Structural Requirements for Active Intestinal Transport

THE NATURE OF THE CARRIER-SUGAR BONDING AT C-2 AND THE RING OXYGEN OF THE SUGAR

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Several weakly transported sugars were tested for transport by the Na^+ -dependent sugar carrier with slices of everted hamster intestinal tissue. Sugars were assumed to be transported by this carrier if the accumulation was diminished in the absence of Na^+ and in the presence of the competitive inhibitor 1,5-anhydro-D-glucitol. The extent of accumulation was correlated with the number of hydroxyl groups in the D-*gluco* configuration if the ring oxygen was placed in the normal D-glucose position. 5-Thio-D-glucose, with a sulphur atom in the ring, was transported at about the same rate as D-glucose and had a similar K_t for D-galactose transport, but myoinositol was poorly accumulated. It is suggested that there is no hydrogen bonding at the ring oxygen atom, but that the oxygen atom is found at this position as a result of steric constraints. No sugar without a hydroxyl group in the D-*gluco* position at C-2 of the sugar, including D-mannose, 2-deoxy-D-glucose, 2-chloro-2-deoxy-D-glucose and 2-deoxy-2-fluoro-D-glucose, was transported by the Na^+ -dependent carrier, but these sugars and L-fucose weakly and competitively inhibit the Na^+ -dependent accumulation of L-glucose into slices of everted hamster intestinal tissue. It is concluded that the bond between the carrier and C-2 of the sugar may be covalent, and a possible mechanism for active intestinal transport is proposed.

It has been proposed (Barnett, Jarvis & Munday, 1968) and partially confirmed (Barnett, Ralph & Munday, 1969) that actively transported sugars bind to the Na^+ -dependent sugar carrier in hamster intestine by hydrogen bonds that can form only when the sugar has hydroxyl groups or in some cases electronegative atoms other than oxygen, in the D-*gluco* configuration. The observation that L-glucose can be weakly actively transported (Neale & Wiseman, 1968, 1969) led us to undertake a survey of some weakly transported sugars to distinguish those transported by the Na^+ -dependent active transport mechanism, and to discover whether the hypothesis of binding only to those positions that have a hydroxyl group in the D-*gluco* configuration can explain their relative rates of transport, or whether some modification is needed. During these experiments it was found necessary to investigate the role of the ring oxygen atom and further explore the requirements at C-2 of the sugar. The transport of some of the substances described in this paper has been investigated by using hamster intestinal rings and sacs (Bihler, 1969) and rat intestinal sacs (Neale & Wiseman, 1969). Some of the results have been the subject of a preliminary communication (Barnett, Ralph & Munday, 1970).

MATERIALS

D-Galactose, D-glucose, D-arabinose, D-xylose, 6-deoxy-D-galactose, 6-deoxy-L-galactose, D-mannose, D-mannitol and myoinositol were obtained from British Drug Houses Ltd. (Poole, Dorset, U.K.). L-Xylose, L-glucose and L-rhamnose were obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks, U.K.). L-Galactose, m.p. 165°C, was made by the method of Frush & Isbell (1962), and 1,5-anhydro-D-glucitol, m.p. 141°C, by the method of Ness, Fletcher & Hudson (1950). Methyl 2-chloro-2-deoxy-D-glucopyranoside, m.p. 161–162°C, was made by the method of Fischer, Bergmann & Schotte (1920). D-[1- ^3H]Galactose, [U- ^{14}C]myoinositol, L-[1- ^{14}C]glucose and D-[1- ^{14}C]mannose were obtained from The Radiochemical Centre (Amersham, Bucks, U.K.). D-[1- ^{14}C]Mannitol was made by sodium borohydride reduction of D-[^{14}C]mannose. Sugar purity was tested by paper chromatography and, when necessary, sugars were purified by preparative chromatography on Whatman no. 3MM paper with butan-1-ol-ethanol-water (4:1:5, by vol.; upper layer) as solvent.

2-Chloro-2-deoxy-D-glucose and 2-deoxy-2-fluoro-D-glucose (Adamson, Foster, Hall & Hesse, 1969) were generous gifts from Professor A. B. Foster (Chester Beatty Research Institute, London S.W.3, U.K.) and 5-thio-D-glucose (Rowell & Whistler, 1966) was a generous gift from Professor R. L. Whistler (Department of Biochemistry, Purdue University, Lafayette, Ind., U.S.A.).

