

## Structural Requirements for Active Intestinal Sugar Transport

### THE INVOLVEMENT OF HYDROGEN BONDS AT C-1 AND C-6 OF THE SUGAR

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1. A series of D-galactose derivatives substituted at C-1 and C-6 were tested for active accumulation by everted segments of hamster and rat intestine. 2. D-Galactose and 6-deoxy-6-fluoro-D-galactose were accumulated far more rapidly than 6-deoxy- and 6-chloro-6-deoxy-D-galactose, and this is interpreted as due to hydrogen-bonding at C-6 during the transport process. 3. 6-Bromo-6-deoxy- and 6-deoxy-6-iodo-D-galactose were not actively transported, indicating that the allowed size of substituent at C-6 lies between that of chlorine and bromine atoms. 4. Similar results were obtained at C-1. Both methyl  $\alpha$ -D-galactopyranoside and methyl  $\beta$ -D-galactopyranoside were well transported, but methyl  $\beta$ -D-thiogalactopyranoside and 1-deoxy-D-galactose were not transported; D-galactopyranosyl fluoride was transported, but only poorly. Again hydrogen-bonding is suggested. 5. It is proposed that D-glucose is the ideal structure for active transport and that binding occurs at C-1, C-2, C-3, C-4 and C-6. Loss of two or more of these bonds usually causes loss of active transport. 6. By plotting Lineweaver-Burk plots of the rates of transport of the galactose derivatives, the apparent  $V$  and  $K_m$  values were obtained. With hamster intestine both these values were very reproducible. Contrary to expectation,  $V$  varied for different sugars. 7. The  $K_t$  of some of the analogues modified at C-1 and C-6 was determined with methyl  $\alpha$ -D-glucoside as substrate. 8. An attempt to alkylate the carrier by using methyl 3,4-anhydro- $\alpha$ -D-galactoside was unsuccessful. There was no evidence that this compound was bound to the carrier.

Minimum requirements for the active transport of sugars across mammalian intestine have been suggested (Crane, 1960) (Fig. 1). More recently xylose has been shown to be poorly accumulated against a concentration gradient (Alvarado, 1966), indicating that C-6 is not absolutely obligatory, and Csaky & Lassen (1964) and Csaky & Ho (1966) have shown that both D-xylose and D-mannose are transported by frog intestine. Many substances with these basic structural requirements are not transported and this can often be attributed to the bulk of the substituent groups. However, this is not the universal cause, since several compounds with small substituents are not transported. The data seem to indicate that D-glucose is probably the ideal structure for active transport, and that generally if two or more of the hydroxyl groups (other than that at C-2, which is obligatory) are inverted or converted into the deoxy sugar then the sugar is usually no longer actively transported. Thus D-gulose (inverted at C-3 and C-4) and 1,6-dideoxy-D-glucose are not transported, though D-galactose (inverted at C-4), D-allose (inverted at

C-3), 6-deoxy-D-glucose and 1-deoxy-D-glucose are (Wilson & Landau, 1960; Bihler, Hawkins & Crane, 1962). This implies that most, if not all, of the hydroxyl groups of the sugar may be involved in binding during some part of the active transport process, that at C-2 being particularly important. To investigate the nature of this involvement we prepared, and tested for active transport, a number of D-galactose analogues modified at C-1 or C-6 or both. Galactose, which is transported at about the same rate as D-glucose but is not rapidly metabolized (Wilson & Vincent, 1955), was chosen both for the ease of preparation of substituents, and also since it

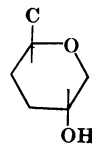


Fig. 1. Minimum structural requirement for active transport (Crane, 1960).

already probably contains one departure from the 'ideal' structure (at C-4) the modification of a second hydroxyl group should have a strong effect.

A preliminary account of part of this work has been published (Jarvis, Barnett & Munday, 1967).

