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Glucose and Gluconate Metabolism in an *Escherichia coli* Mutant Lacking Phosphoglucose Isomerase

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A single gene mutant lacking phosphoglucose isomerase (*pgi*) was selected after ethyl methane sulfonate mutagenesis of *Escherichia coli* strain K-10. Enzyme assays revealed no *pgi* activity in the mutant, whereas levels of glucokinase, glucose-6-phosphate dehydrogenase, and gluconate-6-phosphate dehydrogenase were similar in parent and mutant. The amount of glucose released by acid hydrolysis of the mutant cells after growth on gluconate was less than 2% that released from parent cells; when grown in the presence of glucose, mutant and parent cells contained the same amount of glucose residues. The mutant grew on glucose one-third as fast as the parent; it also grew much slower than the parent on galactose, maltose, and lactose. On fructose, gluconate, and other carbon sources, growth was almost normal. In both parent and mutant, gluconokinase and gluconate-6-phosphate dehydrase were present during growth on gluconate but not during growth on glucose. Assay and degradation of alanine from protein hydrolysates after growth on glucose-*1*-^{14}C and gluconate-*1*-^{14}C showed that in the parent strain glucose was metabolized by the glycolytic path and the hexose monophosphate shunt. Gluconate was metabolized by the Entner-Doudoroff path and the hexose monophosphate shunt. The mutant used glucose chiefly by the shunt, but may also have used the Entner-Doudoroff path to a limited extent.

The properties of a mutant of *Salmonella typhimurium* deficient in a central enzyme of glycolysis, phosphoglucose isomerase, have been described previously (8,9). This mutant grew on glucose about one-fifth as fast as the parent strain, apparently largely by using glucose via the hexose monophosphate shunt. Enzymatic and isotopic data suggested that both parent and mutant strains used the shunt at about the same rate; thus, this pathway appeared to be nonexpandable and, indeed, to limit the growth rate of the mutant. Nevertheless, both parent and mutant grew at similar, high rates on gluconate, although this compound had been thought to be metabolized only via the shunt. However, isotopic experiments and measurement of enzymes (8) showed that gluconate was metabolized in *S. typhimurium* largely via the Entner-Doudoroff path (6); gluconokinase and the first enzyme of the Entner-Doudoroff path, gluconate-6-phosphate dehydrase were found to be induced by gluconate but not by glucose. Thus, when gluconate-6-phosphate arose from glucose its metabolism appeared to be restricted to the shunt, whereas when it arose from gluconate it was able to use the Entner-Doudoroff pathway (Fig. 1 shows the relevant pathways).

We have now also selected mutants of *Escherichia coli* lacking phosphoglucose isomerase. In this paper, we describe the properties of one such mutant. We have paid particular attention to the role of the Entner-Doudoroff path in gluconate and in glucose metabolism, partly because Loomis and Magasanik (12), in experiments with *E. coli* K-12 and a mutant lacking phosphoglucose isomerase derived from it (10), obtained results suggesting that the Entner-Doudoroff path may have a major role in glucose, as well as in gluconate metabolism. Our results differ from theirs in certain respects, and show that the pathways of glucose and gluconate metabolism in *E. coli* are similar to those of *S. typhimurium*.

MATERIALS AND METHODS

Chemicals. Gluconate-*1*-^{14}C was from Nuclear-Chicago Corp., Des Plaines, Ill. Glucose-*1*-^{14}C, L-alanine-*U*-^{14}C and DL-alanine-*1*-^{14}C were from New England Nuclear Corp., Boston, Mass. 2-Keto-3-deoxygluconate-6-phosphate was a generous gift from W. A. Wood. Sodium gluconate was from Eastman Organic Chemicals, Rochester, N.Y., and contained

<0.05% glucose. Glucose-6-phosphate dehydrogenase and lactic dehydrogenase were from Boehringer Mannheim Corp., New York, N.Y.; other biochemicals were from Boehringer or from Sigma Chemical Co., St. Louis, Mo.

