Biochimica et Biophysica Acta, 382 (1975) 230–245

BBA 76888

GALACTOSE TRANSPORT ACROSS THE SEROSAL BORDER OF RABBIT ILEUM AND ITS ROLE IN INTRACELLULAR ACCUMULATION*

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(Received September 23rd, 1974)

SUMMARY

1. Unidirectional fluxes of D-galactose across the brush and serosal border of rabbit ileum were determined using the method described previously (Naftalin, R. J. and Curran, P. F. (1974) J. Membrane Biol. 16, 257–278). With Ringer [Na] = 75 mequiv., the K_m for galactose influx across the brush-border is 5 mM, with 0.1 mM ouabain present K_m = 50 mM, the V (2.0 μmol · cm^{-2} · h^{-1}) remains unaltered. The Michaelis parameters for galactose influx across the serosal border are K_m = 59 ± 9 mM and V = 4.7 ± 0.24 μmol · cm^{-2} · h^{-1} and for efflux K_m = 85 ± 10 mM and V = 6.8 ± 0.7 μmol · cm^{-2} · h^{-1}.

2. 2-Deoxy-D-glucose and methyl β-D-glucopyranoside inhibit galactose entry exclusively at the serosal and mucosal borders respectively, whilst 3-*O*-methyl-D-glucose inhibits galactose influx at both borders. 0.1 mM ouabain increases the K_i of 3-*O*-methylglucose for the mucosal galactose transport system from 50 to 150 mM, whilst the K_i of 3-*O*-methylglucose for the serosal transport system (100 mM) is unaffected by ouabain.

Inhibition of mucosal galactose transport by ouabain or by competition with other sugars results in a reciprocal increase in exit permeability and decrease in entry permeability. Inhibition of serosal galactose transport results in inhibition of both the entry and exit permeability, entry is more affected.

3. There is a small degree of permeability asymmetry at the serosal border to galactose which is reduced by ouabain or removal of Na^+ from the Ringer. Uptake of ^{14}C-labelled galactose from the serosal solution into the tissue is also inhibited by addition of ouabain or Na^+ removal. It is therefore considered that there is a weak active transport system for galactose at the serosal border.

4. Net transepithelial galactose flux is sufficiently high and serosal permeability to galactose sufficiently low to be consistent with the view that galactose is concentrated within the tissue fluid, after convection (Naftalin, R. J. and Holman, G. D. (1974) Biochim. Biophys. Acta., 373, 453–470) across across the mucosal border because it is reflected at the serosal boundary.

* Dedicated to the late Peter F. Curran.

INTRODUCTION

Previous studies of organic solute transport across the serosal boundary of the small intestine have shown that this membrane differs in several respects from the brush-border. Firstly, in contrast to transport across the mucosal border, no gross asymmetry in the unidirectional permeabilities to L-lysine [1], L-alanine [2] or sugars [3, 4] has been observed at the serosal boundary. Secondly, there is no marked Na^+-dependent change in the fluxes of organic solutes [3, 4] and thirdly, the affinities of alanine, lysine and D-galactose for the transport sites are at least an order of magnitude lower at the serosal than at the mucosal border. Bihler & Cybulsky [3] have shown recently with an isolated intestinal cell preparation that 2-deoxy D-D-glucose, D-mannose and D-fructose have a higher affinity for the serosal membrane than the mucosal membrane, hence not only the strength of interaction, but also the specificity range of the sugar-serosal membrane interaction differs from that at the brush-border.

The method described previously [4, 5] for determining the unidirectional permeabilities of sugars across the mucosal and serosal boundaries of rabbit ileum has the advantage that the serosal transport parameters can be obtained without differential inhibition of mucosal transport.

Several questions concerning sugar transport across the serosal border of fully active rabbit ileum remain unresolved, namely, does the serosal border have a measurable affinity towards sugars? Does the activity of the tissue Na^+-pump or [Na] in the tissue and Ringer affect sugar transport across the serosal membrane? Is the serosal border symmetrically permeable to sugars? Does the serosal border have a role in the concentration of sugars within the cell fluid? An attempt will be made to answer these questions in this paper.

MATERIALS AND METHODS

Ringer

The method of estimating the unidirectional fluxes across the mucosal and serosal boundary of rabbit ileum stripped of its serosal muscle layers has been previously described [4, 5]. The experiments described in this paper employ 'Ringer' solutions of different compositions to those used previously. The solutions used were as follows. 75 mequiv. NaCl Ringer: 75 mM NaCl, 10 mM $KHCO_3$, 0.4 mM KH_2PO_4, 2.4 mM K_2HPO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 65 mM choline chloride. Hypertonic serosal solution: 140 mM NaCl, 10 mM $KHCO_3$, 0.4 mM KH_2PO_4, 2.4 mM K_2HPO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 60 mM NaCl or 120mM mannitol. D-galactose and/or the sugars were added as indicated. In solutions where low Na^+ hypertonic Ringer was required choline chloride was substitued for NaCl in the above solution. The solutions were gassed and stirred with 95 % O_2/5 % CO_2 at 37 °C. All the chemicals used were Analar grade, except inhibitor sugars 2-deoxy D-glucose, 3-O-methyl D-glucose and methyl β-D-glucoside which were obtained from Koch-Light Ltd.

Scintillation fluid

Because of difficulties in obtaining Triton X 100 the emulsifying agent was changed to Tergitol 15-S-9 (Union Carbide). Tergitol is a superior emulsifyer of

water/toluene mixtures to Triton X 100. The new scintillation fluid composition is 500 ml toluene: 500 ml Tergitol: 5.0 g PPO (Koch-Light): 10 ml scintillation fluid: 1 ml of salt solution or 0.4 ml 0.1 M HNO_3 tissue extract.