## MATERIALS

**Chemicals.** D-Galactose, D-mannose and D-xylose were obtained from British Drug Houses Ltd. (Poole, Dorset). 6-Deoxy-D-galactose, 6-deoxy-L-galactose, 3-O-methyl-D-glucose and 1,2:3,4-di-O-isopropylidene- $\alpha$ -D-galactopyranose were obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.). Methyl  $\beta$ -D-thiogalactopyranoside was from Calbiochem Ltd. (London, W. 1). 6-Deoxy-6-fluoro-D-galactose, m.p. 156°, and methyl 6-deoxy-6-fluoro- $\alpha$ -D-galactopyranoside, m.p. 135–136° (Taylor & Kent, 1958), 6-chloro-6-deoxy-D-galactose, m.p. 128–129° (Wood, Fisher & Kent, 1966), 6-deoxy-6-iodo-D-galactose, m.p. 108–111° (Raymond & Schroeder, 1948), methyl  $\alpha$ -D-galactopyranoside, m.p. 123°, methyl  $\beta$ -D-galactopyranoside, m.p. 170–171°, and 1,5-anhydro-D-glucitol, m.p. 138–140° (Ness, Fletcher & Hudson, 1950), L-galactose, m.p. 165° (Frush & Isbell, 1962), D-allose, m.p. 128–132° (Theander, 1964),  $\alpha$ -D-galactopyranosyl fluoride, decomp. 127° (Barnett, Jarvis & Munday, 1967b), and methyl 2,3-anhydro- $\alpha$ -D-alloside, m.p. 100–102° (Gut & Prins, 1947), were made as previously described. Methyl 3,4-anhydro- $\alpha$ -D-galactopyranoside was a gift from Dr P. W. Kent (Department of Biochemistry, University of Oxford). 1,5-Anhydro-D-galactitol, m.p. 106–108°, was made by LiAlH<sub>4</sub> reduction of tetra-acetylgalactopyranosyl bromide as described for the glucose analogue.

**6-Bromo-6-deoxy-D-galactose.** 1,2:3,4-Di-O-isopropylidene-6-O-methanesulphonyl- $\alpha$ -galactopyranoside (5g.) and anhydrous LiBr (6g.) were refluxed in dimethylformamide (50ml.) for 1hr. The solution was cooled and poured into methylene dichloride and water. The organic layer was separated, washed with water and NaHCO<sub>3</sub> and dried over anhydrous CaCl<sub>2</sub>. The solvent was removed *in vacuo* and distilled, b.p. 107–108°/0.1mm., giving 6-bromo-6-deoxy-1,2:3,4-di-O-isopropylidene- $\alpha$ -galactopyranose (3g.), which crystallized on standing, m.p. 47–48° (lit. m.p. 45°; Petrov, Nifant'ev, Shchegolev & Tevekhov, 1964). The isopropylidene compound (2g.) was dissolved in methanol (8ml.) and water (20ml.), and 2N-H<sub>2</sub>SO<sub>4</sub> (2ml.) was added. After refluxing for 1hr., the solution was cooled and deionized with Amberlite IR-4B (CO<sub>3</sub><sup>2-</sup> form) and evaporated to dryness *in vacuo*. The syrupy 6-bromo-6-deoxy-D-galactose was chromatographically pure, but could not be induced to crystallize.

**Methyl 6-chloro-6-deoxy- $\alpha$ -D-galactopyranoside.** 6-Chloro-6-deoxy-D-galactose (1g.) was dissolved in dry methanol (50ml.) and refluxed for 6hr. with Amberlite IR-120 (H<sup>+</sup> form) (1g.), previously washed with dry methanol. The resin was filtered off and washed with methanol, and the methanolic solutions were evaporated to dryness. Methyl 6-chloro-6-deoxy- $\alpha$ -D-galactopyranoside was recrystallized from ethanol and then from ethyl acetate, m.p. 160–163°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> +180 ± 1°. Methyl 6-deoxy- $\alpha$ -D-galactoside was made in the same way, m.p. 140–142° (lit m.p. 156–158°; Schmidt, Mayer & Distelmaier, 1944).

