MECHANISMS OF 'INDUCER EXCLUSION' BY GLUCOSE

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1. Introduction

In enteric bacteria such as *Escherichia coli* and *Salmonella typhimurium*, glucose exerts at least three profound but different inhibitory effects on the utilization of other nutrients; the terms catabolite repression [1], transient repression [2] and inducer exclusion [3] have been applied to those phenomena. The first of these terms is used to describe the effect of glucose (or, following Magasanik's demonstration [1] that the effect is not confined to glucose, any other carbon source that is readily utilized for growth) on the expression of an inducible enzyme. It was first recorded nearly 40 years ago by Epps and Gale [4], who demonstrated that glucose inhibited the synthesis of amino acid decarboxylases by *E. coli*. However, since not only glucose but a wide variety of nutrients affect the differential rates of synthesis of enzymes that are normally inducible, even in mutants that form these enzymes constitutively, the effect indicates a general correlation between the intensity of carbon flux through catabolic pathways and the rates at which the genes specifying these enzymes are expressed.

'Transient repression' describes the observation that glucose [5] or some other good carbon source [6], when added to a bacterial culture in which an inducible enzyme is being synthesized gratuitously [7] during growth on a relatively poor carbon source, halts the synthesis of that inducible enzyme for up to 1 doubling of the cells, after which synthesis begins again at a rate characteristic of the rate of expression of the enzyme during growth on the new carbon source. As with 'catabolite repression', 'transient repression' is observed also in cells that form the relevant enzyme constitutively.

'Inducer exclusion', unlike the other two effects of glucose already mentioned, acts not on the expression of genes directly but on the entry of the inducers necessary to activate those genes. This phenomenon was first described in 1959 by Cohn and Horibata [3], who found that the induction of β-galactosidase in succinate-grown *E. coli* by 0.5 mM methyl β-D-thiogalactoside was totally abolished by the simultaneous addition of 1 mM glucose but that this inhibition could be overcome either by increasing the concentration of inducer or by delaying the addition of glucose. Winkler and Wilson [8] confirmed that glucose inhibited the transport of β-galactosides even by cells that formed this latter transport system constitutively; since the glucose analogue methyl α-D-glucoside (αMG) was also effective as an inhibitor, and since this effect was not observed with mutants impaired in the sugar-specific component of the uptake system for glucose and αMG, it was clear that some component of the glucose uptake system was responsible for mediating the exclusion of lactose and other β-galactosides.

Before this interaction can be considered further, it is necessary briefly to compare the transport systems for β-galactosides (and many other sugars, sugar-phosphates and dicarboxylic acids) with those that effect the uptake of glucose (and a variety of other hexoses and hexitols) in enteric bacteria. The former class of substrates are taken up by 'active transport', i.e., they are taken up by processes that depend on the expenditure of metabolic energy, but their substrates traverse the cell envelope to appear in the cytoplasm chemically unchanged. In contrast, the latter group of hexoses and hexitols undergo phosphorylation at the expense of phosphoenolpyruvate (PEP), apparently concomitantly with their uptake into the cell, and thus appear in the cytoplasm either as the 6-phosphate (in the case of glucose, glucosamine, *N*-acetyl glucosamine, mannose and the hexitols) or as the 1-phosphate (in the case of fructose). The mechanism of this transfer of phosphate from PEP to

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Dedicated to Professor Sir Hans Krebs, FRS, on his eightieth birthday

the sugar or sugar alcohol has been elucidated largely by S. Roseman and his colleagues (reviewed [9–12]). The overall process has been termed 'the PEP-dependent phosphotransferase' (or 'PT') system; it involves at least:

(i) An initial transfer of phosphate from PEP to a small phosphoryl carrier protein, HPr; this is catalysed by an enzyme termed enzyme I; and
(ii) A subsequent transfer of phosphate from phospho-HPr to the sugar substrate, catalysed by one or more relatively sugar-specific enzymes II. The enzymes II are firmly bound to the inner membrane of the cell but may also be associated with more easily solubilized proteins ('Factors III') that mediate the transfer of the phosphate moiety from the phospho-HPr to the sugar.