It was shown [4] 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 of R to the specific activity of radioisotope within the tissue compartment 2.

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

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

subscripts 1, 2, 3 refer to the mucosal, cell and serosal compartments respectively; superscripts T and C refer to ^3H and ^{14}C-labelled D-galactose respectively. J_{ij} refers to flux from compartment i to j and $P_{ij} = J_{ij}/C_i$.

It was shown that:

$$J_{12} = J_{31}R - J_{13}$$
$$J_{21} = J_{31}(1 - R)$$
$$J_{23} = J_{13}(1 - 1/R)$$
$$J_{32} = J_{31} - J_{13}/R$$

RESULTS

(*1a*) *Saturation of galactose transport across the mucosal and serosal membranes of rabbit ileum*

In this section the effects of varying Ringer [galactose] on galactose fluxes across the mucosal and serosal borders of rabbit ileum are examined. The unidirectional galactose fluxes across the mucosal and serosal borders of the tissue are calculated as described previously [4] from measurements of transepithelial mucosal-serosal and serosal-mucosal fluxes, together with the specific activity ratio of ^3H ^{14}C labelled galactose within the tissue extract. It was shown [4] that the K_m for galactose influx across the brush-border is 5 mM and that if Ringer [galactose] is raised above 20 mM the transepithelial permeability to galactose in actively transporting tissue is increased, probably because of concomitant enlargement of the paracellular spaces. To avoid difficulties arising from these changes in passive permeability, in the series of experiments described in this section, Ringer [Na] is reduced to 75 mequiv., isotonicity being maintained by isosmotic replacement of NaCl with mannitol. With Ringer [Na] 75 mequiv. the tissue permeability to galactose is still highly asymmetric, but the previously observed increase in transepithelial galactose permeability at high levels of Ringer [galactose] is no longer observed.

Fig. 1 shows Lineweaver-Burk plots of galactose influx across the brush-border J_{12}. The effect of 0.1 mM ouabain on galactose influx is shown in the same figure. The K_m for galactose influx across the brush-border is 5 mM. This is a similar value to the K_m derived previously, but with Ringer [Na] 140 mequiv. [4]. The

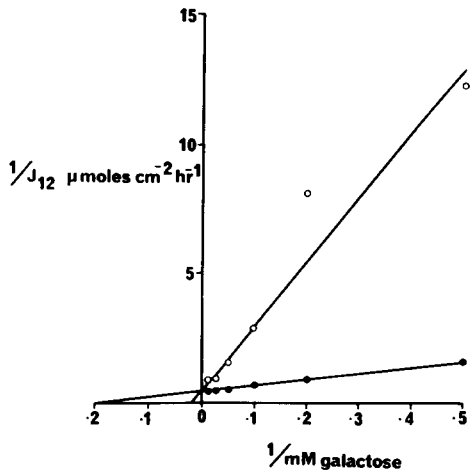

Fig. 1. Lineweaver-Burk plots of galactose influx across the brush-border of rabbit ileum. ●, control; ○, 0.1 mM ouabain present. Data presented are from a single experiment.

major difference between this and the previously obtained result is that there is no significant passive permeability component observed in the present series. With ouabain added to the Ringer, the apparent affinity of galactose influx across the mucosal border is reduced tenfold, $K_m = 50$ mM, but the $V = 2.0$ μmol · cm^{-2} · h^{-1} is unaltered (5 control experiments and 2 with ouabain).

Galactose influx across the serosal border is also a saturable function of Ringer [galactose] (Fig. 2). The K_m for serosal influx of galactose is 59±9.0 mM (4 experiments) and the V is 4.47±0.27 μmol · cm^{-2} · h^{-1} (Fig. 2a insert). In the galactose concentration range 0–10 mM ouabain reduces galactose influx across the serosal border ($P < 0.01$) above this concentration range, ouabain has no measurable effect on galactose entry or exit across the serosal border (Fig. 2a). The exit of galactose across the serosal boundary is also a saturable function of the tissue [galactose] the derived Michaelis parameters for exit are similar to those for entry $K_m = 85$±10 mM, $V = 6.77$±0.68 μmol · cm^{-2} · h^{-1} (3 experiments) (Fig. 2b).

The influence of unstirred layer effects on the kinetics of galactose transport across the serosal boundary can be assessed as follows. Assuming that the submucosal layer is the major diffusion barrier 500 μm thick and the diffusion coefficient of galactose within the submucosa is isotropic and $5 \cdot 10^{-6}$ cm^2 · s^{-1}, then the apparent total resistance of the tissue R_{ms} to unidirectional galactose mucosal-serosal movement with Ringer [galactose] at 2 mM $= 1/P_{\text{tissue}_{ms}} + 1/P_{\text{submucosa}}$.

The resistance to serosal-mucosal galactose flux $R_{sm} = 1/P_{sm} + 1/P_{\text{submucosa}}$ since $R_{ms} = 3.6 \cdot 10^4$ s · cm^{-1}, $R_{sm} = 1 \cdot 10^6$ s · cm^{-1} and $R_{\text{submucosa}} = 1 \cdot 10^4$ s · cm^{-1} it follows that the apparent permeability P_{ms} may be up to 30 % less than the true permeability and the apparent P_{sm} may be 1 % less than the true permeability.

