# The Oxidation of $C^{14}$Galactose by Patients with Congenital Galactosemia*

## *Evidence for a Direct Oxidative Pathway*

STANTON SEGAL, M.D.† *and* PEDRO CUATRECASAS, M.D.
*Bethesda, Maryland*

The oxidation of galactose-1-$C^{14}$ and galactose-2-$C^{14}$ by normal human subjects and patients with congenital galactosemia who lack the enzyme, galactose-1-phosphate uridyl transferase, was measured to determine whether this newly described direct oxidative pathway of galactose metabolism was present. Via this route, galactose is oxidized to galactonate which is subsequently decarboxylated to form D-xylulose, a keto sugar capable of entering the pentose phosphate metabolic pathway. By comparing $C^{14}O_2$ excretory pattern after intravenous administration of galactose-1-$C^{14}$ and galactose-2-$C^{14}$ with those seen after giving glucose correspondingly labeled, it was concluded that normal man oxidizes both small and large amounts of intravenously administered galactose to carbon dioxide by conversion first to glucose via the well known uridine sugar nucleotide pathway. On the other hand, in the Caucasian galactosemic subject with little capacity to oxidize galactose twice as much label is incorporated into carbon dioxide after giving galactose-1-$C^{14}$ than when galactose-2-$C^{14}$ is given, a finding consistent with operation of the oxidative route. The Negro variant galactosemic subject who can metabolize galactose demonstrates patterns of $C^{14}O_2$ formation similar to the normal. The data indicate that the low residual oxidative activity in the Caucasian galactosemic patients is due to operation of the galactonate pathway. The unknown metabolic pathway for extensive galactose oxidation in the Negro galactosemic subject is probably more closely related to one in which there is no differential oxidation of carbon one of galactose, like the conventional sugar nucleotide pathway.

THE capacity of patients with congenital galactosemia to oxidize galactose-1-$C^{14}$ to $C^{14}O_2$ has been reported [*1*]. In these studies it was observed that two groups of patients could be delineated. The first consisted of Caucasian subjects, who could oxidize small and variable amounts of galactose ranging from 0 to 8 per cent of the normal without regard to age. The second, composed of Negro subjects, could oxidize normal or near normal amounts of the intravenously administered sugar during the five hour period of study even though as in the first group red cell galactose-1-phosphate uridyl transferase is absent. An additional Negro patient with similar oxidative capacity has been described [*2*].

Unanswered by these experiments was the intriguing question of the nature of the metabolic pathway whereby patients with congenital galactosemia could oxidize any galactose. In the conventional route of galactose metabolism galactose is converted to glucose by the following reactions:

1. Galactose → Galactose-1-phosphate
2. Galactose-1-phosphate + uridinediphosphoglucose → uridinediphosphogalactose + glucose-1-phosphate
3. Uridinediphosphogalactose → uridinediphosphoglucose
4. Uridinediphosphoglucose + pyrophosphate → uridinetriphosphate + glucose-1-phosphate.

The glucose-1-phosphate then enters the

* From Clinical Endocrinology Branch, National Institute for Arthritis and Metabolic Disease, National Institutes of Health, Bethesda, Maryland. Manuscript received April 12, 1967.

† Present address: Department of Pediatrics, University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