Assays. Glucose was measured with glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N.J.). Protein was measured by the Folin method (14) with bovine plasma albumin as a standard, corrected for moisture content (11).

Enzyme assays. Cell-free extracts were prepared from 250- or 500-ml cultures. The cells were collected by centrifugation, washed once with 0.9% NaCl, and resuspended in 1% of the original volume of buffer containing 0.01 M tris(hydroxymethyl)aminomethane (Tris) chloride, 0.01 M $MgCl_2$, and 0.001 M dithiothreitol (*p*H 7.8). These suspensions were treated for 1 min per ml with an MSE Ultrasonicator, and then were centrifuged at 17,000 × *g* (maximum) for 30 min; the pellets were discarded. Incubation mixtures for all assays contained 0.05 M Tris chloride and 0.01 M $MgCl_2$ (*p*H 7.6). The nicotinamide adenine dinucleotide phosphate (NADP)-linked direct spectrophotometric assays were done with 1-ml final volumes, and contained 0.2 mM NADP. The other additions were as follows: for glucokinase, 0.5 mM glucose, 2mM adenosine triphosphate (ATP), and 1 μg of glucose-6-phosphate dehydrogenase (Boehringer Mannheim Corp.); for phosphoglucose isomerase, 0.4 mM fructose-6-phosphate and 1 μg of glucose-6-phosphate dehydrogenase; for glucose-6-phosphate dehydrogenase, 0.6 mM glucose-6-phosphate; for gluconate-6-phosphate dehydrogenase, 0.4 mM gluconate-6-phosphate; and for gluconokinase, 0.5 mM sodium gluconate and 2 mM ATP. 2-Keto-3-deoxygluconate-6-phosphate (KDGP) aldolase was measured as substrate-dependent reduced nicotinamide adenine dinucleotide ($NADH_2$) oxidation in a mixture with a total volume of 0.2 ml containing the usual buffer, 0.15 mM KDGP (barium salt), 0.1 mM $NADH_2$, and 1 μg of lactic dehydrogenase. In all these assays, the reactions were started by the addition of extract, and the change in absorption at 340 mμ was followed in a Gilford Model 2000 recording spectrophotometer with the cell chamber kept at 25 C. With one exception, the reactions were proportional to the amount of extract and were linear with time for several minutes. The exception was gluconate-6-phosphate dehydrogenase, whose rate fell as much as 40% in the first 2 min, possibly because of inactivation; for this enzyme, initial rates are given.

Gluconate-6-phosphate dehydrase was measured at room temperature in a two-step assay, as previously described (8). This assay depends on the excess of KDGP aldolase present in the extract.

Organisms and media. The HFr strain of *E. coli* K-12, called K-10, was used as the parent strain. Minimal medium 63 (8) was supplemented with thiamine hydrochloride (1 μg/ml) and the carbon source (4 mg/ml). The broth medium was 63 supplemented with 1% tryptone (Difco) and 0.4% glucose. Solid media contained 2% agar. For glucose tetrazolium indicator plates (16), 25.5 g of Antibiotic Medium No. 2 (Difco) and 50 mg of 2,3,5-triphenyl

