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STUDIES ON *IN VITRO* TRANSMURAL POTENTIALS IN RELATION TO INTESTINAL ABSORPTION

III. K^+ INHIBITION OF Na^+-DEPENDENT TRANSMURAL POTENTIAL OF RAT SMALL INTESTINE*

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SUMMARY

The transmural potential difference (PD) measured *in vitro* with paired everted jejunal and ileal sacs of rat small intestine is strongly influenced by the concentration of Na^+ and by the presence and concentration of actively transported sugars. Changes in PD, ΔPD, following the addition of certain sugars have been assumed to reflect changes in the rate of Na^+ entry. Double reciprocal plots, $1/\Delta PD$ *vs.* 1/[sugar], are suggestive of saturation phenomena interpretable in terms of a ternary interaction of Na^+, sugar and a mobile carrier in the brush border membrane. Reasoning along these lines, comparison of the kinetics observed with $Tris^+$, a non-penetrating ion, and with K^+, a penetrating ion, suggest that K^+ is a competitive inhibitor for Na^+ interaction with the sugar carrier. The dissociation constants for the various equilibria which may be assumed to occur in a system containing carrier, Na^+ or K^+ and sugar have been evaluated by extrapolation from plotted values and by computer iterative analysis.

The effect of K^+ replacement of Na^+ with rat preparations differs somewhat from the effect with rabbit ileum reported by others although, in both, PD and short-circuit current measurements rise in response to increases in Na^+ and/or sugar concentrations. The need is thus indicated for consideration of two kinetic models: one in which there is a high degree of interaction between the ion-binding and sugar-binding sites of the carrier, a model which appears to be appropriate for the rat, and another in which interaction between these sites is minimal, a model which appears to fit the data for the rabbit.

Abbreviation: PD, potential difference.

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INTRODUCTION

It is now well established that Na^+ is involved in the active intestinal transport of a variety of substances[1–7], including sugars. Na^+ is required and other cations neither substitute for it nor appear to act in concert with it[1,2,8]. Also, it appears to be involved in substrate entry across the brush border membrane of the mucosal cell[3,9,10].

Studies *in vitro* with hamster small intestine[11], in which Na^+ was replaced progressively with K^+, showed a parallel inhibition of sugar transport, measured by accumulation of 6-deoxy-D-glucose, and of Na^+ entry, measured by flame photometry and $^{22}Na^+$ influx. This apparent relationship between sugar and Na^+ transports was interpreted to mean that entering sugar is accompanied by Na^+ and the effects of K^+ interpreted as a competition between K^+ and Na^+ for an ion-binding site on a carrier. Subsequent studies of the effect of Na^+ concentration on the apparent Michaelis constants for sugar transport[12] provided support for this interpretation. When Na^+ was replaced with $Tris^+$, the apparent K_m for 6-deoxy-D-glucose was increased, but with K^+ the increase was greater.

As discussed previously, sugar entry into the cell, if it occurs as postulated by CRANE *et al.*[13,14], should not be an electrically neutral process and studies on the transmural potential difference, PD, and the short-circuit current, seem to bear this out. PD is dependent upon Na^+ (*cf.* refs. 15–20) and is increased in the presence of any of the glucose group of actively transported sugars[18,19,21–31]. With rabbit ileum[25–28] the increase in PD is accompanied by an increase in the mucosal→serosal $^{22}Na^+$ flux and a numerical equivalence between short-circuit current and $^{22}Na^+$ flux has been established. Moreover, sugar-induced changes in PD and short-circuit current are linearly related[27–31].

The effect on PD of graded concentrations of actively transported sugars has been interpreted[31] in terms of the ternary interaction of sugar and Na^+ with a mobile carrier in the brush border membrane postulated by CRANE *et al.*[13,14] and changes in PD, ΔPD, have been taken as a measure of sugar-dependent entry of Na^+ into the cell. From plots of $1/\Delta PD$ *vs.* 1/[sugar] values of apparent K_m and v_{max} for D-glucose and 6-deoxy-D-glucose could be extrapolated. The kinetics in these studies[31], in which Na^+ was replaced by $Tris^+$, were of the competitive type.

In a recent paper SCHULTZ AND ZALUSKY[28] presented short-circuit current data obtained with rabbit ileum suggesting, in parallel with the above, that Δshort-circuit current is a saturable function of the concentration of sugar in the mucosal medium. However, their data indicated that replacement of Na^+ with K^+ caused a decrease in v_{max} but no substantial change in K_m; that is, the kinetics were non-competitive. These findings are at variance with ours for the rat and may, to some extent be due to species differences. However, preliminary experiments in which replacement by $Tris^+$ and K^+ were compared, suggested that the kinetics with K^+ replacement are indeed, as found by SCHULTZ AND ZALUSKY, substantially non-competitive in character while with $Tris^+$ replacement they are of the competitive type.