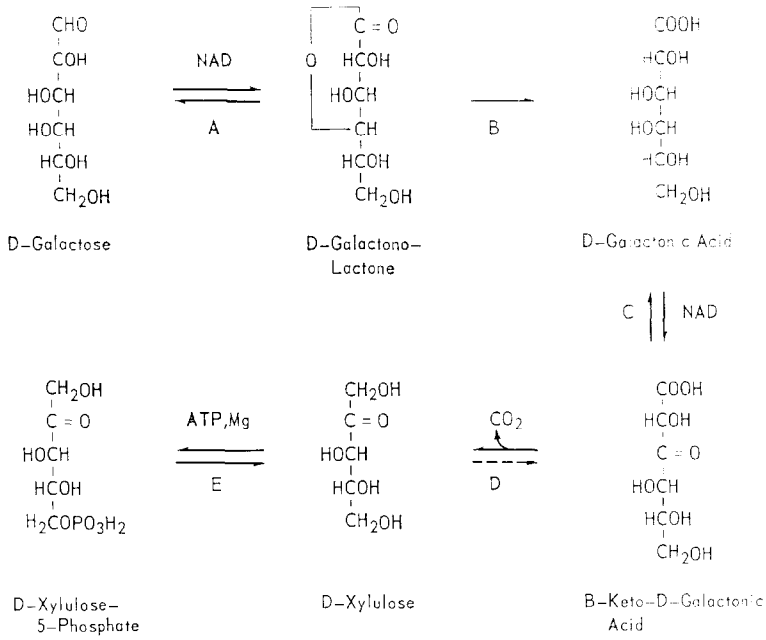


FIG. 1. Proposed scheme of an alternate pathway of galactose metabolism by mammalian liver. Enzymes are galactose dehydrogenase (A), lactonase (B), β-L-hydroxy acid dehydrogenase (C) and D-xylulokinase (E). Decarboxylation (D) may be enzymatic.

pathways of glucose to carbon dioxide. It is the absence of the enzyme catalyzing reaction 2, galactose-1-phosphate uridyl transferase which is deficient in congenital galactosemia [*3*]. Thus, a block of reaction 2 should eliminate the conversion of galactose to glucose phosphates and ultimately to carbon dioxide. One possibility of circumventing the block is through another enzyme [*4*], which catalyzes the reaction:

5. Galactose-1-phosphate + uridinetriphosphate → Uridinediphosphogalactose + pyrophosphate

the product of which could enter reaction 3.

An alternative direct oxidative pathway of galactose has recently been described [*5*] (Fig. 1) which might account for the metabolism in these patients. This pathway has none of the intermediates of the conventional pathway, does not involve phosphorylated intermediates and can convert galactose to D-xylulose which can be used in the pentose phosphate pathway. By this sequence galactose is oxidized to galactonate, which is then oxidized and decarboxylated to xylulose. Galactose labeled in the C-1 position would give off labeled carbon dioxide directly. The second carbon of galactose becomes C-1 of xylulose, which could have several metabolic fates. Indeed, it was observed that in a *in vitro* rat liver preparation which oxidized galactonate-1-$C^{14}$ to $C^{14}O_2$, no labeled carbon dioxide was formed from galactonate-2-$C^{14}$ [*5*]. The first enzyme in the pathway has been found in human liver and it thus seemed possible that the function of this direct oxidative pathway could be studied by the administration of galactose-1-$C^{14}$ and galactose-2-$C^{14}$ to patients with galactosemia to ascertain differences in the production of $C^{14}O_2$ from the two substrates. Theoretically, if the galactose molecule is metabolized via the conventional sugar nucleotide pathway, or the alternate route with UTP (reaction 5), galactose conversion to glucose should occur with the carbon chain intact and the pattern of respiratory $C^{14}O_2$ excretion from both labeled sugars should resemble that obtained from glucose-1-$C^{14}$ and glucose-2-$C^{14}$. However, in the conversion of galactose to xylulose, C-1 may be preferentially excreted and a more rapid excretion of $C^{14}O_2$ from C-1 labeled galactose might be observed. In order to assess these possibilities galactose-1-$C^{14}$ and galactose-2-$C^{14}$ were administered intravenously to normal subjects, three Caucasian patients and one Negro patient with galactosemia, and the oxidation to radioactive carbon dioxide was determined in the expired air for up to eight hours following the injection. This report describes the results of these experiments.

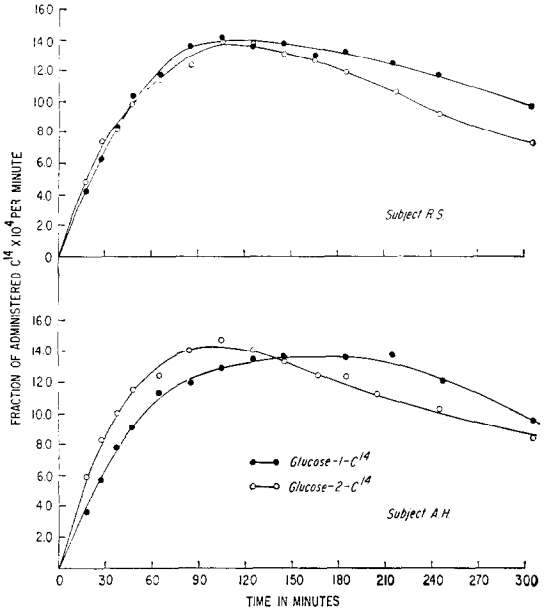


FIG. 2. The excretion of $C^{14}O_2$ in expired air by normal subjects after intravenous administration of glucose-1-$C^{14}$ and glucose-2-$C^{14}$.

