Active Sugar Transport by the Small Intestine

The effects of sugars, amino acids, hexosamines, sulfhydryl-reacting compounds, and cations on the preferential binding of D-glucose to Tris-disrupted brush borders

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ABSTRACT Tris-disrupted and intact brush border membrane preparations from mucosa of hamster jejunum were capable of preferentially binding actively transported D-glucose in a similar manner. Density gradient centrifugation of the Tris-disrupted brush borders indicated that D-glucose was bound to a fraction containing the cores or inner material of the microvilli. The properties of this binding were examined with the Tris-disrupted brush border preparation. Actively transported sugars competitively inhibited preferential D-glucose binding, whereas no effect was observed with nonactively transported sugars. Neither actively nor nonactively transported amino acids affected D-glucose binding. D-Glucosamine, which is not actively transported, was inhibitory to preferential D-glucose binding as well as to the active transport of D-glucose by everted sacs of hamster jejunum. No inhibitory effect was observed with the same concentration of D-galactosamine. Preferential D-glucose binding was also inhibited by sulfhydryl-reacting compounds, Ca^{2+}, and Li^{+} ions. On the other hand, Mg^{2+} was shown to be stimulatory and Na^{+}, NH_4^{+}, and K^{+} had no effect on this phenomenon. The results of these experiments suggest that preferential D-glucose binding to brush borders is related to the initial step in active sugar transport by the small intestine.

INTRODUCTION

The brush border region or the microvilli of mucosal cells is thought to be the site where sugars are actively transported against their own concentra-

tion gradients in the small intestine (1). It has been generally accepted that active sugar transport involves an association or binding of the sugar to a component within the brush border membrane. This initial step apparently requires no metabolic energy (2). A second step, however, which is responsible for the net movement of a sugar against its gradient and which involves the dissociation of the sugar-membrane complex, depends either directly or indirectly upon energy derived from cellular metabolism (3, 4). Although it has been observed that an important part is played by Na^+ in both steps in active sugar transport by the small intestine, the precise nature of this role is still undetermined (1, 5–7).

Recently, Faust et al. (8) reported that brush borders isolated from the mucosa of hamster jejunum and disrupted with tris (hydroxymethyl) aminomethane (9) can preferentially bind actively transported D-glucose. Preferential binding of D-glucose was demonstrated by an increase in the disintegrations per minute (dpm) ratio of D-mannose-^{3}H to D-glucose-^{14}C in the supernatant from incubated Tris-disrupted brush borders. Additional studies indicated that there was no preferential binding of D-glucose with the nuclear, mitochondrial, or ribosomal fractions isolated from the mucosa of hamster jejunum and treated with Tris (Faust and Leadbetter, unpublished observations).

It has been suggested that preferential D-glucose binding to Tris-disrupted brush borders is similar to the initial step in active sugar transport by the small intestine (8) in that this binding is not directly dependent upon energy derived from Na^+-K^+–stimulated, Mg^{2+}-dependent ATPase activity, but is inhibited by: (*a*) phlorizin, a competitive inhibitor of active sugar transport, (*b*) mercuric chloride, a compound which binds sulfhydryl groups and denatures protein, and (*c*) a decrease in temperature, which is indicative of a temperature-dependent complexing between the sugar and the Tris-disrupted brush borders.

The present paper describes experiments which were designed to examine the possible relation of preferential D-glucose binding to Tris-disrupted mucosal brush borders to the initial step in active sugar transport by the small intestine.

MATERIALS AND METHODS

Isolation and Preparation of Brush Borders

The methods employed to isolate the epithelial brush border membranes from hamster jejunum were the same as those previously reported (8) and yielded a substantially pure preparation as is shown in Fig. 1. In most experiments, these isolated and intact brush borders were disrupted with 1 M Tris (pH 8.2) for 45 min at 2°C. In some experiments, the brush borders were exposed to either (*a*) Krebs-bicarbonate-saline

