Journal of General Microbiology (1976), **96**, 383–391
Printed in Great Britain

Uptake of Fructose by the Sorbitol Phosphotransferase of *Escherichia coli* K12

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(*Received* 13 *May* 1976)

SUMMARY

Strains of *Escherichia coli* that are unable to grow on fructose because they lack the phosphoenolpyruvate:fructose phosphotransferases specified by *ptsF* and *ptsX* mutate to grow on media containing fructose as sole carbon source, but do not regain the function of either of the missing phosphotransferases. Instead, fructose is taken up and phosphorylated to fructose 6-phosphate by a phosphoenolpyruvate: sorbitol phosphotransferase which, in wild-type cells, is induced by sorbitol but not by fructose, but which is constitutively expressed in these mutants. The regulatory gene *srlC* controlling enzymes of sorbitol uptake and catabolism has been located on the *E. coli* genome as part of the linkage group *cysI srlC att186 pheA*.

INTRODUCTION

Fructose normally enters *Escherichia coli* by two distinct routes, A and B in Fig. 1 (Ferenci & Kornberg, 1974; Jones-Mortimer & Kornberg, 1974). Both routes require the activity of the phosphotransferase system (Kundig, Ghosh & Roseman, 1964), in which the phosphate group of phosphoenolpyruvate is enzymically transferred, initially to a small histidine-containing protein HPr. The phospho-HPr thus formed can then donate that phosphate group to fructose in one of two ways. In an enzymic reaction characterized by its high affinity for fructose ($K_m < 10^{-4}$ M), fructose 1-phosphate is formed (Fraenkel, 1968; Ferenci & Kornberg, 1971); the gene specifying this enzyme, designated *ptsF*, has been located on the *E. coli* linkage map at minute 41·5 (Jones-Mortimer & Kornberg, 1974). On the other hand, mutants that lack this high-affinity system for the uptake and phosphorylation of fructose can still grow on fructose, albeit in a manner strongly dependent on the concentration of the sugar present in the growth medium. Under these conditions, fructose 6-phosphate arises from the transfer of phosphate from phospho-HPr to fructose (Ferenci & Kornberg, 1974). This system, characterized by its low affinity for fructose ($K_m > 10^{-3}$ M), is specified by a gene, designated *ptsX*, that has been located on the *E. coli* linkage map at minute 36·5 (Jones-Mortimer & Kornberg, 1974, 1976). It is likely that the normal physiological role of this system is to participate in the uptake of glucose, glucosamine and mannose by *E. coli* (Curtis & Epstein, 1975; Kornberg & Jones-Mortimer, 1974) rather than in the utilization of fructose. However, under conditions of batch culture of *E. coli*, where growth substrates are usually supplied at 5 to 10 mM, mutants that lack the high-affinity uptake system for fructose grow readily on fructose by taking up this sugar via the PtsX system. Mutants that lack only this system (*ptsF*$^+$ *ptsX*) grow on fructose in a manner indistinguishable from that of wild-type cells; mutants that lack both systems (*ptsF ptsX*) do not grow even on high concentrations of fructose, and do not take up this sugar when supplied at either 0·1 or 5 mM (Kornberg, 1972).

