The Accumulation of Glucose 6-Phosphate from Glucose and Its Effect in an Escherichia coli Mutant Lacking Phosphoglucoisomerase and Glucose 6-Phosphate Dehydrogenase*

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SUMMARY

When presented with glucose, an Escherichia coli mutant lacking both phosphoglucoisomerase and glucose 6-phosphate dehydrogenase accumulated, internally, high concentrations (approximately 50 mM) of glucose 6-phosphate. It also accumulated glucose in polysaccharide form. Other metabolism of glucose, if any, was very restricted. The accumulation of glucose 6-phosphate was associated with inhibition of growth on those carbon sources the metabolism of which requires functional fructose diphasphatase activity; inhibition of this enzyme in vitro by high concentrations of glucose 6-phosphate was shown.

The isolation of a double mutant of Escherichia coli which lacks activities of both phosphoglucoisomerase (α-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) and glucose 6-phosphate dehydrogenase (β-glucose 6-phosphate:TPN+ oxidoreductase, EC 1.1.1.49) has been recently reported (1). The present paper is a study of glucose metabolism in this mutant.

The selection of this strain was based on the fact that a single gene mutant lacking phosphoglucoisomerase grows slowly on glucose with the use of the hexose monophosphate shunt (2); if phosphoglucoisomerase and glucose 6-phosphate dehydrogenase were the only productive pathways of glucose 6-phosphate catabolism (Fig. 1), a second mutation blocking the shunt would be expected to produce a strain unable to grow on glucose at all. Such a strain ought to be severely restricted in glucose metabolism, perhaps assimilating glucose only in polysaccharide form. It also might be expected to accumulate glucose 6-phosphate. It is a common observation that a substance, the catabolism of which is incomplete because of a mutational block, may inhibit growth on other substances (examples are reviewed in Reference 3). This inhibition may sometimes be correlated with the accumulation of the substrate of the blocked reaction. It will be shown that glucose did inhibit the growth of the double mutant on certain carbon sources. This inhibition reflected the accumulation of glucose 6-phosphate internally. Furthermore, the specificity of the growth inhibition suggested that the site of inhibition might be the fructose diphasphatase reaction (α-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11), and inhibition of this enzyme in vitro by glucose 6-phosphate was demonstrated.

MATERIALS AND METHODS

Bacterial Strains—Strain K10 (Hfr C) of E. coli, the parental strain of our mutants, is wild type in known sugar metabolism. DF40 (formerly called L40 (2)) is a phosphoglucoisomerase mutant derived from K10. DF2000 is a double mutant, derived from DF40, lacking both phosphoglucoisomerase and glucose 6-phosphate dehydrogenase (1). All three strains are prototrophs.

Media—Minimal salts Medium 63 (4) was always supplemented with 1 μg of thiamine-HCl per ml and the indicated carbon source at 4 mg per ml. Solid minimal media contained 2% agar. Rich medium was Medium 63 supplemented with 1% Bacto-tryptone (Difco Laboratories) and other carbon sources as indicated. All growth experiments were performed with adequate aeration (2) at 37°C. Turbidity was measured on a Lumetron model 401 colorimeter with a 580 filter, and readings were converted to bacterial dry weight with the use of a standard curve (2).

Chemicals—Glucose-U-[14C], from New England Nuclear, was used at 9.9 μC per μmole unless indicated otherwise. Glucose-1-[14C] was from the same source. Carbohydrates were Pentastich products.

Chromatography—Paper chromatography was done with Whatman No. 3MM paper. Solvent I was 1 M ammonium acetate,
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The internal concentration of glucose 6-phosphate was calculated with the assumption that 1 mg, dry weight, represents 4 μl of water. In some experiments the cells were extracted from the filter with a mixture of minimal Medium 63 (10 volumes) and 60% perchloric acid (1 volume); after 30 min in an ice bath KOH was added to neutralize the acid, and, after centrifugation, glucose 6-phosphate was assayed as above. The two extraction methods gave similar results, but the second one is less convenient as the final fraction may be slightly turbid. If, instead of filtration, portions of a culture were directly boiled or acidified, glucose 6-phosphate recovered was about the same as the sum of the cell soluble pool and filtrate fraction determined above, thus, during filtration, the pool content was not notably changed. When glucose 6-phosphate was added to the culture immediately before extraction (without filtration), 89% was recovered. Further extractions with water of once extracted cells did not release more than 5% additional glucose 6-phosphate.

