JOURNAL OF BACTERIOLOGY, June 1974, p. 1082-1089
Vol. 118, No. 3

Sugar Metabolism in Transketolase Mutants of *Escherichia coli*

BETTY L. JOSEPHSON[1] AND D. G. FRAENKEL

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received for publication 11 February 1974

This paper continues the description of transketolase mutants of *Escherichia coli*; they are absolutely unable to grow on pentoses, but slightly "leaky" with respect to their aromatic requirement (B. L. Josephson and D. G. Fraenkel, 1969). Several experiments have explored the degree of leakiness and shown it to be low. There is little conversion of radioactive xylose to carbon dioxide. The labeling of ribose in cells grown on [1-^{14}C]glucose and [2-^{14}C]glucose accords with its origin being chiefly by the oxidative pathway. A mutant lacking both transketolase and gluconate-6-phosphate dehydrogenase has been constructed; it requires supplementation with pentose. Pentoses are inhibitory to growth of transketolase mutants, but high levels of pentose phosphates do not accumulate in this situation. Several experimental results are suggestive of regulation of metabolic flow in the oxidative branch of the hexose monophosphate shunt.

We have previously described *Escherichia coli* mutants lacking transketolase (EC 2.2.1.1) activity: their selection, general growth properties, and approximate genetic map position (16). This paper is about further characterization of such mutants. The subjects are (i) their "leakiness", (ii) inhibition of growth by pentoses, (iii) the biosynthesis of ribose in such strains, and (iv) how their properties may reflect metabolic control of the hexose-monophosphate shunt.

MATERIALS AND METHODS

Bacterial strains and media. The wild-type strain in this work is *E. coli* K-10, the HfrC derivative of K-12; it is a streptomycin-sensitive prototroph and carries bacteriophage lambda. BJ501, 502, and 503 are transketolase-deficient mutants of K-10, carrying the alleles *tkt-1*, *tkt-2*, and *tkt-3*, respectively (16). BJ562 is a recombinant (16) carrying *tkt-2* (*tkt*$^-$, *trp*$^-$, *tyr*$^-$). BJ565 is an eductant (*his-gnd*Δ; 18) of strain BJ562. Most media and growth conditions were specified earlier; unless noted otherwise, minimal media for both mutants and wild type were supplemented with shikimic acid or an aromatic amino acid supplement (16). For anaerobic growth on plates, we used the Gas-Pak system (Baltimore Biological Laboratory, Cockeysville, Md.), and in liquid culture we used filled 14-mm tubes sealed with serum stoppers and containing small bar magnets for mixing.

Ribose isolation and degradation. In most experiments cells were grown from small inocula (1:250 dilution from stationary phase) in 25-ml cultures containing [1-^{14}C]glucose or [2-^{14}C]glucose, 2 mg/ml, and harvested at approximately 0.28 mg (dry weight)/ml. Ribose was purified and its specific activity was determined by the method described earlier (19), except for omission of the final chromatographic step. The radioactivity in positions C1, C5, and, by difference, C2-C4, was determined by a procedure involving periodate oxidation and isolation of formaldehyde as the dimedon derivative: when done on ribose itself, this yielded C5; when done after borohydride reduction both C1 and C5 were obtained, and C1 was determined by difference. Borohydride reduction was by the method of Abdel-Akher et al. (1): 0.5 ml (3 to 6 μg) of ribose isolated from BJ502 (from [1-^{14}C]glucose, ca. 600 count/min, and from [2-^{14}C]glucose, ca. 3,000 count/min) was mixed with 1 ml of 0.24 M (non-radioactive) ribose, and 2 ml of fresh 0.12 M $NaBH_4$ was added. The reaction was complete after 2 h at 25 C (determined by loss of ability to reduce Fehling solution), and excess $NaBH_4$ was removed by addition of 0.5 ml of 1 N hydrochloride. The degradation mixtures contained either 2 ml of the 0.06 M ribitol or 2 ml of 0.06 M ribose (isolated material plus carrier), 0.05 ml of 3.5 N NaOH, and 2 ml of 0.2 M $NaIO_4$. The reaction was complete after 2 h at 25 C (determined by dimedon assay of controls for formaldehyde formation), 3 ml of 1 N hydrochloride and 4 ml of 1.2 M $NaAsO_2$ were added to destroy excess periodate, and the mixtures were neutralized with 3.5 N NaOH. Formaldehyde was collected as the dimedon derivative (21) on a tared 2.4-cm membrane filter (Millipore Corp., Bedford, Mass.), dried in air 15 min and then 1 h at 70 C, and weighed, and its radioactivity was determined by scintillation counting, by using either Bray's (2) mixture or a toluene-based mixture; counts per minute in formaldemethone and ribose were additive over a wide range of relative concentrations. With [1-

[1] Present address: Department of Biology, Bard College, Annandale on Hudson, N.Y. 12504.

