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RELATION BETWEEN SODIUM CONCENTRATION, ELECTRICAL POTENTIAL AND TRANSFER CAPACITY OF RAT SMALL INTESTINE

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SUMMARY

1. A study has been made of the effect of Na replacement in the incubation media on the hexose-dependent potential and the capacity of sacs of rat intestine to transfer fluid and galactose.

2. Replacement of NaCl with mannitol or Tris Cl had little effect on the hexose-dependent potential, while replacement with LiCl and KCl had an inhibitory effect on the potential.

3. Replacement with KCl, LiCl, mannitol and Tris Cl all reduced the capacity to transfer fluid and galactose, but the effects of Li and K replacement were greater than mannitol or Tris replacement.

4. There is not a fixed relation between the magnitude of the potential and the amount of galactose transferred.

INTRODUCTION

Barry, Dikstein, Matthews, Smyth & Wright (1964) showed that actively transferred hexoses cause a potential across the wall of the rat intestine, and Barry, Smyth & Wright (1965) considered that this potential was specifically related to the hexose transfer mechanism. Since Riklis & Quastel (1958) showed that sodium is necessary for hexose movement, and since various relationships between sodium and hexose movement have been discussed by different authors it was of interest to study the relation between the potential related to the hexose transfer mechanism and the sodium concentration. Galactose was chosen for this study because it is an actively transported hexose which is not significantly metabolized by the rat intestine. A preliminary account of these results has been published by Barry, Eggenton, Smyth & Wright (1966).

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METHODS

White rats of the Sheffield strain were used and before experiment were maintained on a commercial diet (diet 86-Oxoid, London) in cube form. The cubes and water were made available *ad libitum*. The everted sac preparation was used in all experiments and was made from the middle region of the combined jejunum and ileum. The saline was bicarbonate saline (Krebs & Henseleit, 1932) in which sodium chloride was replaced to a varying extent by potassium chloride, lithium chloride, mannitol or Tris chloride, as shown in Table 1. It will be seen from this Table that the amounts of the different substances were calculated so as to maintain a constant osmolarity. In all cases the saline was in equilibrium with 5% CO_2, 95% O_2.

Measurement of electrical potentials. The object of these experiments was to determine the magnitude of the galactose-induced potential when NaCl was replaced with other solutes. This was done in two different ways.

(1) Two adjacent segments of the intestine were taken comprising approximately the middle fifth of the combined jejunum and ileum and both of these were set up for recording the potential by the method of Barry *et al.* (1964). Each sac contained initially 1 ml. of the saline being studied and was immersed in 125 ml. of the same saline. The potential was continuously recorded and had always reached a stable level within 10 min. The level was now read and this is the value (*a*) in the presence of the particular saline. To the mucosal fluid of one preparation galactose was now added to make a final concentration of 28 mM, and to the mucosal fluid of the other preparation mannitol was added to make a final concentration of 28 mM. Mannitol was used as a control to produce an osmotic effect corresponding to that of galactose. The potentials were recorded in the presence of galactose (*b*) and in the presence of mannitol (*c*). The change in potential due to the osmotic effect of mannitol is *a*–*c*, and if this is subtracted from the value in the presence of galactose we get the potential in the presence of galactose corrected for the osmotic effect of the sugar ($b-a-c$). This corrected value is the one which is shown in Figs. 1–4 for the potential in the presence of galactose. The algebraic difference between the potential in the presence of galactose (*b*) and in the presence of mannitol (*c*) gives the galactose-dependent potential ($b-c$), and this is the potential which is shown in Fig. 5. It will be noted that mannitol is used in these experiments in two different ways. In the first place it is used to replace sodium chloride in one set of experiments; in the other set the NaCl was replaced with Tris Cl, LiCl and KCl. But in whatever way the sodium chloride is replaced mannitol is used in all cases to replace galactose as described above in order to allow for the osmotic effect of the galactose.

