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Structural Requirements for Active Intestinal Transport

SPATIAL AND BONDING REQUIREMENTS AT C-3 OF THE SUGAR

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Analogues of D-glucose modified at C-3, and in some cases at a second position, were prepared and tested for active accumulation by everted segments of hamster intestine. Their relative affinity for the sugar carrier was measured by tissue/medium ratio, Michaelis–Menten kinetics and competitive inhibition of D-galactose or methyl α-D-glucoside transport. D-Glucose and its 3-deoxy-3-fluoro, 3-chloro-3-deoxy and to a smaller extent its 3-bromo-3-deoxy derivatives, bound and were transported more strongly than 3-deoxy-D-glucose and other sugars not containing an electronegative atom in the *gluco* configuration at C-3. 3-Deoxy-D-galactose, 3,6-dideoxy-D-glucose and D-gulose, which have two alterations from the D-glucose structure, were not, or only very weakly, transported. The results are interpreted as indicating the presence of a hydrogen bond from the carrier to the hydroxyl group at C-3 of D-glucose. Spatial requirements are also discussed. New syntheses are reported for 3-chloro-3-deoxy- and 3-bromo-3-deoxy-D-glucose and 3,6-dideoxy-D-glucose.

Barnett, Jarvis & Munday (1968) have shown that sugars were bound to the sugar carrier of the hamster intestine by hydrogen bonds from the carrier to the sugar at C-1 and C-6 of the sugar. The results suggested that D-glucose had the ideal structure for active transport and that it was bound to the carrier by hydrogen bonds at each of the hydroxyl groups. We have now investigated the relative affinities for the carrier of D-glucose analogues modified at C-3 in an attempt to elucidate the nature of the binding at this position.

MATERIALS

Chemicals. D-Galactose and D-glucose were obtained from British Drug Houses Ltd. (Poole, Dorset). 3-*O*-Methyl-D-glucose and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose were obtained from Koch–Light Laboratories Ltd. (Colnbrook, Bucks). Allose, m.p.131–133° (Theander, 1964), D-gulose (m.p. of di-*O*-isopropylidene derivative 104–105°; Meyer zu Reckendorf, 1967), 3-deoxy-D-*ribo*-hexose (3-deoxy-D-glucose), m.p. 110–112° (Anet, 1960), 3-deoxy-3-fluoro-D-glucose, m.p. 112–113°, $[\alpha]_D^{20}$ +59±2° (*c* 0·1 in water) (Foster, Hems & Webber, 1967), 3-deoxy-D-*xylo*-hexose (3-deoxy-D-galactose) (m.p. of di-*O*-isopropylidene derivative 78°; Zimmer, Wulf & Heinatz, 1964) and 3-*O*-ethyl-D-glucose (Glen, Meyers & Grant, 1951) were made as previously described. Several of the sugars had, initially, significant amounts of D-glucose present as an impurity and these 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 and then crystallized where possible.

3-Chloro-3-deoxy-D-glucose. Di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose, m.p. 120° (Brimacombe, Bryan, Husain, Stacey & Tolley, 1967) (6g.), anhydrous LiCl (6g.) and anhydrous $CaCO_3$ (100mg.) were heated in redistilled dimethylformamide (120ml.) at 140–145° on an oil bath with magnetic stirring for 7hr. T.l.c. showed, after location with 5% (v/v) H_2SO_4, two spots, one corresponding to the starting material and the other to 3-chloro-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. The solvent remained colourless to pale yellow under these conditions. The mixture was poured into water (150ml.) and extracted with dichloromethane (3×150ml.). The combined organic layers were washed with $NaHCO_3$ solution and water and dried over $CaCl_2$. The solvent was removed *in vacuo* at 50–60°, and the product carefully fractionally distilled giving *3-chloro-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose*, b.p. 84–88°/0·05mm. Hg. (2·6g.). The syrup (1·3g.) was heated in ethanol (5ml.) and water (15ml.) with Amberlite IR-120 (H^+ form) resin (5g.) with magnetic stirring for 6hr., the resin filtered off and the solvent removed to leave *3-chloro-3-deoxy-D-glucose* (500mg.), recrystalized from aqueous ethanol, m.p. 150–152°, $[\alpha]_D^{22}$ +66° (*c* 0·18 in water) (Found: C, 36·4; H, 5·5; Cl, 18·7. Calc. for $C_6H_{11}ClO_5$: C, 36·3; H, 5·5; Cl, 17·9%). Newth, Overend & Wiggins (1947) give m.p. 155–156°, $[\alpha]_D^{20}$ +64·1°.