^{14}C]ribose (New England Nuclear Corp., Boston, Mass.) in carrier, the mass recovery as C1 + C5 was 95% theoretical, and, as C5, 90% theoretical; the recovery of radioactivity was 95% in C1 + C5 and 0% from C5, as expected for the correct compound. However, the same control done with ribose of low specific activity comparable to the samples from BJ502 on [1-^{14}C]glucose yielded 5 to 7% of the radioactivity apparently in C5, and, therefore, labeling patterns from low activity samples are undoubtedly less accurate than the others.

Chemicals. L-Arabinose, D-xylose, D-ribose, and ribitol were from Pfanstiehl Chemical Co., Waukegan, Ill., D-glucose from Merck & Co., Inc., Rahway, N.J., glycerol from Fisher Scientific Co., Pittsburgh, Pa., and Na-gluconate and Na-succinate from Eastman Organic Chemicals, Rochester, N.Y. D-Xylose-U-^{14}C was from Amersham/Searle Corp., Chicago, Ill., and other radiochemicals were from New England Nuclear Corp.

RESULTS

Inhibition of transketolase mutants by pentoses. We noted earlier (16) that *tkt* mutants had two marked phenotypic characteristics, both expected from the known roles of the reaction (Fig. 1): they did not grow on pentoses, and for growth on other carbon sources, such as glucose, there was a requirement for aromatic amino acid (or shikimic acid) supplementation. The two characteristics were caused by the same lesion, as they reverted together. The requirement for aromatic amino acids was slightly "leaky," and differed somewhat among the several isolates, but the inability to grow on pentoses was complete. We suggested that the low residual transketolase activity in the mutants (in vitro assay showed 0 to 3% wild-type levels) might slightly serve the biosynthetic pathway, but not allow any growth on pentoses, since pentose phosphates might accumulate and be inhibitory.

We have investigated in vivo leakiness of the lesion and possible growth inhibition by pentoses for several reasons. First, conclusions drawn from studies of such a mutant about the normal role of transketolase have to be qualified if leakiness is extensive. Second, marked leakiness would force consideration of the possibility that there exists a second, minor transketolase activity. Third, if pentoses were inhibitory to growth in such mutants, pentose phosphates might accumulate, and the mutants could be a useful source of such compounds. Fourth, the finding of leaky mutants raises the possibility that complete loss of transketolase is lethal or might have a phenotype different from that selected. Table 1 shows plate growth of mutant, parent, and revertant strains on several carbon

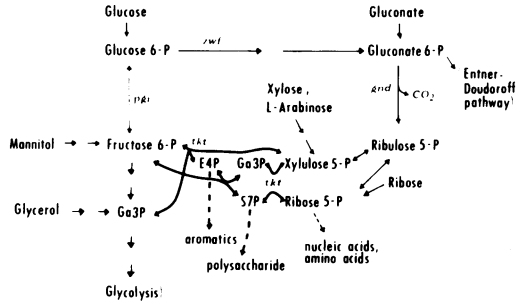

FIG. 1. *Pathways of sugar metabolism in E. coli. The usual formulation of the nonoxidative pentose-phosphate pathway is shown. Abbreviations: E4P (erythrose-4-phosphate), Ga3P (glyceraldehyde-3-phosphate), S7P (sedoheptulose-7-phosphate); genetic abbreviations, tkt (transketolase), gnd (gluconate-6-phosphate dehydrogenase), zwf (glucose-6-phosphate dehydrogenase), and pgi (phosphoglucose isomerase), indicate the sites of blockage in certain mutants.*

sources and the effect of pentoses on such growth. Growth of the mutants on single carbon sources (shikimic acid was present in all cases) was as reported previously: negative on xylose and arabinose and normal on glucose, mannitol, and glycerol. Pentoses were not inhibitory to growth of the mutant on glucose, but were inhibitory on other carbon sources, the effect depending on the carbon source, the pentose concentration, and the particular mutant. BJ502, the "tighter" mutant by other indications, was somewhat more sensitive to pentoses than BJ501. Inhibition also occurred in liquid medium (Fig. 2), but higher concentrations of pentose were needed, and with intermediate concentrations inhibition was transient; a similar situation was seen with glucose inhibition of another mutant (13), the difference probably depending on the very different cell concentrations in the two situations.

Growth inhibition by a sugar whose further metabolism is blocked is a familiar situation with bacterial mutants; the effect is often thought to be somehow related to accumulation of a phosphorylated derivative, e.g., the substrate of the blocked reaction (see ref. 13). Thus, pentose phosphates might accumulate when pentoses are added to *tkt* mutants growing on glycerol. Situations where growth is not inhibited could reflect absence of accumulation or indifference to the accumulated product. So, for *tkt* mutants on glucose, insensitivity to pentoses might merely reflect catabolite repression or inducer exclusion of pentose utilization (7, 8, 12). The fact that BJ562 is inhibited by arabinose, but not by xylose, would accord with its

TABLE 1. *Effect of pentoses on colony size*[a]

