Regulation of Fructose Uptake by Glucose in *Escherichia coli*

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SUMMARY

A mutant, DA1, has been isolated from the *Escherichia coli* K12, strain K2.1t, as a colony resistant to 2-deoxyglucose (DG) when growing on fructose but still sensitive to DG when growing on other sugars. The mutation in DA1 specifically affects the catabolite inhibition of fructose utilization by glucose and glucose-6-phosphate; the affected gene (designated *cif*) is located at min 41 on the *E. coli* linkage map and is highly co-transducible with the genes that specify the uptake of fructose (*ptsF*) and enzymic conversion of fructose-1-phosphate to fructose-1,6-bisphosphate (*fpk*).

INTRODUCTION

The addition of glucose to cultures of *Escherichia coli* growing on a wide variety of other carbon sources rapidly inhibits the utilization of those substrates. This phenomenon, which has been termed 'catabolite inhibition' by McGinnis & Paigen (1969, 1973), is due to interference by glucose with the continued uptake of the other substrates. Non-catabolizable analogues of glucose, such as methyl-α-D-glucoside, and 1- and 2-deoxy-D-glucose, were found to exert a similar inhibitory effect on carbohydrate transport, but only if the organisms had been previously induced for the uptake of such analogues (Kepes, 1960; Kessler & Rickenberg, 1963; Koch, 1964; Boniface & Koch, 1967; Winkler & Wilson, 1967). This suggests that it is either the actual process of translocation of glucose and its analogues into the cells, or elevation of the intracellular concentration of the 6-phosphate esters formed from these materials, that inhibits the uptake of other substrates. In accordance with either explanation, it has been found that, in mutants impaired in glucose-specific components of the phosphotransferase (PT) system (Kundig, Ghosh & Roseman, 1964), glucose and its non-catabolizable analogues do not inhibit the uptake of substances such as galactose (Asensio, Avigad & Horecker, 1963; Kornberg, 1973*a*) and fructose (Kornberg, 1972, 1973*a*).

This paper describes the properties of a mutant of *E. coli* in which specifically the uptake of fructose is much less inhibited by glucose and by its non-catabolizable analogues than it is in the parent organism. The utilization of fructose by the mutant is also less susceptible to inhibition by glucose-6-phosphate. The gene that specifies this altered behaviour is highly co-transducible with those specifying fructose-1-phosphate kinase (*fpk*) and a fructose-specific component of the multicomponent PT-system (*ptsF*) (Jones-Mortimer & Kornberg, 1974). These findings suggest that, in *E. coli*, glucose exerts not only a pleiotropic inhibition on the uptake of many carbohydrates, which is associated with the uptake and phosphorylation of glucose via the PT-system, but can also inhibit

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Table 1. *Strains of E. coli* K12 *used*

Organism	Genetic markers	Mating type	Reference or source
AT2243	*metB ura*	Hfr-C	Gift from A. L. Taylor
AT2243.11^c	*metB ura uhp^c*	Hfr-C	Ferenci & Kornberg (1973)
AT2243.11^c.25	*metB ura uhp^c fpk*	Hfr-C	Ferenci & Kornberg (1973)
KL16	—	Hfr	Gift from B. Low
KL16.21	*ptsF ptsX*	Hfr	Ferenci & Kornberg (1971)
KL16.25	*fpk*	Hfr	Gift from M. C. Jones-Mortimer
KL16.30	*cif*	Hfr	This paper
K2.1t	*his argH thr leu pps str*	F$^-$	Brice & Kornberg (1967)
K2.1t.θ	*his argH thr leu pps thyA str*	F$^-$	Trimethoprim^R derivative of K2.1t
DA1	*his argH thr leu pps cif str*	F$^-$	This paper
DA3	*metB ura uhp^c cif*	Hfr-C	This paper
DA6	*his ura cif str*	F$^-$	This paper
K2.1.22	*his argH thr leu pps umg str*	F$^-$	Kornberg & Smith (1972)
K2.22.25	*argH thr leu fpk umg str*	F$^-$	[AT2243.11^c.25 × K2.1.22]His$^+$ Str^R
K2.1.25	*metB his ura fpk pps str*	F$^-$	[AT2243.11^c.25 × K2.1t^R]Thy$^+$ Str^R
K2.22	*argH thr leu umg str*	F$^-$	This paper
K2.22.30	*argH thr leu cif umg str*	F$^-$	This paper
K2.21.23θ	*argH trp thr leu ptsF ptsX thyA str*	F$^-$	Laboratory stock
K2.21	*argH trp thr leu ptsX str*	F$^-$	This paper
K2.21.30	*argH trp thr leu ptsX cif str*	F$^-$	This paper
K2.21.22.23	*his argH thr leu ptsX umg ptsF str*	F$^-$	[KL16.21 × K2.1.22]Pps$^+$ Str^R
K2.21.22	*his argH thr leu ptsX umg str*	F$^-$	[KL16 × K2.21.22.23]Fru$^+$ Str^R
K2.21.22.30	*his argH thr leu ptsX umg cif str*	F$^-$	[KL16.30 × K2.21.22.23]Fru$^+$ Str^R
0114DFGR-W	*purB umg str*	F$^-$	Kornberg (1972)
2570C	*ptsI-3*	Hfr (KL16 type)	Gift from M. L. Morse

specifically the uptake of at least one sugar – fructose – by reacting (either directly or as glucose-6-phosphate) with a site on the inducible system that effects the uptake of fructose.