As J_{23} and J_{32} are calculated from the following relationships (see Methods and [4]);

$$J_{23} = J_{31}(1 + 1/R)$$
$$J_{32} = J_{31} + J_{13}/R$$

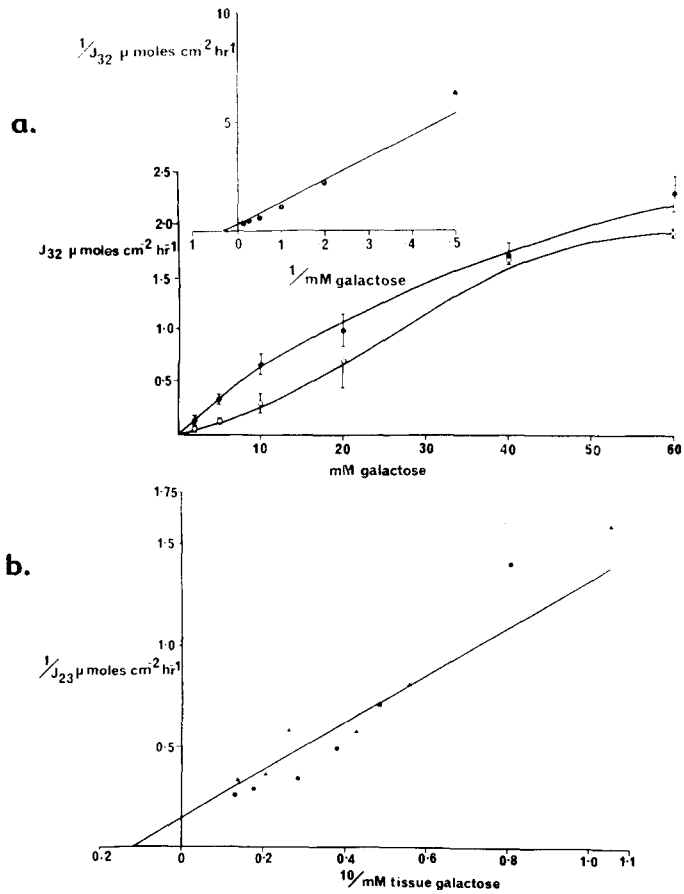

Fig. 2. (a) Calculated galactose influx across the serosal border J_{32} plotted as a function of Ringer [galactose]. ●, control (5 experiments); ○, 0.1 mM ouabain present (2 experiments). Bars represent S.E. Insert: Lineweaver-Burk plot of galactose influx across the serosal border (control data). The line is fitted to the computed K_m and V values. (b) Lineweaver-Burk plot of galactose efflux across the serosal border J_{23} as a function of tissue [galactose]. ○, ●, and △ are data from 3 separate experiments. The line is fitted to the computed mean K_m and V values.

it follows that the error in estimation of J_{32} incurred due to unstirred layer effects will be less than that incurred in the estimate of J_{23}. Yet the operational kinetic parameters for galactose entry and exit across the serosal border are indistinguishable, hence it is unlikely that a large error due to unstirred layer effects is present in actively transporting tissue. When ouabain is added, the tissue permeability asymmetry towards galactose is lost, hence the error due to unstirred layer effects is now the same in both directions, i.e. 10 %. The observed 10-fold increase in the operational K_m for galactose influx across the brush-border following ouabain addition is too large to be ascribed to a change in unstirred layer effect.

These results demonstrate that galactose transport across the serosal membrane is mediated by low affinity binding sites. Previously, owing to the marked increase in shunt permeability at high concentration levels of Ringer galactose, little direct

TABLE I

CALCULATED UNIDIRECTIONAL PERMEABILITIES OF THE MUCOSAL AND SEROSAL BORDERS OF RABBIT ILEUM (RINGER Na = 75 mequiv.) TO GALACTOSE

Significance levels calculated from Student's 't' test unpaired means solution.

	[galactose] mM	P_{12} (cm · h^{-1} ± S.E.)	P_{21} (cm · h^{-1} ± S.E.)	P_{23} (cm · h^{-1} ± S.E.)	P_{32} (cm · h^{-1} ± S.E.)	$\ln \frac{P_{12}}{P_{21}}$	$\ln \frac{P_{32}}{P_{23}}$
A	2+5	0.186±0.027	0.0061±0.0011	0.061±0.0033	0.064±0.0047	3.38±0.34	0.183±0.06
	n	(10)	(10)	(8)	(10)	(10)	(10)
B	40+60	0.035±0.0027	0.016±0.0013	0.047±0.0026	0.041±0.0022	0.79±0.10	−0.12±0.03
	n	(10)	(10)	(10)	(10)	(10)	(10)
	difference (A−B) *P*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
+0.1 mM ouabain							
C	2+5	0.026±0.005	0.013±0.002	0.023±0.005	0.027±0.003	0.677±0.204	0.225±0.145
	n	(4)	(4)	(4)	(4)	(4)	(4)
D	40+60	0.022±0.0015	0.014±0.0015	0.046±0.004	0.036±0.003	0.460±0.165	−0.232±0.107
	n	(4)	(4)	(4)	(4)	(4)	(4)
	difference (C−D) *P*	NS	NS	<0.02	NS	NS	<0.05
	difference (A−C) *P*	<0.01	<0.02	<0.02	<0.001	<0.001	NS
	difference (B−D) *P*	<0.02	NS	NS	NS	NS	NS

TABLE II

EFFECT OF 20 mM INHIBITOR SUGAR ON CALCULATED UNIDIRECTIONAL PERMEABILITIES OF THE MUCOSAL AND SEROSAL BORDERS OF RABBIT ILEUM TO 0.2 mM GALACTOSE

With 3-*O*-methylglucose present Ringer [galactose] in both control and inhibited conditions is 1.0 mM. Student's 't' test paired means solutions.