**Radioactive chemicals.** 1,5-Anhydro-D-[6-<sup>3</sup>H]galactitol

(1-deoxy-D-[6-<sup>3</sup>H]galactose) (2mc/m-mole) was made as described by Barnett (1967). Methyl  $\alpha$ -D-[6-<sup>3</sup>H]glucopyranoside (1.8mc/m-mole) was made from [6-<sup>3</sup>H]glucose (The Radiochemical Centre, Amersham, Bucks.) by the method described above for methyl 6-chloro-6-deoxy-D-galactose.

## METHODS

**Assays of sugars.** Reducing sugars were measured by the method of Nelson (1944). Methyl glycosides, including epoxides and thioglycosides, and  $\alpha$ -D-galactopyranosyl fluoride were assayed by the  $\alpha$ -naphthol-H<sub>2</sub>SO<sub>4</sub> method (Devor, 1950). Good blanks were obtained only with nitrogen-free H<sub>2</sub>SO<sub>4</sub> (British Drug Houses Ltd.). Radioactive sugars (1-deoxy-D-[6-<sup>3</sup>H]galactose and methyl [6-<sup>3</sup>H]glucoside) were measured in a Beckman model 1650 liquid-scintillation counter. For these experiments deproteinized 0.2ml. portions of mucosal or tissue solution containing between 1000 and 30000 counts/min. were added to 5ml. of NE220 scintillation fluid [Nuclear Enterprises (G.B.) Ltd., Sighthill, Edinburgh].

**Tissue accumulation experiments.** One or two hooded rats, or two or three hamsters that had been starved for 48hr., were anaesthetized with ether, and the guts were rinsed with ice-cold Krebs-Henseleit Ringer solution (Krebs & Henseleit, 1932) *in situ* and removed. After eversion of the guts on a glass rod, they were cut into 2–3mm.-wide segments under cold oxygenated Ringer solution. About ten segments (approx. 200–300mg.) were transferred to the incubation flask containing 5ml. of Ringer solution. The segments were added so that each flask received pieces from all parts of the intestine. To the preincubated oxygenated flasks were added the sugar (1ml.) in Ringer solution, and the flasks were shaken at 37° for the required time, generally 10min. for kinetic experiments and 30min. for tissue/medium ratio experiments. The pieces of tissue were then blotted free from excess of fluid, weighed and homogenized in 4ml. of water (for radioactive substances, 3ml.). The mucosal solution and homogenate were analysed for sugar after deproteinization with Ba(OH)<sub>2</sub> and ZnSO<sub>4</sub> (Nelson, 1944). When radioactive sugars were used, the incubation medium contained 10<sup>6</sup>–10<sup>7</sup> counts/min. in the incubation flask and 0.2ml. portions were assayed.

**Chromatography.** The sugars were generally tested for purity by thin-layer chromatography (Barnett, Jarvis & Munday, 1967a) before each sequence of experiments; with the labile  $\alpha$ -D-galactosyl fluoride the tissue homogenate was chromatographed on silica gel after the experiment to ensure that there was no conversion into galactose. All the 6-deoxygalactoses contained a trace of galactose, which was usually less than 0.5%. With 6-deoxy-6-fluoro-D-galactose this rose to 1.0–1.5%.

## RESULTS

**Time-dependence of accumulation of 3-O-methyl-D-glucose.** Flasks containing rat intestinal slices were incubated with 1mm 3-O-methyl-D-glucose for 0–30min. Over this time range the accumulation was linear.

