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Alternate models for shared carriers or a single maturing carrier in hexose uptake into rabbit jejunum in vitro

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The uptake (tissue accumulation) of three hexoses into rabbit jejuum was measured in a flux chamber in conditions of effective stirring. Glucose uptake was inhibited by galactose or 3-*O*-methylglucose: 1–40 mM galactose caused a progressive decline in glucose uptake; 1–5 mM 3-*O*-methylglucose inhibited glucose uptake but higher concentrations of 3-*O*-methylglucose had no further effect. When 1–40 mM 3-*O*-methylglucose was added to glucose plus galactose there was a further decrease in the uptake of glucose; adding 1–40 mM galactose to glucose plus 3-*O*-methylglucose also produced a decrease in glucose uptake. Both glucose and 3-*O*-methylglucose inhibited uptake of galactose but the pattern of inhibition varied between the two sugars. The uptake of 3-*O*-methylglucose was also inhibited by glucose and by galactose, but the uptake of 3-*O*-methylglucose in the presence of either galactose or glucose was no further reduced by adding the third hexose. Graphical analysis and analysis by non-linear regression both showed that neither the single Michaelis-Menten function, nor the single Michaelis-Menten-plus-competitive-inhibition function was appropriate for any of these data. The results are consistent with the hypothesis that either there are multiple (at least three) intestinal carriers for hexoses; alternatively that there is a single carrier whose transport properties for the three hexoses change differentially during cell maturation and migration up the villus.

Introduction

Although it has been generally accepted that monosaccharides of the hexose group are transported in the small intestine by a single, Na^+-dependent, phlorizin-sensitive carrier [1–4], there is also evidence indicating that sugar absorption may be mediated by more than one transport system. This postulate is derived from inhibition studies with uranyl nitrate, fucose and phlorizin, and from studies of the changes in sugar transport induced by sugar-containing diets and differences in sodium-dependence [5–19]. For example, Ponz and Lluck [5] and Newey et al. [6] found that uranyl nitrate at low concentrations inhibited transport of galactose but not of glucose in rat intestine in vivo, and this was confirmed in vitro by Newey et al. [6]. McMichael [14] found a biphasic inhibition of glucose transport by phlorizin in the rat jejunum in vivo, but a monophasic inhibition of galactose transfer. The interpretation of these observations is not straight-forward [9], but the simplest explanation is that there is more than one carrier. Other workers have also reported findings challenging the view that glucose and galactose share

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only a single common intestinal transport pathway [20–22].

L-Fucose (6-deoxy-L-galactose) is of considerable interest, since it is not translocated aprpeciably in hamster small intestine, yet it competitively inhibits glucose absorption [1,7]. Bihler [23] found that the action of phlorizin on sugar transport in vitro was of two types: its inhibition is competitive for sugars whose transport is sodium-dependent, but non-competitive for those whose transport is not sodium-dependent. He suggested the possibility of two transport binding sites on the same sugar carrier. The apparent Michaelis constants (K_m^*) for sugar transport and the monosaccharide-induced changes in potential difference across the rat intestine are affected in different ways by hormones, by fasting, and by different sugars [8,10,17].

Holdsworth and Dawson [24] noted inhibition of galactose uptake by glucose in man in vivo, but glucose uptake was not affected by galactose. McMichael [14] also observed a lack of inhibition by glucose on galactose absorption in rat intestine in vivo. Honegger and Semenza [12] postulated that there is a multiplicity of carriers for glucalogues in hamster small intestine since the uptake of 6-deoxyglucose and of 3-*O*-methylglucose was best described not by a single Michaelis-Menten term but rather by the sum of two such terms.

Kimmich and Randles [17] characterized monosaccharide transport in isolated intestinal epithelial cells from chickens: in addition to the usually accepted Na^+-dependent process, a second process functioned in the absence of sodium, had a higher sensitivity to phloretin and lower sensitivity to phlorizin, had a different substrate specificity, and had a higher K_m and maximal transport rate.

In none of these previous studies was the effect of the intestinal unstirred water layer taken into account. It is now clear that neglect of this factor can introduce qualitative isinterpretation as well as inevitable quantitative errors [25]. Accordingly, the present studies were undertaken to investigate the interactions between glucose, galactose and 3-*O*-methyl-glucose, under conditions of thorough stirring of the bulk phase so as to minimize the effective thickness of the intestinal unstirred water layer.

Methods and Materials

1. Animals. Female New Zealand white rabbits 6–8 weeks of age and weighing 2.0–2.5 kg were used. The guiding principles in the use of laboratory animals, approved by the Canadian Federation of Biological Sciences and by the Council of the American Physiological Society, were observed. The animals were allowed access to water and food ad libitum until the morning of the study.

2. Probe and marker compounds. The compound used to measure the adherent mucosal fluid volume, [G-^{3}H]dextran (molecular weight approx. 15000 to 17000), was obtained from New England Nuclear Corporation, Boston, MA. The unlabelled and ^{14}C-labelled glucose, galactose and 3-*O*-methylglucose were supplied by Sigma Co. and by New England Nuclear, respectively.

3. Tissue preparation. The animals were anesthetized with the intraperitoneal injection of sodium thiopental. As described in detail elsewhere [25–27], a 15 cm length of proximal jejunum was rapidly removed and rinsed gently with 50 ml of cold saline (0.9% NaCl). The intestine was opened along its mesenteric border, and the mucosal surface was carefully washed with a stream of cold saline from a syringe to remove visible mucus and debris. Pieces of intestine cut from the segment were mounted as flat sheets in the incubation chambers and were clamped between two plastic plates so that the serosal and mucosal surfaces were exposed to separate incubation solutions. To the serosal compartment was added 1.2 ml of Krebs-bicarbonate buffer [25]. The preincubation chambers contained oxygenated Krebs-bicarbonate buffer at 37°C and pH 7.4. The discs were preincubated for 30 min to allow the tissue to equilibrate at this temperature and for the intervillous spaces to close [25]. Then the transport chambers were transferred to other incubation beakers for specific experiments. The preincubation and incubation solutions were both mixed with circular magnetic bars, and the stirring rates were accurately adjusted to 600 rpm by means of a strobe light. This stirring rate was selected so that the effective thickness and surface area of the unstirred layer would be as already determined [25].