The present studies are an extension of these preliminary experiments which appear to resolve the differences between the findings of the two laboratories.

METHODS

Tissue preparation

Paired everted sacs[32] of jejunum and ileum were prepared as previously described[31] from Sprague–Dawley MRC rats of either sex, weighing 150±20 g, after a fast of 48 h during which water was available *ad libitum*.

Incubation media

The everted sacs were incubated at 37° in Krebs–Henseleit bicarbonate media[33] modified by replacing 25 mM $NaHCO_3$ with 25 mM $Tris^+$–bicarbonate as a buffer at pH 7.4. The remaining 120 mM, required for isotonicity, were divided between NaCl and either $Tris^+$–HCl or KCl. The ionic compositions of these media and their designations are given in Table I. When D-glucose or D-galactose was added to the mucosal medium, in graded concentrations between 1 and 25 mM, the control solutions contained D-mannitol, in comparable concentrations, to correct for osmotic disequilibria.

TABLE I

IONIC COMPOSITION OF THE BUFFERS USED

Krebs–Henseleit bicarbonate buffer (Concn. in mmoles/l)

Na^+,	145;	K^+,	6;	Ca^{2+},	1.3;	Mg^{2+},	2.5;				
Cl^-,	127;	SO_4^{2-},	2.5;	HPO_4^{2-},	1.2;	HCO_3^-,	25.				

$Tris^+$–bicarbonate buffers

Designation	*Component*	*Concn. in mmoles/l*					
Na^+–Tris	NaCl	120*	96	72	48	24	0
	$Tris^+Cl^-$	0	24	48	72	96	120
Na^+–K^+	NaCl	120*	96	72	48	24	0
	KCl	0	24	48	72	96	120

* Although the composition of these buffers is the same, the appropriate designation is used in a given context for clarity.

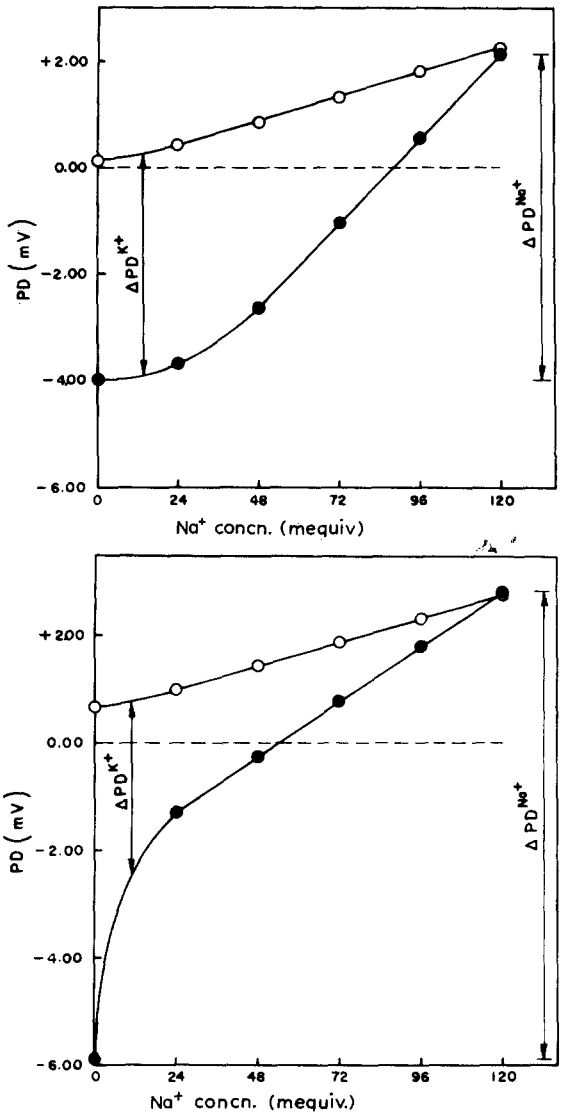
Incubation apparatus and measurement of transmural PD

The apparatus and the method of measurement of PD values have been described in detail in the first report of this series[31].

RESULTS AND DISCUSSION

Effects of K^+ and $Tris^+$ on the Na^+-dependent transmural PD

The spontaneous transmural PD, measured with jejunal sacs, depends upon the Na^+ concentration in the medium (Fig. 1), whether replacement is with K^+ or $Tris^+$. With either replacement ion, PD is a linear function of Na^+ concentration between 24 and 120 mequiv, although the effect of changes in Na^+ was proportionally greater in the presence of $Tris^+$, a non-penetrating ion, than in the presence of K^+, a penetrating ion. The difference in slope of the two curves suggests that K^+, relative to $Tris^+$, reduces Na^+ influx.