METHODS

Assays of sugars. Reducing sugars were measured by the method of Nelson (1944). 2-Deoxy-D-glucose was determined by the method of Waravdekar & Saslaw (1959) and halogenated sugars by the α -naphthol- H_2SO_4 method (Devor, 1950). The sensitivity of 2-deoxy-2-fluoro-D-glucose to this reagent was poor and only by using ice-cold solutions and by taking care not to use excess of $Ba(OH)_2$ during deproteinization were reproducible results obtained, and the colour yield was low. This can be ascribed to the remarkable stability of this sugar to acid attack.

Radioactive sugars were measured with a Beckman liquid-scintillation counter with either NE220 or NE250 scintillation fluid [Nuclear Enterprises (G.B.) Ltd., Edinburgh, U.K.]. Samples of volume 0.2 ml were added to 5 ml of scintillation fluid.

Tissue accumulation experiments. For each experiment the entire small intestine from two hamsters was used. The animals were anaesthetized with ether and the guts were washed *in situ* with ice-cold glucose-free Krebs-Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) gassed with $O_2 + CO_2$ (95:5). In some experiments all Na^+ was replaced by tris. The gut was tied at one end on to a narrow glass rod, and removed from the animal and everted simultaneously. Segments (2–3 mm wide) were cut while the gut was immersed in ice-cold buffer. The slices were added so that each flask contained gut rings from all regions of the small intestine. After preincubation of the flasks at $37^\circ C$ with shaking for 15 min, sugar in buffer (1 ml) was added to the incubation medium (final volume 6 ml). The incubation was stopped after the required time by decanting the incubation medium and washing the tissue in ice-cold buffer. The tissue samples were washed twice, dried between filter papers to remove excess of moisture and weighed. The samples were then homogenized in water (4 ml normally, 2 ml when radio-

active sugars were used) followed by protein precipitation with $ZnSO_4-Ba(OH)_2$ by the method of Nelson (1944). When radioactive sugars were used the incubation medium contained 10^6-10^7 c.p.m.

Tissue accumulation experiments in the presence of fluoride. The methods described above were used, but in the place of the normal buffer one of the following composition was used. Salt solutions (0.154 M) were added in the proportions by volume shown giving the final concentrations shown in parentheses: NaCl, 98.2 (116.8 mm); KCl, 4.0 (4.76 mm); tris-HCl buffer, pH 7.4, 3.0 (3.75 mm); KH_2PO_4 , 1.0 (1.19 mm); Na_2SO_4 , 0.5 (0.6 mm); $NaHCO_3$, 21.0 (25.0 mm); NaF, 1.7 (2.0 mm). This buffer lacks Mg^{2+} and Ca^{2+} and is 145 mequiv. in Na^+ and 2 mequiv. in F^- .

Determination of tissue water content. The water content of each batch of tissue samples was determined. The gut slices were incubated in the same way and blotted dry as described above. They were then dried in an oven at $90^\circ C$ to constant weight.

RESULTS

Tissue/medium ratios. The sugar (1 mm) was incubated for 30 min with 200–300 mg of hamster intestinal slices and the medium and tissue concentrations of sugar were determined by analysis. The water content of the experimental slices was deduced from measurements made of the wet weight and the dry weight of comparable slices. From these two values the concentration of the sugar in the tissue water was deduced. The tissue water contents were $81 \pm 2\%$ irrespective of the sugar used. In all cases D-galactose was tested for accumulation to ensure that the preparation was active and, generally, a sample of both tissue and

Table 1. *Tissue/medium ratios for the accumulation of sugars by hamster intestinal slices in the presence and absence of Na^+*

Sugar	Final tissue/medium ratio (initial concn. in medium 1 mm)		
	With Na^+ (145 mequiv.)	With Na^+ and 1-deoxy-D-glucose	Without Na^+
L-Arabinose	0.71 ± 0.08	0.38 ± 0.02	0.18 ± 0.01
D-Arabinose	0.17 ± 0.04	0.18 ± 0.02	0.18 ± 0.02
D-Xylose	0.75 ± 0.09	0.15 ± 0.01	0.13 ± 0.02
L-Xylose	0.43 ± 0.03	0.21 ± 0.01	0.13 ± 0.02
D-Fucose	0.81 ± 0.12	0.53 ± 0.08	0.19 ± 0.02
L-Fucose	0.15 ± 0.02	—	0.19 ± 0.02
D-Galactose	6.3 ± 0.09	1.8 ± 0.24	0.39 ± 0.04
L-Galactose	0.14 ± 0.01	—	0.19 ± 0.01
L-Glucose	1.01 ± 0.11	0.22 ± 0.02	0.18 ± 0.02
D-Mannose	0.21 ± 0.01	—	0.18 ± 0.02
D-Mannose*	0.14 ± 0.01	—	—
2-Deoxy-D-glucose	0.17 ± 0.02	—	0.17 ± 0.02
myoInositol	0.61 ± 0.09	—	0.24 ± 0.03
D-Mannitol	0.17 ± 0.03	—	—