## METHODS AND MATERIALS

*The Subjects.* The clinical features of the patients studied here have been described in the previous report on the ability of galactosemic patients to metabolize galactose [*1*]. The Caucasian patients with galactosemia were P.R., L.J. and H.T. of that report. P.R., a female patient, was fifteen years old at the time of the present study, and entering into puberty. Two male patients, L.J. and H.T., were nineteen and seventeen years of age, respectively. They were four, four and two years older, respectively, than when reported before but their condition had not changed appreciably. All had been on a galactose-free diet. The Negro patient was T.B., aged thirty-four, the subject of the first report of galactosemia in the American literature [*7*] whose remarkable ability to oxidize galactose has been described [*1*]. Four male and three female volunteer subjects, age eighteen to twenty-three, served as normal control subjects.

*Plan of the Experiments.* Each galactosemic patient received intravenous galactose injections of 1 gm. labeled with 1 μc. of galactose-1 and -2-$C^{14}$, with a minimum interval of one week between the two tests. Two normal control subjects also received these sugars, 1 gm. labeled with 5 μc. In addition, one of these normal subjects was studied after injection of 5 μc. in 20 gm. of the unlabeled sugars. Three normal control subjects also received a tracer dose (10 mg.) of either 5 μc. of galactonate-1-$C^{14}$ or galactonate-2-$C^{14}$ intravenously. Glucose-1-$C^{14}$ and glucose-2-$C^{14}$, 5 μc. each, were also given intravenously in tracer dose (less than 1.0 mg.) to two normal volunteer subjects. The sugars were injected as rapidly as possible into an antecubital vein and at various intervals after the injection, four minute samples of expired air were collected in Douglas bags for assay of $C^{14}O_2$. Each experiment was performed after an overnight fast which was continued throughout the period of expired air collection.

*Materials.* Galactose-1-$C^{14}$ (specific activity = 4.72 μc. per mg.) was purchased from the National Bureau of Standards, galactose-2-$C^{14}$ (specific activity = 3.06 μc. per mg.) from Calbiochem, and glucose-1-$C^{14}$ (specific activity = 2.67 μc. per mg.) and glucose-2-$C^{14}$ (specific activity 5.56 μc. per mg.) from the Volk Co. All these sugars were chromatographically pure. The galactose-1-$C^{14}$ however, had a 0.3 per cent volatile impurity detected by the addition of dilute acid. Galactonate-1-$C^{14}$ and galactonate-2-$C^{14}$ (specific activity = 0.47 μc. per mg.) were prepared by the oxidation of galactose-1-$C^{14}$ and galactose-2-$C^{14}$ according to the procedure of Moore and Link [*8*]. A solution of these radioactive sugars containing 1 μc. per ml. in normal saline solution was prepared by the Radiopharmaceutical Service of the National Institutes of Health. Unlabeled galactose was purchased from the Pfanstiehl Co. and was prepared for intravenous administration as an aqueous solution containing 20 gm. per 100 ml. These solutions were sterile and pyrogen free. Appropriate amounts of radioactive solution and the 20 per cent solution of unlabeled sugar were mixed in the same syringe for injection.

*Methods.* The amounts of carbon dioxide and $C^{14}O_2$ excreted in expired air by galactosemic patients were analyzed by a modification [*9*] of the method of Fredrickson and Ono [*10*]. The latter method was used without the intermediate step of

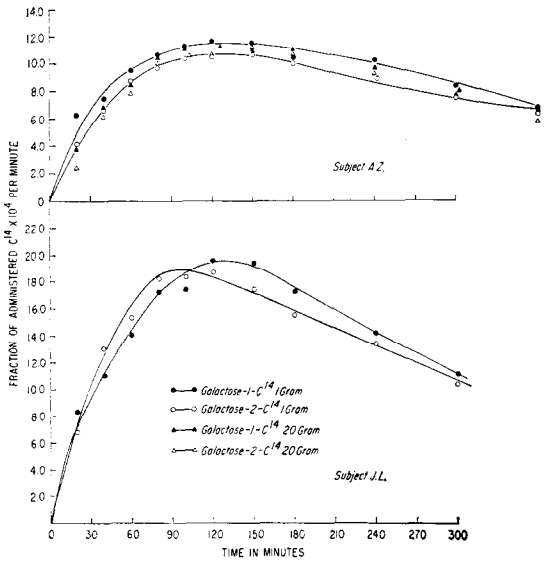


FIG. 3. The excretion of $C^{14}O_2$ in expired air by normal subjects after intravenous administration of galactose-1-$C^{14}$ and galactose-2-$C^{14}$.

barium carbonate precipitation in studies with normal subjects since the yield of $C^{14}O_2$ is much greater than in the patients. $C^{14}O_2$ was assayed in hyamine using a liquid scintillation spectrometer at 60 per cent efficiency. The cumulative excretion of $C^{14}O_2$ was calculated by integration under the excretion curve of $C^{14}O_2$ versus time [*7*].

