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THE ROLE OF Na AS A DETERMINANT OF THE ASYMMETRIC PERMEABILITY OF RABBIT ILEAL BRUSH-BORDER TO D-GALACTOSE

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SUMMARY

1. Raising Ringer [Na] from 0–140 mequiv causes a significant rise in [Na] within rabbit ileal tissue fluid, with 0.1 mM ouabain present the tissue [Na] is raised still further.

2. There is a Na-dependent increase in the calculated entry permeability of the brush-border to galactose and the calculated exit permeability of the brush-border to galactose falls as the tissue [Na] is increased. The maximal permeability ratio of the brush-border to galactose exceeds the inverse Na distribution ratio between tissue water and Ringer by 20-fold.

3. Ouabain both reduces the Na-dependent increase in entry permeability and reduces the Na-dependent decrease in exit permeability of the brush-border to galactose. A model explaining the observed asymmetric permeability of the brush-border to galactose in terms of convective–diffusion flow is proposed. By resolving galactose flux across the brush-border into separate convective and diffusive velocity components (see Appendix), it is shown that both velocity components are increased by increasing Ringer [Na]. Addition of ouabain to the Ringer reduces the convective component to zero without affecting the diffusive component, hence it is deduced that the Na pump activity provides the force required to produce mass flow across the brush-border. As brush-border [galactose] is increased, the convective velocity decreases more than the diffusive velocity. This result is consistent with the view that the permeability asymmetry of the brush-border to galactose is the result of mass flow via narrow channels.

INTRODUCTION

It is well established that there is a linkage between Na and active transport of organic solutes in the small intestine [1, 2]. However, the nature of this linkage is uncertain. The following findings are evidence in favour of the Na gradient hypothesis: there is a 1 : 1 stoichiometric relationship between the sugar-dependent influx of Na and Na-dependent influx of sugars across the brush-border [3]; reversal of the normal Na gradient in ouabain treated tissue accelerates alanine exit [4]; high concentrations

of intracellular alanine and sugars accelerate Na exit [5, 6] and the effects of inhibitors of Na transport on sugar influx into rabbit ileal brush-border may be satisfactorily explained as consequences of the changes in Na gradient across the brush-border [7].

However, studies with isolated chick intestinal cells do not support the Na gradient hypothesis [8]. With this cell preparation Kimmich found that reversal of the normal Na gradient did not prevent net accumulation of D-galactose, and with ouabain present and ion gradients which would be expected to favour sugar accumulation, no accumulation was observed. Because of the heterogeneity and loss of polarity of the chick cell preparation, doubts have been expressed as to the validity of Kimmich's conclusions [9] and it is also difficult to accept his alternative model suggesting that the sugar pump is linked directly to the Na pump via an energised intermediate, since brush-border ATPase activity is not present [10]. With Ehrlich ascites tumour cells several studies have indicated that the total energy available from the electrochemical potential gradients across the cell membrane is insufficient to account for the steady state distribution ratio of certain amino acids [11], but recent studies have shown that there may, in fact be sufficient energy available from the transmembrane Na gradient to support the observed distribution ratio of 2-amino-isobutyric acid between cells and medium [12].

In vivo studies in human, dog and rat small intestines have revealed that total replacement of luminal Na by mannitol does not effect the rate of active sugar absorption in these tissues [13]. However, it has been suggested that unstirred layer or surface charge effects may allow sufficient Na to be recirculated through the cells following passage through the lateral intercellular spaces to maintain the Na-dependent activation of sugar transport across the brush-border [9].

Thus it can be seen that all the data challenging the Na gradient hypothesis is considered inconclusive. Because sugars are accumulated within the cell fluid as a result of the operational permeability asymmetry of the brush-border to sugars, interpretation of studies relying solely on fluxes in a single direction is difficult. The observed linked effects may result from symmetrical changes in membrane permeability, or alternatively the extent of the induced asymmetry may be insufficient to give the observed levels of solute accumulation within the tissue. By measuring the bidirectional brush-border sugar permeabilities simultaneously, together with the solute distribution ratio between the intracellular and extracellular water some of the ambiguity is reduced.

A method by which the bidirectional fluxes of a dual labelled solute can be estimated simultaneously across both the mucosal and serosal boundaries of strips of rabbit ileum has been described [14], when combined with measurements of the tissue solute concentrations, it is possible to estimate the bidirectional permeabilities of the tissue boundaries to the labelled solutes. It was shown using this method that replacement of Ringer Na by choline increases the exit permeability of the brush-border to D-galactose. Lowering Ringer Na reduces intracellular [Na] and hence should, according to the predictions of the Na gradient hypothesis, decrease the exit permeability [1]. This result although inconsistent with the Na gradient hypothesis may shed some light on the mechanism of Na-linked activation of cellular accumulation of sugars, hence it was decided to further investigate this effect.

METHODS

It was shown [14] that the unidirectional fluxes of galactose across mucosal and serosal borders of strips of rabbit ileum can be estimated from groupings of two or three independently measured variables; the mucosal–serosal flux J_{13}, the serosal to mucosal flux J_{31}, and the ratio R of the specific activity of radioisotope within the tissue Compartment 2.

When the concentrations of D-galactose in the mucosal and serosal compartments are identical:

$$R = \frac{(\text{cpm})_2^T}{(\text{cpm})_2^C} \times \frac{(\text{cpm/ml})_3^C}{(\text{cpm/ml})_1^T}$$

subcripts 1, 2, 3 refer to the mucosal, cell and serosal compartments, respectively; superscripts T and C refer to ^{3}H- and ^{14}C-labelled D-galactose, respectively. J_{ij} refers to flux from compartment i to j and $P_{ij} = J_{ij}/C_i$; c_i is the concentration of sugar in compartment i. By making the following three assumptions: (1) that the tissue behaves kinetically as a single compartment towards galactose entering it from both mucosal and serosal solutions, i.e. there is no significant unstirred layer effect or compartmentalization within the tissue, or shunt permeability to sugar; (2) that the estimates of the measured variables, the bidirectional transmural fluxes, the tissue galactose accumulation ratio (distribution ratio) = [galactose/ml tissue water]/[galactose/ml Ringer] and the specific activity ratio, R are in steady state, i.e. that the net flux across the whole tissue is equal to the net flux of sugar across the mucosal and serosal boundaries; (3) that the tissue does not metabolise either ^{3}H- or ^{14}C-labelled galactose to any significant extent.