Further evidence for the involvement of the PT-system in the utilization of sugars taken up by active transport (which, for convenience, will be generically termed 'non-PT-sugars') comes from studies of mutants impaired in enzyme I function. Such mutants are, of course, impaired also in the utilization of all PT-sugars, since they cannot catalyse the process (i) common to their uptake; however, they are also impaired to various degrees in the utilization of non-PT-substrates such as lactose, galactose, melibiose, maltose, glycerol and succinate [13–17]. As was first shown by Gershanovitch et al. [18], this phenomenon does not indicate a role of the PT-system in the intracellular catabolism of the affected substrates but is due to an increased difficulty in inducing the enzymes required for their uptake and catabolism. Mutants lacking enzyme I activity could be induced to grow on lactose if either the non-catabolizable inducer isopropyl β-D-thiogalactoside (IPTG) or cyclic adenosine 3′,5′-monophosphate (cAMP) was also added to the medium [19]; moreover, once the lactose permease of enzyme I-mutants has been induced with IPTG, such mutants continue to grow for many generations on lactose even in the absence of IPTG and maintain their ability to express the *lac* operon [20]. Similarly, the addition of cyclic AMP to cultures of mutants lacking enzyme I activity enables them to grow on glycerol [21] and on all other non-PT-substrates tested [22].

The relationship of cAMP to sugar utilization had been revealed by three main observations in the late 1960s. It was found that cAMP could overcome both the catabolite repression [23,24] and the transient repression [25] caused by glucose; it was also found that mutants impaired in adenylate cyclase (and hence deficient in cAMP) grew only poorly if at all on a variety of sugars but that the addition of cAMP restored the normal utilization of all these carbon sources [26]. The repressive effects of glucose on the induction of other enzyme systems can thus be viewed largely (if not entirely [27]) as a modulation of cAMP levels in the bacterial cells; indeed, glucose has long been known to lower the intracellular concentration of cAMP [28] and to inhibit the membrane-associated adenylate cyclase [29]. The question thus arises whether cAMP is also involved in inducer exclusion.

There are two lines of evidence that raise this possibility. The first comes from studies of the effects of αMG on 'leaky' enzyme I mutants of *S. typhimurium* and *E. coli* [30]. In such mutants, some residual enzyme I activity can still be detected: these mutants could be induced to grow on non-PT sugars such as maltose, melibiose and (*E. coli* only) lactose, and on glycerol, but this induction was sensitive to inhibition by αMG in as low a concentration as 5 μM [31]. This abnormal sensitivity to αMG was overcome by a further mutation, designated *crr* (for carbohydrate repression resistance), which mapped close to the genes *ptsI*, specifying enzyme I, and *ptsH*, specifying HPr, on the *S. typhimurium* genome. A biochemical consequence of the *crr*-mutation in these organisms was a dramatic decrease in the level of a protein (factor III^{Glc}) associated with the enzyme II for the uptake of glucose and of αMG; another was that such *crr*-mutants of *S. typhimurium* were markedly impaired in their ability to form cAMP during growth on a mixture of amino acids and galactose [31]. Saier and Feucht [31] therefore suggested that the *crr*-gene product is a regulatory protein, RPr, which is phosphorylated by PEP via enzyme I and HPr, and that this protein (or its phosphorylated form phospho-RPr) allosterically regulates the activities of adenylate cyclase and the permease proteins for the uptake of non-PT-sugars.