METHODS

Organisms. These were all derivatives of the K12 strain of *E. coli*. Their relevant geno-types, and the sources from which they were derived, are listed in Table 1. The abbreviations used to describe genetical markers are defined in the text, if not among those listed by Taylor & Trotter (1972).

Organisms were grown at 37 °C in media containing salts (Ashworth & Kornberg, 1966), supplemented as appropriate with the required amino acids or pyrimidines to 50 to 100 μg/ml. The genetical methods employed were those compiled by Miller (1972).

Selection of cif mutants. Samples (0·05 to 0·2 ml) of cultures of *E. coli* strain K2.1t that had grown overnight on medium containing 10 mM-fructose and the required amino acids were spread on plates containing this medium supplemented with 10 mM-2-deoxy-D-glucose and solidified with 1·5 % (w/v) agar. The plates were incubated at 37 °C for 2 to 3 days. Colonies that grew up during that time were picked on to similar plates and grown over-night; they were replica-plated on to 'Oxoid' nutrient agar supplemented with 10 μM-methyl-α-D-[^{14}C]glucoside and again incubated at 37 °C until good growth had been achieved (15 to 20 h). The colonies were then transferred to Whatman No. 1 filter paper, by pressing a slightly-trimmed 9 cm disc of that paper directly on to the surface of the agar and peeling it back gently; they were air-dried and left in contact with Kodak 'Blue Brand' X-ray film for 24 h. Mutants that had acquired their tolerance to 2-deoxy-glucose by loss of the quantitatively predominant Enzyme II for the uptake of glucose and its analogues (specified by the *umg* gene; Kornberg & Smith, 1972) did not blacken

the film; these were discarded. 2-Deoxyglucose-tolerant mutants that blackened the film (and were presumably not greatly impaired in glucose uptake) were tested further by transfer from the (fructose + 2-deoxy-D-glucose) media to plates containing 20 mM-glycerol and 10 mM-2-deoxy-D-glucose. One colony that grew on the former but not the latter medium, designated DAI, was purified by repeated isolation of single colonies and was used in this work.

Uptake of labelled substrates. The uptake of radioactively-labelled substrates by cultures of bacteria growing on them was measured as previously described (Kornberg, 1972, 1973*a*). Samples (0·5 ml) of the cultures growing at 37 °C in liquid media were filtered through Millipore filters (0·45 μm pore size); the filters were washed with nitrogen-free medium (Ashworth & Kornberg, 1966) at 25 °C and dissolved in 5 ml of Bray's (1960) fluid in a glass scintillation vial. Samples (0·5 ml) were also filtered through smaller Millipore filters attached to disposable 1 ml syringes: portions (0·1 ml) of the filtrates were added to 5 ml of Bray's (1960) fluid in a glass scintillation vial. The radioactivity in the vials was measured with a Packard 'Tri-carb' model 3385 liquid scintillation spectrometer, a total of at least 5000 counts being recorded for each vial.

The uptake of labelled substrates by washed suspensions of bacteria was measured as previously described (Morgan & Kornberg, 1969; Ferenci & Kornberg, 1974).

[14]C-labelled substrates were purchased from the Radiochemical Centre, Amersham, Buckinghamshire. Other chemicals were of the highest purity readily available commercially.

RESULTS AND DISCUSSION
Effect of glucose and its analogues on the utilization of other sugars

Non-catabolizable analogues of glucose, such as 2-deoxy-D-glucose, methyl-α-D-glucoside, or 3-deoxy-3-fluoro-D-glucose are known to inhibit the utilization, by *E. coli*, of a variety of carbon sources (McGinnis & Paigen, 1969, 1973; Kornberg & Smith, 1972; Kornberg, 1972, 1973*a, b*; Miles & Pirt, 1973). In consequence, there is a rapid cessation of growth after 2 mM-2-deoxy-D-glucose is added to cultures of *E. coli*, such as the strain K2.1t (Fig. 1), growing on 5 mM-lactose, 10 mM-fructose, or 20 mM-glycerol as sole carbon source. This effect was used to select mutants that continue to grow in the presence of the toxic glucose analogues: in many instances, such mutants owe their tolerance to an impaired ability to take up these glucose derivatives and the glucose they mimic. Such mutants, which show a marked increase in their doubling time on media containing glucose as sole carbon source, and an almost total loss of methyl-α-D-glucoside transport, are designated Umg⁻ (from uptake of methyl glucoside). The *umg* gene specifying this character, ascribed to an Enzyme II of the PEP-phosphotransferase system (Kundig *et al.* 1964) specific for glucose and methyl-α-glucoside, has been located at 25 min on the *E. coli* genome (Kornberg & Smith, 1972). As expected from this interpretation, *umg* mutants of *E. coli* are not inhibited by methyl-α-glucoside, or by other glucose analogues such as 2-deoxy-D-glucose and 3-deoxy-3-fluoro-D-glucose, in their growth on any carbon source (other than galactose, which is a special case; H. L. Kornberg and C. L. Riordan, unpublished).

