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2-Deoxy-D-galactose, a Substrate for the Galactose-Transport System of *Escherichia coli*

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The following observations showed that 2-deoxy-D-galactose is a useful tool for the isolation and elucidation of the activity of one system for galactose uptake into *Escherichia coli*. 1. 2-Deoxygalactose, which is not a substrate for growth of *E. coli*, was transported into strains of the organism induced for galactose transport. 2. By using appropriate mutants it was shown that 2-deoxygalactose is a much better substrate for the galactose-transport system than for the methyl galactoside-transport system. This was confirmed by the results of mutual inhibition studies with substrates of each transport system. 3. The glucose-, arabinose- or lactose-transport systems did not effect significant transport of 2-deoxygalactose. 4. Like other substrates of the galactose-transport system, 2-deoxygalactose promoted effective proton uptake into de-energized suspensions of appropriate *E. coli* strains. 5. The S183 series of *E. coli* mutants were found to contain a constitutive galactose-transport system, if 2-deoxygalactose transport is used as one criterion for such activity.

Exposure of *Escherichia coli* to D-galactose causes the induction of two separate systems for uptake of the sugar into the organism (Buttin, 1968; Rotman *et al.*, 1968; Wilson, 1974). One is known as the methyl galactoside-transport system MglP, because, besides the natural substrate galactose, it transports methyl β-D-galactoside (Rotman, 1959; Rotman *et al.*, 1968). The other is known as the galactose-specific transport system GalP, because it does not transport methyl galactoside (Buttin, 1968; Rotman *et al.*, 1968). Elucidation of the properties of the MglP system has been greatly facilitated by its specificity for methyl galactoside [reviewed by Oxender (1972) and Boos (1974)], and the study of the GalP system would be similarly expedited if a sugar analogue were found for which it had a reasonably exclusive specificity.

Evidence has been obtained that 2-deoxy-D-glucose is a substrate for the galactose-specific system and not for the methyl galactoside-transport system (Henderson *et al.*, 1977). However, 2-deoxyglucose is also transported by the mannose phosphotransferase (PtsM) system of *E. coli* (Curtis & Epstein, 1975; Kornberg & Jones-Mortimer, 1975), and so it is not an ideal substrate with which to investigate the galactose-specific transport system. These observations suggested that 2-deoxy-D-galactose could be such a substrate, and the work described below confirms this prediction.

Clearly, the epithet 'galactose-specific' is no longer applicable. Therefore the term galactose-transport system will be used in this paper for the system of designated phenotype GalP coded for by the *galP* gene, which is located at about min 62 on the *E. coli* linkage map (Riordan & Kornberg, 1977). The nomenclature of genes and phenotypes follows the conventions of Demerec *et al.* (1966) and Bachmann *et al.* (1976). D- or L-configuration of a sugar is given on first mention and subsequently is the D-form unless indicated otherwise.

Experimental

The methods of maintenance, growth and harvesting of the *E. coli* strains listed in Table 1 were described by Henderson *et al.* (1977). The S183 series of organisms was a gift from Dr. B. Rotman, Division of Biological and Medical Sciences, Brown University, Providence, RI 02912, U.S.A. Strain JM1150 was obtained as follows. The *mglD* lesion of strain S183-726 was transduced (phage P1-mediated) into strain JM1079 (*fda,ptsF,ptsM,galP,mgl*), and *ptsF*$^+$ transductants were selected. One such transductant was shown to have acquired a constitutive methyl galactoside-transport system. It was then made *fda*$^+$ by infection with phage P1 grown on wild-type strain K10 by selecting for xylose$^+$ transductants, one of which was strain JM1150 (M. C. Jones-Mortimer, unpublished work).

The transport of radioisotope-labelled sugars and sugar-promoted pH changes were measured as described by Henderson *et al.* (1977). A 15s time point was used to calculate initial rates of transport of labelled sugar (concentration 0.05mM unless specified otherwise). Corrections were made for non-specific binding of label to filters if this was significant.