4. Determination of rates of uptake of hexoses. After preincubation, each chambr was transferred to another beaker containing [^{3}H]dextran and the various ^{14}C-probe molecules in oxygenated Krebs-bicarbonate buffer at pH 7.4 and at 37°C. After incubation of the discs for 6 min in the test solutions, the uptake was terminated by removing the chamber and quickly rinsing the tissue in cold saline for approx. 5 s. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch and gently blotted on filter paper. The tissue was placed in tared counting vials for drying overnight in an oven at 75°C. The dry weight was determined, the sample was saponified with 0.75 N NaOH [25], scintillation fluid was added, and the radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes [25]. The amount of ^{14}C radioactivity taken up by each tissue disc was calculated by subtracting the amount present in the adherent mucosal fluid.

5. Individual experiments. In preliminary studies, the rates of uptake of 40 mM glucose, galactose and 3-*O*-methylglucose into the tissue were found to be linear with respect to time over 2–10 min, and extrapolation to zero time gave zero uptake. The volume of adherent mucosal fluid estimated with [^{3}H]dextran was also constant between 2 and 10 min. For uptake experiments, the intestinal tissue was exposed to glucose, galactose, or 3-*O*-methylglucose in concentrations of 0.5, 1.0, 2.0, 5.0, 10.0 or 40.0 mM for an incubation period of 6 min. In some experiments the concentration of one sugar was held constant and the concentraiton of a second sugar was varied. In other studies the effect of variable concentrations of one sugar on the uptake of a second sugar was examined while a fixed concentration of a third sugar was also present.

6. Expression of results. The rate of uptake, J_d, was calculated after correction of the total tissue ^{14}C radioactivity for the mass of the probe molecule present in the adherent mucosal fluid. The uptake rates were expressed as nmol of the probe molecule taken up into the mucosa per min per 100 mg dry weight of tissue (nmol/min per 100 mg). Each data point is reported as the mean ± S.E. of the results for 8–12 animals. The statistical significance of the difference between any two means was determined by an unpaired Student's *t*-test.

7. Kinetic analysis of uptake rates. Each uptake curve (rate of uptake versus hexose concentration) was analysed by weighted non-linear regression as advocated by Gardner and Atkins. The program, based on Marquardt's algorithm permitted fitting of the following models directly to the data: single Michaelis-Menten, single Michaelis-Menten plus linear term, double Michaelis-Menten, double Michaelis-Menten plus linear term, Michaelis-Menten including competitive inhibition, Michaelis-Menten including substrate inhibition, together with the Hill equation and various exponential functions. The program, an extensive modification of that previously used [40], was written in IMP by one of the authors (G.L.A.) and was run interactively on an IBM 2988 computer at the Edinburgh Regional Computer Centre. The main criterion for comparison of models was the weighted sum of squares of residuals, although the runs test and the Akaike information theoretic criterion

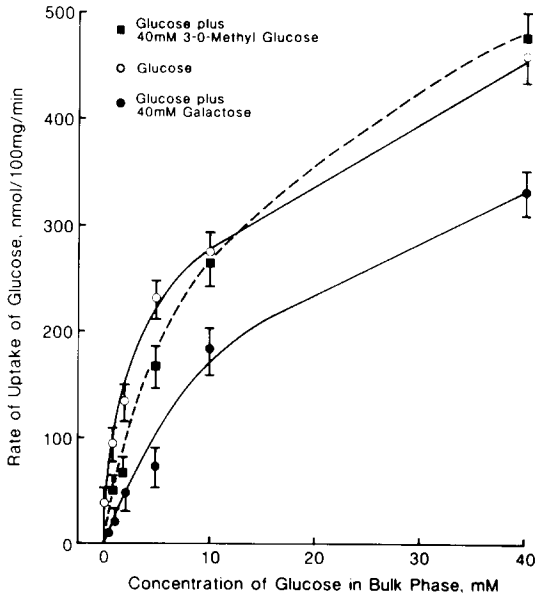

Fig. 1. Effect of 40 mM galactose or 40 mM 3-*O*-methylglucose on jejunal uptake of varying concentrationsof glucose. The concentration of glucose was varied from 0.5 to 40 mM, and a fixed concentration (40 mM) of galactose or 3-*O*-methylglucose was added. The bulk phase was stirred at 600 rpm to reduce the effective resistance of the intestinal unstirred water layer. Each point represents the mean ± S.E. of the results of 9–12 animals.