A second possible association between cAMP and inducer exclusion arises from the effects of glucose on the uptake of PT-sugars. These are operationally analogous to the effects exerted by glucose on non-PT-sugars. Glucose or its non-catabolizable analogues (e.g., αMG, 2-deoxyglucose, 5-thio-D-glucose and 3-deoxy-3-fluoroglucose) powerfully inhibit the uptake of PT-sugars such as fructose or sorbitol by *E. coli*, even when the uptake system for fructose or sorbitol is fully induced and this does not occur if the glucose-specific enzyme II is absent [20,32]. Mutants resistant to inhibition by 5-thio-D-glucose were

selected: in them glucose was still utilized virtually normally but glucose or some of its analogues no longer inhibited the uptake of any PT- or non-PT-sugar [33,34]. Like the *crr*-gene [35], the lesion responsible, designated *tgs* (to indicate loss of thioglucose sensitivity), mapped close to the *ptsI* and *ptsH* genes on the *E. coli* genome; like *crr*-mutants of *S. typhimurium*, *tgs*-mutants of *E. coli* were markedly impaired in adenylate cyclase activity [33]. Does this indicate that the mechanisms of inducer exclusion by glucose on the uptake of non-PT-sugars and of PT-sugars are identical, and that cAMP plays a necessary role in them? It is the purpose of this paper to present evidence that the answer to both these questions is 'No'.

2. Experimental

2.1. *Isolation of mutants*

tgs- and *crr*-Mutants were selected from strains of *E. coli* that carry the temperature-sensitive allele of the gene specifying enzyme I activity, *ptsI*^{ts}. *tgs*-Mutants were obtained as organisms resistant to 5-thio-D-glucose at 30°C on media containing 10 mM fructose and 2 mM 5-thio-D-glucose, as in [33]. *crr*-Mutants were selected as *ptsI*^{ts}-mutants that had regained the ability to grow on maltose or glycerol at 40°C despite the absence of enzyme I activity at the higher temperature [35]. Both the *tgs*- and *crr*-mutations, together with the closely-linked *ptsI*^{ts} marker, were transferred by phage P1-mediated transduction to a *ptsI* derivative of *E. coli* strain HK24 [36] as in [34]; one colony obtained from each transduction was purified and designated PW11 (*tgs*) and PW127 (*crr*). The *ptsI*^{ts} *tgs*$^{+}$ *crr*$^{+}$ strain PW7 [37] was used in control experiments.

The *ptsI purB* double mutant HK623 was isolated in two steps. In the first, strain PA309 (*trp his arg thr leu str*; [38]) was crossed with strain HK548, a derivative of the Hfr-strain KL16 that carried the markers *umgC* (constitutive uptake of αMG [39]) and *purB*; the recombinant HK557 was selected as a Trp^{+} Str^{R} colony and was found to carry the markers *his arg thr leu purB str*. This was in turn conjugated with strain 2570C, a *ptsI*-derivative of strain KL16 [16], recombinants being selected for His^{+} Str^{R}. Many of these had acquired the *ptsI*-marker of the donor strain: one of them was purified by the isolation of single colonies and used as strain HK623. The selection of mutants HK597, 598, 690 and 691 is described in section 3.

2.2. *Growth of cells*

Cultures of *E. coli* were grown aerobically at 30°C or 37°C on defined media containing salts [40] supplemented at 80 mg . l^{-1} with adenine and amino acids as required by the bacterial strains used, plus carbon-sources at the following concentrations: glycerol, 20 mM; hexoses, hexitols, gluconate, 10 mM; and disaccharides, 5 mM. Growth was estimated turbidimetrically as A_{680} in a Unicam SP600 spectrophotometer. With the cultures and instrument used, an A_{680} of 1.0 was found to be equivalent to a bacterial dry mass of 680 μg . ml^{-1}.

2.3. *Measurement of uptake*

The uptake of ^{14}C-labelled sugars by cell suspensions was measured as in [41]. Radiolabelled substrates were purchased from the Radiochemical Centre, Amersham.

2.4. *The phosphotransferase activity*

This was determined in cells rendered permeable with toluene by the method in [42].