The latter two assumptions have been verified. The bidirectional fluxes reach steady state within 20 min following addition of radio-isotope to the mucosal and serosal solutions and no significant fraction of radio-isotope extracted from the tissue migrates with any other material, except D-galactose on paper chromatography [14].

Because of the low tissue permeability to D-galactose it is unlikely that a significant unstirred layer effect exists external to the tissue, the chambers on either side of the tissue are stirred vigorously by gassing to prevent reflux of radio-isotope and the specific activity levels in the unlabelled sides do not exceed 5 % of the labelled side specific activity during the time course of the experiment. Also, when Ringer galactose is less than 5 mM, the serosal–mucosal flux is approximately 1 % of the mucosal–serosal flux in fully active tissue, hence it can be deduced that the shunt permeability to galactose is insignificant.

The first assumption that the tissue behaves kinetically as a single compartment is difficult to verify; the assumption implies that the specific activity ratio R of [^{3}H]galactose/[^{14}C]galactose is homogeneously distributed throughout the entire tissue. An important check on the accuracy of the method of calculating unidirectional fluxes is the comparison of the measured ratio of mucosal–serosal/serosal–mucosal galactose permeability with the calculated ratio, mucosal–cell/cell–mucosal galactose permeability ratio (Fig. 5). Since it is known that almost the entire tissue asymmetry results from asymmetry generated at the brush-border, [14, 15] the measured transmural permeability ratio should be approximately the same as the calculated permeability ratio to galactose entry and exit across the brush-border. As will be shown in Results,

the observed and calculated permeability ratios are similar, hence the assumption that the tissue behaves as a single compartment is partially vindicated. Furthermore, because the method of estimating unidirectional fluxes across the mucosal and serosal borders depends on combinations of two or three independently measured variables, any error resulting from inhomogeneous distribution of isotope within the tissue will propogate a smaller error in the estimates of unidirectional flux [14].

It was previously shown (14) that:

$$J_{12} = J_{31} \cdot R + J_{13}$$

$$J_{21} = J_{31}(1 + R)$$

$$J_{23} = J_{31}(1 + 1/R)$$

$$J_{32} = J_{31} + J_{13}/R.$$

Estimation of cell Na and K. Na and K are estimated from the same 0.1 M HNO_3 extracts as are used to obtain the ratio R and the tissue concentration of galactose. The cell water is assumed to be 0.7 of the total tissue water which is estimated from the weight difference between wet and dry tissue. The tissue weight is estimated to 0.1 mg. The assumption of a 30 % extracellular fluid volume was checked by measuring the inulin space in a few tissues. Because the tissue is washed in ice-cold isotonic choline chloride and blotted to remove extracellular radio-isotope, the estimates of both cell Na and K levels are probably underestimates of the true intracellular cation levels at the end of incubation.

The methods to determine flux used in this paper are similar to those described previously [14], except for two minor improvements which do not materially affect the results.

Flux chambers. The flux chambers were designed to measure bidirectional transmural fluxes across six adjacent pieces of intestine. The temperature of the solutions within the chambers is maintained constant by forced circulation of thermostated water through conduits cut into the lucite block (see Diagram 1). Using

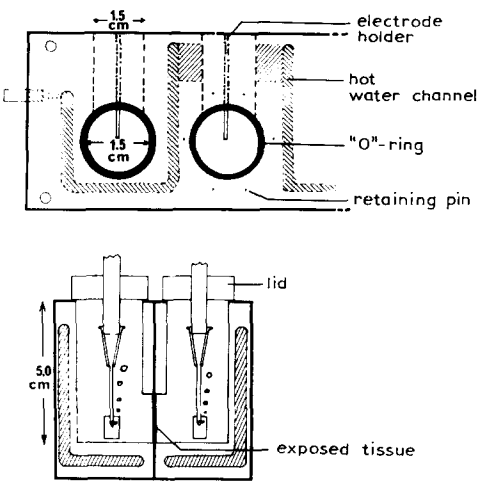

Diagram 1

this device the temperature of solutions within the block can be readily maintained at 37±0.2 °C over a prolonged time period. Gassing and mixing within each chamber is achieved by continuous supply of O_2–CO_2 (95 : 5, v/v) bubbled directly into the chambers. The end of the gas lead is surrounded with a cuff of plastic tubing (0.5 cm diameter) which forms a chimney for the gas bubbles which cause a fluid jet to circulate continuously whilst the tissue is protected from sparge damage. The mixing time within the chambers was observed to be approximately 2 s.

Flux measurement. ^{3}H- and ^{14}C-labelled D-galactose obtained from Radio Chemical Centre Amersham are added to the mucosal and serosal solutions, respectively, in quantities sufficient to give at least 1500 cpm/ml solution count increments/30 min of both labels in the contralateral chambers.

The initial volume of Ringer in each chamber is 7 ml. 1 ml samples are taken from each chamber 20 min, 50 min and 80 min following addition of radio-isotopes. Flux, μmoles · min^{-1} · cm^{-2} area of exposed membrane is calculated from the increment in corrected counts averaged over the last hour. Extraction of the isotopes from the tissue at the end of the flux period is as previously described [14].

Counting radio-isotopes. Samples are counted using standard double label techniques in a Packard Tricarb liquid scintillation counter. The external standard was previously calibrated with samples containing known amounts of single labelled isotopes quenched in chloroform.

Scintillation fluid. Formula as recommended by Fox, 500 ml toluene; 500 ml Triton X-100; 3.5G, 2,5-diphenyloxazole [16].

Statistics. All the experiments described in this paper were designed to test the effects of six levels of [Na] on measured variables in six adjacent pieces of rabbit ileum simultaneously. Usually two sets of six were tested together so that the effect of a single other factor could be determined, e.g. [ouabain] or [galactose].

The statistical significance of the effects of the factors on the determined variables can be tested by analysis of variance using an unweighted means solution with repeated measures of a single factor, i.e. [Na]. The statistical significance levels of the calculated F ratios were obtained from Fisher and Yates F tables [17]. The calculations were all made using the Wang Laboratories Incorp. 700 series software (Package 9A).

