

Table 2. Galactose-1-phosphate uridyl transferase in blood cell hemolysates (micromoles of uridine diphosphoglucose utilized per milliliter of red blood cells, per hour, 4).

Subject	Enzymatic activity
Normal adult	1.0 to 3.0
Normal infant (cord blood)	2.0
Galactosemic adult	< 0.02
Galactosemic infant (cord blood)	< 0.02
Galactosemic infant transfused with normal blood	
First day	0.50
40th day	0.24
200th day	0.01

pressed as micromoles of galactose-1-phosphate or glucose-1-phosphate incorporated per gram of liver, per hour. It can be seen that the galactose-1-phosphate uridyl transferase of liver is greatly lowered in congenital galactosemia. In one case, an infant afflicted with the disease, no detectable incorporation of 1-C<sup>14</sup>-galactose-1-phosphate took place (less than 0.5 percent); the analogous transferase incorporating pyrophosphate (uridine diphosphoglucose pyrophosphorylase) was, however, present. In another case, an adult with the disease, there was a slight but definite ability to incorporate 1-C<sup>14</sup>-galactose-1-phosphate into uridine nucleotides. The rate amounted to about 5 percent of the average normal rate. Differences in the severity of the disease may thus be reflected in the completeness of the metabolic defect in liver tissue. This patient has previously been found, clinically, to manifest some tolerance for galactose. The estimate here is in fair agreement with some metabolic *in vivo* studies performed on the same subject (12). Whether the result found is due to a slight activity of galactose-1-phosphate uridyl transferase or to an alternate, related pathway (compare 13) that is at present unknown cannot be decided by this technique. Neither uridine diphosphoglucuronic acid nor uridine triphosphate produced the same incorporation of 1-C<sup>14</sup>-galactose-1-phosphate as did uridine diphosphoglucose in liver tissue from this patient. It should be noted that the same adult patient showed no detectable galactose-1-phosphate uridyl transferase in red cell hemolysates that were incubated with galactose-1-phosphate and uridine diphosphoglucose (see Table 2).

In the case of uridine diphosphoglucose pyrophosphorylase, the values are minimized by appreciable hydrolysis of both reactants in the homogenates; the levels of activity given for this enzyme are therefore undoubtedly much underestimated. In addition, uridine diphospho-

glucose may be broken down or may undergo exchange with unlabeled glucose-1-phosphate in the homogenates, and incorporation into the nucleotide fraction may therefore be decreased. This would affect the values for both enzymes. However, the activity of galactose-1-phosphate uridyl transferase in galactosemic liver is still clearly only a small percentage of that in nongalactosemic tissue.

Table 2 gives the results of some further studies on galactose-1-phosphate uridyl transferase in red cell hemolysates. One infant with galactosemia was transfused with normal red cells because of bleeding tendencies; this offered an opportunity to follow the disappearance of donor erythrocyte galactose-1-phosphate uridyl transferase. A half-life of about 40 days was found.

As can be seen, the assay can be applied to umbilical cord blood of newborn infants, and the defect is also present in cord blood of galactosemic infants (14). This observation indicates that the disease can be diagnosed at birth and that the proper treatment can be instituted immediately without ever subjecting these patients to the deleterious effects of galactose or milk ingestion.

ELIZABETH P. ANDERSON\*  
HERMAN M. KALCKAR  
KURT J. ISSELBACHER†

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

#### References and Notes

- H. M. Kalckar, *Science*, this issue.
- Abbreviations: G-1-P,  $\alpha$ -glucose-1-phosphate; Gal-1-P,  $\alpha$ -galactose-1-phosphate; PP, inorganic pyrophosphate; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; UTP, uridine triphosphate.
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14. The galactosemic cord blood analyzed was sent to us by Fred I. Dorman, Lakeland, Fla.

\* Fellow in cancer research of the American Cancer Society. Present address: National Cancer Institute, National Institutes of Health, Bethesda, Md.

