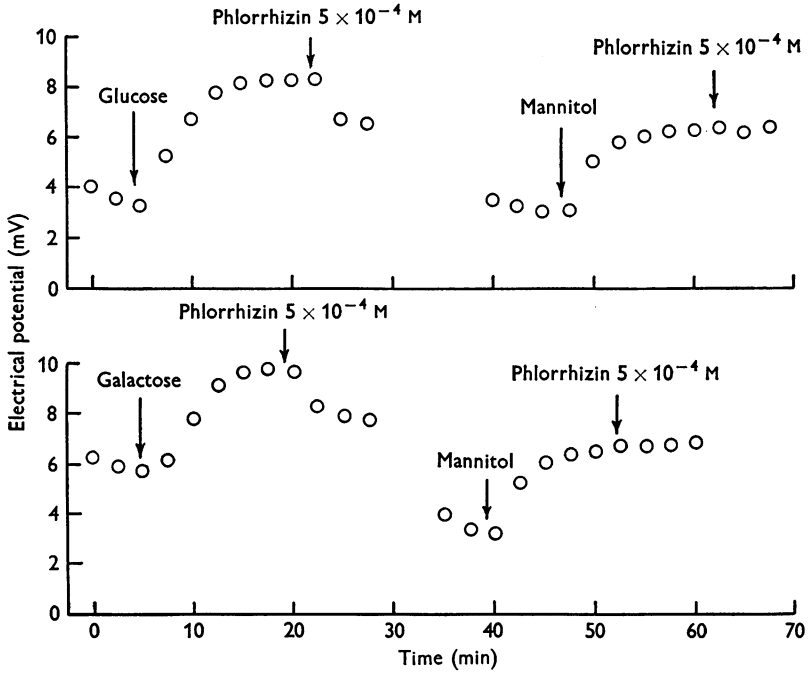

Fig. 15. Effects of adding sugars to the serosal fluid. Two separate experiments are shown. In the upper record glucose (168 mM) was first added to the serosal fluid, and subsequently phlorrhizin 5×10^{-4} M to the mucosal fluid. Both mucosal and serosal fluids were then replaced with bicarbonate saline. Mannitol (168 mM) was then added to the serosal fluid, followed by phlorrhizin 5×10^{-4} M to the mucosal fluid. In the lower record a similar procedure was carried out with galactose instead of glucose.

Fluid transfer

The fluid transfer by different regions of the intestine in presence of different sugars is shown in Table 3. If we first consider sac III, glucose caused by far the greatest fluid transfer, fructose caused a fluid transfer significantly greater than the no-sugar level; galactose, 3-methyl-glucose and α-methyl-glucoside and mannitol caused fluid transfers not significantly different from the no-sugar level, while 2-deoxyglucose gave a significantly smaller fluid transfer than the no-sugar level. Sac I showed a similar pattern, except that the values were all smaller than in sac III. Sac V showed quite a different pattern, and none of the sugars showed a significant increase in fluid transfer.

Sugar metabolism

The amount of various sugars metabolized is seen in Table 4. Glucose was metabolized to a much greater extent than any other substance in all

TABLE 3. Effect of various substances on mucosal fluid transfer by sacs of rat everted intestine. I, III and V indicate fifths of the combined jejunum and ileum numbered from the jejunal end. The sacs contained initially 1 ml. of bicarbonate saline with 28 mM of the substance shown in the first column, and were incubated for 1 hr in 10 ml. of the same solution. The transfer is expressed as ml./g initial wet wt. of gut, and the values given are the means of the number of experiments shown in brackets, with the S.E. of the mean

Test substance	Fluid transfer (ml./g wet wt./hr)		
	I	III	V
No sugar	0·08 ± 0·02 (9)	0·39 ± 0·05 (9)	0·70 ± 0·04 (9)
Glucose	1·14 ± 0·17 (6)	1·69 ± 0·14 (6)	0·86 ± 0·09 (6)
Galactose	0·06 ± 0·03 (9)	0·34 ± 0·03 (9)	0·58 ± 0·07 (9)
3-Methyl-glucose	0·17 ± 0·04 (6)	0·30 ± 0·08 (5)	0·59 ± 0·06 (6)
α-Methyl-glucoside	0·16 ± 0·05 (6)	0·47 ± 0·09 (5)	0·75 ± 0·05 (6)
Fructose	0·28 ± 0·08 (6)	0·61 ± 0·07 (6)	0·47 ± 0·07 (6)
2-Deoxy-glucose	−0·01 ± 0·04 (6)	0·08 ± 0·04 (6)	0·44 ± 0·08 (6)
Mannitol	0·16 ± 0·04 (6)	0·40 ± 0·06 (6)	0·58 ± 0·04 (6)

three sacs. The amount metabolized was, however, much smaller in sac V than in sacs I and III. Fructose was metabolized to a small extent in sacs I and III and possibly to a very small extent in sac V, although the amount involved probably falls within the limits of experimental error. Galactose may be metabolized to a small extent in all three sacs, and α-methyl-glucoside and 3-methyl-glucose not at all.

