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AUTHOR(S):

C. T. Gregg, J. Rudnick, B. B. McInteer, T. W. Whaley, and W. W. Shreeve

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los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87545

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THE EFFECT OF ETHANOL ON GALACTOSE TOLERANCE IN MAN

C. T. Gregg, J. Rudnick, B. B. McInteer, T. W. Whaley

Los Alamos Scientific Laboratory, University of California

Los Alamos, New Mexico 87545

and

W. W. Shreeve

Veterans Administration Hospital

Northport, New York 11768

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ABSTRACT

Galactose-^{13}C was given to 18 subjects; $^{13}CO_2$ excretion in respiratory air was followed for 3 hours. Each subject was given galactose-$^{13}C_6$ (10 g/m^2), then retested some days later with the same amount of labeled sugar and a low level (3.5 g/m^2) of ethanol. On the basis of the $^{13}CO_2$ excretion curves in the presence and absence of ethanol, the subjects were divided into four groups (i.e., subjects considered as normal, probably normal, probable liver damage, and liver damage). Ethanol strongly inhibited galactose metabolism in normal subjects. This effect of ethanol progressively declined in the four groups until, in the last group (liver damage), ethanol had no further effect on the already severely depressed oxidation of galactose. Comparison of the galactose tolerance data with other clinical tests and with the results of a drinking history suggests that the ethanol-primed galactose tolerance test may give good discrimination between groups of people with varying degrees of liver damage short of frank cirrhosis, although alcohol-priming is not necessary to distinguish between normal and cirrhotic subjects.

INTRODUCTION

This work brings together the threads of several series of previous investigations. One of these includes the studies by Tengström in the 1960s (1) and by Shreeve and his colleagues in 1976 (2) on the utility of the galactose tolerance test for evaluating liver function. Tengström pointed out that, in his opinion, the best available test for assessing liver function was bromsulfophthalein (BSP) retention. Tengström went on to conclude, however, that since this procedure occasionally gives rise to serious and even fatal complications, other tests for liver function should be sought. Also, BSP clearance is abnormal in all types of liver disease and cannot distinguish between liver abnormalities. The galactose tolerance test has the advantage of being completely harmless except to those rare victims of hereditary galactosemia, and it does aid in the differential diagnosis of liver diseases. Tengström concluded, on the basis of his experiments, that the intravenous galactose tolerance test gave valuable diagnostic and prognostic information in liver cirrhosis and hepatitis; it was also useful in differentiating between obstructive and parenchymatous jaundice. He found, for example, that the rate of galactose clearance was significantly reduced in 84% of patients with cirrhosis; the amount of decrease correlated well with the degree of liver damage determined by biopsy. More recently, Shreeve and his colleagues gave ^{13}C-labeled galactose orally and measured $^{13}CO_2$ in the breath (2). They found that this test gave better separation between controls and patients with alcoholic cirrhosis than did serum alkaline phosphatase, total bilirubin, or glutamic-oxalacetic transaminase.

The other lineage that contributed to this work was that beginning with the observation of Wagner, nearly 65 years ago, that the excretion of galactose in the urine was enhanced by drinking brandy (3). Bauer and Wozasek (4), some

20 years later, confirmed Wagner's results and showed that urinary galactose excretion was not increased by ethanol in subjects with liver disease. In 1962, Tygstrup and Lundquist (5) studied the effect of ethanol on the intravenous galactose tolerance test; six years later, the utility of this test for detecting fatty liver was investigated by Salaspuro (6). Tygstrup and Lundquist concluded that ethanol had a profound depressant effect on galactose metabolism in normal subjects but much less, if any, effect in cirrhotic patients whose rate of galactose metabolism was already depressed.

Salaspuro (6) decided that none of the liver function tests available to him in 1967 was sufficiently sensitive for the early detection of fatty liver, a condition often considered as a forerunner of cirrhosis. Only blind liver biopsy gave reasonably good results, and this procedure is sometimes dangerous and often unsatisfactory. In contrast to Tygstrup and Lundquist, Salaspuro found that, in the absence of ethanol, the rate of galactose metabolism was the same in controls and patients with demonstrated fatty livers. This difference presumably was due to the selection, by Salaspuro, of patients with less severe liver damage. Salaspuro concluded that fatty liver should always be suspected when the rate of elimination of galactose from the blood in the presence of ethanol was 50% or more below normal (6).