Table 1. *Strains of E. coli used*

Strain	Genotype	Relevant transport phenotype	Reference
K10	Hfr prototroph	GalP$^+$,MglP$^+$	Low (1973)
JM1097*	*ptsG,ptsM,ptsF,mglP* (λ,λd*gal*$^+$)	GalP$^+$,MglP$^-$	British Library Lending Division Supplementary Publication SUP 50074
JM1099*	*ptsG,ptsM,ptsF,mglP*	GalP$^+$,MglP$^-$	
JM1104*	*ptsG,ptsM,galP* (λ,λd*gal*$^+$)	MglP$^+$,GalP$^-$	
JM1109*	*ptsG,ptsM,galP*	MglP$^+$,GalP$^-$	
JM1101*	*ptsM,ptsF,galP,mglP*	MglP$^-$,GalP$^-$,PtsG$^+$	
JM1103*	*ptsG,ptsF,galP,mglP*	MglP$^-$,GalP$^-$,PtsM$^+$	
JM1150*	*ptsM,mglD,galP* (λ,λd*gal*$^+$)	MglP^c†,GalP$^-$	This paper
S183-726	*mglD*	MglP^c†,GalP^c†	Robbins (1975)
S183-27T	*mglB,mglC,mglD*	MglP$^-$,GalP^c†	Robbins & Rotman (1975)
SB5314	*araA*	AraE$^+$,AraF$^+$	Hogg & Englesberg (1968)

* Strains are all Hfr *his,gnd,thyA,galK*.
† Superscript c here and throughout the paper indicates constitutivity.

2-Deoxy-D-galactose was purchased from Koch–Light, Colnbrook, Bucks. SL3 0BZ, U.K. The compound was labelled with ^3H at C-1 by catalytic exchange with $^3H_2$ gas, a service of The Radiochemical Centre, Amersham, Bucks., U.K. Purification and identification was achieved by repeated ascending paper chromatography with butan-1-ol/ethanol/water (52:33:15, by vol.) as solvent. Before use, the label was diluted with unlabelled 2-deoxygalactose to a final concentration of 2mM (4Ci/mol). The sources of other labelled compounds and the method of measuring their radioactivity were described by Henderson *et al.* (1977).

In certain experiments growth of the organisms at 37°C was measured in 10ml cultures contained in loosely capped test tubes (23ml capacity) agitated at 250rev./min (Gallenkamp orbital incubator). A Corning–Eel nephelometer (Evans Electroselenium Ltd., Halstead, Essex, U.K.) was used to record the increase in cell density.

Results and Discussion

Uptake of radioisotope-labelled 2-deoxygalactose into GalP$^+$ strains of E. coli

E. coli strain JM1099 is *galP$^+$,mglP*, whereas strain JM1109 is *galP,mglP$^+$* (Table 1). Labelled 2-deoxygalactose accumulated in strain JM1099 containing the galactose-transport system to a concentration about 10 times that in strain JM1109, which contained the methyl galactoside - transport system (Table 2). Both organisms are galactokinase-negative (Table 1), so the possibility of metabolism of the 2-deoxygalactose was eliminated (see below). In fact, in the corresponding galactokinase-positive organisms the same preferential uptake of 2-deoxygalactose into the strain containing the galactose-transport system was observed (Table 2), and the absolute values were higher (Table 2). 2-Deoxygalactose uptake was 2–5% of the methyl galactoside uptake into strains containing only the methyl galactoside - transport system (Table 2). Furthermore the rate of 2-deoxygalactose uptake was about 2% of the rate of methyl galactoside uptake into strain JM1150 (Fig. 1*a*), which is constitutive for the methyl galactoside-transport system and lacks the galactose-transport system (Table 1). These differences would be even greater if a correction was made for the 0.03–0.14nmol/mg taken up by a corresponding transport-negative (*galP,mglP*) strain (Henderson *et al.*, 1977), an amount expected from passive diffusion of sugars into the cells.

Chemotaxis of *E. coli* towards galactose is mediated by the binding of the sugar to the components of the methyl galactoside - transport system (Adler *et al.*, 1973; Ordal & Adler, 1974; Boos, 1974). 2-Deoxygalactose did not stimulate chemotaxis (Adler *et al.*, 1973), which implies that it does not interact with the methyl galactoside-transport system.

All these results indicate that 2-deoxygalactose is a much better substrate for the galactose-transport system than for the methyl galactoside-transport system of *E. coli*. Table 2 also shows that 2-deoxygalactose uptake into the wild-type organism, K10 (*galP$^+$,mglP$^+$,galK$^+$*) was very similar to that into the *galP$^+$* mutants. When strains K10 or JM1097 were grown on glycerol without fucose, 2-deoxygalactose uptake was decreased by 90–95% (results not shown). This inducibility by fucose further substantiates that 2-deoxygalactose uptake occurs on a system that normally transports galactose (Buttin, 1963, 1968).