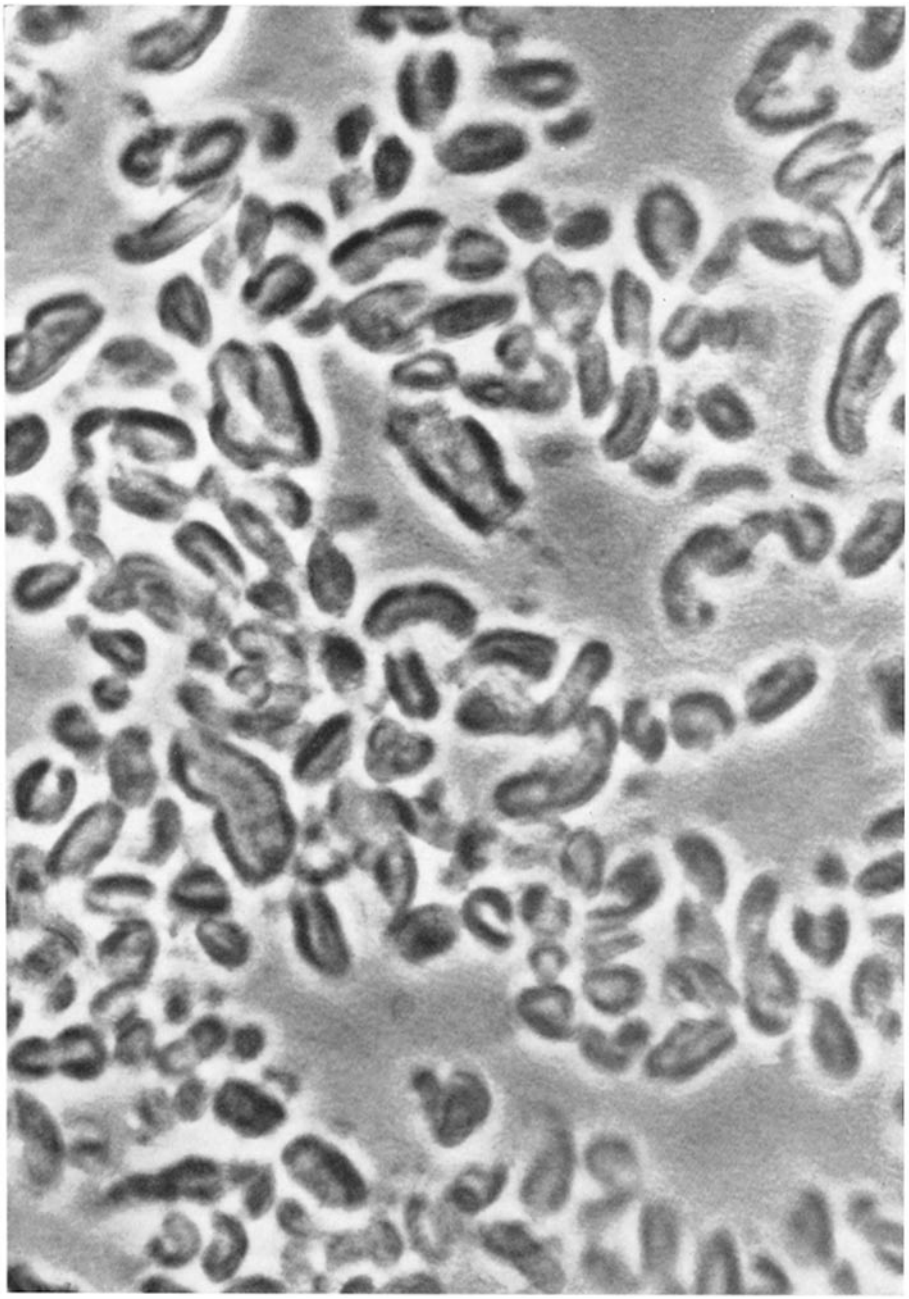

FIGURE 1. Phase contrast photomicrograph of isolated intact brush border membranes from mucosal cells of hamster jejunum. × 800.

(10) for 90 min at 25°C, or (*b*) distilled water for 45 min at 2°C, or (*c*) distilled water for 90 min at 25°C, or (*d*) distilled water and used immediately.

After this preliminary treatment the brush borders were centrifuged (6,000 *g*) and the supernatant was replaced by cold distilled water. The volume of distilled water added depended upon the number of animals originally used, e.g., 2 ml of water was added for every three animals employed. Usually jejuna from 12 animals were used each day.