TABLE 4. Amounts of various substances metabolized by sacs of rat everted intestine. I, III and V indicate fifths of the combined jejunum and ileum numbered from the jejunal end. The sacs contained initially 1 ml. bicarbonate saline with 28 mM of the substance shown in the first column, and were incubated for 1 hr in 10 ml. of the same solution. The amount metabolized is expressed as μ-mole/g initial wet wt. of intestine, and the values given are the means of six experiments, with the S.E. of the mean

Substance tested	Amount metabolized (μ-mole/g initial wet wt./hr)		
	I	III	V
Glucose	88·10 ± 2·61	98·49 ± 4·17	38·27 ± 3·89
Fructose	31·94 ± 1·61	38·11 ± 2·33	4·00 ± 1·67
Galactose	12·22 ± 3·83	12·28 ± 3·44	8·89 ± 3·11
α-Methyl-glucoside	−5·33 ± 3·67	5·61 ± 3·72	−6·39 ± 2·83
3-Methyl-glucose	−1·28 ± 1·78	−2·56 ± 2·28	−1·67 ± 2·61

DISCUSSION

The earliest measurements of potentials across the intestine were those of Nistler (1932), who found a small potential varying considerably in different parts of the gut. During the last few years there has been a resurgence of interest in these potentials in relation to active transport mechanisms and intestinal metabolism. Curran & Solomon (1957) studied the potential across the rat ileum *in vivo* and when the sodium concentration in the intestinal lumen was 146–163 m-equiv/l. the potential was less

than 1 mV. Similarly, Cooperstein & Brockman (1959) and Tidball (1961) found no 'appreciable' potential difference across the wall of the dog small intestine, again *in vivo*. However, using *in vitro* preparations of the small intestine a number of workers have now reported a potential difference across the isolated gut wall. In the absence of glucose, potential differences of 0·5–2·5 mV have been found in the gut of various mammals (Barry, R. J. C. *et al.* 1961; Baillien & Schoffeniels, 1961; Clarkson, Cross & Toole, 1961*a*, Schachter & Britten, 1961; Zalusky & Schultz, 1963).

The potential across the small intestine is greatly increased in presence of glucose (Barry, R. J. C. *et al.* 1961; Clarkson *et al.* 1961*a*; Schachter & Britten, 1961), and this has subsequently been confirmed by Finkelstein & Schachter (1962), Borle, Keutmann & Neuman (1963), Gilman, Koelle & Ritchie (1963), and Sawada & Asano (1963). The serosal side is positive relative to the mucosal, but the size of the potential recorded by different workers has varied from 4 mV (Finkelstein & Schachter, 1962) to the 12 mV recorded in the present series of experiments. This variation could be due to the experimental conditions used, and as shown here both the glucose concentration and the region of the intestine used can affect the size of the potential. Our results show that a definite increase in potential is obtained with a concentration as low as 2 mM, which is probably within physiological limits.

The major interest is the cause of the glucose-stimulated potentials, and when these were first recorded the most obvious line of reasoning was as follows. Glucose stimulates the movement of a fluid which is approximately isotonic. The fluid movement is therefore probably secondary to ionic movement caused by a sodium pump dependent on glucose metabolism; and hence the glucose-dependent potential is due to this sodium pump. This was essentially the argument used by both Clarkson *et al.* (1961*a*) and Barry, R. J. C. *et al.* (1961), and on the then known facts was a reasonable one. The results in the present paper show that this argument is untenable. If we consider the experiments with sac III, where transfer capacity for fluid and hexoses is greatest, glucose and galactose are most effective in causing a potential, 3-methyl-glucose and α-methyl-glucoside are less effective and fructose and 2-deoxyglucose are ineffective. Of these substances glucose, galactose, 3-methyl-glucose and α-methyl-glucoside are capable of movement against a concentration gradient (see review by Crane, 1960). As is shown here, glucose promotes the greatest fluid transfer, fructose causes a small transfer, and the others have little effect on fluid transfer. Hence it appears that those substances which cause a potential are the ones which can be moved against a concentration gradient, and not necessarily those which cause fluid movement. The idea that the potential is related to movement of the sodium with which fluid movement

is associated is thus incorrect, and the potential is obviously linked to the mechanism for sugar transfer. This conclusion would mean that the glucose-dependent and glucose-independent fluid transfer mechanisms have at least one feature in common, i.e. that neither causes a large potential.