It should be noted that the extraction methods used were developed with the use of cultures of strain DF2000 which had accumulated much glucose 6-phosphate, a particularly stable metabolite. They are completely inadequate for determination of the low levels of glucose 6-phosphate in wild type cultures, even though samples of up to 0.5 ml have been used in the final enzymic assay. The few data on low levels in wild type, or mutant grown without glucose, are included only to show the difference in order of magnitude of these concentrations compared with the accumulated levels in the mutant.

RESULTS

Toxicity of Glucose in Double Mutant—Table I shows that strain DF2000 failed to form colonies on minimal media containing glucose as sole carbon source, whereas with other carbon sources (e.g. glucose, glycerol, mannitol, succinate, and xylose) it formed normal or near normal sized colonies. The unexpected finding that no colonies appeared on fructose is the subject of the accompanying paper (8). When the plates contained glucose in addition to one of the utilisable carbon sources, on some media (e.g. glucose) growth was unaffected while on others (e.g. glycerol) it was completely prevented. Qualitatively similar results were seen in liquid culture experiments, glucose addition being without notable effect on growth on gluonate, but rapidly inhibiting growth on glycerol (see Fig. 5, below).

Fate of Radioactive Glucose in DF2000—If Fig. 1 accurately represents the initial pathways of glucose metabolism in E. coli, one would expect that the double mutant might convert glucose to glucose 6-phosphate, intermediates in the fructose or glycerol biosynthetic pathways, and polysaccharides. However extensive such metabolism, one would not expect the formation of the double mutant was not growing. The results in both cases

Another method of obtaining the soluble pool—the rapid chilling and centrifugation of 200-ml cultures and extraction of the pellet with small volumes of perchloric acid—did not give reproducible results, as more than half the internal glucose 6-phosphate was released from the cells during the centrifugation.

Table I

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Colony size</th>
<th>No glucose</th>
<th>Glucose added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluconate</td>
<td>mm</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Glycerol</td>
<td>mm</td>
<td>0.9</td>
<td>NC*</td>
</tr>
<tr>
<td>α-Glycerophosphate</td>
<td>mm</td>
<td>0.6</td>
<td>NC</td>
</tr>
<tr>
<td>Fructose</td>
<td>mm</td>
<td>NC</td>
<td>ND*</td>
</tr>
<tr>
<td>Mannitol</td>
<td>mm</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Succinate</td>
<td>mm</td>
<td>0.8</td>
<td>NC</td>
</tr>
<tr>
<td>Xylose</td>
<td>ca. 1.0</td>
<td>ca. 0.8</td>
<td></td>
</tr>
</tbody>
</table>

* NC, no colonies.
* ND, not done.

pH 5, 95% ethanol, and 0.1 m disodium ethylenediaminetetra-acetic acid, 30:70:1, v/v/v (5); development was ascending, at 37°C for 15 hours. Solvent II was n-butyl alcohol-pyridine-water, 6:4:3, v/v/v; development was descending, at room temperature for 20 hours. Radioactivity on chromatograms was located with a Packard model 7201 radiochromatogram scanner. Standards were located with the periodate-benzidine technique (6).

