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Utilization of Gluconate by *Escherichia coli*

INDUCTION OF GLUCONATE KINASE AND 6-PHOSPHOGLUCONATE DEHYDRATASE ACTIVITIES

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1. A mutant of *Escherichia coli*, devoid of phosphopyruvate synthetase, glucosephosphate isomerase and 6-phosphogluconate dehydrogenase activities, grew readily on gluconate and inducibly formed an uptake system for gluconate, gluconate kinase and 6-phosphogluconate dehydratase while doing so. 2. This mutant also grew on glucose 6-phosphate and inducibly formed 6-phosphogluconate dehydratase; however, the formation of the gluconate uptake system and gluconate kinase was not induced under these conditions. 3. The use of the Entner–Doudoroff pathway for the dissimilation of 6-phosphogluconate, derived from either gluconate or glucose 6-phosphate, by this mutant was also demonstrated by the accumulation of 2-keto-3-deoxy-6-phosphogluconate (3-deoxy-6-phospho-L-*glycero*-2-hexulosonate) from both these substrates in a similar mutant that also lacked phospho-2-keto-3-deoxygluconate aldolase activity. 4. Glucose 6-phosphate inhibits the continued utilization of fructose by cultures of the mutants growing on fructose, as it does in wild-type *E. coli*. 5. The mutants do not use glucose for growth. This is shown to be due to insufficiency of phosphopyruvate, which is required for glucose uptake.

A metabolic route for the dissimilation of glucose by *Pseudomonas saccharophila*, discovered by Entner & Doudoroff (1952), has been shown to be of major importance in glucose catabolism by several pseudomonads (Wood, 1955; Lessie & Neidhardt, 1967; Kersters & de Ley, 1968). In other micro-organisms, this pathway is not important for the utilization of glucose, but its key enzymes are inducibly formed on exposure of microbial cultures to gluconate (Mortlock, 1962; Fraenkel & Horecker, 1964; Eisenberg & Dobrogosz, 1967; Fraenkel & Levisohn, 1967; Brubaker, 1968). This difference was explained by Eisenberg & Dobrogosz (1967); glucose is oxidized to gluconate by pseudomonads, and it is likely that this product is, or is the precursor of, the 'true' inducer of the enzymes of the Entner–Doudoroff pathway in this and other micro-organisms. Evidence that this oxidative formation of gluconate has to occur before glucose can serve to induce these enzymes in *Pseudomonas fluorescens* has been presented (Quay *et al.*, 1972).

The conversion of external gluconate into intermediates of glycolysis, via the Entner–Doudoroff pathway, involves at least four steps (Scheme 1). Gluconate must first be taken up by the cells. There is some evidence that this process is catalysed by a discrete uptake system (Kaback, 1972) and mutants defective in it have been obtained (Faik & Kornberg, 1973). The phosphorylation of gluconate to 6-phosphogluconate is catalysed by one (Cohen, 1951) or possibly more than one (Hung *et al.*, 1970) gluconate kinase (EC 2.7.1.12). This product is then dehydrated to 2-keto-3-deoxy-6-phosphogluconate (3-deoxy-6-phospho-L-*glycero*-2-hexulosonate), which is then split by a specific aldolase (EC 4.1.2.14) to glyceraldehyde 3-phosphate and pyruvate.

Two main lines of evidence illustrate the role that this pathway plays in gluconate utilization by *Escherichia coli*. In the first place, mutants devoid of 6-phosphogluconate dehydrogenase (EC 1.1.1.43) activity (*gnd*; Scheme 1), which are thus unable to catabolize 6-phosphogluconate by any route other than the Entner–Doudoroff pathway, grow at roughly wild-type rates on gluconate (Fraenkel, 1968*a*); in contrast, mutants that contain 6-phosphogluconate dehydrogenase but lack 6-phosphogluconate dehydratase activity (EC 4.2.1.12; *edd*, Scheme 1) grow very slowly on gluconate (Zablotny & Fraenkel, 1967). In the second place, whereas the specific activities of 6-phosphogluconate dehydrogenase in *E. coli* extracts appear to be independent of the nature of the carbon source used for growth of the organism, 6-phosphogluconate dehydratase is present in only trace amounts if the organism has not been previously exposed to gluconate. The location of the