Measurement of Preferential D-Glucose Binding

To 1 ml aliquots of the resuspended brush borders was added 1.2 ml of 10 mM phosphate buffer (pH 7.4), 1.0 ml of a nonradioactive solution containing an experimental compound or, in the case of controls, distilled water, and 0.1 ml of a radioactive sugar mixture containing D-mannose-1-^{3}H and D-glucose-^{14}C (uniformly labeled). The concentration of D-mannose-1-^{3}H was 10^{-5} mM and (U.L.) D-glucose-^{14}C, 10^{-3} mM. The suspensions were incubated for 1 hr at 37°C and centrifuged (17,000 *g*). After centrifugation, 0.1 ml of the supernatant was added to 15 ml of liquid scintillation fluid—7.5 g of 2,5-diphenyloxazole (PPO), 0.3 g of 1,4-bis [2-(5-phenyloxazolyl)]-benzene (POPOP), 1 liter of toluene, and 500 ml of Triton X-100 in vials. The ^{3}H-labeled D-mannose and the ^{14}C-labeled D-glucose were counted with a Nuclear-Chicago liquid scintillation system, Mark I series. Counts per minute (cpm) were converted to disintegrations per minute (dpm). The effects of various substances on preferential D-glucose binding are reported as percentages. The following equations were employed:

$$D_C = \left(\frac{R_{BB}}{R_{H_2O}} - 1\right) 100 \quad \text{and} \quad D_E = \left(\frac{R_{BB} + E}{R_{H_2O} + E} - 1\right) 100$$

where D_C = the percentage increase in the ratio of ^{3}H/^{14}C caused by brush borders in the absence of the experimental compound, R_{BB} is the ^{3}H/^{14}C ratio with brush borders, R_{H_2O} is the ^{3}H/^{14}C ratio without brush borders; D_E = the percentage increase in the ^{3}H/^{14}C ratio caused by brush borders in the presence of the experimental compound, R_{BB+E} is the ^{3}H/^{14}C ratio with brush borders, and the experimental compound, R_{H_2O+E}, is the ^{3}H/^{14}C ratio without brush borders but with the experimental compound.

The percentage inhibition (D_{Inhibit}) produced by the experimental compound on the percentage increase in the ^{3}H/^{14}C ratio by brush borders was calculated as follows:

$$D_{INHIBIT} = \left(\frac{D_C - D_E}{D_C}\right) 100$$

When necessary, quench corrections were applied.

Density Gradient Centrifugation of Radioactive Tris-Disrupted Brush Borders

Tris-disrupted brush borders, previously incubated in D-mannose-^{3}H and D-glucose-^{14}C, were washed by repeated centrifugation (17,000 *g*) and resuspension in cold

distilled water (2°C). This procedure removed the ^{3}H and ^{14}C which were in the original incubation medium and caused little or no loss of the ^{3}H and ^{14}C bound to the disrupted brush borders. These disrupted brush borders, obtained from at least five animals, were resuspended in 0.5 ml of cold distilled water. The suspension was then layered on a 20, 30, 40, 50, and 60% glycerol with 0.5 M $MgCl_2$ gradient in a 6 ml centrifuge tube and centrifuged at 63,000 *g* (Beckman model L Spinco Ultracentrifuge, SW-39L rotor) for 15 min, according to the method of Eichholz and Crane (9). The separated brush border fractions obtained were identified by passing a beam of light through the centrifuge tube (Tyndall effect). These fractions were removed and washed by resuspension in cold distilled water and centrifugation at 100,000 *g* for 20 min. The $^{3}H/^{14}C$ dpm ratios of the separated and washed brush border fractions were determined in the manner previously described.

Purity of Chemicals

All chemicals used were reagent grade and were of the highest purity obtainable. Compounds which inhibited preferential D-glucose binding; i.e., D-glucosamine hydrochloride, D-xylose, 3-*O*-methyl-D-glucose, and D-galactose were demonstrated to be homogeneous by means of paper chromatography (11). In addition, glucose contamination within these compounds, as well as within phlorizin, was determined by the glucose oxidase or the hexokinase and glucose-6-phosphate dehydrogenase assay methods (12). D-Xylose, phlorizin, and 3-*O*-methyl-D-glucose were glucose-free. D-Galactose and D-glucosamine hydrochloride contained 0.0006% and 0.017% of D-glucose, respectively. It was concluded that the extremely low levels of D-glucose within these compounds could not account for their observed effects on preferential D-glucose binding.