Major carbon source	Pentose supplement (μg/ml)	Wild type K-10 (*tkt*⁺)	Mutants			Revertants	
			BJ501 (*tkt-1*)	BJ502 (*tkt-2*)	BJ562 (*tkt-3*)	BJ501R1 (*tkt*⁺)	BJ502R4 (*tkt*⁺)
Glucose	None	>2	>2	>2	ND[b]	>2	>2
Glucose	Arabinose (1,000)	>2	>2	>2	1.3	>2	>2
Mannitol	None	>2	>2	>2	ND	ND	ND
Mannitol	Arabinose (1,000)	>2	1.1	0.9	ND	ND	ND
Gluconate	None	>2	1.6	0.7	0.5	>2	2.0
Gluconate	Arabinose (1,000)	>2	0.9	0.0	0.0	>2	>2
Gluconate	Xylose (1,000)	>2	0.9	0.0	0.2	>2	>2
Succinate	None	1.2	1.4	0.9	1.8	1.5	2.0
Succinate	Arabinose (1,000)	>2	0.7	0.0	0.0	2.0	>2
Glycerol	None	0.9	0.8	0.7	0.4	1.8	1.3
Glycerol	Arabinose (1)	1.0	0.7	0.2	ND	ND	ND
Glycerol	Arabinose (10)	1.5	0.2	0.0	ND	ND	ND
Glycerol	Arabinose (50)	1.5	<0.2	0.0	ND	ND	ND
Glycerol	Arabinose (100)	1.8	0.0	0.0	ND	ND	ND
Glycerol	Arabinose (500)	>2	0.0	0.0	ND	ND	ND
Glycerol	Arabinose (1,000)	>2	0.0	0.0	0.0	2.0	>2
Glycerol	Xylose (100)	1.5	0.0	0.0	0.5	1.7	>2
Glycerol	Xylose (1,000)	1.4	0.0	0.0	0.5	1.7	>2
Arabinose	None	1.6	0.0	0.0	0.0	2.0	2.0
Xylose	None	1.5	0.0	0.0	0.0	1.8	2.0

[a] The strains were grown in broth, diluted, spread so as to give approximately 50 colonies/plate, and incubated for 48 h at 37 C; average colony diameter (millimeters) was estimated. In all cases the minimal media contained the major carbon source, 0.4 mg/ml, and shikimic acid, 25 μg/ml. Plates for BJ562 also contained tyrosine and tryptophan (25 μg/ml each).

[b] ND, not determined.

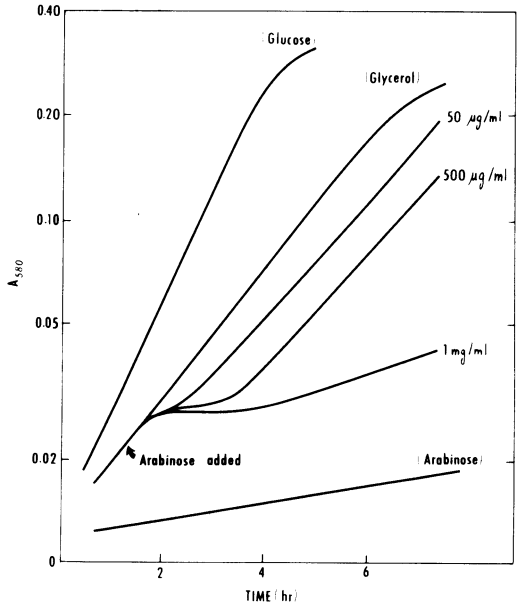

FIG. 2. *Inhibition of BJ502 in liquid medium. L-Arabinose was added at the concentrations indicated to a culture of BJ502 growing on glycerol. Growth on glucose alone, or arabinose alone, is also shown. All media included shikimic acid, 25 μg/ml.*

also containing (like its parental strain, X'121) a specific mutation in the xylose pathway. And, conversely, the slow growth of *tkt* mutants on gluconate (especially BJ502, Table 1) might be caused by endogenous formation (from gluconate-6-phosphate) of the inhibitor(s).

To properly explain growth inhibition, the accumulations would have to be characterized in detail, and the site(s) and mechanism of inhibition explained. We have not pursued the latter problem, since sites of inhibition in such mutants have often been elusive. However, we did some experiments on the metabolism of radioactive pentoses in the mutants, both to see if there were high accumulations of any intermediate and to further probe the leakiness of the transketolase mutations. Figure 3 shows the gross distribution of radioactivity from U-[^{14}C]xylose in parent and mutant grown on glycerol. For the parent, as expected, all the radioactivity disappeared from the medium: more than half became $^{14}CO_2$, and one-third went into macromolecules (the insoluble fraction). By contrast, in the mutant only 12% of the radioactivity was lost from the medium after a 2-h incubation; this small amount of radioactivity was distributed among the three

TABLE 2. *Specific activity of ribose*[a]

Expt	Strain	Position of ^{14}C in glucose	Sp act of ribose (isolated)/sp act of glucose (substrate)
1	BJ502(*tkt-2*)	C1	0.14, 0.14
2	BJ502(*tkt-2*)	C2	0.87
3	K-10 (wild type)	C1	0.79, 0.79
4	K-10 (wild type)	C2	(1.25)[b]
5	DF40 (*pgi-2*)	C1	(0.01)[b]
6	DF2001 (*zwf-2*)	C1	(1.33)[c]
7	K-10, anaerobic	C1	1.34, 1.24