Enzymic Assay of Glucose 6-Phosphate in Soluble Pool—In most of the experiments reported, 2 ml of culture of measured turbidity were filtered through 2.4 cm Millipore membrane filters (pore size, 0.45 μ). The filters were immediately extracted with 2 ml of boiling water for 4 min, and the debris was removed by chilling and centrifugation of 200 ml cultures. The absorbance at 340 μm was determined, 5 μg of glucose 6-phosphate dehydrogenase (Dochringer) were added, and the increase in absorbance was measured. Glucose 6-phosphate in the sample was calculated according to the molar absorbitivity of TPNH, 6.22 × 10^5 M^-1 cm^-1 (7).
were similar, in that release of radioactivity from glucose was clearly more rapid in the wild type and in the isomerase mutant than in the double mutant. Although there was slow apparent release of radioactivity from the mutant, we would conclude

![Figure 2](image-url)  
**Fig. 2.** Glucose respiration in the wild type (K10), in a phosphoglucone isomerase mutant (DF40), and in a mutant lacking both phosphoglucone isomerase and glucose 6-phosphate dehydrogenase (DF2000). The cultures were grown to logarithmic phase in gluconate minimal medium. In A, at a density of about 0.28 mg, dry weight, per ml, glucose-U-14C, 10 μC per μmole, was added to 0.2-ml portions of the cultures so as to be about 0.5 mM. These small cultures were incubated in slanted 25-mm diameter tubes on a water bath shaker at 37°. Periodically, 0.01-ml samples were added to 0.01 ml of 1 N HCl; after mixing, 0.5-ml portions were transferred to 10 ml of scintillation fluid (9) and radioactivity was determined. (In one experiment (upper curve in A) glucose-l-14C, 0.55 mM, 0.0003 μC per μmole, was used; because of the low specific activity the incubation was with a 10-ml culture, and 0.5-ml portions were acidified and counted.) In B, before the addition of radioactive glucose the cells were removed from the gluconate growth medium by centrifugation and resuspended, at about 0.28 mg per ml, in minimal medium without gluconate. The plotted results are expressed as total radioactivity, the units being arbitrary but corresponding initially to micromolar concentration of glucose. All curves were corrected slightly to reflect initial cell densities of 0.28 mg per ml. In an experiment with the double mutant when the radioactive glucose was diluted 10-fold with unlabeled glucose, radioactivity per ml increased slightly during the incubation. On the assumption that this increase was because of evaporation in the small (0.2 ml) incubation mixtures, correction factors (0.97 at 90 min and 0.94 at 120 min) were derived from this experiment and used in the others. Radioactivity was measured with a Nuclear-Chicago Mark I scintillation counter.

![Figure 3](image-url)  
**Fig. 3.** Glucose disappearance with DF2000. Glucose-U-14C, 10 μC per μmole, was added to a 0.2-ml culture in logarithmic growth in glycerol minimal medium at a cell density of 0.28 mg, dry weight, per ml, so as to give an initial glucose concentration of 0.55 mM. After 40, 80, 150, and 240 min, 0.02-ml samples were added to 0.02 ml of acetone; the total suspensions were spotted onto Whatman No. 3MM paper and developed with System I. The migration of standards is shown by the horizontal bars. G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; GLU, glucose.

that total respiration of glucose is probably very restricted in this strain.

Fig. 3 shows that although total respiration was restricted in the double mutant, all the glucose was metabolized. Strain DF2000, growing on glycerol, was exposed to glucose-U-14C, 0.55 mM, and subsequently samples of the culture were treated with acetone and chromatographed in toto. The radioactive glucose completely disappeared in 4 hours, and radioactivity appeared in materials migrating like glucose 6-phosphate, as well as in slower and nonmigrating peaks. Similar results have been obtained with gluconate cultures, although then there was no growth inhibition and glucose disappearance was more rapid.