RESULTS

Preferential Binding of D-glucose to Various Brush Border Preparations

At first, it was important to determine whether various preparations of brush borders incubated in the presence of D-mannose-1-^{3}H and D-glucose-^{14}C (U.L.) were capable of preferentially binding D-glucose. It can be seen in Fig. 2 that brush borders pretreated with either 1 M Tris for 45 min at 2°C or Krebs-bicarbonate-saline for 90 min at 25°C produce a similar increase in the $^{3}H/^{14}C$ dpm ratio. In both cases preferential binding of D-glucose was completely inhibited by phlorizin, and similar ratios were obtained when D-arabinose-5-^{3}H (10^{-5} mM) was substituted for D-mannose-1-^{3}H (10^{-5} mM). When these preparations were observed with a phase contrast microscope, the isolated brush borders pretreated with 1 M Tris were disrupted, whereas the brush borders exposed to Krebs-bicarbonate-saline remained intact.

Fig. 2 also illustrates that there is a large reduction in the preferential binding of D-glucose to brush borders pretreated with distilled water for 45 and 90 min at 2° and 25°C, respectively. Similarly, fresh intact brush borders do not preferentially bind D-glucose as well as Tris-disrupted or Krebs-bicarbonate-saline–treated brush borders.

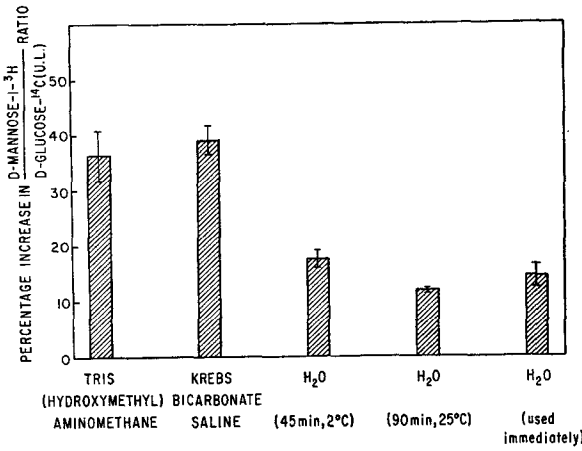

FIGURE 2. The effects of pretreatment on the ability of brush borders to preferentially bind D-glucose. The percentage increase in the $^3H/^{14}C$ dpm ratio was measured in the supernatant from the brush borders incubated for 1 hr at 37°C in a phosphate-buffered medium (pH 7.4) containing 10^{-5} mM D-mannose-1-^{3}H and 10^{-3} mM D-glucose-^{14}C (U.L.). Each bar point represents the mean of at least four experiments. The vertical lines represent 1 SE of the mean above and below the bar points.

TABLE I

EFFECTS OF HEXOSAMINES ON THE PREFERENTIAL BINDING OF D-GLUCOSE-^{14}C (U.L.) TO TRIS-DISRUPTED AND KREBS-BICARBONATE-SALINE-TREATED BRUSH BORDER MEMBRANES

	Percentage inhibition	
Hexosamine (1 mM)	Tris-disrupted brush borders	Krebs-bicarbonate-saline-treated brush borders
D-Glucosamine	93.9±10.4 (3)	90.9±2.0 (4)
D-Galactosamine	−9.8±5.0 (3)	−1.6±4.8 (3)

The number of experiments is given in parentheses. The standard error is given for each mean.

Effects of Hexosamines on Preferential Binding of D-Glucose to Tris-Disrupted and Krebs-Bicarbonate-Saline–Treated Brush Borders

Hexosamines affect the preferential binding of D-glucose to Tris-disrupted and Krebs-bicarbonate-saline–treated brush borders in a similar manner. The effects of 1 mM D-glucosamine and 1 mM D-galactosamine (which structurally resemble their related actively transported hexoses except for the replacement of the hydroxyl group by an amino group on the second carbon atom) on the preferential binding of D-glucose to Tris-disrupted and Krebs-bicarbonate-saline–treated brush borders are shown in Table I. D-Glucosamine is very inhibitory to the preferential binding of D-glucose to both preparations of

brush borders, whereas D-galactosamine has no effect at this concentration. It is of interest to note that these hexosamines have a similar effect on the active transport of D-glucose by everted sacs of hamster jejunum. We have found that the final serosal to mucosal fluid concentration ratios of D-glucose (S/M) with everted sacs which contained an initial concentration of 11 mM in each compartment were 5.23 ± 0.51 (7) for the control, 4.30 ± 0.20 (5) in the presence of 1 mM D-galactosamine, and 2.32 ± 0.15 (5) in the presence of 1 mM D-

