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STUDIES ON THE TRANSPORT OF GLUCOSE FROM DISACCHARIDES BY HAMSTER SMALL INTESTINE *IN VITRO**

I. EVIDENCE FOR A DISACCHARIDASE-RELATED TRANSPORT SYSTEM

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SUMMARY

In the presence of saturating concentrations of free D-glucose, total glucose uptake was enhanced beyond the theoretical V for free glucose uptake when disaccharides were incubated with intestinal rings. This phenomenon was not seen when glucose 1-phosphate was the substrate.

Analogs of D-glucose transport system, galactose and β-methyl glucoside, had an inhibitory effect on glucose uptake from sucrose and their uptake was in turn inhibited by the disaccharide. This inhibition was non-competitive.

The effect of phlorizin on glucose uptake from sucrose was 2-fold, competitive at low concentrations and non-competitive at high concentrations.

Sucrose did not induce counterflow of the preloaded β-methyl glucoside.

These observations indicate that with a disaccharide as the substrate, there is a component of glucose transport which is in addition to the monosaccharide-transport system and that this could arise as a consequence of the association of disaccharidases with the brush border membrane.

INTRODUCTION

It may by now be taken to be firmly established that the terminal enzymatic steps in the digestive release of monosaccharides and the carrier-mediated steps initiating their absorption both occur at the brush border membrane. The basis for this view is rather broad. With respect to the enzymes, studies of the distribution within intact tissue of the products of hydrolysis by sucrase, maltase and alkaline phosphatase, with transport inhibited as compared with controls, have indicated that the enzymes are external to the transport processes and presumably located at the outer surface of the membrane[1–4]. This indication has been corroborated by

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histochemical studies of several kinds[5–7] as well as by the continued presence and increased specific activity of these enzymes in isolated microvillus membranes[8–10]. With respect to the carrier-mediated steps, studies by microdissection and assay of incubated tissue showed that sugar accumulated by *in vitro* preparations of hamster small intestine was present at its highest concentration within the epithelial cells, consistent with the absorptive process for sugars lying within the brush border region[11]. The application of autoradiographic techniques at the level of the light and electron microscopes have shown the plasma membrane covering the microvilli to be the most probable site where the concentration gradient accompanying sugar absorption is actually created[12–14].

Taking it as established that the functional components subserving the sequential processes of digestion and transport are in the same membrane, it has also been inferred that they are more than randomly oriented to one another[15]. In early studies Miller and Crane[2] found that incubation of hamster intestinal tissue with sucrose gave rise in early time periods to an accumulation of fructose, suggesting that hydrolysis had occurred at a locus where diffusion of free monosaccharide into the tissue was more efficient than diffusion into the medium. They also found that addition of glucose oxidase to the medium led to a large reduction of the tissue accumulation of glucose released from glucose 1-phosphate by the action of alkaline phosphatase whereas the accumulation of glucose released from sucrose by sucrase was not appreciably affected. These results were interpreted to indicate a locus for sucrase closer to the transport carriers than for alkaline phosphatase[15].

Further studies on the relationship of the digestive enzymes to glucose transport are the subject of this report. Our current results indicate the presence of a disaccharidase-related component of monosaccharide transport which is in addition to and different from the well documented Na^+-dependent glucose-transport system[16]. Generally, we have observed that under conditions in which the glucose carrier is made maximally active by addition of a high concentration of glucose, the further addition of any one of several disaccharides leads to an additional accumulation of glucose within the tissue. The phenomenon does not occur with glucose 1-phosphate. Our observations differ from those of studies made with an *in vitro* perfused preparation from amphibian small intestine[17] wherein the results indicate that the processes of disaccharide hydrolysis and transfer of released hexose units can be readily and completely dissociated from one another.

MATERIALS AND METHODS

All compounds except isomaltose were obtained from the following commercial sources: D-glucose from Matheson, Coleman and Bell; β-methyl glucoside and D-galactose from Sigma Chemical Co.; phlorizin from Calbiochem; sucrose and fructose from Fisher Scientific Corp.; maltose from General Biochemicals, trehalose and other sugars from Pfanstiehl Labs, Inc.; D-[U-^{14}C]glucose, D-[U-^{14}C]galactose, [^{14}C]sucrose–([U-^{14}C]glucose) and D-[U-^{14}C]mannitol were obtained from New England Nuclear Corp. [^{14}C]glucose had a specific activity of 14.1 Ci/mole, [^{14}C]-galactose 42.4 Ci/mole and [^{14}C]sucrose 243 Ci/mole. β-Methyl [U-^{14}C]glucoside was obtained from Calbiochem and had a specific activity of 55 Ci/mole.

