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Transport of Galactose, Glucose and their Molecular Analogues by *Escherichia coli* K12

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1. Strains of *Escherichia coli* K12 were made that are unable to assimilate glucose by the phosphotransferase system, since they lack the glucose-specific components specified by the genes *ptsG* and *ptsM*. 2. Derivative organisms lacking the methyl galactoside or galactose-specific transport system were examined for their ability to transport galactose, D-fucose, methyl β-D-galactoside, glucose, 2-deoxy-D-glucose and methyl α-D-glucoside. 3. Galactose, glucose and to a lesser extent fucose are substrates for both transport systems. 4. 2-Deoxyglucose is transported on the galactose-specific but not the methyl galactoside system. 5. The ability of sugars to elicit anaerobic proton transport is associated with the galactose-specific, but not with the methyl galactoside transport activity. Hence a chemiosmotic mechanism of energization is likely to apply to the former but not to the latter. Alternatively the methyl galactoside system may be switched off under certain conditions, which would indicate a novel regulatory mechanism. 6. Details of the procedure for the derivation of strains may be obtained from the authors, and have been deposited as Supplementary Publication SUP 50074 (8 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1977), **161**, 1.

In *Escherichia coli* there are at least seven systems capable of transporting galactose (reviewed by Kornberg, 1976). Only two of these are specifically induced by galactose, and also effect its active transport (see reviews by Buttin, 1968; Boos, 1974). They are the methyl galactoside and galactose-specific systems which have been designated the 'MglP' and 'GalP' transport systems respectively (Buttin, 1968; Rotman *et al.*, 1968; Wilson, 1974*a*), terms consistent with the recommended nomenclature of Demerec *et al.* (1966) and Bachmann *et al.* (1976). The present paper gives evidence that the galactose-specific system operates by galactose–H^+ symport and the methyl galactoside system does not; also, it will be shown that 2-deoxyglucose is a substrate for the galactose-specific and not the methyl galactoside system. Because the methyl galactoside system has been shown to contain at least four genetically separable components (Ordal & Adler, 1974*a*,*b*; Robbins, 1975; Robbins & Rotman, 1975), and because the number of components of the galactose-specific system has not yet been elucidated, we shall identify the transport systems as the 'methyl galactoside' and 'galactose-specific' systems respectively. We emphasize here that at least one of the systems is specified by more than one cistron.

The genes for each system, designated *mglP* and *galP*, are widely separated on the *E. coli* genome: those for the methyl galactoside system are at about min 45 of the recalibrated map (Bachmann *et al.*, 1976; Ganesan & Rotman, 1965) and the locus for the galactose-specific system is at about min 61 (Riordan, 1976). The aim of the present work was to separate unequivocally methyl galactoside and galactose-specific activities by using genetical techniques, in strains as isogenic as possible (see the plea by Oxender, 1972, that comparisons of different transport systems should be made in such strains); the mode of energization of each could then be separately elucidated. The entry of galactose into *E. coli* cells on the glucose phosphotransferase (Kornberg & Riordan, 1976; Postma, 1976) was eliminated by obtaining *mglP* and *galP* lesions in a uniformly *ptsG ptsM* background; *ptsG* and *ptsM* are genes for the glucose-specific components of the glucose transport systems (Curtis & Epstein, 1975; Kornberg & Jones-Mortimer, 1975). Other genetic symbols are those defined by Bachmann *et al.* (1976).

Evidence that a transport system may be energized by a chemiosmotic mechanism is provided by the observation of an alkaline pH change during carrier-mediated diffusion of a sugar into de-

energized cells (Mitchell, 1970, 1973). Such observations have been reported for transport of lactose, galactose, arabinose and tentatively for maltose in *E. coli* (West, 1970; West & Mitchell, 1972, 1973; Henderson, 1974; Henderson *et al.*, 1975) and for some sugar-transport systems in other organisms (see review by Hamilton, 1975).

By using this experimental approach we have previously observed in wild-type strains that methyl β-galactoside failed to elicit an alkaline pH change under conditions where galactose did so (Henderson, 1974; Henderson *et al.*, 1975). The implication that the methyl galactoside system is not a proton-symport type is substantially confirmed by the observations below on *mglP*$^+$, *galP* and *mglP,galP*$^+$ strains. Other interpretations will be considered, but it seems likely that the galactose-specific system is energized by a chemiosmotic mechanism and that the methyl galactoside system is not. The operation of the methyl galactoside system will be discussed in terms of 'direct energization' by ATP or another glycolytic product, a mechanism proposed by Berger and others (Berger, 1973; Berger & Heppel, 1974; Curtis, 1974; Wilson, 1974*b*).

D-Fucose (6-deoxy-D-galactose) is an inducer and substrate of both systems, but is not metabolized by *E. coli* (Buttin, 1963*a*; Rotman *et al.*, 1968; Lengeler *et al.*, 1971; Wilson, 1974*a*). Fucose is therefore used extensively in the present work to define the activity of the galactose-transport systems. We have confirmed that glucose is a substrate for both systems (Boos, 1969; Silhavy *et al.*, 1974; Wilson, 1974*a*), but the unexpected finding that 2-deoxyglucose is a substrate for the galactose-specific system and not for the methyl galactoside system will be discussed in detail. The term 'galactose-specific' transport system is now clearly a misnomer, but it is retained in this paper to preserve continuity with previous studies.

Experimental

Organisms and growth conditions

The strains of *E. coli* listed in Table 1 were maintained on slopes of 2.6% CM1 nutrient broth/1.5% agar (Oxoid Ltd., London SE1 9HF, U.K.). Inocula were prepared from slopes by growing cells aerobically at 37°C for 6–8h in 10ml of 2.6% nutrient broth, and used for 1 week before preparation of a fresh inoculum. Larger amounts of cells were grown overnight (16–18h) at 37°C in a 500ml conical flask agitated at 250rev./min (Gallenkamp orbital incubator). The 400ml of growth medium [42mM-sodium/potassium phosphate (pH7.1)/42mM-NH_4Cl/2.5μg of thiamin per ml/<0.5mM trace elements (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, SO_4^{2-}, Cl^-)] contained 22mM-glycerol as carbon source, 1–3ml of inoculum, 1mM-D-fucose as inducer supplemented with 100μg of thymine/ml, 90μg of L-histidine/ml, or 80μg of L-methionine/ml as necessary. Under these conditions of low aeration the bacteria grew exponentially, with a doubling time of about 2h, until A_{680}^{1cm} of the culture reached 0.4–0.5. The growth rate then gradually decreased and the cells were harvested by centrifugation when A_{680} reached 0.7–0.8; 1ml of a suspension of $A_{680} = 1.0$ contained 0.68mg dry mass of bacteria.