Figs. 1 and 2. Influence of the replacement ion on the Na^+-dependent transmural PD in the jejunum (Fig. 1) and in the ileum (Fig. 2). Upper curves, K^+ replacement; lower, $Tris^+$ replacement. ΔPD^{K+} and ΔPD^{Na+}, K^+-dependent and Na^+-dependent increments of PD, respectively.

It may be noted that when Na^+ is replaced by $Tris^+$, PD is positive toward the serosa only when external Na^+ is in excess of 88 mequiv; a reversal in polarity is seen at lower concentrations. There is no reversal in polarity with K^+, presumably because an increasing portion of the PD is supported by the movement of K^+ as it replaces Na^+ (Fig. 1).

The relationship between the Na^+ concentration in the medium and the PD measured with ileal sacs (Fig. 2) is similar to that described for jejunal sacs except that reversal in polarity occurs at a Na^+ concentration of 54 mequiv.

Effect of [Na^+] in Na^+–$Tris^+$ buffers on sugar-dependent increment of transmural PD

The increment in PD, ΔPD, induced by mucosal sugar is a function of the concentrations of both Na^+ and sugar. For example, as the concentration of Na^+ was increased from 0 to 120 mequiv, ΔPD induced by sugar rose as shown in Fig. 3. These curves are typical of the general pattern observed with jejunal and ileal sacs and with graded concentrations of glucose or galactose. With either sugar, ileal ΔPD values were generally higher than jejunal over the range of Na^+ concentrations. Increments in PD with glucose were greater than those with galactose in both jejunal and ileal preparations.

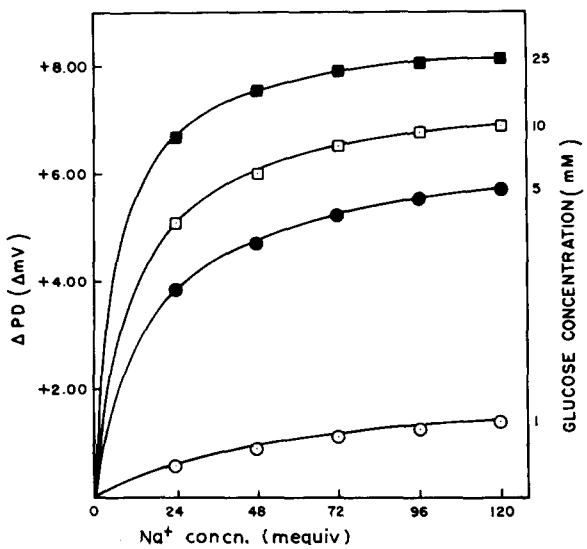


Fig. 3. Influence of Na^+ and sugar concentrations on transmural PD values in ileum with $Tris^+$ replacement. Each point represents the mean of 6 experiments; each percentage figure is the average standard deviation (S.D.) from the mean for all values obtained with a given concentration of sugar: ■—■, ± 18%; ⊡—⊡, ± 21%; ●—●, ± 11%; ○—○, ± 20%.

Effect of [Na^+] in Na^+–K^+ buffers on sugar-dependent increment of transmural PD

The mutual influence of Na^+ and sugar on the increment of PD is clearly to be seen also when K^+ is used to replace Na^+. However, the way in which ΔPD changes is markedly influenced by the replacement ion (Fig. 4). The same pattern was observed with jejunal and ileal sacs and with glucose and galactose. Again, the glucose-induced increments tended to exceed those with galactose and they were higher in the ileum.

Kinetics of Na^+ flux in Na^+–$Tris^+$ buffers

If it is correct to assume that PD is a measure of energy-dependent Na^+ translocation across the basal membrane of the epithelial cell*, then the maximal rate of

* This assumption appears to be a valid one since sugar-dependent changes in PD and short-circuit current, measured in the same everted sac, parallel one another. Thus, the tissue conductance ratio, change in short-circuit current:change in PD ratio, has been found to be a constant whose numerical value depends upon the Na^+ concentration of the medium.