**Tissue/medium ratios.** The sugar (1mm) was incubated for 30min. with 200–300mg. of hamster

Table 1. *Data for the accumulation of sugars by hamster and rat intestinal slices*

Compound	Tissue/medium ratio (initial concn. 1 mM)	<i>v</i> (mm/10 min.) (initial concn. 10 mM)	<i>K<sub>m</sub></i> (mM)	<i>V</i> (mm/10 min.)	<i>K<sub>t</sub></i> (mM)
<b>Hamster slices</b>					
L-Galactose*	0.3 ± 0.05	2.8	—	—	—
D-Galactose†	12.0 ± 4.5	25.0	9	44	1.5
6-Deoxy-D-galactose‡	1.45 ± 0.15	6.5	7	6	16
6-Deoxy-6-fluoro-D-galactose	4.3 ± 0.8	12.0	14	23	7
6-Chloro-6-deoxy-D-galactose	1.7 ± 0.1	6.5	7	6	—
6-Bromo-6-deoxy-D-galactose	< 0.6	3.0	—	—	—
6-Deoxy-6-iodo-D-galactose*	< 0.7	2.8	—	—	—
Methyl α-D-galactopyranoside	10 ± 1.5	25	9	44	—
Methyl 6-deoxy-α-D-galactopyranoside	< 0.6	3.0	—	—	—
Methyl 6-deoxy-6-fluoro-α-D-galactopyranoside	2.2 ± 0.3	5.0	8	25	—
Methyl 6-chloro-6-deoxy-α-D-galactopyranoside	1.1 ± 0.3	—	—	—	—
Methyl β-D-galactopyranoside	5.6 ± 0.2	16.0	15	32	—
Methyl β-D-thiogalactopyranoside	0.5 ± 0.03§	3.5	—	—	—
1,5-Anhydro-D-galactitol (1-deoxy-D-galactose)	0.4 ± 0.1	2.8	—	—	—
α-D-Galactopyranosyl fluoride	1.1 ± 0.4	8.4	75	44	16
D-Allose†	2.8 ± 1.1	—	—	—	—
D-Xylose	—	4.0	—	—	—
Methyl α-D-glucoside*	—	29.0	3.0	40	—
<b>Rat slices</b>					
L-Galactose	0.15	1.5	—	—	—
D-Galactose	2.5	6.2	10	—	—
6-Deoxy-D-galactose	1.0	—	—	—	—
6-Deoxy-6-fluoro-D-galactose	1.25	2.9	26	—	—
6-Chloro-6-deoxy-D-galactose	1.0	—	—	—	—
Methyl α-D-galactopyranoside	2.7	—	—	—	—
Methyl 6-deoxy-6-fluoro-α-D-galactopyranoside	1.4	—	—	—	—
Methyl 3,4-anhydro-α-D-galactopyranoside	0.7	—	—	—	—
3-O-Methyl-D-glucose	2.7	3.2	42	—	—
D-Mannose	0.3	—	—	—	—
1,5-Anhydro-D-glucitol (1-deoxy-D-glucose)	—	—	—	—	67

\* Previously studied by Wilson & Landau (1960).

† Previously studied by Fisher & Parsons (1953).

‡ Previously studied by Wilson & Crane (1958).

§ This determination was carried out by Miss A. Smedley. The tissue/medium ratio of D-galactose was 7.5.

|| The tissue/medium ratio of D-galactose in this experiment was 3.7.

or rat intestinal slices and the mucosal and tissue concentrations were assayed by the appropriate method. The water content of the tissue was assumed to be 80% (Alvarado & Crane, 1964) and each value is the average of three or four determinations. In all cases D-galactose was tested for transport to ensure that the preparation was active. The tissue/medium ratios, with standard errors where possible, of several sugars with hamster and rat intestinal slices are shown in Table 1. In many

cases the standard errors are calculated from only three to five points.