The harvested bacteria were resuspended in 400ml of 150mM-KCl/5mM-glycylglycine buffer (pH6.8)/1mM-mercaptoethanol, and incubated at 37°C on the shaker for 1h to deplete endogenous energy sources; this procedure was not obligatory for subsequent observation of sugar-promoted pH changes, but was found to increase the rate and extent of the pH change and decrease the slow excretion of acid by the cells. After one wash in 400ml of 150mM-KCl/2mM-glycylglycine, pH6.8, the bacteria were resuspended in this medium to an A_{680} of 45–55 (about 30–38mg dry mass/ml). It was often convenient to grow 200ml of cells in a 250ml flask, in which case volumes were halved.

If the cells were grown in a 2-litre baffled flask to permit optimal aeration, but otherwise treated exactly as described above, the growth rate was much faster but the sugar-promoted pH change did not always appear. The reason for this variability has not been established, but use of the limited-aeration conditions yielded absolutely reproducible appearance of the sugar-requiring alkaline pH change. When the bacteria were grown under completely anaerobic conditions on 11mM-galactose, the pH changes still occurred, but were partially obscured by prolonged secretion of acid from the cells.

Continuous measurement of pH and oxygen consumption

To observe an alkaline pH change elicited by sugar transport, the proton ejection caused by respiration must be prevented (West, 1970; West & Mitchell, 1972; Lawford & Haddock, 1973). The experiments described below were therefore performed under anaerobic conditions in a glass cell. An oxygen electrode (YSI 5331; Yellow Springs Instrument Co., c/o V. A. Howe Ltd., London S.W.6, U.K.) was sealed into the cell through a horizontal gas-tight screw-thread joint (Quickfit SQ 18) and a microcombination pH electrode (Jena 401 M5, or Beckman CECAR electrode) through a second horizontal joint (Quickfit SQ 13). Access to the cell was maintained through a vertical glass extension tube of 5mm diameter, about 40mm long. Through this port the cell and electrodes were washed several times with water, once with anoxic 150mM-KCl/2mM-glycylglycine, pH6.8, and then air was flushed from the empty cell by a stream of argon continued

until complete lack of oxygen was indicated by the electrode measuring system (see below). Then 17–18mg dry mass of bacteria was introduced as a measured volume of the stock suspension, and the cell filled with anoxic 150mM-KCl/2mM-glycylglycine, pH6.8. The entry port was then closed by insertion of a tight-fitting polyethylene plug 40mm long, which contained a narrow hole (diam. 0.5mm) along its length; through this, additions could be made to the suspension (total vol. 6.0ml) with Hamilton precision syringes. The temperature of the suspension was maintained at 25°C by water flowing through a spiral-glass-tube heat-exchanger inside the cell, and rapid stirring was achieved with a Teflon-covered metal bar driven by a rotating external magnet.

A pH-meter built according to a design kindly given to us by Professor P. B. Garland (Department of Biochemistry, University of Dundee) was connected to the pH electrode, and used to drive one channel of a three-pen recorder (Rikadenki Kogyo Co., TEM Sales Ltd., Crawley, Sussex RH10 2R6, U.K.) via a selective resistance circuit for backing-off and obtaining a convenient sensitivity. The oxygen electrode was connected to a second channel of the same recorder via a second polarizing and backing-off circuit. Recorded changes of the pH of the suspension were converted into $\Delta[H^+]$ by calibration with 3μl of air-free standard 0.01M-NaOH, and sugars were added as 20μl of air-free 0.5M solutions. Additions were normally made when the pH recording indicated a very low rate of drift some 10–15min after introduction of the bacteria, and the pH of the suspension was between 6.5–6.8; pH changes after the addition of a sugar were not greater than 0.1pH unit.

Transport of radioactively labelled sugars

Strains of *E. coli* were grown exactly as described above but the final wash and resuspension were carried out in 150mM-KCl/5mM-Hepes [2-(*N*-hydroxyethylpiperazin-2′-yl)ethanesulphonic acid] pH6.5. The use of 5mM-Hepes instead of 2mM-glycylglycine made little difference to the extent of sugar uptake, but the increased buffering capacity prevented undesirable changes in pH when metabolizable sugars were used. Volumes (1ml) of a final suspension, $A_{680} = 1.8–2.2$, were exposed to 10mM-glycerol for 3min at 20–22°C, and then 50nmol of labelled sugar was added with mixing. Portions (0.2ml) were withdrawn and discharged into approx. 6ml of KCl/Hepes at 15s and at 2min. The 2min rather than 15s points were chosen for comparison between different strains and sugars, as they reflect more clearly accumulation above the external concentration. The diluted suspension was filtered immediately through Sartorius cellulose nitrate filters (0.45μm pore size), washed once in an equal volume of KCl/Hepes, and then the radioisotope retained in the filtered bacteria was measured by liquid-scintillation counting to 1–2.5% standard error (Packard model 3385 instrument) in toluene (80%), 2-methoxyethanol (20%), 2,5-diphenyloxazole (4g/l), 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.2g/l). Air was bubbled through the bacterial suspension throughout the experiment, and sampling was done with a Compupet 100 (General Diagnostics, Eastleigh, Hants. SO5 3QZ, U.K.) and automatic probe (Newton Instruments, Hoylake, Merseyside L47 4AY, U.K.) controlled by a Ramstetter card reader (Engel and Gibbs, Borehamwood, Herts. WD6 1SQ, U.K.).

Background measurements were made with identical scintillant and counting radioactivity of filters not exposed to radioisotopes; similarly 4nmol of the radioisotope-labelled sugars with filters were counted to find specific radioactivities, which were used to convert counts in the experimental vials into mol of sugar. No significant variation in quenching between vials occurred.