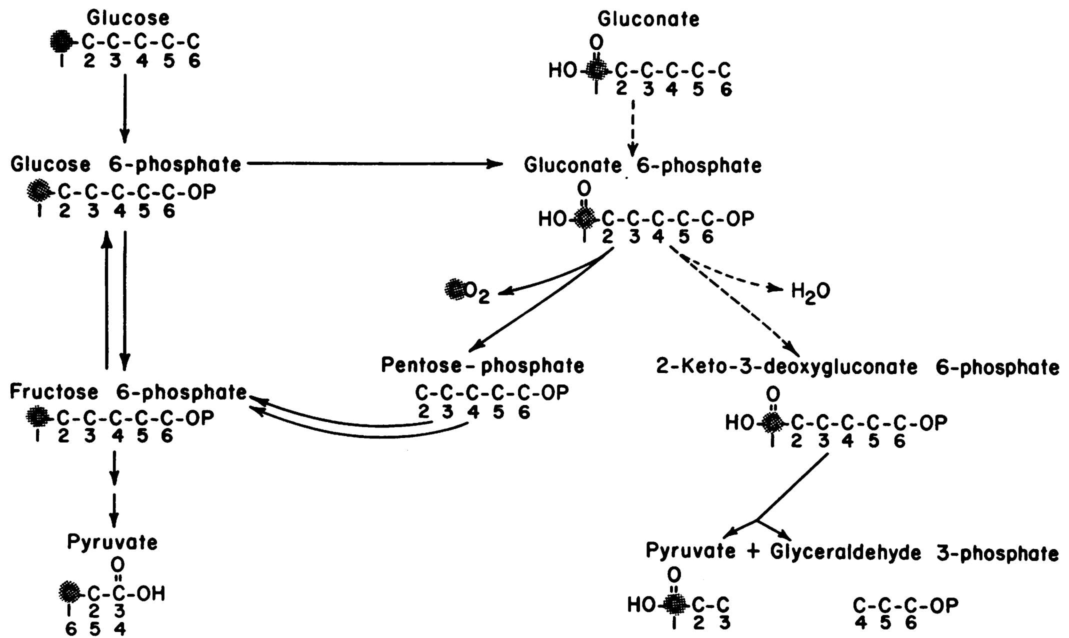

FIG. 1. *Pathways of glucose and gluconate metabolism. This figure outlines the major pathways of glucose and gluconate metabolism in Escherichia coli. Dashed arrows represent reactions known to be catalyzed by inducible enzymes. The distribution of the carbon atoms of glucose-1-^{14}C and gluconate-1-^{14}C are also sketched. The numbering is of the carbon atoms in the parent compounds.*

tetrazolium chloride (Sigma Chemical Co.) were dissolved in 950 ml of water and autoclaved for 30 min at 120 C; 50 ml of 20% glucose was added. Cultures were always incubated at 37 C.

Growth rates. Flasks (125-ml) fitted with side arms 14 mm in diameter and baffles on the bottom to increase aeration were used with 10 ml of culture, shaking on a New Brunswick Gyrotory water bath shaker at 37 C, at 220 cycles per min of 1.1-cm excursion. Turbidity was followed on a Lumetron colorimeter model 401 with a 580 filter; the readings were corrected for their deviation from proportionality to bacterial concentration and were translated to bacterial dry weight on the basis of a dry-weight determination of a measured quantity of K-10 in logarithmic growth on glucose. The turbidity range in growth rate experiments was 0.025 to 0.75 mg (dry weight) per ml. Growth rates were determined from the slopes of plots of bacterial dry weight (on a logarithmic scale) versus time.

Glucose utilization. Cultures were grown in a medium containing, initially, 2 mg of glucose per ml. Samples (0.5 ml) were immediately frozen and were later thawed and centrifuged in the cold to remove cells. Glucose was assayed on portions of the supernatant fluid, and the values obtained were plotted against dry weight as determined from the turbidity measurements.

Glucose content of hydrolysates. Strains K-10 and L40 were inoculated in dilutions of 1:500 and grown to stationary phase in minimal media containing (i) 0.4% sodium gluconate and (ii) 0.4% sodium gluconate plus 0.4% glucose. The cells were harvested and washed three times with water. Portions corresponding to 50 mg (dry weight) were suspended in 1.0 ml of 1.0 N HCl; the tubes were closed with rubber stoppers and placed in a boiling-water bath for 3 hr. The debris was separated by centrifugation, the pellets were washed once with 1 ml of water, and the combined supernatant fluid and wash were neutralized with 10 N KOH. Glucose was assayed on portions of these hydrolysates. In an internal control, when 1 mg of glucose was added before hydrolysis to a sample of L40 grown on gluconate, 96% was recovered. Also, the quantities of hydrolysate used did not inhibit the glucose assay.