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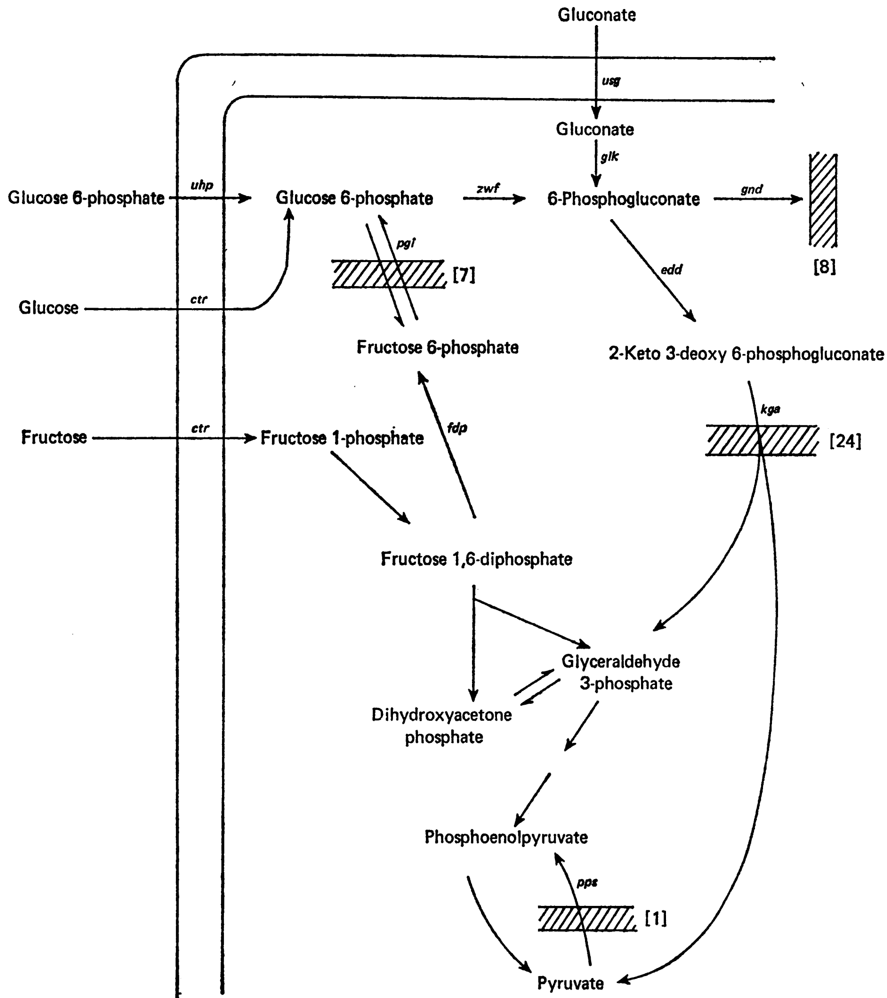


Scheme 1. *Metabolic pathways in Escherichia coli considered in this paper*

The maps shows, in outline, the main routes for the entry of a number of C_6 compounds into *E. coli* and for their catabolism. The genetic markers that specify appropriate metabolic steps (Taylor, 1970) are *ctr*: carbohydrate uptake catalysed by phosphoenolpyruvate phosphotransferases; *edd*: 6-phosphogluconate dehydratase; *fdp*: fructose 1,6-diphosphatase; *glk*: gluconate kinase; *gnd*: 6-phosphogluconate dehydrogenase; *kga*: phospho-2-keto-3-deoxygluconate aldolase; *pgi*: glucosephosphate isomerase; *pps*: phosphopyruvate synthetase; *uhp*: uptake of hexose phosphates; *usg*: uptake of gluconate; *zwf*: glucose 6-phosphate dehydrogenase. Hatched bars indicate the positions on this map of enzymic lesions in the mutants used; the code numbers describing mutants carrying these lesions (Table 1) are given in square brackets. (Thus, for example, the code number K2.1.7.8 designates a derivative of strain K2 that carries the markers *pps*, *pgi* and *gnd*.)

genes that specify the activity of the dehydratase (*edd*; Peyru & Fraenkel, 1968) and of phospho-2-keto-3-deoxygluconate aldolase (EC 4.1.2.14) (*kga*; Faik *et al.*, 1971), so close to each other on the *E. coli* genome as to be over 95% co-transducible (Faik *et al.*, 1971; Pouysségur, 1971; Fraenkel & Banerjee, 1972), might suggest that these enzymes are controlled as one metabolically regulated unit. However, studies

with *E. coli* mutants defective in a regulator gene have shown that phospho-2-keto-3-deoxygluconate aldolase can be de-repressed under conditions where the 6-phosphogluconate dehydratase is formed in only low activity (Pouysségur & Stoeber, 1972).

Although both the activities of the uptake system for gluconate and of gluconate kinase are, of course, also induced by exposure of *E. coli* to gluconate, this does not necessarily mean that these two activities are regulated co-ordinately with that of either 6-phosphogluconate dehydratase or phospho-2-keto-3-deoxygluconate aldolase. The genes specifying the gluconate uptake system and the kinase(s) have not yet been located on the *E. coli* genome; further, since 6-phosphogluconate cannot be taken up as such by *E. coli*, there is no direct way of determining whether the enzymes of 6-phosphogluconate catabolism (the dehydratase and aldolase) can be induced without induction of the enzymes of 6-phosphogluconate formation [the gluconate uptake system and kinase(s)].

It is the main purpose of this paper to describe experiments with several *E. coli* mutants, defective in a variety of enzymes of carbohydrate metabolism, that show that the enzymes of the Entner–Doudoroff pathway concerned with the catabolism of 6-phosphogluconate can be induced without also inducing the gluconate uptake system and gluconate kinase(s). Our experiments also provide an explanation for the inability of some *E. coli* mutants, blocked in both glucosephosphate isomerase (EC 5.3.1.9) (*pgi*, Scheme 1) and 6-phosphogluconate dehydrogenase activities, to utilize glucose via the Entner–Doudoroff pathway despite their ready utilization of glucose 6-phosphate.