Although the mutant DAI of strain K2.1t was selected as able to grow on fructose in the presence of 2-deoxy-D-glucose, it differed in many properties from *umg* mutants. For example, although the growth of strain DAI on 10 mM-fructose was only slightly retarded by the addition of 2 mM-2-deoxy-D-glucose, 0·5 mM-methyl-α-D-glucoside, or 0·2 mM-3-deoxy-3-fluoro-D-glucose, growth was still rapidly arrested by these agents when lactose or glycerol was the carbon source (Fig. 2). Moreover, unlike *umg* mutants, the mutant
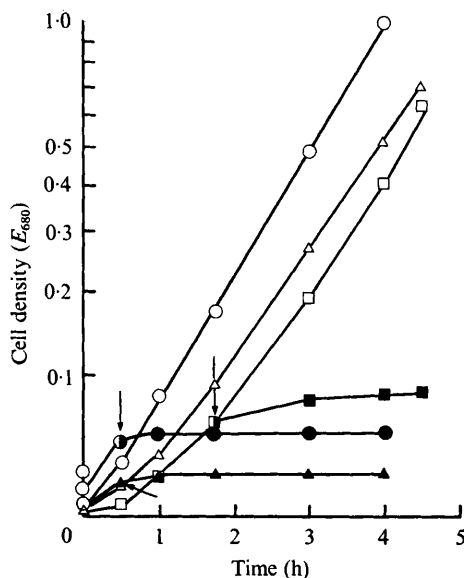
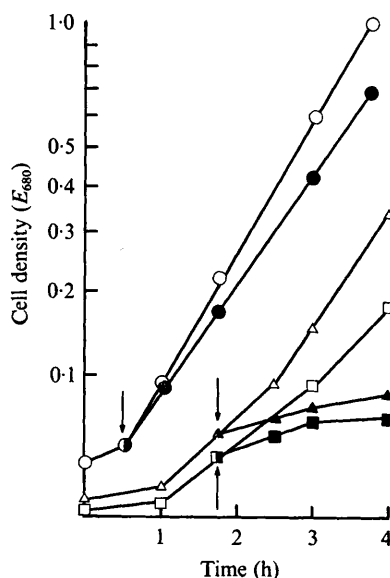Fig. 1   Fig. 2

Fig. 1. Effect of 2 mM-2-deoxy-D-glucose on the growth of *E. coli* strain K2.1t on 5 mM-lactose (□, ■), 10 mM-fructose (○, ●), and 20 mM-glycerol △, ▲). Duplicate flasks of cultures growing on these carbon sources were shaken at 37 °C; 2-deoxy-D-glucose was added to one of each pair (solid symbols) at the times indicated by arrows.

Fig. 2. Effect of 2 mM-2-deoxy-D-glucose on the growth of the *E. coli* mutant DA1 on 5 mM-lactose (□, ■), 10 mM-fructose (○, ●) and 20 mM-glycerol (△, ▲). Duplicate flasks of cultures growing on these carbon sources were shaken at 37 °C; 2-deoxy-D-glucose was added to one of each pair (solid symbols) at the times indicated by arrows.

DA1 grew at wild-type rates on medium containing glucose as sole carbon source, and when grown in the presence of methyl-α-D-[^{14}C]glucoside it readily took up this labelled material. Thus the ability to take up glucose and its analogues had not become generally impaired, which suggests that DA1 differs from its wild-type parent specifically in the manner in which glucose affects the uptake of fructose. This is supported by measurements of the rates of utilization of glucose by cultures growing on this hexose in the presence of other carbohydrates.