[a] In the aerobic experiments (#1–6), strains were grown from small inocula (1:250) in 25 ml of medium 63 containing [^{14}C]glucose (2 mg/ml) and harvested at approximately 0.28 mg (dry wt)/ml; for the anaerobic experiment (#7), the inoculum was 1:50 and the culture volume was 30 ml; the final specific activity of ribose was corrected for the amount of inoculum. The specific activity of the glucose in the various experiments ranged between 30,000 and 150,000 counts/min per μmol. For experiments 1 and 2, the medium included shikimic acid, 25 μg/ml. Ribose was isolated (see Materials and Methods). Each value is from a separate culture.

[b] D. G. Fraenkel, unpublished data.

[c] Data from ref. 19.

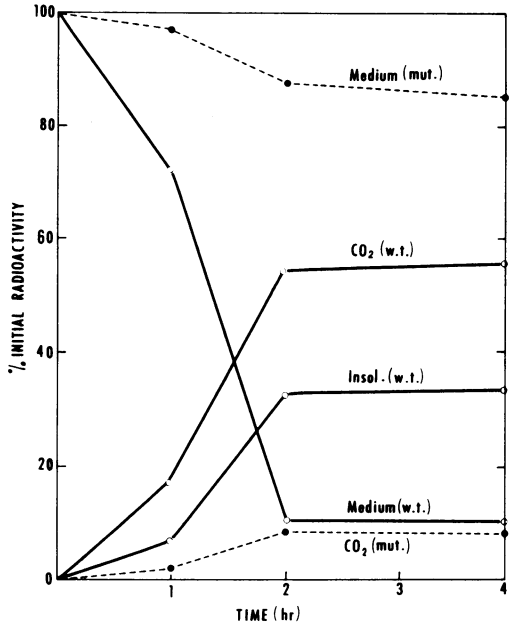

FIG. 3. *Use of U-[^{14}C]xylose. To cultures (ca. 0.2 mg (dry wt)/ml) growing in minimal medium with glycerol as sole carbon source, U-[^{14}C]xylose was added (final concentration: 100 μg/ml, 3 × 10^6 counts/min per ml), and during subsequent incubation 1-ml samples were taken and fractionated as follows. The medium was recovered by filtrations.*

fractions. The main conclusion from these results is that, as expected, transketolase mutants do not use much pentose. The finding of some radioactivity in the insoluble pool is not surprising, since pentose phosphates still can be used for nucleotide and amino acid biosynthesis. Release of any $^{14}CO_2$ was unexpected, however, and could reflect leakiness of the block or another minor pathway of pentose metabolism. Or, one could speculate that, if abnormal concentrations of pentose phosphates were accumulating in the mutant, transaldolase might act on them to transfer C1-C3 to other compounds; transaldolase is known to act on a variety of "unusual" substrates at high concentrations (O. Tsolas, Ph.D. thesis, Albert Einstein College of Medicine, 1967). The data do not permit distinction among these several possibilities.

Many chromatographic experiments were done to detect directly abnormal levels of pentose phosphates or other metabolites in the transketolase mutants, after addition of pentoses to cultures growing on glycerol. Using U[-^{14}C]xylose only radioactive xylose could be found in the medium, whereas in the soluble pool there was too little radioactivity to detect specific compounds. The same results were found with [1-^{14}C]ribose. (For xylose, such failure to detect intracellular accumulations might merely reflect catabolite repression [7], but there is known to be some ribose utilization even in the presence of glucose [10]). The failure to find accumulation of pentose phosphates in transketolase mutants does not necessarily show that growth inhibition is unrelated to abnormally high levels of certain metabolites, for it is possible that small increases have large effects. But the results do suggest that there may be some mechanism, other than interference with induction, preventing accumulation of pentose phosphates from pentoses in the transketolase mutants. This mechanism is not known, but probably involves some control of

The cells were resuspended in 1 ml of 0.3 N perchloric acid and centrifuged after 15 min at 0 C, and the supernatant, (the acid-soluble fraction) was neutralized with KOH; the insoluble fraction was washed with perchloric acid and hydrolyzed for 16 h at 105 C in 0.25 ml of 1 N H_2SO_4. Radioactivity was determined in each fraction, by using Bray's mixture (6). The curves labeled CO_2 are the calculated differences between input and recovered radioactivity. The fractions were also chromatographed (results not shown). Wild type, solid lines; BJ502 (tkt$^-$), dashed lines. Fractions not shown (acid soluble, both strains and insoluble, mutant) had <5% of the radioactivity at any time.

an early step in the catabolic pathways. (For an analogous situation, the absence of ribose-5-phosphate accumulation from ribose in a mutant lacking the "degradative" ribose-phosphate isomerase, one suggested explanation was feedback inhibition of ribose kinase by its product [10]).