Isolation and degradation of alanine. Amounts of 0.01 ml from overnight cultures were inoculated in 25 ml of minimal medium containing 15.2 μmoles of glucose-*1*-^{14}C or gluconate-*1*-^{14}C per ml and incubated at 37 C on a shaker until fully grown. The cells (11 to 21 mg, dry weight, total) were harvested by centrifugation after acidification to 0.14 N trichloroacetic acid. Protein was prepared from them by the fractionation procedure of Roberts et al. (15). The protein fractions were hydrolyzed in 1.5 ml of 6 N HCl in sealed tubes at 109 C for 14 hr. The small amounts of debris were removed by centrifugation and washed with water; the washes and supernatant fluids were combined and taken to dryness several times in vacuo over solid KOH and concentrated H_2SO_4. Alanine was isolated from these hydrolysates by paper chromatography on Whatman no. 1 paper, descending, 19 hr by use of the *meta*-cresol-water system in an atmosphere of 1% NH_3 (3). Standards were located with a ninhydrin spray, and radioactivity in the samples was located with a Packard 4π chromatogram scanner. The band corresponding to alanine was eluted and chromatographed in a second system, *n*-butanol-acetic acid-water, 65:15:25 (18), Whatman no. 1 paper, descending, 12 hr. Alanine was eluted from the paper with 0.01 M acetic acid; its concentration was determined by the quantitative ninhydrin method of Troll and Cannon (18), and its radioactivity was determined in Bray's solution (2).

Radioactivity in the carboxyl group of alanine was determined by decarboxylation in the presence of carrier by the quantitative ninhydrin method done in a closed system, as follows. To a Thunberg tube 21 mm in diameter were added 0.1 ml (ca. 0.1 μmole) of radioactive sample, 0.05 ml of L-alanine-^{12}C (10 μmoles/ml), 0.8 ml of the KCN-pyridine reagent (18), and 0.8 ml of 80% phenol (18). After chilling in ice, 0.2 ml of cold ninhydrin (50 mg/ml in ethyl alcohol) was quickly added, and the top, whose side arm contained 0.5 ml of 1 N KOH, was put on. The system was evacuated with a water pump and sealed when the liquid in the trap started to bubble (the reaction mixture being still yellow at this point). The reaction was started by putting the bottom 2.5 cm of the tube in a boiling-water bath. After 5 min, the tube was removed from the bath, and allowed to cool for a few minutes with occasional swirling to promote CO_2 trapping. The content of the trap, with two water washes, was transferred to a test tube, and the carbonate was precipitated by addition of 1 ml of saturated $Ba(OH)_2$. The precipitate was collected on B-6 membrane filters (Schleicher & Schuell Co., Keene, N.H.) and washed twice with 1 ml of water. The filters were dried for 15 min under a heat lamp, and their radioactivity was determined in a toluene scintillation system containing 2,5-diphenyloxazole (4 g per liter) and *p*-bis[2-(5-phenyloxazolyl)]-benzene (50 mg per liter). Radioactivity was measured with a Nuclear-Chicago Mark I scintillation counter equipped with an external standard which allowed all activities of samples counted in solution to be corrected to disintegrations per minute (dpm).

RESULTS

Selection of mutants lacking phosphoglucose isomerase. Both the *S. typhimurium* (9) and the *E. coli* (10) mutants lacking phosphoglucose isomerase were first recognized as abnormal galactose-negative mutants. Our "rational" isolation of such mutants was based on the assumption that they would likely be negative or pseudonegative on glucose indicator plates, and on minimal medium might show the growth pattern characteristic of the *Salmonella* mutant—slow growth on glucose (or galactose or maltose) and normal growth on carbon sources which are not metabolized via glucose-6-phosphate (fructose, gluconate, or glycerol).