* In the presence of fluoride.

Table 2. *Tissue/medium ratios and K_t for the accumulation of D-galactose or L-glucose into hamster intestine for sugars modified at C-2 and C-5*

Sugar	Final tissue/medium ratio (initial concn. in medium 1 mM)	K_t (substrate D-galactose) (mM)	K_t (substrate L-glucose) (mM)
D-Glucose	5.05 ± 0.6	2.3	—
D-Glucose*	16.4 ± 0.4	—	—
3-Chloro-3-deoxy-D-glucose	24.2 ± 2.8	3.55	—
5-Thio-D-glucose	8.2 ± 0.4	2.9	—
5-Thio-D-glucose*	12.5 ± 0.7	—	—
D-Mannose	0.21 ± 0.01	∞	65
2-Deoxy-2-fluoro-D-glucose	0.51 ± 0.18	∞	—
2-Chloro-2-deoxy-D-glucose	0.21 ± 0.03	—	65
Methyl 2-chloro-2-deoxy-D-glucoside	0.21 ± 0.02	—	—
2-Deoxy-D-glucose	0.17 ± 0.02	—	65
L-Fucose	0.15 ± 0.02	—	65

* In the presence of fluoride.

medium fluids was chromatographed to ensure that the sugar was not altered. The tissue/medium ratios were determined in the presence and absence of Na^+ and in the presence of the competitive sugar 1,5-anhydro-D-glucitol (1-deoxy-D-glucose) (5 mM). The values and the standard errors are shown in Tables 1 and 2. The standard errors were calculated for three to six determinations, usually on two separate occasions. The percentage recovery (not shown) was calculated in each case to ensure that undetected metabolism did not take place. 5-Thio-D-glucose (73.5% recovery) and D-glucose (33% recovery) were the only sugars significantly metabolized. The accumulation of these sugars was also measured in 2 mequiv. of F^- . It is noteworthy that the recovery of 5-thio-D-glucose in the urine after intraperitoneal injection into rats was high (97%) (Hoffman & Whistler, 1968).

Inhibition of D-galactose accumulation. D-Galactose (1–5 mM) was incubated with everted intestinal slices (200–300 mg) for 10 min. The tissue concentration, corrected for dry weight content, was measured. Since the accumulation of D-galactose in this system is linear over 30 min (Barnett *et al.* 1969), the concentration of sugar after 10 min was proportional to the initial rate of accumulation by the tissue. Apparent sugar-carrier competitive inhibitor constants, K_t , were calculated from Lineweaver-Burk plots of the reciprocal of the final tissue concentration against the reciprocal of the initial D-galactose concentration with and without inhibiting sugar (20 mM). The K_t values are given in Table 2.

Inhibition of L-glucose accumulation. L-Glucose (1–5 mM) was incubated for 10 min, in the presence and absence of the potential inhibitor (20 mM), with slices of everted hamster intestinal tissue (approx. 200 mg). The concentration in the tissue fluid was

determined and corrected for accumulation into extracellular space (the mannitol space) by subtraction of the accumulation of D-mannitol into comparable slices of tissue. The apparent percentage of the tissue water available for equilibration with D-mannitol was 18.5%.

The initial rate of accumulation was assumed to be proportional to the accumulation in 10 min, and Lineweaver-Burk plots were constructed of the reciprocal of the final concentration in the tissue against the reciprocal of the initial L-glucose concentration in the medium. These are shown in Fig. 2. Points were very reproducible and the points for L-glucose without inhibitor show the standard error for five different occasions. The standard error was calculated at each concentration, and the reciprocal of the limits is represented by a bar on Fig. 2.