Animals. Male New Zealand white rabbits weighing 2–3 kg each were fed normally. They were killed by intravenous injection of Nembutal.

RESULTS

The effects of equimolar replacement of Ringer Na by choline on the measured bidirectional transmural fluxes of 2 mM galactose, the tissue galactose concentrations and the specific activity ratio R of ^{3}H-labelled galactose: ^{14}C-labelled galactose within the tissue

The effects of equimolar replacement of Ringer Na by choline on the flux of ^{3}H-labelled galactose from mucosal–serosal solution J_{13} and of ^{14}C-labelled galactose from serosal–mucosal solution J_{31} are shown in Fig. 1. The fluxes are plotted logarithmically to illustrate the relative changes in transmural fluxes in both directions. J_{13} the mucosal–serosal galactose flux increases hyperbolically, the K_t for Na-dependent activation of J_{13} is 25±5 mequiv Na. Increasing Ringer [Na] from zero decreases

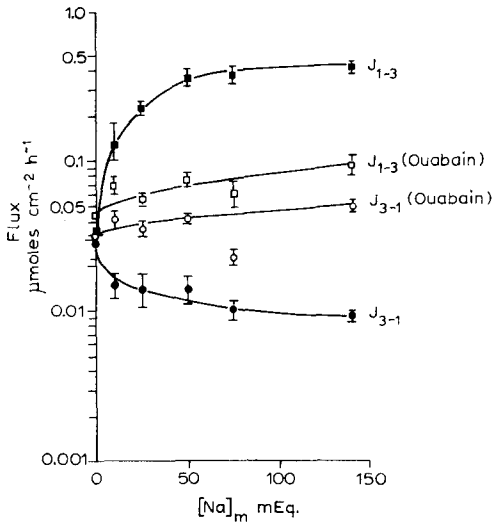

Fig. 1. Effects of variation of Ringer [Na] on transmural galactose fluxes J_{13} and J_{21} in the presence and absence of 0.1 mM ouabain. Note that the ordinate is logarithmic. Ringer [galactose] = 2 mM. I, S.E. values of seven control experiments and of five experiments with ouabain, six levels of Ringer [Na] per experiment; ○, flux J_{31}; □, flux J_{13}; open symbols, +0.1 mM ouabain; closed symbols, controls. Lines drawn by eye.

the serosal–mucosal galactose flux ($P < 0.001$). Fig. 1 shows that the fall in J_{31} reciprocates with the rise in J_{13}. With 0.1 mM ouabain added to the Ringer, both the Na-dependent increase in J_{13} and the Na-dependent decrease in J_{31} are reduced ($P < 0.001$ for both effects).

Thus 0.1 mM ouabain reduces the flux asymmetry of galactose ($P < 0.001$) and virtually abolishes net galactose absorption ($P < 0.001$) (Figs 2 and 5).

Fig. 3 shows the effects of variation of Ringer [Na] on the specific activity ratio R of [^{3}H]galactose/[^{14}C] galactose in the tissue water and also the total con-

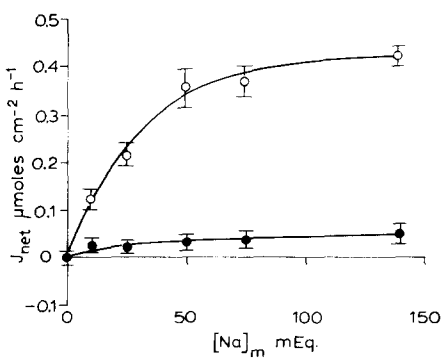

Fig. 2. Effect of varaition of Ringer [Na] on net galactose flux across rabbit ileum. Closed symbols, +0.1 mM ouabain; open symbols, controls; I, S.E. values of seven control experiments and of five experiments with ouabain. Lines drawn by eye.

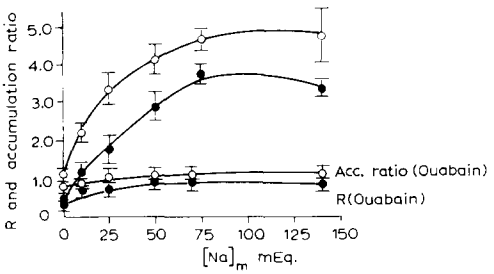

Fig. 3. Effect of variation of Ringer [Na] on tissue accumulation ratio of galactose with Ringer [galactose] = 2 mM (○—○); ^3H: ^{14}C specific activity ratios (●—●); I, S.E. values of seven control experiments (upper two lines); and 5 experiments with 0.1 mM ouabain present (lower two lines).

centration of galactose within the cells at the end of the 90 min incubation period. The tissue galactose concentration is plotted as an accumulation ratio = [galactose cells]/[galactose Ringer].

Raising Ringer [Na] results in a hyperbolic increase in both functions. The Ringer [Na] giving half maximal increments is approximately 25 mequiv for both functions. With 0.1 mM ouabain added to the Ringer the Na-dependent increase in galactose accumulation and in the ratio R is almost abolished ($P < 0.001$ for both effects), as was shown previously [14].

The effects of variation of Ringer Na on the calculated entry and exit permeabilities of the brush-border to galactose

The data shown in Figs 1–3 can be utilized to calculate the bidirectional fluxes of galactose across both the mucosal and serosal boundaries of the tissue. Since it is not possible to control the tissue galactose concentration exactly, the permeability ratio P_{ij}/P_{ji}, where $P_{ij} = J_{ij}/c_i$, is a better index of membrane asymmetry than the flux ratio J_{ij}/J_{ji}. However the estimate of the exit permeability of galactose across the brush-border P_{21} is liable to error in flux measurement and also in the tissue sugar concentration. As the latter parameter is particularly liable to be underestimated, with the washing procedure employed, the calculated exit permeability of galactose is likely to be an overestimate of the true permeability.