Kinetics of 2-deoxygalactose transport

It was possible that the difference in uptake of 2-deoxygalactose by each transport system was only apparent at the short exposure time (2min) and low concentration (0.05mM) used in the above experiments. However, this was not so. Fig. 1(*a*) shows that

Table 2. *Uptake of 2-deoxygalactose into different strains of E. coli*

The strains were grown on glycerol with 1 mM-fucose added in every case except for strain S183-27T. Transport of 2-deoxygalactose was measured as described by Henderson *et al.* (1977). In this and other Tables the results are expressed as means ±S.E.M., with the numbers of measurements in parentheses; duplicate measurements were always made on at least two batches of each strain.

Strain	Relevant phenotype	2-Deoxygalactose uptake (nmol/2 min per mg)	Methyl β-galactoside uptake (nmol/2 min per mg)
K10	$GalP^+,MglP^+,GalK^+$	1.74±0.07 (4)	5.08±0.90 (12)
JM1097	$GalP^+,MglP^-,GalK^+$	1.74±0.19 (6)	0.09±0.01 (6)
JM1099	$GalP^+,MglP^-,GalK^-$	0.96±0.06 (4)	0.60±0.27 (6)
JM1104	$GalP^-,MglP^+,GalK^+$	0.46±0.04 (4)	9.00±0.60 (6)
JM1109	$GalP^-,MglP^+,GalK^-$	0.11±0.03 (6)	5.61±0.34 (26)
S183-27T	$GalP^c,MglP^-$	8.33±0.65 (8)	0.02±0.02 (6)

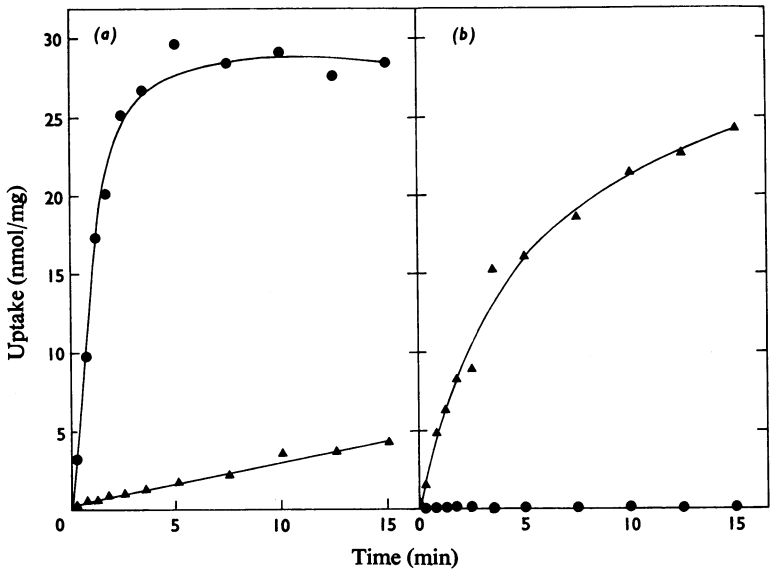

Fig. 1. *Uptake of 2-deoxygalactose or methyl β-galactoside into E. coli strains constitutive for the methyl galactoside-transport system (a) or the galactose-transport system (b)*

Labelled 2-deoxygalactose (▲) or methyl β-galactoside (●) (0.05 mM) were added to 1.5 ml of bacterial suspension containing 2–2.2 mg dry mass of strain JM1150 (*a*) or strain S183-27T (*b*). Samples (0.1 ml) were taken at the indicated intervals. Details of the method were described by Henderson *et al.* (1977).

even after 15 min 2-deoxygalactose uptake into the $mglP^c,galP$ strain was less than 15% of the plateau value of methyl galactoside uptake. A similar difference was maintained with inducible strains when uptake was followed for 15 min (results not shown). In a strain constitutive for 2-deoxygalactose transport (see the discussion below) the methyl galactoside uptake was negligible (Fig. 1*b*); this shows that the methyl galactoside system is a less effective discriminator between methyl galactoside and 2-deoxygalactose than the galactose-transport system.

The rate of 2-deoxygalactose transport into $galP^+$ strains increased markedly when the sugar concentration was raised (Fig. 2). By contrast, concentrations up to 0.6 mM still failed to drive significant uptake into the $mglP^+$ strains, and even above this concentration the uptake of 2-deoxygalactose was still much slower than into the $galP^+$ strains (Fig. 2).