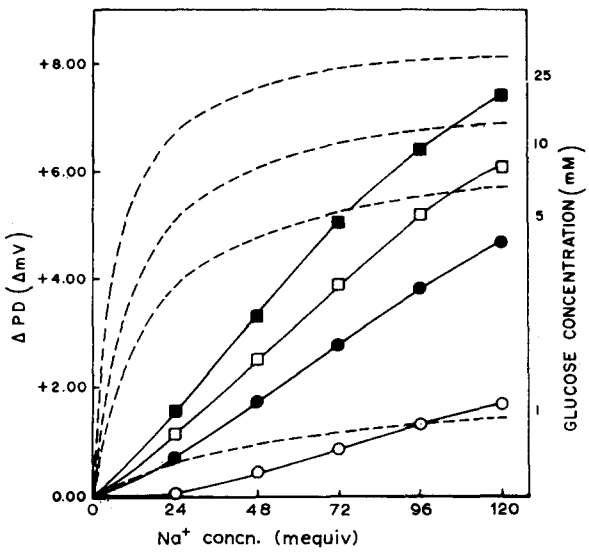


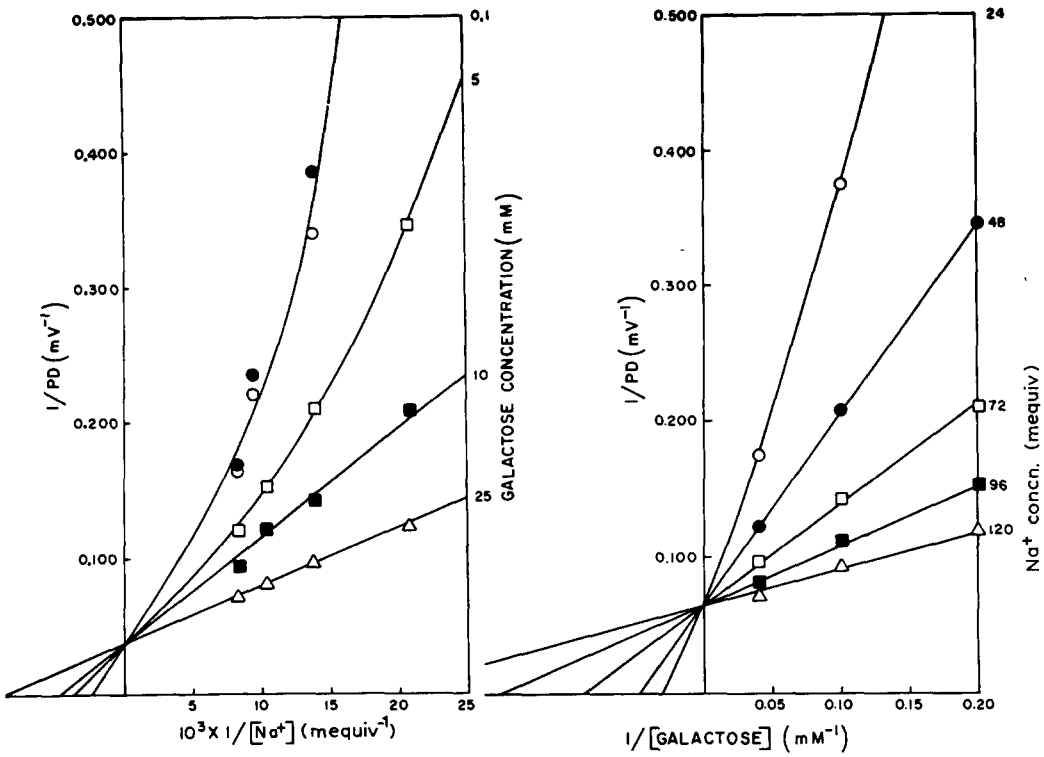
Fig. 4. Influence of the replacement ion on the Na^+-dependent PD in the ileum in the presence of sugar. The dashed curves are for $Tris^+$ replacement and are taken from Fig. 3; the continuous curves with experimental points are for K^+ replacement. All curves were translated to extend from the origin. Each point on the continuous curves represents the mean of 6 experiments; each percentage figure is the average S.D. for all values obtained with a given concentration of sugar: ■—■, ± 5%; □—□, ± 16%; ●—●, ± 15%; ○—○, ± 24%.

this translocation should be the maximal rate of mucosal→serosal Na^+ flux. However, the possibility exists that Na^+ entry at the mucosal membrane may exceed this. By analogy with enzyme kinetics and along the lines of mobile carrier concepts[34], it is assumed that Na^+ interacts with the sugar carrier in the brush border membrane at a specific ion-binding site and that this carrier-mediated transport of Na^+ is reflected by increases in PD and short-circuit current. Compounds (*e.g.* sugars) whose movement is Na^+-dependent and which consequently enhance Na^+ movement may thus be regarded as "non-essential activators"[35] which interact with a second specific site on the carrier[31].