Our own studies were a natural extension of the ^{13}C studies of Shreeve *et al.* (2) and had two principal goals. The first was to determine whether the simultaneous addition of ethanol in the oral galactose tolerance test increased its sensitivity sufficiently to detect subjects with mild liver damage (that is, with liver damage that could still be reversed by moderating their drinking habits, for example). The second goal was simply to enhance our understanding of the metabolic effects of ethanol in man, a task for which the use of stable isotopes seems particularly well suited.

In common with most clinical applications of stable isotopes, we report here on a relatively small number of subjects. Thus, this is really a preliminary report, but we feel that our initial findings are sufficiently encouraging that they may be of interest to this group.

METHODS

The methods we use are similar to those described by Shreeve et al. (2). Subjects are fasted overnight, a blood sample is taken the following morning, after which the subject exhales into a balloon and his expired breath is transferred to an evacuated mass spectrometer bulb. Galactose is then administered orally in orange juice, with or without a small quantity of ethanol. The administered galactose is 2.1 mol % ^{13}C. The dose of galactose is 10 g/m^2, or about 18 g for the average male subject. The test is administered to each subject once without ethanol and then, not less than 2 and not more than 10 days later, it is readministered with a small amount of ethanol. We originally used a level of ethanol comparable to that used by other workers (that is, about 14 g/m^2). We found, however, that reducing the ethanol level by a factor of 4 had no effect on the ethanol depression of galactose metabolism and that it made the galactose, ethanol, and orange juice cocktail much more palatable on an empty stomach. The total volume of the cocktail is 93 ml/m^2; the solution is consumed within two minutes. Subsequent breath samples are taken at half-hour intervals up to three hours. The blood sample is used for a variety of clinical chemical measurements.

Under the conditions of this test (that is, with a loading dose of galactose), the sugar is metabolized almost exclusively in the liver; this organ is also the site of the first two steps of alcohol metabolism. The effect of ethanol on galactose metabolism is usually rationalized on the basis that the enzyme UDP-galactose epimerase, that converts galactose to glucose as the first

step of its further metabolism, is inhibited by the high intracellular NADH/NAD ratio resulting from the two NAD-dependent steps that convert ethanol first to acetaldehyde, then to acetate. That this is part of the story is clear, but it is probably not the whole reason for the effect of ethanol on galactose metabolism.

RESULTS

Eighteen patients, including both men and women, were selected by my clinical associates. I was not told which subjects were in which group. On the basis of the kinetics of excretion of $^{13}CO_2$ from galactose, I divided the 18 subjects into four groups. Later, when I had access to the results of their physical examinations, clinical laboratory data, and drinking histories, I was able to compare the predictive value of the ethanol-primed galactose tolerance test with other tests for liver function.

Figure 1 shows the curves of $^{13}CO_2$ excretion for four normal subjects. Normal should perhaps be in quotation marks; I said they were normal on the basis of this set of curves. The upper curve is that obtained in the absence of ethanol. The lower curve is that resulting from the simultaneous administration of labeled galactose and a small quantity of ethanol. Several features of these curves should be emphasized. First, the unprimed (upper) curve peaks at 90 minutes; the ethanol-primed curve never peaks at all. Up to 90 minutes, the primed and unprimed curves are separated by three or four standard deviations. It is also significant, for comparison with the other curves that I will show, that the peak of the unprimed curve is close to a ^{13}C content of 1.15 mol %.

Figure 2 shows a second group of five subjects that I have chosen to call probably normal. Here there is a modest shift in the direction of abnormality, but the two curves are still reasonably well separated. The unprimed curve in this set peaks at 120 minutes rather than at 90 minutes, as in the preceding

group. The ethanol effect is much less pronounced, but the primed and unprimed curves remain well separated, and the height of the unprimed curve is in the same region as for that group of subjects considered to be normal.

The next figure (Fig. 3) shows a group of subjects that I considered, on the basis of their carbon dioxide excretion pattern, to have probable liver damage. As in the preceding case, the peak of $^{13}CO_2$ excretion is delayed to 120 minutes and is nearly as high as the normal group, but the ethanol effect on galactose oxidation is nearly abolished. In the studies of Shreeve et al. (2) and DaCosta et al. (7), fatty infiltration of the liver either lowered or delayed the peak of isotope excretion from galactose, although these effects were not seen in the intravenous galactose tolerance test by Salaspuro (6).