In Fig. 4 the entry and exit permeabilities of the brush-border to galactose are plotted logarithmically as functions of Ringer [Na]. Raising Ringer [Na] from 0–140 mequiv has the following effects on the unidirectional permeabilities: the mean entry permeability P_{12} is increased from 0.02–0.12 cm · h^{-1} ($P < 0.001$). The Ringer [Na] giving half maximal activation of P_{12} is 23±2.5 mequiv Na. The permeability of the brush-border to galactose exit from the tissue to the mucosal solution P_{21} is decreased from 0.02—0.004 cm · h^{-1} ($P < 0.001$). With 0.1 mM ouabain added to the Ringer, the Na-dependent increase in P_{12} is reduced ($P < 0.001$) P_{12} increases from 0.02—0.035 cm · h^{-1} ($P < 0.001$) there is also a small increase in the exit permeability P_{21} as Ringer [Na] is raised from 0–140 mequiv, thus ouabain reverses the effects of Na on the exit permeability of the brush-border to galactose. A similar Na-dependent rise in L-alanine efflux across the brush-border of rabbit ileum with ouabain present has been directly observed [4].

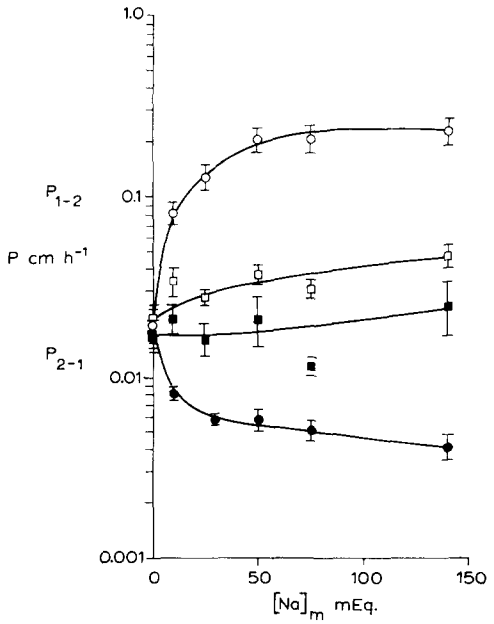

Fig. 4. Effects of variation of Ringer [Na] on calculated entry and exit permeability of galactose across the brush-border. Results were computed from the data shown in Figs 2, 3, and 4. Ringer [galactose] = 2 mM. I, S.E. values of the permeabilities, individual results were calculated separately. Seven control experiments (○); five experiments: +0.1 mM ouabain (□); entry permeabilities P_{12} (○); exit permeabilities P_{21} (■). The lines were drawn by eye.

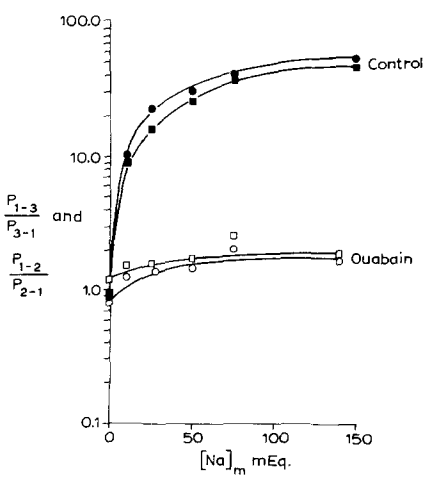

Fig. 5. Effects of variation of Ringer [Na] on the permeability ratio of galactose across the whole tissue P_{13}/P_{31} (□); and across the brush-border P_{12}/P_{21} (○). Points shown are the mean values of seven control experiments (closed symbols); and five experiments with ouabain (open symbols).

The ratio of galactose entry: exit permeability P_{12}/P_{21} and the ratio of the bidirectional transmural permeability P_{13}/P_{31} are plotted logarithmically as functions of Ringer Na (Fig. 5). As Ringer [Na] is increased from 0–140 mequiv the galactose permeability ratio across the brush-border and across the whole tissue rise together from approximately unity to about 60. With 0.1 mM ouabain present raising Ringer [Na] has little effect on either ratio.

The effects of varying concentrations of galactose on the brush-border Na-dependent galactose entry and exit permeabilities

The comparative effects of varying Ringer [Na] from 0–140 mequiv on the bidirectional permeabilities of the brush-border to galactose, with Ringer [galactose] held at either 0.2 or 20 mM are shown in Fig. 6. The primary data from which the permeabilities are calculated are similar to those in Figs 1–3 and are not included here.

The Na-dependent rise in P_{12} is less when Ringer [galactose] is 20 mM than with Ringer galactose held at 0.2 mM ($P < 0.001$). Furthermore, the Na-dependent decrease in galactose exit permeability is less with Ringer [galactose] 20 mM than with Ringer [galactose] 0.2 mM ($P < 0.001$). However, although smaller, the Na-dependent increase and decrease in P_{12} and P_{21} with Ringer [galactose] 20 mM are still significant ($P < 0.001$ for both effects).

The Na concentration giving half maximal activation of galactose entry permeability with Ringer galactose 0.2 mM is less than with Ringer [galactose] 20 mM; the K_t values are 18.9 ± 6.9 and 101 ± 16 mequiv, respectively. The K_t for Na-dependent activation of galactose entry permeability with Ringer [galactose] held at 20 mM is similar to the K_t observed for Na-dependent activation of 20 mM 3-*O*-methylglucose influx into rabbit ileum as measured directly [3].

These last experiments were undertaken primarily to determine whether the

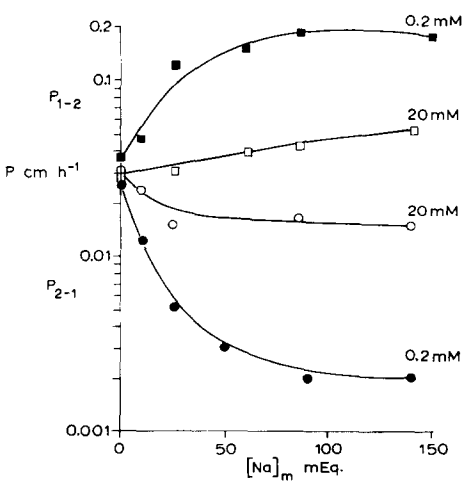

Fig. 6. Effect of variation of Ringer [Na] on exit permeability P_{21} (○) and entry permeability P_{12} (□); with Ringer [galactose] = 0.2 mM (closed symbols); and Ringer [galactose] = 20 mM (open symbols). Three experiments performed with each concentration, points are mean values at each level of Na.

fall in exit permeability is due to a saturation effect at the inner surface of the brush-border, as a result of active sugar accumulation. Because no substantial change in the brush-border permeability to galactose is seen in the concentration range 0.2–2.0 mM and because the tissue accumulation ratio is never observed to exceed 7, the experiment indicates that the Na-dependent decrease in exit permeability of the brush-border to galactose is not due to saturation at the inner surface of the membrane.