All the sugars tested, 2-deoxy-D-glucose, D-mannose, 2-chloro-2-deoxy-D-glucose (not shown) and L-fucose, showed inhibition that was assumed to be competitive. The extent of the inhibition was similar in each case, giving an apparent K_t about 65 mM. D-Mannitol was not inhibitory (Fig. 2).

DISCUSSION

The observation of glucose-galactose malabsorption (Lindquist, Meeuwisse & Melin, 1962; Dahlqvist, Lindquist & Meeuwisse, 1968), in which glucose and galactose are only very slowly absorbed by the small intestine, but fructose is absorbed at a normal or even at an elevated rate, suggests that there are two carriers for mammalian intestinal sugar transport. It is well known that fructose absorption is not stimulated by Na^+ (Crane, 1968). Therefore, to test for transport by the Na^+ -dependent carrier of active transport, the accumulation of sugars

Fig. 1. Comparison of the structures of weakly transported sugars with their accumulation into hamster intestinal slices under various conditions. The sugars (initial concentration 1 mM) were incubated for 1 h in the presence of Na⁺ (145 mequiv.) (□), in the presence of Na⁺ and 1,5-anhydro-D-glucitol (▨) and in the absence of Na⁺ (■). Sugars are drawn so that the oxygen falls in the position usually occupied by the oxygen in D-glucose, so that the hydroxymethyl group falls either at the position normally occupied by C-6 of D-glucose or in the aglycone position of a β-D-glucopyranoside. If the reducing group is in the position occupied by C-1 of D-glucose, no preference is indicated for the α- or β-anomers, but if the reducing group falls at the position occupied by C-5 of D-glucose the β-anomer is drawn since this occupies the C-6 position of D-glucose.

(1 mM) into everted hamster intestinal slices was measured in the presence and the absence of Na⁺ and in the presence of a known substrate for active intestinal transport, 1,5-anhydro-D-glucitol (1-deoxy-D-glucose). The specificity of the passive

transport system of the mammalian erythrocyte (LeFevre, 1961) suggests that 1-deoxy-D-glucose may not interact with the second, Na⁺-independent, carrier if the specificity requirements of this carrier are similar to that of the erythrocyte. Thus

accumulation that is both dependent on Na^+ and inhibited by 1-deoxy-D-glucose may be considered to be due to the active carrier system. The results are shown in Table 1.

Three categories of sugars may be distinguished: D-galactose, which was actively accumulated; those poorly transported but by an Na^+ -dependent mechanism and inhibited by 1-deoxy-D-glucose; and those which do not appear to interact with the Na^+ -dependent carrier. D-Mannitol, usually regarded as measuring extracellular space, was accumulated to about the same extent as this last group. D-Galactose, like other well-transported sugars (Barnett *et al.* 1968) has only one deviation from the D-glucose structure. The structures of the sugars weakly accumulated are represented in Fig. 1. The sugars are shown in the Haworth formulae, both for clarity and because the actual conformation of the sugar bound to the carrier is not known. If the conformation on the carrier is different from that in solution, a simple equilibrium will exist between the forms. The methyl or hydroxymethyl group at C-6 of the L-hexoses is placed in the large space known to be available on the carrier at the position corresponding to the phenoxy group of the well-transported phenyl β -D-glucopyranoside (Alvarado & Crane, 1964). This formulation has also been used by Caspary, Stevenson & Crane (1969). When the oxygen of the sugar ring is placed in the same relative position as in D-glucose, as in Fig. 1, the extent of the Na^+ -dependent transport correlates reasonably well with the similarity to the D-glucose structure. Loss of two hydroxyl groups from the *gluco* configuration gives only very weak accumulation, and loss of three hydroxyl groups gives no Na^+ -stimulated accumulation. Thus D-fucose (6-deoxy-D-galactose) and L-arabinose are weakly accumulated by the Na^+ -dependent carrier but L-rhamnose (6-deoxy-L-mannose) is not. L-Arabinose is not actively transported by dietary-restricted rats (Neale & Wiseman, 1969), and Bihler (1969) has reported that the transport in hamster is not Na^+ -stimulated, so this sugar was carefully purified by chromatography and the accumulation determined 12 times. The principles outlined above have been previously established (Barnett *et al.* 1968).

The notable exception to these general rules is that no substance, even D-mannose, which does not have a hydroxyl group in the *gluco* configuration at C-2, is transported at all. This rationalization suggested that a further investigation of the role of the ring oxygen atom, which seems to take up an obligatory position, and the hydroxyl group at C-2, which appears to be essential, was necessary. The results are shown in Table 2.