Finally, Fig. 4 shows a group of subjects classified by me as having definite liver damage. These curves are bizzare compared with those obtained with putatively normal subjects. There is no peak in either the primed or unprimed excretion curves. The levels of isotopic enrichment in the breath are very low in both cases; the ethanol effect on galactose metabolism is not only lost, but the unprimed curves in some of these subjects are actually above the primed curves.

Figure 5 simply shows a comparison of the unprimed galactose tolerance curves for the four subjects considered to have frank liver damage with the other 14 subjects--all of whom, for the purpose of this figure, are considered to be normal. Thus, this curve can be compared with that obtained by Shreeve et al. (2) when they compared normal and cirrhotic patients. Our results are essentially the same as theirs. Ethanol priming clearly is not necessary to distinguish normal from cirrhotic patients.

Having classified the subjects into tentative groups on the basis of their CO_2 excretion curves, we were naturally interested to see how this

classification compared with that based on a greater variety of standard liver function tests. These data are tabulated in Table 1. Here the subjects are arranged into groups on the basis of the breath test, as shown on the left. This classification is compared with the results of measuring the serum enzymes γ-glutamyltranspeptidase, or GGTP, glutamic-pyruvic and glutamic-oxalacetic transaminases, alkaline phosphatase, and also with the results of a drinking history.

It is clear that, unbeknownst to me, my clinical colleagues were selecting a subject sample with a very much higher than usual incidence of elevated GGTP. This abnormality appears in less than 6% of the Los Alamos population, whereas it appears in more than half of our subjects. Of the nine persons with a positive drinking history, seven showed an elevated GGTP. However, an elevated GGTP was also found in three subjects with no other signs of liver abnormality. The only other parameter that correlated well with the galactose tolerance test, elevated GGTP, and drinking history was the glutamic-pyruvic transaminase. Neither the glutamic-oxalacetic transaminase nor the alkaline phosphatase levels were of value in our investigations, although other laboratories have found them useful. Likewise, the measurement of serum albumin showed no correlation with the other parameters we tested--in contrast to the findings of Shreeve et al. (2).

One normal subject showed an elevated total serum bilirubin level, and one subject in the liver-damaged group had an elevated total serum protein level. None of the rest of the subjects in any class had abnormal values of these or other measures of liver function, including urinary bilirubin. Prothrombin times and serum protein flocculation tests were not performed.

DISCUSSION

In general, the other data support the classification of subjects on the basis of the galactose tolerance test alone although, as always, there are data that do not fit. One example is the individual with an elevated GGTP and a positive drinking history whose galactose excretion curve is normal. Even more striking is the individual whose carbon dioxide excretion curve caused him to be classified as having liver damage but who is a teetotaler with no other indications of any liver problems. We are attempting to determine whether he has any other history of hepatitis or possible exposure to liver toxins that might explain this finding.

It is clear that ethanol-priming is not required in the two extreme cases (that is, either in normal subjects or in those with frank liver damage). The utility of ethanol-priming is that it appears to enhance the sensitivity of the galactose tolerance test in intermediate cases. Thus, subclinical liver damage may be noted early enough so that, if liver damage is the result of alcohol abuse, for example, a moderation in drinking habits may permit the damage to be reversed. Since liver cirrhosis is the fourth leading cause of death in Americans between the ages of 45 and 64, any aid in reducing the number of people who progress to this level of liver damage is welcome.

In summary then, the assessment of liver function remains a problem to the solution of which galactose-^{13}C tolerance tests make a valuable contribution. This test gives better results than most of the other common liver function assays and is without the hazards of some. Moreover, the galactose tolerance test is the only test for liver function that directly measures the metabolic response of the liver to alcohol ingestion; this direct measurement seems to have diagnostic utility.

Ethanol-priming of the galactose tolerance test appears to enhance its

sensitivity for detecting intermediate levels of liver damage, although further work clearly is necessary to establish this point. Both increased numbers of subjects need to be examined and the subjects already tested need to be re-tested over a period of some years to determine whether or not, with the passage of time, they move from a group of mild liver damage to more extensive damage. In a two-year follow-up study of their subjects, Shreeve et al. (2) found that, of five who subsequently developed hepatic failure, four excreted carbon dioxide from galactose at a lower rate than controls. If our subsequent data show such a pattern, it would strongly support the use of the ethanol-primed galactose tolerance test, or some variant, as a useful test for liver function. Automation of sample handling and possibly use of a less expensive substrate could reduce the cost of this test to a level competitive with other, possibly less valuable, measures of liver integrity.

