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STREAMING POTENTIALS IN THE RAT SMALL INTESTINE

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SUMMARY

1. The effect of adverse osmotic pressure gradients on fluid transfer and electrical potential across the wall of sacs of rat everted small intestine was investigated.

2. Addition of mannitol to the mucosal fluid produced a potential change of 0·062 mV/m-osM and a decrease in fluid transfer of 0·015 ml./m-osM/hr. This is consistent with the production of streaming potentials due to fluid movement through negatively charged pores in the intestine.

3. The solute-linked fluid movement does not pass through these negatively charged pores which are responsible for the streaming potentials.

4. From the magnitude and polarity of the streaming potential a value of -50 mV has been calculated for the zeta potential at the phase boundary in the pores.

5. Streaming potentials have been used to measure the equivalent pore radius, and a value of 4 Å has been obtained.

6. It is concluded that electro-osmosis is not responsible for fluid transfer by the intestine, and the potential difference associated with hexose transfer is not electrokinetic in origin.

INTRODUCTION

Electrokinetic phenomena have long been described in relation to biological processes, since Porret (1816) considered that minute electrical currents may have an influence in regulating the flow of water through minute pores in living tissues. In spite of the enormous application of electrokinetic phenomena to biological problems, e.g. use of electrophoresis, the actual existence of electrokinetic phenomena in the body in physiological conditions has not often been demonstrated. One of these phenomena is streaming potentials, first recognized by Quincke (1861) as the converse of electro-osmosis. Recently Diamond (1962), Pidot & Diamond (1964) and Dietschy (1964) demonstrated streaming potentials in the gall-bladder. Smyth & Wright (1964) also demonstrated streaming

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potentials in the small intestine, following the suggesting of Barry, Dikstein, Matthews, Smyth & Wright (1964) that they were responsible for certain electrical changes in the gut. The following is an extended account of these preliminary observations in the intestine.

METHODS

The recognition of streaming potentials depends on the demonstration of electrical potentials produced in response to a movement of fluid through pores. Applying this to the rat intestine therefore involves measurement of the potential across the intestinal wall in response to a force causing fluid movement. Smyth & Taylor (1957) have shown that it is very difficult to cause fluid movement through the intestine by means of hydrostatic pressure and therefore osmotic forces were used.

Procedure. Male white rats of the Sheffield strain weighing between 230 and 250 g were used. Before experiments they were maintained on a standard commercial diet (diet 86 Oxoid, London) in cube form, and both the cubes and water were available *ad libitum*. The experiments were carried out using everted sacs of the middle fifth of the combined jejunum and ileum. This preparation is not convenient for studying electrical potentials and for measurement of fluid movement simultaneously. Two sets of experiments were therefore done, one in which electrical potential was measured and the other in which fluid movement was measured. In interpreting the results it is assumed that the conditions which were shown to cause fluid movement would cause the same movement in the experiments where a potential was measured. The potential difference across the wall of the everted sac of intestine was measured as described by Barry *et al.* (1964). The fluid transfers were determined by the technique described by Barry, Matthews & Smyth (1961). The parameter of fluid transfer used was the mucosal fluid transfer and this is defined as the decrease in the mucosal fluid during incubation. It was calculated from the weights of the sacs as described by Parsons, Smyth & Taylor (1958). In both types of experiment the osmotic-pressure gradient across the intestine was produced by the addition of mannitol to the mucosal fluid.

Chemical. The saline used was either bicarbonate saline (Krebs & Henseleit, 1932) or phosphate saline (Robinson, 1949). The bicarbonate saline was maintained in equilibrium with a 5% CO_2/95% O_2 gas mixture and the phosphate saline with 100% O_2. The pH of the phosphate saline was varied by (*a*) altering the ratio of Na_2HPO_4 to KH_2PO_4 or (*b*) replacing the phosphate buffer with either NaOH/phthalate or HCl/phthalate buffers.