Table 1. *Escherichia coli strains used in this work*

Strain no.	Genetic markers	Reference and source
KL16.21	Hfr *ptsF1 ptsX1 kdgR*^{c}	Ferenci & Kornberg (1971)
JM463	F$^-$ *fpk galT lac pro metB cysI his str uhp*^{c}	Jones-Mortimer & Kornberg (1974)
JM474	F$^-$ *ptsX1 kdgR*^{c} *fpk galT lac pro metB cysI str uhp*^{c}	KL16.21 × JM463 → His$^+$
JM477	F$^-$ *ptsF1 gnd kga leu str*	Jones-Mortimer & Kornberg (1974)
JM485	Hfr *ptsF1 ptsX1 kdgR*^{c} *srlC1*	KL16.21 (fructose positive)
JM486	F$^-$ *ptsF2 ptsX1 kdgR*^{c} *fpk galT lac pro metB cysI str uhp*^{c}	JM474 (fructose resistant on glycerol)
JM733	Hfr *fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$	Jones-Mortimer & Kornberg (1976)
JM737	Hfr *ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$	JM733 (glucosamine resistant on glycerol); Jones-Mortimer & Kornberg (1976)
JM739	F$^-$ *ptsF1 ptsX2 gnd leu str*	P1.JM737 × JM477 → Kga$^+$
JM759	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$	JM737 (fructose resistant on glycerol)
JM760	F$^-$ *ptsF2 ptsX1 kdgR*^{c} *fpk galT lac srlC2 pro metB cysI str*	JM486 (fructose positive)
JM763	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$ *srlA*	JM759 (sorbitol resistant on glycerol); Jones-Mortimer & Kornberg (1976)
JM781	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$ *srlC3*	JM759 (fructose positive)
JM784	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$ *srlC3 srlA*	JM781 (sorbitol resistant on glycerol)
JM794	F$^-$ *ptsF2 ptsX1 kdgR*^{c} *fpk galT lac pro metB cysI srlC4 str*	JM486 (fructose positive)
JM795	F$^-$ *ptsF2 ptsX1 kdgR*^{c} *fpk galT lac pro metB cysI srlC5 str*	JM486 (fructose positive)
JM803	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$ *srlC3 ptsI*	JM781 (*N*-acetylglucosamine and mannitol resistant on lactate)
JM1058	Hfr *ptsF3 ptsX2 fda*^{ts} *hisgnd*$^\Delta$ *galbio*$^\Delta$ *mtlA*	JM759 (mannitol resistant on glycerol)
JM1069	Hfr *pheA* (186)	186 lysogen of KA197 (Hoekstra *et al.*, 1974)
JM1072	F$^-$ *ptsF2 ptsX1 kdgR*^{c} *fpk galT lac pro metB cysI srlC4 str* 186^R	JM794 (186 resistant)

The main purpose of this paper is to describe the properties of derivatives of *ptsF ptsX* strains, readily obtained by selecting phenotypic revertants to growth on 20 mM-fructose, that grow on this sugar because they effect its uptake and phosphorylation through a phosphotransferase normally used for sorbitol transport which, in these mutants, has become constitutive.

METHODS

The *E. coli* strains used in this work are listed in Table 1: their isolation or construction is indicated in Table 1 or described in the text.

Organisms were grown on media and under conditions previously described (Ashworth & Kornberg, 1966; Ferenci & Kornberg, 1974). The genetical methods used were those compiled by Miller (1972).

The uptake of ^{14}C-labelled substrates was measured as described by Ferenci & Kornberg (1974). Sorbitol-6-phosphate dehydrogenase (EC. 1.1.1.140) was assayed by the method of Horwitz (1966).

D-Glucosaminitol borate ester was prepared by sodium borohydride reduction of D-glucosamine.

RESULTS AND DISCUSSION

Evidence that the fructose-positive derivatives of strain KL16.21 *are not revertants of ptsX or ptsF*

From the Hfr strain KL16.21 (*ptsF ptsX kdgR*^{c}) that does not grow on 20 mM-fructose, numerous colonies that grew on plates containing 20 mM-fructose as sole carbon source were isolated. Of these, one (designated JM485), and four other independent isolates of the same phenotype, were mated with the F$^-$ strain JM477 (*ptsF ptsX*$^+$ *kga str*); Kga$^+$ recombinants were isolated on plates containing gluconate as sole carbon source, the donor organism being counter-selected by inclusion of streptomycin. Each of the crosses of the five mutants with the recipient strain yielded both Kga$^+$ recombinants that (like the parental organisms) grew on 20 mM-fructose and others that (like the original strain KL16.21) did not. This showed that although the five Hfr mutants tested grew on 20 mM-fructose, they still contained the *ptsX* marker and were able to transfer it to *ptsF ptsX*$^+$ recipients to render these *ptsF ptsX* and hence unable to utilize fructose.