(2) Two preparations of everted sacs were set up as already described in the previous paragraph except that galactose (28 mM) was initially present in both the mucosal and serosal solutions of one preparation, while mannitol (28 mM) was initially present in the mucosal and serosal solutions of the other preparation. After 10 min phlorrhizin was added to each mucosal solution to give a final concentration of 5×10^{-4} M, which has been shown by Barry *et al.* (1964) to abolish the hexose-dependent potential. The algebraic difference between the potentials in the presence and absence of phlorrhizin was taken to be the galactose-dependent potential. The sacs with mannitol present showed that addition of phlorrhizin had no effect on the osmotic potential.

Measurement of galactose transfer. Galactose transfer was determined by the method of Barry, Matthews & Smyth (1961). A sac made from the middle fifth of the combined jejunum and ileum was suspended in 25 ml. bicarbonate saline with 28 mM galactose (mucosal fluid) and contained 1 ml. of the same solution (serosal fluid). The incubation lasted for 30 min. The following parameters were used for expressing transfer. The mucosal fluid transfer is the diminution in volume of mucosal fluid during the period of incubation. The mucosal galactose transfer is the amount of galactose disappearing from the mucosal fluid

during incubation. The final galactose concentration difference is the final concentration of galactose in the serosal fluid minus the final concentration of galactose in the mucosal fluid.

RESULTS

Potential measurements. In these experiments salines were used both for the mucosal and serosal fluid in which the NaCl was replaced by mannitol, Tris Cl, KCl, or LiCl as shown in Table 1. The results of these experiments are seen in Figs. 1–4 which show the effect of replacement with mannitol,

TABLE 1. Compositions of the saline solutions used. In addition to the concentration of Na, K, Li, Cl, mannitol or Tris shown, all solutions contained Ca 2·5 m-equiv/l., Mg 1·2 m-equiv/l., SO_4 1·2 m-equiv/l., HCO_3 24·9 m-equiv/l., H_2PO_4 1·2 m-equiv/l. The concentrations for the ions are in m-equiv/l. and for mannitol mM

Saline	Na	K	Li	Tris	Mannitol	Cl
A	143·4	5·9	—	—	—	125·7
B	80	69·3	—	—	—	125·7
C	40	109·3	—	—	—	125·7
D	25	124·3	—	—	—	125·7
E	80	5·9	63·4	—	—	125·7
F	40	5·9	103·4	—	—	125·7
G	25	5·9	118·4	—	—	125·7
H	80	5·9	—	63·4	—	125·7
J	40	5·9	—	103·4	—	125·7
K	25	5·9	—	118·4	—	125·7
L	80	5·9	—	—	126·8	62·3
M	40	5·9	—	—	206·8	22·3
N	25	5·9	—	—	236·8	7·3

Tris Cl, KCl and LiCl respectively. The results obtained with mannitol and Tris were very similar to each other. Lowering the Na concentration in the absence of galactose caused the potential to decrease and the polarity was reversed between 143 m-equiv/l. Na and 80 m-equiv/l. Na. A similar decrease was found in the presence of galactose. When the sodium concentration was plotted logarithmically the relation between the potential and sodium concentration was a linear one, and this was the case both in the presence and absence of galactose. Details of the equations for the regression lines in Figs. 1–4 are given in Table 2. For both mannitol and Tris Cl replacement the regression lines in the presence and absence of galactose are approximately parallel indicating that the galactose-dependent potential was little affected by lowering the Na concentration. Figures 3 and 4 show the experiments with K and Li replacement. These are plotted on a different ordinate scale from Fig. 1 as the values in presence and absence of galactose are much closer together. On replacement of Na with K or Li there was also an approximately linear relationship between the log [Na] and the potential in the absence of galactose. The slopes of these regression lines are very similar, but very different from the regression line for mannitol or Tris replacement. In the presence of galactose in both cases the relationship was not a linear one, and the difference