	P_{12} (cm · h^{-1})	P_{21} (cm · h^{-1})	P_{32} (cm · h^{-1})	P_{23} (cm · h^{-1})	$\frac{P_{32}}{P_{23}}$	Mucosal accumulation ratio	Serosal accumulation ratio
Control	0.182 ± 0.018	0.0048 ± 0.0002	0.086 ± 0.0068	0.046 ± 0.0053	1.87 ± 0.16	4.02 ± 0.47	1.89 ± 0.10
n	(10)	(10)	(14)	(14)	(14)	(10)	(14)
% of Control ± S.E.							
2-Deoxy D-glucose	104 % ± 11 (NS)	208 % ± 44 ($p < 0.001$)	63 % ± 4.3 ($p < 0.001$)	114 % ± 11 (NS)	65 % ± 5.8 ($p < 0.001$)	101 % ± 11 (NS)	61 % ± 2.8 ($p < 0.001$)
n	(10)	(10)	(11)	(11)	(11)	(10)	(11)
3-*O*-methyl D-glucose*	83 % ± 4.4 ($p < 0.001$)	210 % ± 28 ($p < 0.001$)	73 % ± 5.8 ($p < 0.001$)	107 % ± 9.7 (NS)	60 % ± 10 ($p < 0.001$)	62 % ± 16 ($p < 0.001$)	65 % ± 10 ($p < 0.001$)
n	(5)	(5)	(6)	(6)	(6)	(6)	(6)
methyl-β-D-glucoside	19.5 % ± 2.4 ($p < 0.001$)	310 % ± 45 ($p < 0.001$)	104 % ± 7.6 (NS)	107 % ± 8 (NS)	99 % ± 10 (NS)	12 % ± 1 ($p < 0.001$)	98 % ± 9 (NS)
n	(8)	(8)	(11)	(11)	(11)	(8)	(11)

* galactose flux measured with 1 mM present in Ringer

evidence of a saturable component to serosal galactose transport was obtained [4]. The close similarity between the serosal entry and exit flux parameters, shown here, indicates that transport across the serosal membrane is more symmetrical than across the mucosal membrane. In Table I it is shown that whilst raising Ringer [galactose] from low to high concentrations reduces the entry permeability of P_{12} of the brush-border to galactose ($P < 0.001$), the exit permeability P_{21} is raised ($P < 0.001$). The entry and exit permeabilities of the serosal membrane to galactose differ from the mucosal permeabilities in that both are significantly reduced when Ringer [galactose] is raised or following the addition of ouabain to the Ringer.

(1b) Inhibition of galactose transport across the mucosal and serosal border of rabbit ileum by D-*galactose analogues*

Unidirectional fluxes of 0.2 mM D-galactose across the mucosal and serosal membranes are determined in the presence of 20 mM additional sugar or 20 mM mannitol in controls. Table II shows the effects of various sugars when added to the mucosal or serosal solutions, on the entry and exit permeabilities of the serosal and brush-borders to galactose, and on the ratio of unidirectional permeabilities and the tissue accumulation of ^{3}H and ^{14}C-labelled galactose from the mucosal and serosal solutions respectively. 2-deoxy D-glucose, 3-*O*-methyl D-glucose both inhibit galactose influx across the serosal membrane; 3-*O*-methyl D-glucose and methyl β-D-glucoside inhibit galactose entry across the mucosal membrane. Hence 3-*O*-methyl D-glucose inhibits galactose transport across both membranes, whereas 2-deoxy D-glucose and methyl β-D-glucoside act exclusively on serosal and mucosal entry permeabilities respectively. It can be seen that the inhibition of galactose transport across the brush-border by competing sugars has similar effects to either raising the Ringer [galactose] or adding ouabain, namely to decrease the entry permeability, raise the exit permeability of the brush-border to galactose and reduce the amount of tissue galactose accumulated from the mucosal solution.

Raised galactose mucosal exit permeability is also seen with 2-deoxyglucose present (Table II). This increase cannot be attributed to accelerated exchange since it is also observed when 2-deoxyglucose is added only to the serosal solution (P_{21} 150 % of control ($P < 0.02$, $n = 6$)). This effect is being further investigated.

Inhibitors of brush-border galactose transport also have a much reduced affinity for the transport system when ouabain is present (see below). Sugars which inhibit galactose transport across the serosal boundary do so by decreasing entry permeability without affecting the exit permeability, thus reducing the permeability ratio P_{32}/P_{23} and the amount of galactose accumulated from the serosal solution.

Since 3-*O*-methyl D-glucose interacts with both the mucosal and serosal galactose transport systems and is not metabolised [6] by rabbit ileum, the effects of ouabain on the affinities of sugar for the transport systems at both boundaries may be obtained by simultaneously determining the effects of increasing concentrations of 3-*O*-methylglucose on galactose transport across both boundaries. The method of determining the unidirectional fluxes of galactose across the mucosal and serosal membranes is similar to that previously used in Section 1a. To avoid possible complications due to osmotic imbalance, the Ringer [Na] is reduced to 75 mequiv. as previously but the remaining 130 mosmol required to bring the Ringer to isotonicity is supplied by addition of suitable combinations of sugars and mannitol. Figs 3c and