Materials

D-Fucose, 2-deoxy-D-glucose, D-galactose (glucose-free grade), glycylglycine and Hepes were obtained from Sigma, Kingston-upon-Thames, Surrey KT2 7BH, U.K., D-glucose and methyl α-D-glucoside from BDH Chemicals Ltd., Poole, Dorset BH12 4NN, U.K., and methyl β-D-galactoside was from Koch–Light, Colnbrook, Bucks. SL3 0BZ, U.K., and Schwartz/Mann, Orangeburg, NY, U.S.A. All were of the highest available purity. D-[1-^{3}H]Fucose, D-[U-^{14}C]galactose, D-[1-^{3}H]galactose, D-[U-^{14}C]-glucose, methyl α-D-[U-^{14}C]glucoside and L-[1-^{3}H]-arabinose were from The Radiochemical Centre, Amersham, Bucks., U.K.; [^{14}C]methyl β-D-galactoside, [^{14}C]methyl β-D-thiogalactoside, and 2-deoxy-D-[1-^{14}C]glucose were from New England Nuclear Corp., Swindon, Wilts., U.K. All standard labelled solutions contained 2mM sugars, 2.5–12.5Ci/mol. Chemicals used for growth cultures were of reagent grade, but all others were of the highest purity commercially available.

Construction of strains

It proved to be convenient to start from the strain h8, an *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced mutant of strain K10 (Böck & Neidhardt, 1966), which does not carry any of the lesions comprising the *gal*$_b$ (*gal 6*) mutation. All further mutations in this strain arose spontaneously, and were selected by appropriate modifications of the method of Jones-Mortimer & Kornberg (1976). The procedure for the derivation of the strains may be obtained from the authors, and has been deposited with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., as Supplementary Publication SUP 50074.

Bacteriophage P1kc-mediated transduction was carried out as described by Lennox (1955), and heteroimmune superinfection curing of lysogens was achieved with bacteriophage *λimm 21 b2* (Kaiser & Masuda, 1970). Strains were screened for λ lysogeny by using bacteriophage strains *λvir* and *λCI26* or *λCI857*.

Galactose-transport-negative strains were screened for their *galK* or *galK*/*λgal K*$^+$genotype on 10mM-galactose +0.5mM-isopropyl β-D-thiogalactoside, which causes induction of the lactose-transport system, for which galactose is a substrate. In choosing this route for preparing the strains we were guided by three considerations: first that we start from a strain in which galactose–H$^+$ symport was well characterized; secondly, that rather than selecting galactose-positive recombinants directly, we obtain them by screening clones which are recombinant for a closely linked marker; in this way we not only know which transport system we have reintroduced, but also avoid the possibility of selecting mutants with novel transport systems for galactose (for example, galactose-transport-negative mutants mutate readily to grow on galactose by becoming constitutive for the lactose operon); thirdly, that we should be able to obtain galactokinase-negative derivatives of galactose-transport-deficient strains. The organisms used are listed in Table 1.

Results

Transport of sugars into different mutants

All strains were grown with glycerol as carbon source and fucose to induce galactose-transport systems as required. Standard conditions for measuring sugar transport with replicate measurements on different batches of cells were used, so that valid comparisons could be made. Table 2 shows the amounts of different sugars transported into strains K10 (wild type), JM1097 (*mglP*), JM1109 (*galP*), JM1100 (*mglP*, *galP*), and ts19-1Δ (*ptsI* deletion). Methyl β-galactoside was transported into the wild type and strain JM1109 (*mglP*$^+$, *galP*), but not into *mglP* strains (Table 2). Taken together with the transport of galactose, glucose and fucose into strain JM1109 (Table 2) this is consistent with the previously reported specificity of the methyl galactoside system (Rotman *et al.*, 1968; Boos, 1969). In fact, uptake of each of the four sugars into strain JM1109 was not significantly different from uptake into the wild-type strain K10 (Table 2), which implies that activity of the methyl galactoside system on its own could account for all the observed transport in wild-type strains.

Consistent with the specificity of the galactose-specific system for sugars (Wilson, 1974*a*), galactose and fucose, but not methyl β-galactoside, were accumulated by strain JM1097 (*mglP*, *galP*$^+$) (Table 2). The amounts of sugars accumulated in 2min were significantly lower than in the wild-type or JM1109 (*mglP*$^+$, *galP*) strains, which conforms with the reported lower activity of the galactose-specific system in comparison with the methyl galactoside system (Wilson, 1974*a*). It is intriguing that glucose and 2-deoxyglucose were better substrates for the galactose-specific system than were galactose or fucose, and this is further discussed below.

Possible involvement of the lactose and arabinose

Table 1. *Strains of E. coli used*

Strain	Genotype	Relevant transport phenotype	Obtained from	Reference
h8	Hfr *fda*^{ts}	Wild-type	R. A. Cooper	Böck & Neidhardt (1966)
20SOK$^-$	*F$^-$ galP galK mgl*	MglP$^-$ GalP$^-$	W. Boos	Buttin (1963*a*,*b*)
ML308-225	*lac iz*	Lac y$^+$	I. C. West	West (1970)
ts19-1Δ	Hfr *metB pts crr*	PtsI$^-$ GalP$^+$		Jones-Mortimer & Kornberg (1974)
JM173	F$^-$ *lacX74 thyA*	Lac y$^-$	*E. coli* RV	Yudkin (1969)
K10	Hfr prototroph	MglP$^+$ GalP$^+$		Low (1973)
JM1090*	*ptsG ptsM ptsF mgl* (*λ*,*λdgal*$^+$) *galP fda*	MglP$^-$ GalP$^-$	h8	British Library Lending Division Supplementary Publication SUP 50074
JM1097*	*ptsG ptsM ptsF mgl* (*λ*,*λdgal*$^+$)	MglP$^-$ GalP$^+$	JM1090 by transduction	
JM1099*	*ptsG ptsM ptsF mgl*	MglP$^-$ GalP$^+$	JM1097 by heteroimmune superinfection	
JM1098*	*ptsG ptsM ptsF mgl galP* (*λ*,*λdgal*$^+$)	MglP$^-$ GalP$^-$	JM1090 by transduction	
JM1100*	*ptsG ptsM ptsF mgl galP*	MglP$^-$ GalP$^-$	JM1098 by heteroimmune superinfection	
JM1104*	*ptsG ptsM galP* (*λ*,*λdgal*$^+$)	MglP$^+$ GalP$^-$	JM1098 by transduction	
JM1109*	*ptsG ptsM galP*	MglP$^+$ GalP$^-$	JM1104 by heteroimmune superinfection	

* Strains are all Hfr *his gnd thyA galK*.

Table 2. *Uptake of sugars into different strains of E. coli induced with fucose*
The growth conditions and method of measuring nmol of sugar uptake/2min per mg dry mass are described in the Experimental section. The values are means±S.E.M. of the numbers of measurements in parentheses. Duplicate measurements were made on each of at least two batches of cells.