E. coli strain K-10, 7 × 10^7 viable cells per ml in medium 63 was treated with 0.035 volumes of ethyl methane sulfonate (13) at 37 C for 20 min,

and dilutions were plated on glucose-tetrazolium plates. (On such plates, wildtype "fermentations" give pale colonies, whereas mutants are varying shades of red.) The viable count did not decrease significantly with mutagenesis, but the indicator plates from the mutagenized culture contained a small proportion of red colonies of various types. One hundred of these were purified and streaked on glucose, galactose, fructose, and gluconate minimal plates. Several classes of mutants could be recognized: some grew normally on glucose-minimal medium, others failed to grow on minimal medium at all, and yet others grew normally on some media but not on others. Among the latter were two, L40 and L93, which showed the same pattern previously seen with *Salmonella pgi* mutants. Enzyme assays on extracts prepared from cultures grown to stationary phase in broth showed that the parent strain had a phosphoglucose isomerase activity of 600 mμmoles per min per mg of protein, whereas L40 and L93 had activities of 2 and <1, respectively. Studies of revertants and mapping experiments with these strains show that they are both single gene mutations (7). In accord with the convention suggested by Demerec et al. (5) the mutant genes in these isolates will be called *pgi-2* and *pgi-3*. L40 (*pgi-2*) was chosen for further study.

Growth studies. Strains K-10 and L40 were inoculated on a variety of minimal media to give about 50 colonies per plate. As Table 1 shows, L40 formed substantially smaller colonies than K-10 on glucose, galactose, maltose, and lactose; on the other media, the colony sizes were more similar, though the mutant generally formed slightly smaller colonies than the parent strain.

Table 2 shows the growth rates of the two strains in liquid minimal media. The data are given from several determinations because the behavior of L40 on glucose was unusually variable. The cause of this variation is not known. In several experiments, L40 was subcultured repeatedly in glucose minimal medium, and the doubling times always increased, for example, from 144 min initially to 195 min after 16 generations. Further subcultures usually gave outgrowth of revertants. In a control with gluconate instead of glucose, the initial doubling time was 80 min, which after 24 generations was down to 64 min. Each single cycle of growth in such an experiment looked logarithmic. We conclude that L40 grows from one-half to one-third as fast as K-10 on glucose, slightly more slowly than K-10 on gluconate, and at an equal rate on fructose. The slow growth of the mutant on glucose is characterized by slow glucose utilization, since the efficiency of glucose utilization is similar in mutant and parent (Table 3).

Glucose residue content. Phosphoglucose isomerase has two roles: in growth on glucose, it must function in the direction of the conversion of glucose-6-phosphate to fructose-6-phosphate; in growth on gluconate or glycerol, on the other hand, it must function to convert fructose-6-phosphate to glucose-6-phosphate, which is a necessary step in the biosynthesis of several sugars

TABLE 1. *Colony size on several carbon sources*[a]

Carbon source	Diam of colonies (mm)	
	Parent strain (K-10)	Mutant strain (L40)
Glucose	>2	0.5
Galactose	2.0	0.3
Lactose	1.8	—[b]
Maltose	1.5	0.3
Acetate	0.4	0.35
Arabinose	1.8	1.8
Fructose	1.4	1.3
Gluconate	2.0	1.3
Glycerol	1.2	0.9
Succinate	1.3	1.1

[a] Cultures were spread on minimal medium plates so as to give ca. 50 colonies per plate, and average colony size was estimated after 48 hr of incubation. All sugars, with the exception of (L) arabinose, were of the D configuration.

[b] Colonies on lactose appear with longer incubation.

TABLE 2. *Growth rates in minimal medium*[a]

Doubling time (min)					
Glucose		Fructose		Gluconate	
Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)
68	149	77	74	59	88
63	218	79	76	54	84
65	206		95		
63	121				
	156				
	150				
	177				
Avg: 65	168	78	82	56	86

[a] The cultures were first grown to logarithmic phase in fructose minimal medium, and then were centrifuged and resuspended in minimal medium with the indicated carbon source. Each value represents a separate run. The rates were not different when the inocula were from stationary-phase cultures on fructose.