## RESULTS

*$C^{14}O_2$ Excretion after Glucose-1-$C^{14}$ and Glucose-2-$C^{14}$ Administration in Normal Subjects.* The excretion curves of $C^{14}O_2$ by two normal subjects given tracer amounts of glucose-1-$C^{14}$ and glucose-2-$C^{14}$ is shown in Figure 2, in which the fraction of the injected $C^{14}$ excreted per minute is plotted as a function of time. In R.S., a male, the initial rate of $C^{14}O_2$ excretion from these differentially labeled glucose molecules was the same. A slightly faster rate of fall of carbon dioxide after the peak was the only difference noted. A.H., a female, demonstrated a somewhat faster initial rate of $C^{14}O_2$ formation and an earlier peak in radioactive activity following glucose-2-$C^{14}$ injection than after glucose-1-$C^{14}$ injection. Thus, in the conversion of glucose to carbon dioxide via various pathways [*11*] $C^{14}$ derived from glucose-2-$C^{14}$ appears in the expired air at a rate equal to or greater than that from glucose-1-$C^{14}$.

*$C^{14}O_2$ Excretion after Galactose-1-$C^{14}$ and Galactose-2-$C^{14}$ Administration to Normal Subjects.* Figure 3 shows the pattern of $C^{14}O_2$ labeling by two normal control subjects given C-1 and C-2 labeled galactose. A.Z., a male subject, was

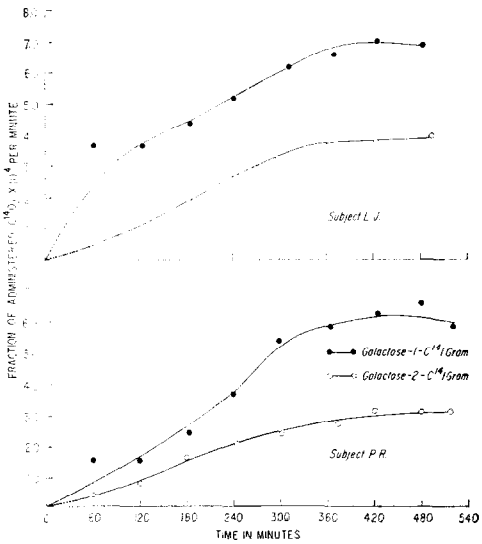


FIG. 4. The excretion of $C^{14}O_2$ in expired air by two Caucasian patients with galactosemia after intravenous administration of galactose-1-$C^{14}$ and galactose-2-$C^{14}$.

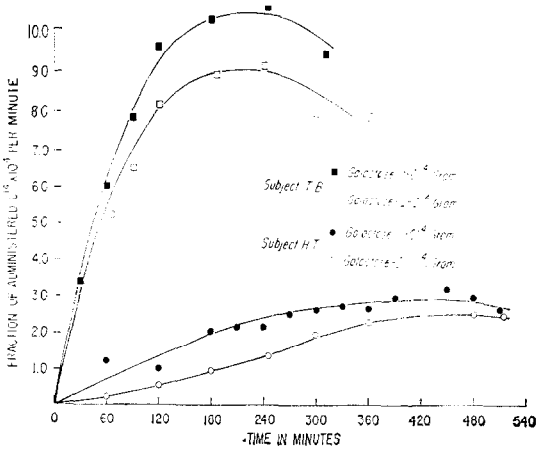


FIG. 5. The excretion of $C^{14}O_2$ in expired air by a Caucasian patient (H.T.) and a Negro patient (T.B.) with galactosemia after intravenous administration of galactose-1-$C^{14}$ and galactose-2-$C^{14}$.

given these sugars in both 1 and 20 gm. amounts. These curves reveal a slightly higher excretion of the label after galactose-1-$C^{14}$. Since the affinity of the first enzyme in the galactose oxidative pathway, galactose dehydrogenase, is relatively low (Km = 24 millimolar [*6*]), loading with 20 gm. of galactose was performed with the possibility that a greater difference between the $C^{14}O_2$ labeling patterns would result. Only the same small and questionably significant difference between the curves was seen. (The specific activity curves of the $C^{14}O_2$ in counts per minute per millimole was essentially the same for these four studies, the difference in the curves shown being due to slight differences in the minute millimolar carbon dioxide output in each of the studies.)