The kinetics of Na^+ flux with regard to Na^+ concentration, when varied by $Tris^+$ replacement, are those of a relatively simple association–dissociation phenomenon. The same or nearly the same maximal velocity is approached either (Fig. 5) at a given sugar concentration when the concentration of Na^+ is increased without limit or (Fig. 6) at a given Na^+ concentration when the concentration of sugar is increased without limit. A kinetic model consistent with these findings has been visualized for the hamster[12] and for the rat[31] in terms of the ternary complex mentioned above consisting of carrier, Na^+ and sugar in which there is a high degree of interaction between the ion-binding and sugar-binding sites. As formulated, this is perhaps an example of mutual allosteric activation[36].

Kinetics of Na^+ flux in Na^+–K^+ buffers

There is a decided difference in the kinetics of Na^+ flux, as measured by PD, when K^+, rather than $Tris^+$, is used as the replacement ion (compare Fig. 7 with Fig.



Figs. 5 and 6. Reciprocal plots of 1/PD *vs.* 1/[Na^+] at various galactose concentrations (Fig. 5) and of 1/PD *vs.* 1/[galactose] at various Na^+ concentrations (Fig. 6), obtained with paired rat jejunal sacs incubated at 37° in $Tris^+$–bicarbonate buffers containing graded sugar concentrations and $Tris^+$ as a replacement for Na^+. Each point represents the mean of 6 experiments.

6). Replacement by $Tris^+$ (Fig. 6) resulted in a progressive increase in the apparent dissociation constant for carrier–sugar interaction from about 4 mM galactose at 120 mequiv Na^+ to about 33 mM sugar at 24 mequiv, while the maximal rate remained unchanged at 15.4 mV. Replacement by K^+ (Fig. 7) had no obvious effect on the affinity of the carrier for galactose but the maximal rate was sharply decreased from 15.6 mV at 120 mequiv Na^+ to 6.1 mV at 24 mequiv Na^+.

The effects of K^+ noted here in terms of total PD, seem to be in line with the results obtained by SCHULTZ AND ZALUSKY for the rabbit ileum[28]. A more direct comparison, however, is that based on double reciprocal plots of ΔPD and [sugar] (Figs. 8 and 9). Since ΔPD is the difference between the velocities determined in the presence (v) and absence (v_0) of sugar or Na^+, *i.e.* it is equivalent to $v-v_0$, it follows from theory that a plot of $1/\Delta PD$ *vs.* 1/[sugar] or 1/[Na^+] should extrapolate to infinity since v_0 will approach v as 1/[sugar] or 1/[Na^+] approaches zero. Therefore, such plots are not expected to yield straight lines, although these, in fact, do (Figs. 8 and 9).

In any case, with $Tris^+$ as replacement ion (Fig. 8) the curves extrapolate to intercept the ordinate at a common point representing the reciprocal of an increment in v_{max} of 7.9 mV. The apparent K_m for carrier–galactose interaction varies, in this instance, from about 14 mM at 24 mequiv Na^+ to less than 4 mM at 120 mequiv.

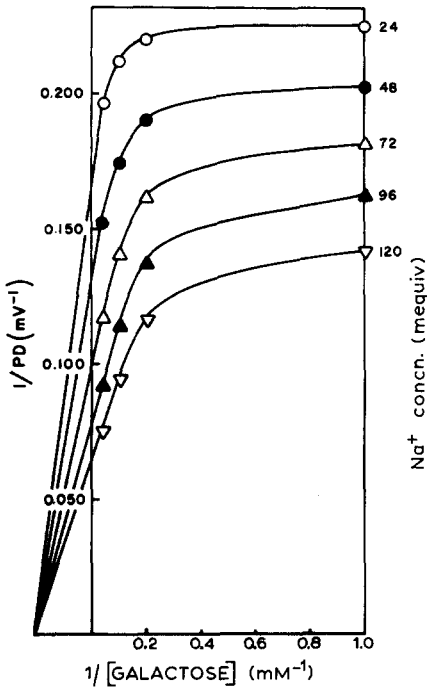
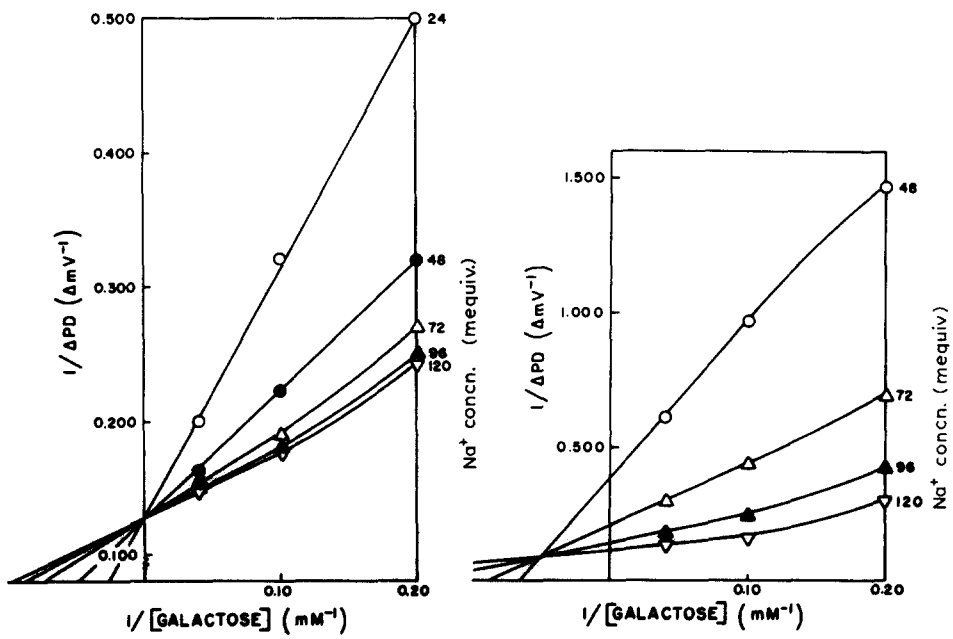