3. Results

3.1. *Rates of growth in the presence and absence of enzyme I function*

Since all the strains PW7, PW11 and PW127 contain temperature-sensitive enzyme I function, active at 30°C but inactive (and rapidly destroyed) at 37°C [15], growth in the presence and in the absence of enzyme I activity could be measured at these two temperatures (table 1). On gluconate, the utilization of which is not dependent on the PT-system, all the strains grow faster at 37°C than at 30°C. However, the control strain PW7 and the *tgs*-mutant PW11 grew much faster at 30°C than at 37°C both on the PT-sugars glucose and fructose and on the non-PT-carbohydrates maltose, lactose and glycerol. In contrast, the strain containing the *crr*-mutation grew as rapidly, or more rapidly, at 37°C than at 30°C on all substrates except the PT-sugars. The rate of growth on each substrate at 30°C was not substantially changed by either mutation. The *crr*-mutant thus behaved like the mutant described in [35] whereas, on these criteria, the *tgs*-mutant PW11 was barely distinguishable from the control strain PW7 that also carried the *ptsI*^{ts}-marker.

Table 1
Growth rates of *tgs* and *crr* mutants

Strain and markers	Temp. (°C)	Doubling time (h) of growth on:					
		Glucose	Fructose	Maltose	Lactose	Glycerol	Gluconate
PW7 (+)	30	2.5	2.5	2.5	2.0	2.0	2.0
	37	>10	>10	>10	>10	>10	1.75
PW11 (*tgs*)	30	2.75	2.25	2.75	2.5	2.25	2.5
	37	>10	>10	4.0	4.75	4.0	2.0
PW127 (*crr*)	30	2.25	2.25	2.25	2.5	3.0	2.25
	37	>10	>10	2.5	2.0	1.5	1.25

Cells were grown in minimal medium containing gluconate at 30°C, harvested and resuspended in fresh medium containing the carbon source shown. Growth was measured at 30°C and 37°C

3.2. *Rates of growth in the presence and absence of glucose analogues*

Growth on fructose (fig.1) and on maltose (fig.2) was measured in the presence of the glucose analogue αMG, which is taken up and phosphorylated by the enzyme II specified by *ptsG* [43], and of 2-deoxyglucose, which is predominantly a substrate for the enzyme II specified by *ptsM* [44]. As reported in [41],

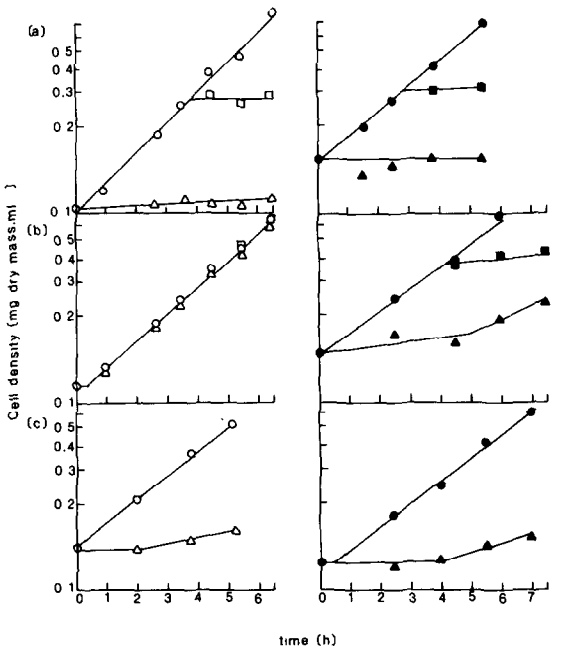

Fig.1. Effect of glucose analogues on the growth on fructose of *tgs*- and *crr*-mutants. Strains (a) PW7 (wild-type), (b) PW11 (*tgs*) and (c) PW127 (*crr*) were grown in minimal medium containing gluconate at 30°C, then harvested and resuspended in fresh medium containing fructose at 30°C. Growth was measured when methyl α-glucoside (10 mM) (open symbols) or 2-deoxyglucose (2 mM) (closed symbols) were: (○,●) absent; (△,▲) added at the same time as the substrate; or (□,■) added after 1 doubling of the cells.