The results with the $galP^+$ strains gave linear Lineweaver–Burk replots (inset, Fig. 2). Calculation of the apparent kinetic parameters and their standard deviations by the computerized method of Cleland (1967) yielded a K_m for 2-deoxygalactose of 1.36 (S.D. ±0.33) mM and $V_{max.}$ of 18.8 (S.D. ±2.3) nmol/min per mg in the inducible strain, and K_m 0.70 (S.D. ±0.13) mM, $V_{max.}$ 88.2 (S.D. ±6.7) nmol/min per mg in the

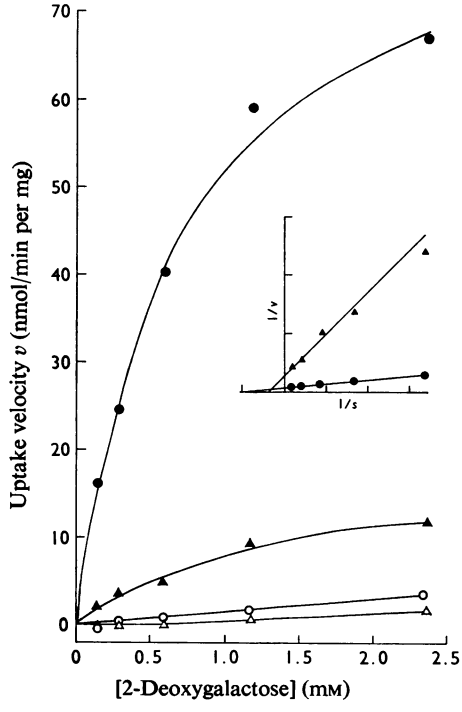

Fig. 2. *Concentration-dependence of rates of 2-deoxygalactose uptake*

Uptakes of 2-deoxy [1-^{3}H]galactose (12.5 Ci/mol) at the indicated concentrations were measured for 15 s in 0.55 ml suspensions containing 0.66–0.70 mg dry mass of strain S183-27T (●), fucose-induced strain JM1097 (▲), strain JM1150 (○), or fucose-induced strain JM1109 (△). Closed symbols are GalP$^+$,MglP$^-$ strains and open symbols are GalP$^-$,MglP$^+$ strains (Table 1). Each point is the mean of duplicate measurements. The lines show the best fits of all data points to the Michaelis–Menten equation (Cleland, 1967). The inset is a Lineweaver–Burk replot of the same points.

constitutive strain. The corresponding K_m values for galactose were about one-third of these, 0.45 (S.D. ± 0.06) and 0.22 (S.D. ±0.02) mM in the two strains, as might be expected for the natural substrate. $V_{max.}$ for galactose was similar to the $V_{max.}$ for 2-deoxygalactose in each strain.

To obtain optimal discrimination between the two transport systems for galactose, 2-deoxygalactose should be used at concentrations up to 0.5 mM and transport measured for less than 5 min. For the following experiments, in which the ability of other sugar-transport systems to transport 2-deoxygalactose was measured, it was convenient to use 0.05 mM for comparative purposes.

Confirmation of selectivity by inhibition experiments

If a compound is a substrate for a particular transport system, then its uptake should be inhibited by other substrates of the same system. Galactose, fucose and 2-deoxyglucose are substrates for the galactose-transport system, and methyl β-galactoside is not (Rotman *et al.*, 1968; Wilson, 1974; Henderson *et al.*, 1977). Therefore, if 2-deoxygalactose is a substrate for the galactose-transport system and not for the methyl galactoside-transport system, its uptake would be inhibited by galactose, fucose and 2-deoxyglucose, but not by methyl β-galactoside. This prediction was exactly fulfilled (Table 3) by using strain S183-726, which is constitutive for both transport systems.

By a similar argument, 2-deoxygalactose would be expected to inhibit uptake of another substrate of the galactose-transport system but not uptake of a substrate of the methyl galactoside - transport system. This prediction was confirmed with the constitutive strain, when unlabelled 2-deoxygalactose inhibited uptake of labelled 2-deoxyglucose, but not uptake of labelled methyl β-galactoside (Table 3); in fact, the initial rate of methyl β-galactoside uptake was en-

Table 3. *Inhibition of uptakes of labelled sugars into a GalP$^+$,MglP$^+$ strain*

Strain S183-726 was grown on glycerol and uptakes of 2-deoxy[1-^{3}H]galactose, [^{14}C]methyl β-galactoside or 2-deoxy-[1-^{14}C]glucose were measured in the absence or presence of the indicated compounds added 3 min before the labelled sugar. Both labelled and unlabelled sugars were used at 0.05 mM concentration, except for the unlabelled 2-deoxygalactose, which was 1 mM. Values are the means of duplicate measurements on one batch of cells.