Experimental

Organisms used

All mutants were induced in derivatives of the *E. coli* K12 strain K2.1t (Brice & Kornberg, 1967). The identity and origins of these mutants are listed in Table 1.

Wherever possible, the abbreviations used are those employed by Taylor (1970) and conform to the general recommendations of Demerec *et al.* (1966). Thus, differences in genotype from wild-type characters are designated by italicized three-letter symbols; phenotypic characters are designated by the same three letters but these are not italicized, the first is capitalized, and the abbreviation is followed by '−' or '+'. (For example, the phenotype Pps$^-$ is associated with a mutation in the *pps* locus; the wild-type genotype is restored by the introduction of the *pps*$^+$-allele.)

Growth of cells

Cultures were grown aerobically at 37°C on defined media containing salts (Ashworth & Kornberg, 1966) and a carbon source at 10mM unless otherwise stated; the media were supplemented with L-amino acids as required, at 40–100μg/ml. The absorbance of cell suspensions at 680nm multiplied by 0.68 was taken to equal mg dry wt./ml. (Ashworth & Kornberg, 1966; Kornberg & Reeves, 1972).

Assay of enzymes

Cells were harvested (unless otherwise stated) in the early phase of exponential growth (0.2–0.6mg dry wt./ml) by centrifuging at 20000*g* for 5min at 15°C.

Table 1. *Organisms used in this work*

The genetic symbols used indicate a requirement for *his* = L-histidine, *leu* = L-leucine, *thr* = L-threonine; *str* denotes resistance to streptomycin. Other abbreviations are listed under Scheme 1. Recombinants from genetic crosses were prepared, selected and purified by repeated isolation of single colonies as previously described (Brice & Kornberg, 1967).

Organism	Relevant genetic markers	Mating type	Origin or reference
DF 40	*pgi*	Hfr-C	Fraenkel (1968*a*)
DF 1071	*gnd*	Hfr-C	Fraenkel (1968*a*)
DF 1071-2B	*gnd,kga*	Hfr-C	Faik *et al.* (1971)
AT 2571	—	Hfr (*O-pps-his.*)	Gift from A. T. Taylor (Denver, Col., U.S.A.)
K2.1.5^c.7	*pps,pgi,his,thr,leu,str*	F$^-$	Kornberg (1970)
K2.1.5^c.7.8	*pps,pgi,gnd,thr,leu,str*	F$^-$	[DF 1071 × K2.1.5^c.7]His$^+$ *str*
K2.5^c.7.8	*pgi,gnd,thr,leu,str*	F$^-$	[AT 2571 × K2.1.5^c.7.8]Pyruvate$^+$ *str*
K2.1.5^c.7.8.24	*pps,pgi,gnd,kga*	F$^-$	[DF 1071-2B × K2.1.5^c.7.8]His$^+$ *str*

They were suspended, at approx. 9mg dry wt./ml of suspending buffer, in 50mM-Tris–HCl, pH7.6, to which fresh $FeSO_4$ and 2-mercaptoethanol solutions were then added to final concentrations of 0.8 and 50mM respectively (Pouysségur & Stoeber, 1972). The cell suspensions were disrupted by exposure at 0°C for 30s to the output of an MSE 100W ultrasonic oscillator, operating at maximum peak output of 10mV, and were centrifuged at 20000g for 30min at 0°C. The activities of gluconate kinase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydratase and 6-phosphogluconate dehydrogenase in the supernatant solutions obtained were measured essentially as described by Pouysségur & Stoeber (1972). Owing to the presence of interfering materials in these solutions, it was not possible to measure protein by the usual procedures (Lowry *et al.*, 1951): enzyme specific activities, such as those used in Fig. 2, are thus expressed as the enzymic activity (μmol of substrate reacting/min) per mg dry wt. of cells disrupted.

Assay of 2-keto-3-deoxy-6-phosphogluconate

After the experiment illustrated by Fig. 3, the 50ml cultures were harvested by centrifugation at 20000g for 15min at 0°C. The amounts of 2-keto-3-deoxy-6-phosphogluconate in portions of the supernatant solutions were assayed in two ways. The first procedure was essentially that described by Meloche & Wood (1966) and involved incubating samples of the supernatant solutions with phospho-2-keto-3-deoxygluconate aldolase (contained in extracts of *E. coli* that had not been grown on gluconate and that therefore contained little 6-phosphogluconate dehydratase activity), lactate dehydrogenase and NADH; the decrease in extinction at 340nm was measured with a Unicam SP. 1800 recording spectrophotometer. In the second procedure, the supernatant solutions were incubated with the *E. coli* extract containing phospho-2-keto-3-deoxygluconate aldolase for 30min at 30°C. The pyruvate formed from 2-keto-3-deoxy-6-phosphogluconate was isolated as the 2,4-dinitrophenylhydrazone and identified by paper chromatography as described by El Hawary & Thompson (1953).