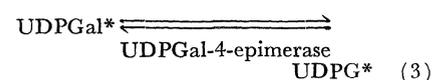
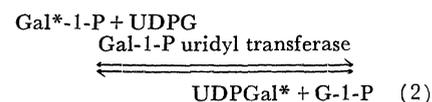
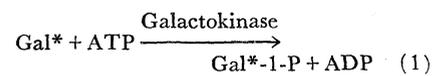
† Present address: Department of medicine, Massachusetts General Hospital, Boston, Mass.

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### Enzyme Formation in Galactose-Negative Mutants of *Escherichia coli*

Morse, Lederberg, and Lederberg recently reported the transduction of Gal<sup>+</sup> genes from galactose-positive to galactose-negative cells of *Escherichia coli* strain K-12 by the bacteriophage lambda (1). They also described the transduction of Gal<sup>-</sup> genes from galactose-negative to galactose-positive cells (2). This report (3) deals with the correlation between the various galactose loci and the formation of galactose-metabolizing enzymes.

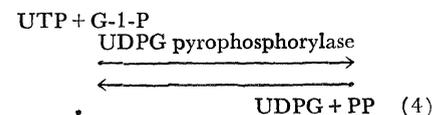
Galactose (Gal) (4) is metabolized by *E. coli* adaptively much as it is by *Saccharomyces fragilis* (5) through  $\alpha$ -galactose-1-phosphate (Gal-1-P), uridine diphosphogalactose (UDPGal), and uridine diphosphoglucose (UDPG) as follows:



The sum of reactions 1, 2, and 3 is



As is shown in these equations, it is necessary that the three enzymes in steps 1, 2, and 3 operate in order to convert free galactose to a glycolytic intermediate. The catalytic amount of uridine diphosphoglucose needed for this conversion could be supplied by another enzyme, uridine diphosphoglucose pyrophosphorylase, as follows:



Galactokinase activity was measured by determining the amount of galactose-1-phosphate formed during the incubation of galactose and adenosine triphosphate (ATP) with the enzyme solution. The galactose-1-phosphate formed was measured by the reduction of triphosphopyridine nucleotide in a coupled re-

action of purified galactose-1-phosphate uridyl transferase, glucomutase, and *Zwischenferment* (6). Galactose-1-phosphate uridyl transferase activity was measured by determining spectrophotometrically the reduction of triphosphopyridine nucleotide in a coupled reaction with phosphoglucomutase and *Zwischenferment* (7). Uridine diphosphogalactose 4-epimerase activity was measured by determining diphosphopyridine nucleotide reduction spectrophotometrically in a coupled reaction with uridine diphosphoglucose dehydrogenase (8). Uridine diphosphoglucose pyrophosphorylase activity was measured in the same way as galactose-1-phosphate uridyl transferase assay except that pyrophosphate was substituted for galactose-1-phosphate (7).

The mutants of *E. coli* K-12 were grown in 300 ml of glycerol-complete medium [10 g of casein digest (NZ case), 5 g of yeast extract, 3 g of  $K_2HPO_4$ , 1 g of  $KH_2PO_4$ , and 5 g of glycerol per liter] for 12 to 16 hours at 37°C (9). After harvest by centrifugation, the cells were resuspended in 300 ml of galactose-complete medium (as described in the previous sentence except that galactose was substituted for glycerol) and incubated for 6 to 10 hours at 37°C. The crude cell-free extracts were prepared by grinding the washed cells with alumina powder or by disintegration in a mechanical cell disintegrator (10).

The distribution of the four enzymes concerned with galactose metabolism in the galactose mutants of *E. coli* is summarized in Table 1. On the basis of this table, the mutants that were tested can be classified into two groups: one lacks galactose-1-phosphate uridyl transferase and the other lacks galactokinase. Uridine diphosphogalactose-4-epimerase and uridine diphosphoglucose pyrophosphorylase were present in all the mutants tested. In fact, the latter two enzymes are not induced enzymes because they are present in the cells grown on glycerol synthetic medium without previous induction to galactose (11).