Three kinds of explanation could account for the fructose-positive phenotype of strain JM485 and others like it. It might be that a second mutation within the *ptsF* gene restored its catalytic activity but caused an increase in the K_m of the system for fructose. If so, and in the absence of PtsX function, fructose would be taken up and phosphorylated to fructose 1-phosphate: the introduction of the *fpk* gene (Fig. 1) should then abolish utilization of fructose. This, however, is not the case. When the mutant JM485 was mated with strain JM486, which carries not only *ptsF* and *ptsX* but also *fpk* (besides *uhp*^{c} *pro cysI metB galT str*), 9 out of 120 Pro$^+$ Lac$^+$ Str^R recombinants tested grew on fructose and on glycerol, but growth on the latter substrate was inhibited by fructose 1-phosphate. This showed that recombinants were able to take up the phosphate ester directly (and were thus Uhp^c) but were devoid of fructose-1-phosphate kinase activity; the growth on fructose observed could therefore not have involved formation and catabolism of fructose 1-phosphate, and hence could not have been achieved in consequence of an altered *ptsF* gene. On the other hand, when one such fructose-positive recombinant was transduced with phage P1 propagated on an *E. coli* mutant devoid of fructose-6-phosphate kinase activity (*pfk*) and Met$^+$ transductants were selected, a high proportion of such transductants failed to grow on fructose or mannitol. Since *pfk* is known to be highly cotransducible with *metB* (Morrissey & Fraenkel, 1969; Kornberg & Smith, 1970), this result strongly indicates that fructose is utilized in these mutants via fructose 6-phosphate.

The cross between strains JM485 and JM486 that has been described also eliminates a second possible explanation for the growth of JM485 on fructose, namely that it is due to the presence of an *amber* suppressor mutation. Both the *cysI* and *galT* markers of the recipient strain JM486 are known to be due to *amber* mutations but many of the fructose-positive recombinants obtained from the cross with strain JM485 were either Cys$^-$, or Gal$^-$, or both.

A third possible explanation for the growth on fructose of mutants still carrying the *ptsF ptsX* genes is that this phenotype could arise by another type of translational suppression. However, mutants carrying *ptsX2*, an independently isolated allele of *ptsX* different from the *ptsX1 kdgR* deletion carried by strain KL16.21 and its derivatives (Jones-Mortimer & Kornberg, 1976), were also suppressed by the gene that permitted *ptsX1* mutants to grow on fructose. Thus a cross of strain JM485 (*ptsF1 ptsX1 kdgR* Fructose$^+$) and strain JM739 (*ptsF1 ptsX2 leu str*) gave Leu$^+$ Str^R recombinants, some of which had the donor phenotype for growth on fructose but did not grow on 2-keto-3-deoxygluconate: such recombinants had

Table 2. *Analysis of* Cys$^+$ Str^R *exconjugants from an interrupted mating between strains* JM1069 [Hfr P045 *pheA* (186)] *and* JM1072 [F$^-$ *cysI srlC ptsF ptsX* 186^R Str^R]

Recombinants were tested for their ability to grow with fructose as the carbon source, for their phenylalanine requirement and for being lysogenic for phage 186. If the gene order is *cysI srlC att186 pheA* then recombinants of classes B, D, G and H must arise from four crossings-over.

Class	Phenylalanine	Fructose	186	No. of recombinants
A	−	−	+	40
B	−	−	−	2
C	+	+	−	133
D	+	+	+	11
E	+	−	+	31
F	+	−	−	51
G	−	+	−	0
H	−	+	+	10

thus not inherited the *kdgR* (Pouysségur & Stoeber, 1974), and hence also not the *ptsX1*, allele. The ability of the suppressor to suppress the fructose negativity of such *ptsF1 ptsX2* mutants is therefore not allele-specific; since it is also rather efficient, it is highly unlikely to be a translational suppressor. The mutation that confers ability to grow on fructose, via fructose 6-phosphate, on these *ptsF ptsX* strains must therefore represent a novel route of fructose utilization.