Measurement of utilization of ^{14}C-labelled substrates

For the experiment of Fig. 4, sufficient radioactive materials were added to growing cultures to give a final isotopic concentration of 0.2μCi/ml; unlabelled carriers were added to give the desired chemical concentrations. Samples (3ml) were taken at intervals and the cell density was measured in 3ml quartz cuvettes (1cm light-path). A portion (0.5ml) of each suspension was now filtered rapidly, with suction, through a Millipore filter (0.45μm pore size), the filter was washed with 2×5ml of N-free basal salts medium (Ashworth & Kornberg, 1966) at room temperature, and the filter was transferred to 5ml of Bray's (1960) fluid, for measurement of radioactivity in a Packard model 4000 liquid-scintillation spectrometer. For measurement of the removal of labelled substrates from growth media, portions (1ml) were filtered through the Millipore filter into clean test tubes; samples (0.1ml) of these filtrates were transferred to 5ml of Bray's (1960) fluid and assayed for radioactivity.

The uptake of labelled materials by washed suspensions of cells was measured as previously described (Morgan & Kornberg, 1969). All labelled substances were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Results and Discussion

Mutants of *E. coli* devoid of 6-phosphogluconate dehydrogenase activity grow on gluconate as sole carbon source at virtually the same rates as do their wild-type parents (Fraenkel, 1968*a*), which indicates the predominant role of the Entner–Doudoroff pathway in the catabolism of gluconate. Since neither glucose 6-phosphate nor fructose 6-phosphate lie on that pathway, the presence of a further mutation, *pgi*, that renders the cells unable to form phosphoglucoseisomerase, does not affect the utilization of gluconate. However, a *pgi,gnd* double mutant would not be able to catabolize glucose or glucose 6-phosphate except via 6-phosphogluconate and its subsequent dehydration and cleavage by the appropriate enzymes of the Entner–Doudoroff pathway. As shown in Fig. 1, a *pgi,gnd* double mutant of *E. coli*, designated K2.1.5^{c}.7.8, which is also devoid of phosphopyruvate synthetase activity (*pps*), can indeed utilize glucose 6-phosphate for growth, both when this is the major carbon source supplied to cells pre-grown on gluconate (in which 6-phosphogluconate dehydratase and phospho-2-keto-3-deoxygluconate aldolase activities are already fully induced) and when it is supplied to cells pre-grown on fructose [in which the dehydratase is present in very low activity and that of the aldolase is also at its basal, though high (Zablotny & Fraenkel, 1967), activity]. This implies that the 6-phosphogluconate generated internally from glucose 6-phosphate serves respectively to maintain the induction of, or to induce, the appropriate enzymes of the Entner–Doudoroff pathway. In contrast, glucose does not support the growth of this *pgi,gnd* mutant even when it is pre-grown on gluconate, although under the latter conditions there is evidence of a small 'sparing' effect, in that the total yield of cells during growth on 10mM-glucose plus 1mM-gluconate is greater than on the latter alone. A second point requires further comment: whereas there is no obvious 'diauxie' (Monod, 1942) in the growth of this mutant on 1mM-gluconate plus 10mM-

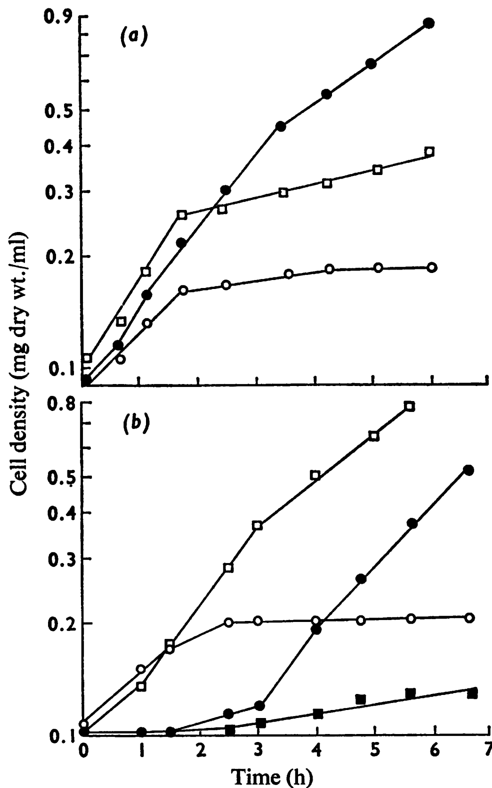


Fig. 1. *Growth of E. coli strain K*2.1.5c.7.8 (*pps,pgi, gnd*) *on gluconate, glucose and glucose 6-phosphate*

The inocula used had been grown on media containing (*a*) 10mM-sodium gluconate and (*b*) 10mM-fructose as sole carbon sources; growth continued on media containing (*a*) 1mM-sodium gluconate only (○); 1mM-sodium gluconate plus 10mM-glucose (□); 1mM-sodium gluconate plus 10mM-glucose 6-phosphate (●); or (*b*) 1mM-fructose only (○); 1mM-fructose plus 10mM-glucose (■); 1mM-fructose plus 10mM-glucose 6-phosphate (●); 1mM-fructose plus 10mM-sodium gluconate (□).

glucose 6-phosphate, there is a lag of over 2h before fructose-grown cells begin to resume growth on a mixture of 1mM-fructose plus 10mM-glucose 6-phosphate. These observations are discussed separately below.