To further identify the materials formed from glucose, glucose-U-14C, 0.55 mM, was added to cultures of mutant and of wild type growing on gluconate, and, after 1 hour of incubation, the cells were recovered by filtration. They were treated with cold perchloric acid to yield soluble and insoluble fractions. The insoluble fractions were hydrolyzed in 1 N H2SO4, and portions were chromatographed (Fig. 4, A and B). The soluble fractions (only results for mutant are shown) were chromatographed both directly and after treatment with phosphatase (Fig. 4, C and D). In the hydrolysate of the insoluble fraction from the wild type (Fig. 4A) there were no discrete peaks; as this fraction represents total insoluble cell material and there is no impediment in glucose metabolism, all cell constituents may be radioactive. In striking contrast, almost all the radioactivity in the corresponding fraction from the mutant was in a discrete peak which (not shown), upon chromatography in System II, migrated like glucose. (We cannot, however, exclude the possibility that it also contained some galactose.) The only other noticeable peak migrated before galactose, perhaps like glucosamine. It should be noted
There was relatively little radioactivity in the soluble fractions from the wild type, and the chromatograms (not shown) were unremarkable. In the mutant soluble fraction (Fig. 4C), however, the main peak migrated like glucose 6-phosphate (and on co-chromatography was coincident with it). Alkaline phosphatase treatment (Fig. 4D) converted the glucose 6-phosphate peak to one moving like glucose. (In this solvent system glucose 1-phosphate moves only slightly faster than glucose 6-phosphate, and the presence of a small amount of the former is not excluded by these experiments.) The peak at the origin is probably the result of incomplete separation of soluble and insoluble fractions, and was smaller in experiments with other fractionations. The slowly migrating material in the soluble fraction is at present unidentified. The nature or origin of the small peak migrating ahead of glucose after phosphatase treatment is also unknown; rhamnose migrates similarly in this system.

The experiments on the fate of radioactive glucose in the mutant have been done with a variety of extraction techniques: stronger perchloric acid, trichloroacetic acid, acetone, and ethanol. The chromatograms of the hydrolyzed insoluble fraction have always resembled Fig. 4B: 90% or more glucose. With the soluble fraction, results have been more variable. This variability does not merely reflect that the initially low concentrations of glucose are eventually completely used (Fig. 3). Rather, there was variability in the amount of early migrating material and in its exact migration pattern. The chromatogram presented in Fig. 4C is typical in the amount of this material, but similar experiments have sometimes revealed far less of it. It is unlikely to be nucleotide sugars, as it was insensitive to 15-min treatment with 1 M HCl at 100°. Indeed, 15-hour treatment of the soluble fraction with 1 M HCl largely removed the glucose 6-phosphate, but most of the early migrating material remained.

In spite of the presence of unidentified components derived from glucose in the soluble material of the mutant, several conclusions may be drawn from this experiment. Very little, if any, material migrating like free glucose is seen in the wild type or mutant. The mutant accumulated substantial glucose 6-phosphate in the soluble fraction and, in the insoluble fraction, a material which after hydrolysis migrated like glucose and therefore might have been glycogen.

**Accumulation of Glucose 6-Phosphate**—Since the experiments with radioactive glucose showed that a large amount of glucose 6-phosphate accumulated in strain DF2000, we have used an enzymic method to specifically assay its concentration in the soluble pool under various conditions. The data in Table II illustrate several points. When the mutant was grown in glucose, mannitol, or glycerol the assayed amounts of glucose 6-phosphate were near zero, and it is expected that more accurate assay techniques would in fact reveal no glucose 6-phosphate in the mutant grown without glucose. In contrast, when the mutant was grown with glucose, high concentrations of glucose 6-phosphate accumulated inside the cells, up to about 30-fold the amount in the wild type growing on glucose. The accumulated levels were higher in the glycerol culture (where growth is inhibited) than in the glucose culture (where growth is not inhibited) or the mannitol culture (where growth is partially inhibited in liquid culture, although in long term experiments on plate there was no inhibition). The accumulation was rapid and the level of glucose 6-phosphate seemed relatively indepen-
ent of the external glucose concentration; thus, with an initial external concentration of 0.55 mM the internal level was 40 mM at 10 min. However, with such low initial external concentrations, the glucose was eventually used up (not shown) and the internal pool fell to zero (Table II), probably because of glycogen formation and other reactions of the glucose 6-phosphate. This pattern is illustrated in Fig. 5, which shows that addition of either low or high concentrations of glucose resulted in growth inhibition. The internal level of glucose 6-phosphate was high during the inhibition (the value of 60 mM was found in both of the inhibited cultures). With the low initial concentration of glucose, inhibition was eventually released, and this release was associated with a fall in the internal concentration to 2 mM. Similar results have been obtained with other amounts of glucose, and a family of growth curves may be seen with varying displacements from the control, depending on initial concentration. With the particular initial cell density used, no inhibition of growth is evident with an initial glucose concentration of 0.02 mg per ml or less.

