

NOTES

CO₂ Production from Galactose in Galactose-1-Phosphate Uridyl Transferase-Deficient *Escherichia coli*

ROBERT J. LAPOLLA,* MARK R. GEIER, THOMAS B. FRIEDMAN,¹ AND CARL R. MERRILL
Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014

Received for publication 25 June 1975

Escherichia coli K-12 deficient in galactose-1-phosphate uridyl transferase is capable of converting significant amounts of D-[1-¹⁴C]galactose to ¹⁴CO₂, whereas strains deficient in other enzymes of the Leloir pathway cannot do so.

The LeLoir pathway is a major metabolic route for the metabolism of galactose in eukaryotes and in many prokaryotes (4). This pathway utilizes three sequential reactions that are catalyzed by galactokinase (EC 2.7.1.6), galactose-1-phosphate uridyl transferase (gal-transferase; EC 2.7.7.12), and 5'-uridine diphosphate-galactose-4-epimerase (EC 5.1.3.2). Human deficiencies in gal-transferase result in a clinical disorder known as galactosemia (9). The absence of galactokinase results in another metabolic disorder characterized by juvenile cataracts (8). Human fibroblasts that lack detectable gal-transferase activity are known to be capable of converting significant amounts of D-[1-¹⁴C]galactose to ¹⁴CO₂ despite their inability to grow on galactose (3, 7). Galactokinase-deficient fibroblasts, on the other hand, have little or no ability to metabolize galactose (3, 7). The additional pathway(s) that the gal-transferase-deficient human cells utilizes must, therefore, require phosphorylation of galactose for further metabolism (3). Neither galactokinase- nor gal-transferase-deficient human fibroblasts will grow on galactose (3, 5).

Bacterial mutants of *Escherichia coli* K-12 that lack similar enzymes have been isolated and characterized extensively (1). These strains do not grow in minimal galactose medium and are not fermenters, thus forming white colonies on MacConkey galactose indicator plates. Galactose fermenters produce red colonies on this indicator plate.

In this study, the ability of each of these bacterial mutants to convert D-[1-¹⁴C]galactose to ¹⁴CO₂ was explored. This project was also

undertaken to determine whether gal-transferase-deficient *E. coli*, as has been found in human gal-transferase-deficient fibroblasts, might be capable of converting galactose to CO₂ despite their similar inability to grow on galactose. The ability of the bacterial strains to convert D-[1-¹⁴C]galactose to ¹⁴CO₂ is shown in Fig. 1 and Table 1. The galactokinase-deficient, the epimerase-deficient, and the galactose operon deletion strains make almost no ¹⁴CO₂ from D-[1-¹⁴C]galactose, whereas the gal-transferase-deficient strain was able to metabolize significant amounts of galactose. The gal-

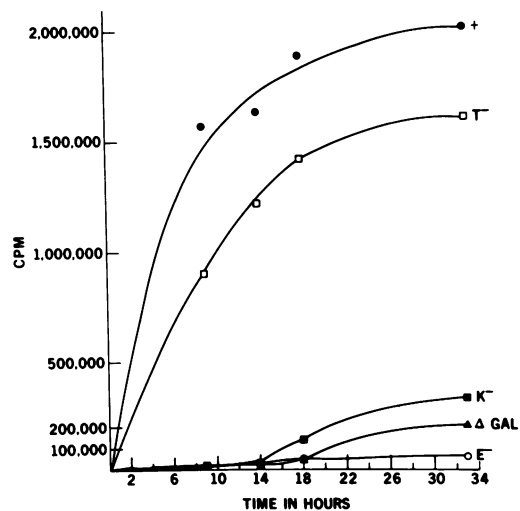


FIG. 1. Galactose metabolism as reflected by the amount of ¹⁴CO₂ produced by gal-deficient *E. coli* K-12 from D-[1-¹⁴C]galactose. Techniques employed here are the same as those described in footnote a of Table 1.

¹ Present address: Department of Biological Sciences, Oakland University, Rochester, Mich. 48063.