3,6-Anhydro-α-D-glucofuranose. In an early experiment, the $CaCO_3$ was omitted from the preparation of 3-chloro-3-deoxyglucose described above. The mixture was refluxed for 4hr. and it turned very dark. After the mixture had been worked up as described above, the product, 3,6-anhydro-1,2-di-*O*-isopropylidene-α-D-glucofuranose (1·2g.), was distilled at 120°/0·05mm. Hg. Removal of the isopropylidene group as described above led to 3,6-anhydro-α-D-glucose

(400mg.), recrystallized from aqueous ethanol, m.p. 119°, $[\alpha]_D^{20}$ +71°→+54° (*c* 0·2 in water, 1→5min.). Ohle & Wilcke (1938) give m.p. 119°, $[\alpha]_D^{20}$ +49° (Found: C, 45·1; H, 5·9. Calc. for $C_6H_{10}O_5$: C, 44·6; H, 6·1%).

3-Bromo-3-deoxy-D-glucose. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (6g.), LiBr (8g.) and $CaCO_3$ (200mg.) was stirred at 140–145° in dimethylformamide (100ml.) for 6hr. The mixture was worked up as for the chloro compound, but, since t.l.c. showed that about 50% of the starting material remained, ether (20ml.) was added after removal of the solvent and the precipitated, crystalline, starting material (2·5g.) was filtered off and washed with a little dry ether. The mother liquors were distilled, giving 3-bromo-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1·8g.), b.p. 92–96°/0·05mm. Hg, and, after removal of the isopropylidene groups, 3-bromo-3-deoxy-D-glucose (600mg.), m.p. 148°, $[\alpha]_D^{22}$ +62° (*c* 0·09 in water). Newth *et al.* (1947) give m.p. 151°, $[\alpha]_D^{20}$ +60·9° (Found; C, 29·5; H, 4·6; Br, 33·0. Calc. for $C_6H_{11}BrO_5$: C, 29·7; H, 4·6; Br, 33·0%).

3-O-Toluene-p-sulphonyl-α-D-allose. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (1g.) was suspended in methanol (10ml.), water (10ml.) and Amberlite IR-120 (H^+ form) (5g.), and stirred at 70° for 6hr. The resin was removed by filtration, the solution washed with diethyl ether and evaporated to leave 3-*O*-*toluene-p-sulphonyl-D-allose* (200mg.), recrystallized from aqueous ethanol, m.p. 143°, $[\alpha]_D^{22}$ +5·7±1·0° (*c* 0·1 in water) (Found: C, 46·2; H, 5·4; S, 11·5. $C_{13}H_{18}O_8S$ requires C, 46·6; H, 5·4; S, 9·6%).

3-O-Methyl-D-allose. 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose, m.p. 76° (Ondera, Hirano & Kashimura, 1965) (5g.), was dissolved in acetone (20ml.) and powdered NaOH (3g.) and dimethyl sulphate (3ml.) were added dropwise with stirring over 30min. The well-stirred mixture was refluxed for 6hr., poured into chloroform, washed with water and dried over Na_2SO_4. After removal of solvent, the syrup was heated at 100°/0·05mm. Hg for 1hr. to remove acetone polymers. The pale-yellow syrup had no absorption band in the hydroxyl region of the i.r. spectrum. The syrup (2g.) was hydrolysed as described for 3-chloro-3-deoxy-D-glucose, giving 3-O-*methyl-D-allose*, m.p. 88–92°, $[\alpha]_D^{22}$ +8·5±2° (*c* 0·1 in water) (Found: O·CH_3, 18·5. $C_7H_{14}O_6$ requires O·CH_3, 17·1%).