In J.L., a female subject, the $C^{14}O_2$ excretion curves mimic very closely those seen after the administration of glucose-1-$C^{14}$ and glucose-2-$C^{14}$ to A.H. (Fig. 2), with a somewhat faster labeling and earlier peak after the administration of galactose-2-$C^{14}$.

The comparison of $C^{14}O_2$ liberation from both glucose and galactose labeled in the one and two positions (Fig. 2 and 3) in normal versions indicates that galactose probably was oxidized by the same metabolic pathways as glucose. This would occur if galactose was first converted to glucose with the carbon chain intact. Previous studies from this laboratory have shown that in normal subjects intravenously injected galactose-1-$C^{14}$ is rapidly and extensively converted to circulating blood glucose [*12*].

*$C^{14}O_2$ Excretion after the Administration of*

TABLE I
PER CENT OF THE ADMINISTERED $C^{14}$ IN EXPIRED $C^{14}O_2$

| Subjects | Age (yr.) and Sex | $C^{14}$ | Hours | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *Galactosemic Patients* | | | | | | | | | | | |
| P.R. | 15, F | C-1 | 0.27 | 1.02 | 2.32 | 4.16 | 6.79 | 10.03 | 13.68 | 17.55 | 21.19 |
| | | C-2 | 0.09 | 0.45 | 1.20 | 2.32 | 3.73 | 5.40 | 7.26 | 9.22 | 11.20 |
| H.T. | 17, M | C-1 | 0.28 | 0.97 | 1.98 | 3.25 | 4.77 | 6.49 | 8.35 | 10.19 | 11.77 |
| | | C-2 | 0.05 | 0.25 | 0.65 | 1.29 | 2.25 | 3.52 | 4.96 | 6.47 | ... |
| L.J. | 19, M | C-1 | 0.91 | 2.79 | 5.10 | 7.88 | 11.15 | 14.85 | 18.90 | 23.06 | ... |
| | | C-2 | 0.14 | 0.69 | 1.63 | 2.99 | 4.73 | 6.81 | 9.06 | 11.42 | ... |
| T.B. | 35, M | C-1 | 1.87 | 6.83 | 13.22 | 19.88 | 26.22 | ... | ... | ... | ... |
| | | C-2 | 1.72 | 5.66 | 10.89 | 16.33 | 21.62 | 26.33 | ... | ... | ... |
| *Normal Subjects* | | | | | | | | | | | |
| J.L. | 21, F | C-1 | 5.22 | 15.82 | 27.10 | 36.47 | 43.90 | ... | ... | ... | ... |
| | | C-2 | 5.73 | 16.68 | 27.20 | 35.47 | 42.84 | ... | ... | ... | ... |
| A.Z. | 18, M | C-1 | 3.10 | 9.07 | 15.35 | 21.12 | 26.12 | 30.22 | ... | ... | ... |
| | | C-2 | 3.10 | 9.07 | 15.35 | 21.12 | 26.12 | 30.22 | ... | ... | ... |
| | | C-1b | 3.10 | 9.07 | 15.35 | 21.12 | 26.12 | 30.22 | ... | ... | ... |
| | | C-2b | 2.64 | 8.57 | 14.84 | 20.62 | 25.61 | 29.72 | ... | ... | ... |

NOTE: All subjects received 1 gm. of galactose except A.Z. who received 20 gm. in studies marked b. Normal subjects received 5 μc. and all galactosemic subjects received 1 μc. except T.B. who received 2.5 μc. in each study.