RESULTS

Effect of mannitol on the electrical potential

In these experiments the tonicity of the mucosal fluid was varied by addition of mannitol both in the presence and absence of glucose. Figure 1 shows an experiment in which the mannitol concentration was increased in a step-wise manner. In the presence of glucose (28 mM) in both the mucosal and serosal fluids the potential difference across the intestine was about 10 mV, the serosal side being positive, and the additions of mannitol to the mucosal fluid caused a step-wise reduction in the potential. Each addition of mannitol (28 mM) caused a change in the potential of about 1·5 mV. This figure also shows the effect of mannitol in the absence of glucose. The potential difference across the gut was about 2 mV, the serosal side being positive, and with the lower mannitol concentrations the effect

was similar to that obtained in the presence of glucose. Since the initial potential was small, this resulted in an actual reversal of potential so that the mucosal fluid became positive. With higher concentrations of mannitol the proportional change in potential was not maintained, and the potential tended to drift back towards zero.

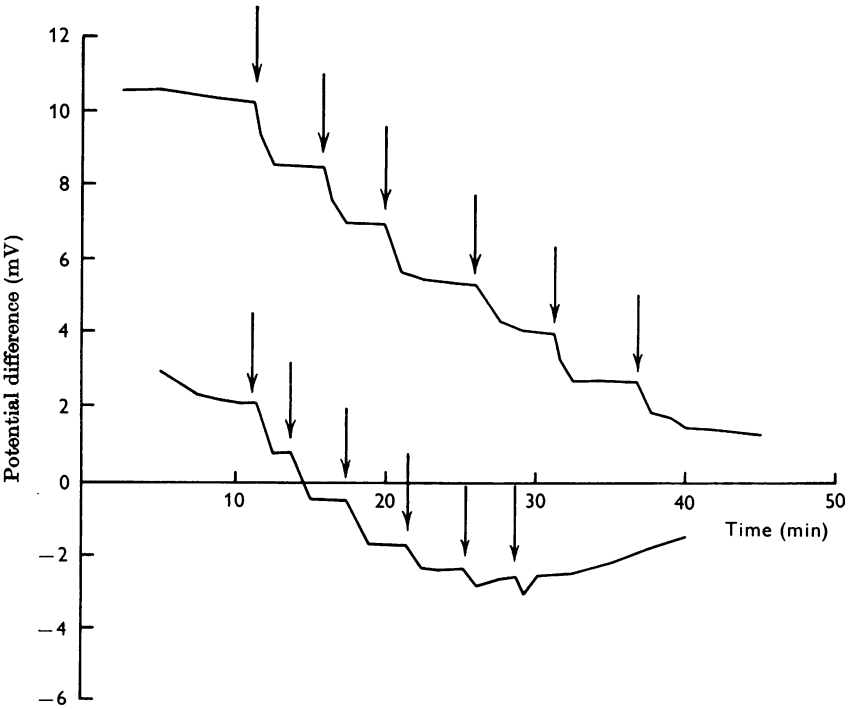

Fig. 1. Effect of addition of mannitol to the mucosal fluid on the electrical potential across the wall of sacs of rat everted intestine. At each arrow the concentration of mannitol was increased by 28 mM. In the upper curve glucose was initially present in both mucosal and serosal fluids in a concentration of 28 mM, while in the lower curve glucose was absent. Ordinate: potential in mV, positive values meaning that the serosal side is positive to the mucosal side. Abscissa: time in min.

One explanation of this effect may be the instability of the potential in the absence of glucose. Figure 2 shows the effect of mannitol (168 mM) over a longer period in the presence of glucose and Fig. 3 shows the effect in the absence of glucose. In the presence of glucose the potential was fairly stable, and even after 1 hr when the mucosal solution was replaced with a mannitol-free solution the potential approached the level seen in a control experiment where no mannitol was added. In the absence of glucose (Fig. 3) addition of mannitol produced an initial effect very similar

to the effect in the presence of glucose, but this potential was not maintained.

On account of this instability all subsequent experiments were carried out only in the presence of glucose (28 mM) in both the mucosal and serosal fluids. In a series of five experiments the potential produced by the addition of mannitol (84 mM) to the mucosal fluid was $5{\cdot}2 \pm 0{\cdot}1$ mV, i.e 0·062 mV/m-osM or $8{\cdot}03 \times 10^{-12}$ stat V/dyne/cm^2.