An observation, which may be noteworthy, is the fact that the loci which are all concerned with the development

Table 2. Incorporation of galactose-1- $C^{14}$  into uridine nucleotides by extracts of *E. coli* mutants.

Strain	Genetic notation	Additions of extracts (ml)									
		Single extract					Mixed extract				
W3100	Gal +	0.1									
W3091	Gal <sub>1</sub> -		0.1			0.1	0.1	0.1			
W3094	Gal <sub>4</sub> -			0.1			0.1		0.1	0.1	
W3092	Gal <sub>2</sub> -				0.1			0.1			0.1
W3142	Gal -*					0.1			0.1		0.1
Total counts† incorporated into uridine nucleotides (10 <sup>-8</sup> count/min)		33.9	0	0	0	0	0	37.6	36.2	29.6	39.0

\* See the footnote of Table 1. † The counts have been corrected for the control, the reaction of which was stopped at zero time.

of galactokinase activity are not located in the same cluster of genes. As reported by Morse, Lederberg, and Lederberg (1, 2), the galactose loci, Gal<sub>1</sub> to Gal<sub>8</sub> (W3091 to W3097 and W3178) are closely linked to one another, whereas the Gal- locus in the mutant W3142 is separated from the afore-mentioned cluster of genes and not closely linked to the phage lambda. It appears from Table 1 that the loci Gal<sub>2</sub>, Gal<sub>3</sub>, and the more distant Gal- locus of W3142 are all involved in the development of galactokinase activity.

In order to confirm these results by an independent technique, experiments with  $C^{14}$ -labeled galactose were carried out. The principle of these experiments was that extracts lacking either galactokinase or galactose-1-phosphate uridyl transferase would fail to incorporate free galactose-1- $C^{14}$  into uridine nucleotides, as indicated by the asterisks in Eq. 1, 2, and 3. If, however, the extracts of a mutant in group 1 and of another in group 2 are mixed, the incorporation of free galactose-1- $C^{14}$  into uridine nucleotides should take place.

One micromole of galactose-1- $C^{14}$  (2.25 × 10<sup>5</sup> count/min μmole) (12), 1.7 μmole of adenosine triphosphate, 5 μmole of  $MgCl_2$ , 100 μmoles of tris buffer (pH 7.5), 0.3 μmole of uridine diphosphoglucose, 25 μmole of NaF, 10 μmole of cysteine, and 0.1 ml of crude extract of

*E. coli* (about 1 mg of protein) in a total volume of 1.0 ml were incubated for 1 hour at 37°C. After the reaction had been stopped by heating the reaction mixture at 100°C for 1.5 minutes, the nucleotides were adsorbed on charcoal and treated as described by Kalckar *et al.* (13).

The results of these experiments are presented in Table 2. The first part of the table shows the results obtained with unmixed extracts of single mutants, and the second part of the table presents the results obtained with mixtures of the extracts of two different mutants. It should be noted that the mixture of extracts of two mutants which lack the activity of the same enzyme failed to show any incorporation of free galactose into uridine nucleotides, whereas the mixture of extracts of two mutants which lack the activity of galactokinase and galactose-1-phosphate uridyl transferase, respectively, showed the incorporation of free galactose at the level observed with the extract of the Gal+ mutant.

These results not only confirmed the conclusions derived from Table 1, but also ruled out the possibility that the inactivity of galactokinase or galactose-1-phosphate uridyl transferase is the result of the presence of some inhibitor in the extracts of these mutants. It cannot be decided at this point whether the lack of the enzymatic activity observed in these mutants is due to a complete loss of the ability to form the enzyme protein molecule or whether it is due to the formation of an incomplete enzyme protein molecule as in the *Neurospora* mutants that have defects in tryptophan synthesis, as described by Yanofsky (14).