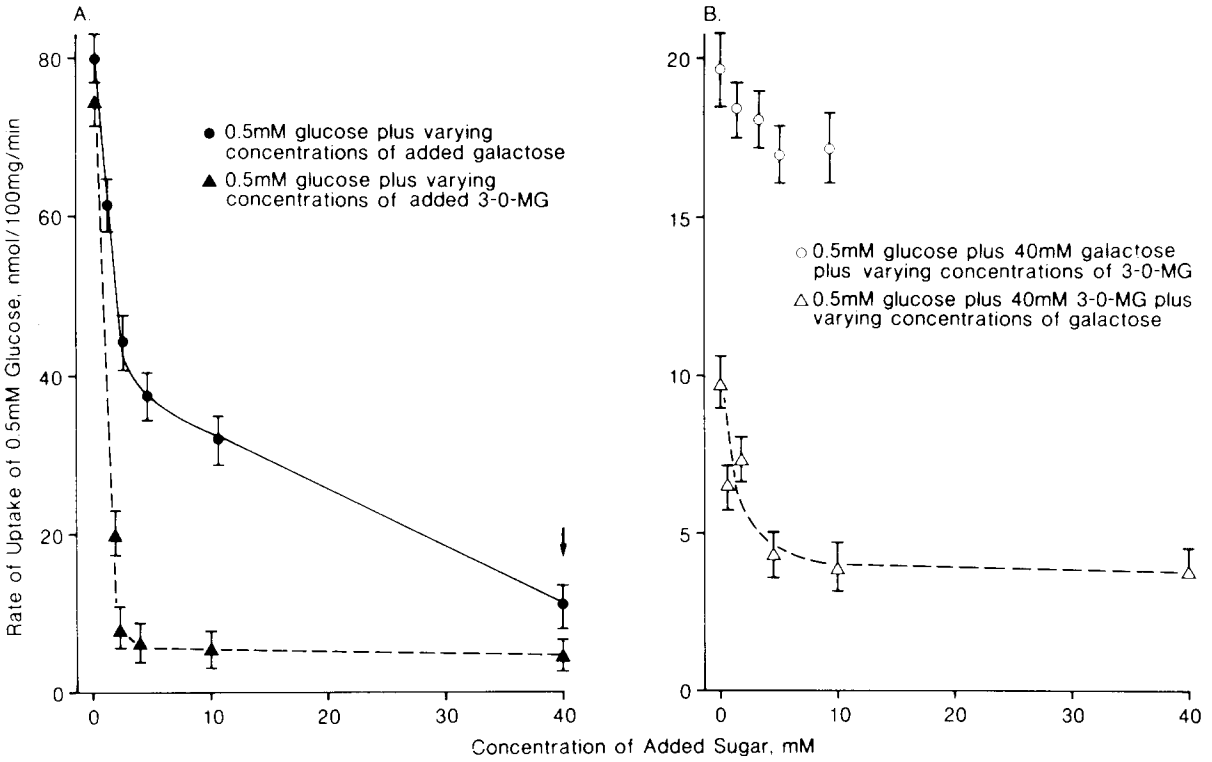

Fig. 2. Effect of varying concentrations of galactose or 3-*O*-methylglucose (3-O-MG) on uptake of 0.5 mM glucose. In panel A, the concentration of added galactose or of added 3-*O*-methylglucose was varied from 1–40 mM, and the concentration of glucose was fixed at 0.5 mM. In panel B is shown both the effect of varying concentrations of 3-*O*-methylglucose on the uptake of 0.5 mM glucose plus 40 mM galactose, and the effect of varying concentrations of galactose on the uptake of 0.5 mM glucose plus 40 mM 3-*O*-methylglucose.

were also taken into account. Each uptake curve was examined also by replotting the data according to Lineweaver-Burk, Hanes and Hofstee linear transformations. Curvilinearity of these, assessed by variance ratio tests and a non-parametric method as well as visual inspection, was taken to indicate inadequacy of the single Michaelis-Menten function with or without an additional term for competitive inhibition, although work in progress suggests that this criterion is sensitive to the error structure in the data and so may not invariably be infallible (Gardner, M.L.G. and Atkins, G.L., unpublished data).

Results

1. Uptake of glucose

(a) Glucose alone. The concentration of glucose in the bulk phase was varied from 0.5 to 40 mM and the rate of uptake of glucose into the rabbit jejunum was determined with the bulk phase stirred at 600 rpm (Fig. 1). An apparently saturable relationship was noted between glucose concentration and uptake (Fig. 1), but kinetic analysis showed that the single Michaelis-Menten equation was not appropriate. In particular, Hofstee, Hanes and Lineweaver-Burk plots were each curvilinear.

(b) Glucose plus one other sugar. The addition of 40 mM galactose or 40 mM 3-*O*-methylglucose caused a decline in the rate of uptake of glucose at each concentration of glucose, but the relationship between glucose concentration and uptake remained curvilinear. Galactose (40 mM) inhibited glucose uptake at each concentration of glucose, whereas an inhibitory effect of 40 mM 3-*O*-methylglucose was seen only at the lowest concentration of glucose (0.5 mM).

The concentration of glucose was then held constant at 0.5 mM, and either galactose or 3-*O*-methylglucose at various concentrations between 1

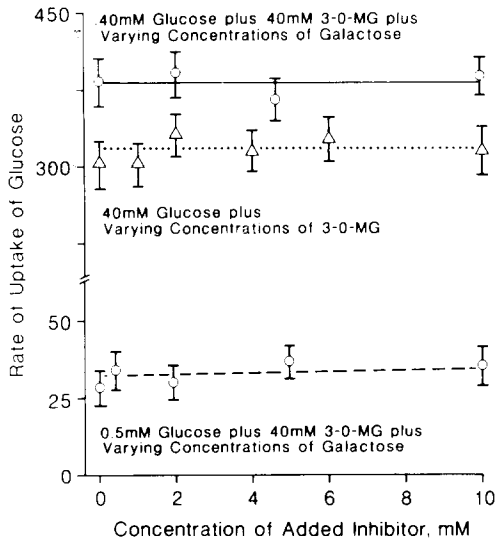

Fig. 3. Effect of varying concentrations of galactose or 3-*O*-methylglucose (3-O-MG) on uptake of 40 mM glucose. The concentration of glucose was fixed at 40 mM, and 1–40 mM 3-*O*-methylglucose or 1–40 mM galactose plus 40 mM 3-*O*-methylglucose were added.

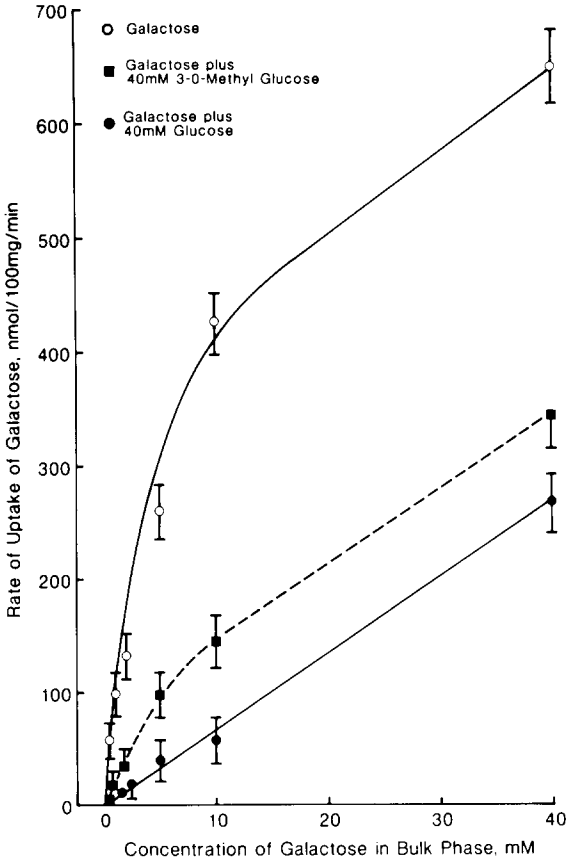

Fig. 4. Effect of 40 mM glucose or 40 mM 3-*O*-methylglucose on uptake of varying concentrations of galactose. The concentration of galactose was varied from 0.5 to 40 mM, and either 40 mM glucose or 3-*O*-methylglucose was added.

mM and 40 mM was added to the bulk phase (Fig. 2A). The jejnal uptake of glucose from the 0.5 mM solution was reduced by the addition of galactose or 3-*O*-methylglucose at each concentration between 1 and 40 mM, but the pattern of the reduction by the two sugars was different: 3-*O*-methylglucose had a dramatic effect on glucose uptake when low concentrations (1–5 mM) of 3-*O*-methylglucose were added, but at higher concentrations (10 and 40 mM) of 3-*O*-methylglucose, there was little further inhibition in the uptake of glucose from the 0.5 mM solution. In contrast, the addition of galactose (1–40 mM) caused a progressive and continued decline in glucose uptake as the galactose concentration was increased to 40 mM. Glucose uptake from the 0.5 mM glucose medium was lower in the presence of 40 mM 3-*O*-methylglucose than in the presence of 40 mM galactose (Fig. 2A).