While the potential is fundamentally related to sugar transfer, it is dependent on a source of metabolic energy. Of the sugars causing a potential, glucose differs from the others in being metabolized in substantial amounts by the intestine. While glucose and galactose behave similarly in causing a potential in aerobic conditions, they behave very differently in anaerobic conditions. In the presence of glucose a potential is maintained anaerobically, although it is much smaller than the aerobic potential. With galactose, however, the potential very quickly falls away when anaerobic conditions are established, and hence it appears that in anaerobic conditions the intestine is able to derive energy from glucose metabolism to produce a potential. Fluoroacetate (10^{-2} M) in the presence of glucose does not have much effect on the potential, but is very effective in the presence of galactose. This suggests that the energy for maintenance of the potential in the case of galactose depends on the citric-acid cycle, while in the presence of glucose energy can also be obtained from either the pentose cycle or the glycolytic pathway. Since glucose is partly effective in anaerobic conditions, this would suggest that the glycolytic pathway is able to produce at least part of the energy necessary for the potential. The potential is reduced but not abolished by 2,4-dinitrophenol, and following the argument of Matthews & Smyth (1961) this would suggest either (*a*) that the energy for the potential is not completely dependent on ATP or (*b*) that dinitrophenol uncouples oxidative phosphorylation, but not substrate-linked phosphorylation, and the ATP generated by substrate-linked phosphorylation is available for the potential. Certainly two components are involved in the production of the potential: (1) the presence of a transferable sugar and (2) the source of energy derived from *either* aerobic metabolism of endogenous substrates *or* glycolysis.

The action of phlorrhizin is of special interest in locating activities in the epithelial cell. Parsons *et al.* (1958) found that phlorrhizin in concentrations of less than 5×10^{-4} M had little effect on oxygen consumption of the intestine, and Newey, Parsons & Smyth (1959) showed that at these concentrations phlorrhizin inhibited the entry of glucose into the cell from the luminal side, with only a small effect on metabolism. These findings showed that phlorrhizin had effects on cellular processes involved in glucose transfer, quite apart from its effect on glucose metabolism. Our results support this conclusion by showing that phlorrhizin in a concentration of 5×10^{-4} M almost completely abolishes the glucose-dependent

potential. The abolition of the galactose potential by phlorrhizin further establishes that the phlorrhizin-sensitive mechanism is independent of metabolism, since galactose is poorly metabolized. Miller & Crane (1961) located this phlorrhizin-sensitive entry mechanism in the brush border of the cell just inside a zone containing maltase, and Newey *et al.* (1963) showed that the phlorrhizin-sensitive mechanism lies between the maltase zone and the glucose-dependent fluid-transfer mechanism. The proximity to the luminal border is now confirmed by using the argument put forward by Clarkson *et al.* (1961*a*). They found that the effect of glucose on the intestinal potential was seen in less than 3 sec, and comparing this with the time (10 sec) found by Chinard, Taylor, Nolan & Enns (1959) for transit of glucose across the renal epithelial cell, they concluded that the potential was produced 'soon after glucose entered the cell'. Since phlorrhizin produced its inhibitory effect within 3 sec, the phlorrhizin-sensitive mechanism must also be near the luminal border of the cell. In these experiments the potential across the whole thickness of the gut wall is measured, but since it is related to sugar transfer it is a reasonable assumption that this potential exists across the epithelial cell. This transcellular potential is the resultant of at least two potential steps, i.e. at the membrane on the luminal side of the cell, and at the membrane on the serosal side of the cell. If the argument quoted above about time relations is valid, the change in transcellular potential is probably associated with the potential step at the luminal border of the cell.