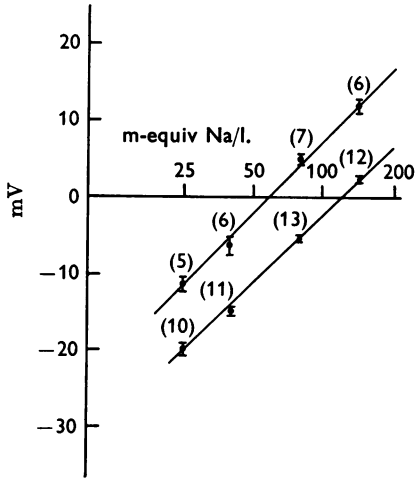

Fig 1.

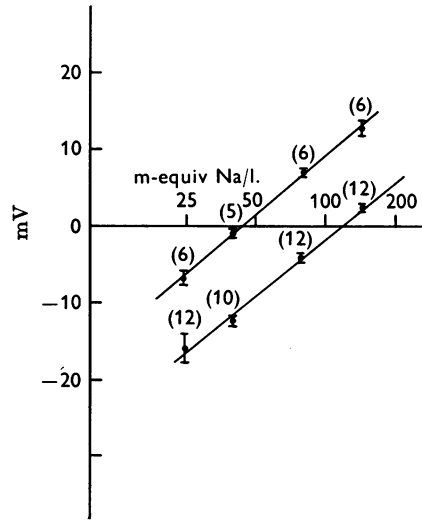

Fig 2.

Fig. 1. Relation between potential (ordinate) and [Na] (abscissa) when NaCl is replaced with mannitol, and [Na] is plotted on a logarithmic scale. The upper line shows values in presence of galactose (28 mM), the lower line in absence of galactose. In all cases the value given is the mean ± S.E.M. with the number of experiments in brackets. The solutions used in these experiments were solutions *A*, *L*, *M* and *N* in Table 1.

Fig. 2. Relation between potential (ordinate) and [Na] (abscissa) when NaCl is replaced with Tris Cl, and [Na] is plotted on a logarithmic scale. The upper line shows values in the presence of galactose (28 mM), the lower line in absence of galactose. In all cases the value given is the mean ± S.E.M. with the number of experiments in brackets. The solutions used in these experiments were solutions *A*, *H*, *J* and *K* in Table 1.

TABLE 2. Data for equations of regression lines given in Figs. 1–4. The equations have the form $y = bx + a$ where y is the potential in mV and x is log[Na] expressed in m-equiv/l. The table shows the values of b (the regression coefficient), the S.E. of the regression coefficient, and the value of the constant a

Experimental conditions	Degrees of freedom	b	S.E. of b	a
Mannitol replacement without galactose	44	29·64	±0·86	−61·99
Mannitol replacement with galactose	22	31·81	±1·46	−56·56
Tris Cl replacement without galactose	44	24·25	±0·68	−50·45
Tris Cl replacement with galactose	21	24·70	±1·13	−40·97
KCl replacement without galactose	44	2·67	±0·19	−3·63
LiCl replacement without galactose	46	2·22	±0·25	−2·76

between the values for each Na concentration in the presence and absence of galactose decreased with lowered Na concentration. This means that the galactose-dependent potential was decreased at lower Na concentrations. The point is better seen by plotting the galactose-dependent potential

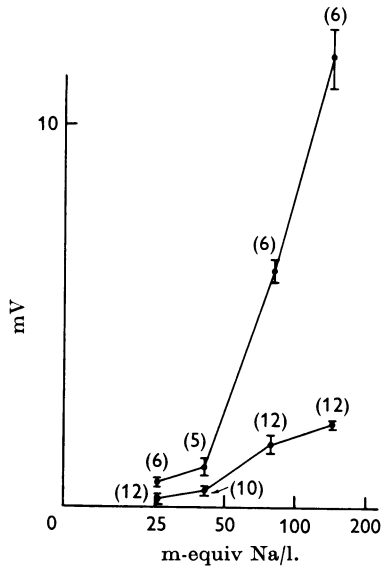

Fig 3.