3,6-Dideoxy-α-D-ribo-hexose (3,6-dideoxy-D-glucose). Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexoside, m.p. 175° (recrystallized from ethanol but not sublimed; cf. Vis & Karrer, 1954) (3g.), was dissolved in pyridine (20ml.) and benzoyl chloride (2ml.) added. After standing at room temperature overnight, the solution was poured into ice–water and was extracted with chloroform, washed with 6M-HCl and $NaHCO_3$ solution and dried over Na_2SO_4. The solvent was removed and the product crystallized after addition of a little ethanol. *Methyl 4,6-O-benzylidene-2-O-benzoyl-3-deoxy-α-D-ribo-hexoside* was recrystallized from ethanol (2·1g.), m.p. 116–120°, $[\alpha]_D^{21}$ +91·5±2·0° (*c* 0·25 in $CHCl_3$) (Found: C, 67·6; H, 6·2. $C_{21}H_{22}O_6$ requires C, 68·1; H, 6·0%). The benzoyl derivative was dissolved in sodium-dried benzene (20ml.), and *N*-bromosuccinimide (1g.) and $CaCO_3$ (1g.) were added. The mixture was refluxed for 2hr., when t.l.c. on silica gel with toluene–ether (2:1, v/v) showed one spot running slightly faster than the starting material. The solution was poured into chloroform and washed with Na_2CO_3 solution and water, then dried over Na_2SO_4. Removal of solvent gave a chromatographically pure syrup, which could not be induced to crystallize, but had the i.r. spectrum corresponding to *methyl 2,4-di-O-benzoyl-6-bromo-3,6-dideoxy-α-D-ribo-hexoside.* The syrup was dissolved in dry tetrahydrofuran (20ml.) added to $LiAlH_4$ (3g.) in dry tetrahydrofuran (50ml.) and refluxed for 16hr. with magnetic stirring. After cooling, water was added dropwise and, when all the hydride was destroyed, a further 50ml. was added. The precipitated aluminium oxide was filtered off and the solution made acid by addition of Amberlite IR-120 (H^+ form). At this point a test for Br^- ion was positive. The solution was finally deionized with Amberlite MB_1 (20g.) and evaporated to dryness *in vacuo* to give methyl 3,6-dideoxy-α-D-*ribo*-hexoside (520mg.). The syrup was dissolved in 0·4M-H_2SO_4 and refluxed for 1hr., deionized with Amberlite MB_1 and evaporated to give 3,6-dideoxy-D-*ribo*-hexose as a syrup (210mg.), $[\alpha]_D^{22}$ −8±4° (*c* 0·1 in water), which contained no bromine, and gave a single spot on paper chromatography, R_{Glc} 2·9 in butan-1-ol–ethanol–water, R_F 0·71 in butan-1-ol–pyridine–water (Fouquey, Polonsky, Lederer, Westphal & Lüderitz, 1958).

METHODS

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). The gut was tied at one end on to a narrow glass rod, and removed from the animal and everted simultaneously. Segments (2–3mm. wide) were cut while the gut was immersed in ice-cold buffer. The rings were added so that each flask contained gut rings from all regions of the small intestine. After preincubation of the flasks at 37° with shaking for 15min., sugar in buffer (1ml.) was added to the incubation media (final volume 6ml.). The incubation was stopped after the required time by decanting off 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 (4ml. normally, 3ml. when radioactive 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.

Determination of tissue water content. The water content of each batch of tissue samples was determined. The gut rings were incubated in the same way and dried as described above. The gut rings were then dried in an oven at 90° to constant weight.

Assay of sugars. Radioactive sugars D-[1-^{3}H]galactose and methyl [6-^{3}H]glucoside were measured with a Beckman liquid scintillation counter with NE220 scintillation fluid [Nuclear Enterprises (G.B.) Ltd., Edinburgh]; 0·2ml. samples were added to 5ml. of scintillation fluid.

Reducing sugars were measured by Nelson's (1944) method and non-reducing sugars and halogenated derivatives were determined with Molisch reagent (Devor, 1950). 3-Deoxy sugars were determined by the method of Waravdekar & Saslaw (1959).

Chromatography. All sugars were tested for purity by descending paper chromatography on Whatman no. 1 paper

with butan-1-ol–ethanol–water (4:1:5, by vol.; upper layer) or butan-1-ol–pyridine–water (9:2:1, by vol.) (for allose and 3,6-dideoxyglucose) as solvent. Sugars containing a significant quantity of glucose were purified on Whatman no. 3MM paper with these solvents. Sugars were localized by alkaline $AgNO_3$. T.l.c. was on Kieselgel G (nach Stahl) with benzene–ether (9:1, v/v) as solvent. Sugars were localized by 5% (v/v) H_2SO_4 in ethanol and heating for 15min. All sugars used in accumulation experiments were chromatographically pure.