KIYOSHI KURAHASHI\*

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

#### References and Notes

1. M. L. Morse, E. M. Lederberg, J. Lederberg, *Genetics* 41, 143 (1956).
2. ———, *ibid.* 41, 758 (1956).
3. This investigation was aided in part by a grant from the Jane Coffin Childs Memorial Fund for Medical Research. I am grateful to M. L.

Table 1. Distribution of the enzymes metabolizing galactose in *E. coli* mutants.

Strain	Genetic notation	Galactokinase	Gal-1-P uridyl transferase	UDPGal-4-epimerase	UDPG pyrophosphorylase
W3100	Gal +	+	+	+	+
W3091	Gal <sub>1</sub> -	+	-	+	+
W3092	Gal <sub>2</sub> -	-	+	+	+
W3094	Gal <sub>4</sub> -	+	-	+	+
W3096	Gal <sub>6</sub> -	+	-	+	+
W3097	Gal <sub>7</sub> -	+	-	+	+
W3178	Gal <sub>8</sub> -	-	+	+	+
W3142	Gal -*	-	+	+	+

\* No designation for this mutant, which is not linked in the same cluster as the others, has yet been published (15).

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4. Abbreviations: ADP and ATP, adenosine di- and triphosphate, respectively; G-1-P,  $\alpha$ -glucose-1-phosphate; Gal, D-galactose; Gal-1-P,  $\alpha$ -galactose-1-phosphate; PP, inorganic pyrophosphate; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; UTP, uridine triphosphate.
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  11. K. Kurahashi, unpublished observation.
  12. The  $C^{14}$ -labeled galactose was synthesized by H. Isbell and his coworkers at the National Bureau of Standards.
  13. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Biochim. et Biophys. Acta* 20, 262 (1956).
  14. C. Yanofsky, in *Enzymes: Units of Biological Structure and Function*, O. H. Gaebler, Ed. (Academic, New York, 1956), p. 147.
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- \* Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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### Studies on Metabolism of Carbon-14-Labeled Galactose in a Galactosemic Individual

It is frequently reported (1) that in congenital galactosemia only about 60 to 80 percent of the galactose administered can be accounted for on the basis of urinary excretion. The fate of the remaining galactose has, so far, been virtually unknown. However, Schwartz *et al.* (2) have recently demonstrated that galactose administration to galactosemic children brings about a significant accumulation of a hexose phosphate identified as galactose-1-phosphate. This poses the question whether the galactose retained in the body as galactose-1-phosphate might not account for the major part, if not the total, of the fraction of the galactose that is not excreted.

We have found (3-5) that in hemolysates, as well as in liver homogenates (6) from galactosemic subjects, the enzyme that catalyzes the metabolic step immediately succeeding galactose-1-phosphate formation (galactose-1-phosphate uridyl transferase) is defective or totally absent.

We were therefore interested in studying the galactose metabolism in man with special reference to: (i) galactose-1-phosphate accumulation and (ii) residual metabolism beyond the galactose-1-phosphate stage. Concerning the latter problem, it was felt that although enzyme studies reveal a defect of major propor-

tion, a study of galactose metabolism in the intact human organism might detect the presence of appreciable metabolism beyond simple phosphorylation. Highly sensitive methods for detecting the conversion of galactose to the glucose of glucose derivatives were based on two principles: (i) The use of  $C^{14}$ -labeled galactose and (ii) the trapping of galactose as a glucosiduronic acid. The latter principle was used for several reasons.

Studies during the last few years have shown that the irreversible conversion of glucose to glucosiduronic acids involves the very same uridine nucleotides that are operating in the conversion of galactose to glucose derivatives (7). Moreover, the conversion of galactose and glucose compounds to alcohol glucosiduronic acids gives rise to compounds that can readily be isolated as crystalline precipitates.