TABLE II

EFFECTS OF SUGARS AND AMINO ACIDS ON THE PREFERENTIAL BINDING OF D-GLUCOSE-^{14}C (U.L.) TO TRIS-DISRUPTED BRUSH BORDERS

Compound	Percentage inhibition at the concentration of 1 mM	Percentage inhibition at the concentration of 10 mM
Actively transported sugars		
D-Glucose	100.0 (3)	—
3-*O*-Methyl-D-glucose	45.5±6.2 (3)	91.7±1.3 (3)
D-Xylose	18.8±2.0 (4)	46.7±4.9 (3)
D-Galactose	1.7±2.2 (4)	46.8±0.9 (3)
Nonactively transported sugars		
L-Sorbose	−0.5±6.7 (3)	6.0±8.2 (3)
D-Ribose	7.4±8.5 (3)	−10.7±5.4 (3)
D-Arabinose	5.5±9.4 (6)	−3.3±4.1 (3)
L-Arabinose	−1.3±10.0 (4)	−2.2±3.4 (3)
Actively transported amino acids		
L-Histidine	−10.5±12.5 (5)	19.3±5.0 (4)
L-Alanine	1.8±5.1 (3)	4.5±1.8 (4)
L-Hydroxyproline	9.1±10.5 (5)	6.1±3.1 (3)
L-Proline	0.4±7.2 (4)	2.7±2.6 (4)
L-Methionine	−10.9±15.7 (4)	−5.7±8.8 (4)
L-Tryptophan	−1.3±1.3 (5)	1.5±3.6 (4)
L-Cysteine	6.6±9.2 (4)	2.9±2.2 (3)
L-Arginine	6.7±8.5 (4)	−17.8±6.8 (4)
Nonactively transported amino acids		
L-Aspartic acid	−3.3±6.8 (6)	−20.7±11.3 (3)
L-Glutamic acid	−7.3±8.1 (4)	−7.9±19.1 (3)

The number of experiments is given in parentheses. The standard error is given for each mean.

glucosamine. Only D-glucosamine significantly reduced the final S/M ratio of D-glucose ($P < 0.01$). The sacs were incubated for 1 hr at 37°C. A S/M value greater than 1.00 is indicative of active D-glucose transport against a concentration gradient toward the serosal compartment.

Effects of Sugars and Amino Acids on Preferential Binding of D-Glucose to Tris-Disrupted Brush Borders

Since the properties and extent of preferential D-glucose binding are similar in Tris-disrupted and Krebs-bicarbonate-saline–treated brush borders, only the

disrupted membrane preparation was employed for the remainder of this investigation.

The effects of actively and nonactively transported sugars and amino acids on the preferential binding of D-glucose-^{14}C (U.L.) to Tris-disrupted brush borders are presented in Table II. Only sugars which are actively transported by the hamster jejunum inhibit the preferential binding of D-glucose-^{14}C, although D-galactose, like D-galactosamine, is not inhibitory at the lower (1 mM) concentration. Neither actively transported amino acids nor nonactively transported sugars and amino acids significantly affect the preferential binding of D-glucose-^{14}C to Tris-disrupted brush borders prepared from mucosa of hamster jejunum.

Effects of Sulfhydryl-Reacting Compounds on Preferential Binding of D-Glucose to Tris-Disrupted Brush Borders

The importance of SH^- groups for preferential binding of D-glucose is illustrated in Table III. All the sulfhydryl reagents employed reduced the pref-

TABLE III

EFFECT OF SULFHYDRYL-REACTING COMPOUNDS ON THE PREFERENTIAL BINDING OF D-GLUCOSE-^{14}C (U.L.) TO TRIS-DISRUPTED BRUSH BORDERS

Sulfhydryl-reacting compound	Number of experiments	Percentage inhibition
Iodoacetate	3	41.6±5.9
N-Ethylmaleimide	3	54.0±7.6
O-Iodosobenzoate	3	54.2±5.0
p-Hydroxymercuribenzoate	3	90.6±3.2
p-Hydroxymercuribenzoate + L-cysteine (1 mM)	4	−4.6±10.5

All inhibitors are at a concentration of 1 mM. The standard error is given for each mean.

erential binding of D-glucose to the Tris-disrupted brush borders. A greater reduction, however, was observed with *p*-hydroxymercuribenzoate than with the other inhibitors. Furthermore, the SH^- groups of an equimolar concentration of L-cysteine protected the Tris-disrupted brush borders from the inhibitory effect of *p*-hydroxymercuribenzoate.