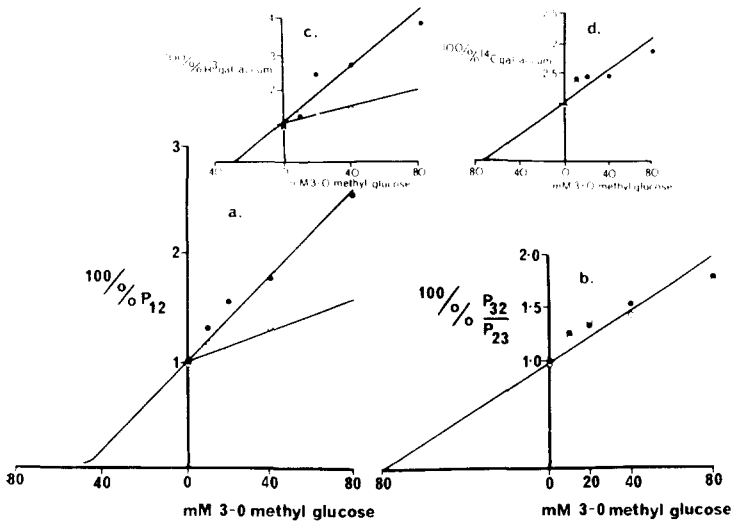

Fig. 3. (a) Dixon plot of fractional inhibition of the calculated galactose entry permeability of the brush-border as a function of Ringer [3-*O*-methyl-D-glucose]. The data is obtained from the same experiments as were used to obtain data for Figs 3c and 3d. (*b*) Dixon plot of the fractional percentage inhibition of the serosal permeability ratio to galactose P_{32}/P_{23} as a function of serosal [3-*O*-methylglucose] (6 paired experiments). (c) Dixon plot of ^{3}H-labelled galactose accumulated by the tissue from the mucosal solution (Ringer [galactose] = 1 mM, [Na] = 75 mequiv.). Inhibition of galactose accumulation in the presence (○) and absence (●) of 0.1 mM ouabain is expressed as a percentage of the uninhibited control accumulation (6 paired experiments). (d) Dixon plot of ^{14}C-galactose accumulated from the serosal solution (the experimental conditions were identical to those described in 3c).

3d show the effects of varying [3-*O*-methylglucose] in the presence and absence of ouabain on the tissue uptake of ^{3}H and ^{14}C-labelled galactose from the mucosal and serosal solutions respectively with ouabain present in both solutions. Dixon plots of the effects of 3-*O*-methylglucose on galactose influx across the brush-border are shown in Fig. 3a. The data are obtained from 6 separate experiments normalised by expressing influx in the presence of inhibitor as a percentage of flux with inhibitor absent. With ouabain present the data are normalised against the ouabain treated inhibitor-free controls. These results indicate that the K_I of 3-*O*-methylglucose for inhibition of 1 mM galactose entry across the mucosal border is approx. 50 mM, with ouabain present the K_I is raised to approx. 150 mM. Fig. 3b shows a Dixon plot of the effect of 3-*O*-methylglucose on the normalised ratios of galactose entry: exit P_{32}/P_{23} across the serosal border. The operational K_I inferred from 3b and 3d for inhibition of galactose entry across the serosal border is 80 mM, and ouabain does not materially affect the K_I at the serosal border.

Active transport of galactose across the serosal membrane

In ref. 4 it is shown that permeability ratio of galactose across the serosal border P_{32}/P_{23} is reduced when ouabain is added to the Ringer, also that the tissue accumulation of ^{14}C-labelled galactose from the serosal solution was reduced following addition of ouabain. These effects are indicative of a weakly active galactose

transport system at the serosal border of the tissue. However, the observed serosal permeability ratio to galactose rarely exceeds 3.0 whilst the permeability ratio of the brush-border to galactose can exceed 250. Thus the galactose transport system at the serosal border may simply be an enfeebled form of the mucosal system. In order to optimise the observed changes in Na^+-dependent serosal transport of galactose in the present study, the serosal solutions were made hypertonic (400 mosmol) by addition of mannitol. The serosal solution was made hypertonic for two reasons, firstly, to allow an increased range of [Na] to be used so that larger increments in intracellular [Na] could be obtained; secondly, the permeability of the serosal membrane is increased in these conditions, probably because access to the serosal surface of the epithelium is improved as a result of expansion of the submucosal and paracellular spaces [4]. This manoeuvre has the disadvantage that the transepithelial