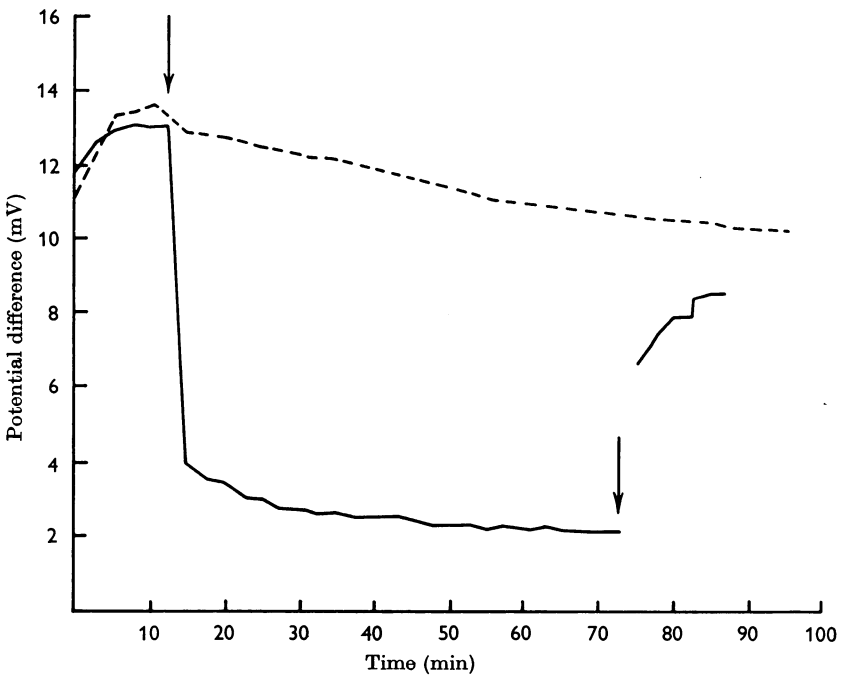

Fig. 2. Effect of mannitol on potential across the intestine with initial concentration of glucose 28 mM in both mucosal and serosal fluids. In the experiment shown by the continuous line mannitol (168 mM) was added to the mucosal fluid at the first arrow, and at the second arrow the mucosal fluid was replaced by a mannitol-free solution. The time required for this operation is responsible for the break in the record. The interrupted line shows a control experiment where no mannitol was added. Ordinate and abscissa as in Fig. 1.

Effect of mannitol on fluid movement

Figure 4 shows experiments in which the effect of mannitol was studied on fluid movements in the presence of glucose. With no mannitol present there was an average fluid movement of 2·91 ml./hr. Addition of mannitol reduced this, but even at the highest concentration used did not actually cause net movement of fluid towards the mucosal side. The change in fluid movement was roughly proportional to the mannitol concentration, and

the slope of the regression line corresponds to a change in fluid movement produced by mannitol of $0{\cdot}015 \pm 0{\cdot}002$ ml./m-osM/hr.

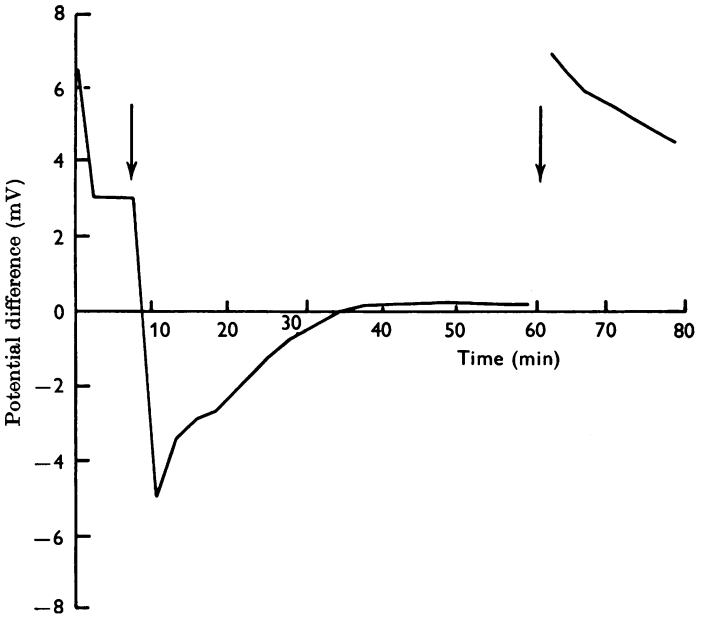

Fig. 3. Effect of addition of mannitol on potential across the gut in absence of glucose. At the first arrow mannitol (168 mM) was added to the mucosal fluid and at the second arrow the mucosal fluid was replaced by a mannitol-free solution containing glucose (28 mM). Ordinate and abscissa as in Fig. 1.