(e.g., glucose, galactose, and rhamnose) found in polysaccharides. Thus, if strain L40 is indeed deficient in phosphoglucose isomerase activity in vivo, in certain media this should be reflected in its polysaccharide content. Table 4 shows that, when the strains were grown on gluconate, the glucose found in an acid hydrolysate of washed cells in the mutant was about 2% of that in the parent, whereas after growth in the presence of glucose they had similar contents.

Enzymes of glucose and gluconate metabolism. Table 5 presents the activities of some enzymes of glucose metabolism in extracts prepared from cultures harvested from logarithmic growth on glucose, fructose, or gluconate. Phosphoglucose isomerase activity was constitutive in the parent strain, but it was absent in the mutant. The levels of glucokinase, glucose-6-phosphate dehydrogenase, and gluconate-6-phosphate dehydrogenase were similar in parent and mutant strain, and did not vary much with these growth conditions.

The mutant grew on gluconate only slightly more slowly than the parent strain (Table 2). Table 6 shows the levels of some enzymes of gluconate metabolism. Gluconokinase and gluconate-6-phosphate dehydrase were found, in both strains, only after growth on gluconate. KDGP aldolase, the second enzyme of the Entner-Doudoroff pathway, was found in high levels in all media, although there was some increase in the gluconate cultures. Thus, the metabolism of gluconate was effectively inducible by gluconate, since the kinase was inducible; and the metabolism of gluconate-6-phosphate by the Entner-Doudoroff path also would be expected to occur only in the presence of gluconate, since one of its enzymes was induced only in the presence of gluconate.

These results suggest that, in *E. coli*, glucose is metabolized via both phosphoglucose isomerase reaction and the hexose monophosphate shunt, whereas gluconate can be metabolized via the hexose monophosphate shunt and the Entner-Doudoroff pathway. In a *pgi* mutant, there was no evidence from these enzyme assays for derepression of either the hexose monophosphate shunt or the Entner-Doudoroff pathway, and the growth on glucose of such a mutant indeed appeared to be limited somehow by the capacity of the shunt.

TABLE 3. *Growth yields with glucose*[a]

Expt	Cells formed (μg, dry wt) per mg of glucose used	
	Parent strain (K-10)	Mutant strain (L40)
1	302	371
2	338	298
3	308	338
Avg	316	336

[a] The values were determined from plots of glucose in medium versus corrected turbidity (see Materials and Methods).

TABLE 4. *Content of glucose residues*[a]

Medium	Glucose (μg/mg dry wt cells)	
	Parent (K-10)	Mutant (L40)
Gluconate	13.1	0.2
Gluconate and glucose	44	37

[a] Glucose was determined in acid hydrolysates of cultures grown as indicated (see Materials and Methods).

Tracer experiments. The enzymatic data alone do not conclusively show that the Entner-Doudoroff pathway has no role in glucose metabolism, since it is conceivable that gluconate-6-phosphate dehydrase is present, but particularly unstable, in glucose-grown cells. We have therefore used specifically labeled radioactive substrates to independently estimate the use of several pathways in glucose and gluconate metabolism. The Entner-Doudoroff pathway was discovered in *Pseudomonas saccharophila* through experiments showing that gluconate labeled in the C-1 position could give rise to carboxyl-labeled pyruvate (6). Gluconate-*1*-^{14}C used by the hexose monophosphate shunt ought to give unlabeled pyruvate, whereas glucose-*1*-^{14}C used by glycolysis would give methyl-labeled pyruvate (see Fig. 1).