Induction of enzymes of the Entner–Doudoroff pathway

The ability of glucose 6-phosphate and of gluconate to induce enzymes of the Entner–Doudoroff pathway was tested by following the appearance of two enzymes characteristic of gluconate catabolism in the *pgi,gnd* mutant K2.1.5c.7.8 after either glucose 6-phosphate or gluconate had been added to cells growing on fructose. Gluconate kinase activity was assayed, since this enzyme is essential for gluconate catabolism by *E. coli*; 6-phosphogluconate dehydratase activity was measured because this enzyme is the first enzyme specific to the Entner–Doudoroff pathway. Glucose 6-phosphate dehydrogenase activity was also assayed as an internal standard, although it was known (Fraenkel & Banerjee, 1971) that this other enzyme of 6-phosphogluconate formation is constitutively synthesized by *E. coli*. No attempts were made to measure phospho-2-keto-3-deoxygluconate aldolase activity; it has been established that, although this enzyme is particularly abundant in extracts of cells grown on gluconate or hexuronates (Pouysségur & Stoeber, 1971), it is also formed in high activity by *E. coli* under all other growth conditions (Zablotny & Fraenkel, 1967).

No gluconate kinase activity was detected in ultrasonic extracts of the fructose-grown cells used. However, this enzymic activity rapidly appeared after continued growth of the mutant in the presence of gluconate: as shown in Fig. 2, the differential rate of synthesis (Monod, 1956) of this enzyme was high and constant with increase in cell mass. In contrast, there was only a small increase in gluconate kinase activity during growth in the presence of glucose 6-phosphate. The total activity of the dehydratase, which was low though readily measurable in fructose-grown cells, also greatly increased during growth in the presence of gluconate (Fig. 2).

Although the addition of glucose 6-phosphate had not stimulated gluconate kinase synthesis, it had a marked effect on the rate of 6-phosphogluconate dehydratase formation. This effect was ascribable to the formation (and, presumably, accumulation) of 6-phosphogluconate from glucose 6-phosphate in the double mutant: the addition of glucose 6-phosphate to fructose-grown strain K2.1.5c.7, which is devoid of glucosephosphate isomerase but which contains 6-phosphogluconate dehydrogenase and in which 6-phosphogluconate would be rapidly oxidized via this enzyme, did not induce dehydratase synthesis.

Washed suspensions of fructose-grown cells took up very little ^{14}C when incubated with 0.05mM-[^{14}C]gluconate under the experimental conditions described by Morgan & Kornberg (1969). This low rate of gluconate uptake was markedly stimulated by exposure to, and subsequent growth on, gluconate, but was not increased by growth in the presence of glucose 6-phosphate (P. Faik & H. L. Kornberg, unpublished work). It must therefore be concluded that the intracellular generation of 6-phosphogluconate from glucose 6-phosphate, which clearly can induce the synthesis of its dehydratase, does not induce either the kinase that catalyses its formation from gluconate or the system that effects the entry of gluconate, from low external concentrations, into the cells.

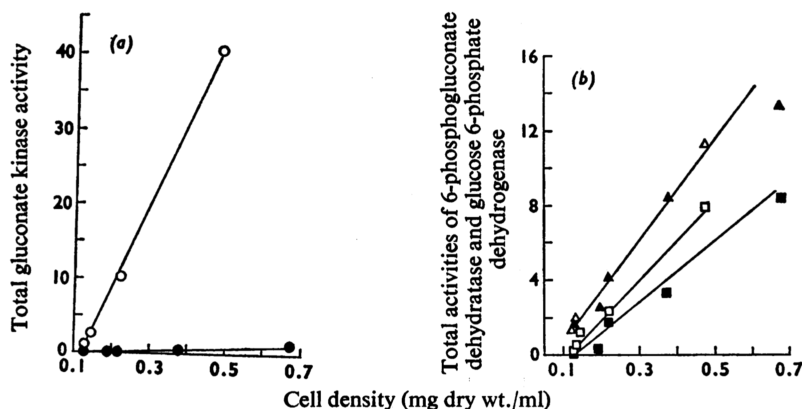


Fig. 2. *Induction of enzymes of* 6-*phosphogluconate metabolism by gluconate and by glucose* 6-*phosphate*

Cultures of the fructose-grown *pgi,gnd* mutant K2.1.5^c.7.8 were suspended to approx. 0.1 mg dry wt./ml in fresh growth media containing either 10 mM-gluconate (open symbols) or 10 mM-fructose (closed symbols) as carbon source. Samples of the culture on gluconate were taken immediately and at intervals after growth at 37°C; all such samples were transferred to chloramphenicol (final concentration 100 μg/ml) and were assayed as described in the Experimental section. After one generation at 37°C, glucose 6-phosphate to 5 mM was added to the culture on fructose; samples were taken thereafter and treated as for the gluconate culture. The total activities (defined as the product of the cell density and the enzyme activities/mg dry wt. of cells) of (*a*) gluconate kinase (○,●) and of (*b*) 6-phosphogluconate dehydratase (□,■) and glucose 6-phosphate dehydrogenase (△,▲) are plotted against the cell densities at which the samples were taken, as a measure of the rates of induced synthesis of these enzymes (Monod, 1956).