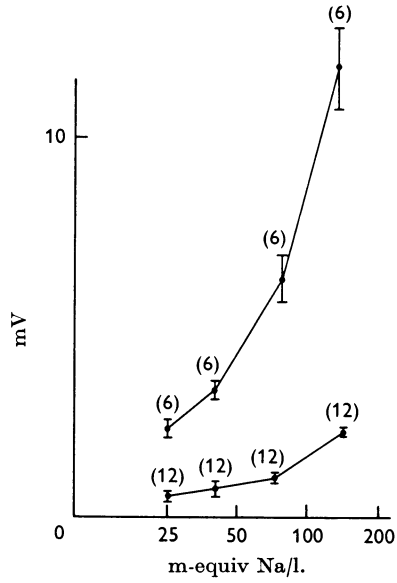

Fig 4.

Fig. 3. Relation between potential (ordinate) and [Na] (abscissa) when NaCl is replaced with KCl, and [Na] is plotted on a logarithmic scale. The upper line shows values in presence of galactose (28 mM), the lower line in absence of galactose. In all cases the value given is the mean ±S.E.M. with the number of experiments in brackets. The solutions used in these experiments were solutions *A*, *B*, *C* and *D* in Table 1.

Fig. 4. Relation between potential (ordinate) and [Na] (abscissa) when NaCl is replaced with LiCl, and [Na] is plotted on a logarithmic scale. The upper line shows values in presence of galactose (28 mM), the lower line in absence of galactose. In all cases the value given is the mean ±S.E.M. with the number of experiments in brackets. The solutions used in these experiments were solutions *A*, *E*, *F* and *G* in Table 1.

against [Na], and this is seen in Fig. 5. In order to indicate the concentration of the replacing solute as well as the sodium concentration, the latter is plotted on a linear scale. It is evident that K and Li replacement both greatly reduced the galactose-dependent potential, while mannitol or Tris replacement had little effect down to 25 m-equiv/l. Na.

Galactose and fluid transfer. The effect of sodium replacement on fluid and galactose transfer is shown in Tables 3–5. The fluid movement was

reduced by lowering the Na concentration, but mannitol and Tris produced effects different from those caused by K and Li replacement. With Tris there was no effect at 80 m-equiv/l. Na, but a definite depression was seen at lower concentrations. With mannitol there was a small depression at 80 m-equiv/l. Na and a greater depression at the lower concentrations.

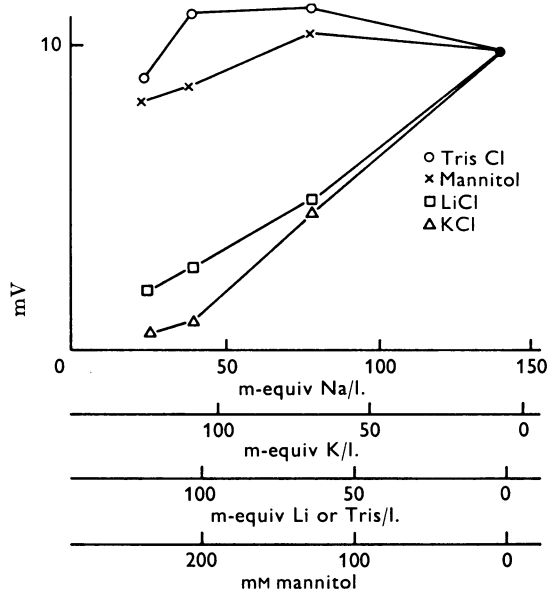

Fig. 5. Relation between sodium concentration and hexose-dependent potential when sodium is replaced by various substances. The values are obtained from Figs. 1–4 and are the algebraic difference between the potential in the presence of galactose and the potential in the absence of galactose. In order to indicate the concentration of the replacing substance as well as that of sodium, the concentrations are plotted on a linear scale.