A few figures have been included on the external concentration of glucose 6-phosphate. Although no systematic study has been made, these and other data show that there is some apparent excretion of glucose 6-phosphate, which lags behind the internal accumulation. When the initial external level of glucose was high, high concentrations of glucose 6-phosphate were eventually found outside the cells. In an experiment in which the mutant was grown for many generations in a medium initially containing

**TABLE II**  
Glucose 6-phosphate accumulation

Cultures were in logarithmic growth, in minimal medium containing 1 mg of the specified carbon source per ml. Glucose was added as indicated, and glucose 6-phosphate was determined in cells and filtrate by an enzymic method (see "Materials and Methods"). Values in parentheses are below the limit of resolution of the assay method. The data in the last part of the table on the glucose 6-phosphate concentration as a function of time were collected from several experiments.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium</th>
<th>Glucose 6-phosphate outside</th>
<th>Glucose 6-phosphate inside</th>
<th>Time after glucose addition</th>
<th>Initial glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>K10 (wild type)</td>
<td>Glucose</td>
<td>22 (1.5)</td>
<td>ND</td>
<td>0.56</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>(0.5)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>(0.6)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF2000</td>
<td>Glucarate</td>
<td>5.5 (0.25)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
<tr>
<td>(double mutant)</td>
<td>Glucarate</td>
<td>5.5 (0.25)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mannitol</td>
<td>5.5 (0.25)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>5.5 (0.25)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
<tr>
<td>DF2000</td>
<td>Glycerol</td>
<td>0.55 (0.3)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>0.55 (0.3)</td>
<td>(0)</td>
<td>0.56</td>
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<tr>
<td></td>
<td>Glycerol</td>
<td>0.55 (0.3)</td>
<td>(0)</td>
<td>0.56</td>
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<td>Glycerol</td>
<td>0.55 (0.3)</td>
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<td>5</td>
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<tr>
<td></td>
<td>Glycerol</td>
<td>0.55 (0.3)</td>
<td>(0)</td>
<td>0.56</td>
<td>5</td>
</tr>
</tbody>
</table>

* ND, not done.

**Fig. 5.** Glucose 6-phosphate accumulation inside and outside cells after addition of glucose, 0.1 or 2.0 mg per ml, to a culture of DF2000 growing on glycerol. Growth was followed by turbidity measurement, and is plotted as dry weight. At the times indicated by the arrows, 2-ml portions of the culture were filtered, and glucose 6-phosphate in the cells and in the filtrates was determined as described in "Materials and Methods." The pairs of numbers circled are the assayed glucose 6-phosphate concentrations (millimolar inside, and micromolar outside).

**Fig. 6.** Inhibition of fructose diphosphatase. Fructose diphosphatase was purified 4-fold from strain DF40 (which lacks phosphoglucone isomerase), grown in Medium 63 supplemented with 1% Bacto-tryptone (Difco), 0.4% yeast extract (Difco), and 0.4% sodium gluconate. Only the first three steps of the described procedure (11) were used, and they were modified so that the final fraction was a 40 to 50% ammonium sulfate fraction (rather than a 50 to 60% fraction); the preparation used had a specific activity of 0.04 pmole per min per mg of protein. Activity was measured by the phosphate release assay: 1-ml incubation mixtures contained 0.05 M Tris-HCL, 0.01 M MgCl2, 4 mM cysteine-HCl, 0.18 mM fructose 1,6-diphosphate, 0.13 mg of protein, and the indicated concentrations of inhibitor. The reactions were terminated after 25 min at 25° by the addition of 0.05 ml of 6.5% trichloracetic acid, the precipitates were removed by centrifugation, and inorganic phosphate in the supernatants was measured. To correct for phosphate in, or released from, the inhibitor, controls were run with the inhibitors alone. The reaction being measured was fructose diphosphatase according to the following criteria: (a) the rate of phosphate release was one-half the rate of TPN reduction in the spectrophotometric assay (as was found earlier for such preparations (11)), (b) the reaction was strictly dependent on MgCl2 (phosphate release was only 4% without it), and (c) the sensitivity to AMP was characteristic of this enzyme.
glucose 6-phosphate was added to a culture of strain DF2000 growing on glycerol is inhibitory to growth. However, the external concentration required (with the standard initial cell density of 0.28 mg per ml) was higher than with glucose: 1 mM had little effect, and 5 mM was strongly inhibitory.