Location of the lesion in ptsF ptsX strains which allows growth with fructose as the carbon source

The mating described between strains JM485 and JM486 suggested that the lesion in JM485 might lie between the origin of the Hfr strain used (at min 55) and the *pro* and *lac* loci (at min 10) on the *E. coli* chromosome. Analysis of recombinants from interrupted conjugation of these two strains showed that 75 to 80 % of the *cys*$^+$ recombinants selected within a few minutes of the beginning of mating had inherited the fructose-positive character of the donor strain, which suggested that the lesion is located close to *cysI*. This was confirmed by transduction. Bacteriophage P1 was propagated on strains KL16.21 (*ptsF ptsX*, Fructose$^-$) and JM485 (*ptsF ptsX*, Fructose$^+$) and used to transduce the *cysI* strains JM760 (*ptsF ptsX*, Fructose$^-$) and JM486 (*ptsF ptsX fpk*, Fructose$^-$) respectively to *cysI*$^+$. Of 128 recombinants from the first cross, 10 (8 %) were fructose-negative, and of 536 recombinants from the second cross, 49 (9 %) were fructose-positive. Since we were unable to demonstrate co-transduction of the lesion with *thyA* it is probable that the lesion is located at about min 51·5 rather than at min 53·5.

To test this conclusion, the Hfr strain JM1069 (*pheA*, lysogenic for phage 186) was mated with the fructose-positive strain JM1072 (*cysI ptsF ptsX* 186^R). Conjugation was interrupted 15 min after mixing and *cysI*$^+$ recombinants were selected on lactate minimal medium containing phenylalanine. These recombinants were scored for ability to grow on fructose, for phenylalanine requirement and for the inheritance of prophage. The results of this experiment (Table 2) indicate that the recombinant classes arising from four crossings-over are B, D, G and H, so that the gene giving the Fructose$^+$ phenotype (which, as shown below, was identified as an altered regulatory gene of sorbitol uptake, *srlC*), is located between *cysI* and the integration site for phage 186; the gene order is therefore *cysI srlC att186 pheA*.

Genetical evidence that the sorbitol phosphotransferase is involved in the utilization of fructose

Epstein (cited in Taylor & Trotter, 1972) and Lengeler (1975) showed that genes involved in sorbitol catabolism map between *cysI* and *pheA*. Since this is also the location of the mutation which allows *ptsF ptsX* mutants to grow on fructose, fructose might enter these cells via the sorbitol phosphotransferase. If this is so, two predictions can be made. Firstly, mutants that lack the sorbitol phosphotransferase (*srlA*) as well as the two fructose uptake systems (*ptsF ptsX*) should not mutate to grow on fructose; secondly, *srlA* mutants selected in a fructose-positive *ptsF ptsX* strain should not be able to grow on fructose. Both these predictions have been borne out by experiments.

Strain JM759 (*fda*^{ts} *ptsF ptsX*) was isolated from strain JM737 (*fda*^{ts} *ptsX*) as a mutant that grew on glycerol at 41 °C in the presence of fructose. Strains carrying the *fda*^{ts} marker are devoid of fructose-1,6-bisphosphate aldolase activity at 41 °C but not at 30 °C; the uptake and catabolism of fructose at the higher temperature thus leads to the formation and accumulation of fructose 1,6-bisphosphate and growth stasis (Böck & Neidhardt, 1966). Many of the mutants resistant to inhibition by fructose under these conditions (Jones-Mortimer & Kornberg, 1976) owe this phenotype to the loss of fructose uptake system(s); since strain JM737 was already devoid of PtsX function, its derivative strain JM759 must also have lost PtsF activity. Like other *ptsF ptsX* mutants, strain JM759 does not grow on fructose at either 30 or 41 °C; however, it readily mutates to do so at the lower temperature. One such mutant able to grow on fructose at 30 °C but not at 41 °C, and presumably *srlC*, was designated JM781. It was mutated further for loss of the gene for sorbitol uptake (*srlA*) in a manner analogous to that used for selection of *ptsF* in a *fda*^{ts} background, by selecting mutants able to grow on glycerol at 41 °C in the presence of sorbitol. Strain JM784 is one such *fda*^{ts} *ptsF ptsX srlC srlA* derivative. It differs from its parent JM781 in being unable to grow on sorbitol and on fructose at 30 °C. This shows that the utilization of fructose by strain JM781, which lacks the 'normal' systems for fructose uptake specified by *ptsF* and *ptsX* but is constitutive for sorbitol transport, occurs initially by sorbitol phosphotransferase.