TABLE 3. Effect of NaCl replacement on mucosal fluid transfer by sacs of rat everted intestine. Sacs of mid-intestine contained initially 1 ml. of saline containing 28 mM galactose and were incubated in 15 ml. of the same solution for 30 min. The saline used in the various groups of experiments is indicated by the letter in brackets, the letters referring to the solutions in Table 1. The number of experiments in each group is shown by the figure in brackets. The values are the mean ± S.E.M. and are expressed as ml. fluid/g initial wet wt. intestine

Na concn. (m-equiv/l.)	KCl replacement (ml.)	LiCl replacement (ml.)	Tris Cl replacement (ml.)	Mannitol replacement (ml.)
140	(*A*) 0·49 ± 0·02 (20)	(*A*) 0·49 ± 0·02 (20)	(*A*) 0·49 ± 0·02 (20)	(*A*) 0·49 ± 0·02 (20)
80	(*B*) 0·11 ± 0·01 (5)	(*E*) 0·19 ± 0·01 (5)	(*H*) 0·50 ± 0·04 (6)	(*L*) 0·38 ± 0·03 (9)
40	(*C*) 0·12 ± 0·01 (6)	(*F*) 0·13 ± 0·01 (6)	(*J*) 0·25 ± 0·03 (6)	(*M*) 0·22 ± 0·02 (9)
25	(*D*) 0·16 ± 0·01 (6)	(*G*) 0·15 ± 0·01 (5)	(*K*) 0·22 ± 0·02 (6)	(*N*) 0·14 ± 0·01 (9)

In contrast, with K and Li replacement the maximum effect was produced at a concentration of 80 m-equiv/l. Na and this was as great as the maximum mannitol or Tris effect.

The effects on galactose transfer were roughly analogous to those on fluid transfer in that K and Li replacement caused a greater depression of transfer than did mannitol replacement. In the case of mannitol and Tris

TABLE 4. Effect of NaCl replacement on mucosal galactose transfer by sacs of rat everted intestine. The data are from the experiments referred to in Table 3. The transfers are expressed as μmoles galactose/g initial wet wt./30 min of gut, and are the means $\pm$ S.E.M.

Na concn. (m-equiv/l.)	KCl replacement	LiCl replacement	Tris Cl replacement	Mannitol replacement
140	71·1 ± 2·8	71·1 ± 2·8	71·1 ± 2·8	71·1 ± 2·8
80	33·3 ± 4·6	21·4 ± 4·4	72·4 ± 8·8	75·3 ± 5·3
40	19·3 ± 3·6	17·2 ± 2·8	38·2 ± 5·7	47·4 ± 3·5
25	17·8 ± 2·5	24·0 ± 3·9	29·8 ± 2·3	30·1 ± 2·1

TABLE 5. Effect of NaCl replacement on final galactose concentration difference in serosal and mucosal fluids. A positive value indicates a higher galactose concentration in the serosal fluid. The experiments are those referred to in Table 3

Na concn. (m-equiv/l.)	K replacement (mM)	Li replacement (mM)	Tris Cl replacement (mM)	Mannitol replacement (mM)
140	23·3 ± 1·1	23·3 ± 1·1	23·3 ± 1·1	23·3 ± 1·1
80	0·4 ± 0·3	3·1 ± 0·6	20·7 ± 2·0	22·8 ± 1·6
40	−3·0 ± 0·5	−1·1 ± 0·8	9·5 ± 1·2	12·8 ± 1·2
25	−4·7 ± 0·5	−2·1 ± 0·8	6·4 ± 1·0	6·4 ± 0·9

there was no depression of hexose transfer at a sodium concentration of 80 m-equiv/l. Na but there was a reduction at the two lower concentrations. With K and Li replacement there was a large effect on galactose transfer even at 80 m-equiv/l. Na, particularly in the case of Li where the maximum effect appeared to be reached at this concentration. In the case of K a somewhat greater depression was obtained at lower Na concentrations.