When fructose-grown cultures of strain K2.1t were placed in media containing either 5 mM-[^{14}C]fructose + 5 mM-unlabelled glucose, or 5 mM-unlabelled fructose + 5 mM-[^{14}C]-glucose, over 85 % of the total carbon incorporated during the growth of the organism on these sugars was derived from glucose, although the fructose-grown cells had not previously been exposed to glucose. In contrast, *umg* mutants derive less than 25 % of their total carbon from glucose when grown upon this mixture (Kornberg, 1972, 1973*b*; Kornberg & Jones-Mortimer, 1975). The behaviour of strain DA1 is intermediate between these extremes: as with *umg* mutants, glucose was no longer utilized preferentially to fructose but over 35 % of the total carbon incorporated from the mixture of the two hexoses was derived from glucose (Fig. 3). When cells grown on the non-PT sugars glycerol or lactose, or on the PT sugar mannose, were allowed to grow on similar mixtures of glucose and the respective sugar, there was no significant difference between the behaviour of strain K2.1t and its mutant DA1: in both, glucose virtually abolished the utilization of the sugar on
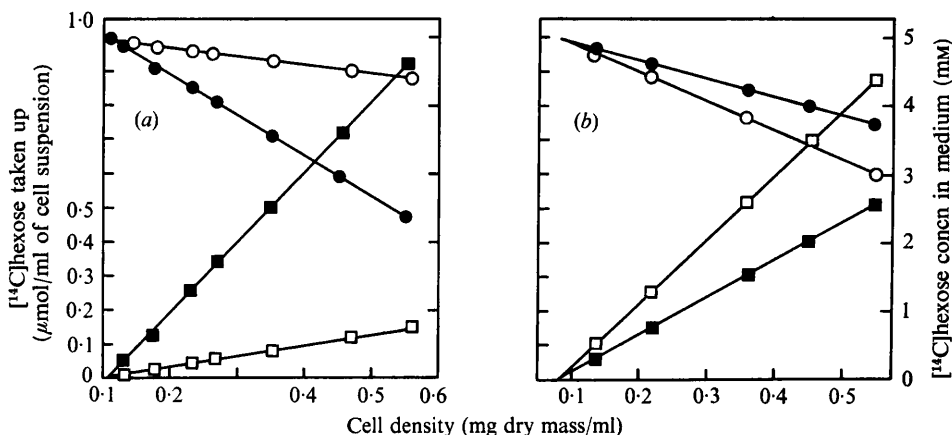
Fig. 3. Utilization of fructose and glucose by (*a*) *E. coli* strain K2.1t, and (*b*) its mutant DAI, growing on mixtures of these sugars. Fructose-grown cells continued to grow on media containing 5 mM-[^{14}C]fructose + 5 mM-unlabelled glucose, or 5 mM-unlabelled fructose + 5 mM-[^{14}C]glucose as carbon source. The incorporation of ^{14}C derived from fructose (□) or from glucose (■) into the growing cells, and the removal of labelled fructose (○) and labelled glucose (●) from the media, were measured as described in Methods.
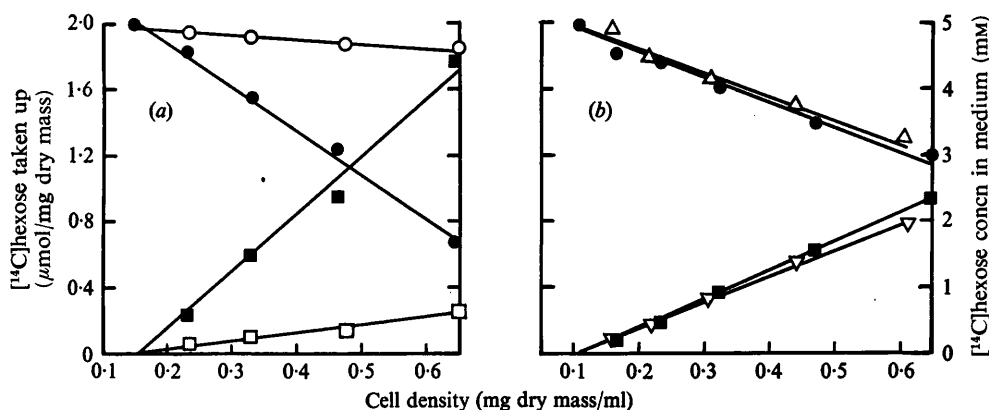

Fig. 4. Utilization of (*a*) mannitol and glucose, and (*b*) fructose and glucose, by a Mtl$^+$ revertant of strain DAI. Mannitol-grown cells continued to grow on media containing either (*a*) 5 mM-[^{14}C]mannitol + 5 mM-glucose or 5 mM-mannitol + 5 mM-[^{14}C]glucose, or (*b*) 5 mM-[^{14}C]fructose + 5 mM-glucose or 5 mM-fructose + 5 mM-[^{14}C]glucose, as carbon sources. (*a*) The incorporation into cell materials (□, ■), and removal from the medium (○, ●), of mannitol (open symbols) and glucose (closed symbols). (*b*) The incorporation into cell materials (▽, ■), and removal from the medium (△, ●), of fructose (open symbols) and glucose (closed symbols).

which the inocula had grown. A similar experiment was done with an Mtl$^+$ revertant of strain DAI. As shown in Fig. 4, glucose strongly inhibited the utilization of mannitol but had much less effect on the utilization of fructose.