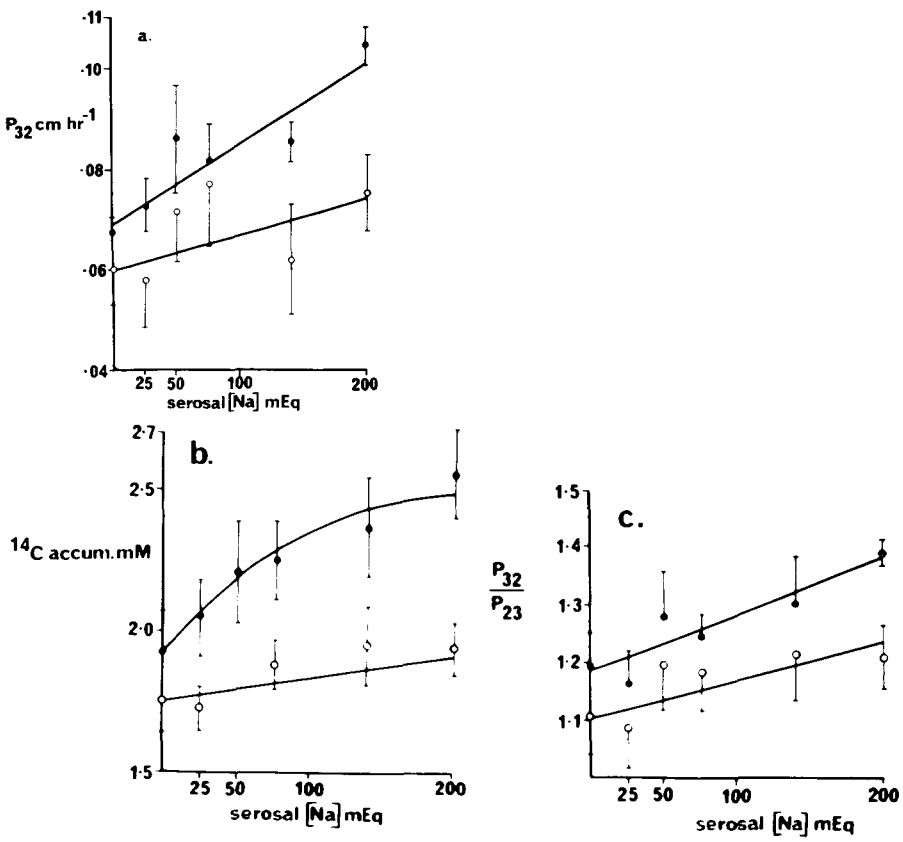

Fig. 4. (a) The effect of varying serosal [Na] on the serosal entry permeability (Ringer [galactose] = 2 mM) in the presence (○) and absence (●) of 0.1 mM ouabain. Bars represent S.E. of 6–8 observations. The lines drawn are the least squares linear regression lines to the data. (b) The effect of varying serosal [Na] on the tissue accumulation of ^{14}C-labelled galactose from the serosal solution (Ringer [galactose] = 2 mM) in the presence (○) and absence (●) of 0.1 mM ouabain. The bars represent S.E. of 6–10 observations. Lines are fitted by eye. (c) The effect of varying serosal Ringer [Na] on the serosal permeability ratio to galactose P_{32}/P_{23}, in the presence (○) and absence (●) of 0.1 mM ouabain. Bars represent S.E. of 6–8 observations. The lines shown are least squares linear regression lines throughout the data.

permeability is increased, hence any real changes in serosal influx permeability P_{32} may be obscured by alterations in the transepithelial permeability P_{31}.

Unidirectional fluxes and galactose accumulation were measured with serosal Ringer [Na] 0–200 mequiv. at two constant mucosal concentrations of Na^+ 25 and 140 mequiv. The mucosal Ringer [Na] was replaced by choline because it was felt that high levels of tissue galactose might obscure any serosal asymmetry to galactose.

In the experiments reported in this section serosal Ringer NaCl was replaced by isosmotic amounts of mannitol. (A few experiments were carried out with choline replacing mannitol with Na^+, but there was no detectable difference between the effects of mannitol and of choline.)

Fig. 4a shows the effects of varying serosal Ringer [Na] on the calculated galactose entry permeability P_{32} across the serosal border. When Ringer [Na] is increased from 0 to 200 mequiv., P_{32} increases from 0.065 to 0.105 cm · h^{-1} ($P < 0.01$). Because of the high transepithelial shunt permeability to galactose in this experimental situation, the effects of ouabain on the serosal influx permeability are obscured. However, the other two indices of serosal "active transport" activity, the ^{14}C-labelled galactose accumulation level within the tissue, and the serosal permeability ratio to galactose P_{32}/P_{23}, are significantly reduced by ouabain ($P < 0.001$ and $P < 0.025$ respectively). (Figs 4b and 4c).

DISCUSSION

The ratio of galactose unidirectional permeabilities at the serosal border in all conditions examined here and previously [4] is close to unity. This contrasts markedly with the galactose permeability ratio at the brush-border [4, 5] and indicates that galactose is subjected to a much weaker vectorial driving force at the serosal than at the mucosal membrane.

Concentration polarization of galactose at the serosal border of intestinal epithelial cells

Operationally, the intestinal epithelial cell may be considered as a double membrane in series array, with cytoplasm occupying the intermembrane zone (see Diagram i). The mucosal membrane, unlike the serosal membrane, permits convective sugar flow across it when the tissue Na^+ pump is activated [5]. However, passive permeabilities of both membranes to galactose are similar ([4] and Table 1). Since, at steady state, net galactose movement across the whole tissue is the same as the net movement across both mucosal and serosal membranes, it follows from the

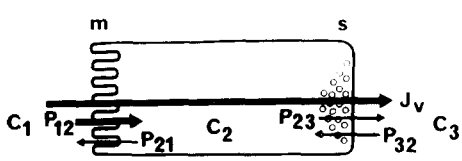

Diagram i. Analogue of intestinal epithelial cell. The conditions necessary for accumulation of solute within the tissue fluid, i.e. $C_2/C_1 > 1$, where C_1 ... C_3, are; that the reflection coefficient of the mucosal border, σ_m < the serosal reflection coefficient, σ_s and the rate of transepithelial water flow across the tissue, $J_v > 0$. Broad arrow = inwardly directed convective-diffusion flow of solute. $P_m \approx P_s$.

previous statements that the reflection coefficient of the brush-border to galactose σ_m is less than the reflection coefficient at the serosal membrane σ_s[4].

Kedem & Katchalsky [7] showed that convective flow across a series membrane array can give either solute concentration or depletion within the inter-membrane region according to the following relationship: (Note: the subscripts used by Kedem & Katchalsky have been altered for convenience)

$$C_2 = C_1 \exp(-\lambda J_v) \tag{1}$$

where C_1 and C_2 are the solute concentrations, outside and between the membranes respectively, J_v is the volume flow (positive when directed from mucosa-serosa) $\lambda = (\sigma_m - \sigma_s)/(P_m + P_s)$, P_m and P_s are the passive permeabilities of the mucosal and serosal membranes to solute (galactose). Thus, when $\sigma_s > \sigma_m$ and J_v is directed from mucosal-serosal solutions $(-\lambda J_v)$ will have a positive sign according to equation (1); hence the entrained galactose will accumulate within the inter-membrane (intracellular) zone. Accumulation in the inter-membrane region results from concentration polarization of solute at the membrane with the higher reflection coefficient. Hence mass flow directed from mucosa-serosa gives net accumulation between the membranes; convective flow in the opposite direction should give rise to solute depletion in the inter-membrane zone.