The experiments on pentose metabolism in *tkt* mutants may therefore be summarized as follows. (i) There is little pentose metabolism. (ii) Pentoses are inhibitory to growth on some sugars, but the mechanism of inhibition is not clear. (iii) High accumulations from pentoses of pentose phosphates—the substrates of transketolase—do not occur. And (iv) the transketolase lesions are not very leaky, although some leakiness is possible.

The matter of pentose sensitivity of transketolase mutants also raises questions about glucose metabolism: if slow growth on gluconate were related to endogenous formation of pentose phosphates, then the same problem might occur with glucose. However, growth rates on glucose appeared relatively normal, and glucose utilization was not accompanied by accumulation of pentose or pentose phosphates in the medium. We considered the possibility, too, that pentose accumulations from glucose might be avoided by induction of the Entner-Doudoroff pathway, but, as tested by gluconokinase assay, this did not occur (B.L. Josephson, Ph.D. thesis, Harvard University, 1972). Thus, the normality of the transketolase mutants on glucose might reflect some control of the hexose-monophosphate shunt.

The origin of ribose in a transketolase mutant. The transketolase mutants do not require exogenous ribose for growth, presumably making it entirely by the oxidative pathway. If that is the case, then in growth on [1-^{14}C]glucose cellular ribose should be nonradioactive, whereas on [2-^{14}C]glucose the ribose should have the same specific activity as the glucose. Table 2 shows that the actual relative specific activities were 0.14 and 0.87, respectively, in general accord with the prediction; these values differ substantially from those in the wild type. Table 2 also cites results of similar experiments with other strains. A *pgi* mutant appears to make ribose exclusively by the oxidative pathway, with a relative specific activity of 0.01 from [1-^{14}C]glucose. Data from a *zwf* mutant are most simply interpreted as exclusive origin of ribose via the nonoxidative pathway (relative specific activity, 1.33).

Although the specific activities of ribose in the transketolase mutants were, on the whole, consistent with its origin from glucose by the oxidative pathway, the data show some deviation from the predicted values (0.14 and 0.87, found, and 0.00 and 1.00, predicted). Some of the difference may be caused by experimental errors, which might be as much as 20% for ribose samples having a low radioactivity (see Materials and Methods). In addition, for the predictions, metabolism was oversimplified; in particular, it was assumed that labeling in the hexose-phosphate pool is identical with that of the input glucose. In the wild type many factors might influence this labeling. These include cycling in the hexose-monophosphate shunt (17), exchange reactions catalyzed by transaldolase (20) and transketolase (5), non-equilibration of hexose phosphates, triose phosphates (24), or pentose phosphates (9, 26), and resynthesis of hexose phosphates in reverse glycolysis, as well as the possible presence of unknown reactions.

In a transketolase mutant certain of these complications (e.g., cycling and transketolase exchange reactions) should not occur, whereas others are still possible. To assess the labeling data better, ribose samples from the *tkt* mutant were degraded to C1, C2-C4, and C5 fragments (Table 3). The low radioactivity of ribose from [1-^{14}C]glucose proved to be almost all in the 5-position. This distribution could be accounted for by transaldolase exchange or reverse glycolysis, since these reactions could place label from C1, C2, and C3 of hexose phosphate into its C6, C5, and C4 positions, respectively; some C6-labeled hexose phosphate would thus be made from [1-^{14}C]glucose, and ribose derived by the oxidative pathway would be labeled at C5. For the case of [2-^{14}C]glucose, most label was, as expected, in the 1-position of ribose. The reactions just considered would label the 5-position of hexose phosphate, thus giving some label also in the 2-4C fragment of ribose.

Although these mechanisms could account for some of the deviations from simple prediction, they are not entirely satisfactory, particu-

TABLE 3. *Labeling pattern of the ribose from BJ502*[a]

Carbon position in ribose	Radioactivity in carbon position (%)	
	From [1-^{14}C]-glucose	From [2-^{14}C]-glucose
C1	0.15	0.80
C2, 3, 4	0.01	0.19
C5	0.84	0.01

[a] Ribose from experiments 1 and 2, Table 2, was degraded (see Materials and Methods).

larly since the exchange reactions placing label in the lower three carbons of hexose phosphate would tend to increase its overall specific activity, and thus might be expected to raise the specific activity of ribose from [2-^{14}C]glucose to more than 1.0. To further study ribose synthesis, the radioactive experiments would also have to include determination of the labeling of a hexose-phosphate derivative (17). It also would be ideal to have a genetically proven deletion of the *tkt* locus. In spite of these reservations, the present experiments certainly accord with ribose synthesis in a *tkt* mutant occurring mainly via the oxidative branch of the hexose-monophosphate shunt.

Mutants blocked in both pathways. The discussion of the origin of ribose has been based on another implicit assumption that there are only two possible pathways for ribose formation, the oxidative and nonoxidative branches of the hexose-monophosphate shunt. The data on growth and labeling are most easily interpreted as showing that, in mutants, one or the other pathway may suffice, and one would expect that a strain blocked in both pathways ought to require exogenous pentose for growth.