*Galactose-1-$C^{14}$ and Galactose-2-$C^{14}$ to Galactosemic Patients.* Unlike the similarity of the patterns of carbon dioxide labeling seen after giving normal subjects different galactose substrates, those observed in Caucasian galactosemic patients, P.R., L.J. and H.T., reveal differences in the amount of radioactivity appearing in the expired air. As shown in Figures 4 and 5, $C^{14}O_2$ from galactose-1-$C^{14}$ oxidation was labeled more rapidly and to about twice the extent as that seen from galactose-2-$C^{14}$ oxidation. On the other hand, the curves of $C^{14}O_2$ production in the experiments with the Negro galactosemic patient, T.B. (Fig. 5), indicate a response similar to that found in normal subjects with rapid and extensive labeling of carbon dioxide from C-2 labeled galactose.

The data in Table I include quantification of the galactose oxidation in per cent of the administered dose in both normal and galactosemic patients as derived from integration of the areas under the curves. It is quite evident that in Caucasian subjects the $C^{14}O_2$ derived from C-1 of the sugar is approximately twice that from C-2. The pattern in T.B. is almost the same as in the normal subjests.

An important point can be made from these data. In our previous studies a standardized $C^{14}O_2$ excretion time of five hours was adhered to and Caucasian galactosemic patients excreted up to 8 per cent of the dose in that time. Here the collections were made for nearly eight to nine hours and it is apparent that these patients can excrete much more as $C^{14}O_2$ if followed for longer periods. P.R., for example, oxidizes galactose-1-$C^{14}$ in nine hours to the same extent as the normal subject A.Z. does in four hours. T.B. is again found to be almost normal in the extent of his galactose oxidation. Also, the quantitative $C^{14}O_2$ yields by the different galactosemic patients vary considerably, which suggests some heterogeneity in the Caucasian galactosemic group.

*Oxidation of Galactonate-1-$C^{14}$ and Galactonate-2-$C^{14}$ by Normal Subjects.* In order to determine if the oxidation of galactonate, the metabolite of galactose via the oxidative pathway (Fig. 1) occurred, 5 μc. of galactonate-1-$C^{14}$ was given intravenously to two normal subjects and galactonate-2-$C^{14}$ to one normal subject and carbon dioxide assayed for radioactivity. None could be detected in the carbon dioxide and between 85 and 95 per cent of the administered $C^{14}$ was excreted in the urine in eight hours. Paper chromatography of the urine showed this $C^{14}$ to be in biochemically unaltered galactonate. Since rat liver homogenates [*5*] and rat kidney cortex slices [*13*] can oxidize galactonate-1-$C^{14}$ to

$C^{14}O_2$, it seems likely that renal excretion removes the compound from the body much faster than it can be oxidized. This is substantiated by experiments with nephrectomized or ureter-ligated rats which show oxidation of the compound to $C^{14}O_2$ whereas normal rats quantitatively excrete the labeled substrate in the urine [*13*].

## COMMENTS

The direct oxidative pathway of galactose metabolism is analogous to that observed for glucose except that no phosphorylated hexose is involved. The so-called pentose phosphate pathway of glucose metabolism exists in man [*11*] but the total per cent of glucose metabolism in the fasting state due to this direct oxidation is small, about 8 per cent. Apparently due to the nature of the citric acid cycle and the labeling of pyruvate derived from glucose-2-$C^{14}$ by the Emden-Meyerhof pathway the second carbon of glucose is as rapidly excreted in expired carbon dioxide as the first carbon (Fig. 2). Administered galactose-1- or -2-$C^{14}$ would have the same metabolic fate as glucose labeled in the same positions, if, as is known, galactose is rapidly converted to blood glucose [*12*] with an intact carbon chain by the uridine nucleotide pathway of galactose metabolism. This appears to be the case from the data of Figure 3. However, a small fraction of galactose metabolism by a direct oxidation of carbon 1 to $C^{14}O_2$ might very well go undetected in the presence of a large fraction of sugar entering the nucleotide sugar pathway. It seems likely that, in normal man, both small and large amounts of galactose given intravenously are metabolized chiefly by the conventional nucleotide pathway.

In the Caucasian galactosemic subject the fates of the first and second carbons are markedly divergent. As might be expected from operation of a direct oxidative pathway, C-1 of galactose is preferentially excreted. This is seen in the patients with very slow galactose metabolism in whom the uridine nucleotide pathway is deficient due to the low activity of the transferase enzyme. It is impossible with our data of Figure 4 to quantitate the amounts of galactose metabolized by this newly recognized pathway. It may well be that all the galactose is metabolized to carbon dioxide by this route in these patients. The excretion of $C^{14}O_2$ from galactose-2-$C^{14}$ would represent the operation of the oxidative cycle with production of D-xylulose-1-$C^{14}$ which may be converted to blood glucose via the pentose phosphate pathway. Not enough radioactivity could be given to our patients to make it feasible to isolate and analyze the blood glucose for randomization of the label to C-1 or C-3 position of glucose [*11*]. As an alternate route of galactose metabolism this "galactonate" pathway operates only at a fraction of the rate of the nucleotide sugar pathway and does not appear to compensate for loss of the transferase enzyme.