In the previous paper [5] it was shown that the asymmetric permeability of the brush-border to galactose could be ascribed to convective-diffusion flow via narrow channels. Although convective-diffusion by itself cannot accomplish uphill sugar flow, with a double membrane array interposed, selective retardation of the entrained galactose at the serosal membrane, which does not permit convective flow of galactose, will give rise to solute accumulation within the tissue fluid.

At steady state, net solute movement across the mucosal membrane must equal net movement across the serosal membrane, i.e.

$$J_{net} = J_{12} - J_{21} = J_{23} - J_{32} \tag{2}$$

If it is assumed that there is negligible solute convection at the serosal border, i.e. $\sigma_s = 1$, and therefore the serosal membrane is approximately symmetrical, i.e.

$$P_{32} = P_{23} = P_s \tag{3}$$

(P_s is serosal permeability coefficient to galactose), then

$$J_{net} = P_s(C_2 - C_3) \tag{4}$$

C_3 and C_2 are the external and cell sugar concentrations, respectively. Hence, from Eqns (2), (3) and (4) it follows that

$$J_{net} = J_{23} - J_{32} = P_{32}(C_2 - C_3) \tag{5}$$

and hence that

$$\frac{C_2}{C_3} = \frac{J_{23}}{J_{32}} \tag{6}$$

from equations 8–13 of ref. 4 it can be shown that

$$J_{23} \quad (1 - 1/R) \quad [\text{galactose}]_{\text{tissue}}$$
$$J_{32} \quad J_{31} \quad 1 \quad [\text{galactose}]_{\text{Ringer}} \qquad (7)$$
$$J_{13} \quad R$$

Hence the hypothesis that solute is accumulated within the cell fluid as a result of concentration polarization at the serosal border may be checked by comparing the predicted accumulation according to Eqn (7) with the measured level of galactose accumulation. Fig. 5 shows a plot of the predicted intracellular level of [galactose] against the directly observed concentration. There is a significant linear correlation between the observed and predicted values ($P < 0.001$). However, there is a small shortfall in the observed compared to the predicted intracellular galactose concentration. Two possible explanations for this shortfall are, firstly, that the washing procedure following flux measurement removes a significant portion of the intracellular galactose without affecting the specific activity ratio R of $^3H/^{14}C$-labelled galactose remaining in the tissue: alternatively, the reflection coefficient of galactose at the serosal border may be less than unity. The main point is that net transepithelial galactose flux is shown to be sufficiently large and the serosal permeability to galactose, sufficiently small, to be consistent with the hypothesis that galactose accumulation within tissue fluid results from concentration polarization at the serosal boundary.

The specificity of the serosal and mucosal membrane towards sugars

Bihler & Cybulsky [3] have shown that the serosal membrane of intestinal epithelial cells has a different range of affinities towards sugars to the mucosal membrane. The results shown in Table II confirm that 2-deoxy D-glucose and also 3-*O*-methyl D-glucose reduce unidirectional galactose influx J_{32} into the tissue from the serosal solution: they also reduce the unidirectional permeability ratio of the serosal

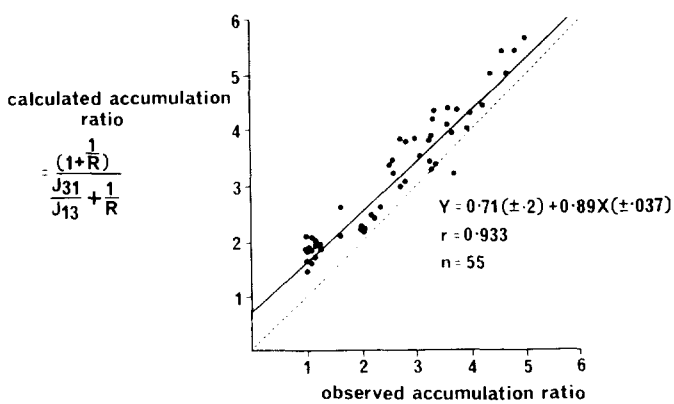

Fig. 5. Plot of calculated galactose accumulation versus observed accumulation within the tissue fluid. The data are taken from the experiments described in ref. 5, i.e. galactose accumulation is varied by (1) reducing Ringer [Na] by replacement with choline, (2) addition of 0.1 mM ouabain, (3) varying Ringer [galactose]. Only a small proportion of the data where accumulation is close to unity is included. The solid line is the least squares linear regression line through all the data; the dotted line is the line of identity between the predicted and observed accumulation.