Such a strain, BJ565, lacking both transketolase and gluconate-6-phosphate dehydrogenase (*tkt*$^-$, *gnd*$^-$) was constructed (see Materials and Methods). Table 4 shows that the double mutant, unlike either single mutant, required pentose for growth. On glucose the requirement was satisfied by ribose or uridine, but not by arabinose or adenosine. No supplement was found which allowed growth on glycerol or on other carbon sources (not shown); this result might be related to the inhibition by pentoses of growth of transketolase mutants. Clearly, however, the main prediction is met in that a doubly blocked mutant does require pentose.

The origin of ribose under anaerobic conditions. The experiments described thus far have involved aerobic growth conditions, i.e., vigorous agitation of cultures in air, and the conclusion that ribose synthesis might occur by either pathway only applies to that condition. Many experiments with [^{14}C]glucose and [^{18}O]-glucose have indicated that flow in the oxidative branch of the hexose-monophosphate shunt is somehow regulated, being relatively less under conditions of slow growth or anaerobiosis (17, 22). Certain results with mutants might also reflect such a control. For example, in mutants whose primary carbon metabolism is via the oxidative pathway (e.g., phosphoglucose isomerase mutants on glucose, and gluconate-6-phosphate dehydrase mutants on gluconate)

TABLE 4. *Pentose requirement*[a]

Supplement, μg/ml	Strain (colony diameter [mm])		
	BJ562 (*tkt*$^-$, *gnd*$^+$)	BJ565 (*tkt*$^-$, *gnd*$^-$)	BJ565R10[b] (*tkt*$^+$, *gnd*$^-$)
None	1.8	0.0	1.4
Ribose, 1	1.8	0.4	1.3
Ribose, 5	1.8	0.7	1.2
Ribose, 10	1.9	0.9	1.2
Ribose, 20	1.7	1.0	1.2
Arabinose, 1	1.8	0.0	2.0
Arabinose, 5	1.8	0.0	2.0
Arabinose, 10	1.8	<0.2	1.9
Arabinose, 20	1.8	<0.2	1.8
Adenosine, 20	1.4	0.2	1.2
Uridine, 20	1.8	1.0	1.9

[a] Plates contained 0.4% glucose, shikimic acid, histidine, tyrosine, and tryptophan (25 μg/ml each), and colony size was measured after 48 h at 37 C.

[b] BJ565R10 is an arabinose$^+$ (*tkt*$^+$) revertant of BJ565.

growth occurs aerobically but not anaerobically (unpublished data, this laboratory). And when a wild-type strain was grown anaerobically on [1-^{14}C]glucose, the relative specific activity of its ribose was 1.29, similar to that (1.33) for a glucose-6-phosphate dehydrogenase mutant (*zwf*$^-$) grown aerobically (expt. 7, Table 2), as if in both cases the oxidative pathway were not used.

If the oxidative pathway is not used anaerobically, then a transketolase mutant, which grows on glucose aerobically, should not grow anaerobically unless supplemented with pentose. In fact, none of the three transketolase mutants grew anaerobically on glucose or gluconate (solid media were used, and details are given in Table 16 of B.L. Josephson, Ph.D. thesis, Harvard University, 1972). Unfortunately, we could not show that the failure to grow was indeed related to a pentose deficiency, since none of several pentose supplementations (ribose, arabinose, or uridine) restored growth. The latter results are difficult to interpret in view of the previous difficulties in supplementing the *tkt*$^-$, *gnd*$^-$ mutants with pentose, and it may be that proper supplementation would require special conditions. In rich media, such as broth, the mutants did grow normally under anaerobic conditions, so they do not have a lesion preventing anaerobic growth altogether.

DISCUSSION

The "leakiness" of transketolase mutations. The transketolase mutants were shown earlier to be completely unable to grow

on pentoses, but somewhat leaky with respect to their aromatic amino acid requirement (16). Eidels and Osborn have reported transketolase mutants in *Salmonella typhimurium*, modifying our selection procedure to also demand resistance to phage C21 and including 2,3-dihydroxybenzoic acid in the aromatic supplement (11). The mutants they obtained were similar to the *E. coli* mutants, being unable to grow on pentose, leaky with respect to the aromatic requirement, and having low (0 to 4%) residual enzyme activity. In addition, they showed that transketolase mutants in both species were deficient in lipopolysaccharide heptose, and the deficiency could be repaired by including sedoheptulose-7-phosphate in the medium. This demonstration that heptose probably arises from sedoheptulose-7-phosphate thus indicates another function for transketolase and extends the possible explanations for why transketolase mutants are always slightly leaky. As mentioned earlier, the leakiness might represent a second transketolase of low activity or reflect that complete loss of the activity would be lethal. It is still not possible to distinguish between these alternatives, since there are no deletions or conditionally lethal *tkt* mutants yet. It is still possible that some unknown metabolite is essential and requires transketolase for its formation. (That compound is unlikely to be the heptose of lipopolysaccharide, since lipopolysaccharide mutants are known which completely lack heptose [23]). A different explanation for slight leakiness might be that it is necessary to prevent excessive accumulation of inhibitory metabolites in transketolase mutants. Such metabolites need not necessarily be substrates of transketolase; for example, they might be precursors of lipopolysaccharide. (A selection procedure which included sedoheptulose-7-phosphate in the permissive medium has not, to date, yielded nonleaky *tkt* mutants [C. C. Carren and B. L. Josephson, unpublished data]).