Streaming potential and fluid flow

The magnitude of the streaming potential and electro-osmotic flow of fluid are related by the equation

$$H/P = v/I, \tag{1}$$

where H is the streaming potential in stat V, P the pressure responsible for this potential expressed in dynes/cm^2, v the electro-osmotic flow in ml./sec/cm^2, and I the current during electro-osmosis in stat A/cm^2. This relation has been shown to be independent of the structure of the membrane (Mazur & Overbeek, 1951). In the streaming potential experiments reported here the value of H/P was found to be $8{\cdot}03 \times 10^{-12}$ stat V/dyne/cm^2. The rate of fluid movement in the presence of glucose was found to be 2·91 ml./sac/hr, which corresponds to $3{\cdot}37 \times 10^{-5}$ ml. sec/cm^2 (the serosal area of a sac of intestine was 24 cm^2). If this solute linked fluid movement was the result of electro-osmosis across the tissue the necessary current flow would be $v/H/P 4{\cdot}2 \times 10^6$ stat A. Since the resistance of the intestine is 29 Ω cm^2 (Barry, Smyth & Wright, 1965) this current should produce a

potential difference of -41 mV, the serosal side of the intestine negative. In fact the observed potential was 10 mV, serosal side positive, and so it is clear that electro-osmosis cannot be the mechanism of fluid movement in the small intestine.

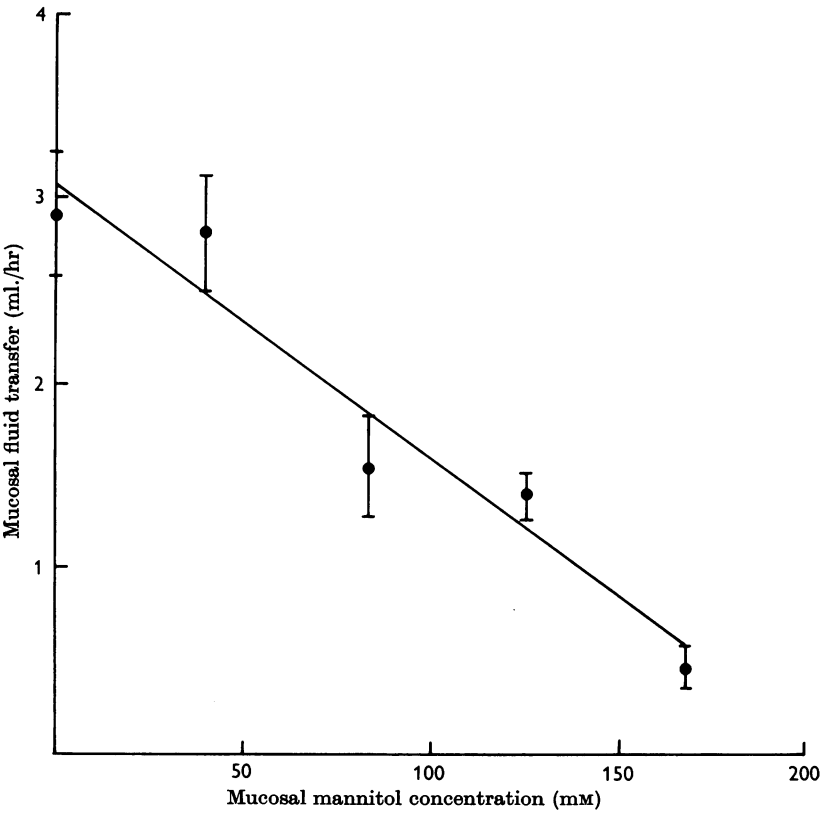

Fig. 4. Effect of mannitol on fluid transfer by sacs of rat everted small intestine. The ordinate shows the fluid transfer in ml./sac/hr, and the abscissa the initial concentration of mannitol in the mucosal fluid. The points are the means of six experiments with ±S.E. of the means.