Galactose-1- $C^{14}$  (8) was administered intravenously over a period of 30 minutes to a 24-year-old male with galactosemia in a dose of 5  $\mu$ c. In this experiment, 1 g of nonisotopic galactose was added to the galactose-1- $C^{14}$  being infused. Concurrent with the administration of the isotope, the subject ingested 1 g of menthol over a period of 24 hours. Urine was collected at 2-hour intervals during this time. From each collection, menthyl glucosiduronic acid was isolated as the ammonium salt (9) and purified (10).

An aliquot of the urine was taken prior to isolation, acidified, extracted with redistilled ether, and total menthyl glucosiduronic acid was determined on the ether extract by the orcinol reaction using the conditions described by Dische (11). The purified menthyl glucosiduronic acid was counted in solution in a Packard Tri-Carb liquid scintillation spectrometer. A sample of the substrate galactose-1- $C^{14}$  was counted in the same way.

From the radioactivity of the pure menthyl glucosiduronic acid and the quantity of the compound excreted, total counts were calculated. From this figure and the counts administered as galactose-1- $C^{14}$ , the percentage conversion of the substrate to glucosiduronic acid was computed. Urea was isolated for the purpose of sampling the  $CO_2$  pool. Urinary galactose was measured by the method of Nelson (12) as the reducing sugar remaining after treatment of the urine with glucose oxidase (13). Galactose and galactose-1-phosphate in blood were detected by indirect methods. No detectable counts were found in the blood plasma. However, in the erythrocytes, appreciable amounts of counts were found (see Table 1).

The presence of galactose-1-phosphate was inferred for the following three reasons. (i) The  $C^{14}$ -labeled material was confined to the erythrocytes, with no

radioactive material present in the plasma fraction. Free galactose would be distributed fairly evenly between the two fractions. (ii) The reported observation of galactose-1-phosphate accumulation in the erythrocytes from congenital galactosemia has been reported (2, 3). (iii) Enzymatic assay, although it was too low to be considered quantitative, revealed traces of galactose-1-phosphate (4).

Excreted galactose and glucosiduronic acid were measured as described. The amount of suspected galactose-1-phosphate present in the relatively small blood specimens was too minute to isolate. The radioactivity measurements were therefore performed directly on small samples of crude filtrates, and corrections for self-absorption were made. Counts for a known sample of  $C^{14}$ -labeled galactose were taken under identical conditions—that is, as an internal standard. In this way counts could be expressed as micromoles of galactose.

Filtrates from plasma were found to be nonradioactive, whereas filtrates from erythrocytes showed distinct radioactivity. The latter could not be attributed to free galactose because galactose would distribute itself freely between plasma and cells. It was therefore classified as "cellular" galactose, for any cellular incorporation of radioactivity would figure on the balance as galactose retained in the body. This is actually the essential term in the balance. From the studies by Schwartz *et al.* (2) as well as those by us (5), it seems likely that all of the cellular galactose is identical with galactose-1-phosphate.

The distribution of galactose which was found over a period of 4 hours is shown in Table 1. As can be seen, out of the 1 g of galactose administered, 75 to 80 percent was not metabolized beyond the galactose-1-phosphate stage, 3 percent was metabolized to the glucosiduronic acid stage, and 20 to 25 percent was not accounted for. The latter fraction was probably metabolized to carbon dioxide or lactic acid but diluted by carbon from the general carbohydrate pool so as to escape detection, for the urea

Table 1. Balance of galactose compounds (after the infusion of 1 g of galactose and 5  $\mu$ c of galactose-1- $C^{14}$  to a 24-year-old male with galactosemia).

Item	Amount (mg)
Galactose excreted in urine	700
Galactose metabolized to glucosiduronic acid	30
Galactose accumulating as "cellular" galactose (galactose-1-phosphate and so forth)	50 to 100*

\* All tissues with the exception of muscle and bone.