(c) Glucose plus two other sugars. The addition of galactose (1–40 mM) to solutions containing 0.5 mM glucose together with 40 mM 3-*O*-methylglucose caused a further decline in glucose uptake (Fig. 2B); the relationship between the galactose concentration and the inhibition of glucose uptake in the presence of 3-*O*-methylglucose was curvilinear. The addition of 3-*O*-methylglucose (1–40 mM) to solutions containing both 0.5 mM glucose and 40 mM galactose produced no further decrease in glucose uptake (Fig. 2B).

Various concentrations (0.5 and 10 mM) of 3-*O*-methylglucose had no effect on the rate of glucose uptake at a glucose concentration of 40 mM (Fig. 3). Various concentrations of galactose in the presence of 40 mM 3-*O*-methylglucose had no inhibitory effect on glucose uptake at either 40 mM or 0.5 mM glucose concentration. Likewise, various concentrations of galactose had no significant effect on the uptake of 40 mM glucose and 2–40 mM 3-*O*-methylglucose had no significant effect on the uptake of 40 mM glucose in the presence of 40 mM galactose (not shown).

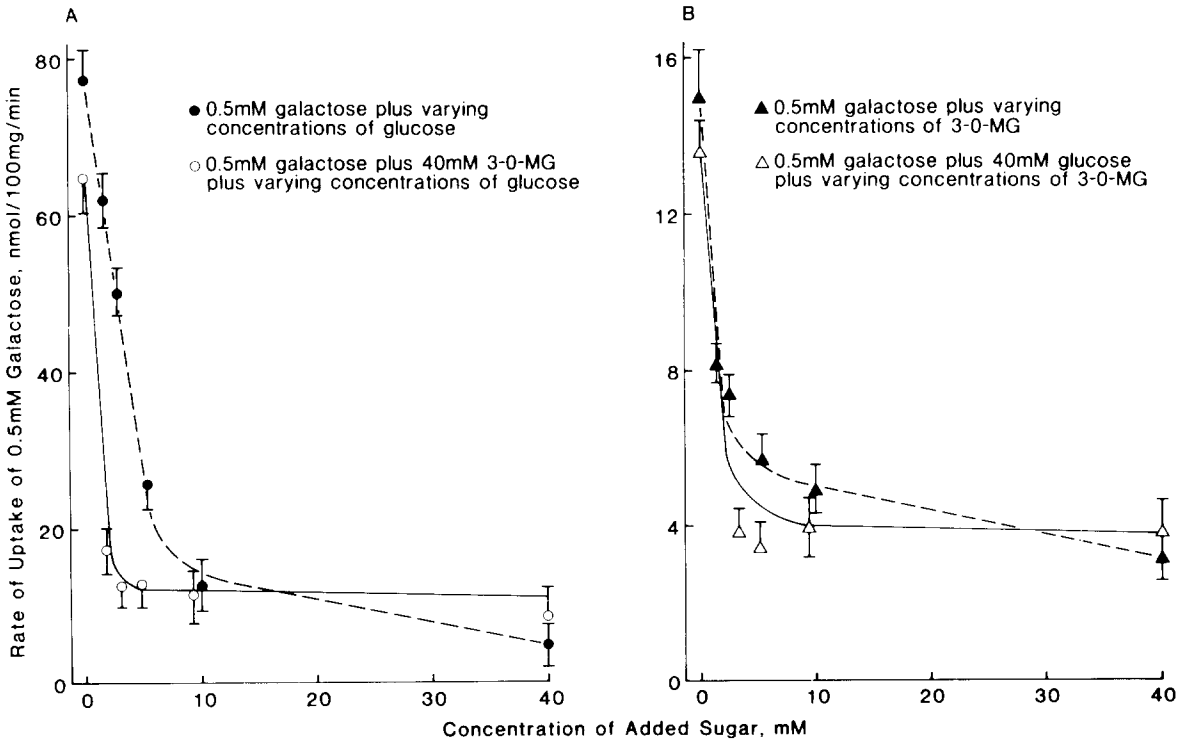

Fig. 5. Effect of varying concentrations of glucose or 3-*O*-methylglucose (3-O-MG) on uptake of 0.5 mM galactose. In panel A, the concetration of galactose was fixed at 40 mM and the concentration of added glucose or 3-*O*-methylglucose was varied from 1 to 40 mM. In panel B is shown the effect of varying concentrations of glucose on the uptake of 0.5 mM galactose plus 40 mM 3-*O*-methylglucose, and the effect of varying concentrations of 3-*O*-methylglucose on the uptake of 0.5 mM galactose plus 40 mM glucose.

2. Uptake of galactose

(a) Galactose alone. The relationship between the concentration of galactose (0.5–40 mM) and rat of galactose uptake was curvilinear (Fig. 4), but Hofstee, Hanes and Lineweaver-Burk plots were each curvilinear, indicating non-conformity to a single Michaelis-Menten equation.

(b) Galactose plus one other sugar. When the experiments were repeated in the presence of 40 mM glucose there was a reduction in galactose uptake rate at each concentration of galactose and the relationship between galactose uptake and concentration became linear. The uptake of galactose at each concentration was inhibited also by 40 mM 3-*O*-methylglucose, but the inhibition of galactose uptake by 40 mM 3-*O*-methylglucose was less than that by 40 mM glucose: i.e. glucose (40 mM) was a stronger inhibitor of galactose uptake than 3-*O*-methylglucose (40 mM).