Calculations of the zeta potential

The magnitude of the streaming potential depends on the force causing movement of fluid, and the zeta potential of the phase boundary in the pore. The relation is given by the equation formulated by Briggs (1928).

$$\zeta = 4\pi\eta\, KsH/(\epsilon P) \qquad (2)$$

where ζ = zeta potential in stat V, η = coefficient of viscosity in poise, Ks = the specific electrical conductivity of the fluid in the pores in reciprocal stat Ω, ϵ = the dielectric constant, and H/P the same ratio as in

eqn. (1). To calculate the value of the zeta potential η has been taken as 0·01 poise, Ks as $1{\cdot}5 \times 10^{-2}$ mhos, and ϵ as 80. This gives a value of 50 mV. From the polarity of the potential across the intestine and the direction of flow of fluid this potential must be regarded as negative, a point discussed more fully subsequently.

The surface microclimate

Hartley & Roe (1940) considered the effect of this zeta potential on the pH of the solution in close contact with the charged surface (pH_s), and derived the following equation to relate this to the pH of the bulk phase (pH_B):

$$pH_s = pH_B + (\zeta/60), \tag{3}$$

at 25° C, where ζ = zeta potential in mV. At 37° C and with a bulk phase pH of 7·4 eqn. (3) becomes

$$pH_s = 7{\cdot}4 - (50/61{\cdot}5),$$

which gives a value of 6·6 for the pH of the microclimate.

The effect of pH *on the streaming potential*

The relation between the magnitude of the streaming potential and the bulk phase pH is shown in Fig. 5. The streaming potentials produced by the addition of mannitol to the mucosal fluid are plotted against the initial pH of the saline. It can be seen that there was no significant effect until the pH was lowered to below pH 5·4. At pH 3·7 and 4·4 there was a considerable reduction in the potential, and the polarity of the potential was actually reversed at pH 2·3. From the figure an apparent pK_a of 4·0 and isoelectric point of 2·7 can be obtained for the end groups responsible for the streaming potential.

It is appreciated that another factor which could influence potential changes in these experiments is the nature of the buffer. Above pH 6 the buffer was phosphate and below pH 6 phthalate. Phthalate behaves as a weak acid with a pK of 4 so that, at the lowest pH on Fig. 3, most of the phthalic acid would be in the undissociated form, which is not very soluble in water but more soluble in lipid. The importance of these facts is difficult to evaluate, but should be borne in mind in interpreting the results.

The effect of various lipid-insoluble solutes

Staverman (1951) has pointed out that for solutes that can cross a membrane the osmotic pressure deviates from the classical van't Hoff osmotic pressure. The effective osmotic pressure developed is related to the theoretical osmotic pressure by the following equation:

$$\sigma = \frac{\pi_{\text{expt.}}}{\pi_{\text{theor.}}} = \frac{\pi_{\text{expt.}}}{RTc},$$

where σ = the Staverman coefficient (reflexion coefficient), $\pi_{expt.}$ = the effective osmotic pressure, $\pi_{theor.}$ = the ideal osmotic pressure, R = the gas constant per mole, T = the absolute temperature, and c = the molal concentration. It is apparent that when the membrane is impermeable to the solute then $\pi_{expt.} = RTc$ and consequently $\sigma = 1$, but when the membrane is permeable $\pi_{expt.} < RTc$ and $\sigma < 1$. Since the potentials are proportional to the osmotic pressure gradient they can be used to determine σ, and if