	Additions	Rate of labelled sugar uptake (nmol/min per mg)
Expt. 1	2-Deoxy[1-^{3}H]galactose	5.1
	Galactose before 2-deoxy[1-^{3}H]galactose	0.9
	Fucose before 2-deoxy[1-^{3}H]galactose	2.4
	Methyl β-galactoside before 2-deoxy[1-^{3}H]galactose	5.1
Expt. 2	[^{14}C]Methyl β-galactoside	8.8
	2-Deoxygalactose before [^{14}C]methyl β-galactoside	12.3
Expt. 3	2-Deoxy[1-^{14}C]glucose	55.5
	2-Deoxygalactose before 2-deoxy[1-^{14}C]glucose	20.3

hanced by 2-deoxygalactose (Table 3), although the uptake after 2 min was not significantly different (results not shown). A very similar pattern of results has been obtained with strains inducible for each of the transport systems that were also *ptsM*, to eliminate interference by 2-deoxyglucose uptake on the mannose phosphotransferase system (results not shown).

These inhibition experiments added further support to the conclusion that 2-deoxygalactose is a substrate for the galactose-transport system, but not for the methyl galactoside-transport system.

2-*Deoxygalactose is not a substrate for the glucose phosphotransferase transport systems*

Glucose is taken up into *E. coli* K12 by the glucose phosphotransferase, PtsG, system (Kundig *et al.*, 1964) or by the mannose phosphotransferase, PtsM, system (Curtis & Epstein, 1975; Kornberg & Jones-Mortimer, 1975). The separation of these systems into $PtsG^+$,$PtsM^-$ and $PtsG^-$,$PtsM^+$ derivative mutants, known as JM1101 and JM1103 respectively, has been effected by Jones-Mortimer [Supplementary Publication SUP 50074, available from 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]. These organisms are deficient in both galactose-transport systems.

Radiolabelled 2-deoxygalactose was not significantly accumulated by either of the strains in which the appropriate glucose phosphotransferase had been induced by growth on glucose (Table 4), as evidenced by the high uptakes of glucose, 2-deoxyglucose and methyl glucoside (Table 4).

It can therefore be concluded that 2-deoxygalactose is not a substrate for either of the glucose-transport systems of *E. coli* K12. At the substrate concentration used (0.05 mM), the phosphotransferases clearly discriminate between glucose and galactose, and between 2-deoxyglucose and 2-deoxygalactose (Table 4), so the configuration of the 4-hydroxyl group is a critical substrate feature. This contrasts with the facility of both galactose-transport systems to take up glucose at least as efficiently as galactose (Boos, 1969; Wilson, 1974; Henderson *et al.*, 1977).

Table 4. *Failure of glucose-uptake systems to transport 2-deoxygalactose*

Strains JM1101 (*ptsM*) and JM1103 (*ptsG*) were grown on glucose and sugar transport measured in the usual manner.

Sugar	Uptake (nmol/2 min per mg) JM1101 $PtsG^+$	JM1103 $PtsM^+$
2-Deoxygalactose	0.04 ± 0.01 (6)	0.06 ± 0.04 (6)
2-Deoxyglucose	0.23 ± 0.06 (6)	20.7 ± 0.9 (8)
Galactose	0.30 ± 0.06 (6)	0.37 ± 0.10 (6)
Glucose	15.6 ± 1.0 (8)	6.11 ± 0.23 (5)
Methyl α-glucoside	5.49 ± 0.63 (10)	0.47 ± 0.04 (6)

2-*Deoxygalactose is not a substrate of the active-transport systems for* L-*arabinose or lactose*

It has been reported that galactose and fucose are substrates for the L-arabinose-transport systems as well as for the galactose-transport systems of *E. coli* (Novotny & Englesberg, 1966). This was also observed under our experimental conditions (Table 5). However, the extent of uptake of 2-deoxygalactose was only 2–3% of that of L-arabinose, galactose or fucose into an *E. coli* strain induced for the L-arabinose-transport systems (Table 5). 2-Deoxygalactose also failed to promote effective H^+ uptake into the same organism, confirming that it is not a substrate of the L-arabinose-transport system (results not shown).