Some of the data in the present paper are germane to the problem of leakiness and are additional indications that the leakiness is not severe. Thus, the gross distribution of counts from radioactive xylose is in accord with the determined lesion; the appearance of a small amount of radioactivity in carbon dioxide might, but does not necessarily, indicate leakiness. Likewise, the composition of ribose formed from radioactive glucose accords with the lesion, and deviations from the theoretical expectation for a complete transketolase block might partly reflect exchange reactions. It is interesting that Johnson, Krasna, and Rittenberg (15) have recently reported an experiment on the labeling of ribose from [1-^{18}O]glucose in the transketolase mutant strain BJ502, and detected no ^{18}O whatsoever in ribose positions 2 to 5. They concluded that the strain forms ribose exclusively by the oxidative pathway, and, in addition, that there was no equilibration between the 1 and 6 positions of hexose. (The absence of complications to the ^{18}O-labeling pattern is a little surprising and might reflect different sensitivities of the ^{18}O and ^{14}C experiments.) In general, however, all the new results on transketolase mutants support the assignment of the lesion to that enzymatic step and attest to its relative tightness.

Inhibition of tkt mutants by pentoses. Pentoses do inhibit growth in certain media, and considerations of the media make it likely that the inhibition is related to formation of pentose phosphates. But techniques which would have detected high accumulations failed to reveal them. There was no indication that toxicity was related to inhibition at any single site.

Ribose synthesis in E. coli. A single gene mutant blocked in either the oxidative or the nonoxidative pentose-phosphate pathway does not have a nutritional requirement for ribose; the doubly blocked mutant does have such a requirement. Thus, either pathway is sufficient for ribose synthesis, and it is unlikely that a third effective pathway of ribose synthesis exists. The general question of how ribose is made in the wild-type strain is not addressed by these experiments; this matter has been discussed elsewhere (14, 17). It should also be noted that our double mutant which requires ribose is not the first such phenotype to be reported in *E. coli*. Skinner and Cooper selected this phenotype and found a strain which lacked one (the "biosynthetic") of two apparent ribose-phosphate isomerase activities (26). David and Wiesmeyer also presented evidence for two physiologically different ribose-phosphate isomerases (9). The relationship and control of the activities of these two isomerases is not yet clear (14).

Control of the oxidative branch of the hexose-monophosphate shunt. Two of the experiments in this paper bear on this problem. It is clear that, when a *tkt* mutant grows aerobically on glucose, ribose is formed by the oxidative pathway and it is not formed in great excess to its use—its accumulation was detected neither intracellularly nor extracellularly. This result might be an indication of control of flow in the oxidative pathway, particularly since analysis of data from wild-type strain *E. coli* B indicated that flow in the oxidative pathway

was greatly in excess of the requirement for biosynthesis (17). (However, the corresponding analysis has never been done with *E. coli* K-12.) We have also mentioned that a considerable literature implies that the oxidative pathway may be used much less anaerobically than aerobically, and that the failure of *tkt* mutants to grow anaerobically on glucose might accord with such a control. Neither control is understood. There have been reports that ribose 5-phosphate (10) and reduced nicotinamide adenine dinucleotide (25; but see 4) inhibit *E. coli* glucose-6-phosphate dehydrogenase, and that fructose-1,6-diphosphate dehydrogenase inhibits gluconate-6-phosphate dehydrogenase (3).

Other pathways of pentose catabolism. Both this paper and the previous one have supported the dual role of transketolase in pentose degradation and erythrose-4-phosphate synthesis in *E. coli*. Recently, Williams and his colleagues have suggested that the usual formulation of the pentose-phosphate pathway may be incorrect, at least for rabbit liver, and have proposed a new reaction scheme (6, 27). We will discuss in another report whether the new scheme could apply to *E. coli*. The present data on the role of transketolase accord with either scheme.