TABLE 1. Ability of *E. coli* K-12 strains to metabolize D-[1-¹⁴C]galactose^a

Strain	Galactose phenotype	Growth on minimal galactose plates	Color on MacConkey galactose indicator plates	Rate of galactose utilization ^c	Final % utilization of input galactose \pm SD ^c	Mating type	Genotype
SA814	Wild type	+	Red	106,300 \pm 9,000	19.3 \pm 2.7	HrfH	<i>thi str</i> ⁺
SA622	Transferase deficient	-	White	80,700 \pm 3,300	14.0 \pm 0.6	F ⁻	<i>galT151 (amber)</i> <i>his str</i> ⁺
N99	Kinase deficient	-	White	14,300 \pm 6,700	3.2 \pm 0.4	F ⁻	<i>galK 2 str</i> ⁻
N14	Epimerase deficient	-	Dies	2,900 \pm 300	0.5 \pm 0.2	HfrH	<i>thi str</i> ⁺ <i>galE PL2</i>
SA758	Gal deletion	-	White	5,300 \pm 2,700	1.4 \pm 0.4	F ⁺	Δ <i>gal str</i> ⁻ <i>his</i>

^a Cells were grown in a liquid minimal salts medium containing (per milliliter): thiamine, 12.5 μ g; adenine, 0.16 mg; biotin, 4.0 μ g; and casein acid hydrolysate, 125 mg. Stocks of each strain were grown up overnight at 37 C in 5 ml of this medium with a final concentration of 1% glucose. The phenotype of all strains was verified on MacConkey galactose indicator plates. In addition, all strains were checked for galactokinase (2), gal-transferase (6), and uridine 5'-diphosphate-galactose-4-epimerase (2) activity, and all exhibited the expected phenotype (Table 2). The experimental procedure for determining ¹⁴CO₂ production from D-[1-¹⁴C]galactose or D-[1-¹⁴C]glucose was performed in plastic tissue culture flasks (Falcon Co., 75 cm²). Each flask contained 20 ml of defined medium and 5 μ Ci of the appropriate hexose; 5 μ Ci corresponded to 0.0170 mg of D-[1-¹⁴C]galactose and 0.0166 mg of D-[1-¹⁴C]glucose, both of which had a specific activity of 52.9 μ Ci/mmol and were obtained from New England Nuclear Corp. Each flask was inoculated with an equal number of viable cells (10⁶ colony-forming units). Flasks were then sealed with sterile, silicone rubber stoppers (no. 2, Arthur Thomas Co.). Through the course of the experiment, 5-ml gas samples were withdrawn from each of the flasks by using a 6-ml greased, silicone plastic syringe and a 19-gauge needle. A 5-ml volume of air was replaced after each sample was taken. The amount of ¹⁴CO₂ produced was determined by the method of Pettriciani et al. (7). The efficiency of trapping by silica gel-phenethylamine columns (7) was found to be 99.9% by passing ¹⁴CO₂ through two columns in series and then determining the amount of radioactivity in each. During the experimental procedure and at its conclusion, small samples of medium were taken, and the number and phenotype of cells present in each flask were determined. This demonstrated that the observed effects were not due to aberrant differences in cell numbers or to selection of galactose fermentors. All strains used in this experiment were graciously provided by Sankar L. Adhya, National Cancer Institute, Bethesda, Md.

^b Counts/minute per hour \pm standard deviation.

^c SD, Standard deviation.

TABLE 2. Specific activities of galactokinase, gal-transferase, and uridine 5'-diphosphate-galactose-4-epimerase (epimerase) in strains employed^a

Strain	Galactose phenotype	Galactokinase activity	Gal-transferase activity	Epimerase activity
SA814	+	75.8	32.7	345.4
SA622	T ⁻	97.8	<0.1	170.7
N99	K ⁻	0.7	25.6	516.3
N14	E ⁻	18.5	8.2	42.5
SA758	Δ <i>gal</i>	8.9	<0.1	<0.1

^a Galactokinase and epimerase were assayed by the method of Chacko et al. (2). The method described by Merrill et al. (6) was used to assay gal-transferase activity. The specific activity of galactokinase is expressed as nanomoles of galactose phosphorylated per hour per milligram of protein. The specific activity of gal-transferase is expressed as nanomoles of uridine 5'-diphosphate-galactose formed per hour per milligram of protein. Epimerase activity is expressed as nanomoles of uridine 5'-diphosphate-glucose formed per hour per milligram of protein.

transferase-deficient strain produced 0.667 \pm 0.027 nmol of ¹⁴CO₂/h per flask compared to 0.880 \pm 0.075 nmol of ¹⁴CO₂/h per flask produced by the wild-type strain.