That this altered behaviour was associated with the uptake of fructose and glucose, and not with some metabolic step subsequent to the entry of these hexoses into *E. coli*, is shown in Table 2. The wild-type strain K2.1t and its mutant DAI took up [^{14}C]glucose and [^{14}C]fructose at similar rates, which confirmed that strain DAI had not suffered any gross change in the transport of either sugar. However, whereas the presence of an excess

Table 2. *Uptake of [^{14}C]glucose and [^{14}C]fructose by washed cell*
suspensions of fructose-grown E. coli

Cells were harvested from the mid-exponential growth phase and suspended at 1·4 mg dry mass/ml in nitrogen-free basal salts (Ashworth & Kornberg, 1966). To portions (1 ml) of these suspensions, shaken in a water bath at 25 °C, were added water and substrates to a final volume of 2 ml. Samples (0·2 ml) were removed 30, 60, 90 and 120 s after addition of [^{14}C]-labelled material; these were filtered and their radioactivity was assayed as described in Methods.

	Rates of uptake (nmol ^{14}C/min/mg dry mass)			
Substrates	K2.1t (*cif*+)	DAI (*cif*−)	K2.21.22 (*cif*+ *ptsX umg*)	K2.21.22.30 (*cif*− *ptsX umg*)
[^{14}C]glucose (0·2 mM)	31	29	2	1
[^{14}C]fructose (0·2 mM)	13	12	10	11
[^{14}C]fructose (0·2 mM) + unlabelled glucose (1 mM)	5·5	10	4	12

of unlabelled glucose reduced by over 50 % the rate at which strain K2.1t took up [^{14}C]-fructose, it had only a slight effect on this process in strain DAI.

These results suggest that the loss of preference for glucose by the mutant DAI, which was indicated by its tolerance of usually toxic analogues (Fig. 2), by the relative rates of hexose utilization during growth on mixtures of glucose and fructose (Figs. 3 and 4), and by its behaviour in washed suspensions (Table 2), was due to an alteration in the manner in which glucose entered the cells and was associated specifically with the simultaneous uptake of fructose; no such departure from wild-type behaviour was evident when other sugars, whether taken up by the PT-system or not, were also being utilized.

Location of the gene specifying the loss of catabolite inhibition exerted by glucose on the utilization of fructose (*cif*)

The finding that, in contrast to wild-type strains, the mutant DAI took up and incorporated labelled fructose well although unlabelled glucose was also present, formed the basis of a method for detecting the presence of this lesion in recombinants and transductants derived from strain DAI. Three types of transductant were selected after infection of suitable recipients with phage P1 propagated on the mutant DAI.

(i) Since the mutant DAI was tolerant of methyl-α-D-glucoside (as well as of other glucose analogues) during growth on fructose, it was possible that the lesion was associated with the uptake system for this analogue (*umg*), particularly since this marker is known to be associated with a regulatory component of the PEP-phosphotransferase system that effects glucose utilization (Kornberg & Smith, 1972) and has been implicated in glucose-mediated catabolite repression (Tyler *et al.* 1969; Curtis & Epstein, 1970; Bourd *et al.* 1974). Accordingly, the phage was used to infect the Umg⁻ strain 0114DFGR-W which also carried the *purB* allele co-transducible with *umg*; PurB⁺ Umg⁺ transductants were selected as colonies that grew on glucose in the absence of adenine. However, none of the transductants thus obtained and tested had the DAI phenotype.

(ii) The possibility that the lesion in mutant DAI was associated with that region of the *E. coli* genome in which are located the genes specifying the Enzyme I and the HPr components of the PEP-phosphotransferase system (Wang, Morse & Morse, 1969; Epstein, Jewett & Fox, 1970) was tested by infecting the *ptsI* mutant 2570C with phage P1 propagated on mutant DAI, and selecting Pts⁺ transductants as colonies that grew on mannitol. None of the transductants thus obtained and tested had the DAI phenotype.
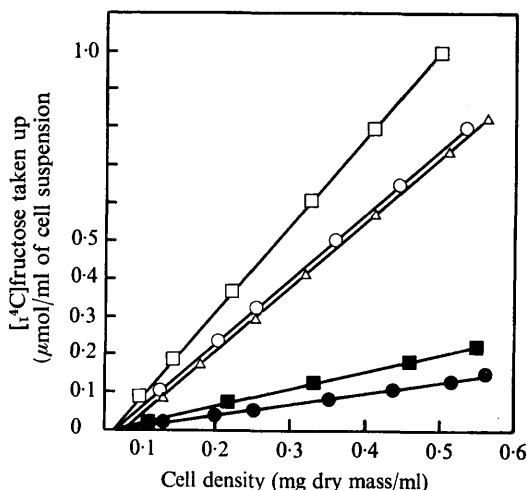
Fig. 5. Incorporation of carbon from fructose by strains of *E. coli* growing on a mixture of 5 mM-[¹⁴C]fructose + 5 mM-unlabelled glucose as carbon source. ■, Strain AT2243.11ᶜ (parent of AT2243.11ᶜ.25); □, its derivative DA3. ●, Strain K2.1t (parent of K2.1.25); △, its mutant DA1; ○, its derivative DA6.