This conclusion is reinforced by an experiment in which strain JM759 (*fda*^{ts} *ptsF ptsX*) was mutated to sorbitol resistance on glycerol at 41 °C; the mutant JM763 thus obtained was devoid of sorbitol phosphotransferase activity (*srlA*). In contrast to *srlA*$^{+}$ strains that carried *ptsF* and *ptsX*, this *srlA* mutant did not give rise to any further mutants when plated on medium containing fructose as sole carbon source at 30 °C.

In order to establish that phosphotransferase activity was required for the function of the novel transport system for fructose, a mutant (JM803) was selected from strain JM781 (*fda*^{ts} *ptsF ptsX srlC*) as a colony resistant at 41 °C on lactate minimal medium simultaneously to both *N*-acetylglucosamine and mannitol: this mutant had lost the activity of Enzyme I of the phosphotransferase system (*ptsI*). In consequence, this strain (*fda*^{ts} *ptsI ptsF ptsX srlC*) did not grow on fructose at 30 °C. This was not due to loss of the mutation (*srlC*) that had conferred on the parent strain JM781 the ability to grow on fructose since, when bacteriophage P1 propagated on strain JM803 was used to transduce strain JM486 (*ptsF ptsX fpk cysI*) to Cys^{+}, 8 % of the transductants were fructose-positive. Mutants devoid of Enzyme I activity sometimes fail to grow on substrates that are not utilized via the phosphotransferase system as well as on substrates that are; this phenotype can be overcome by selecting further mutants that, whilst still devoid of Enzyme I activity, carry a *crr* gene highly cotransducible with *ptsI* (Saier & Roseman, 1972; Jones-Mortimer & Kornberg, 1975). When such *crr*

Table 3. *Uptake of fructose and sorbitol, and sorbitol-6-phosphate dehydrogenase activity, in strain* KL16.21 *and its fructose-positive derivative strain* JM485

The rates of uptake of ^{14}C-labelled substrates by washed cells are expressed as nmol ^{14}C incorporated min^{-1} (mg dry mass)$^{-1}$; sorbitol-6-phosphate dehydrogenase activity is expressed as μmol NADH formed min^{-1} (mg protein)$^{-1}$.

			Uptake of			Sorbitol-6-phosphate dehydrogenase activity
Strain	Genotype	Grown on	0·1 mM-sorbitol	0·1 mM-fructose	5 mM-fructose	
KL16.21	*ptsF ptsX*	Sorbitol	18	1	63	15
		Glycerol	2	0·1	5	0·5
JM485	*ptsF ptsX srlC1*	Sorbitol	18	1	26	18
		Glycerol	10	0·1	30	4
		Fructose	12	0·1	35	4

mutants were selected from strain JM803 as colonies able to grow on maltose, they still failed to grown on fructose. We thus conclude that the growth of mutants of *ptsF ptsX* strains on fructose requires an intact gene for sorbitol phosphotransferase and a functional Enzyme I. Consequently, fructose does not enter the cells as free fructose (by a system of active transport or of facilitated diffusion) subsequently to be phosphorylated by ATP in a reaction catalysed by a fructo(manno)kinase (Sebastian & Asensio, 1967). The system of fructose utilization in these strains therefore also does not resemble that for galactose utilization in *mglP galP* Umg^c strains described by Kornberg & Riordan (1976). However, the possibility of analogy with this system suggested that mutation to growth on fructose might be the result of constitutive synthesis of the sorbitol phosphotransferase rather than an alteration in its specificity. To test this hypothesis we isolated a mannitol-negative mutant (strain JM1058) from strain JM759 (by selection for mannitol resistance on glycerol at 41 °C). From strain JM1058 sorbitol-constitutive mutants (Lengeler & Lin, 1972) were selected on mannitol medium at 30 °C. Since such Mtl$^+$ Srl^c mutants have simultaneously acquired the ability to grow on fructose, we conclude that the suppression of the PtsX$^-$ phenotype in *ptsF ptsX* mutants is the result of mutation in the regulatory system of the sorbitol phosphotransferase.