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TABLE 1. Comparison of the Classification of Subjects on the Basis of the Oral Galactose Tolerance Test Alone, with Classification on the Basis of Other Clinical Tests and Drinking History

Group (by GTT)[a]	GGTP[b]	SGPT[c]	SGOT[d]	AP[e]	History
Normal					
	-	-	-	-	-
	-	-	-	-	-
	+	-	-	-	+
	-	-	-	-	-
Probably Normal					
	+	-	-	+	-
	-	-	-	-	-
	-	-	-	-	+
	+	-	-	-	+
	+	-	-	-	-
Probable Liver Damage					
	+	+	+	-	-
	-	-	-	-	+
	-	-	-	-	+
	+	+	-	-	+
	-	-	-	-	-
Liver Damage					
	-	-	-	-	-
	+	+	-	-	+
	+	+	+	-	+
	+	-	-	-	+
	+	+	-	-	+

[a] GTT, the oral galactose-^{13}C tolerance test.

[b] GGTP, serum γ-glucamyltranspeptidase.

[c] SGPT, serum glutamic-pyruvic transaminase.

[d] SGOT, serum glutamic-oxalacetic transaminase.

[e] AP, serum alkaline phosphatase.

FIGURE LEGENDS

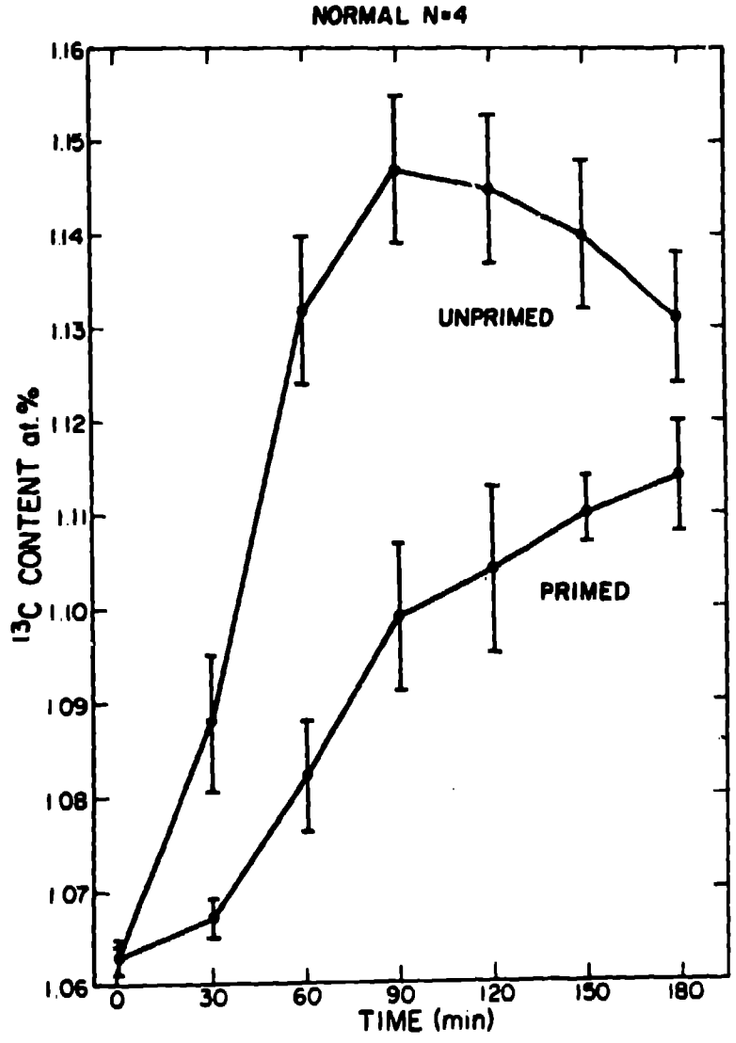
Fig. 1. Oral galactose tolerance test in the presence (primed) and absence (unprimed) of ethanol in four normal subjects. Subjects were given galactose-U-$^{13}C_6$ enriched to 2.1 mol % ^{13}C at a dose of 10 g/m^2 of body surface area. The sugar was given in orange juice either with (primed) or without (unprimed) a small amount of ethanol (3.5 g/m^2). Each subject was given both tests from a few days to a week apart. Breath samples were collected at zero time (when a blood sample was also taken), then every 30 minutes for 3 hours. The breath samples were measured on an isotope ratio mass spectrometer to determine the degree of ^{13}C enrichment in the respired air.