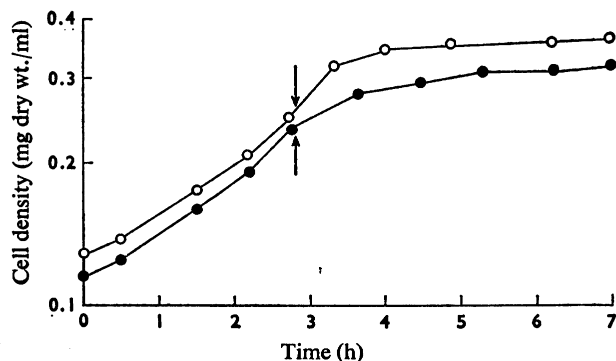


Fig. 3. *Effect of glucose* 6-*phosphate* (●) *and gluconate* (○) *on the growth of the E. coli mutant K*2.1.5^c.7.8.24 (*pgi,gnd,kga*) *on a medium containing glycerol as sole carbon source*

The inhibiting materials were added after 2.75 h (at arrows). After 7 h, the cells were removed by centrifugation and the residual media were analysed for the presence of 2-keto-3-deoxy-6-phosphogluconate (see the text).

Further evidence for the induction of 6-phosphogluconate dehydratase after addition of glucose 6-phosphate to cultures growing on fructose is provided by an experiment in which a triple mutant was used. This organism, K.2.1.5^c.7.8.24, carries the genetic markers *pgi,gnd* and *kga*: it lacks not only glucose-phosphate isomerase and 6-phosphogluconate dehydrogenase activities but is also devoid of phospho-2-keto-3-deoxygluconate aldolase. It is known that the addition of gluconate to cultures of mutants devoid of this aldolase speedily arrests their growth (Fig. 3): this is a consequence of the formation and

accumulation of 2-keto-3-deoxy-6-phosphogluconate (Faik *et al.*, 1971; Fradkin & Fraenkel, 1971; Pouysségur, 1971). If the 6-phosphogluconate arising from the oxidation of glucose 6-phosphate were also to induce dehydratase activity, then addition of glucose 6-phosphate to cultures of *pgi,gnd,kga* mutants should also arrest their growth and lead to the accumulation of 2-keto-3-deoxy-6-phosphogluconate. Such is found to be the case. As shown in Fig. 3, both gluconate and glucose 6-phosphate arrest the growth on glycerol of the triple mutant, which, unlike otherwise similar but Kga$^+$-mutants (Fig. 1), does not recover from this inhibition. When the media collected at the end of the experiment shown in Fig. 3 were incubated with an extract containing phospho-2-keto-3-deoxygluconate aldolase, and the products that reacted with 2,4-dinitrophenylhydrazine hydrochloride were extracted and chromatographed in the solvent system of El Hawary & Thompson (1953), pyruvate was found to be present both in the medium to which gluconate has been added and in that to which glucose 6-phosphate had been added. Quantitative analysis confirmed that 2-keto-3-deoxy-6-phosphogluconate had been formed to 0.36mM in the former, and to 0.13mM in the latter, conditions. None was found in the medium obtained from a culture of the triple mutant grown on glycerol as sole carbon source.

Preferential use of glucose 6-phosphate

It is apparent from Fig. 1 that glucose 6-phosphate is used preferentially to fructose, since continued growth of fructose-grown cells on 1mM-fructose plus 10mM-glucose 6-phosphate appears to be inhibited until the enzymes required for glucose 6-phosphate utilization by the double mutant have been induced. The implication of this observation, that glucose 6-phosphate interferes with the continued utilization of fructose, is borne out by measurement of the rates at which [^{14}C]fructose and [^{14}C]glucose 6-phosphate are incorporated by cells growing on media initially containing only fructose but to which glucose 6-phosphate is added after one doubling of the cells. As shown in Fig. 4, the addition of unlabelled glucose 6-phosphate to a culture of the *pgi,gnd* mutant K2.1.5^c.7.8 growing on [^{14}C]fructose sharply decreases the rate at which radioactive material is removed from the growth medium and is incorporated into the growing cells. Similarly, the rate at which [^{14}C]glucose 6-phosphate, added to cultures of the organisms growing on unlabelled fructose is removed from the mixture and is incorporated into the cells is so high, and so closely approximates to the rate observed during growth on [^{14}C]fructose as sole carbon source, as to indicate that the presence of glucose 6-phosphate largely prevents the further utilization of fructose. This preferential use of glucose 6-phosphate is not confined to mutants lacking 6-phosphogluconate dehydrogenase activity: a culture of the Gnd$^+$ mutant K2.1.5^c.7, in which the Entner–Doudoroff pathway is not operative during growth on glucose 6-phosphate, was found to behave identically. Indeed, this sudden switch from the utilization of fructose to that of glucose 6-phosphate by *E. coli* growing on fructose has been previously described for wild-type cells: it is a manifestation of the inhibition by hexose phosphates of the entry of fructose into *E. coli* (Kornberg, 1972, 1973).