Isomaltose was prepared by incubating 100 g Dextran-40 (Pharmacia) in 500 ml

of 0.17 M potassium phosphate buffer, pH 6.0, with 5 mg of Dextranase (21 units/mg; Worthington Biochemical Corp.) at 37 °C for 12 h. This was followed by chromatography on carbon–celite columns and elution of the glucose-free isomaltose with 5% ethanol as described by Whistler and Durso[18].

Incubation methods

Small intestines from hamsters, 9–10 weeks old, that had free access to food and water were used in two kinds of everted preparation; namely, (1) rings of everted intestine as conventionally used in our laboratory[20] and (2) everted segments of intestine tied off so that the serosal side was rendered inaccessible to substrate. The latter were made by everting the intestine onto a polyethylene tubing (PE 280), tying off small segments to the tubing and then cutting between ties. Use of the tubing which remained in place during incubation made segment preparation easier and more rapid than with the more common everted sac technique. However, rings are still much easier and more rapid to use. As we could find no observable difference between the results with the two procedures, the rings were most frequently used.

Certain modifications of the incubation procedures as ordinarily used in this laboratory[20] were developed. About 0.3 g total weight of the everted preparations were incubated in 50.0 ml of buffer contained in 125 ml of erlenmeyer flasks gassed with O_2. Incubations were made in a shaking incubator having a total horizontal excursion of about 1.5 inches at the maximal rate of 200 cycles/min. These special details were introduced in efforts, (1) to minimize local build-up of the concentration of glucose released by disaccharidase activity at the membrane and, (2) to insure ready access of substrate to the transport system and the enzymes of the membrane. The duration of incubation was rarely made longer than 2 min and frequently less, inasmuch as a short time period would minimize the influence of intracellular accumulation on the results. Corrections for "extracellular" monosaccharide were made by use either of [^{14}C]mannitol as before[20] or of phlorizin. For the latter, control flasks were arranged to contain, in addition to the test substrates, concentrations of phlorizin that inhibit glucose uptake completely but are without effect on disaccharidase activity. Neither method is ideal. When disaccharides were used as substrates, extracellular glucose measured by the use of mannitol is surely an undercorrection owing to the higher concentration of glucose at the site of its release by hydrolysis than in the medium. On the other hand, the use of phlorizin to prevent glucose entry and thus to estimate the local glucose concentration in the medium adhering to the tissue is just as surely an overcorrection owing to the higher local concentration of glucose at the membrane surface which would result from eliminating entry into the tissue as one route for dissipation of this concentration. Phlorizin correction was preferred because it was an over- rather than an undercorrection. When using monosaccharides, the phlorizin correction and the mannitol correction were virtually the same.

When higher concentrations of substrate were used, mannitol was added to control flasks to maintain comparable osmolarity. All incubations were done in duplicate and repeated at least three times.

Analytical methods

Tissue and media were processed for assay as described by Crane and Mandel-

stam.[20] Glucose was assayed by the glucose oxidase method[21,22] and reducing sugar according to Somogyi[23]. Radioactivity was measured by a Beckman liquid scintillation counter, using the medium prepared as described by Patterson and Green[24]. Results are all expressed as rate of entry in μmoles of sugar accumulated per ml of tissue water in a specified time, assuming a water content of 80% of the wet tissue weight[20]. All the values were corrected for endogenous and extracellular glucose.

RESULTS AND DISCUSSION

Maximal rate of glucose transport

In order to carry out the projected studies with glucose and added disaccharide, it had first to be demonstrated that a maximal transport rate could actually be achieved with high concentrations of glucose, though it is well known that the process of glucose accumulation in hamster intestine *in vitro* has a K_m of about 1.5 mM[25]. This was not previously done. Hence, hamster intestinal rings were incubated in buffer containing increasing concentrations of D-glucose and the uptake was measured over a 2-min period. As shown in Fig. 1, uptake reached a maximum at 30 mM and did not further increase with increases in the concentrations of glucose up to at least 75 mM. In a separate experiment, not shown, maximal rate did not further increase with increases to 100 mM. This result is approximately to be predicted from a K_m for glucose transport of 1.5 mM, using the expression

$$v = \frac{V[S]}{K_m + S}$$

and provides a measure of confidence that extra glucose which may be found in the