Fig. 7. Reciprocal plot of 1/PD *vs.* 1/[galactose] obtained with paired rat jejunal sacs incubated at 37° in Tris$^+$–bicarbonate buffers containing graded sugar concentrations and K^+ as a replacement for Na^+. Each point represents the mean of 6 experiments.



Figs. 8 and 9. Reciprocal plots of 1/ΔPD *vs.* 1/[galactose] obtained with paired rat ileal sacs incubated at 37° in Tris$^+$–bicarbonate buffers containing graded sugar concentrations and Tris$^+$ (Fig. 8) or K^+ (Fig. 9) as the replacement for Na^+. Each point represents the mean of 6 experiments.

On the other hand, with K^+ as replacement for Na^+ (Fig. 9), the curves do not extrapolate to a common intercept on the ordinate; there is not only a change in K_m, there is one also in Δv_{max}. The point of intersection of the curves suggests that K^+ has two effects in this system; namely, (1) to lower the maximal rate of sugar-dependent Na^+ flux (non-competitive inhibition) and (2) to depress interaction between sugar and its binding sites on the membrane carrier (competitive inhibition).

Although PD and short-circuit current values are enhanced by increases in the concentrations of Na^+ or sugar, with both rat as seen here and rabbit as found by SCHULTZ AND ZALUSKY[28], the difference between Fig. 9 and Fig. 4 of ref. 28, indicates the need to consider two different kinetic models; namely, one in which there is a high degree of interaction between the ion-binding and sugar-binding sites of the carrier—a model which appears to be appropriate for the rat; and another one in which interaction between these sites is minimal—a model which appears better to fit the rabbit data.

Kinetic analysis

The concept of a ternary interaction such as that postulated in transport systems has some novelty and for this reason requires being inspected thoroughly from many viewpoints. Thus, we are interested in developing a thorough kinetic analysis of the system and a continuing attempt is being made to evaluate the relevant parameters. The following analysis is entirely based on concepts and formulations developed by Dr. G. SEMENZA which are being prepared for publication elsewhere and which have been generously provided to us as a personal communication. The analysis is presented at some length in order that it may be readily understood.

The various presumed interactions in the system under study have been defined by the following list of equilibria, where C indicates the carrier, S the substrate (Na^+), A the non-essential activator (sugar) and I the inhibitor (K^+). The respective dissociation constants are indicated.

$$C + S \rightleftharpoons CS \xrightarrow{k_3} PD;\ I_{sc}\ (\text{"Products"}) \tag{1}$$

$$K_S = \frac{[C]\,[S]}{[CS]} \tag{1a}$$

$$C + A \rightleftharpoons CA \not\longrightarrow \tag{2}$$

$$K_A = \frac{[C]\,[A]}{[CA]} \tag{2a}$$

$$CS + A \rightleftharpoons CSA \xrightarrow{k_3'} \text{"Products"} \tag{3}$$

$$K_A' = \frac{[CS]\,[A]}{[CSA]} \tag{3a}$$

$$CA + S \rightleftharpoons CSA \xrightarrow{k_3'} \text{"Products"} \tag{4}$$

$$K_S' = \frac{[CA]\,[S]}{[CSA]} \tag{4a}$$

$$C + I \rightleftharpoons CI \xrightarrow{k_3''} \text{"Products"} \tag{5}$$

$$K_I = \frac{[C]\,[I]}{[CI]} \tag{5a}$$

$$CI + A \rightleftharpoons CIA \xrightarrow{k_3'''} \text{"Products"} \tag{6}$$

$$K_A'' = \frac{[CI]\,[A]}{[CIA]} \tag{6a}$$

$$CA + I \rightleftharpoons CIA \xrightarrow{k_3'''} \text{"Products"} \tag{7}$$

$$K_I' = \frac{[CA]\,[I]}{[CIA]} \tag{7a}$$

$$CS + I \rightleftharpoons S + CI \xrightarrow{k_3''} \text{"Products"} \tag{8}$$