(iii) Since the lesion in strain DA1 became apparent only under conditions in which fructose was also being utilized, it was conceivable that it was part of, or closely associated with, the high-affinity uptake system for fructose inducibly formed by *E. coli* (Fraenkel, 1968; Ferenci & Kornberg, 1971, 1974). The gene specifying this system, *ptsF*, is co-transducible with the *fpk* marker for fructose-1-phosphate kinase (Jones-Mortimer & Kornberg, 1974). Since *fpk* mutants do not grow on low concentrations of fructose (Ferenci & Kornberg, 1973) although they may give rise to phenotypic revertants at higher fructose concentrations (Ferenci & Kornberg, 1974), transductants were selected that grew rapidly on media containing 3 mM-fructose as sole carbon source: such transductants must have received the *fpk*⁺ allele, and almost certainly also the *ptsF*⁺ allele, of the phage donor strain DA1. When such transductants were selected after infection of the *fpk* strain AT2243.11ᶜ.25, it was found that all the *fpk*⁺ transductants tested had acquired the phenotype of strain DA1, in so far as labelled fructose was taken up readily despite the presence of unlabelled glucose. The behaviour of one such transductant (DA3) is shown in Fig. 5. When the experiment was repeated but the phage recipient was the *fpk* strain K2.1.25 which, like the mutant DA1, had been derived from strain K2.1t, it was again found that all the *fpk*⁺ transductants tested had the phenotype of the mutant DA1. The behaviour of one such (DA6), of the donor strain DA1, and of the grandparent strains AT2243.11ᶜ and K2.1t, are also shown in Fig. 5.

It is evident from these results that the altered phenotype of the mutant DA1 is attributable to a change in an amount of genetic material so small that it can be transferred via phage P1. Hence it is probably a change in one gene, *cif*, which is highly co-transducible with the genes specifying the uptake of fructose into the cell (*ptsF*⁺) and the first metabolic step taken by the fructose-1-phosphate that thus appears inside the cell (*fpk*⁺). Since the character specified by the *cif* marker affects both the simultaneous utilization of fructose and glucose in the presence of each other by growing cells, and the uptake of these hexoses by washed cell suspensions, it is likely that the Cif⁻ phenotype, exhibited by the mutant
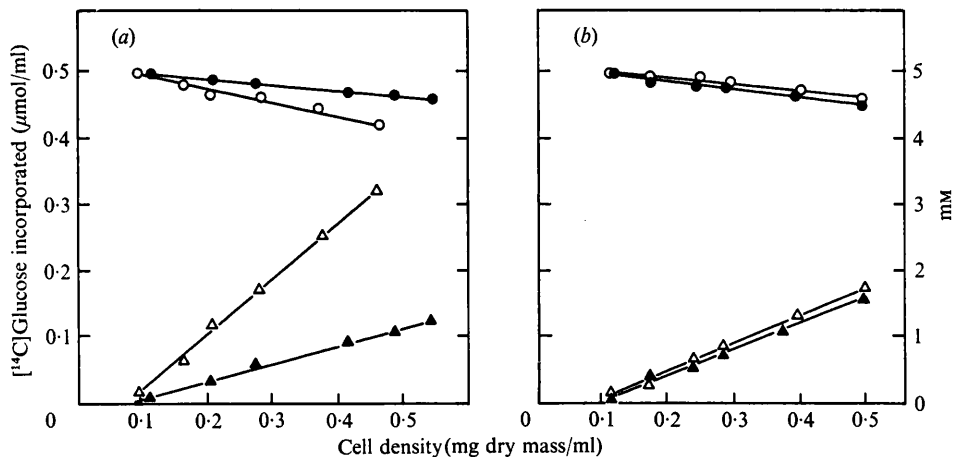
Fig. 6. Incorporation of [^{14}C]glucose by cif^+ ($\triangle$) and cif^- ($\blacktriangle$) strains of *E. coli* that lack Umg activity. (*a*) Cells growing on 5 mM-fructose + 5 mM-[^{14}C]glucose; (*b*) cells growing on 5 mM-mannitol + 5 mM-[^{14}C]glucose. The removal of [^{14}C]glucose from the growth media by cif^+ ($\bigcirc$) and cif^- ($\bullet$), cells is indicated.

DAI and the transductants derived from it, denotes an alteration in a glucose-specific component of the fructose-uptake system specified by $ptsF^+$.

A third route of entry of glucose into E. coli

There are two major routes for the entry of glucose into the K2 strain of *E. coli*. The wild-type organism, and the *pps* mutant K2.1t derived from it, double every hour when shaken at 37 °C in media containing basal salts and 10 mM-glucose. Mutants lacking the Enzyme II component of the PT-system that specifies the uptake of methyl-α-D-glucose (*umg*) still grow on glucose but take about 3 h to double; the rate at which such cells take up labelled glucose during growth on glycerol plus [^{14}C]glucose is 27 % of the rate at which the *umg*$^+$ strain K2.1t incorporates that isotope under these conditions (Kornberg & Jones-Mortimer, 1975). This lower rate of glucose utilization is reduced still further by a mutation *ptsX*, that also affects the utilization of glucosamine and mannose (Epstein & Curtis, 1972; W. Epstein, personal communication) and the utilization of fructose at high concentrations (Ferenci & Kornberg, 1974; Jones-Mortimer & Kornberg, 1974). Derivatives of strain K2.1t that lack both the activities specified by *umg* and *ptsX* require over 18 h to double on glucose growth medium, and, during growth on glycerol plus [^{14}C]glucose, incorporate [^{14}C]glucose at only 4 % of the rate observed with wild-type cells (Kornberg & Jones-Mortimer, 1975).