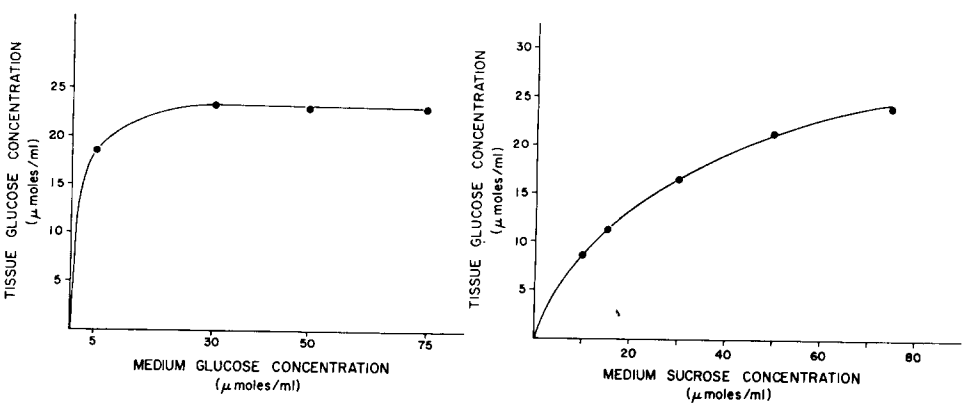

Fig. 1. Uptake of glucose by intestinal rings incubated with high concentrations of glucose. Incubation was in 50.0 ml of the modified Krebs–phosphate buffer[19] for 2 min. Corrections for extracellular space were made from controls incubated with similar concentrations of D-[1-^{14}C]-mannitol. The portion of the curve from 0–5 mM was constructed on the assumption of a K_m of 1.5 mM.

Fig. 2. Glucose uptake from sucrose as a function of concentration. Conditions as in Fig. 1. Corrections for extracellular space were made using controls containing 2.5 mM phlorizin as inhibitor.

tissue when other substrates are added in addition to a saturating concentration of glucose does not enter the tissue by the same route.

Uptake of glucose released from sucrose at the brush border membrane

Monosaccharides released at the brush border membrane seem to be efficiently captured by the transport mechanisms, at least *in vivo*, as relatively little escapes into the lumen[26,27]. Also, studies *in vitro* using everted hamster intestinal rings[2] have shown considerable tissue accumulation of glucose derived from sucrose, which seemed not explainable simply as a result of accumulation from free glucose in the medium. However, the studies are not clearly comparable. Studies of intestinal absorption in man using a double lumen tube have indicated a high K_m both for sucrose hydrolysis and for absorption of the released glucose. For example, Gray and Ingelfinger[26] report a K_m value of 142 mM for sucrase, which is within the range of concentrations of sucrose found in the intestinal contents after an oral dose of sucrose[28] and a similarly high K_m for glucose absorption. The sucrase K_m, *in vitro*, is much lower. Our studies *in vitro* using rings of hamster intestine suggest that the rate of uptake measured over 2 min reaches a maximum at about 75 mM of sucrose (Fig. 2) with an apparent K_m value, calculated from these results, in the range of 30 mM. Even this value, however, is to be viewed with caution, inasmuch as high concentrations of released glucose such as may be presumed to be present at the membrane have been shown to have an inhibitory effect on hydrolysis[29]. Moreover, the apparent K_m value varied with various batches of hamsters used. Therefore, in order to carry out the following experiments in some sensible way, a concentration of 30 mM sucrose was fixed on simply because experiments showed that glucose uptake from sucrose at this concentration was generally about the same as glucose uptake from 30 mM glucose under the conditions used. The question of efficient capture, *in vitro*, was then approached.

The modified incubation procedure used in these studies and described above; namely, high shaking rate, short time of incubation and large volume of the bathing medium (50 ml) would be expected not only to keep the average concentration of free glucose in the medium at a low level but also to reduce the concentration at the membrane during the course of the incubation. The possibility that the observed uptake of glucose is from the bulk medium is easily dealt with. For example, in a representative experiment, a value of 17.2 mM for glucose accumulation from sucrose was obtained while the concentration of glucose in the medium had reached only 0.12 mM at the end of incubation. Taking the average concentration of glucose during the incubation as 0.06 mM, much previous experience such as exhibited in Fig. 1 and elsewhere[2] tells us that the high value for tissue glucose could not have accumulated through transport from the extracellular pool during 2 min only of incubation.

The possibility that the observed uptake of glucose is from a local high concentration at the membranes, however, is less easy to deal with and will be discussed later.

Uptake from mixture of glucose with disaccharides or glucose 1-phosphate

As shown above, maximal rate of glucose uptake was reached at 30 mM glucose. This observation is fundamental to the others and was, hence, further reaffirmed. Tissue was incubated with [U-^{14}C]glucose and [1-^{3}H]glucose alone (30 mM) and