*Variation of rate of accumulation with concentration.*

The sugar (5–20 mM) was incubated with either rat or hamster intestinal slices for 10 min. With hamster (but not rat) tissue the rate of accumulation in separate experiments was extremely reproducible, especially at the higher values. The rates shown (Figs. 2 and 3) are the averages of four points taken on two separate occasions. In Fig. 2 the average

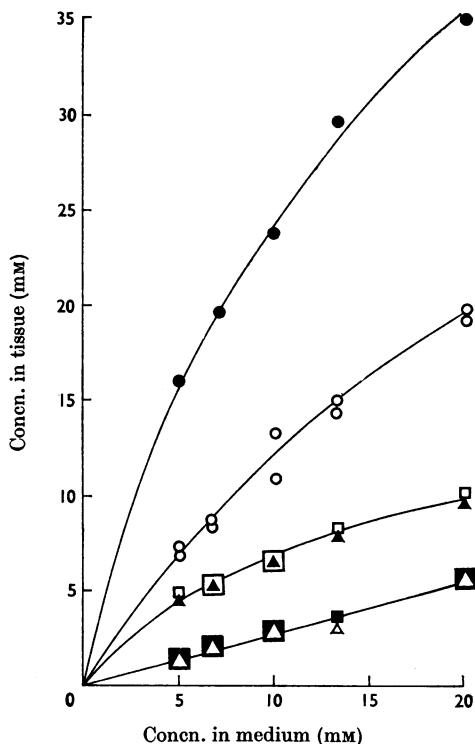


Fig. 2. Molar concentration of sugar accumulated by everted hamster intestinal slices in various medium concentrations of D-galactose derivatives modified at C-6, incubated for 10 min. at 37° in oxygenated Ringer solution. ●, D-Galactose; ○, 6-deoxy-6-fluoro-D-galactose; □, 6-deoxy-D-galactose; ▲, 6-chloro-6-deoxy-D-galactose; △, 6-deoxy-6-iodo-D-galactose; ■, L-galactose.

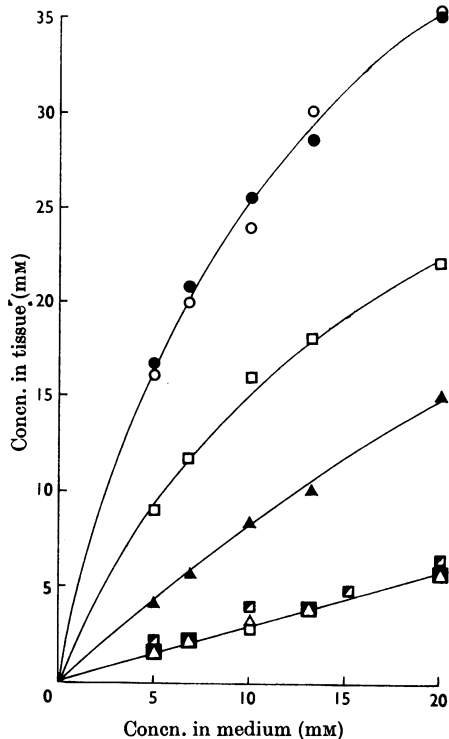


Fig. 3. Molar concentration of sugar accumulated by everted hamster intestinal slices in various medium concentrations of D-galactopyranosides modified at C-1, incubated for 10 min. at 37° in oxygenated Ringer solution. ○, D-galactose; ●, methyl α-D-galactopyranoside; □, methyl β-D-galactopyranoside; ▲, D-galactopyranosyl fluoride; △, 1-deoxy-D-galactopyranose (1,5-anhydro-D-galactitol); ■, L-galactose; ▣, methyl β-D-thioglucopyranoside.

points for two separate occasions are shown for 6-deoxy-6-fluoro-D-galactose. Each point was the average of duplicate determinations on the same sample. The Lineweaver-Burk plots for the determination of  $K_m$  and  $V$  values were corrected for diffusion by subtraction of the L-galactose penetration rate. Alvarado & Crane (1964) have suggested that almost the same values for  $K_m$  are obtained whether rates are corrected or not. We found that this was true only for the more rapidly transported sugars. We also found that the apparent  $K_m$  varied with the concentration range and for this reason the same range was always used.