Effects of Various Cations on Preferential Binding of D-Glucose to Tris-Disrupted Brush Borders

The effects of cations on preferential D-glucose binding also were investigated. It can be seen in Table IV that both Ca^{2+} and Li^{2+} ions are inhibitory to preferential D-glucose-^{14}C (U.L.) binding, whereas Mg^{2+} is stimulatory and K^+, NH_4^+, and Na^+ ions are without effect.

TABLE IV

EFFECTS OF CATIONS ON THE PREFERENTIAL BINDING OF D-GLUCOSE-^{14}C (U.L.) TO TRIS-DISRUPTED BRUSH BORDERS

Cation	Number of experiments	Percentage inhibition
Ca^{2+}	3	64.0±5.1
Li^{+}	3	40.0±11.7
Mg^{2+}	4	−33.4±5.7
K^{+}	3	0.5±7.9
NH_4^{+}	5	6.0±5.7
Na^{+}	4	9.4±7.1

All cations are chloride salts at a concentration of 100 mM. The standard error is given for each mean.

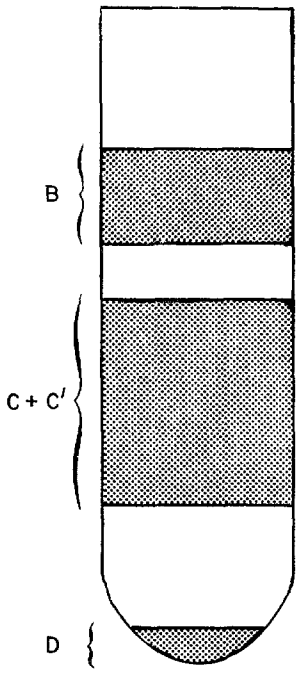

FIGURE 3. Schematic diagram of fractions obtained after glycerol density gradient centrifugation of washed, Tris-disrupted, brush borders as visualized by the Tyndall effect. Fraction B has not been morphologically identified, but fraction C + C′ contains microvillous membranes and fraction D contains the inner or core material of the microvilli (9, 13).

Location of Preferentially Bound D-Glucose within the Tris-Disrupted Brush Border Preparation

Knowledge pertaining to the structural organization of the mucosal brush border membrane gained by the method of density gradient centrifugation of Tris-disrupted brush borders has been provided by other investigators (9, 13). Consequently, these techniques were employed to locate and identify the specific region within the disrupted brush border preparation which preferentially binds D-glucose.

A schematic diagram of the fractions of washed, radioactive Tris-disrupted

brush borders obtained after centrifugation on a 20, 30, 40, 50, 60% glycerol with 0.5 M $MgCl_2$ gradient is illustrated in Fig. 3. The bands formed are similar to those originally reported by Eichholz and Crane (9), except that band A does not appear because this less dense material was removed by our washing procedure. However, previous experiments had shown that fraction A contained very little ^3H or ^{14}C. Similarly, fractions B and C + C′ were relatively free of any radioactivity. Most of the D-glucose-^{14}C (U.L.) which was preferentially bound appeared in fraction D.

In a typical study, a 40.2% increase in the initial $^3H/^{14}C$ dpm ratio was observed in the supernatant from Tris-disrupted brush borders (an increase in ratio of 1.84 to 2.58) and a corresponding $^3H/^{14}C$ dpm ratio of 0.29 was measured in fraction D after the radioactive brush borders were separated by glycerol density gradient centrifugation. In some experiments fraction D was purified (it may contain some partially disrupted microvilli) by reexposure to 1 M Tris and recentrifugation on a glycerol density gradient (14). Determination of the $^3H/^{14}C$ dpm ratio of this fraction indicated that the preferentially bound D-glucose-^{14}C (U.L.) remained in fraction D which contains microvillous cores.

DISCUSSION

The data presented in this investigation indicate that Tris-disrupted and intact Krebs-bicarbonate-saline–treated brush borders are capable of preferentially binding D-glucose in a similar manner. Pretreatment of the brush borders with distilled water instead of Tris or Krebs-bicarbonate-saline may not expose as many D-glucose binding sites. It was evident that temperature during these preparatory procedures is not critical within the range of 2 to 25°C.