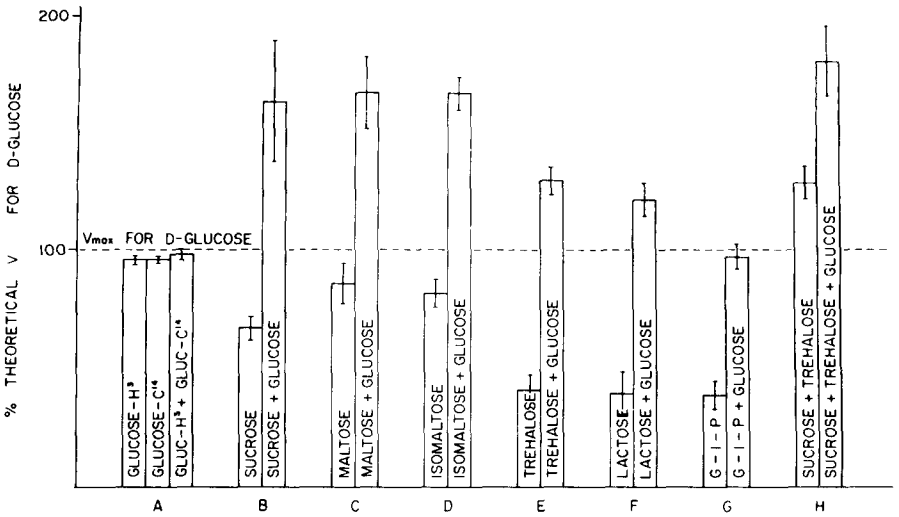

Fig. 3. Glucose uptake from mixtures of D-[U-^{14}C]glucose and a disaccharide or D-[1-^{3}H]glucose or glucose 1-phosphate. Intestinal rings were incubated in 50.0 ml of Krebs–phosphate buffer with single or mixed substrates in concentrations as follows: D-[U-^{14}C]glucose, sucrose and maltose, isomaltose and glucose 1-phosphate (G-1-P), 30 mM; trehalose and lactose, 50 mM. Corrections for extracellular glucose were made from appropriate controls containing 2.5 mM phlorizin. Incubations were for 2 min. When D-[U-^{14}C]glucose and D-[1-^{3}H]glucose were used in combination, the two isotopes in mixture were assayed according to Okita *et al.*[39]. Results were expressed as percentage of the theoretical maximal rate derived for D-[U-^{14}C]glucose, assuming a K_m of 1.5 mM. The standard deviation of the mean of at least 4 experiments is indicated in the figure for each variation, A–H. For B–F the difference between disaccharide *plus* glucose and the theoretical V has a P value by Student's t test of less than 0.0005. In G, glucose 1-phosphate *plus* glucose does not differ from A. In H, only two experiments were done.

together (total = 60 mM). The results (Fig. 3) are those predicted from Fig. 1, that is, there was no increase over the theoretical maximum uptake of glucose and the tissue radioactivity was evenly divided between ^{3}H and ^{14}C. Tissue was then incubated in the presence of 30 mM glucose or 30 mM disaccharide or both together (total = 60 mM). As seen in Fig. 3, there is a component of glucose uptake from each alone as would be expected. When both are together, however, these individual components are additive. In the presence of both substrates, there is a substantial component of glucose uptake in addition to that attributable to a saturating concentration of free glucose. The theoretical maximum uptake of glucose is exceeded by 63% with 30 mM sucrose, 60% with 30 mM maltose, 30% with 50 mM trehalose and 23% with 50 mM lactose. The point is made more substantially by the observation that when glucose 1-phosphate, a substrate for brush border alkaline phosphatase, is used together with glucose in the same kind of experiment, there is no additive component. In a separate experiment, not shown, it was found that entry of glucose contributed by glucose 1-phosphate was almost completely inhibited by 30 mM [^{14}C]glucose. These results with an alkaline phosphatase substrate accentuate the results with disaccharides as well as confirm the conclusions from the previous studies of the glucose oxidase effect[15].

The phenomenon of an additional glucose loading from sucrose was also found

using segments of intestine where the serosal side was rendered inaccessible to substrate (Table I). It can be calculated from the results presented in Table I, that the theoretical maximum uptake of glucose was exceeded by 62% at 15 s and by 52% at 30 s with 30 mM sucrose.

TABLE I

GLUCOSE UPTAKE FROM SUCROSE USING EVERTED SAC PREPARATIONS

The standard deviation of the mean is indicated. *P* value comparing glucose *plus* sucrose with glucose only was less than 0.0025 for both experiments.