membrane to galactose P_{32}/P_{23} and the tissue level of ^{14}C-galactose accumulation which is taken up exclusively from the serosal solution. 2-deoxyglucose is a more potent inhibitor of serosal than of mucosal galactose transport, 3-*O*-methylglucose affects mucosal and serosal galactose transport to approximately the same extent methyl β-D-glucose inhibits galactose transport exclusively at the brush-border. Although 3-*O*-methylglucose inhibits galactose transport across both the mucosal and serosal borders, by reducing galactose influxes J_{12} and J_{32} respectively and also the galactose permeability ratios at the mucosal and serosal membranes P_{12}/P_{21} and P_{32}/P_{23}, there are two differences between the effects of the sugar inhibitors which suggest that the mode of galactose transport across the mucosal membrane differs from that at the serosal membrane. Firstly, addition of 3-*O*-methylglucose to the mucosal solution causes a reciprocal increase in the exit permeability of the mucosal membrane P_{21} as the entry permeability P_{12} is reduced; whereas addition of 3-*O*-methylglucose to the serosal solution decreases the apparent serosal galactose entry permeability P_{32} without affecting P_{23}. A second difference is that addition of ouabain reduces the apparent affinity of 3-*O*-methylglucose for the galactose transport system across the brush-border, whereas ouabain has no effect on the inhibition of galactose transport across the serosal membrane by 3-*O*-methylglucose (Figs 3b and 3d) or 2-deoxyglucose (unpublished results). The effects of competing sugars on galactose transport across the mucosal and serosal membranes are similar to the previously observed effects of raised Ringer [galactose] on both these transport systems, i.e. mucosal galactose transport is reduced by a reciprocal fall in P_{12} and rise in P_{21} [5] whereas both the serosal entry permeability P_{32} and P_{23} are reduced (Table I) by raising Ringer [galactose].

The relative order of affinities of the serosal membrane sugar transport system 2-deoxyglucose $>$ 3-*O*-methylglucose $\gg$ methyl β-D-glucose resembles the passive facilitated sugar transport systems in the human red cell [9, 10] the antiluminal border of the renal proximal tubular epithelial cells, ascites tumour cells [11, 12] and rat brain synaptosomes [13]. There is also resemblance between the sugar specificity requirements of the brush-border sugar transport systems in small intestinal and renal proximal tubular sugar transport systems [11].

The apparent selectivity of the mucosal and serosal membranes towards methyl β-D-glucose and 2-deoxy D-glucose as shown by differential inhibition of galactose influx across the brush-border and serosal border further substantiates the assumptions on which the present method of estimating unidirectional sugar fluxes is based.

Evidence for weak active serosal transport of galactose

The following results suggest that there is a weakly active transport system at serosal boundary for galactose. (1) Raising [Na] in hypertonic serosal Ringer from 0 to 200 mequiv. causes a significant increase in the serosal unidirectional permeability ratio to galactose ($P < 0.01$). With ouabain present no Na^+-dependent increase in the permeability ratio P_{32}/P_{23} occurs. Hence the rise in the serosal permeability ratio is ouabain sensitive ($P < 0.025$). (2) The tissue level of ^{14}C-labelled galactose accumulated from the serosal solution using the incubation conditions as above, is increased when the serosal Ringer [Na] is raised from 0 to 200 mequiv., ($P < 0.001$) no Na^+-dependent increase in galactose uptake is seen when ouabain

is present ($P < 0.001$). Although a small Na^+-dependent ouabain sensitive increase in galactose influx P_{32} across the serosal border is observed, because the galactose permeability P_{32}/P_{23} never exceeds 3.0, it cannot be stated with certainty that galactose transport across the serosal membrane is active.

An alternative possible explanation for the above data is that activation of the tissue Na^+ pump increases the volume of the paracellular shunt and submucosal space [4, 14] thus allowing increased amounts of ^{14}C-labelled galactose to accumulate passively in the extracellular space. Although there is a weakly positive correlation between the ratio of wet: dry tissue weight and net galactose flux across the tissue ($P < 0.01$) and a strong correlation between net transepithelial galactose flux and accumulation of [^{14}C] galactose from the serosal solution ($P < 0.001$), suggesting that rabbit ileum stripped of its serosa swells when the tissue Na^+ pump is activated, there is no significant correlation between the ratio of wet: dry tissue weight and tissue accumulation of [^{14}C] galactose. ($P > 0.1$). Thus, it is our present view that the most likely focus of serosal [^{14}C] galactose accumulation is within the epithelium and that a weak Na^+-dependent, ouabain-sensitive active transport system is the most likely explanation for its accumulation there.

The route of galactose transport across the tissue

If galactose is actively transported across the serosal membrane, the simple two-membrane series array analogue of the intestinal epithelial cell must be abandoned in favour of a more complex conformation (see Diagram ii). If the Na^+ pump is situated exclusively at the lateral boundary of the epithelial cell, then net galactose uptake into the cells could be via both mucosal and basal membranes whilst net exit occurs exclusively via the lateral boundary. This scheme would permit active sugar transport across both mucosal and serosal borders whilst the overall direction of net sugar flow remains towards the serosa. Other evidence for this scheme is that competing sugars added to the serosal solution affect the influx permeability of galactose across the serosal membrane without altering exit, in contrast to their effects on galactose flux across the mucosal membrane.

The findings suggest that the route for galactose passage across the serosal boundary of rabbit ileum is circuitous.

ii

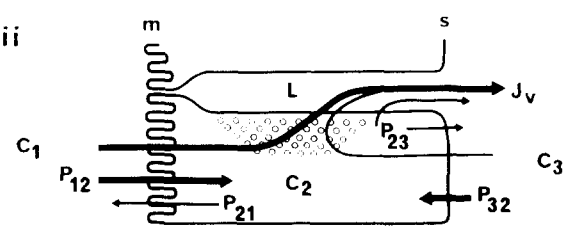

Diagram ii. Analogue of intestinal epithelial cell permitting inwardly directed convective-diffusion flow of solute across both mucosal and serosal borders. The reflecting barrier is now assumed to be the lateral border of the cell L, hence, $1 > \sigma_1 > \sigma_s > \sigma_m$.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Robert Norledge for his skilled assistance and the Medical Research Council for financial support.

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