Site of Glucose Inhibition of Growth—The data on the accumulation of glucose 6-phosphate have shown a correlation between inhibition of growth of the mutant on glycerol and the presence of high internal levels of glucose 6-phosphate. On some other carbon sources, however, growth was not inhibited by glucose (Table I), although glucose 6-phosphate likewise accumulated (Table II). Thus, if growth inhibition were the result of the inhibition of a single enzyme reaction by glucose 6-phosphate, that reaction would have to be essential for growth on some substances (e.g. glycerol or succinate) but not on others (e.g. gluconate, mannitol, or xylose). The only such reaction in E. coli is fructose diphosphatase. During growth on glycerol and other gluconeogenic substances, hexose monophosphates (necessary materials for pentose, gluconate, and polygalacturonic acid) arise solely from the action of the enzyme on fructose 6-1,6-diphosphate. On gluconate, mannitol, or xylose, on the other hand, hexose monophosphates arise differently (Fig. 1) and fructose diphosphatase activity is not necessary for growth. Indeed, this rationale was used for the selection of fructose diphosphatase mutants (10). Thus, the simplest explanation for the effects of glucose on the growth of strain DF2000 would be that the accumulated glucose 6-phosphate inhibited the fructose diphosphatase reaction in vitro. In that case the inhibition ought to be demonstrable in vitro. This prediction was verified (Fig. 6). The activity of a slightly purified preparation of this enzyme from a phosphogluco isomerase mutant of E. coli was strongly inhibited by glucose 6-phosphate at high concentrations. Fig. 6 also shows that gluconate 6-phosphate was an equally effective or better inhibitor, and that both were far less effective inhibitors than AMP.


discussion

Glucose Metabolism in E. coli—According to the formulation of Fig. 1, glucose metabolism should be very restricted in the double mutant. The apparent slight loss of radioactivity from glucose upon incubation with this strain (Fig. 2) is not at present accounted for. Therefore, the existence of some other, minor, pathway of glucose oxidation is not completely excluded. There are reports of apparent glucose oxidase activity in vitro in E. coli (12), and of the formation of gluconic acid from glucose in vitro in an E. coli fructose diphosphate aldolase mutant (3). However, the fact that the mutant fails to grow at all on glucose but grows rapidly on gluconate makes it unlikely that glucose oxidase could be functionally useful in this strain. No gluconic acid was detected in the chromatograms of radioactive material in the soluble pool.

The radioactivity in insoluble material derived from glucose in the mutant was, after hydrolysis, mostly glucose. Although lipopolysaccharide synthesis ought to be occurring during such incubations (growth being normal in the presence of gluconate), it cannot account for a major fraction of the incorporated radioactivity, since one would then expect much galactose in the hydrolysate. The glucose-containing material has not yet been isolated or characterized, and it is not known whether it is mainly glucose homopolymers. This mutant might be useful for studying glycon synthesis in vivo.

In the acid-soluble pool, the major component present during the incubation time studied was glucose 6-phosphate. Other components are unidentified; as discussed in "Results," they are probably not nucleotide sugars. This fact, together with the occurrence of some apparent respiration of glucose, prevents complete description of the pathways of glucose metabolism in the mutant. Nonetheless, the results confirm Fig. 1 as a generally correct representation. Because of the trivial possibility that "leakiness" of a block in vivo could account for some of the present uncertainties, it would be interesting to repeat this study with a mutant containing genetic deletions, rather than point mutations. Such a strain is not yet available.