ACKNOWLEDGMENTS

This work was supported by grant GB15958 from the National Science Foundation. B. L. J. was supported by a Public Health Service training grant, and D. G. F. by a career development award, from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Abdel-Akher, M., J. K. Hamilton, and F. Smith. 1951. Reduction of sugars with sodium borohydride. J. Amer. Chem. Soc. **73**:4691-4692.
2. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1**:279-285.
3. Brown, A. T., and C. L. Wittenberger. 1971. Mechanism for regulating the distribution of glucose carbon between the Embden-Meyerhof and hexose-monophosphate pathways in *Streptococcus faecalis*. J. Bacteriol. **106**:456-467.
4. Cavalieri, R. L., and H. Z. Sable. 1973. Enzymes of pentose biosynthesis. II. Evidence that the proposed control of glucose 6-phosphate dehydrogenase by reduced diphosphopyridine nucleotide is an instrumental artifact. J. Biol. Chem. **248**:2815-2817.
5. Clark, M. G., J. F. Williams, and P. F. Blackmore. 1971. The transketolase exchange reaction *in vitro*. Biochem. J. **125**:381-384.
6. Clark, M. G., J. F. Williams, and P. F. Blackmore. 1972. The new pentose phosphate pathway: the fate of (1-^{14}C) glucose in rabbit liver *in situ*. Search **3**:87-88.
7. David, J. D., and H. Wiesmeyer. 1970. Control of xylose metabolism in *Escherichia coli*. Biochim. Biophys. Acta **201**:497-499.
8. David, J., and H. Wiesmeyer. 1970. Regulation of ribose metabolism in *E. coli*. I. The ribose catabolic pathway. Biochim. Biophys. Acta **208**:45-55.
9. David, J., and H. Wiesmeyer. 1970. Regulation of ribose metabolism in *E. coli*. II. Evidence for two ribose 5-phosphate isomerase activities. Biochim. Biophys. Acta **208**:56-67.
10. David, J., and H. Wiesmeyer. 1970. Regulation of ribose metabolism in *E. coli*. III. Regulation of ribose utilization *in vivo*. Biochim. Biophys. Acta **208**:68-76.
11. Eidels, L., and M. J. Osborn. 1971. Lipopolysaccharide and aldoheptose biosynthesis in transketolase mutants of *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U.S.A. **68**:1673-1677.
12. Engelsberg, E., R. L. Anderson, R. Weinberg, N. Lee, P. Hoffee, G. Huttenhauer, and H. Boyers. 1962. L-Arabinose-sensitive, L-ribulose 5-phosphate 4-epimerase-deficient mutants of *Escherichia coli*. J. Bacteriol. **84**:137-146.
13. Fraenkel, D. G. 1968. The accumulation of glucose 6-phosphate and its effect in an *Escherichia coli* mutant lacking phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. J. Biol. Chem. **243**:6451-6457.
14. Fraenkel, D. G., and R. T. Vinopal. 1973. Carbohydrate metabolism in bacteria. Annu. Rev. Microbiol. **27**:69-100.
15. Johnson, R., A. I. Krasna, and D. Rittenberg. 1973. ^{18}O studies on the oxidative and nonoxidative pentose phosphate pathways in wild type and mutant *Escherichia coli* cells. Biochemistry **12**:1969-1977.
16. Josephson, B. L., and D. G. Fraenkel. 1969. Transketolase mutants of *Escherichia coli*. J. Bacteriol. **100**:1289-1295.
17. Katz, J., and R. Rognstad. 1967. The labelling of pentose phosphate from glucose-^{14}C and estimation of the rates of transaldolase, transketolase, the contribution of the pentose cycle, and ribose-phosphate synthesis. Biochemistry **6**:2227-2247.
18. Kelly, B. L., and M. G. Sunshine. 1967. Association of temperate phage P2 with the production of histidine negative segregants by *Escherichia coli*. Biochem. Biophys. Res. Commun. **28**:237-243.
19. Kupor, S. R., and D. G. Fraenkel. 1972. Glucose metabolism in 6-phospho-gluconolactonase mutants of *Escherichia coli*. J. Biol. Chem. **247**:1904-1910.
20. Ljungdahl, L., H. G. Wood, E. Racker, and D. Couri. 1961. Formation of unequally labelled fructose 6-phosphate by an exchange reaction catalyzed by transaldolase. J. Biol. Chem. **236**:1622-1625.
21. MacFayden, D. A. 1945. Estimation of formaldehyde in biological mixtures. J. Biol. Chem. **158**:107-133.
22. Model, P., and D. Rittenberg. 1967. Measurement of the activity of the hexose monophosphate pathway of glucose metabolism with the use of (^{18}O) glucose. Variations in its activity in *Escherichia coli* with growth conditions. Biochemistry **6**:69-79.
23. Osborn, M. J. 1969. Structure and function of the bacterial cell wall. Annu. Rev. Biochem. **38**:501-538.
24. Rose, I. A., R. Kellermeyer, R. Stjernholm, and H. G. Wood. 1962. The distribution of C^{14} in glycogen from deuterated glycerol-C^{14} as a measure of the effectiveness of triosephosphate isomerase *in vivo*. J. Biol. Chem. **237**:3325-3331.
25. Sanwal, B. D. 1970. Regulatory mechanisms involving nicotinamide adenine nucleotides as allosteric effectors. III. Control of glucose 6-phosphate dehydrogenase. J. Biol. Chem. **245**:1626-1631.
26. Skinner, A. J., and R. A. Cooper. 1971. The regulation of ribose 5-phosphate isomerization in *Escherichia coli* K12. FEBS Lett. **12**:293-296.
27. Williams, J. F., and M. G. Clark. 1972. An error in metabolism: the pentose phosphate cycle. Search **2**:80-88.