	K10 wild-type	JM1097 *mglP galP*$^+$ *ptsG ptsM*	JM1109 *mglP*$^+$ *galP ptsG ptsM*	JM1100 *mglP galP ptsG ptsM*	*ts19-1Δ ptsI*
Galactose	8.22±1.90 (12)	3.83±0.28 (12)	7.74±1.46 (10)	0.06±0.01 (4)	2.37±0.15 (9)
Fucose	2.48±0.29 (18)	1.39±0.10 (10)	2.50±0.25 (8)	0.14±0.02 (4)	1.37±0.27 (9)
Methyl β-galactoside	5.08±0.89 (12)	0.09±0.01 (6)	5.61±0.34 (26)	0.03±0.01 (4)	0.55±0.18 (8)
Glucose	6.66±1.66 (6)	7.63±0.52 (4)	7.80±1.94 (4)	0.98±0.10 (6)	4.75±0.80 (8)
2-Deoxyglucose	9.12±2.43 (6)	7.85±0.51 (14)	0.42±0.03 (4)	0.13±0.03 (4)	1.18±0.13 (12)
Methyl α-glucoside	1.41±0.10 (4)	0.19±0.02 (4)	0.45±0.13 (4)	0.10±0.02 (4)	0.16±0.01 (6)
Methyl β-thiogalactoside	0.16±0.04 (5)	0.22±0.02 (6)	0.62±0.20 (4)	—	0.14±0.03 (5)
Arabinose	0.43±0.19 (5)	0.32±0.03 (4)	0.36±0.03 (4)	—	0.17±0.02 (4)

transport systems in our experiments was eliminated by the following observations. The maximum uptake of methyl β-D-thiogalactoside by any of the strains was 0.62nmol/2min per mg (Table 2), confirming the relative absence of the lactose permease, and the maximum uptake of L-arabinose was 0.43nmol/2min per mg (Table 2), confirming that transport of sugars by the arabinose system could not be a significant factor. These activities are less than 5% of the values for organisms induced for lactose and arabinose transport (R. A. Giddens & P. J. F. Henderson, unpublished work). The use of fucose as inducer of galactose-transport systems aids the elimination of the arabinose systems, because fucose represses biosynthesis of the latter (Beverin *et al.*, 1971). Similarly, the low rates of methyl α-glucoside uptake established the relative absence of the glucose transport system specified by *ptsG* under the conditions of our experiments.

If the uptakes of sugars in Table 2 were by passive diffusion, then the concentration reached inside the cells from an external concentration of 0.05mM would approach 0.14nmol/mg, assuming there to be 2.7μl of intracellular water per mg dry mass of cells (Winkler & Wilson, 1966). Clearly, the accumulation of galactose, fucose or methyl β-galactoside by strain JM1109 is much greater than this in only 2min, and represents active transport against a concentration gradient by the methyl galactoside system, since none of these sugars is further metabolized (strain JM1109 is galactokinase-negative). Similarly, transport of fucose and galactose in *galP*$^+$ strains is active transport; strain JM1097 in Table 2 is *galK*$^+$, but galactose accumulation occurred to very similar extents, 3.28±0.32nmol/2min per mg, into strain JM1099 (Table 1), which is *galK*, *galP*$^+$. In fact Table 2 gives a minimum estimate of the potential accumulation, since uptake continued for some time after the 2min sample taken, to the point where depletion of the external substrate greatly enhanced the gradient achieved. As expected of active transport, uptakes of galactose, fucose, 2-deoxyglucose and methyl β-galactoside were inhibited by 80–90% by the uncoupling agents dinitrophenol (2mM), tetrachlorosalicylanilide (20μM) or carbonyl cyanide *m*-chlorophenylhydrazone (20μM).

Table 3. *Sugar-promoted alkaline pH changes with different strains of E. coli induced with fucose*
The growth conditions and methods of measuring pH changes are described in the Experimental section. Values are means±S.E.M. of measurements on at least three separate batches of cells.

	Effective H^+ uptake (nmol)		
	K10 *mgl*$^+$ *galP*$^+$	JM1097 *mgl galP*$^+$	JM1109 *mgl*$^+$ *galP*
Galactose	44.7±5.0 (8)	30.6±3.9 (9)	0.0 (4)
Fucose	44.8±8.0 (19)	48.0±8.8 (6)	0.0 (4)
Methyl β-galactoside	0.7±0.6 (5)	2.2±1.9 (4)	1.0±0.8 (3)

At least three levels of information are available from the experiments in this section. First, the specificity of the methyl galactoside and galactose-specific systems for different sugars was confirmed and extended. Secondly, the observation of the expected phenotypic properties authenticated the genotypic properties. Thirdly, each system was shown independently to effect accumulation of sugar against a concentration gradient, i.e. active transport.

Sugar-promoted effective proton uptake into different mutants

Once the ability to effect active transport was established, the strains could be examined for sugar–H^+ symport; in many cases the same batch of cells was used for both types of experiment. The results are presented in Table 3.

The addition of fucose or galactose to an anaerobic suspension of the fucose-induced wild-type strain

K10 elicited an alkaline pH change corresponding to proton uptake (Table 3; see also Henderson, 1974; Henderson *et al.*, 1975); methyl β-galactoside failed to promote an alkaline pH change (Table 3). Similar results were obtained with strain JM1097 (Figs. 1 and 2 and Table 3), which is *galP*$^+$, *mglP*. With galactose the initial alkaline pH change reversed to a continuous acidification (Fig. 1). This was presumably due to end-products of galactose metabolism, since it did not occur with *galK* strains (Henderson, 1974) or with the non-metabolizable sugar, fucose (Fig. 1). The measurements in Table 3 reflect the initial alkaline change with no correction for the subsequent acid production. As required by a chemiosmotic mechanism (Mitchell, 1961, 1970, 1973), the pH changes with strains K10 and JM1097 were abolished by each of the uncoupling agents mentioned above (results not shown). With both strains (cf. Henderson *et al.*, 1975), the pH changes failed to appear when fucose was omitted from the growth medium; this inducibility by fucose substantiates the association of the pH changes with a galactose-transport system (see Table 4). No pH changes were observed (Table 3) when these sugars were added to a suspension of fucose-induced