Rotman *et al.* (1968) reported that galactose, but not fucose, was a substrate for the lactose-transport system of *E. coli*. With our conditions, galactose uptake was approx. 20% of methyl β-D-thiogalactoside uptake (the latter compound is a good substrate for the lactose-transport system; Cohen & Monod, 1957; Rotman *et al.*, 1968). Fucose or 2-deoxygalactose uptake was about 5% of the methyl β-D-thiogalactoside uptake (Table 6). When H^+ uptake into cells induced for lactose transport was examined as

Table 5. *Failure of* L-*arabinose-uptake systems to transport 2-deoxygalactose*

Strain SB5314 (*araA*) was grown on glycerol and 1 mM-L-arabinose.

Sugar	Uptake (nmol/2 min per mg)
2-Deoxygalactose	0.40 ± 0.14 (4)
6-Deoxygalactose (fucose)	18.5 ± 1.6 (10)
Galactose	13.9 ± 2.2 (5)
L-Arabinose	24.0 ± 1.5 (13)

Table 6. *Relative inability of the lactose-uptake system to transport 2-deoxygalactose*

E. coli K10 (wild-type) was grown on glycerol + 0.5 mM-isopropyl β-D-thiogalactoside to induce the lactose-uptake system.

Sugar	Uptake (nmol/2 min per mg)
2-Deoxygalactose	0.41 ± 0.05 (4)
6-Deoxygalactose (fucose)	0.30 ± 0.01 (6)
Galactose	2.03 ± 0.39 (8)
Methyl β-D-thiogalactoside	8.32 ± 0.86 (13)

Table 7. *Alkaline pH changes elicited when 2-deoxygalactose and related sugars were added to de-energized suspensions of E. coli*

The strains were prepared and effective proton movements measured as described by Henderson *et al.* (1977). Values are means ±S.E.M. of measurements on at least three separate batches of cells.

	Effective H^+ uptake (nmol)		
	K10 *galP*$^+$,*mglP*$^+$	JM1097 *galP*$^+$,*mglP*	S183-27T *galP*^{c},*mglP*
2-Deoxygalactose	84±15 (7)	44±4 (6)	133±14 (4)
6-Deoxygalactose (fucose)	45± 7 (22)	49±5 (10)	67±14 (3)
Galactose	48± 5 (10)	31±4 (9)	73±15 (4)

described by Henderson *et al.* (1975), galactose was about 80% as effective as methyl β-D-thiogalactoside, whereas fucose and 2-deoxygalactose were 5–15% as effective (results not shown).

These results show that 2-deoxygalactose is a very poor substrate for the arabinose- or lactose-transport systems of *E. coli*.

Association of proton movements with 2-deoxygalactose transport

Evidence has been obtained by Henderson *et al.* (1977) that the galactose-transport system, but not the methyl galactoside - transport system, of *E. coli* can effect sugar–H^+ symport, a characteristic predicted for transport systems that are energized by a chemiosmotic mechanism (Mitchell, 1963, 1970, 1973). Therefore, if 2-deoxygalactose is indeed a substrate for the galactose-transport system, it should elicit an alkaline pH change when added to non-respiring suspensions of GalP$^+$ strains of *E. coli*. This prediction was verified with uninduced strain S183-27T (*galP*$^+$,*mglP*) (see Fig. 3) and with the fucose-induced strains JM1097 (*galP*$^+$,*mglP*) and K10 (*galP*$^+$,*mglP*$^+$) (Table 7). Van Thienen *et al.* (1977) have used the same criterion to show that 2-deoxygalactose is a substrate for the *Salmonella typhimurium* GalP system.

There were, however, quantitative differences in the pH changes observed with the different sugars and different strains of *E. coli*. Firstly, although the rates of effective proton uptake were very similar whether galactose, fucose or 2-deoxygalactose was added to the S183 strains (see, e.g., Fig. 3), with strains JM1097 and K10 galactose promoted about twice the rate obtained with the other two sugars (results not shown). This could mean that the galactose-transport system from different organisms has different specificities for individual sugars, a conclusion substantiated by the different K_m values (Fig. 2), by measurements of the energized uptakes of radioisotope-labelled sugars into these strains (R.A. Giddens and P. J. F. Henderson, unpublished work) and by previous reports of the varied specificities of the galactose-transport systems of other strains (Buttin, 1963; Rotman *et al.*, 1968; Wilson, 1974). Secondly, the extent of effective proton uptake was twice as great with 2-deoxygalactose as with the other sugars in strains S183-27T and K10 (Table 7). Thirdly, no such difference in extents was observed with strain JM1097 (Table 7). Two explanations could be offered for the last two observations. The stoicheiometry of the sugar–H^+ movement could be strain- and/or sugar-dependent, or there could be strain-dependent limited internal metabolism of 2-deoxygalactose, which would tend to draw more sugar into the cells. These possibilities require further investigation.