We have, therefore, done experiments which estimate the specific activity of pyruvate and the location of the radioactivity in the pyruvate molecule, during growth of strain K-10 on glucose-*1*-^{14}C and on gluconate-*1*-^{14}C and of strain L40 on glucose-*1*-^{14}C. To estimate the specific activities during growth, rather than in a nonphysiological situation, such as that of the arsenite-treated resting cells (6, 8), we have assumed that alanine comes from the transamination of pyruvate (19) and have isolated alanine from a protein hydrolysate prepared from cells grown for many generations on the various labeled carbon sources. The alanine was then degraded with ninhydrin to determine the proportion of radioactivity in the carboxyl group. The results are presented in Table 7. When K-10 was grown on glucose-*1*-^{14}C, the specific activity of the alanine was one-third that of the substrate, and a very small fraction of the counts in the alanine

were in the carboxyl group. When the same strain was grown on gluconate-*1*-^{14}C, the alanine had a specific activity about half that of the gluconate, and virtually all its counts were in the carboxyl group. When the mutant was grown on glucose-*1*-^{14}C, the alanine had a specific activity 8% that of the substrate; most of these counts were in the carboxyl group.

To interpret these data, it is assumed that pyruvate derived by different routes is sampled uniformly for alanine biosynthesis; i.e., there is no compartmentation. The interpretation is then as follows. Glucose is used by the wild-type strain largely by glycolysis, and partly by the hexose monophosphate shunt. Because only 3% of the counts in alanine are in the carboxyl group, the

TABLE 5. *Some enzymes of glucose metabolism*

Enzyme	Carbon source					
	Glucose		Fructose		Gluconate	
	Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)
Glucokinase	89	101	128	159	81	104
Phosphoglucose isomerase	1,070	<1	1,280	<1	954	<1
Glucose-6-phosphate dehydrogenase	195	229	216	234	143	147
Gluconate-6-phosphate dehydrogenase	109	88	86	91	124	136

[a] Extracts were prepared from aerobic logarithmic phase cultures at 37 C. Enzyme activities are expressed as millimicromoles per minute per milligram of protein.

TABLE 6. *Some enzymes of gluconate metabolism*[a]

Enzyme	Carbon source					
	Glucose		Fructose		Gluconate	
	Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)	Parent (K-10)	Mutant (L40)
Gluconokinase	1	4	1	1	98 (213)[b]	85 (211)[b]
Gluconate 6-phosphate dehydrase	4	6	6	4	130	139
KDGP aldolase	208	96	131	86	370	465

[a] Extracts were prepared from aerobic logarithmic phase cultures at 37 C. Enzyme activities are expressed as millimicromoles per minute per milligram of protein.

[b] Values in parentheses were found when the assay system included 250 μg of protein of an extract from glucose-grown K-10 to supply excess gluconate-6-phosphate dehydrogenase (8).

TABLE 7. *Origin of alanine*[a]

Strain	Carbon source	Specific activity of carbon source	Specific activity of alanine isolated	Per cent dpm in carboxyl group of alanine[b]
		dpm/μmole	*dpm /μmole*	
Parent (K-10)	Glucose-*1*-^{14}C	4.33×10^4	1.47×10^4	3.6, 3.3
Parent (K-10)	Gluconate-*1*-^{14}C	4.27×10^4	1.97×10^4	94, 97
Mutant (L40)	Glucose-*1*-^{14}C	4.33×10^4	0.35×10^4	74, 78

[a] Alanine was isolated from cultures grown on the indicated carbon source; its specific activity and per cent radioactivity in the carboxyl group were determined (see Materials and Methods). When the decarboxylation was done on L-alanine-*U*-^{14}C and *DL*-alanine-*1*-^{14}C, duplicate determinations gave 29.8 and 28.8% recovery of radioactivity from the former, and 89.0 and 96.8% from the latter.

[b] Duplicate determinations.

use of the Entner-Doudoroff path by the wild-type growing on glucose must be very small. The fraction using the shunt dilutes the specific activity of fructose-6-phosphate, and thus the specific activity of the pyruvate derived from glycolysis is lower than the 50% expected had al! metabolism been via glycolysis.