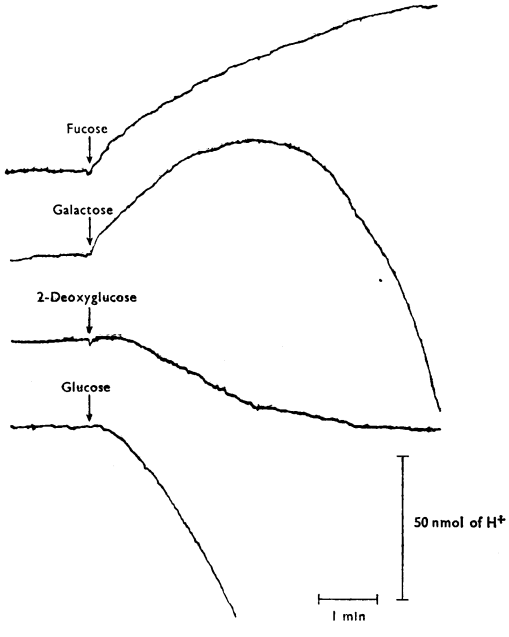

Fig. 1. *pH changes on addition of different sugars to fucose-induced E. coli strain JM* 1097 (*galP*$^+$)
Four separate experiments are shown, performed as described in the Experimental section. An upward deflexion indicates an alkaline pH change in this and subsequent Figures.

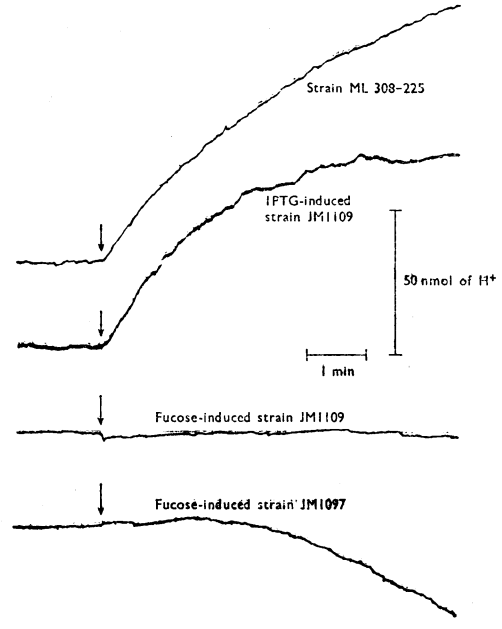

Fig. 2. *pH changes on addition of methyl β-galactoside to different strains of E. coli*
Methyl β-galactoside was added at the points indicated by the arrows. Each strain was grown on glycerol with inducers (isopropyl β-D-thiogalactoside, IPTG) as shown. Otherwise, each was prepared for the experiments in the same manner (see the Experimental section).

strain JM1109 (*galP*, *mglP*$^+$) or strain JM1100 (*galP*, *mglP*). The implication is that the galactose-specific system effects sugar–H$^+$ symport, and that the methyl galactoside system does not.

Three other explanations for the failure to demonstrate proton symport with galactose and fucose in strain JM1109 may be considered. This strain might be inherently incapable of showing sugar–proton symport because of some other more general deficiency, e.g. an enhanced permeability of the cell membrane to protons. Secondly, since the methyl β-galactoside used in the sugar-uptake experiments is labelled only in the methyl group, our measurements might indicate the accumulation of methanol rather than of methyl β-galactoside. If so, a methyl β-galactoside molecule could enter the cell accompanied by a proton and be hydrolysed to galactose and methanol, presumably by residual β-galactosidase activity, an enzyme for which methyl β-galactoside is known to be a substrate (Sinnott & Viratelle, 1973). The galactose molecule so produced might now leave the cell, taking the proton with it, thus setting up an exchange-diffusion transport system with no net pH change. Thirdly, the methyl

galactoside permease system might be switched off under the conditions required for the experiment (anoxia and high sugar concentrations). The following experiments argue against these possibilities.

Strain JM1109 has a functional lactose operon that was induced by growing the organism on glycerol supplemented with 0.5mM-isopropyl β-D-thiogalactoside. After the usual depletion procedure, the addition of methyl β-galactoside, a substrate for the lactose permease (Rotman *et al.*, 1968), to an anaerobic suspension of isopropyl β-D-thiogalactoside-induced strain JM1109 yielded an immediate and extensive alkalinization (Fig. 2). Methyl β-D-thiogalactoside, a substrate of the lactose permease but not of methyl galactoside permease (Rotman *et al.*, 1968; Table 2), gave the same effect (result not shown; see Henderson *et al.*, 1975). Hence strain JM1109 could not be 'leaky' to H^+, and is capable of manifesting a sugar–H^+ symport system. Methyl β-galactoside–H^+ symport on the lactose permease also occurred with isopropyl β-D-thiogalactoside-induced strain K10 (Henderson *et al.*, 1975), and with the *lac* constitutive strain ML308-225 (Fig. 2). Isopropyl β-D-thiogalactoside-induced cultures of strains K10 and JM1109 contain high activities of β-galactosidase, and so any diminution of the sugar–H^+ symport by exchange diffusion would be expected to be maximal in these organisms. That the presence of β-galactosidase does not apparently interfere emphasizes that exchange diffusion of methyl β-galactoside against galactose is not responsible for the absence of methyl β-galactoside–H^+ symport from fucose-induced cells. Further, fucose-induced strain JM173, which carries a deletion of the *lac* operon (Table 1), and thus had no β-galactosidase activity (confirmed by assays with *o*-nitrophenyl β-galactoside), showed a galactose–H^+ or fucose–H^+ but not methyl β-galactoside–H^+ symport activity, even though this strain accumulated radioactive methyl β-galactoside to an extent comparable with that shown by strain JM1109. Consequently, the failure of methyl β-galactoside to elicit effective proton uptake in fucose-induced cells is not the result of interference by enzymes of the *lac* operon.

Unfortunately, because radioisotope-labelled methyl β-galactoside is not available at a sufficiently high specific radioactivity and because completely anaerobic sampling and filtration are difficult to achieve, it was not possible to test unequivocally whether the methyl galactoside system is inoperative under the conditions of the sugar–H^+ experiments. However, the following experiment indicated that galactose at least entered on the methyl galactoside system without causing H^+ uptake. Strain JM1104 is *mglP*$^+$, *galP*, *galK*$^+$. Hence, galactose entry can be followed by metabolism, which, under anaerobic conditions, is manifested as an efflux of acid. In this strain, in which galactose can enter only by the methyl galactoside system, addition of galactose did not promote an alkaline pH change, but an acid one (Fig. 3); this would indicate that the sugar could enter on the methyl galactoside system without an obligatory H^+ uptake. This experiment could still be equivocal, since any 'leakage' of galactose inwards through the cell membrane would produce a similar effect; however, under aerobic conditions no such leakage apparently occurs, since strain JM1098 (*mglP*, *galP*, *galK*$^+$, Table 1) showed no growth whatsoever after many days incubation on galactose minimal medium, except as a result of mutation.