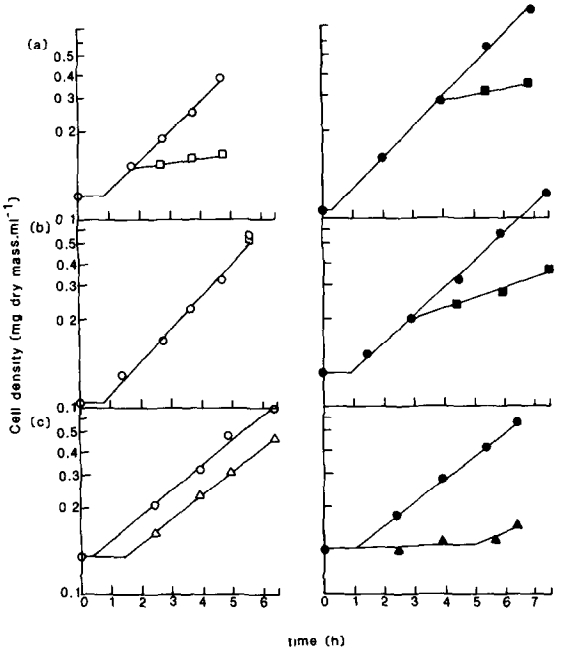

Fig.2. Effect of glucose analogues on the growth on maltose of *tgs*- and *crr*-mutants. Strains (a) PW7 (wild-type), (b) PW11 (*tgs*) and (c) PW127 (*crr*) were grown in minimal medium containing gluconate at 30°C, then harvested and resuspended in fresh medium containing maltose at 30°C Growth was measured when methyl α-glucoside (10 mM) (open symbols) or 2-deoxyglucose (2 mM) (closed symbols) were: (○,●) absent; (△,▲) added at the same time as the substrate; or (□,■) added after 1 doubling of cells.

the growth of the wild-type strain on either sugar was inhibited by these analogues. However, the mutants behaved differently. The *tgs*-strain PW11 was unaffected by the addition of αMG, irrespective of the carbon source on which it was growing. The growth of the *crr*-strain PW127, on the other hand, was strongly inhibited by αMG when fructose was the carbon source (fig.1) but not when maltose was the growth substrate (fig.2). Where mutants grew in the presence of analogues, this growth took place even if the analogue was added at the same time as was the substrate, which indicates that the mutations permitted the induction, and not merely the continued function, of the fructose and maltose transport systems.

Since all the strains were inhibited by 2-deoxyglucose in their growth on either fructose or maltose, these results imply that both the *tgs*- and the *crr*-mutations specifically modify regulation effected by materials that enter the cells via the PtsG system; however, the two mutations differ in that the *crr*-mutation abolished the exclusion by glucose of non-PT-sugars whereas the *tgs*-mutation protected against αMG on all substrates. This has been confirmed by direct measurement of the uptake of a variety of carbohydrates in the presence of glucose. The *tgs*-mutation specifically abolishes the ability of glucose and αMG to exclude other substrates whereas the *crr*-mutation affects only the ability of glucose and αMG to exclude non-PT-sugars [34,45].

3.3. *Uptake and phosphotransferase activity*

Since both the *tgs*- and *crr*-mutations affect the PtsG-system, albeit in different ways, the rates of uptake and of phosphoenolpyruvate-dependent phosphorylation of the PtsG-specific substrate αMG were determined, with fructose as a control (table 2). The substrate concentrations employed in these experiments were much lower than those used for the measurement of growth: whereas 10 mM sugars were used in growth experiments, 100 μM were used for the measurement of uptake of [^{14}C]hexose by washed cell suspensions and 1 mM for the phosphorylation of sugars by permeabilized cells. All the organisms studied were ultimately derived from strain PA309, which

Table 2
Uptake and phosphotransferase activities of *tgs*- and *crr*-mutants

(a) Strain	Marker	Growth substrate	Uptake (nmol . min^{-1} . mg dry mass^{-1}) of:			
			αM[^{14}C]G with		[^{14}C]Fructose with	
			none	fructose	none	αMG
PW7	+	Glucose	17	–	2	–
		Fructose	28	25	28	2
PW11	*tgs*	Glucose	7	–	2	–
		Fructose	8	5	22	11
PW127	*crr*	Glucose	23	–	2	–
		Fructose	19	–	13	1