**Na<sup>+</sup>-dependence of transport.** The effect of replacing Na<sup>+</sup> in the mucosal solution by K<sup>+</sup> on the rate of accumulation was determined for D-galactose and 6-deoxy-6-fluoro-D-galactose. In both cases the rate with 145m-equiv. of Na<sup>+</sup>/l. was far greater than with 50m-equiv./l. The Lineweaver-Burk plots approximated to apparent competitive

stimulation by Na<sup>+</sup> (Fig. 4). The transport of L-galactose was unaffected.

**Competitive inhibition of methyl α-D-glucoside transport.** For some C-1- and C-6-substituted galactoses the apparent  $K_t$  for the substrate-carrier complex was determined with methyl α-D-glucoside as the accumulated sugar and hamster intestinal slices. The  $K_m$  of methyl α-D-glucoside (range 1-5mm) was determined in the presence and absence of the sugar to be tested (20mm) (Fig. 5). The apparent  $K_t$  values are given in Table 1. The  $K_t$  for 1,5-anhydro-D-glucitol (rat) was determined by inhibition of D-galactose accumulation.

**Attempt to alkylate the carrier by sugar analogues.** Everted rat intestine was preincubated with methyl 3,4-anhydro-α-D-galactopyranoside (10mm) or methyl 2,3-anhydro-α-D-allopyranoside (10mm) for 5-30min. at 37° and the tissue/medium ratio of D-galactose (1mm) was measured in the usual way.

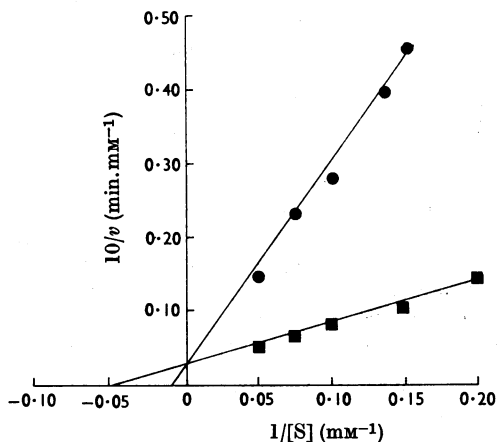


Fig. 4. Effect of  $\text{Na}^+$  concentration: Lineweaver-Burk plot of reciprocal of concentration accumulated into everted hamster intestinal slices per min. at  $37^\circ$  against reciprocal of medium concentration for 6-deoxy-6-fluoro-D-galactose. ●, With 50 m-equiv. of  $\text{Na}^+$ /l.; ■, with 145 m-equiv. of  $\text{Na}^+$ /l.

No statistical difference was observed from a control preincubated alone. The anhydro sugars were not actively accumulated.

## DISCUSSION

The initial experiments, with either hamster or rat intestinal slices, in which the tissue/medium ratios of galactose derivatives modified at C-1, C-6 or both were determined (Table 1), clearly show that if the oxygen function is removed from the optimum configuration then there is a considerable fall in the rate of active transport, or absence of active transport completely. Thus 6-deoxy-D-galactose and 6-chloro-6-deoxy-D-galactose were poorly accumulated, and the corresponding methyl D-galactosides were not actively accumulated at all. 6-Bromo-6-deoxy- and 6-deoxy-6-iodo-D-galactose were not actively accumulated, probably owing to the size of the substituent at C-6, which is comparable with that in 6-O-methyl-D-glucose, which is also not actively accumulated (Wilson & Landau, 1960). This indicates that the size of the substituent at C-6 that allows transport lies between that of chlorine and that of bromine, since the chloro derivative was transported at exactly the same rate as the 6-deoxy-D-galactose.