Constitutivity of 2-deoxygalactose transport in certain E. coli strains

Robbins and co-workers (Robbins, 1975; Robbins & Rotman, 1975; Robbins *et al.*, 1976) explored the activity of the methyl galactoside - transport system with the S183 series of mutants impaired in the *mglA*, *mglB*, *mglC* and *mglD* genes (see also Ordal & Adler, 1974). The first three of these genes apparently code for structural components of the methyl galactoside-transport system (Ordal & Adler, 1974; Robbins & Rotman, 1975; Robbins *et al.*, 1976), whereas *mglD* is a regulatory gene, a lesion in which causes constitutivity of the transport system (Robbins, 1975). Under our experimental conditions 2-deoxygalactose uptake occurred at rapid rates into all these strains, regardless of the number or nature of the lesions in the *mgl* region. One example is given in Fig. 1(*b*), where 2-deoxygalactose uptake occurred into an *mglB*,*mglC* mutant unable to accumulate methyl β-galactoside. Thus 2-deoxygalactose transport occurred independently of the presence or absence of individual components of the methyl galactoside - transport system, as expected if it were not a substrate for this system.

Surprisingly, it was not necessary to induce any of these strains with galactose or fucose for the 2-deoxygalactose uptake to occur (Tables 2 and 3). Also, the rates of 2-deoxygalactose uptake into these strains were about 5-fold greater than into other GalP$^+$ strains grown on the same carbon source (glycerol)

and induced with 1 mM-fucose (Table 2); this is also reflected in the higher $V_{max.}$ value deduced from Fig. 2. Consistently, 2-deoxygalactose, galactose and fucose, but not methyl β-galactoside, elicited H^+ uptake into all the strains (Fig. 3) without the necessity for induction; also the rates of H^+ uptake were higher (3–6-fold; results not shown) than the rates in the inducible strains. The uptakes of radioisotope-labelled galactose, fucose and 2-deoxyglucose, the other substrates of the galactose-transport system, were also correspondingly high in the S183 series of organisms, whatever their *mgl* genotype. It is important to note that the galactose-transport system displayed the same high activity in those strains (S183 and S185, Robbins, 1975; Robbins *et al.*, 1976) that were inducible, i.e. *mglD*$^+$, for the methyl galactoside system. Thus the *mglD* gene did not apparently regulate expression of the galactose-transport system.

It may be concluded that the S183 series of organisms is constitutive for the galactose-transport system, possibly because they all have a lesion in the *galR* regulator gene (Wilson, 1974).

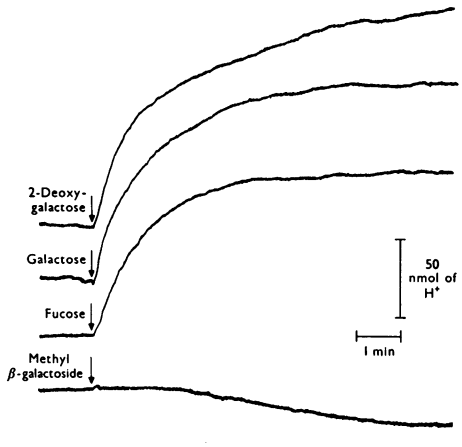

Fig. 3. *pH changes elicited by the addition of sugars to de-energized suspensions of E. coli strain S*183-27*T*
The indicated sugars (10 μmol) were added to anaerobic suspensions of 17 mg of strain S183-27T under the conditions described by Henderson *et al.* (1977). An upward deflexion is an alkaline pH change.

Influence of 2-deoxygalactose on the growth of E. coli and on induction of the galactose-transport systems

When the strains of *E. coli* used in the present paper were incubated with 10 mM-2-deoxygalactose as the only source of carbon, either in liquid culture or on plates, no significant growth occurred. Therefore, like 6-deoxygalactose (fucose), 2-deoxygalactose is not a substrate for growth of *E. coli*.