(b) Strain	Marker	Growth substrate	PT-activity (nmol PEP . min^{-1} . mg dry mass ml^{-1}) with	
			α-MG	Fructose
PW7	+	Glucose	11	0.5
		Fructose	70	38
PW11	*tgs*	Glucose	7	4
		Fructose	7	40
PW127	*crr*	Glucose	16	2
		Fructose	64	73

Cells were grown in minimal medium containing the hexose shown, at 30°C. Measurements of (a) uptake and (b) phosphotransferase activity were performed as in section 2. In the uptake experiments, the inhibiting sugars shown (2.5 mM) were added immediately before the [^{14}C]hexose

synthesizes the PtsG-system constitutively. One feature of the 'constitutive' PtsG-system is that the rate of uptake and of phosphorylation of αMG is greater in cells grown on fructose than in cells grown on glucose [42]. The uptake and phosphorylation of fructose are inducible, irrespective of the constitutivity or inducibility of the PtsG-system.

The *crr*-mutation produced no substantial change in any of the activities measured. Although the *tgs*-mutation did not affect the capacity of the cells to take up and phosphorylate fructose, it considerably reduced the corresponding activities with αMG when supplied at these low concentrations. Furthermore, *tgs*-mutants grown on fructose had uptake and phosphorylation activities no greater than those grown on glucose. The measurements of uptake were repeated in the presence of a 25-fold excess of unlabelled sugar (fructose for the uptake of α-M[^{14}C]G and αMG for the uptake of [^{14}C]fructose) added immediately before the radio-labelled substrate. In both the *crr*-mutant and in the wild-type strain, αMG inhibited the uptake of [^{14}C]fructose by >90%, but αMG inhibited by only 50% in the *tgs*-mutant. Excess fructose impaired the uptake of α-M[^{14}C]G by only 10% in the control strain; however, fructose reduced the uptake of methyl α-glucoside by 37% in the *tgs*-mutant.

3.4. *Adenylate cyclase activities*

One important difference in the properties of *tgs*- and *crr*-mutants is their ability to grow on substrates whose uptake systems are inducible with difficulty, such as C_4-dicarboxylates. *tgs*-Mutants grow only poorly and after a long lag on fumarate or succinate; this reluctance can be overcome by the addition of cAMP. Thus, whereas the *ptsI*^{ts}-strain PW7 grew at 30°C on 10 mM fumarate with a mean doubling time of 2.7 h both in the presence and in the absence of 2.5 mM cAMP, the *tgs*-mutant PW11 took >10 h to double on this C_4-substrate in the absence of cAMP; it took only 2.8 h in its presence. In contrast, the *crr*-mutant PW127 behaved like strain PW7 under these conditions. These findings suggest that only the *tgs*-mutant is significantly impaired in adenylate cyclase activity: as shown in table 3, this was borne out in practice. Moreover, whereas the adenylate cyclase activities of the wild-type and *crr*-strains were inhibited by αMG, and this inhibition was overcome by PEP [47], the low activity of the *tgs*-mutant was much less responsive to these additives.

3.5. *Adenylate cyclase activity and glucose transport*

The correlation between the activity of adenylate cyclase in various *E. coli* mutants and their ability to utilize C_4-dicarboxylates for growth focused our attention on a serendipitous observation. In order to obtain a *ptsI*-mutant of the F$^-$ strain PA309 (*trp his arg thr leu str*) routinely used in our laboratory, the Hfr strain 2570C (a *ptsI* derivative of strain KL16 [13]) was conjugated with strain PA309 and recombinants were selected for Trp$^+$, the donor being counter-selected by the inclusion of streptomycin, and were screened for the absence of enzyme I function by determining their ability to grow on the PT-sugar alcohol, sorbitol. The *ptsI*-recombinants thus obtained were also replica-plated onto fumarate. Rather to our surprise, there was a sizeable proportion of recombinants that, whilst clearly *ptsI*, grew on fumarate; single-colony isolates of each of the fumarate$^+$ and fumarate$^-$ class (designated HK597 and 598, respectively) were studied further.