(c) Galactose plus two other sugars. In the next series of experiments, varying concentrations (1–40 mM) of either glucose or 3-*O*-methylglucose were added to 0.5 mM galactose (Fig. 5). A curvilinear relationship was noted between the rate of uptake of the galactose and the concentration of added glucose or of added 3-*O*-methylglucose (Figs. 5A and 5B). The inhibition of galactose uptake by glucose was greater than that by 3-*O*-methylglucose at concentrations of 10 and 40 mM, whereas the converse was true at concentrations of 1 and 2 mM. The addition of 3-*O*-methylglucose (1 to 40 mM) to solutions containing both 0.5 mM galactose plus 40 mM glucose produced a progressive decline in galactose uptake as the 3-*O*-methylglucose concentration was increased (Fig. 5B). Similarly, when glucose was added to solutions containing 0.5 mM galactose plus 40 mM 3-*O*-methylglucose, there was a progressive decline in galactose uptake as the glucose concentration was increased from 1 to 40 mM, but the extent of the

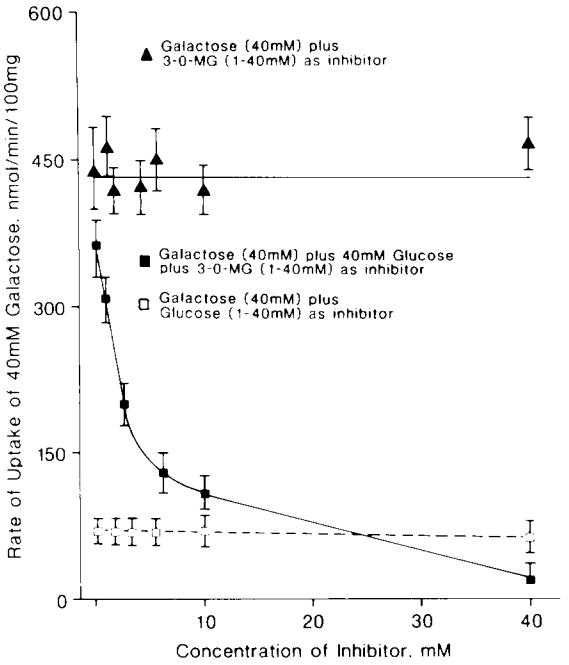

Fig. 6. Effect of varying concentrations of glucose or 3-*O*-methylglucose (3-O-MG) on uptake of 40 mM galactose. The concentration of added glucose or 3-*O*-methylglucose was varied from 1 to 40 mM, and the concentration of galactose was fixed at 40 mM. In one study, the concentration of galactose was 40 mM, and 1–40 mM 3-*O*-methylglucose plus 40 mM glucose was added (lower broken line).

inhibition was greater (Fig. 5A).

The addition of 3-*O*-methylglucose at concentrations up to 40 mM had no effect on the uptake of 40 mM galactose (Fig. 6, upper unbroken line). Also, the addition of 3-*O*-methylglucose (1–40 mM) had no effect on the uptake of 40 mM galactose in the presence of 40 mM glucose (Fig. 5A, lower dashed line). In contrast, the addition of glucose (1–40 mM) caused a reduction in the uptake of 40 mM galactose (Fig. 6, middle curve). This inhibition was proportionally greater for lower than for higher concentrations of glu-

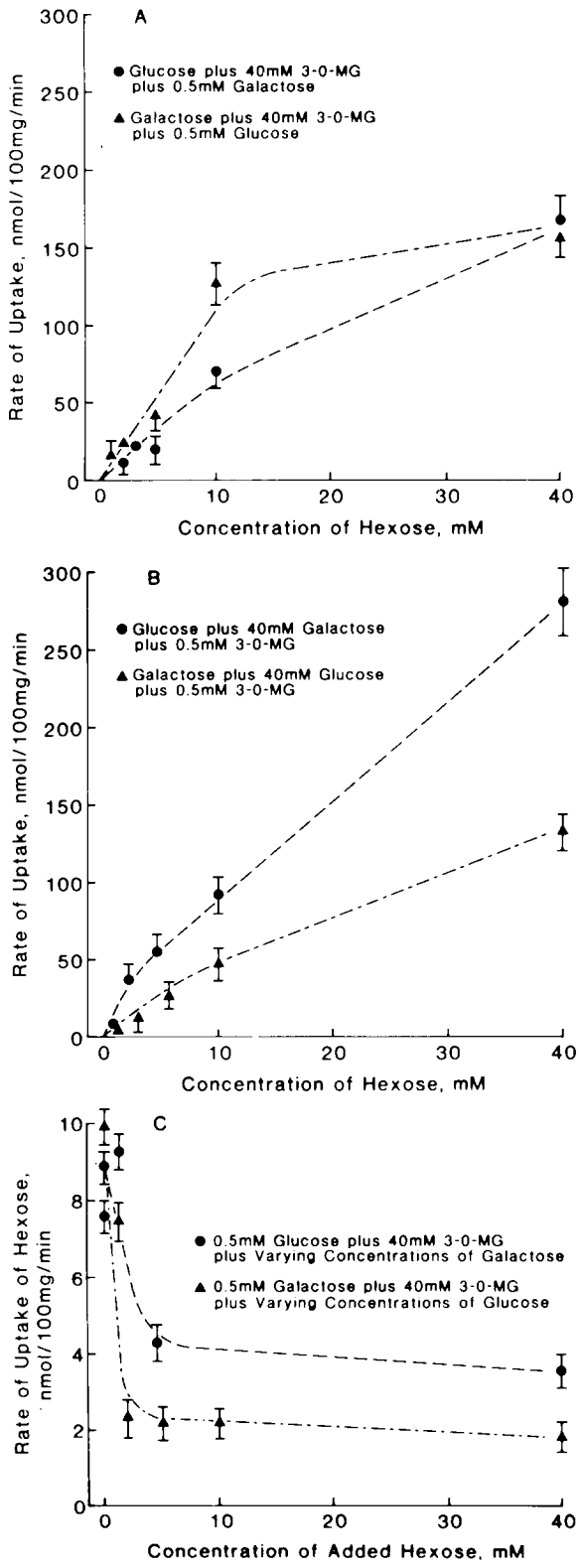

Fig. 7. Comparison of uptake of glucose and galactose in presence of two sugars. The concentration of glucose or galactose was varied from 0.5 to 40 mM and combinations of two sugars were added: 0.5–40 mM glucose plus 40 mM galactose plus 0.5 mM 3-*O*-methylglucose (3-O-MG); 0.5–40 mM glucose plus 40 mM 3-*O*-methylglucose plus 0.5 mM galactose; 0.5–40 mM galactose plus 40 mM glucose plus 0.5 mM 3-*O*-methylglucose; or galactose plus 40 mM 3-*O*-methylglucose plus 0.5 mM glucose.