The effects of replacement of Ringer Na by choline on the tissue levels of Na and K (Ringer [galactose] is 2 mM)

The effects of varying Ringer [Na], ([galactose] is 2 mM) on the tissue levels of Na and K following incubation in the flux chambers then removal of the extracellular Na by washing in ice-cold choline are shown in Fig. 7.

The following effects are noted: with Ringer [Na] $>$ 25 mequiv, addition of 0.1 mM ouabain allows Na to accumulate within the tissue to a significantly higher level than in controls ($P < 0.001$), also tissue K loss is increased ($P < 0.025$). However, because of the prolonged incubation followed by the washing procedure, the observed levels of tissue K are less than those obtained by Knoopman and Schultz [18]. With Ringer [Na] $<$ 25 mequiv, no significant effect of ouabain is observed on either tissue Na or K. As expected, raising Ringer [Na] for 0–140 mequiv results in a significant increase in the tissue [Na], both with or without ouabain added to the Ringer ($P < 0.001$).

Finally the distribution ratio of tissue [Na]: Ringer [Na] falls from unity as Ringer [Na] is raised from 10 mequiv, to 0.3 at Ringer [Na] is 140 mequiv. With ouabain present the Na distribution ratio, is increased, but remains below unity.

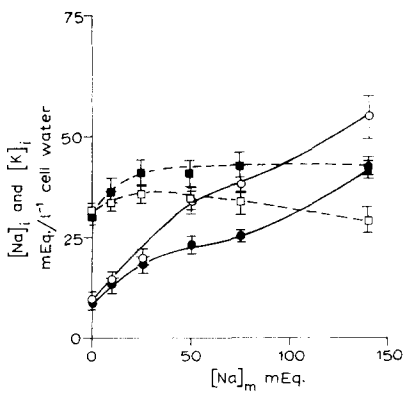

Fig. 7. Effects of variation of Ringer [Na] on intracellular K (□); Na (○); +0.1 ouabain (open symbols); controls (closed symbols). I. S.E. values of nine control experiments and of five experiments with ouabain present. Each experiment involves measurement at six levels of Ringer Na. Lines were drawn by eye.

DISCUSSION

The following relationships derived from Results are consistent with the view that the dissipation of the Na gradient across the brush-border provides the kinetic energy

necessary for sugar accumulation within the tissues. (1) Increasing Ringer[Na] increases the entry permeability of the brush-border to galactose; (2) the accumulation of galactose in control tissue is inversely proportional to the Na distribution ratio between tissue and Ringer; (3) addition of 0.1 mM ouabain to Na Ringer increases tissue [Na] and also exit permeability of the brush-border to galactose. Also (4), with ouabain added to Na Ringer, the steady-state accumulation ratio of galactose is decreased. In the experiments reported here, however, the effect of ouabain on galactose accumulation is greater than on the Na distribution ratio. As previously explained, the tissue ion levels may be underestimates of the true levels, hence this result cannot be considered, by itself, as firm evidence either in favour or against the Na gradient hypothesis.

The following results differ from the predictions of the Na gradient hypothesis and suggest that the previous catalogue gives only specious support to this hypothesis: (1) the exit permeability of the brush-border to galactose decreases as cell [Na] increases over the range 10–60 mequiv/l cell water, this effect is observed over a wide range of tissue [galactose] 0.2–60 mM, and hence is unlikely to be caused by saturation of the inner surface of the brush-border by accumulated sugar.

This relationship between tissue [Na] and galactose exit permeability as previously reported [14] is directly contrary to the Na gradient hypothesis, which predicts that the exit permeability should increase as the tissue [Na] increases. It was suggested previously [14] that an explanation for the Na-dependent decrease in the observed galactose permeability, might be that the exiting sugar is recaptured from an extracellular space. The rate of recapture is assumed to increase as [Na] within the extracellular space is raised. This hypothesis may now be rejected for the following reasons: the Na-dependent decrease in exit permeability is observed with Ringer [galactose] 20 mM, albeit at a reduced level. Competition between the exiting ^{14}C-labelled galactose coming from the serosal solution and ^{3}H-labelled galactose from the mucosal solution would be expected to reduce the rate of recapture to negligible proportions; secondly, no Na-dependent decrease in ^{14}C-labelled galactose exit is observed when 0.1 mM ouabain is present in the Ringer as would be expected if recapture were simply a function of the extracellular Na.

(2) The Na gradient hypothesis predicts that brush-border permeability ratio P_{12}/P_{21} to galactose should never exceed the inverse Na distribution ratio Na_{cell}/Na_{Ringer} yet the observed permeability ratio can exceed the observed inverse Na distribution ratio by 20-fold. Even allowing for a 50 % error in the tissue [Na], it is clear that the brush-border permeability asymmetry to sugars cannot be entirely ascribed to the asymmetric distribution of Na between the Ringer and tissue fluid. And hence this effect is a second divergence from the predictions of the Na gradient hypothesis.

A third relationship which deviates from the predictions of the Na gradient hypothesis is that ouabain, when added to Na Ringer containing low galactose concentrations ($\leqslant$ 5mM) inhibits galactose flux by up to 80 %. At higher galactose concentrations the ouabain sensitive component of galactose influx falls. If it is assumed that a mobile Na–sugar carrier can be distributed between the inside and outside of the brush-border according to the ratio of the [Na] at either surface, then with total equilibration of Na and sugar across the brush-border galactose influx should be inhibited by no more than 50 % of the maximal influx. Goldner et al. [7] found that ouabain reduced influx of 20 mM 3-*O*-methylglucose into rabbit ileum only

by 50 %. Since their results were obtained by direct measurement, whereas, the influxes reported in this paper are measured indirectly, no firm conclusion as to the validity of the Na gradient hypothesis can be based solely on the effects of ouabain on sugar influx. However, there are two other discrepancies between the observed and predicted effects, too large to be ascribed to error or artifact, which lend weight to the conclusion that the Na gradient hypothesis is insufficient to explain all the data.