Biochemical evidence that the sorbitol phosphotransferase is involved in the utilization of fructose

If fructose can be utilized via the sorbitol phosphotransferase then *ptsF ptsX* cells in which this function has been induced should utilize fructose. To test this hypothesis, strains KL16.21 (*ptsF ptsX srlC*$^+$) and JM485 (*ptsF ptsX srlC*) were grown overnight in minimal medium with either sorbitol or glycerol as the carbon source and were subcultured into minimal medium with either fructose as the carbon source or the original carbon source. Cultures of strain KL16.21 that had been grown on glycerol did not adapt to grow on fructose, whereas those that had been grown on sorbitol grew on fructose, but at a rate that decreased with increase in cell mass: when plotted, this growth pattern was linear with time instead of exponential. In contrast, strain JM485 grew exponentially on fructose regardless of previous conditions of culture. The results obtained with strain KL16.21 suggest that fructose is a substrate for, but not an inducer of, the sorbitol phosphotransferase; the results for JM485 further suggest that this mutant, which grows on fructose, is constitutive for the sorbitol phosphotransferase.

We have therefore determined the activities of the sorbitol transport system in cells of various strains, and of the sorbitol-6-phosphate dehydrogenase in extracts of those cells,

Table 4. *Sorbitol-6-phosphate dehydrogenase activity in fructose-negative strains and their fructose-positive (sorbitol-constitutive) derivatives*

Sorbitol-6-phosphate dehydrogenase activity is expressed as μmol NADH formed min^{-1} (mg protein)$^{-1}$.

Strain	Genotype	Growth temperature (°C)	Grown on	
			Glycerol	Sorbitol
JM486	*ptsF ptsX*	37	0·33	7·8
		22	0·50	13
JM795	*ptsF ptsX srlC5*	37	2·3	13
		22	0·68	11
JM794	*ptsF ptsX srlC4*	37	8·6	7·5
JM760	*ptsF ptsX srlC2*	37	1·5	5·0
JM759	*ptsF ptsX*	30	0·33	5·1
JM781	*ptsF ptsX srlC3*	30	7·1	6·3

obtained from cultures grown under inducing and non-inducing conditions. The results are summarized in Table 3. Strains such as JM485, predicted from the genetic evidence to be constitutive for the utilization of sorbitol, synthesize both the sorbitol uptake system and the dehydrogenase that acts on the product of sorbitol uptake and phosphorylation, in the absence of inducer.

The nature of the control system

Lengeler (1975) has shown that the constitutive allele of *srlC* (designated *gutC* by him) is *cis* dominant, since diploids of constitution F′ $C^+A^+D^+/C^cA^-D^-$ are mannitol-negative, whereas diploids of the type F′ $C^+A^+D^+/C^cA^+D^+$ are mannitol-positive. This implies that the mutations to constitutivity are of the operator or initiator type. We have isolated a mutant, strain JM795, whose properties are indicated in Table 4. This strain grows exponentially on fructose at 37 °C, albeit rather slowly, but does not grow at 22 °C; growth on glycerol at either temperature is not impaired. The lesion in this strain (*srlC5*) is 7·3 % cotransducible with *cysI*, and therefore maps in the same region as other *srlC* mutants. If it is a mutation of the same type as those described by Lengeler (1975), the strain must either have a temperature-sensitive operator (which cannot recognize the repressor at 37 °C, but can do so at 22 °C) or a cold-sensitive initiator. It seems more plausible that the mutation is of a different type, resembling the thermosensitive *lacI* mutants described by Sadler & Novick (1965) in being a temperature-sensitive mutant in a negative control gene. This view is supported by the rate of spontaneous mutation of *ptsF ptsX* strains to *srlC*, which is of the order of 10^{-7} per cell per generation, a value which would appear to be too high for the rather specific base changes required to produce an operator-constitutive mutant or any kind of constitutive mutant in a positive control system.