Transport of glucose and 2-deoxyglucose on the galactose-specific system

According to Curtis & Epstein (1975), 2-deoxyglucose is a substrate for the phosphotransferase Enzyme II specified by *ptsM*, methyl α-glucoside for the phosphotransferase Enzyme II specified by *ptsG*, and glucose for both. However, from the results of Kornberg & Jones-Mortimer (1975) and Amaral & Kornberg (1975), it would appear that 2-deoxyglucose is a substrate for both Enzymes II. The contradictory interpretations may be due to strain differences, or to the failure of the glucose derivatives to act as inducers of either transport system; sub-

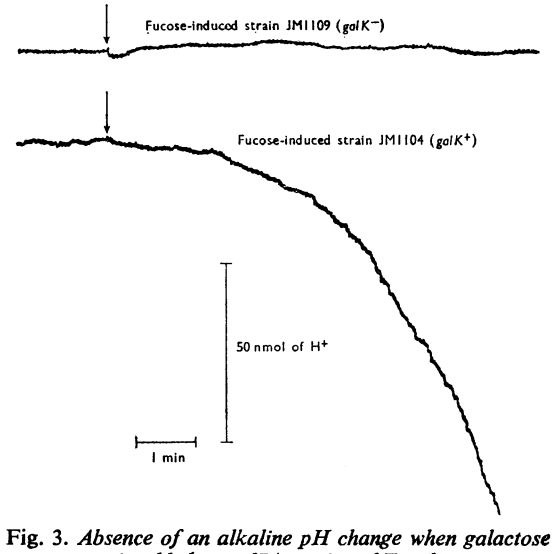

Fig. 3. *Absence of an alkaline pH change when galactose is added to mglP*$^+$ *strains of E. coli*
Both strains JM1109 (*galP*, *galK*, *mgl*$^+$) and JM1104 (*galP*, *galK*$^+$, *mgl*$^+$) were grown on glycerol with fucose as inducer, and prepared in the usual manner (see the Experimental section). Galactose was added at the points indicated by the arrows.

Table 4. *Inducibility of sugar uptakes in different strains of E. coli*

Experiments were performed as described in Table 2, except that fucose was omitted from the growth medium. The induction ratio is the mean value in Table 2 (induced cells) divided by the mean value in this Table (uninduced cells).

	K10		JM1097		JM1109	
	Uptake (nmol/2min per mg)	Induction ratio	Uptake (nmol/2min per mg)	Induction ratio	Uptake (nmol/2min per mg)	Induction ratio
Galactose	2.22±0.06 (8)	3.7	0.36±0.03 (4)	10.6	0.20±0.09 (4)	38.7
Fucose	0.32±0.06 (6)	7.8	0.27±0.02 (4)	5.1	0.30±0.08 (4)	8.3
Methyl β-galactoside	0.63±0.18 (6)	8.1	0.01±0.00 (4)	9.0	0.09±0.04 (4)	62.3
Glucose	6.05±1.26 (4)	1.1	1.47±0.15 (4)	5.2	0.47±0.05 (4)	16.6
2-Deoxyglucose	12.81±0.66 (4)	0.7	0.78±0.02 (4)	10.0	0.13±0.05 (4)	3.2
Methyl α-glucoside	0.50±0.13 (4)	2.8	0.12±0.02 (4)	1.6	0.12±0.04 (4)	3.8

Table 5. *Inhibition by sugars of transport of galactose and 2-deoxyglucose into E. coli JM1097*

Uptakes of galactose and 2-deoxyglucose into fucose-induced *E. coli* JM 1097 were performed in the usual way except that 1mM-unlabelled sugars as indicated were added 3min before the labelled sugar.

	Uptake	
Addition	(nmol/2min per mg)	(% of control)
[^{14}C]Galactose	4.52±0.29 (6)	100
+2-Deoxyglucose	0.50±0.04 (4)	11
+Methyl β-galactoside	2.63±0.17 (4)	58
+Fucose	1.40±0.31 (4)	31
2-Deoxy[^{14}C]glucose	7.93±0.38 (6)	100
+Galactose	0.31±0.02 (4)	4
+Methyl β-galactoside	7.91±0.24 (4)	100
+Fucose	2.42±0.09 (4)	31

sequent experiments indicate that 2-deoxyglucose may enter on the phosphotransferase system specified by *ptsG* when present at 10mM, the concentration used by Amaral & Kornberg (1975) to obtain mutants resistant to 2-deoxyglucose that turned out to be *ptsG*. At the concentration of 0.05mM used in the present study, then 2-deoxyglucose enters almost exclusively by the route specified by *ptsG*. At 0.05mM concentration, methyl α-glucoside can be assumed to enter exclusively by the route specified by *ptsM*. In any case, it would be expected that all our mutant strains (*ptsG*, *ptsM*) would lack the ability to transport glucose, 2-deoxyglucose or methyl α-glucoside. In fact, from Table 2 it is clear that uptake of glucose and 2-deoxyglucose occurs to the same extent into fucose-induced strain JM1097 (*ptsG*, *ptsM*, *galP*$^+$) as into the wild-type strain K10. Further, Table 2 shows that 2-deoxyglucose was not transported into fucose-induced JM1109 (*ptsG*, *ptsM*, *galP*, *mglP*$^+$).