Previously we have investigated the binding at specific positions of the sugar molecule by comparing

analogues in which oxygen is replaced by hydrogen or sulphur, which cannot form part of a hydrogen bond. Thus methyl β -D-galactopyranoside was well transported whereas methyl β -D-thiogalactopyranoside and 1-deoxy-D-galactose were not (Barnett *et al.* 1968). 5-Thio-D-glucose, in which the ring oxygen atom is replaced by sulphur and which therefore cannot hydrogen-bond at this position, was tested. The tissue/medium ratio was high (8.2) and, in the presence of fluoride ion to inhibit metabolism, was raised to a value (12.5) comparable with that for D-glucose in the presence of fluoride ion (16.4). Similarly the K_i for the thio sugar was comparable with that of D-glucose. Thus it seems that there is no hydrogen-bonding to the ring oxygen atom and that the justification for placing the oxygen atom always in the same position must be on steric grounds. This hypothesis is partially supported by the poor accumulation of *myo*inositol, which has a hydroxyl-carrying carbon atom at this position, but otherwise has a structure similar to that of D-xylose or L-glucose. Since it is not transported as well as either of these sugars there is some evidence for a steric restriction at this position preventing a good fit of a group carrying substituents. Indeed, since this work was completed, Caspary & Crane (1970) have suggested that the Na^+ -dependent accumulation of *myo*inositol is by a separate carrier. However, a second steric restriction may be as important. This is that the C-6 of hexoses must be at one of the positions normally occupied by the substituent at C-5 (the hydroxymethyl group) or at C-1 of D-glucose. The space around the other carbon atoms is very restricted (Barnett *et al.* 1968, 1969). It seems possible that the poor accumulation of both D-xylose and L-arabinose compared with 6-deoxy-D-glucose and 6-deoxy-D-galactose may be due to the fact that without this steric constraint they form abortive complexes with the carrier.

Replacement of the hydroxyl group at C-3 of D-glucose by chlorine or fluorine gives substances that are very rapidly accumulated by the Na^+ -dependent carrier (Barnett *et al.* 1969). We therefore studied the accumulation of 2-deoxy-D-glucose, 2-deoxy-2-fluoro-D-glucose and 2-chloro-2-deoxy-D-glucose. In no case was there any Na^+ -dependent accumulation, and 2-deoxy-2-fluoro-D-glucose (20mM) and D-mannose (20mM) gave no detectable inhibition of D-galactose transport (1–5mM). The hydroxyl group at each other position of the D-glucose molecule can be replaced by hydrogen with only diminution rather than complete loss of Na^+ -dependent transport. 2-Deoxy-D-glucose (Caspary *et al.* 1969) and D-mannose are well known as non-transported sugars, despite a recent observation (Bihler, 1969) of slight Na^+ stimulation of the accumulation of the latter that we could not confirm

in our preparation. The hydroxyl group at C-2 of D-glucose therefore seems unique, as suggested by Crane (1960), and it therefore seems probable that the carrier-sugar bond at C-2 is of a different nature to the hydrogen bonds previously proposed for the interactions at the other positions (Barnett *et al.* 1968, 1969). We therefore propose a covalent bond between C-2 of the sugar and the carrier protein, which would also explain the marked variations in the maximum rate of transport, V , found in the previous investigations.

Crane (1965) has proposed that the driving force for active intestinal transport is the Na^+ gradient across the brush-border membrane, maintained by the sodium pump. The sugar binds strongly to the carrier in the presence of Na^+ and weakly in its absence. The concentration of sugar in equilibrium with the carrier is therefore inversely related to the Na^+ concentration, and the low Na^+ concentration inside the cell relative to that outside allows a high sugar concentration inside relative to that outside. In a modification of this theory, Caspary *et al.* (1969) have suggested a multi-stage process involving conformational change without a covalent bond between sugar and carrier. Any covalent carrier-sugar complex that will fit with the important, well-established, features of this mechanism must be formed and broken without net utilization of energy. A possibility for such a system, which is consistent with the observation that ^{18}O is retained at C-2 of the sugar during transport (Crane & Krane, 1959), is shown in Scheme 1. The sugar is adsorbed on the carrier in the presence of Na^+ , the hydroxyl group at C-2 then cleaves an intramolecular ester on the membrane to form an ester bond between sugar and membrane, and finally the intramolecular ester is regenerated with release of the sugar to the inside of the cell in a low- Na^+ environment.