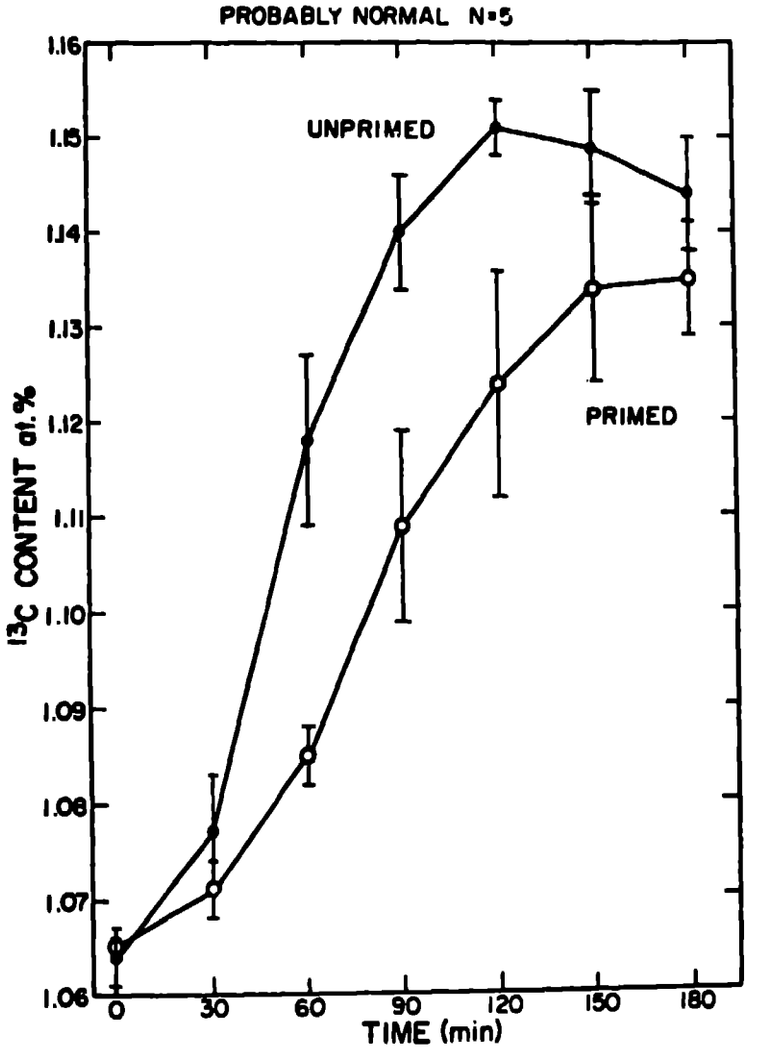
Fig. 2. Oral galactose tolerance test in the presence and absence of ethanol in five probably normal subjects. The conditions were as described in Fig. 1.