At least three explanations could account for the failure to grow on 2-deoxygalactose. It might not be a substrate for the relevant metabolic enzymes, probably those for galactose utilization. The 2-deoxygalactose, or a metabolic derivative, might be toxic,

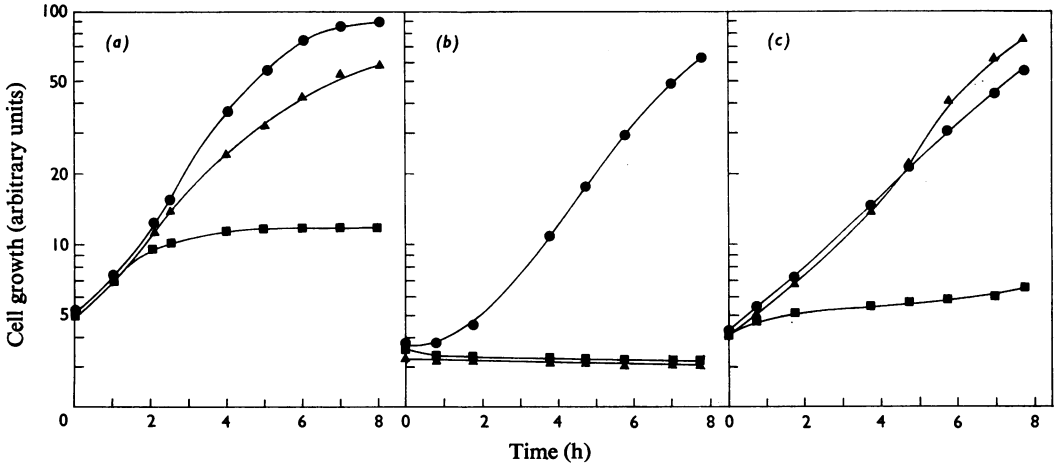

Fig. 4. *Influence of 2-deoxygalactose on the growth of E. coli strain K*10
Inocula were grown overnight in 5 ml of salts medium containing 20 mM-glycerol or 10 mM-galactose. After centrifugation and two washes in salts medium without carbon source, 0.5 ml of the glycerol-grown cells were resuspended with 10 ml of salts medium+glycerol (*a*), whereas galactose-grown cells were resuspended in salts medium+glycerol (*b*) or galactose (*c*). In different tubes 0 (●), 10 (▲) or 500 (■) μmol of 2-deoxygalactose was also present. Growth was measured by nephelometry (see the Experimental section).

or it might not be an inducer of the enzymes required for its utilization. Fucose is known to be a substrate and inducer of the systems for galactose transport, but not a substrate for the enzymes of galactose metabolism, although they are induced by fucose (Buttin, 1963, 1968; Rotman *et al.*, 1968; Wilson, 1974). In contrast with 1 mM-fucose, the effectiveness of 1 mM-2-deoxygalactose as an inducer of the transport systems varied considerably between different strains of *E. coli*. In general, it was more effective with *galK* than with *galK*$^+$ organisms, but the interpretation was complicated by the ability of 2-deoxygalactose to inhibit growth of *E. coli* on glycerol. When an inoculum was grown on glycerol, and then transferred to media containing the same amount of glycerol but different concentrations of 2-deoxygalactose, about 1 mM-2-deoxygalactose produced some inhibition of growth, and progressively higher concentrations were more effective (Fig. 4*a*). However, the inhibition was greatly potentiated when the inoculum was grown on galactose (Fig. 4*b*); this would presumably result from the preinduction by galactose of the uptake system for 2-deoxygalactose. Much higher concentrations (Fig. 4*c*) were required to inhibit subsequent growth on 10 mM-galactose, as would be expected of a competitive interaction between galactose and 2-deoxygalactose. Substitution of identical concentrations of fucose (6-deoxygalactose) for 2-deoxygalactose in these experiments did not cause any impairment of growth (results not shown).

Generally sugar analogues do not inhibit growth of *E. coli* on glycerol unless they accumulate internally as phosphorylated derivative(s) (Fraenkel, 1968). The results above would be consistent with limited metabolism of 2-deoxygalactose to a phosphorylated form by *E. coli*, and would accord with the ability of yeast (Jaspers & Van Steveninck, 1976), renal-cortical cells (Kleinzeller & McAvoy, 1976) and ascites-tumour cells (Smith & Keppler, 1977) to phosphorylate this sugar. Although this may render 2-deoxygalactose less useful than fucose as a gratuitous inducer in *E. coli*, it is proving of value in obtaining resistant mutants that are altered specifically in their ability to utilize galactose.

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