It seems reasonable to assume that the Negro patient with galactosemia differs from the Caucasian patient and is a true variant. Besides the finding in four Negro patients that $C^{14}$ galactose can be oxidized to $C^{14}O_2$ in a near normal fashion [*1,2*], there is the observation in two of these patients that liver obtained by needle biopsy can also oxidize galactose-1-$C^{14}$ to $C^{14}O_2$ [*2,14*]. Other tissues cannot perform this oxidation. The pattern presented here of $C^{14}$-galactose oxidation in a Negro subject more closely resembles that of the normal control subjects than of the Caucasian galactosemic patients, suggesting that the pathway involved in the Negro subject may be the conventional uridine pathway. There is some evidence to support this, namely, the very rapid and seemingly normal conversion of galactose to circulating blood glucose previously seen in patient T.B. [*1*]. A more definitive answer to the nature of the pathway in the Negro subject must await careful analysis of liver enzymes.

The evidence for the operation of the oxidative pathway would have been enhanced by finding the oxidation of an intermediate like galactonate. However, in the experiments in which labeled galactonate was given, essentially all the administered material was rapidly cleared by the kidney and excreted in the urine, leaving little to be metabolized. It is known, however, that galactonate is oxidized by rat liver slices, *in vitro* [*5*] and that rats with ligated ureters will oxidize some of the compound [*13*].

The first enzyme which converts galactose to galactonate in the oxidative pathway has been purified and its properties studied [*6,15*]. Although referred to as "galactose dehydrogenase," it acts upon other sugars as well. It is a soluble enzyme with a relatively high Km. Still, its maximal activity under optimal conditions appears equal to that of galactokinase, the first enzyme in the conventional pathway. The

dehydrogenase, principally found in the liver, has been demonstrated in human tissue [*6*]. In fact, an analysis of isoenzymes revealed that human liver had one enzyme activity in contrast to the rat which appears to have five bands on starch gel electrophoresis [*16*].

Ever since Isselbacher described the uridinediphosphogalactose pyrophosphorylase enzyme which could circumvent the deficiency of transferase and postulated that "maturation" of the enzyme could account for increasing galactose metabolism in older galactosemic patients, there has been an interest in alternate pathways. With regard to the pyrophosphorylase, this appears to be of very low activity, several magnitudes lower than galactose dehydrogenase. Its operation would cause galactose to be converted to glucose according to the regular uridine sugar nucleotide pathway. The data observed in Caucasian galactosemic patients tend to rule this out.

The alternate pathway which has received most prominence is that in which galactose is reduced by the enzyme aldose reductase to form the sugar, alcohol galactitol [*17,18*]. Galactitol has been isolated from tissues and urine of galactosemic patients [*19,20*] and from the tissues of rats fed a diet high in galactose [*21,22*]. The accumulation of galactitol in the lens and its metabolic effect in that tissue make it seem highly likely that this compound is the causal factor in cataract formation [*23*]. There is no evidence that the sugar alcohol is further metabolized or converted to carbon dioxide. After the administration of $C^{14}$ galactitol to normal subjects, all this material is excreted in the urine with no label appearing as $C^{14}O_2$ [*24*].

The question of whether the operation of alternate pathways in galactosemic patients is beneficial and aids in eliminating galactose, especially as age increases, has been discussed previously [*1*]. The Caucasian galactosemic patients do not seem to increase their capacity to oxidize galactose as they get older. The Negro patients have a considerable capacity to oxidize which is independent of age [*1,2*]. Whether the direct oxidative pathway of galactose is present only in older Caucasian galactosemic patients who have been the subject of this report is not known. It is known that quite young Caucasian patients also can oxidize some galactose. Data from rat liver experiments are available indicating that galactose dehydrogenase increases sharply at birth, reaches peak activity within the first few days of life and then tapers to much lower levels in adult rat liver [*6*].

There is no information at present to incriminate any of the intermediates of the direct oxidation pathway, such as galactonate, in the manifestations of the galactose toxicity syndrome.

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