Since means were available for the genetic transfer of the *cif* marker to suitable recipients, the possibility could be tested that some glucose, not taken up via the Umg and PtsX systems, might enter cells via the fructose uptake system(s). Transduction of the *cif* marker from the mutant DAI into the Hfr strain KL16.25 (*fpk*) gave a strain KL16.30 (*fpk*$^+$ *cif*) that transferred its genome in the direction *O–thy-[cif, fpk, ptsF]-his-ptsX-*... and was thus well suited to the construction of appropriate recombinants by interrupted conjugation. When this donor strain was crossed with the recipient K2.21.23θ (F$^-$, *ptsF ptsX thyA str*), recombinants (K2.21.30) could be readily obtained after 15 min incubation that grew on 3 mM-fructose (*ptsF*$^+$) in the absence of thymine (*thy*$^+$) but did not grow readily on either

glucosamine or mannose (*ptsX⁻*). Similar recombinants (K2.21) were obtained from a cross of the grandparent Hfr strain KL16 (*cif⁺*) with K2.21.23θ. Both the *cif⁺ ptsX⁻* and *cif⁻ ptsX⁻* strains grew readily on mixtures of fructose and glucose. When one of these hexoses was supplied labelled with ¹⁴C in the presence of the other, which was unlabelled, the results obtained did not differ significantly from those obtained with strains K2.1t and DAI: the absence of the PtsX system had thus not affected the expression of the Cif character.

A similar conclusion can be drawn from experiments with Umg⁻ strains. Such strains, that were *ptsX⁺*, were readily constructed by transduction of the *fpk⁺* allele from either donor strain K2.1t (*cif⁺*) or DAI (*cif⁻*) into a recipient K2.22.25 (*umg fpk*). As expected, these Umg⁻ strains took up much less [¹⁴C]glucose than did wild-type strains when growing on this hexose in the presence of another, unlabelled, substrate (Fig. 6). No difference was observed in the amounts of [¹⁴C]glucose taken up by the *cif⁺* and the *cif⁻* strains when mannitol was that unlabelled substrate, or (not shown in Fig. 6) when glycerol or lactose were the unlabelled substrates. However, in the presence of fructose, the *cif⁺ umg⁻* strain incorporated 0·8 μmol glucose/increase in cell mass of 1 mg, whereas the *cif⁻ umg⁻* transductant took up only 0·26 μmol under these conditions. This suggested that the induction of the PtsF system for fructose uptake had opened up a route (albeit a quantitatively minor one) for the uptake of glucose in *cif⁺* organisms, but this did not occur in *cif⁻* cells.

Qualitatively similar results were obtained with mutants in which both the PtsX and the Umg systems for glucose uptake were impaired. During growth on 5 mM-fructose + 2·5 mM-[¹⁴C]glucose, the *cif⁺ ptsX umg* mutant K2.21.22 incorporated 0·31 μmol isotope/increase in cell mass of 1 mg, whereas during growth on 10 mM-glycerol + 2·5 mM-[¹⁴C]-glucose only 0·18 μmol ¹⁴C was incorporated. In contrast, the otherwise isogenic but *cif⁻* mutant K2.21.22.30 incorporated 0·2 μmol [¹⁴C]glucose during growth on this labelled hexose with either fructose or glycerol, unlabelled, as the other carbon source. This difference in the uptake of [¹⁴C]glucose is shown also by fructose-grown suspensions of the *cif⁺* and *cif⁻* mutants (Table 2). The *cif⁺* mutant took up glucose at twice the rate of the *cif⁻* cells; unlabelled glucose competed for entry with [¹⁴C]fructose in the former but not the latter cells. Since no differences were apparent in the rates of [¹⁴C]fructose uptake *per se*, it is likely that these differences reflect the ability of the fructose-specific Enzyme II, specified by *ptsF*, to react also with, and to a minor extent translocate, glucose.

Inducer exclusion

Apart from the inhibition that glucose exerts over the continued utilization of fructose by cells grown on fructose, and which is largely overcome by the *cif* mutation, glucose is known to inhibit the induction of enzymes of fructose utilization by cells exposed simultaneously to both these hexoses. The results illustrated in Fig. 7, obtained with the otherwise isogenic *ptsX* strains K2.21 (*cif⁺*) and K2.21.30 (*cif⁻*), show that the change to *cif⁻* overcomes this effect of glucose also. They strengthen the view (Adhya & Echols, 1966; Lengeler, 1966) that the failure to induce the enzymes of fructose utilization if glucose is also present is due, at least in part, to the inability of fructose sufficiently to enter the cells under these conditions.