To verify that the ability or inability of these bacterial strains to metabolize galactose was due to galactose metabolic defects and not to nonspecific differences between strains with respect to glucose metabolism, we used D-[1-¹⁴C]glucose as the hexose source in a control study. In this experiment, all strains produced very nearly the same amount of ¹⁴CO₂ from D-[1-¹⁴C]glucose (0.624 \pm 0.076 nmol of ¹⁴CO₂/h per flask).

The low levels of ¹⁴CO₂ production from D-[1-¹⁴C]galactose observed in the galactokinase-deficient, the epimerase-deficient, and the galactose operon deletion strains (0.118 \pm 0.055, 0.024 \pm 0.002, and 0.044 \pm 0.022 nmol of ¹⁴CO₂/h per flask, respectively) may have been due to a nongalactose contaminant in the D-[1-¹⁴C]galactose used. In an effort to eliminate this possibility, we purified the D-[1-¹⁴C]galactose

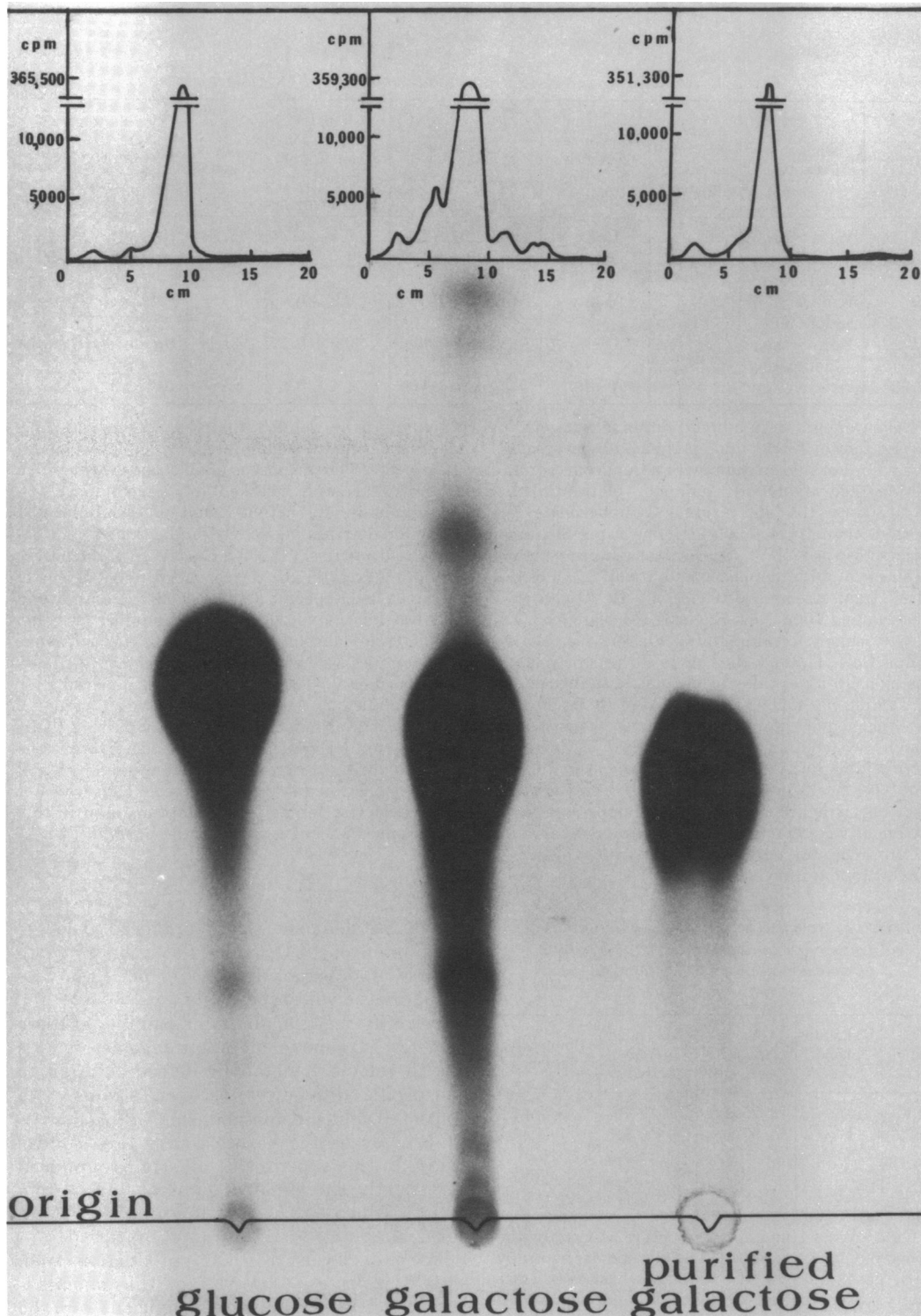


FIG. 2. Autoradiogram of thin-layer chromatographic separation of commercial D-[1-¹⁴C]glucose, commercial D-[1-¹⁴C]galactose, and purified D-[1-¹⁴C]galactose. Above each separation is a plot of counts per minute per centimeter of distance from the base of the thin-layer chromatographic plate for that separation. The thin-layer chromatographic plates used were Eastman silica gel without fluorescent indicator. Plates were not washed prior to use. Separation was effected by the Waldi solvent system (n-butanol-acetone-phosphate buffer, pH 5; 40:50:10 [10]). The autoradiogram was made with Kodak No-Screen medical X-ray film. Exposure time was 48 h at 2 C.

by thin-layer chromatography, using the solvent system described by Waldi (10) for the separation of monosaccharides. The purified galactose was subsequently eluted from the thin-layer chromatographic plate, after localization by autoradiography, and used experimentally with no change in the results. A 0.5- μ Ci portion of the purified D-[1- 14 C]galactose was rechromatographed along with 0.5 μ Ci of commercial D-[1- 14 C]galactose and 0.5 μ Ci of commercial D-[1- 14 C]glucose. An autoradiogram was subsequently made and is shown in Fig. 2. As can be seen, nearly all of the impurities previously present have been removed from the galactose.