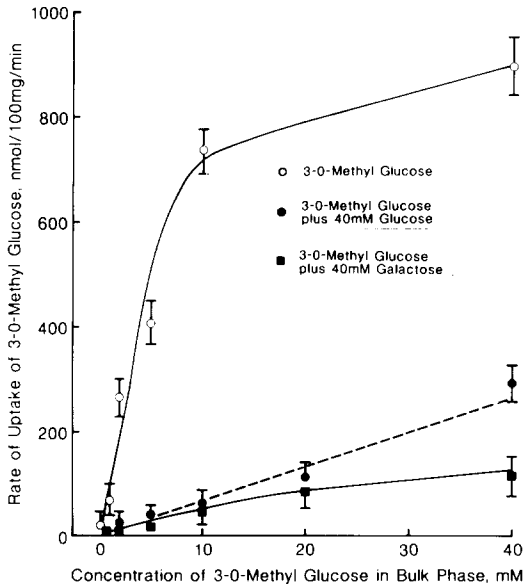

Fig. 8. Effect of addition of 40 mM glucose or 40 mM galactose on the jejunal uptake of varying concentrations of 3-*O*-methylglucose. The concentration of 3-*O*-methylglucose was varied from 0.5 to 40 mM and 40 mM glucose or galactose was added to each of the different concentrations of 3-*O*-methylglucose.

cose. The uptake of 40 mM galactose was greater in the presence of 40 mM 3-*O*-methylglucose than in the presence of 40 mM glucose or 40 mM glucose plus 40 mM 3-*O*-methylglucose.

The uptake of galactose at a concentration of 40 mM was greater in the presence of 40 mM 3-*O*-methylglucose than in the presence of 40 mM glucose (Fig. 4). The uptake of glucose at each concentration below 40 mM was lower in the presence of 40 mM 3-*O*-methylglucose plus 0.5 mM galactose than was the uptake of galactose at the corresponding concentration in the presence of 40 mM 3-*O*-methylglucose plus 0.5 mM glucose (Fig. 7). The uptake of glucose at each concentration up to 40 mM was higher in the presence of 0.5 mM 3-*O*-methylglucose plus 40 mM galactose than was the uptake of 1–40 mM galactose in the presence of 0.5 mM 3-*O*-methylglucose plus 40 mM glucose (Fig. 7B). However, the relative ratios of the uptake of combinations of the three sugars depended on their concentrations. For example, when the concentration of glucose was 0.5 mM and 3-*O*-methylglucose (40 mM) plus varying concentrations (1–40 mM) of galactose was added, the uptake of glucose was reduced in a curvilinear fashion (Fig. 8C, upper curve). The uptake of 0.5 mM galactose was also inhibited in a curvilinear fashion by the addition of 1–40 mM glucose in the presence of 40 mM 3-*O*-methylglucose (Fig. 8B, lower curve). Lesser inhibition was produced on the uptake of 0.5 mM glucose in the presence of 40 mM 3-*O*-methylglucose when 1–40 mM galactose was added.

3. Uptake of 3-O-methylglucose

(a) 3-O-Methylglucose alone. There was a curvilinear relationship between the concentration and uptake rate of 3-*O*-methylglucose (Fig. 8). Hofstee, Hanes and Lineweaver-Burk plots were curvilinear.

(b) 3-O-Methylglucose plus one other sugar. The addition of either 40 mM glucose or 40 mM galactose inhibited the uptake of 3-*O*-methylglucose. Glucose and galactose at varying concentrations up to 40 mM each had a similar inhibitory effect on the uptake of 0.5 mM 3-*O*-methylglucose; this effect was proportionally greater for lower than for higher concentrations of inhibitory sugar (Fig. 9A).

(c) 3-O-Methylglucose plus two other sugars. The uptake of 3-*O*-methylglucose (0.5 mM) was no further inhibited when 1–40 mM glucose was added to the solutions already containing 40 mM galactose together with the 0.5 mM 3-*O*-methylglucose. Similarly, the uptake of 3-*O*-methylglucose was not further inhibited when 1–40 mM galactose was added to the solutions containing 0.5 mM 3-*O*-methylglucose plus 40 mM glucose (data not shown). At a 3-*O*-methylglucose concentration of 40 mM either glucose or galactose at varying concentrations up to 40 mM caused a reduction in the rate of 3-*O*-methylglucose uptake (Fig. 9B). This is in sharp contrast to the lack of inhibitory effect of galactose or 3-*O*-methylglucose on the uptake of 40 mM glucose (Fig. 3), and to the lack of effect of 3-*O*-methylglucose on the uptake of 40 mM galactose (Fig. 6). The relationship between the concentration of added glucose or galactose and the rate of uptake of 40 mM 3-*O*-methylglucose was curvilinear, and the uptake of 40 mM 3-*O*-methylglucose in the presence of glucose was similar to the uptake of 40 mM 3-*O*-methylglucose in the presence of galactose.