The mechanism of glucose–fructose interaction

The observation (Kornberg, 1972) that the change from *umg⁺* to *umg⁻* also abolished the utilization of glucose preferentially to fructose indicates that in wild-type organisms, either glucose and fructose compete for some common component of the PEP-phospho-
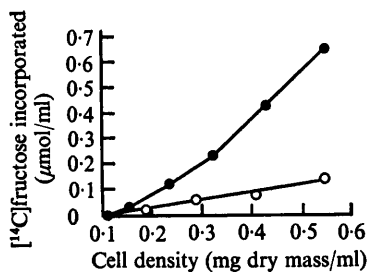
Fig. 7. Incorporation of [^{14}C]fructose by glucose-grown cif^+ (○) and cif^- (●) cells continuing to grow on 5 mM-glucose + 5 mM-[^{14}C]fructose as carbon sources.
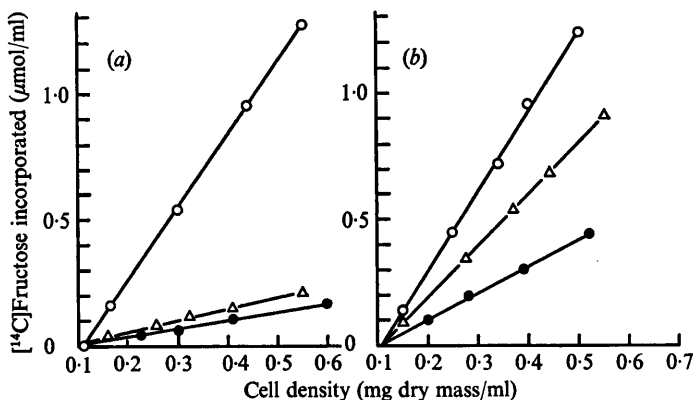

Fig. 8. Effect of glucose and glucose-6-phosphate on the incorporation of [^{14}C]fructose by growing cif^+ and cif^- cells. (a) The incorporation of [^{14}C]fructose by cif^+ cells growing on (○) 5 mM-fructose alone, or in the presence of (△) 5 mM-glucose or (●) 5 mM-glucose-6-phosphate. (b) The data obtained with cif^- cells under the same conditions.

transferase system, or the inhibition of uptake of fructose is exerted not by glucose itself but by the glucose-6-phosphate formed by the action of the Umg system. There are several lines of evidence that favour this latter explanation. For example, metabolic mutations that restrict the catabolism of glucose-6-phosphate (such as impairment of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase activities) increase the degree of inhibition of fructose uptake exerted by glucose, and glucose-6-phosphate inhibits the utilization of fructose by *umg* mutants in which glucose is no longer effective (Kornberg, 1972). Furthermore, the accumulation of methyl-α-D-glucoside-6-phosphate by fructose-grown wild-type cells profoundly inhibits their ability to take up [^{14}C]fructose, but this ability is regained in full when the accumulated phosphate ester is washed out of the cells (Kornberg, 1973a).

Since the inhibitory effect of glucose on fructose uptake was largely overcome by mutation at the locus specified by *cif*, despite the unimpaired activity of the Umg system, it is possible that it is not glucose *per se* but glucose-6-phosphate that interacts with fructose (or fructose-1-phosphate). This conclusion receives some support from the experiment shown in Fig. 8. The incorporation of [^{14}C]fructose by *cif ptsX* cells growing on 5 mM-fructose (3·2 μmol/increase in cell mass of 1 mg) is not significantly different from that of *cif$^+$ ptsX* cells under the same circumstances (2·9 μmol/increase in cell mass of 1 mg). The presence of 5 mM-unlabelled glucose reduced this rate of fructose utilization

to 0·5 μmol/mg increase in *cif*$^+$ cells, but by only a small amount (to 2 μmol/mg increase) in *cif*$^-$ cells. Unlabelled glucose-6-phosphate produced an even greater effect in *cif*$^+$ cells: fructose utilization was reduced to 0·35 μmol/mg increase, which is only 12 % of the rate observed during growth on fructose as sole carbon source. However, although the utilization of fructose by the *cif*$^-$ mutant was also affected by the presence of unlabelled glucose-6-phosphate, the rate of [^{14}C]fructose incorporation observed (1·04 μmol/mg increase) was about three times greater than that observed with *cif*$^+$ cells. The simultaneous (albeit only partial) relief that the *cif*$^-$ mutation produces on the inhibition of fructose utilization by glucose and glucose-6-phosphate emphasizes the interrelationship of these two events. Since glucose-6-phosphate is known also to inhibit fructose-1-phosphate kinase activity, the effect of the high concentrations of glucose-6-phosphate added to the cells, which is illustrated in Fig. 8, may be exaggerated in this experiment: elucidation of its mechanism will require study with the purified membrane proteins specified by the *cif* alleles.

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