An alternative model for Na-dependent asymmetric sugar transport across the brush-border

The reciprocal rise and fall of galactose entry and exit permeability across the brush-border as the tissue Na pump is activated indicates that a vector force acts across the brush-border causing mass flow across it.

Kedem and Katchalsky [19] showed that solute flux across a membrane may be described as follows:

$$J_i = P_i(c_1 - c_2) + J_v(1-\sigma)\bar{c} \tag{1}$$

where J_i = net solute flux; P_i = membrane permeability = ωRT; ω = the solute measured at zero volume flow = J_v across the membrane; R = the gas constant; T the absolute temperature; σ = the solute reflection coefficient; $\bar{c}$ = the mean membrane concentration of solute i; c_1 and c_2 are the solute concentrations in compartments 1 and 2, respectively. If the convective and diffusive components of solute flow are confluent, and J_v is directed from Compartment 1 to Compartment 2, it follows, provided that $\sigma < 1$, that solute flux will be increased in the direction of the convective flow and retarded, when in the opposite direction to the mass flow.

In the Appendix it is shown that the Peclet number (Pe), the dimensionless ratio of solute convective velocity : diffusive velocity within the membrane flow channels can be determined from the bidirectional permeabilities across the brush-border as follows:

$$Pe = J_v \frac{(1-\sigma_i)}{P_i} = \ln \frac{(P_{12})}{-(P_{21})} \tag{2}$$

The Peclet number is zero when convective flow is zero, i.e. $P_{12} = -P_{21}$; $Pe < 0$ when $P_{12} < -P_{21}$ and $Pe > 0$ when $P_{12} > -P_{21}$ (note $-$ve sign for P_{21} because it has the opposite direction to J_v)

The relationships between net flux and the unidirectional permeabilities outlined above, provide a frame within which asymmetric permeability of the brush-border to galactose may be further examined.

The equation of Kedem and Katchalsky (Eqn 1) suggests that within limits the steady-state net solute flux will increase linearly as the volume flow J_v is increased from zero, (the limits are that $\bar{c}$, c_1, c_2, P_i and σ, remain unchanged as J_v is increased). It follows that the Peclet number will increase as J_i increases, and furthermore, the unidirectional coefficients in and opposing the direction of mass flow will increase and decrease logarithmically as the Peclet number is raised (see Appendix). The unidirectional permeability ratios of galactose across the brush-border are plotted logarithmically as functions of the steady state net galactose flux (Ringer [galactose] 2 mM) (Fig. 8).

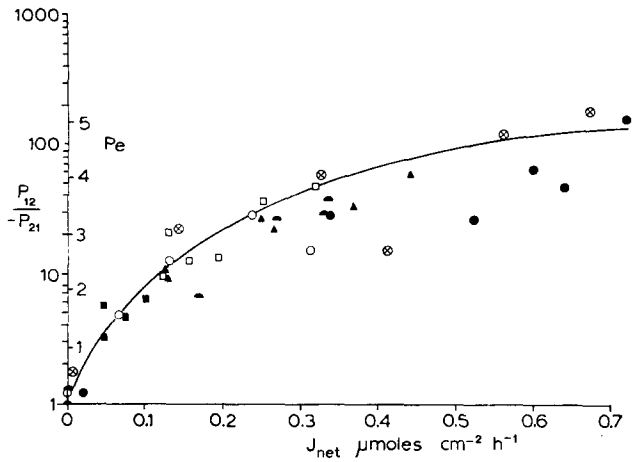

Fig. 8. Plot of calculated permeability ratio of galactose across the brush-border of rabbit ileum versus net galactose flux across the same tissue. Ringer [galactose] = 2 mM. Seven control experiments shown by individual symbols. The independent variable not shown is Ringer [Na]. Note that the permeability ratio scale is logarithmic. A linear Peclet number scale is also shown on the ordinate. The line is drawn by eye.

The ordinate also indicates the Peclet number scale. The net galactose flux is varied by altering the Ringer [Na]. As net flux increases, the Peclet number increases continuously ($P < 0.001$). When net flux exceeds 0.1 μmole cm$^{-2} \cdot$ h^{-1} the relationship becomes markedly convex (see below). At the highest levels of net galactose flux, the Peclet number is seen to exceed 5, i.e. the convective velocity of galactose within the brush-border is five times higher than the diffusive velocity.

The normalised entry and exit permeabilities are shown plotted as logarithmic functions of the Peclet number of galactose across the brush-border (Fig. 9); again the Peclet number is varied by changing Ringer [Na]. The figure shows the linear regression lines through the data, both lines have slopes which differ significantly from zero ($P < 0.001$ for both effects); the entry permeability increases and the exit permeability decreases as the Peclet number is increased. This result, like those already shown in Figs 4 and 6, illustrates the correlation between the rise and fall of entry and exit permeability and supports the view that asymmetric sugar flux across the brush-border results from a convective flow of solute across this membrane.

Without further assumptions being made, convective–diffusion flux of sugar across the brush-border may be resolved into its separate velocity components given the following data; net flux, the bidirectional fluxes and the sugar concentrations on both sides of the brush-border (see Appendix).

The changes in convective and diffusive velocity of galactose across the brush-borders of control tissue resulting from variation of the Ringer [Na] or Ringer [galactose] are shown in Figs 10a and 10b and Figs 11a and 11b.