Analysis of these two strains confirmed that, as commonly found in *ptsI*-mutants [49], their adenylate cyclase activities were considerably lower than those of wild-type cells; however the fumarate$^+$ strain HK597 formed 7.5 pmol cAMP . min^{-1} . mg protein^{-1}

Table 3
Adenylate cyclase activities in strains of *E. coli*

Strain	Marker	Activity in the presence of:		
		No addition	1 mM αMG	1 mM PEP
PW7	+	10	3	12
PW11	*tgs*	1.6	1	1.8
PW127	*crr*	7	2	7

Adenylate cyclase activity was measured [46] in toluenized cells. Activities are expressed as pmol cAMP formed . min^{-1} . mg protein^{-1}

whereas the fumarate$^-$ strain HK598 formed <1 pmol . min^{-1} . mg protein^{-1}.

This difference in activity affected also the properties of *tgs*-mutants derived from these two strains. Phage P1 was propagated on a *tgs*-strain of *E. coli* and was used to infect strains HK597 and 598; PtsI$^+$-transductants were selected as colonies able to grow on sorbitol. It is known [34] that *ptsI* and *tgs* are co-transducible to nearly 100%; the transductants selected would thus be highly likely to be Tgs$^-$. This was found to be the case: in cultures of both the transductant from strain 597 (designated HK690) and of that from strain 598 (designated HK691), glucose was used simultaneously with fructose but was not used preferentially. However, the elevated adenylate cyclase activity of strain 690 (>3 pmol cAMP . min^{-1} . mg protein^{-1}) was associated not only with its ability to grow on fumarate but also with an obvious preference for fructose over glucose (fig.3); strain HK691, which formed only about 1 pmol cAMP . min^{-1} . mg protein^{-1} behaved like other *tgs*-mutants reported [34] in utilizing fructose and glucose to the same extent and in being unable to grow overnight on plates containing fumarate as carbon source, unless cAMP was also added.

These findings suggested an association between adenylate cyclase activity and the rate at which glucose enters the cell. Evidence in support of this suggestion was obtained by analysis of PurB$^+$ transductants obtained when phage P1, propagated on the original *ptsI*-strain 2570C, infected the *ptsI purB* double mutant HK623 (see section 2). As mentioned earlier, the enzyme II specific for the uptake of glucose and αMG may be formed by different strains of *E. coli* either inducibly (as in the phage donor strain) or constitutively (as in the recipient strain); the gene *ptsG* specifying this enzyme II is about 20% co-transducible with *purB* [43]. It is therefore highly suggestive that about 15% of the PurB$^+$ transductants obtained were able to grow on fumarate whereas the remainder retained the fumarate$^-$ phenotype of strain HK623; of course, all the transductants were still devoid of enzyme I activity. Whether the character, present in the donor strain, that permits the *ptsI*- or *tgs*-mutants to grow on fumarate and that, in *tgs*-mutants, reduces