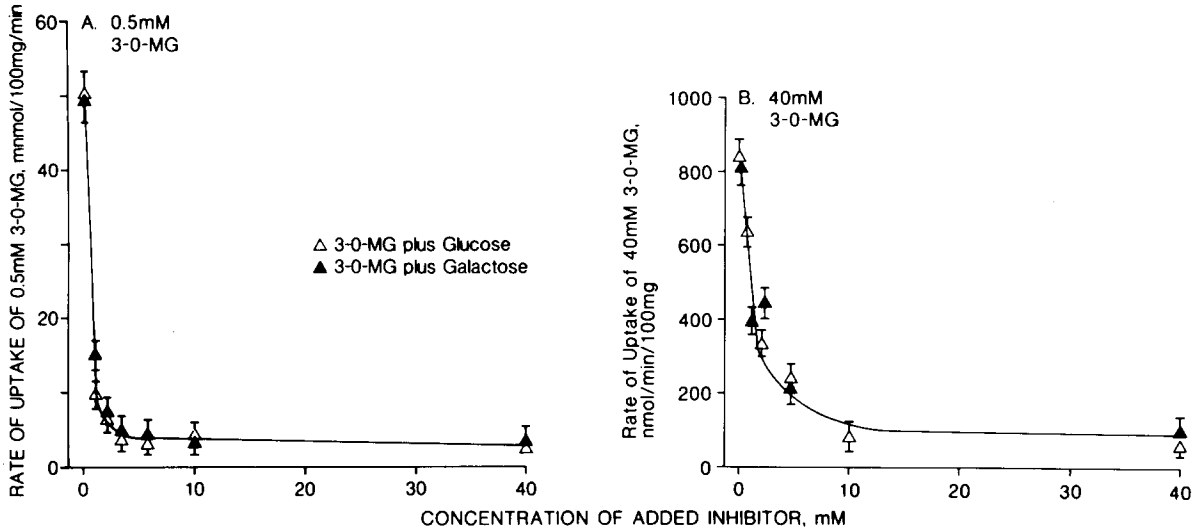

Fig. 9. Effect of varying concentrations of glucose or galactose on uptake of 3-*O*-methylglucose (3-O-MG). In panel A, the concentration of 3-*O*-methylglucose was 0.5 mM, and in panel B the concentration of 3-*O*-methylglucose was 40 mM. The concentration of added glucose or galactose was varied from 1 to 40 mM, and the concentration of 3-*O*-methylglucose was fixed at 0.5 mM or 40 mM.

The uptake of 0.5 mM 3-*O*-methylglucose in the presence of 40 mM galactose was no further inhibited by adding glucose up to 40 mM. Also, the uptake of 0.5 mM 3-*O*-methylglucose in the presence of 40 mM glucose was not further inhibited by adding galactose at up to 40 mM concentration (Fig. 10, lower solid line). The uptake of 40 mM 3-*O*-methylglucose in the presence of 40 mM galactose was no further inhibited by adding 1–40 mM glucose (Fig. 10, upper dashed line). Similarly, the uptake of 40 mM 3-*O*-methylglucose in the presence of 40 mM glucose was no further inhibited by adding 1–40 mM galactose (Fig. 10, middle dotted line).

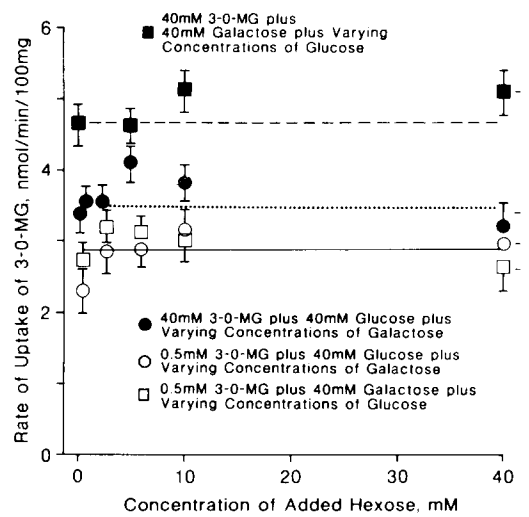

Fig. 10. Effect of combinations of galactose and glucose on uptake of 3-*O*-methylglucose (3-O-MG). The concentration of 3-*O*-methylglucose was fixed at either 0.5 mM or 40 mM, and either 40 mM galactose plus 1–40 mM glucose or 40 mM glucose plus 1–40 mM galactose was added.

4. Kinetic analysis

Lineweaver-Burk, Hanes and Hofstee linear transformations of the data were examined in order to assess conformity to the single Michaelis-Menten model. Because the three linearizations each emphasize a different region of the 'raw' curve, they were all examined. Each plot was obviously non-linear, suggesting that the single Michaelis-Menten function or single Michaelis-Menten function plus competitive inhibition was not appropriate for the data, and this was confirmed by the non-linear regression analyses. None of the other models examined were consistently satisfactory fits to the data; hence it is not appropriate to provide values for 'apparent K_m' or 'apparent V_{max}' etc. Since the linear transformations produced smooth curves, it is likely that this

represents real incompatibility between the simple models examined and the data rather than this being simply a reflection of data error.

Discussion

This work shows that complex interactions occur between the three sugars (glucose, galactose and 3-*O*-methylglucose) in their transport into rabbit jejunal mucosa under conditions of low unstirred layer resistance. These conditions allow a more accurate analysis of the transport interactions than is possible if the unstirred layer is neglected, as has been the case in almost all previously published work on interactions in sugar transport. Indeed, the present experiments give a qualitatively (as well as quantitatively) different pattern of interactions to that previously reported. As explained below, there are inhibitory cross-interactions between each of the three hexoses, but these cannot be simply explained on the basis of one or even two carriers – at least not within the constraints of conventional membrane-carrier theory.

Uptake of glucose, galactose and 3-*O*-methylglucose might in principle be mediated by: (i) three separate and mutually exclusive membrane-carriers, (ii) a single common carrier, or (iii) two or more carriers, some or all of which are shared by the three hexoses. The first of these possibilities, i.e. three separate carriers, one for each hexose, can probably be eliminated immediately: the kinetics of uptake of each hexose by itself do not conform to a single Michaelis-Menten equation. This is clear both from the non-linear Lineweaver-Burk, Hanes and Hofstee plots and from the kinetic analysis by non-linear regression. Further, the sugars do mutually reduce each other's uptake. Theoretically, this could be due to effects of one sugar (the putative inhibitor) on the trans-membrane potential and/or the Na^+-ion influx, rather than due to competition for a common binding site on a carrier. However, the absence of single-carrier Michaelis-Menten kinetics for each of the three sugars studied alone suggests strongly that this cannot be the explanation and therefore that there is more than one carrier.

The second possibility, that there is a single common carrier, can equally be eliminated from consideration: again, kinetics conforming to a single Michaelis-Menten equation would be expected. Also, the interactions between pairs of sugars would be expected to be competitive, although secondary effects mediated via changes in trans-membrane potential and/or the Na^+-ion influx could be superimposed on this.