Raising Ringer [Na] from 0–140 mequiv with Ringer [galactose] held at either 2 or 20 mM results in a convex functional relationship between V = the convective velocity of galactose cm $\cdot$ h^{-1} and Ringer [Na]. The K_t for the Na-dependent activation of V is about 25 mequiv at both galactose concentrations. Since addition of ouabain to, or alternatively, complete removal of Na from, the Ringer reduces net flux and

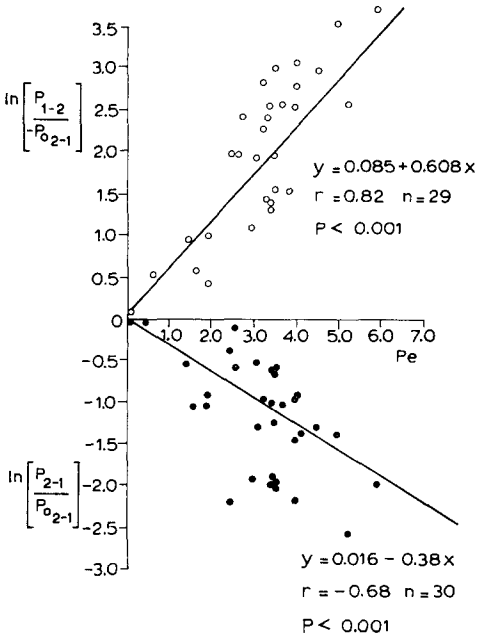

Fig. 9. Plot of the normalised natural logarithm of entry permeabilities (○) and exit permeabilities (●) versus the Peclet number *Pe* of galactose movement across the brush-border of rabbit ileum. The figure illustrates the combined results of six experiments Ringer [galactose] = 2.0 mM. The independent variable is Ringer [Na]. The lines are linear regression lines. r = correlation coefficient. $-P_{O_{2-1}}$ is exit permeability in Choline Ringer.

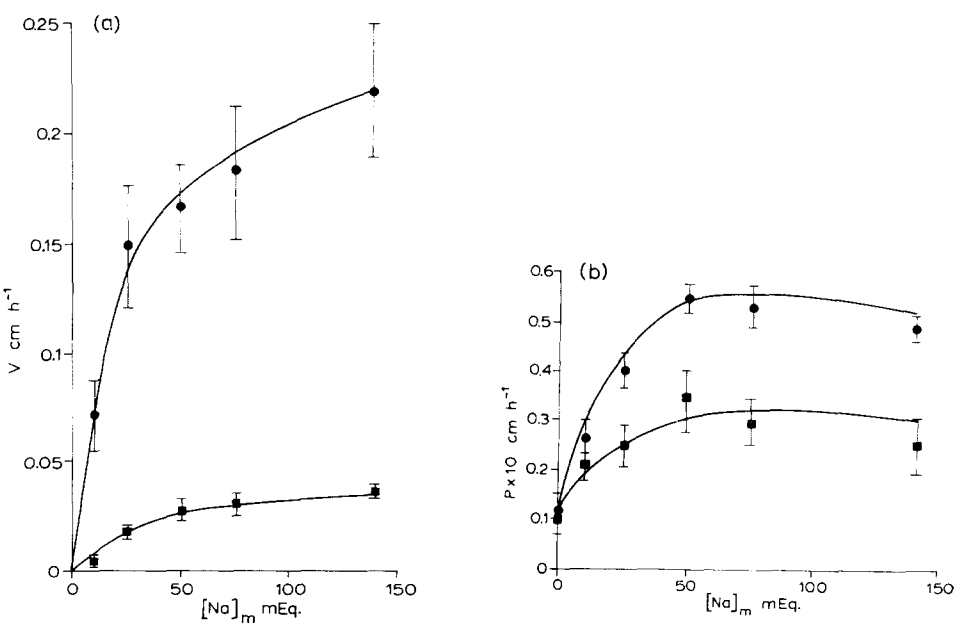

Fig. 10. (a) Effect of variation of Ringer [Na] on calculated convective velocity of galactose across the brush-border when present in the Ringer at 2.0 mM (●); I, S.E. values for seven experiments; with Ringer [galactose] = 20 mM (■); I, S.E. values for three experiments. (b) Effect of variation of Ringer [Na] on the calculated diffusive permeability of the brush-border to galactose present in the Ringer at 2.0 mM (●); I, S.E. values of seven experiments, also with Ringer [galactose] = 20 mM (■); I, S.E. values of three experiments.

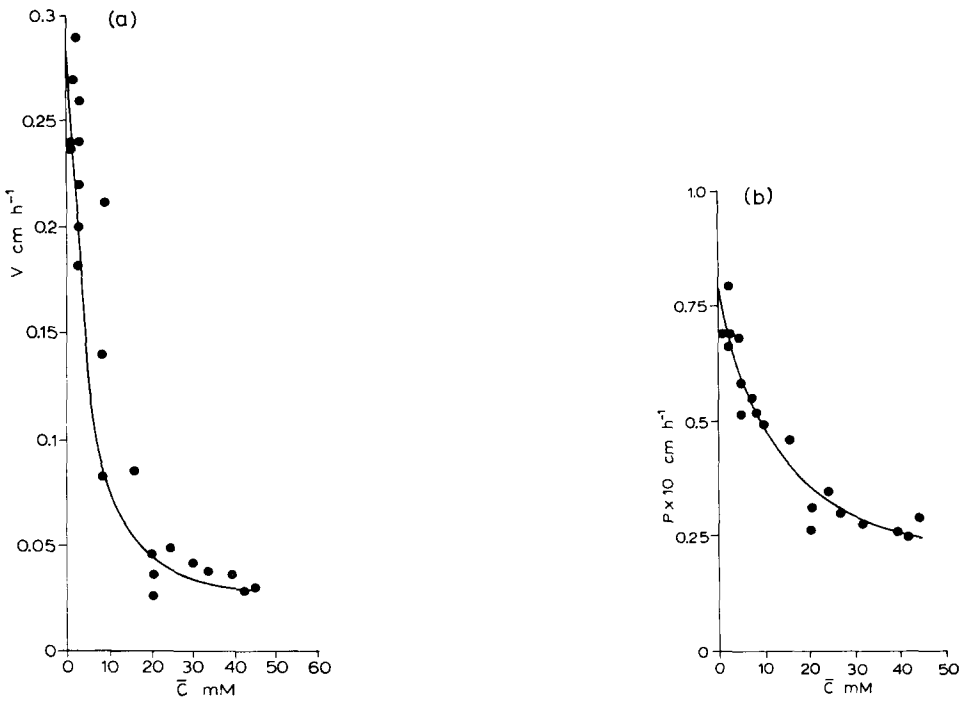

Fig. 11. (a) Effects of variation in brush-border [galactose] $\bar{c}$ on convective velocity of galactose across the brush-border. The points were obtained from experiments in which Ringer [Na] was held constant at 140 mequiv and Ringer [galactose] was varied. $\bar{c}$ was calculated as shown in the Appendix. The line was drawn by eye. (b) Effects of variation in brush-border [galactose] $\bar{c}$ on the calculated value of the galactose diffusive velocity P_1 in control tissues. The points were obtained from the same experiments shown in (a). The line was drawn by eye.

hence convective velocity to zero, the hyperbolic relationship between convective velocity and Ringer Na is likely to be caused by activation of the tissue Na pump.