When the wild-type grew on gluconate-*1-*^{14}C, its alanine was highly labeled in the carboxyl group, showing that a major portion of gluconate metabolism must be via the Entner-Doudoroff pathway. (It is not possible to estimate from these data the exact fraction using this pathway, because the extent of triose phosphate conversion to the pyruvate pool is not known.)

Finally, these data show that, when the mutant was grown on glucose-*1-*^{14}C, most of the glucose was metabolized by a pathway involving the loss of the 1-carbon atom: presumably, the hexose monophosphate shunt. However, since the counts in alanine were largely in the carboxyl group, a small fraction probably uses the Entner-Doudoroff path.

DISCUSSION

The experiments reported in this paper have shown that *gluconate* metabolism in *E. coli* K-10 and in a phosphoglucose isomerase mutant derived from it is partly via the hexose monophosphate pathway and partly via the Entner-Doudoroff pathway. Earlier work with *S. typhimurium* (8) led to similar conclusions, which are also in accord with the report of Eisenberg and Dobrogosz on gluconate metabolism in *E. coli* ML30 (Bacteriol. Proc., 1966, p. 77).

Glucose metabolism in *E. coli* K-10 is via both the phosphoglucose isomerase reaction and the hexose monophosphate pathway. The *pgi*-negative mutant uses glucose chiefly by the hexose monophosphate pathway. The possible role of the Entner-Doudoroff pathway in *glucose* metabolism has been studied both by enzyme measurements and isotopic tracer experiments. There is no evidence for this pathway being used any more than very slightly by strain K-10 during growth on glucose. However, the mutant, after growth on glucose-*1-*^{14}C, contained a small but significant amount of radioactivity in the carboxyl group of alanine. According to results obtained with another mutant (20), no exchange reaction between radioactive CO_2 and unlabeled pyruvate would produce this much labeling in alanine, which must therefore be ascribed to minor use, by the mutant, of the Entner-Doudoroff pathway during growth on glucose. According to Table 6, the level of gluconate-6-phosphate dehydrase is very low, in both parent and mutant, during logarithmic growth on glucose. It is not known whether this low level is sufficient to account for the minor use of the Entner-Doudoroff pathway by the mutant.

Indeed, the main discrepancy between our results and those of Loomis and Magasanik (12) concerns the inducibility of gluconate-6-phosphate dehydrase. They used stationary-phase cultures and found substantial activities in glucose-grown cells, whereas we used logarithmic-phase cultures and did not. Several results would be reconciled if it could be shown that gluconate-6-phosphate dehydrase appears only after logarithmic growth on glucose is over. However, in one experiment, we found no activity of this enzyme in a culture of our mutant in stationary phase on glucose. Thus, either it appears late and transiently, or there may be strain differences between derivatives of *E. coli* K-12.

The other differences between our results and Loomis and Magasanik's (12) concern the growth rates of phosphoglucose isomerase-negative mutants. These differences probably are not significant, for two reasons: (i) we found much variability in the growth rate of our mutant, and (ii) the mutant of Loomis and Magasanik, which we confirmed as *pgi*-negative, was derived in two steps (12) and may differ from the parent strain in more than one gene.

Since glucose was utilized with equal efficiency by our *pgi*-negative mutant and by its parent, the slow growth of the mutant reflected slow utilization of glucose, primarily via the hexose monophosphate pathway. It is not known why this pathway can only be used relatively slowly. Perhaps some enzyme is rate-limiting. Another possibility is that regeneration of nicotinamide adenine dinucleotide phosphate is the limiting step. We must also suggest a third factor possibly contributing to slow growth on glucose: the accumulation of some inhibitory metabolite. For, as might be expected from the properties of some other mutants blocked in catabolic pathways after the primary phosphorylation (e.g., references 1 and 4), in strain L40 glucose inhibits growth on fructose (but does not cause stasis). This problem is now being studied.

Further experiments on the role of the Entner-Doudoroff pathway in glucose and gluconate metabolism are presented in one accompanying paper (20), and genetic mapping of the phosphoglucose isomerase locus is presented in another (7).

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