Hence, it appears that each of the three hexoses must be transported by multiple (at least two) carriers, at least one of which is shared. All carriers are not, however, necessarily shared by all hexoses. The first common carrier with different affinities for the three sugars would explain the mutual inhibitory action of the sugars. However, there is a need to postulate a second carrier for galactose. First, previous work has shown that the maximal transport rate (J_d^m) is higher for galactose than for glucose [27,28], suggesting more transport sites for galactose. Second, at a substrate concentration of 40 mM, the previously suggested value for the Michaelis constant would be exceeded by over 10-fold and all transport sites would be occupied [29]. Although 3-*O*-methylglucose inhibited galactose uptake from 0.5 mM galactose (Fig. 5), the uptake of 40 mM galactose was unaffected by adding up to 40 mM 3-*O*-methylglucose (Fig. 6). This suggests that there is a second carrier for galactose with some affinity for glucose but little affinity for 3-*O*-methylglucose. If this were the case, then why did 1–40 mM galactose, 1–40 mM 3-*O*-methylglucose, or 1–40 mM galactose plus 40 mM 3-*O*-methylglucose, not have any effect on the uptake of 40 mM glucose (Fig. 3)? Presumably the second carrier for galactose has a suffiicently low affinity for glucose that relatively little glucose is transported by this pathway, and thus galactose would have little demonstrable effect on the uptake of high concentrations of glucose. If there are two carriers for glucose, then presumably the 'major' glucose carrier has the higher J_d^m value. Most glucose will be transported on the high-affinity, high-capacity carrier, which is also likely to be the carrier shared by glucose, galactose and 3-*O*-methylglucose. From this study, it was not possible to estimate values for the kinetic constants for the two postulated galactose carriers.

One further problem remains for 3-*O*-methylglucose. The estimated value for the apparent

maximal transport rate for 3-*O*-methylglucose is higher than that for glucose [27,28], suggesting that there may be more carrier for 3-*O*-methylglucose than for glucose. However, the inhibitory effect of these sugars on the uptake of 3-*O*-methylglucose is similar for low (Fig. 9A) and for high (Fig. 9B) concentrations of 3-*O*-methylglucose. This would be surprising, given the suggested differences in the values of the apparent Michaelis constants, unless there were an additional sugar carrier for 3-*O*-methylglucose, with directionally dissimilar affinities for glucose and galactose. In this way, glucose and galactose would demonstrate a comparable inhibitory effect on 3-*O*-methylglucose, despite the differences in their apparent affinity for the first carrier shared by glucose, galactose and 3-*O*-methylglucose. Indeed, the finding of the lack of further inhibitory effect of 1–40 mM glucose plus 40 mM galactose on the uptake of 0.5 mM 3-*O*-methylglucose, and the observation of a lack of an inhibitory effect of 1–40 mM galactose plus 40 mM glucose on the uptake of 3-*O*-methylglucose (Fig. 10), supports this suggestion for a second carrier for 3-*O*-methylglucose. However, if there were a separate carrier for 3-*O*-methylglucose with low affinity for glucose and galactose, then it would have been expected that the addition of 1–40 mM glucose plus 40 mM galactose to 40 mM 3-*O*-methylglucose would have resulted in further inhibition of 3-*O*-methylglucose uptake. Such was not the case. Previous studies have suggested that the J_d^m for 3-*O*-methylglucose is as great as the J_d^m for galactose [27,28], yet as outlined above, 3-*O*-methylglucose does not appear to share the second carrier for galactose. Thus, it is necessary to postulate that there is a third sugar carrier which is predominantly used by 3-*O*-methylglucose and which has a very low affinity for glucose and for galactose.

The simplest model compatible with all these observations is one with three carriers, and the following model is proposed. Carrier I is a common carrier that transport glucose, galactose, and 3-*O*-methylglucose. Carrier II transports predominantly galactose, but also some glucose and even less 3-*O*-methylglucose. Carrier III transports mainly 3-*O*-methylglucose and little or none of the other two sugars. This model allows for a number of predictions. Intestinal carriers appear to be influenced by dietary intake [30,31–34]; for example, feeding additional glucose increases the J_d^m for glucose and galactose but not for fructose, whereas feeding fructose increases the intestinal uptake of fructose but not of glucose [34]. Second, this model predicts that the ratio of the J_d^m for the three sugars will reflect differences in the numbers of their carriers. Such differences have already been described in differet species [27,28,35]. Third, this model predicts that the effect of certain manipulations may very in their effect on the uptake of the different sugars. This has been observed for the effect of ethanol on sugar intake [36], and for the effect of intestinal resection [37]. Finally, the model is compatible with the known defect in congenital galactose malabsorption in which neither glucose nor galactose absorption occurs [38,39].

Proof of this model, however, will not be easy and is almost certainly beyond the power of kinetic analysis [40]. A three-carrier model with each carrier being shared could lead to an equation with at least nine parameters (J_d^m, K_m, K_i); it is very unlikely that this could reliably be fitted even with large numbers of experimental observations of exceptionally high precision, because the 'contour map' of the sum of squares of residuals for such a complex non-linear model almost certainly has very gradual gradients and a 'shallow trough' in the vicinity of the minimum which therefore cannot be located with confidence. Thus, at best, interpretation of a kinetic model in terms of an actual mechanism of transport is fraught with hazard [40].

Finally, another type of model appears to be consistent with these data: it is novel and merits consideration. It is known that the ability of intestinal cells to transport sugars and other solutes develops and increases as cells mature during their passage up the villus. The nature of this maturation is not known, but it does not necessarily simply reflect de novo synthesis of further carriers and a corresponding increase in J_d^m while K_m remains constant. The presence of these developing carrier systems during cell maturation constitutes a heterogeneous carrier-system. If the affinity of a carrier for a particular sugar does change during the villus migration, then a true one-carrier system would behave kinetically as a

multiple-carrier system. Hence it is possible that the three-carrier model postulated above could be accounted for by a single-carrier model in which the mature carrier in cells at the tips of the villi transports all three sugars, i.e. behaves as carrier I above. The carrier in the relatively immature cells at the base of the villi would behave as carrier III, while carrier II would represent an intermediate degree of maturity.

While these experiments do not permit us to conclude with a definitive model, they do provide further insight into the complexities of transport interactions. Also, they make clear the need for studies of this nature to be performed under conditions of effective luminal mixing so as to diminish the unstirred water layer as far as possible. Neglect of this leads to qualitative (as well as quantitative) distortion of interactions. We suggest that either a three-carrier model or one in which the affinities of a single common carrier develop or change differentially for each substrate during cell maturation are the simplest models consistent with these observations.

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