$$K_I'' = \frac{[CS]\,[I]}{[CI]\,[S]} \tag{8a}$$

$$CI + S \rightleftharpoons I + CS \xrightarrow{k_3} \text{"Products"} \tag{9}$$

$$K_S'' = \frac{[CI]\,[S]}{[CS]\,[I]} \tag{9a}$$

$$S + A \not\longrightarrow \tag{10}$$

$$S + I \not\longrightarrow \tag{11}$$

$$A + I \not\longrightarrow \tag{12}$$

From Eqns. 1a–4a, the following relationships may be derived:

$$\frac{K_A}{K_A'} = \frac{K_S}{K_S'} \tag{13}$$

and from Eqns. 2a and 5a–7a, the relationship

$$\frac{K_A}{K_A''} = \frac{K_I}{K_I'} \tag{14}$$

may be obtained. Other useful relationships such as

$$\frac{K_S}{K_S''} = K_I \tag{15}$$

and

$$\frac{K_I}{K_I''} = K_S \tag{16}$$

may be readily derived from Eqns. 13 and 14 and from the remaining dissociation constants. The total amount of carrier available may be expressed as the sum of free carrier and the various bound forms of the carrier as indicated in the equilibria shown above:

$$[C]_{total} = [C] + [CS] + [CA] + [CI] + [CSA] + [CIA] \tag{17}$$

Dividing both sides of Eqn. 17 by [CSA] and substituting the appropriate terms from Eqns. 1a–5a and 7a we derive:

$$\frac{[C]_{total}}{[CSA]} = 1 + \frac{K_A}{[A]} \cdot \frac{K_S'}{[S]} + \frac{K_A'}{[A]} + \frac{K_S'}{[S]} + \frac{K_A}{[A]} \cdot \frac{[I]}{K_I} \cdot \frac{K_S'}{[S]} + \frac{K_S'}{[S]} \cdot \frac{[I]}{K_I'} \tag{18}$$

From Eqn. 18 the following reciprocal rate equation was developed:

$$\frac{1}{v} = \frac{1 + \frac{K_A'}{[A]} + \frac{K_S'}{[S]}\left\{1 + \frac{[I]}{K_I'} + \frac{K_A}{[A]}\left(1 + \frac{[I]}{K_I}\right)\right\}}{V + \Phi \cdot \frac{K_A'}{[A]} + \frac{K_S'}{[S]}\left\{F \cdot \frac{K_A}{[A]} \cdot \frac{[I]}{K_I} + \partial \cdot \frac{[I]}{K_I'}\right\}} \tag{19}$$

where

$$v = k_3[CS] + k_3'[CSA] + k_3''[CI] + k_3'''[CIA] \tag{20}$$

and the maximal velocity terms are defined as follows:

$$\Phi = k_3[C]_{total} \quad (Na^+ = \infty, K^+ = 0, \text{sugar} = 0) \tag{21}$$

$$V = k_3'[C]_{total} \quad (Na^+ = \infty, K^+ = 0, \text{sugar} = \infty) \tag{22}$$

$$F = k_3''[C]_{total} \quad (Na^+ = 0, K^+ = \infty, \text{sugar} = 0) \tag{23}$$

$$\partial = k_3'''[C]_{total} \quad (Na^+ = 0, K^+ = \infty, \text{sugar} = \infty) \tag{24}$$

from which the relationships

$$\frac{\Phi}{V} = \frac{k_3}{k_3'}, \quad \frac{F}{V} = \frac{k_3''}{k_3'} \text{ and } \frac{\partial}{V} = \frac{k_3'''}{k_3'} \tag{25}$$

may be derived.