Microanalyses. These were performed by Dr G. Weiler and Dr F. B. Strauss, Oxford.

RESULTS

Time-dependence of accumulation of D-*galactose.* Flasks containing everted hamster intestinal slices were incubated with 1mM-D-galactose for 0–60min. Over 30min. the accumulation was linear.

Tissue/medium ratios. The sugar (1mM) was incubated for 30min. with 200–300mg. of hamster intestinal slices and the mucosal and tissue concentrations were assayed by the appropriate method. The water content was determined on a sample of tissue. It was $81 \pm 2\%$ irrespective of the sugar used, and tissue concentrations were corrected by the appropriate value. In all cases D-galactose was tested for accumulation to ensure that the preparation was active and a sample of both tissue and medium fluids was chromatographed to ensure that the sugar was not altered. The tissue/medium ratios, calculated from the final concentrations of sugar after 30min., and the standard errors are shown in Table 1. 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 no undetected metabolism took place. Despite the previous observation that 3-deoxy-D-glucose was metabolized by hamster intestinal sacs (Wilson & Landau, 1960), departure ($<90\%$) from 100% recovery was found only with D-glucose (30% recovered after 30min.).

Variation of rate of accumulation with concentration. The sugar (5–20mM) was incubated with everted intestinal slices for 10min. The tissue concentration, corrected for dry-weight content, was measured. Since the accumulation for D-galactose was linear over 30 min., the concentration of sugar in the tissue after 10min. was proportional to the initial rate of accumulation by the tissue. This relationship was assumed for the other sugars. The rates shown (Fig. 1) are the averages of five points taken on two separate occasions. Each point was the average of duplicate determinations on the same sample. The Lineweaver–Burk plots of the reciprocal of the tissue concentration after 10min. (initial rate) against the reciprocal of the initial sugar concentration in the medium for the determination of the K_m and V values shown in Table 1 were not corrected for diffusion, since most of the sugars were rapidly transported (cf. Barnett *et al.* 1968). The short incubation time of 10min. was

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

Compound	Final tissue/medium ratio (initial concn. in medium 1mM)	v* (mM/10min.) (initial concn. in medium 10mM)	K_m (mM)	V (mM/10min.)	K_i (galactose as substrate)
D-Glucose‡§	∞	—	—	—	2·3 (1·5†)
D-Galactose§	8·3±0·25	27	5·0	50	(4·2†)
3-Deoxy-D-glucose‖	2·1±0·2	15	16·6	41	24·0
3-Deoxy-3-fluoro-D-glucose	26·6±3·6	51	4·6	71	2·5
3-Chloro-3-deoxy-D-glucose	24·2±2·8	58	6·6	87	3·2 (1·3†)
3-Bromo-3-deoxy-D-glucose	9·0±0·2	34	10·0	60	20·0
3-*O*-Methyl-D-glucose‖	4·3±1·3	5	40·0	27	30·0 (39·0†)
3-*O*-Ethyl-D-glucose‖	<0·3	—	—	—	—
D-Allose‡‖	1·7±0·1	20	13·5	47·5	40·0
3-*O*-Methyl-D-allose	<0·3	—	—	—	—
3-*O*-Toluene-*p*-sulphonyl-D-allose	<0·3	—	—	—	—
3-Deoxy-D-galactose	1·0±0·04	—	—	—	—
D-Gulose‖	0·3±0·01	—	—	—	—
3,6-Dideoxy-D-glucose	0·5±0·05	—	—	—	—
3,6-Anhydro-D-glucose	<0·3	—	—	—	—

* v is the tissue concentration after 10min. with an initial concentration of 10mM in the medium and is taken from Fig. 1.
† With methyl glucoside as substrate.
‡ Previously studied by Fischer & Parsons (1953).
§ Previously studied by Crane & Mandelstam (1960).
‖ Previously studied by Wilson & Landau (1960).