	Tissue uptake as mM glucose	
	15 s	*30 s*
30 mM glucose	3.7 ± 0.9	6.97 ± 0.8
30 mM sucrose	3.4 ± 0.8	7.6 ± 2.1
30 mM glucose + 30 mM sucrose	7.7 ± 1.7	10.8 ± 1.1

On the face of it, the additive phenomenon seen in Fig. 3 argues strongly for the existence of mode of membrane transfer in addition to the Na^+-dependent glucose-transport system. However, there are a number of counter arguments which come to mind and should be considered. First, there is the question of the local concentration of glucose at the membrane and whether by being especially high due to disaccharidase splitting, the results are thus explainable. The answer to this question is that an effect of a local high concentration would go against kinetic theory and the observed maximal rate with added free glucose. Moreover, the splitting of glucose 1-phosphate by alkaline phosphatase surely gives rise to a local high concentration, but there was no additional glucose uptake when glucose 1-phosphate was the substrate. Second, there is the question of diffusibility at the membrane and the influence thereon of the mucopolysaccharide "fuzzy coat"; an influence, for example, such as was proposed by Hamilton and McMichael[30] on the basis of an assumption that the extensive fuzzy coat serves as a barrier to the diffusion of disaccharide into the intermicrovillus spaces to the site of the disaccharidase and of liberated glucose out of the spaces into the lumen. The answer in this case seems also to be that saturation of transport with 30 mM glucose obviates the proposal on theoretical grounds. Thirdly, there is the question of whether disaccharidases and alkaline phosphatase may not be localized to different regions of the microvillus and that results with their substrates should not be compared in the way that has been done above. This question would be of greatest relevance were disaccharidases known to be localized deep within the intermicrovillus spaces and alkaline phosphatase localized at the tips of the microvilli. However, from what evidence is available, such seems not to be the case. The histochemical technique applied at the level of the electron microscope[5,31] seems to show alkaline phosphatase to be as prevalent deep within the intermicrovillus spaces as it is more toward the surface. The limited information provided by the studies of Gitzelman *et al.*[32] using ferritin antibody con-

jugates suggests similarly that rabbit intestinal sucrase is present along the length of the microvillus.

From these considerations, it seems then possible to proceed on the assumption that the additive phenomenon is dependent upon events at or close to the molecular level of the membrane and not upon gross cellular structure or organization.

Uptake from media containing mixtures of sucrose and D-[*U*-^{14}C]*galactose or β-methyl* [*U*-^{14}C]*glucoside*

Inasmuch as the monosaccharide transport system is shared by several analogs of D-glucose[1,33], we studied the additive phenomenon with some of these, especially D-galactose and β-methyl glucoside which have affinities for the system comparable to that of glucose. The results are summarized in Fig. 4. It will be seen by comparison with glucose that β-methyl glucoside and galactose give somewhat different results. The additive phenomenon so clearly seen for the pair, glucose and sucrose, is very small for the other pairs. There is a mutual inhibition of uptake between sucrose and β-methyl glucoside and between sucrose and galactose which is not apparent between sucrose and glucose. The reason for this difference in behavior not being obvious, a test was made to see whether the accumulation of these analogs might affect the additive phenomenon. Tissue was preincubated with β-methyl glucoside or with sucrose for 2 min and then transferred to a medium containing either sucrose

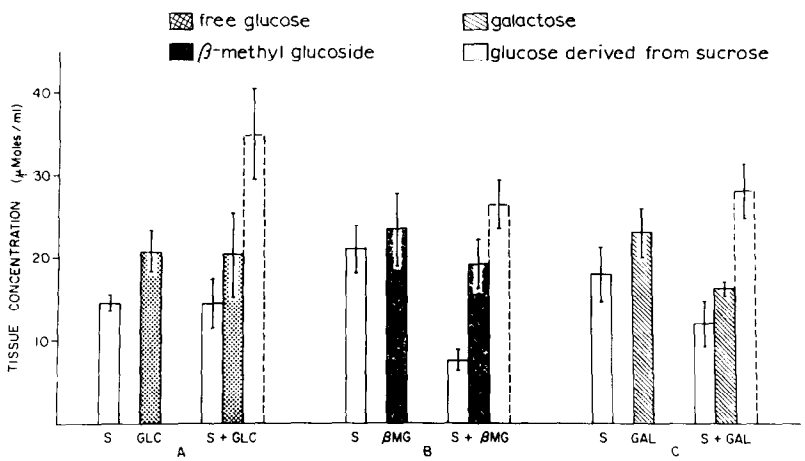

Fig. 4. Tissue uptake from mixtures of sucrose and D-[U-^{14}C]glucose, D-[U-^{14}C]galactose and β-methyl [U-^{14}C]glucoside. Incubations were for 2 min in 50 ml of Krebs–phosphate buffer, using substrates at a concentration of 30 mM. Uptake of glucose derived from sucrose was measured by the glucose oxidase method and uptake of other sugars was determined from radioactivity measurements. Corrections for extracellular sugars were made with phlorizin controls. When sucrose and monosaccharide were used together, uptake of each is denoted by the bars linked to each other. The dotted bar represents the total uptake, that is, the sum of uptakes of monosaccharide and glucose derived from sucrose and is given to denote the extent of additive effect seen with the pairs. The three sets of experiments were done with different batches of animals and hence the differences in the uptake values of glucose from sucrose. The standard deviation of the mean of 4 experiments is indicated in the figure for each variation. The P value by Student's t test of the difference between sucrose *plus* monosaccharide and monosaccharide alone is <0.0025 in variation A. The differences in B and C are not statistically significant ($P<0.1$).