The considerable decrease in the rate of transport of 6-deoxy- and 6-chloro-6-deoxy-D-galactose suggests that the hydroxyl group at C-6 is involved in the transport process. The nature of this involvement is indicated by the relatively high rates of transport of 6-deoxy-6-fluoro-D-galactose and its methyl glycoside. Both of these compounds can form a hydrogen bond at C-6, as can D-galactose itself. One can therefore suggest that one of the binding sites of sugar to carrier is at C-6, and furthermore that this is a hydrogen-bond formation by donation of a hydrogen by the carrier to the sugar, since this is the only sense in which such a bond can be formed with the fluoro derivative. It is noteworthy that 6-deoxy-6-fluoro-D-glucose appears to be transported more readily than 6-deoxy-D-glucose, though the published data are poor (Wilson & Landau, 1960).

Examination of the substituents at C-1 shows a similar loss of transportability. Here 1-deoxy-D-galactose is completely inert, in contrast with 1-deoxy-D-glucose, which is still transported. (This suggests that C-4 is also involved in binding.) 1-Deoxy-1-fluoro-D-galactose (galactosyl fluoride) is transported more readily than 1-deoxy-D-galactose, and can just be shown to be actively accumulated. This is in marked contrast with methyl  $\alpha$ - and  $\beta$ -D-galactoside, which are both transported very well, the former as readily as D-galactose itself. Again one can suggest hydrogen-

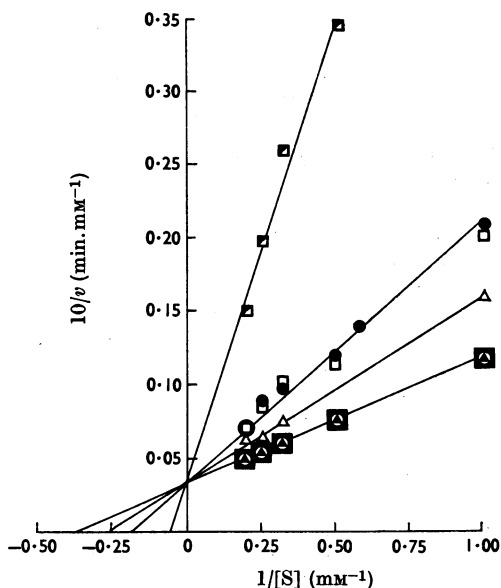


Fig. 5. Inhibition of methyl  $\alpha$ -D-glucopyranoside accumulation by D-galactose derivatives: Lineweaver-Burk plot of reciprocal of concentration of methyl  $\alpha$ -D-glucopyranoside accumulated per min. into hamster intestinal slices against reciprocal of medium concentration in the presence of 20 mM-inhibitor. ■, D-Galactose; □, D-galactopyranosyl fluoride; ●, 6-deoxy-6-fluoro-D-galactose; △, 6-deoxy-D-galactose; ○, 1-deoxy-D-galactopyranose (1,5-anhydro-D-galactitol); ▲, 6-deoxy-L-galactose; ■, methyl  $\alpha$ -D-glucopyranoside alone.

bonding at C-1 from the carrier to the sugar oxygen (or fluorine), and this is supported by the lack of transport of methyl  $\beta$ -D-thiogalactopyranoside, which cannot form this hydrogen bond. The hydrogen bond must be sterically situated so that it can form a bond almost as well with the  $\beta$ - as with the  $\alpha$ -anomer of the methyl glycoside. Hitherto, since glycosides that can still form hydrogen bonds have generally been used, the impression has arisen that C-1 is unimportant in the binding. This is clearly not true.

D-Allose, in which the hydroxyl group at C-3 is inverted from the D-glucose configuration, is transported less well than D-glucose or D-galactose, but 3-O-methyl-D-glucose (rat) is well transported. Here again the presence of an oxygen function at C-3 in the D-glucose configuration seems to be important. D-Mannose, in which the hydroxyl group at C-2 is inverted, was not actively transported in this system, confirming previous observations. However, this compound has been shown to be weakly transported in frog intestine (Csaky & Ho, 1966).