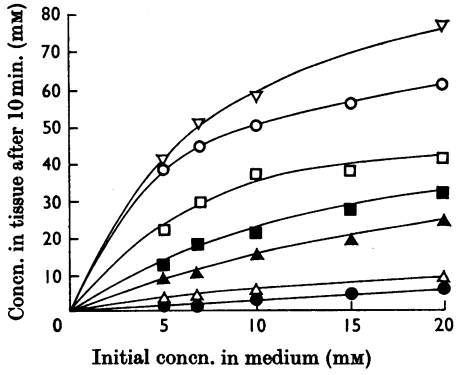

Fig. 1. Molar concentration of sugar accumulated by everted hamster intestinal slices in various concentrations in the medium of D-glucose analogues modified at C-3, incubated for 10 min. at 37° in oxygenated buffer solution. ○, 3-Deoxy-3-fluoro-D-glucose; ▽, 3-chloro-3-deoxy-D-glucose; □, 3-bromo-3-deoxy-D-glucose; ■, D-allose; ▲, 3-deoxy-D-glucose; △, 3-*O*-methyl-D-glucose; ●, L-galactose.

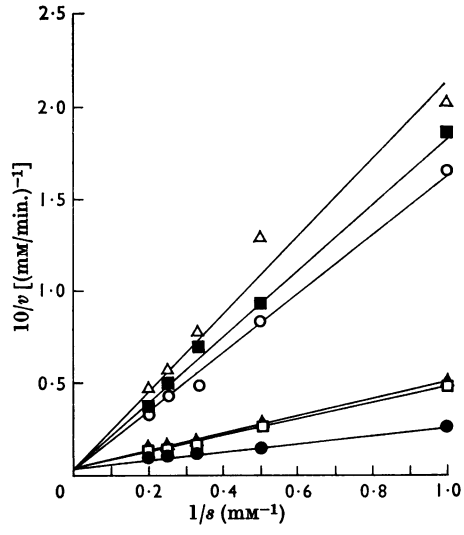

Fig. 2. Inhibition of D-galactose accumulation by analogues of D-glucose modified at C-3: Lineweaver–Burk plot of reciprocal of concentration of D-galactose accumulated/min. into hamster intestinal slices against reciprocal of concentration in the medium in the presence of 20 mM-inhibitor (3-deoxy-3-fluoro-D-glucose, 17·5 mM). △, D-Glucose; ■, 3-deoxy-3-fluoro-D-glucose; ○, 3-chloro-3-deoxy-D-glucose; ▲, 3-bromo-3-deoxy-D-glucose; □, 3-deoxy-D-glucose; ●, D-galactose with no inhibitor.

chosen to ensure the measurement of a true initial rate of transport and leads to relatively low tissue concentrations. Thus 3-*O*-methylglucose, which is clearly actively transported after 30 min. with 1 mM initial concentration in the medium (Table 1), gives tissue concentrations at 10 min. that are usually well below the initial concentration in the medium (Fig. 1).

Competitive inhibition of methyl α-D-glucoside or D-galactose transport. The apparent K_i of the substrate–carrier complex was determined by the inhibition of either methyl α-D-[6-^{3}H]glucopyranoside or D-[1-^{3}H]galactose accumulation over 10 min. (Table 1). The K_m of these two radioactive sugars over the range 1–5 mM was determined in the presence and absence of the sugar to be tested (20 mM, except for 3-deoxy-3-fluoro-D-glucose, which was 17·5 mM). The Lineweaver–Burk plots for D-galactose accumulation are shown in Fig. 2. The values were reproducible and appeared to give good values for D-glucose. Thus, despite the extensive metabolism of D-glucose inside the tissue, the K_i, which is a function of the concentration in the medium, appears to be comparable with those of the non-metabolized sugars.

DISCUSSION

Most of the sugars were subjected to three types of test: determination of the tissue/medium ratio at 1 mM concentration in the medium, determination of the initial rate of accumulation at different concentrations in the medium (in the range 5–20 mM) with subsequent calculation of K_m and V, and determination of the apparent inhibition constant, K_i, with D-galactose (or methyl α-D-glucopyranoside) as substrate.