or β-methyl glucoside, respectively. As seen in Table II, preloading the tissue had no effect on subsequent uptake of glucose from sucrose or of β-methyl glucoside. Galactose and β-methyl glucoside were also tested for any effect on the hydrolysis of sucrose. The results which will be presented in a later paper showed that they are not inhibitory. Hence, studies on the kinetics of sucrose inhibition of the uptake of D-galactose and of β-methyl glucoside were done. The results are presented in Figs 5 and 6, respectively. The Lineweaver–Burk plots[34] in Figs 5a and 6a indicated clearly the non-competitive nature of this inhibition. Using the more sensitive method of Hanes[35] $[S/V]$ *vs* $[S]$ plots (Figs 5b and 6b) were obtained confirming the non-competitive nature of the inhibition.

The kinetic analysis of the effects of D-galactose and β-methyl glucoside on

TABLE II

EFFECT OF PRELOADING THE TISSUE ON UPTAKE FROM β-METHYL [U-^{14}C]-GLUCOSIDE AND SUCROSE

Tissue was preloaded with β-methyl [U-^{14}C]glucoside or 30 mM sucrose in Krebs–phosphate buffer for 2 min. The tissue loaded with β-methyl glucoside was transferred to 50.0 ml of buffer containing 30 mM sucrose and the tissue loaded with sucrose was transferred to 50.0 ml of buffer containing 2 mM β-methyl [U-^{14}C]glucoside and reincubated for 2 min. Corrections for extracellular sugars were made as before.

	Glucose (*μmoles/ml tissue water*)	*β-Methyl [U-^{14}C] glucoside* (*μmoles/ml tissue water*)
2 mM β-methyl [U-^{14}C]glucoside	—	14.24
Preload with 30 mM sucrose; transfer to 2 mM β-methyl [U-^{14}C]glucoside	—	15.15
30 mM sucrose	21.65	—
Preload with 30 mM β-methyl glucoside; transfer to 30 mM sucrose	21.70	—

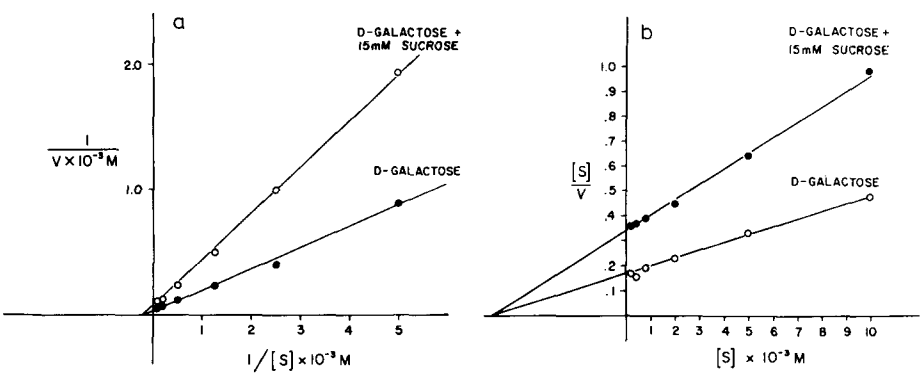

Fig. 5. (a) Lineweaver–Burk plots for D-[U-^{14}C]galactose uptake in the presence and absence of sucrose. Incubations were in 50.0 ml Krebs–phosphate buffer for 2 min. Sucrose was at 15 mM. Corrections for extracellular galactose were made from corresponding mannitol controls. (b) Hanes plots for sucrose inhibition of D-[U-^{14}C]galactose uptake. The data are from a.

glucose uptake from sucrose were made according to Dixon[36] (Figs 7a and 7b). Sucrose at 5 and 15 mM was incubated with different concentrations of the inhibitor analogs. Figs 7a and 7b again reveal the non-competitive nature of inhibition by galactose and β-methyl glucoside.

From these kinetic data, it may be inferred that the transfer of glucose by disaccharidase related transport is affected by the functioning of the monosaccharide transport systems for analogs other than D-glucose but not for glucose itself. As will be seen in a later paper, there are conditions; for example, the absence of Na^+ where such an inhibition can be eliminated. However, the reasons for such differences in the effects of these analogs compared to glucose are not understood.