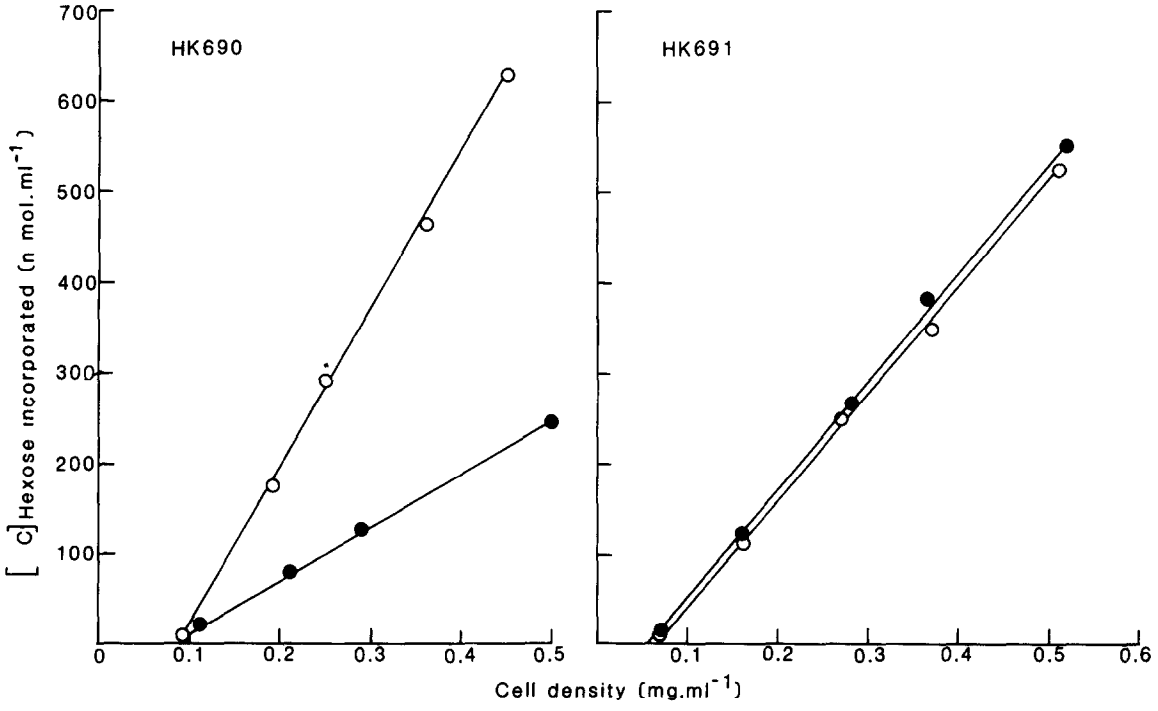

Fig.3. Utilization of glucose and fructose by the *tgs*-mutants. HK690 (fumarate$^+$) and HK691 (fumarate$^-$), growing on a mixture of these two sugars. Fructose-grown inocula were transferred to flasks containing 5 mM [^{14}C]glucose and 5 mM unlabelled fructose, or 5 mM glucose and 5 mM [^{14}C]fructose. The incorporation of ^{14}C derived from glucose (•) and fructose (○) during subsequent growth was measured as in [41].

the entry into the cell of glucose in the presence of another PT-sugar, is indeed associated with an altered enzyme II for glucose, is currently being investigated.

4. Discussion

These findings show that glucose exerts 'inducer exclusion' on the uptake both of PT- and non-PT-sugars, but that the mechanisms whereby this effect is achieved are not identical. In particular, and in confirmation of the work of Saier and his colleagues with *S. typhimurium* [30], mutants can be isolated in *E. coli* that exhibit the Crr^--phenotype. In them, glucose no longer excludes non-PT-sugars; however, glucose still excludes PT-sugars. As expected from the method of their selection (the ability to grow on glycerol, maltose or C_4-dicarboxylates in the absence of enzyme I function) *crr*-mutants of *E. coli* are also not significantly impaired in adenylate cyclase activity. Glucose-mediated 'inducer exclusion' of PT sugars, on the other hand, can be overcome by a *tgs*-mutation that not only differs from *crr* in all the respects mentioned but maps at a site on the *E coli* genome separated by at least two known genes from *crr* [34,50].

The findings that the adenylate cyclase activities of *ptsI*- and *tgs*-mutants (though always lower than their wild-type parents) can be altered by a gene that also affects the entry into the cells of glucose in the presence of other PT-sugars suggests that it is the close association of these membrane-associated processes that determines the partition of phosphate, from PEP via the other components of the PT-system, between adenylate cyclase and the sugar-specific enzyme II. Although much needs to be learned about the details of this process, it is evident that the formation and function of cAMP is casually, not causally, related to the phenomena of 'inducer exclusion'.

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