Specificity of the sorbitol uptake system

The utilization of fructose that can occur through its uptake and phosphorylation by the sorbitol phosphotransferase if that activity is first induced (Fig. 1) also shows that the stereochemical requirements for phosphotransferase induction are more stringent than those exhibited by its activity. This supports the findings of Lengeler & Lin (1972) and Lengeler (1975) who showed that sorbitol, mannitol and 2-deoxyarabinohexitol are substrates for the sorbitol phosphotransferase, but, of these, only sorbitol is an inducer of the system. We have

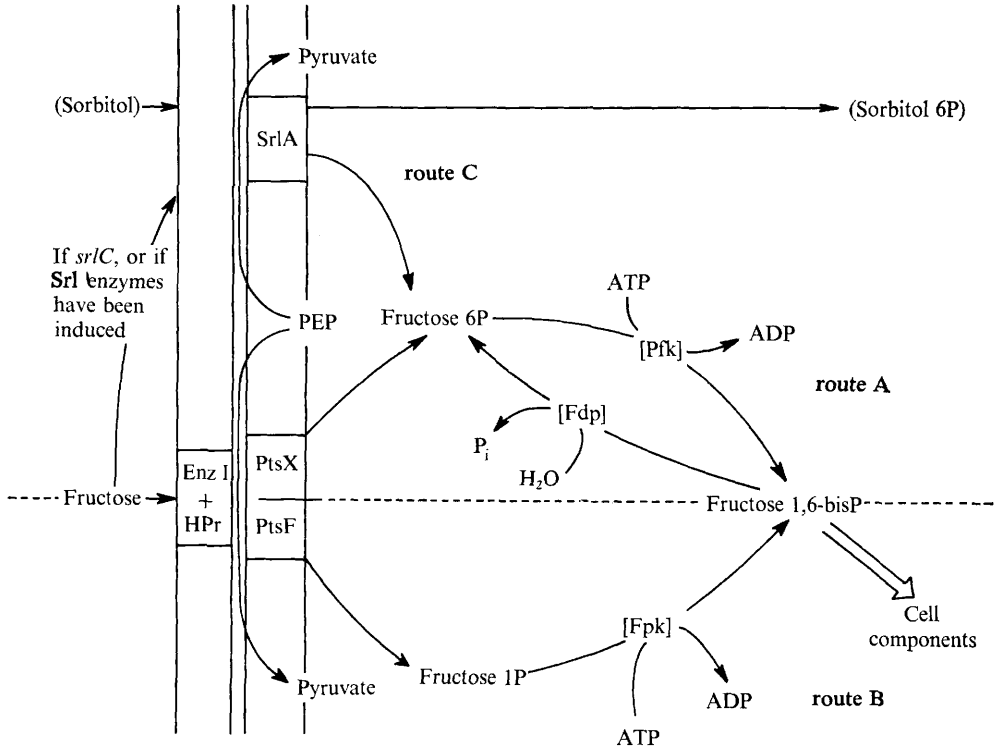

Fig. 1. Postulated pathway of fructose utilization in *ptsF ptsX* mutants in which the enzymes of sorbitol uptake and catabolism have been induced.

shown above that fructose is a substrate for the system but not an inducer. No uptake of glucose could be demonstrated in *E. coli* strains which lack all the known systems for glucose transport (Kornberg & Jones-Mortimer, 1974) but which are constitutive for the sorbitol phosphotransferase. Furthermore, mutants which are inducible for the sorbitol phosphotransferase are not inhibited by glucosaminitol during growth on glycerol, whereas mutants which are constitutive for the system are sensitive, but give rise to resistant clones which have lost the ability to grow on sorbitol. It therefore appears that a hydroxyl group on the C-1 atom is essential for transport by this system, but that, though the nature of the groups attached to the C-2 atom is relatively unimportant in this respect, a hydroxyl group in the D-gluco configuration at C-2 is essential for induction.

We are grateful to Drs B. Bachmann and R. A. Cooper for providing strains of bacteria, to Drs F. Jacob and M. D. Yudkin for strains of bacteriophage, and to Mr J. W. Keyte for technical assistance. We also gratefully acknowledge the support received from the Science Research Council and The Royal Society through research grants.

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