In contrast to the effects on convective velocity, the Na-dependent increases in diffusive velocity P_i are independent of the tissue Na pump activity; with ouabain present the Na-dependent rise in galactose influx permeability (see Fig. 4) is similar to the calculated rise in diffusive permeability in control tissue.

The effects of varying brush-border galactose on the convective and diffusive velocities of galactose

Raising Ringer [galactose] from 0.2–20 mM reduces both the convective and diffusive velocities of galactose across the brush-border ($P < 0.001$ for both effects; Figs 10a and 10b). The mean brush-border galactose concentration $\bar{c}$ (see Appendix for method of determination) causing half maximal decreases in both the convective and diffusive velocities of galactose across the brush-border is approximately 5 mM, (Ringer [Na] is 140 mequiv), the same as the K_t for galactose influx [14]. However, the absolute and relative decrease in convective velocity, 0.3–0.03 cm · h^{-1} as $\bar{c}$ is raised from 0.4–45 mM exceeds the decrease in diffusive velocity, 0.08–0.03 cm · h^{-1} over the same concentration range.

A possible reason for this difference between the effects of increasing galactose on convective and diffusive velocity may be that the sugar partially occludes channels within the brush-border which presumably contain stereospecific binding sites for both Na and organic solutes. Since mass flow is proportional to the (channel radius)^4 whereas diffusional velocity is proportional to the (channel radius)^2 [20, 21] partial occlusion of the sugar permeation channels will retard convective flow more than diffusive flow. This effect may explain two otherwise puzzling relationships: the convex relationship between the Peclet number and net galactose flux and the decrease in the tissue accumulation ratio as Ringer [galactose] is increased, since it explains why the membrane asymmetry is reduced as the intracellular [sugar] is raised.

Resolution of the composite influx permeability of galactose into its convective and diffusive components also simplifies the interpretation of the kinetics of Na activation of sugar influx; i.e. Na-dependent activation of convective velocity of galactose across the brush-border is due to activation of the tissue Na pump activity as tissue [Na] is increased. Hence the K_t values for Na-dependent activation of convective velocity should be similar at all levels of Ringer [galactose]. The K_t values for Na-dependent activation of both the convective and diffusive velocities with Ringer [galactose] held at 2 or 20 mM are approximately the same, 25 mequiv Na whereas the K_t for the composite influx functions at 2 and 20 mM galactose are 23 and 101 mequiv Na, respectively. The proposed mechanism for asymmetric brush-border sugar permeability implies, firstly that the energy liberated by the action of the Na pump at the basal–lateral border of the intestinal cells may be transduced and transmitted across the cell to produce a pressure gradient across the brush-border. A double membrane system such as that proposed by Durbin [22] or Curran and McIntosh [23] or Diamond and Bossert [24] could provide this. Secondly, since convective diffusion can explain the asymmetry at the brush-border, but not uphill sugar flow, the above mechanism for brush-border sugar transport implies that a further concentrating stage exists. This question will be considered in the following paper (submitted to Biochim. Biophys. Acta).

APPENDIX

If it is assumed that solute diffusive and convective flux are confluent within the membrane, then integration across the membrane from Compartment 1 to Compartment 2 of the equation of Kedem and Katchalsky [1] (Eqn 1)

$$J_i = P_i(c_1 - c_2) + J_v(1-\sigma_1)\bar{c} \tag{1}$$

gives

$$J_i = \frac{c_1 - c_2 e^{(-J_v(1-\sigma)/P_i)}}{1 - e^{(-J_v(1-\sigma)/P_i)}} J_v(1-\sigma_i) \tag{2}$$

This equation has been previously derived by Patlak et al. [25], Bean [20] and recently by Anderson and Quinn [21]. Eqn 2 is preferable to Eqn 1 because it avoids approximations of $\bar{c}$.

The unidirectional fluxes of solute as measured by isotope movements from an infinite pool across a membrane into the contralateral compartment devoid of the same label are:

$$J_{12} = \frac{V \cdot c_1}{1 - e^{-Pe}} \tag{3}$$

$$J_{21} = \frac{-V \cdot c_2 e^{-Pe}}{1 - e^{-Pe}} \tag{4}$$

V = convective velocity of solute 1, Pe = Peclet number, the ratio of mass: diffusional velocity of solute within the membrane channels, i.e.

$$Pe = J_v(1-\sigma_i)/P_i = \frac{V}{P_i}$$

The Ussing [26] flux ratio of solute across the membrane is then:

$$\frac{J_{12}}{-J_{21}} = \frac{c_1}{c_2 e^{-Pe}} \tag{5}$$

$$\frac{J_{12}}{c_1} \Big/ \frac{-J_{21}}{c_2} = \frac{1}{e^{-Pe}} \tag{6}$$

hence

$$\ln\left(\frac{P_{12}}{-P_{21}}\right) = Pe \tag{7}$$

Both Hoshiko and Lindley [27] and Kedem and Essig [28] have derived Eqn 7. However, Hoshiko and Lindley assume that

$$\bar{c} = \frac{c_1 + c_2}{2}$$

and Kedem and Essig that

$$\bar{c} = \frac{c_1 - c_2}{\ln c_1/c_2}$$

Because neither approximation allows for the variation of $\bar{c}$ with the magnitude and direction of mass flow across the membrane, Eqn 7 could have been considered valid, only over a limited range of Pe. However, the present derivation of Eqn 7 should be generally valid where $\bar{c}$ as derived from Eqns 1 and 2 is now

$$\frac{c_1 - c_2 e^{-Pe}}{1 - e^{-Pe}} - \frac{c_1 - c_2}{Pe} \tag{8}$$

Rearrangement of Eqn 2 gives:

$$V = \frac{J_i(1 - e^{-Pe})}{c_1 - c_2 e^{-Pe}} \tag{9}$$

and since $Pe = V/P_i$, then $P_i = V/Pe$ (10)

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