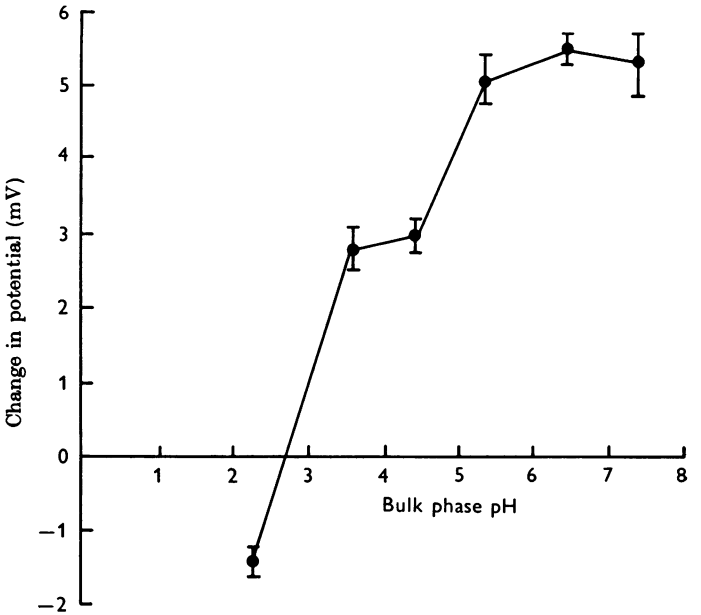

Fig. 5. Relation between initial pH of the mucosal and serosal fluids and the change in potential produced by addition of 84 mM mannitol to the mucosal fluid. The abscissa shows the initial pH, and the ordinate the change in potential caused by addition of mannitol. Positive values indicate that the mucosal side becomes less negative to the serosal side on addition of mannitol. The values are the means of five experiments with ±S.E. of the means.

lipid-insoluble substances are used the value of σ can be related to the pore radius. Lindemann & Solomon (1962) have found that mannitol has a σ value of 0·99 in the rat intestine, and we have determined σ for other substances by comparing the potential produced by them with that produced by the same concentration of mannitol. The results are shown in Table 1, which also includes estimated values for the molecular radii of the substances investigated. These values are taken from Schultz & Solomon (1961) and Lindemann & Solomon (1962). In each case the substance studied was compared with mannitol, and the table shows the significance of the difference between the mannitol potential and that of

the substance being studied. The last column shows the ratio of these potentials and this is regarded as an estimate of the Staverman coefficient. It is seen from Table 1 that substances with a molecular radius of less than 4 Å gave a σ value of less than 1, the value of σ decreasing with decreasing molecular radius.

The determination of the Staverman coefficient for lipid-insoluble substances shown in Table 1 suggests that the pore radius of the membrane is about 4 Å. A similar value is obtained by following the procedure of Goldstein & Solomon (1960) and Durbin (1960) in which the values of $(1-\sigma)$ are plotted against the radius of the probing molecule.

TABLE 1. Effect of addition of various solutes on the potential across the wall of sacs of rat everted intestine in phosphate saline containing 28 mM glucose. The substances were added to the mucosal solution to give a concentration of 84 mM, and in each case the potential was compared with that produced by addition of mannitol (84 mM). The significance of the difference between the mannitol potential and that due to the substance tested is shown. The ratio of these two potentials gives the reflexion coefficient. The values shown are the mean values of five experiments ±S.E. of the means. The molecular radii values for the test solutes were not determined but taken from sources indicated in the text

Solute	Molecular radius (Å)	Potential (−mV)	Mannitol potential (−mV)	P	σ
Lactose	5·4	5·3 ± 0·2	5·5 ± 0·1	> 0·20	0·97 ± 0·05
Erythritol	3·2	4·5 ± 0·2	5·2 ± 0·1	< 0·0025	0·87 ± 0·02
Urea	2·3	4·1 ± 0·2	5·0 ± 0·2	< 0·005	0·82 ± 0·01
Formamide	2·2	1·2 ± 0·2	5·0 ± 0·2	< 0·0005	0·24 ± 0·03
Ethylene glycol	2·3	0·9 ± 0·2	5·2 ± 0·1	< 0·0005	0·17 ± 0·03

DISCUSSION

These experiments show that changes in the electrical potential across the wall of the small intestine can be produced by increasing the osmotic pressure of the mucosal fluid. In the presence of glucose this osmotic pressure gradient also reduced the volume of fluid transported by the tissue. The most probable explanation is that streaming potentials are being produced by the movement of fluid through negatively charged pores, the argument being analogous to that used by Diamond (1962) with the gall-bladder. The calculated value of the zeta potential at the phase boundary in the pores is −50 mV. This is higher than the −19 mV reported by Abramson (1929) for the rat red-cell membrane, or the value of −14 mV which can be calculated from the data of Hays & Lipman (1964) for the epithelial cells of the toad urinary bladder.