Therefore, since transport of glucose and 2-deoxyglucose is also induced by fucose in strain JM1097 (Table 4), these sugars are very probably substrates for the galactose-specific system. This was expected for glucose (see Wilson, 1974*a*) but not for 2-deoxyglucose. However, the conclusion is substantially confirmed by the observation that galactose and 2-deoxyglucose are good mutual inhibitors of each others transport into fucose-induced strain JM1097 (Table 5); consistently, in the same experiment methyl β-galactoside, a substrate for the methyl galactoside system and not the galactose-specific transport system, was not a good inhibitor (Table 5), whereas fucose, a substrate for both systems, was. 2-Deoxyglucose would therefore be expected to promote H^+ uptake into *galP*$^+$, *ptsM* strains such as JM1097. However, addition of 2-deoxyglucose to anaerobic suspensions of such strains elicited a variable and relatively small acid or alkaline pH change (see, e.g., Fig. 1). One explanation of this anomaly could be that the high 2-deoxyglucose concentration necessary for these experiments permitted significant entry on a residual phosphotransferase activity; note that in uninduced strain JM1097 there is significant transport of 2-deoxyglucose and glucose (Table 2), which may reflect the presence of such activity. Glucose also failed to elicit an alkaline pH change with strain JM1097, but it could be that the rapid acid efflux after metabolism of the glucose obscured any H^+ uptake (Fig. 1).

We therefore turned to *E. coli* strain *ts19-1*Δ, an organism devoid of phosphotransferase activity because of a gene deletion (Jones-Mortimer & Kornberg, 1974). When induced with fucose or galactose, strain *ts19-1*Δ exhibited rates of uptake of these two sugars comparable with the rates obtained with the strain JM1097 (Table 2); also, galactose and fucose elicited effective proton uptake, albeit to approximately half the extent seen with strain JM1097 (Tables 3 and 6). Glucose and 2-deoxyglucose were as efficient as galactose and fucose in promoting effective proton uptake (Fig. 4 and Table 6), in contrast with their ineffectiveness with strain JM1097 (Fig. 1). An acidification followed

Table 6. *Sugar-promoted alkaline pH changes with fucose-induced E coli ts*19–1Δ
Conditions were as described in Table 3.

	Effective H^+ uptake (nmol)
Galactose	15.0±3.5 (7)
Fucose	30.7±3.4 (6)
Methyl β-galactoside	5.0±2.4 (3)
Glucose	16.3±2.1 (8)
2-Deoxyglucose	30.4±4.9 (9)
Methyl α-glucoside	2.0±1.2 (5)

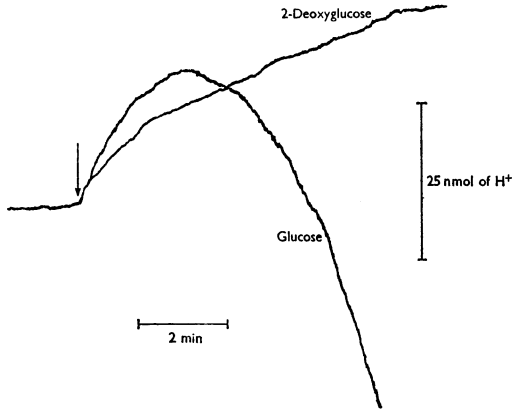

Fig. 4. *Ability of 2-deoxyglucose and glucose to elicit alkaline pH changes with the pts E. coli strain ts*19-1Δ
pH recordings from two separate experiments on one batch of cells have been combined to facilitate comparison. The strain was grown on glycerol with fucose as inducer (see the Experimental section).

the proton uptake with glucose but not with 2-deoxyglucose (Fig. 4), presumably because only the former is a metabolic substrate for *E. coli*; this also indicated that the 2-deoxyglucose did not contain glucose as a contaminant. Both the proton uptakes and the energized transport of these sugars were fucose-inducible (Henderson *et al.*, 1975, and Table 4), again indicating that transport of glucose and 2-deoxyglucose occurred on the galactose-transport system in this strain. As with the other organisms, methyl β-galactoside did not promote effective H^+ uptake into strain *ts19-1*Δ (Table 6), but neither was it a good substrate for energized transport (Table 2).

Despite its failure to elicit effective proton-uptake activity into strain JM1097, it appears that 2-deoxyglucose is transported by the galactose-specific but not by the methyl galactoside system. Therefore, in the same way as methyl β-galactoside has been invaluable for detecting and measuring the methyl galactoside transport function, 2-deoxyglucose should be useful for assessing the galactose-specific system activity, provided that glucose phosphotransferase activity is absent.

We decided to determine whether glucose is taken up by the galactose rather than the glucose system in the wild type. Accordingly, fucose was omitted from the growth medium so that the transport systems for galactose, fucose and methyl β-galactoside would not be induced (see Table 4). This severely impaired transport of glucose and 2-deoxyglucose into strain JM1097, but their transport into strain K10 was not significantly decreased (in fact, 2-deoxyglucose uptake was enhanced; Table 4), indicating that their transport in the wild type is independent of the galactose-transport systems. The apparent extent of 2-deoxyglucose uptake is in fact higher than that of glucose (this may be more apparent than real, since some of the label may be lost from the glucose as $^{14}CO_2$), and the rate with methyl α-glucoside is lower (Tables 2 and 4). When *E. coli* K10 was grown on glucose, the uptake of glucose and 2-deoxyglucose about doubled, whereas the uptake of methyl α-glucoside increased about fivefold (R. A. Giddens, unpublished work). Therefore it may be concluded that uptake of glucose (from 0.05mM concentration) into strain K10 occurs predominantly by the route specified by *ptsM* under the conditions of our experiments where the system specified by *ptsG* is not induced.

Discussion

A number of reports have suggested that transport of certain amino acids and sugars into *E. coli* can be energized under conditions where a transmembrane pH and/or electrical gradient could not be generated (Berger, 1973; Berger & Heppel, 1974; Curtis, 1974; Wilson, 1974*b*). A chemiosmotic mechanism of energization would not therefore be operative in these cases. The experiments were performed in ATPase (adenosine triphosphatase)-negative strains, and transport required provision of glucose, presumably to generate ATP or some other intermediate which then directly energized the transport (Berger, 1973; Berger & Heppel, 1974). From a series of experiments it emerged that transport systems with a binding-protein component (see reviews by Oxender, 1972; Boos, 1974) operate by direct energization, and those without a binding protein utilize energy generated by respiration or ATP hydrolysis, and so are compatible with a chemiosmotic mechanism (Berger, 1973; Berger & Heppel, 1974; Curtis, 1974; Wilson, 1974*b*).