The dissociation constants for the various interactions and the maximal rates under the conditions specified above have been evaluated by computer iterative analysis between the limits 0 and ∞ for [S], [A] and [I] and the values so obtained are presented in Table II. These are preliminary figures to indicate the degree of fit. Full computer analysis will require some months further time and final results will be reported in a subsequent publication. The values for the pairs of dissociation constants K_S—K_S' and K_A—K_A' indicate mutual allosteric activation effects of sugar on the Na^+–carrier interaction and of Na^+ on the sugar–carrier interaction[31]; the corresponding values for the K^+–carrier interaction, K_I—K_I' do not indicate any significant influence due to the presence of sugar. The value for F ($Na^+ = 0$, $K^+ = \infty$, sugar = 0) indicates that K^+ can support a considerable PD in the absence of Na^+ and sugar, a result which is clearly evident in Figs. 1 and 2. In theory, the value for ∂ ($Na^+ = 0$, $K^+ = \infty$, sugar = ∞) should approach the value for V if the effect of K^+ were solely that of an ion competing against Na^+ for the cation-binding site of the carrier. However, while K^+ appears to be competitive *vs.* Na^+ when simply compared with $Tris^+$ at the same Na^+ concentrations, the total effect of K^+ seems to be partly competitive and partly non-competitive (Fig. 9). The value of 3.24 for ∂ (Table II) probably reflects this dual effect of K^+ and such a value would indeed be expected if the K^+ effect were approx. 10% competitive and 90% non-competitive.

From the influence of sugar on the carrier–Na^+ interaction and of Na^+ on the carrier–sugar interaction, it would appear that the relationship expressed in Eqn. 13

$$\frac{K_A}{K_A'} = \frac{K_S}{K_S'}$$

holds both for the rat and for the rabbit. However, contrary to the data obtained for the rat, the rabbit data[28] indicate little or no change in the apparent dissociation constant for the interaction between carrier and sugar over a wide range of Na^+ concentrations, *i.e.* $K_A = K_A'$ and, therefore, $K_S = K_S'$. This result suggests little or no

TABLE II

KINETIC PARAMETERS FOR EQUILIBRIA DESCRIBING INTERACTIONS OF SUBSTRATE (Na^+), NON-ESSENTIAL ACTIVATOR (SUGAR) AND INHIBITOR (K^+) WITH THE CARRIER

Parameter	*Graphical analysis**	*Computer analysis***
	(1)	(2)
Dissociation constants		
Substrate interactions		
K_S	211.0 mequiv	435.0 mequiv
K_S'	1.50 mequiv	1.00 mequiv
K_S''	4.44 mequiv	14.8 mequiv
Activator interactions		
K_A	526.0 mM	655.0 mM
K_A'	1.55 mM	1.50 mM
K_A''	620.0 mM	648.0 mM
Inhibitor interactions		
K_I	47.5 mequiv	29.4 mequiv
K_I'	56.0 mequiv	29.0 mequiv
K_I''	0.22 mequiv	0.07 mequiv
Maximal velocities		
V	16.0 mV	17.1 mV
Φ	16.0 mV	19.7 mV
F	?	32.1 mV
∂	?	3.24 mV
*Rate constants****		
k_3'	—	1.00 t^{-1}
k_3	—	1.15 t^{-1}
k_3''	—	1.87 t^{-1}
k_3'''	—	0.19 t^{-1}

* The values in Column 1 include estimates from data presented in ref. 31. K_I and K_I' were estimated by Dr. G. SEMENZA from data obtained in kinetic studies of intestinal sucrase, an enzyme which is Na^+-activated and inhibited by K^+ (*cf.* ref. 37). K_S'', K_A'' and K_I'' were calculated using Eqns. 14–16. With the reciprocal rate equation (Eqn. 19) the data in Column 1 yield the experimentally determined values of $1/v$.

** The values in Column 2 were determined by Dr. W. BEST through iterative analysis on a computer programmed according to Eqn. 19 and for experimental values of $1/v$ obtained with graded concentrations of Na^+, sugar and K^+. K_S, K_S'', K_A'' and K_I'' were calculated using Eqns. 13–16. With the data in Column 2 Eqn. 19 yielded the experimentally determined values for $1/v$.

*** The values for the rate constants were calculated from the relationships expressed in Eqn. 25 and the value for k_3' was arbitrarily set at 1.00.

allosteric interaction between the cation-binding and sugar-binding sites. In the rat $K_A \neq K_A'$ and, therefore, $K_S \neq K_S'$ (Table II) which suggests, as has been previously pointed out[31], a significant degree of interaction between the cation-binding and sugar-binding sites. Because of this apparent mutual allosteric activation effect in the rat, the inhibitory influence of K^+ on the apparent dissociation constants for Na^+ (K_S and K_S') and for sugar (K_A and K_A') cannot be expected to yield kinetic parameters which are equivalent quantitatively to those obtained in the absence of Na^+. The kinetic model suggested for the rat may represent the more general case while that suggested for the rabbit may constitute a simpler one.

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