Effect of phlorizin on glucose uptake from sucrose

Phlorizin is a potent competitive inhibitor of the glucose-transport system[37] and studies of effects of phlorizin on glucose transfer from sucrose were done with a view to comparing these with its known effect on glucose transfer. As a control for the effects of phlorizin on glucose transfer from sucrose, concentrations of glucose higher than used in previous studies from our laboratory and a 2-min time

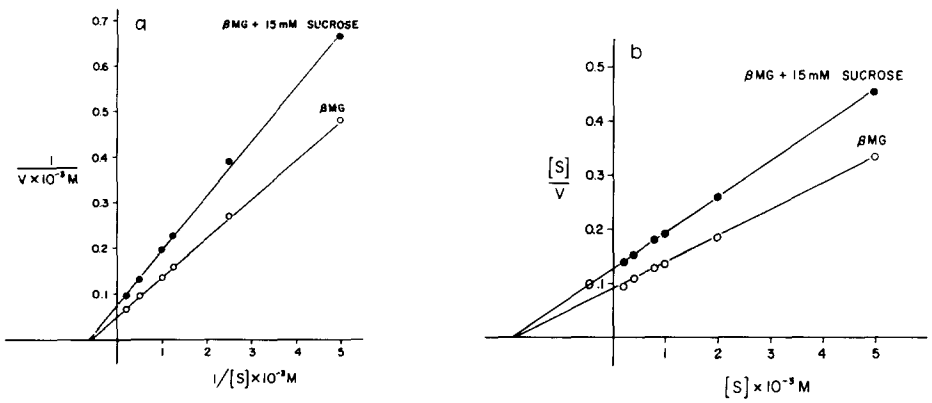

Fig. 6. (a) Lineweaver–Burk plots for β-methyl [U-^{14}C]glucoside (βMG) uptake in the presence and absence of sucrose. The experimental details are as in Fig. 5a. (b) Hanes plots for sucrose inhibition of β-methyl [U-^{14}C]glucoside uptake. The data are from a.

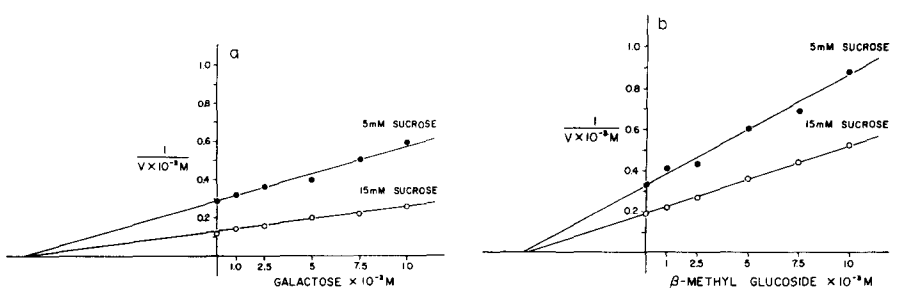

Fig. 7. (a) Dixon plots for galactose inhibition of glucose uptake from sucrose. Incubations were in 50.0 ml medium for 2 min. Corrections were made from phlorizin-inhibited controls as before. (b) Dixon plots for β-methyl [U-^{14}C]glucoside inhibition of glucose uptake from sucrose. Details as in a.

period of incubation were used. As seen in Fig. 8 and as would be predicted from previous knowledge, phlorizin is a competitive inhibitor with high affinity. Compared to this, it is seen in Fig. 9, that when sucrose is the source of glucose, the effect of phlorizin is not simple. It may be separated into two individual components. At low concentrations, the typical high affinity competitive inhibition of Na^+-dependent free glucose entry is seen. This we would take to be the component of tissue loading which is by way of carriers for free glucose. K_i was calculated to be $5.0 \cdot 10^{-6}$ M which is very close to that reported for the competitive inhibition of monosaccharide uptake by phlorizin. At higher concentrations of sucrose, phlorizin inhibition is no longer competitive and resembles the inhibition caused by β-methyl glucoside and galactose.

Counterflow experiments

According to the mobile carrier theory, substrates that utilize the same carrier exhibit counter transport[38]. If the tissue is allowed to accumulate a substrate, addition of a second substrate to the bathing medium should induce outflow of the accumulated substrate against its concentration gradient, provided the second substrate utilizes the same carrier system for entry. If, however, the second substance is not a substrate but only an inhibitor reacting at a different locus to cause inhibition, then the rate

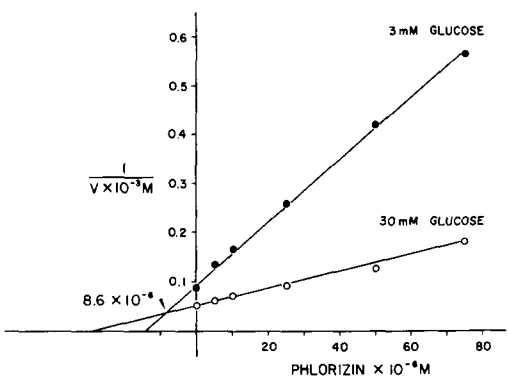

Fig. 8. Dixon plots for phlorizin inhibition of glucose uptake from D-[U-^{14}C]glucose. Period of incubation, 2 min.