That *pgi,gnd* mutants grow on glucose 6-phosphate at all calls into question the physiological significance of the observation (Fraenkel, 1968*b*) that high concentrations of glucose 6-phosphate and 6-phosphogluconate inhibit fructose 1,6-diphosphatase (EC

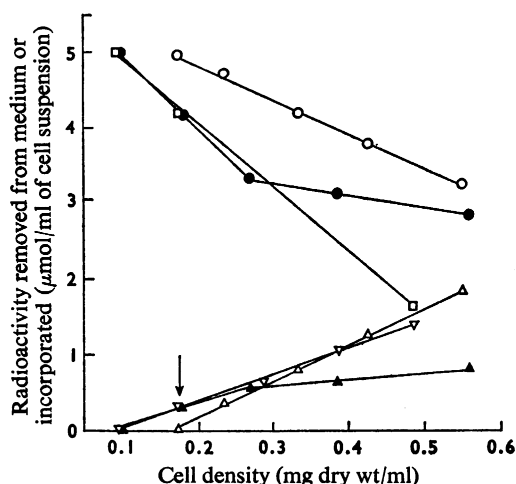


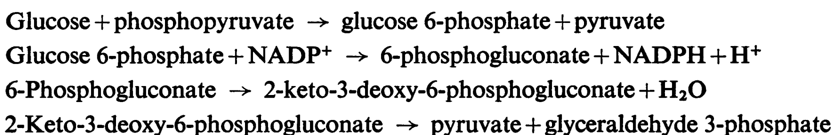
Fig. 4. *Use of glucose 6-phosphate in preference to fructose by the fructose-grown E. coli mutant K2.1.5^c.7.8 (pgi,gnd)*

The rates of utilization of fructose and glucose 6-phosphate, supplied either alone or in presence of each other, by *E. coli* strain K2.1.5^c.7.8 were compared as follows. One culture grew on 10mM-[U-^{14}C]fructose as sole carbon source; the incorporation of ^{14}C into the cells (▽) and its removal from the medium (□) are plotted against the increase in cell mass. To a second culture, identical with the first, were added (at arrow) unlabelled glucose 6-phosphate to 5mM; the incorporation of ^{14}C into the cells (▲) and its removal from the medium (●) was measured. A third culture, growing on unlabelled 10mM-fructose, received [U-^{14}C]glucose 6-phosphate to 5mM at the point indicated by the arrow; the incorporation of ^{14}C from this source (△) and its removal from the medium (○) are plotted in the same manner.

3.1.3.11). It is apparent from Scheme 1 that, in mutants lacking 6-phosphogluconate dehydrogenase activity, the fructose 6-phosphate required for the synthesis of cell wall amino sugars, for example, can arise from 6-phosphogluconate only by a process in which fructose 1,6-diphosphatase plays a necessary role. Since the induction of the appropriate enzymes of the Entner–Doudoroff pathway during growth on glucose 6-phosphate involves the presence of that sugar phosphate as well as the maintenance in the cells of inducing concentrations of 6-phosphogluconate, the escape from the lag and subsequent growth illustrated in Fig. 1 show that fructose 1,6-diphosphatase must function under these conditions.

Reluctant use of glucose

The inability of the *pgi,gnd* double mutant to grow readily on glucose contrasts with the ease with which glucose 6-phosphate is used as carbon source, and implies that some barrier exists to the entry of glucose into the cells and its phosphorylation to glucose 6-phosphate. It is now well established (Roseman, 1969, 1972) that the entry and phosphorylation of glucose are effected by the same system, in which phosphopyruvate serves as phosphate donor. The sequence of reactions whereby external glucose enters the *pgi,gnd* mutant and is catabolized via the Entner–Doudoroff pathway (Scheme 1) may thus be written:

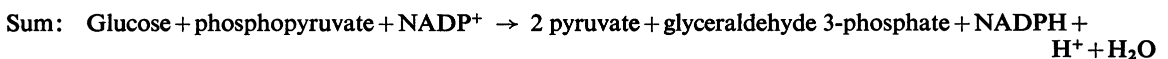


Glucose + phosphopyruvate → glucose 6-phosphate + pyruvate

Glucose 6-phosphate + $NADP^+$ → 6-phosphogluconate + NADPH + H^+

6-Phosphogluconate → 2-keto-3-deoxy-6-phosphogluconate + H_2O

2-Keto-3-deoxy-6-phosphogluconate → pyruvate + glyceraldehyde 3-phosphate



Sum: Glucose + phosphopyruvate + $NADP^+$ → 2 pyruvate + glyceraldehyde 3-phosphate + NADPH + H^+ + H_2O

This emphasizes the need for phosphopyruvate to be available if glucose is to be able to enter the metabolic pathways of its catabolism. However, the only products of the sequence are pyruvate, which in *E. coli* can re-form phosphopyruvate only if phosphopyruvate synthetase (Cooper & Kornberg, 1967) is present, and glyceraldehyde 3-phosphate, which per mol unit yields an equivalent amount of phosphopyruvate via enzymes of glycolysis. Since the *pgi,gnd* mutant K2.1.5^c.7.8 used was devoid of phosphopyruvate synthetase activity (Table 1), the catabolism of one mol unit of glucose would yield only the one mol unit of phosphopyruvate that is necessary for glucose uptake. There is strong evidence that, for growth on carbohydrates, phosphopyruvate must in addition be available to act as an anaplerotic CO_2 acceptor, and that pyruvate cannot be carboxylated by *E. coli* to serve this purpose (Ashworth & Kornberg, 1966; Cánovas & Kornberg, 1966; Kornberg, 1966). The failure of *pps,pgi,gnd* mutants to grow on glucose may be thus ascribed to an insufficiency of phosphopyruvate. This explanation is similar to that advanced (Kornberg & Smith, 1970) to account for the failure of *E. coli* mutants devoid of phosphofructokinase (EC 2.7.1.11) to grow on glucose though growth on glucose 6-phosphate occurs readily. It has been tested in two ways.