The methyl galactoside system contains a binding protein and is said to be directly energized (Boos & Sarvas, 1970; Wilson, 1974*b*). Our failure to observe proton translocation under conditions where the

methyl galactoside system was expected to be operative further supports the implication that this system does not utilize a chemiosmotic mechanism. However, failure to observe H^+ symport could be attributed to at least two other causes. The methyl galactoside system might be 'switched off' by some metabolic regulation under the conditions of anaerobiosis, energy-depletion and high sugar concentration required to detect proton movements (see also Silhavy *et al.*, 1974). Alternatively, operation of the methyl galactoside system might be obligatorily linked to a transmembrane electrical potential and not to a pH gradient, but this would raise the further question of how such a potential might be generated in non-respiring, ATPase-negative strains. It should be recalled that use of the term methyl galactoside system obscures the existence of the products of the genes *mglA* and *mglC*, both of which are integrated with the binding protein to effect active methyl β-galactoside transport (Robbins, 1975; Robbins & Rotman, 1975; Robbins *et al.*, 1976). Their mechanism of interaction is the subject of speculation (Silhavy *et al.*, 1974), and it may well be that the primary function of the system is to effect chemotaxis (Ordal & Adler, 1974*a*,*b*; Adler, 1975) rather than transport. If so, it could make metabolic sense to turn off the methyl galactoside system when high concentrations of sugar are available, as is essential in experiments designed to detect proton symport. The genes *mglA*, *mglB* and *mglC* were not distinguished until after the preparation of our organisms from *E. coli* strain 20SOK$^-$ (Table 1), now reported to be MglA$^-$, MglC$^-$ (Ordal & Alder, 1974*a*). Hence, our *mglP* strains may still contain the binding protein, but subsequent studies have indicated that this would not alter our conclusions concerning the operation of the methyl galactoside and galactose-specific systems.

Rotman *et al.* (1968) reported that the galactose-specific system is induced by and transports galactose and that fucose is neither inducer nor substrate, whereas Buttin (1963*a*) deduced that both sugars are inducers. Wilson (1974*a*) showed that galactose, fucose and probably glucose were substrates for the galactose-specific system and galactose was an inducer, but apparently did not look for induction by fucose. The galactose-specific carrier that we have studied is induced by galactose or fucose, transports glucose, galactose and fucose but not methyl β-galactoside, and by these criteria alone is the galactose-specific carrier studied by Buttin (1963*a*, 1968), Rotman *et al.* (1968) and Wilson (1974*a*). Mapping of a gene for the galactose-specific system at min 61.5, close to *fda*, has been accomplished by Riordan (1976) by using *E. coli* W4345, the *galP*$^+$, *mglP* strain characterized by Rotman *et al.* (1968) as the starting point. Our experiments relied on selecting a mutant in a galactose permeation system, the gene for which was closely linked to *fda* (see Bachmann *et al.*, 1976, for genetic nomenclature) and thus it is almost certainly the same galactose-specific system. Since *galP* is located between *fda* and *lysA* (Riordan, 1976) close to the regulatory gene *galR* (Buttin, 1963*b*, 1968), we cannot be certain that the lesion in our strains is in a structural, rather than a regulatory, component of the galactose-specific system (see also the discussion in Riordan, 1976). It is possible that this phenotype resulted from a mutation in the repressor gene *galR*, which permitted expression of the methyl galactoside system [this system is not regulated by *galR* (Lengeler *et al.*, 1971; Wilson, 1974*a*)], but not of the galactose-specific system. However, a mutation of this type would be as suitable as a structural gene mutant for the purposes of our present experiments, which rely only on the measured phenotypic properties. The membrane-bound transport system that is retained in subcellular vesicles has been assumed to be the galactose-specific system (Kerwar *et al.*, 1972; Wilson, 1974*a*; but see Wilson, 1974*b*) but it could represent the function of the products of *mglA* or *mglC* (Robbins, 1975; Robbins & Rotman, 1975; Robbins *et al.*, 1976) or of both, in the absence of the binding protein.

Wilson (1974*a*) provided evidence that operation of the galactose-specific system did not involve a binding protein, but then reversed this conclusion (Wilson, 1974*b*) when he deduced that the galactose-specific system operated by the 'direct energization' mechanism. Since we observe that transport on the galactose-specific system can be accompanied by proton translocation, the system would appear to have a chemiosmotic mechanism of operation. Of course, the two mechanisms need not be exclusive of each other, and one carrier may use both. A chemiosmotic mechanism is supported by a second type of experiment if sugar transport is demonstrably driven by an artificially imposed transmembrane pH or electrical gradient. This has primarily been shown for *Streptococcus lactis* 7962 (Kashket & Wilson, 1973), but note that this strain is atypical in that it is the only one of the species that does not utilize a phosphotransferase mechanism for lactose transport (McCay *et al.*, 1970). The same approach has since been extended to *E. coli* (Flagg & Wilson, 1976), but it requires the use of ATPase-negative mutants, and so was beyond the scope of the present work.

The simplest interpretation of all our results is that galactose can enter *E. coli* by using a chemiosmotic mechanism (galactose-specific system) or direct energization (methyl galactoside system). Whether these systems operate independently in the wild type, or whether they interact so that the proportion of galactose entering by each is regulated (cf. Wilson, 1974*a*; Silhavy *et al.*, 1974),

are questions that remain to be answered. It seems possible that two energization modes could also operate for transport of arabinose and maltose, as these also involve multi-component systems including binding proteins.

It has been shown that glucose can be transported by both the methyl galactoside and the galactose-specific systems (Boos, 1969; Wilson, 1974*a*); our results confirm this. Were it not for the induction of these two systems by galactose and fucose and not glucose, they could well be glucose transport systems: this is possibly an indication of their evolutionary origin. When a wild-type organism is presented with both glucose and another sugar, the established phenomena of catabolite repression and inducer exclusion (see review by Magasanik, 1970) combine to ensure that the glucose is utilized first. Our observations suggest that a third mechanism additionally operates when the second sugar is galactose, namely that glucose prevents entry of the inducers of the galactose systems by simple competition for the transport mechanisms.

Our results indicate which parts of the hexose molecule are distinguished by the galactose-specific and methyl galactoside transport systems. Replacement of the hydroxyl group on C-6 by hydrogen does not abolish transport by either carrier, nor does inversion of configuration at C-4. Removal of C-6 abolishes transport, since the homologous pentose, L-arabinose, is not a substrate. Replacement of the hydroxyl group on C-2 by hydrogen prevents entry via the methyl galactoside system, and β-glycoside formation prevents entry via the galactose-specific system. The effects of modification at C-3 have not been investigated. The observation that modification at C-2 renders the hexose unsuitable for the methyl galactoside system while it remains a substrate for the galactose-specific system may provide a useful tool for further investigation of these activities.

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