The initial experiments, in which the tissue/medium ratios (Table 1) of the various sugars were determined, showed that the C-3 hydroxyl group was indeed involved in the active transport of D-glucose in the hamster intestine, as had previously been suggested (Barnett *et al.* 1968). Those sugars with the hydroxyl group at C-3 either missing or in the *allo* configuration, 3-deoxy-D-glucose and D-allose, have a decreased accumulation (ratios respectively of 2·1 and 1·7); sugars with a further alteration from the D-glucose structure, 3-deoxy-D-galactose, D-gulose and 3,6-dideoxy-D-glucose, are not accumulated at all (ratios respectively 1·0, 0·3 and 0·5). 3-Deoxy-3-fluoro-, 3-chloro-3-deoxy- and to a smaller extent 3-bromo-3-deoxy-D-glucose were all well transported (ratios respectively 26·0, 24·2 and 9·0). All the rapidly transported substances contain an atom in the *gluco* configuration that has been shown to form a hydrogen bond (Pauling, 1940).

All compounds that gave a tissue/medium ratio greater than 1·0, including D-glucose, were tested as an inhibitor of either D-[1-^{3}H]galactose transport or methyl α-D-[6-^{3}H]glucopyranoside transport.

All behaved as competitive inhibitors. Although the values found for K_i may not be the absolute values of the decomposition constant of the carrier–sugar complex, the values determined, under identical conditions and with the same sugar as substrate, must reflect the relative affinities of the inhibitors and their ability to compete for the carrier. The magnitude of the apparent K_i must also be an indication of this affinity. D-Glucose, 3-deoxy-3-fluoro-D-glucose and 3-chloro-3-deoxy-D-glucose all had very low K_i values, near 3mM. The 3-bromo analogue had a higher K_i, 20mM, indicating a much lower affinity for the carrier, and 3-deoxy-D-glucose and D-allose had even higher K_i values, 24mM and 40mM respectively.

The K_m values can probably not be used as a measure of affinity of the sugar for the carrier (Barnett *et al.* 1968), but the accumulation curves with various substrate concentrations (Fig. 1) show again that the 3-chloro and 3-fluoro analogues of glucose are accumulated far better than 3-deoxyglucose and allose, and the K_m values calculated from these data are slightly lower for the former than for the latter sugars.

These results confirm the suggestion that D-glucose binds to the sugar carrier by a hydrogen bond from the carrier to the hydroxyl group at C-3 of the sugar, and support the hypothesis that D-glucose has the 'ideal' structure for active transport in the hamster intestine, binding at each hydroxyl group of the sugar (Barnett *et al.* 1968).

It is noteworthy that, in a similar series of substituents at C-6 of the sugar molecule, 6-chloro-6-deoxy-D-galactose had an affinity similar to that of 6-deoxy-D-galactose, in contrast with the fluoro analogue, which resembled D-galactose (Barnett *et al.* 1968). The discrepancy between the behaviour of the C-6 series and the C-3 series may be accounted for by one or more of several factors. Since 6-bromo-6-deoxy-D-galactose was not accumulated, presumably owing to its size, the enhanced attraction for the carrier of the chlorine atom compared with the hydrogen atom may have been cancelled out by its increased size. Alternatively the free rotation of the C-6 position, compared with the exact stereochemistry at C-3, may have led to a situation in which the stronger bonds with fluorine in 6-deoxy-6-fluoro-D-galactose and oxygen in D-galactose still formed, but the weaker bond with chlorine did not. Finally, the relative strength of the hydrogen bonds might be markedly affected by the hydrophobic nature of the environment.

Unexpectedly 3-*O*-methyl-D-glucose had a low tissue/medium ratio and a high K_i similar to that of 3-deoxy-D-glucose. It might have been expected to show a higher affinity since the methoxyl group can form a hydrogen bond. The discrepancy is probably attributable to steric factors, since 3-*O*-ethyl-D-glucose is not transported at all. 3-*O*-Methyl-D-allose and 3-*O*-toluene-*p*-sulphonyl-D-allose were not transported, indicating that the size of substituent allowed at this position is small.

Contrary to the prediction of the Crane (1965) hypothesis for the mechanism of active intestinal transport, considerable variations in V were observed; such variations have been previously observed and discussed (Barnett *et al.* 1968).

Since the 3-fluoro and 3-chloro analogues of D-glucose have the exact requirements for active transport in the hamster intestine and yet are not metabolized, they are the most highly accumulated sugars yet found.

We thank Professor A. B. Foster for a seed of 3-deoxy-3-fluoro-D-glucose and Professor W. G. Overend and Dr E. J. Hedgley for a seed of 3-deoxy-D-*ribo*-hexose, and the Science Research Council for their support; we thank Mr D. J. Howland for technical assistance in the preparation of some of the compounds.

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