While we know something of the factors influencing the potential and the approximate position of the mechanism in the cell, we know little about its actual production. Glucose transfer is dependent on the presence of sodium, and Clarkson, Cross & Toole (1961*b*) have shown that the potential is also dependent on the presence of sodium, but not of other cations. Hence it is tempting to think that movement of sodium and glucose are linked together. Crane, Miller & Bihler (1961) have in fact put forward a theory of sugar transfer based on this concept. According to this, sugar and sodium are transported together and subsequently sodium is freed from the complex and is then transported in the reverse direction by a mechanism sensitive to strophanthin. Since, however, this results in no net transfer of sodium it would not implicate sodium in causing the potential. Recently Barry, Smyth & Wright (1963) have shown that the short-circuit current across the intestine is not accounted for by the net movement of sodium, and this supports the view already expressed that the potential is not due to the same cause as fluid transfer. We do not wish to discuss further the discrepancy between net sodium transfer and potential, pending further work on the short-circuit current, but one possibility is that a neutral pump, as postulated by Diamond

(1962*a*) in the gall bladder, is responsible for part of the sodium transfer.

The potential varies in different parts of the intestine, and is greatest in the middle fifth. These results are consistent with those of Clarkson *et al.* (1961*b*) and Gilman *et al.* (1963). Barry, B. A. *et al.* (1961) have shown variations in different parts of the gut as regards the capacity to transfer glucose and fluid. The middle fifth shows the greatest capacity for glucose transfer and also has the greatest potential. This correlation between potential size and capacity for glucose transfer is not, however, seen in the upper jejunum and lower ileum. In both the potential is the same size, but the upper jejunum has considerably greater capacity than the lower ileum for glucose transfer. No explanation can be offered for this at present, but the other evidence discussed above makes it clear that the potential is related to sugar transfer.

Glucose initially present in the serosal fluid in high concentrations is capable of causing a potential. This potential consists of two components, one depending on the presence of a transferable sugar and one depending on the increased osmolarity of the serosal fluid. Lifson & Parsons (1957) and Newey *et al.* (1963) showed that glucose in the serosal fluid stimulated fluid movement, and this indicated that the glucose could diffuse through the gut wall to the site of glucose-dependent fluid-transfer mechanism. Our results indicate that glucose diffuses as far as the phlorrhizin-sensitive glucose-entry mechanism, on the luminal side of the glucose-dependent fluid-transfer mechanism. Newey *et al.* (1959) have shown that if glucose is actually present in the serosal fluid, phlorrhizin in the mucosal fluid does not prevent its metabolism, and this further supports our conclusion that the potential is generated by the transfer of glucose rather than by its metabolism. This metabolism of glucose from the serosal side is able to maintain fluid transfer, and the fact that phlorrhizin does not abolish this, but abolishes the potential, is further evidence of the independence of the potential from fluid transfer.

Changes in osmolarity also cause a potential as indicated by the increase in potential on addition of mannitol to the serosal fluid, and a decrease in potential on addition of mannitol to the mucosal fluid, and the addition of glucose in the presence of phlorrhizin. Curran & Solomon (1957) obtained changes in potential with different sodium concentrations, but total osmolarity as distinct from sodium concentration can cause a potential. These potentials caused by osmolarity could be streaming potentials, as suggested by Diamond (1962*b*) in case of the gall bladder.

The experiments show also that the potentials are fundamentally very similar *in vivo* and *in vitro*, although there are small differences, e.g. the potential in the absence of glucose is somewhat greater *in vivo* than *in*

vitro. This is hardly surprising, as *in vivo* the gut may have better sources of endogenous metabolites. This similarity of the *in vivo* and *in vitro* preparations further strengthens confidence in the *in vitro* gut preparation, and in particular the everted sac, as a suitable preparation for study of transport problems, and the measurement of potentials offers a possibility of exploring the time relations of the events concerned in transport in addition to their over-all effect.

SUMMARY

1. Techniques are described for measurement of the potential across the wall of the rat intestine both *in vivo* and *in vitro*.

2. The serosal side of the intestine is positive to the mucosal. The difference in potential is increased by addition to the mucosal fluid of glucose, galactose, 3-methyl-glucose and α-methyl-glucoside, but not by fructose nor by 2-deoxyglucose.

3. Phlorrhizin has no effect on the potential in the absence of sugars, but in the presence of transferable sugars very rapidly reduces or abolishes the potential. Anoxia and 2,4-dinitrophenol reduce the potential, and the effect is much greater in the absence of glucose.

4. Of the substances tested, glucose and fructose are the only two which are appreciably metabolized and support fluid transfer.

5. It is concluded that the potential is related to the transfer of sugars and not to fluid transfer, and the possible mechanisms involved are discussed.

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