Krebs-bicarbonate-saline homogenates of intestinal mucosa also bind D-glucose as was demonstrated by Fernie et al. (15). However, isolated brush border membranes which have been disrupted are perhaps more advantageous for experiments on the mechanism of active sugar transport by the small intestine because they can be separated into morphologically identifiable fractions (9, 13). Density gradient centrifugation of Tris-disrupted brush borders, which had preferentially bound D-glucose, has indicated that the D-glucose is bound to the inner or core material of the microvilli which is in fraction D. The disaccharidase activity of this fraction is relatively low in relation to the specific enzymatic activity found in the nonradioactive fraction C + C′ containing microvillous membranes (14). Therefore, it is unlikely that the phlorizin-inhibited preferential D-glucose binding to brush borders that we have observed is caused by the binding of D-glucose to disaccharidases. It is also unlikely that we are observing transport of D-glucose into closed vesicles instead of D-glucose binding to a specific component of the mucosal brush border membrane. Electron photomicrographs (13) have indicated that fraction D is composed of compact rods and not closed membranous vesicles as are found in fraction C + C′.

The results of these studies support our previous work (8) in which we suggested the possibility that preferential D-glucose binding to Tris-disrupted brush borders is related to the initial step in active sugar transport by the small intestine. We have shown that only sugars which are actively transported by the small intestine inhibit preferential D-glucose binding, presumably by competing for similar sites on a "carrier" or a component within the brush border membrane of mucosal cells. The affinities of these sugars for intestinal transport (16) correspond reasonably well with their ability to inhibit preferential binding of D-glucose to Tris-disrupted brush borders.

Actively transported amino acids do not compete for the D-glucose binding sites within Tris-disrupted brush borders. This is in accord with the observations by Munck (17) who found no competitive inhibition between the intestinal transport of amino acids and sugars. Therefore, factors other than competition for similar binding sites are apparently responsible for the inhibitory effects observed by other investigators (18, 19) with actively transported amino acids on the active transport of sugars by the small intestine.

Although D-glucosamine is not actively transported by the small intestine (20), it is inhibitory to preferential D-glucose binding to brush borders as well as to the active transport of D-glucose by everted sacs of hamster jejunum. It seems that D-glucosamine competes with D-glucose for the sugar binding site, but may not be readily dissociated. In this regard, the action of D-glucosamine on preferential D-glucose binding to brush borders, as well as on the active transport of D-glucose by everted sacs of jejunum, is similar to that observed with phlorizin (8, 21, 22). It also is of interest that D-glucosamine and unlabeled D-glucose are equally inhibitory to the preferential binding of D-glucose-^{14}C (U.L.), although only D-glucose is actively transported. This may indicate that the OH^- group on the second carbon atom of D-glucose is not essential during the binding or association phase in the active transport of this sugar by the small intestine, but is of primary importance during the second phase which involves the dissociation of the sugar-membrane complex. D-galactosamine, like D-galactose, at a concentration of 1 mM, is not inhibitory to preferential D-glucose binding probably because of its lower affinity for the sugar binding site.

Preferential D-glucose binding to Tris-disrupted brush borders depends on SH^- groups. It is possible that either SH^- groups are closely associated with the sugar binding sites or that they are important for maintaining the integrity of the protein portion of the membrane responsible for the preferential binding of D-glucose. The precise nature of this binding is unknown. It has been observed, however, that preferential D-glucose binding to Tris-disrupted brush borders is a temperature-dependent process (8) which is not reversed by washing with distilled water at 2°C. If the washing procedure is carried out at 37°C (unpublished observation), then dissociation of D-glucose occurs more readily. These intriguing binding properties require further investigation.

We have no evidence demonstrating that Na^+ is required for the preferential binding of D-glucose to Tris-disrupted brush borders. There is also no evidence to illustrate that D-glucose binding does not require Na^+ because there is sufficient Na^+ within the brush border preparation to satisfy a possible Na^+-dependent requirement for this binding (8). Further experimentation is necessary to determine whether Na^+ is indeed necessary for preferential D-glucose binding to Tris-disrupted brush borders. Additional studies are also required to define the role of other cations in the mechanism of active sugar transport by the small intestine. The K^+ is of particular interest because it does not affect preferential D-glucose binding but it is inhibitory to the active transport of sugars by the small intestine (7, 23). It seems that K^+ may be involved in the second or dissociation step of this mechanism.

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