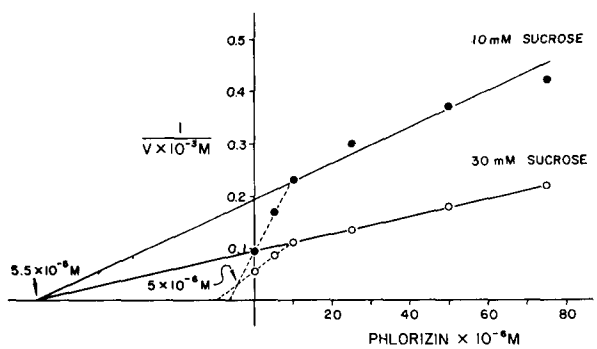

Fig. 9. Dixon plots for phlorizin inhibition of glucose uptake from sucrose. Incubation time, 2 min.

of accumulation of the first compound would be expected to be reduced without an induction of counterflow. Counterflow experiments were therefore designed with D-glucose and sucrose as elicitors and β-methyl glucoside as substrate. Fig. 10 shows that while D-glucose induces exit of β-methyl glucoside against a concentration

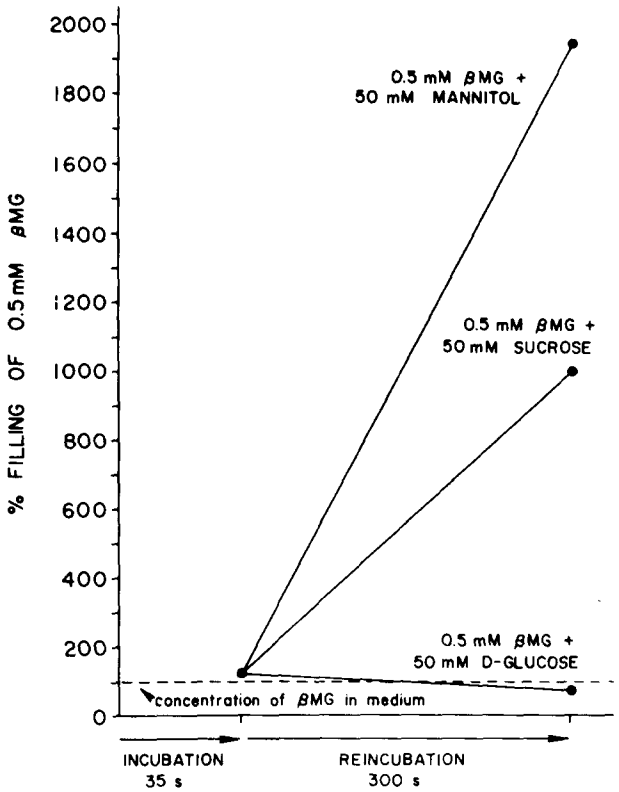

Fig. 10. Countertransport of β-methyl[U-^{14}C]glucoside (βMG). Intestinal rings were incubated for 35 s. in Krebs–phosphate buffer containing 0.2 mM β-methyl [U-^{14}C]glucoside. The tissue was then transferred to 75.0 ml of the same buffer containing 0.5 mM β-methyl [U-^{14}C]glucoside alone or in combination with either 50 mM sucrose or 50 mM glucose as elicitors and reincubated for 5 min. The large medium volumes (75.0 ml) were used to dilute free glucose derived from sucrose and leaking into the medium. In other experiments, the tissue was transferred to fresh incubation medium at the second minute of reincubation to keep the concentration of glucose in the medium at a negligible minimum. The results obtained were the same as given in this figure.

gradient, sucrose at concentrations which give rise to similar rates of entry of glucose as from the free glucose, itself, did not induce counterflow. This result again indicates that the mode of entry into the tissue of glucose from disaccharides is different from that of free monosaccharides although it may be argued that since sucrose does not inhibit uptake of β-methyl glucoside completely, any counterflow that it is capable of inducing is masked. However, it must be stressed that the glucose uptake rates from D-glucose and sucrose are comparable (35.6 and 34.8 mM, respectively) in

these experiments and therefore, if a common carrier were involved, similar effects should have been observed on countertransport.

Conclusions

On the basis of the evidence presented above, it seems reasonable to conclude that under certain conditions when *in vitro* small intestine is presented with a disaccharide, there is a component of glucose transport which can not be attributed to the expected sequential steps of surface hydrolysis and transfer by the Na^+-dependent monosaccharide carrier.

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