In summary, galactokinase- and gal-transferase-deficient *E. coli* show the same pattern of galactose utilization as do their human fibroblast counterparts, with similar inborn errors of galactose metabolism. Galactokinase-deficient *E. coli* and human cells make little or no CO₂ from galactose. Gal-transferase-deficient *E. coli* and human cells can produce 14 CO₂ from D-[1- 14 C]galactose although at a reduced rate compared with normal cells. The observation that galactokinase- and epimerase-deficient *E. coli* cells can make little 14 CO₂ from D-[1- 14 C]galactose suggests that the alternative route(s) first requires phosphorylation of galactose by galactokinase and later requires uridine 5'-diphosphate-galactose-4-epimerase. These data suggest that the other route(s) for galactose me-

tabolism in *E. coli* can only circumvent the enzymatic step requiring gal-transferase.

LITERATURE CITED

1. Adhya, S. L., and J. A. Shapiro. 1969. The galactose operon of *E. coli* K-12. I. Structural and pleiotropic mutations of the operon. *Genetics* **62**:231-247.
2. Chacko, C. M., L. McCrone, and H. L. Nadler. 1972. A study of galactokinase and glucose-4-epimerase from normal and galactosemic skin fibroblasts. *Biochim. Biophys. Acta* **284**:552-555.
3. Friedman, T. B., R. J. Yarkin, and C. R. Merrill. 1975. Galactose and glucose metabolism in galactokinase deficient, galactose-1-phosphate uridyl transferase deficient, and normal human fibroblasts. *J. Cell. Physiol.* **85**:569-578.
4. Kalchar, H. M. 1965. Galactose metabolism and cell "sociology." *Science* **150**:305-313.
5. Krooth, R. S., and E. K. Sell. 1973. Characteristics of fibroblasts cultured from patients with galactosemia or galactokinase deficiency, p. 633-638. *In* P. F. Kruse and M. K. Patterson (ed.), *Tissue culture methods and applications*. Academic Press Inc., New York.
6. Merrill, C. R., M. R. Geier, and J. C. Pettricciani. 1971. Bacterial virus gene expression in human cells. *Nature (London)* **233**:398-400.
7. Pettricciani, J. C., M. K. Binder, C. R. Merrill, and M. R. Geier. 1972. Galactose utilization in galactosemia. *Science* **175**:1368-1370.
8. Pickering, W. R., and R. R. Howell. 1972. Galactokinase deficiency: clinical and biochemical findings in a new kindred. *J. Pediatr.* **81**:50-55.
9. Segal, S. 1972. Disorders of galactose metabolism, p. 174-195. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson (ed.), *The metabolic basis of inherited disease*. McGraw-Hill Book Co., New York.
10. Waldi, D. 1965. Dunnschicht-Chromatographie einiger Zucker und Zuckeralkohole. *J. Chromatogr.* **18**:417-418.