The effects on galactose transfer can also be seen from the final concentration differences (Table 5). In the case of mannitol and Tris replacement there was some galactose movement against a gradient even at the lower sodium concentration, whereas in the case of K and Li the gradient was almost zero at 80 m-equiv/l. and at lower Na concentrations was negative. A negative value for the concentration difference means that the final serosal concentration was smaller than the final mucosal and is probably mainly due to the fact that some galactose diffused from the serosal fluid into the subepithelial space in the gut wall.

DISCUSSION

The results show, in agreement with those of Riklis & Quastel (1958) and other workers, that reduction of sodium concentration in the fluid bathing the gut affects the capacity for hexose transfer. They also show that not only is the sodium concentration important but also the nature of the replacing substance. Mannitol or Tris replacement had less effect than replacement with lithium or potassium, and this is in agreement with the results of Bosackova & Crane (1965) with the hamster gut. In contrast to these workers we obtained very similar results with Tris and mannitol replacement. In agreement with Clarkson & Rothstein (1960) we found that a maximum effect on hexose transfer was produced with potassium and lithium when the sodium concentration was reduced to 80 m-equiv/l.

It is also seen that mannitol and Tris replacement had less effect on fluid transfer than K or Li, and this is in agreement with the results of Parsons & Wingate (1961). In these experiments no measurements were made on sodium movement, but the strong inhibition of fluid transfer by potassium is consistent with the inhibitory effect on sodium influx found by Bosackova & Crane (1965).

Replacement of NaCl by mannitol or Tris Cl on the one hand and by KCl and LiCl on the other had quite different effects on the potential. In the absence of galactose, mannitol or Tris Cl replacement caused a much greater decrease in potential across the gut wall than did KCl or Li Cl replacement. The potential caused by mannitol and Tris Cl replacement in the absence of galactose is presumably a diffusion potential due to the different ionic concentrations in the different compartments, i.e. mucosal fluid, intracellular fluid, subepithelial fluid and serosal fluid. Even the muscle layer cannot be excluded as contributing to this. It should be remembered that although the mucosal and serosal fluids have initially the same composition, the serosal fluid volume is small and its composition can change considerably by diffusion through the muscle layer. It is not the purpose of this paper to study the precise origin of these potentials, which has been discussed in detail by Wright (1966). What concerns us here is the effect of sodium replacement on the galactose-dependent potential, and it is clear that K or Li affect this much more than mannitol or Tris. The potential is thus not merely dependent on the availability of Na in the external medium. Presumably K and Li, which can enter the cell, cause some fundamental disturbance of ionic balance in the cell, which is responsible for the fall in the hexose potential. It is also possible that the differences in the galactose-dependent potential in the presence of mannitol, Tris, Li or K may be, at least in part, attributed to differences in tissue conductance (Schultz, Curran & Wright, 1967).

It is evident that there is not a simple relationship between the magnitude of hexose potential and the amount of hexose transferred. In the mannitol and Tris replacement the hexose-dependent potential is little affected at 25 m-equiv/l. concentration of Na, at which level the hexose transfer is considerably reduced. The maintenance of hexose-dependent potential at lowered Na concentration is in agreement with the results of Lyon & Crane (1966) who found that the hexose-dependent potential was maintained down to a Na concentration of 24 m-equiv/l. In the case of Li and K a maximum effect on transfer is produced at 80 m-equiv/l. Na, and although the potential is also reduced this is not abolished. In all cases therefore the galactose transfer seems to be affected more than the galactose-dependent potential.

The relation between Na and hexose movement, and the electrical potential across the gut is still not settled. While our results agree with those of other workers that hexose movement is dependent on the presence of Na, they do not suggest a simple relation between Na concentration and hexose movement, nor a simple relation between potential and magnitude of hexose movement.

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