In contrast with the behaviour of the *pps,pgi,gnd* mutant K2.1.5^c.7.8, the growth of which on fructose is arrested for at least 4h by the addition of glucose (Figs. 1 and 5), the growth on fructose of the *pgi,gnd* mutant K2.5^c.7.8, to which phosphopyruvate synthetase activity had been restored by the introduction of the *pps*$^+$ allele, was only transiently inhibited by glucose (Fig. 5). Subsequent growth on the mixture of glucose and fructose occurred with a doubling time of about 2.5h, which was only slightly slower than the doubling time of this mutant on glucose 6-phosphate (2.1h). Extracts of this mutant, after growth on fructose in the presence of glucose, were still devoid of phosphoglucose isomerase and 6-phosphogluconate dehydrogenase activities, but contained considerable 6-phosphogluconate dehydratase activity. They did not contain significant gluconate kinase activity. Since it is known that glucose is used in preference to fructose by *E. coli* (Kornberg, 1972, 1973), this shows that the acquisition of the ability to form more phosphopyruvate from the products of glucose catabolism also overcomes the reluctance of *pgi,gnd* mutants to use glucose as growth substrate; under these circumstances, the onset of glucose catabolism is also accompanied by the induction of 6-phosphogluconate dehydratase but not of gluconate kinase.

A second indication of phosphopyruvate insufficiency is provided by measurement of the rates at which washed suspensions of malate-grown K2.1.5^c.7.8 take up 0.1mM-[^{14}C]glucose. As shown in Fig. 6, the rate of [^{14}C]glucose uptake by the cells is low, and is not stimulated by the addition of unlabelled pyruvate. However, the addition of unlabelled L-malate, which yields phosphopyruvate by oxidation to oxaloacetate and subsequent decarb-

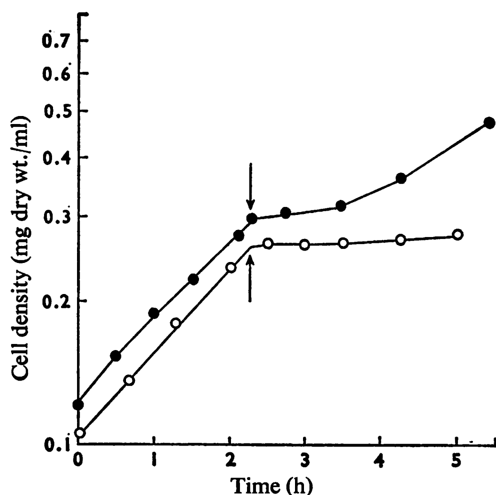


Fig. 5. *Effect of glucose on the growth on fructose of E. coli pgi,gnd mutants K2.1.5^c.7.8 (Pps$^-$) and K2.5^c.7.8 (Pps$^+$)*

After approx. two doublings on 10mM-fructose, 5mM-glucose was added, at the point marked by the arrow, to the cultures of the Pps$^-$ (○) and the Pps$^+$ (●) mutants.

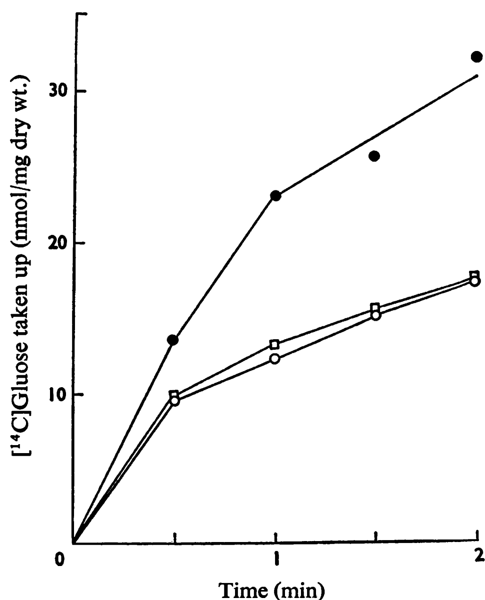


Fig. 6. *Uptake of [U-^{14}C]glucose by washed suspension of the E. coli pps,pgi,gnd mutant K2.1.5^c.7.8*

The cells, grown on L-malate as carbon source, were incubated at 0.68mg dry wt./ml for 15min at 30°C in salts medium either (○) alone, or together with (□) 1mM-sodium pyruvate or (●) 1mM-sodium L-malate, before addition of 0.1mM-[U-^{14}C]glucose.

oxylation by the ATP-linked oxaloacetate carboxykinase (phosphopyruvate carboxykinase) (EC 4.1.1.32), greatly stimulated the rate and extent at which [^{14}C]glucose was taken up by the mutant. Since it is known (Kornberg & Smith, 1970) that pyruvate stimulates [^{14}C]glucose uptake only if the organism contains the allele specifying functional phosphopyruvate synthetase, these observations provide further evidence that glucose cannot readily enter the K2.1.5^c.7.8 mutant used because there is insufficent phosphopyruvate for this purpose.

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