Further evidence that these potential changes produced in response to osmotic gradients are streaming potentials is provided by the experiments showing the effect of pH on these potentials. Below pH 5·4 the magnitude of the potential varies with pH, and at pH 2·3 the sign of the potential is

actually reversed. This would be consistent with the idea of an isoelectric point at approximately pH 2·7 and an apparent pK_a of about 4·0. In the epithelial cells of the urinary bladder there is an isoelectric point at pH 3·4 (Hays & Lipman, 1964). On the other hand Bangham, Pethica & Seaman (1958) could not show an isoelectric point in the red-cell membrane. We appreciate that in our experiments the cells are being exposed to grossly unphysiological conditions, but nevertheless the results are consistent with the view that changes in the membrane are the result of dissociation of basic and acidic groups in the protein forming the wall of the pores. In view of the experimental conditions we would not like to speculate further about identifying the protein end-groups.

The streaming potentials are much more stable in the presence of glucose than in its absence and, furthermore, in the absence of glucose there is no longer a linear relation between magnitude of potential and osmotic gradient at the higher mannitol concentrations. In the presence of fructose a sugar which can be metabolized, the action of mannitol is similar to that in the presence of glucose, and so it is possible that the stability of the potentials is related to energy available from hexose metabolism.

Since we have demonstrated electrokinetic phenomena in the intestine the question arises as to how far these phenomena can play a role in (1) intestinal transfer of fluid, (2) the stimulation of fluid transfer by glucose, or (3) the potential produced by hexose transfer. All these questions can be answered fairly definitely in the negative.

For electro-osmosis to account for the fluid movement across the intestine the serosal side would have to be negative to the mucosal side, but in fact the observed potential is in the opposite direction. It is also evident from the use of equation (1) that electro-osmosis cannot possibly account for the fluid movements observed in the presence of glucose. It is widely believed that the fluid transported from the mucosal to the serosal surface of the intestine does so as a result of a local osmotic gradient maintained by active transport of solutes, mainly Na and other ions. Barry *et al.* (1965) have stressed that the sodium pump is a neutral one and that the potential across the intestine is related to the active transfer of hexoses. This would imply that solute linked fluid movement does not occur through the charged pores responsible for the streaming potentials. Additional evidence in support of this is that fructose stimulates solute-linked fluid movement without generating a potential difference across the intestine (Barry *et al.* 1964). A similar conclusion was reached by Pidot & Diamond (1964) about passive and solute linked fluid movement in the gall bladder.

Can the potential caused by transferable hexoses have an electrokinetic origin? The serosal side of the intestine is positive, and we have already established that the side of the intestine to which fluid is transported

becomes positive. Is then the potential associated with the actively transported hexoses generated by fluid movement? This seems unlikely as Barry *et al.* (1964) have already established that the potential is not associated with the ability of the hexoses to stimulate fluid transfer. Galactose, 3-methyl glucopyranose and α-methyl glucoside all support potentials across the intestine but do not stimulate fluid transfer, while fructose stimulates fluid transfer but does not increase the potential.

The calculation of the pH of the microclimate close to the charged surface of the luminal membrane is of some interest in view of the postulation by Hogben, Tocco, Brodie & Schanker (1959) of a microclimate with a pH different from that in the bulk phase. On the basis of their measurements of the distribution of weak acids and bases across the intestine, they calculated that a region existed close to the epithelial cells in which the pH was about two units of pH lower than the hydrogen-ion concentration in the bulk phase. Using the value of the zeta potential calculated here and the equation of Hartley & Roe (1940) we conclude that the pH of the microclimate in the pores of the wall of the intestine is about 1 unit of pH less than the bulk phase. We would not claim that these calculations offer a final answer to the question of pH of the microclimate, but this is one approach to this particular problem.

The measurement of streaming potentials also provides a method for determining the Staverman coefficient, and if lipid-insoluble substances are used as the probing molecule they can be used for determination of equivalent pore radius. As seen in Table 1, this gives a pore radius of about 4 Å, which agrees remarkably well with the determination by Lindemann & Solomon (1962) by an entirely different method. It is appreciated that there are certain reservations about pore size as pointed out by Goldstein & Solomon (1960), e.g. that the degree of hydration of the molecules is not considered in determination of molecular radius from models, but recognizing these limitations the determination is of value in relation to problems of intestinal absorption.

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