Summarizing, it appears that D-glucose binds to the carrier, probably by hydrogen-bonding at C-1, C-2, C-3, C-4 and C-6, and if one of these bonds is missing, as in D-galactose and those of its derivatives that can still hydrogen-bond, or in D-allose, 1-deoxy-D-glucose or 6-deoxy-D-glucose, the sugar is still actively accumulated. C-2 is more important and alteration of the hydroxyl group appears to give loss of transport. Loss of two or more of the bonds, as in those D-galactose derivatives that have a further bond missing such as 6-deoxy-D-galactose, 1-deoxy-D-galactose, methyl  $\beta$ -D-thiogalactopyranoside, or 1,6-dideoxy-D-glucose, D-gulose etc., usually gives complete loss of active transport.

Since the transport of sugars into the intestine need not be regarded as a true enzymic process requiring active groups oriented in a specific way to the substrate, it follows that the oxygen of the pyranose ring is probably involved in bonding also. If this bonding is of the same order as that of the other groups, then, if most of the other groups are in bonding positions, it may be possible for the oxygen to be at a different point in the pyranose ring. Such a orientation could account for the observation (Neale & Wiseman, 1968) that L-glucose can be weakly transported against a concentration gradient in rat intestine. One of the possible orientations in which only two bonds are missing is that in which  $\beta$ -L-glucose is arranged so that the oxygen is at C-4 of the pyranose ring of D-glucose; then C-1, C-2, C-3 and C-6 of D-glucose are coincident with C-3, C-2, C-1 and C-6 of L-glucose.

Crane (1965) has studied the affinity of sugar for carrier in various  $\text{Na}^+$  concentrations and has shown that  $V$  remains constant but  $K_m$  varies, so that the

affinity of sugar is only high in the presence of high  $\text{Na}^+$  concentration. He suggested that the cause of active transport of sugars is the  $\text{Na}^+$  gradient across the membrane, which gives high affinity of sugar for carrier on the outer surface of the membrane but a low affinity on the inside. Owing to the low internal  $\text{Na}^+$  concentration, the  $\text{Na}^+$ -carrier-sugar complex formed on the outer surface decomposes on the inner surface. All sugars appear to be carried on the same carrier, since competition can be demonstrated. We therefore expected that a kinetic analysis of the initial rate of sugar transport would show that the  $V$  of all the sugars would be the same (all the carrier saturated), but that the affinity of the sugars for the carrier would vary so that the more rapidly transported sugars at 1mM concentration would be expected to have a higher affinity. The best criterion of affinity was considered to be  $K_t$ , measured in this case by the inhibition of methyl  $\alpha$ -D-glucopyranoside transport (Fig. 5), since the  $K_m$  was shown not to be a true constant for the system. The variation in  $K_m$  with sugar concentration is probably due to variations in the rate term demanded by the Briggs-Haldane theory, which will be influenced by several factors such as the 'leakiness' of the tissue slice and its proportion of inactive cells. The inhibition constants,  $K_t$ , all determined under identical conditions may not give correct absolute values, but must reflect the relative affinities of the inhibitors and their ability to compete.

The inhibition constants,  $K_t$ , of D-galactose, 6-deoxy-6-fluoro-D-galactose and 6-deoxy-D-galactose were 1.5, 7 and 16mM respectively. This indicates that the affinity for the carrier of the hydroxy and fluoro compounds was greater than that of 6-deoxy-D-galactose, in agreement with expectation. However, the maximum velocities of accumulation,  $V$ , of these compounds were not identical as predicted (Table 1 and Fig. 2). If enzyme kinetic concepts are valid for a system as complex as tissue accumulation this suggests that saturation of the carrier is not the only variable and that some subsequent process can alter the rate of transport of the sugar.

An attempt to alkylate the carrier by using anhydro sugars was unsuccessful. The anhydro sugars were not actively accumulated and there is no evidence that they could bind with the carrier, even though methyl 3,4-anhydro- $\alpha$ -D-galactoside has Crane's minimum structure (Fig. 1). This is not entirely unexpected, since bonding at C-3 and C-4 is lost, as in D-gulose.

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