There are several anomalies of the previous concept of active transport involving sugar binding

only by hydrogen bonds that such a scheme might explain.

Although it has been suggested that many sugars are transported at the same maximum rate (Bihler, 1969), in our previous investigations large variations have been noted. Thus V for 3-chloro-3-deoxy-D-glucose and 3-deoxy-3-fluoro-D-glucose was 87 and 71 mm/10 min respectively whereas V for 3-deoxy-D-glucose was only 41 mm/10 min (Barnett *et al.* 1969). Although such a large difference in rate is difficult to explain for such sterically similar sugars by a mechanism in which the sugar is hydrogen-bonded to the carrier, the formation and breaking of an ester at C-2 could readily explain the differences by electronic effects.

By analogy with an enzyme reaction, if transport is by a mechanism similar to that outlined in Scheme 1, it might be expected that sugars of similar structure to D-glucose but lacking the hydroxyl group at C-2 would have a weak affinity for the carrier, but would not be transported. Although we could not demonstrate any competitive inhibition with either D-mannose or 2-deoxy-2-fluoro-D-glucose (20 mM) with the strongly bound substrate D-galactose, Caspary *et al.* (1969), using 3-O-methyl-D-glucose as substrate, and Caspary & Crane (1968), using L-glucose as substrate, have shown that L-fucose (6-deoxy-L-galactose) behaves exactly in this way. We therefore tested the non-transported sugars as inhibitors of L-glucose accumulation, which has an apparent K_m about 65 mM and is therefore only weakly bound to the Na^+ -dependent carrier.

All the tested non-transported C-2 analogues of D-glucose inhibited L-glucose accumulation; D-mannitol did not (Fig. 2). The apparent K_i was about 65 mM in each case. This is higher than the value of 20 mM reported by Caspary *et al.* (1969), using 3-O-methyl-D-glucose as substrate, but this discrepancy may be accounted for by the different

Scheme 1.

Fig. 2. Lineweaver-Burk plots of L-glucose accumulation into slices of everted hamster intestinal tissue in the presence of various sugars (20 mM), corrected for D-mannitol space. The L-glucose points for four separate occasions are shown as bars, the limits of which represent the reciprocals of the limits of the standard error for the points, in the absence of inhibiting sugar. ●, D-Mannitol; ○, 2-deoxy-D-glucose; ■, D-mannose; ▲, L-fucose.

range of substrate concentrations as well as by the different substrate.

The extension of the observation of inhibition of Na^+ -dependent transport by non-transported sugars to these closely related D-glucose analogues removes L-fucose from its apparently anomalous unique position, and supports the suggestion that the carrier-sugar bonding at C-2 of the sugar is of a different type from that at the other positions. Sugars unable to form the bond at C-2 bind weakly to the carrier but cannot be transported.

Conversely a substance that can form the covalent bond at C-2 but that cannot pass through the membrane and so be discharged in the low- Na^+ environment on the inside of the membrane might be expected to have a particularly high apparent affinity for the carrier. Such a substance is phlorrhizin (Alvarado & Crane, 1962).

By combining the results obtained in this paper with those in the previous papers (Barnett *et al.* 1968, 1969), it is possible to construct a possible model for the binding in the carrier-sugar complex (Fig. 3). It is known that loss of the hydroxyl group at C-4 leads to a weakening of the binding, indicating a bond at this position, but since no sugar with a halogen in the D-glucose configuration at C-4 has been tested it is not possible to distinguish the direction of the hydrogen bond. The bond to the oxygen at C-1 must be capable of formation between the

Fig. 3. Diagram of the possible binding of D-glucose to the hamster intestinal sugar carrier. Broken lines represent hydrogen bonds.

carrier and both α - and β -sugars, since both α - and β -glycosides are equally well transported.

The concept of covalent carrier-sugar binding suggested in this paper for the Na^+ -dependent sugar carrier may be of general occurrence. Examination of the structural requirements for sugar transport in the human erythrocyte (LeFevre, 1961) shows that a similar scheme may operate if the ester bond is reversibly formed by cleavage of an ester or thiol ester on the membrane by the hydroxyl group at C-1 of the sugar in the ring form.

A similar complex for active intestinal transport of amino acids would involve the reversible cleavage of a membrane acid anhydride by the carboxylate ion of the amino acid.

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