Origin of Glucose 6-Phosphate—The data of this paper make it seem likely that glucose 6-phosphate is an early intermediate in glucose metabolism in E. coli. However, there is interesting uncertainty about its enzymic origin. According to mutant analysis, two different enzymic reactions can initiate glucose metabolism in E. coli: the phosphoenolpyruvate phosphotransferase system (13) and glucose kinase. The product of the first reaction is glucose 6-phosphate (14), as is probably the product of the second (E. coli glucose kinase has not been highly purified (15), but a similar enzyme from Aerobacter aerogenes has been isolated (16). The lack of glucose kinase has little effect on the growth of E. coli on glucose (17). However, a mutant lacking Enzyme I (P-enolpyruvate-HPr phosphotransferase) of the P-enolpyruvate phosphotransferase system grows very slowly on glucose (17, 18); another E. coli mutant lacking this enzyme does not grow on glucose at all (19). The fact that a strain lacking both Enzyme I and glucose kinase, when presented internally with glucose derived from lactose hydrolysis, excretes it all (17) seems to exclude any third functional system for glucose phosphorylation. These results implicate the P-enolpyruvate phosphotransferase system as the main system responsible for productive glucose phosphorylation in E. coli.

They would rule out any model (20) according to which this system be involved in sugar transport only, and not in utilization. But they do not conflict with the idea that some component of such a system be involved in transport, or that the phosphorylation accompanies transport (14, 21).

Accumulation and Excretion of Glucose 6-Phosphate—As might have been expected, the double mutant, strain DF2000, accumulated glucose 6-phosphate when presented with glucose.4 This resulted in extraordinarily high intracellular levels of this compound, far exceeding those found in the wild type in usual circumstances (e.g. growth on glucose or glycerol). Such accumulation makes it unlikely that glucose phosphorylation in E. coli is regulated by the level of glucose 6-phosphate. The exact internal level in the mutant probably depends on the rate of growth of the culture, on the use of the glucose 6-phosphate for

4 The idea that glucose 6-phosphate might cause catabolite repression (22) was not supported by the results of an experiment in which DF2000 was grown for many generations in two glucose minimal medium cultures containing 1 mM isopropyl thiogalacto-side, one of which contained 11 mM glucose (and an internal glucose 6-phosphate concentration of 27 mM). The specific activities of β-galactosidase, asayed by orthonitrophenyl galacto-side hydrolysis in toluene-treated cells, were 11.7 and 10.3 (units per min per mg of protein), respectively.
polysaccharide synthesis, and, possibly, on its excretion. It is not known how the excretion occurs. Although we have found (not shown) that viability of an inhibited culture remains constant, it is possible that some glucose 6-phosphate leaves the cell because of cell rupture or nonspecific leakage. A more interesting possibility would be that glucose 6-phosphate permease (17, 23, 24) is involved.5

Inhibition of Fructose Diphosphatase by Glucose 6-Phosphate—In the present experiments inhibition by glucose of growth of the double mutant was associated with glucose 6-phosphate accumulation. The types of carbon sources on which inhibition occurred implicated fructose diphosphatase as the site of the effect, and inhibition of this enzyme by glucose 6-phosphate was shown in vitro. Is glucose 6-phosphate a physiological effector of this enzyme in the wild type? The present data do not support this idea, for the concentrations of glucose 6-phosphate accumulated from glucose in the mutant were far higher than those found under any conditions in the wild type, and only high concentrations inhibited fructose diphosphatase in vitro. This argument does depend on the hypothesis that the measured pool size properly reflects concentration at the enzyme. Further more, as discussed elsewhere (11), it is not easy to understand why AMP should be a physiological inhibitor of this enzyme in E. coli. Glucose 6-phosphate or gluconate 6-phosphate could be considered as “end products” of the reaction, and it is conceivable that they may be natural effectors functioning, at normal concentrations, synergistically with AMP. It would be interesting to test this possibility on a highly purified preparation of the E. coli enzyme.

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REFERENCES