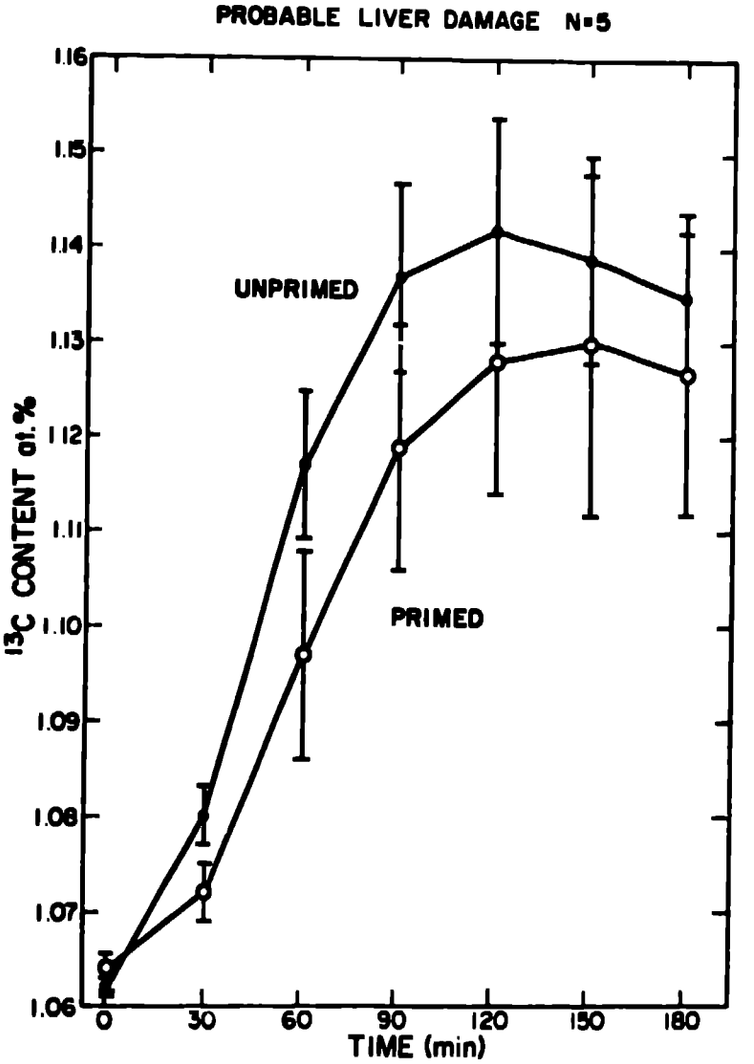
Fig. 3. Oral galactose tolerance test in the presence and absence of ethanol in five subjects with probable liver damage. The conditions were as described in Fig. 1.

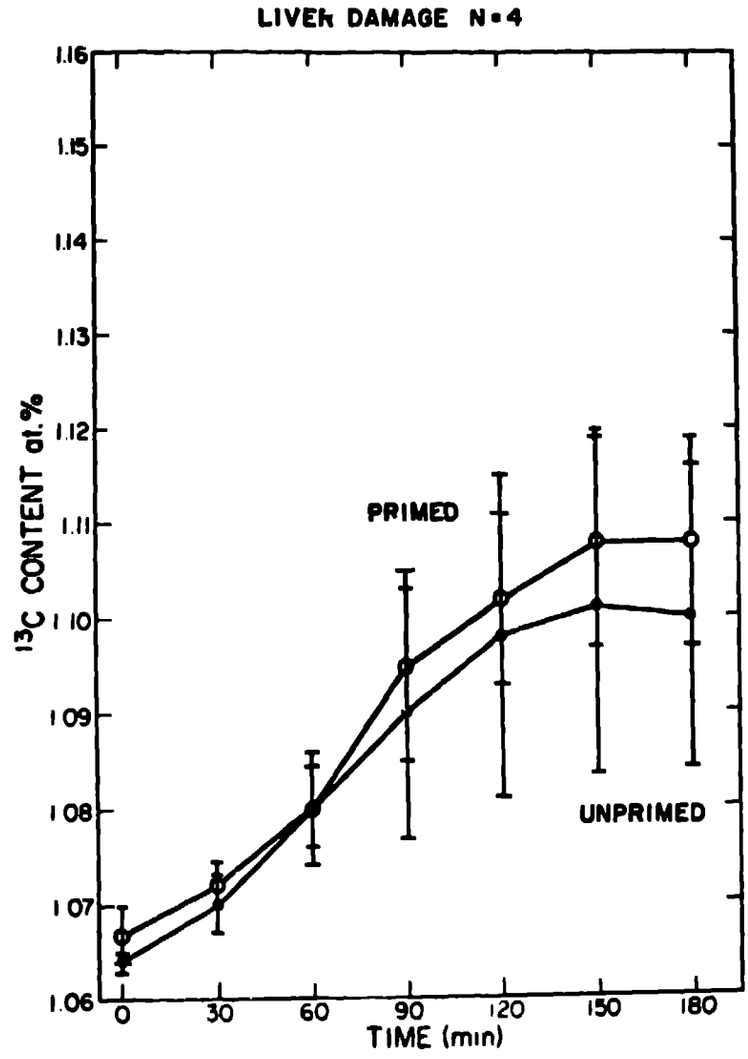
Fig. 4. Oral galactose tolerance test in the presence and absence of ethanol in four subjects with liver damage. The conditions were as described in Fig. 1.

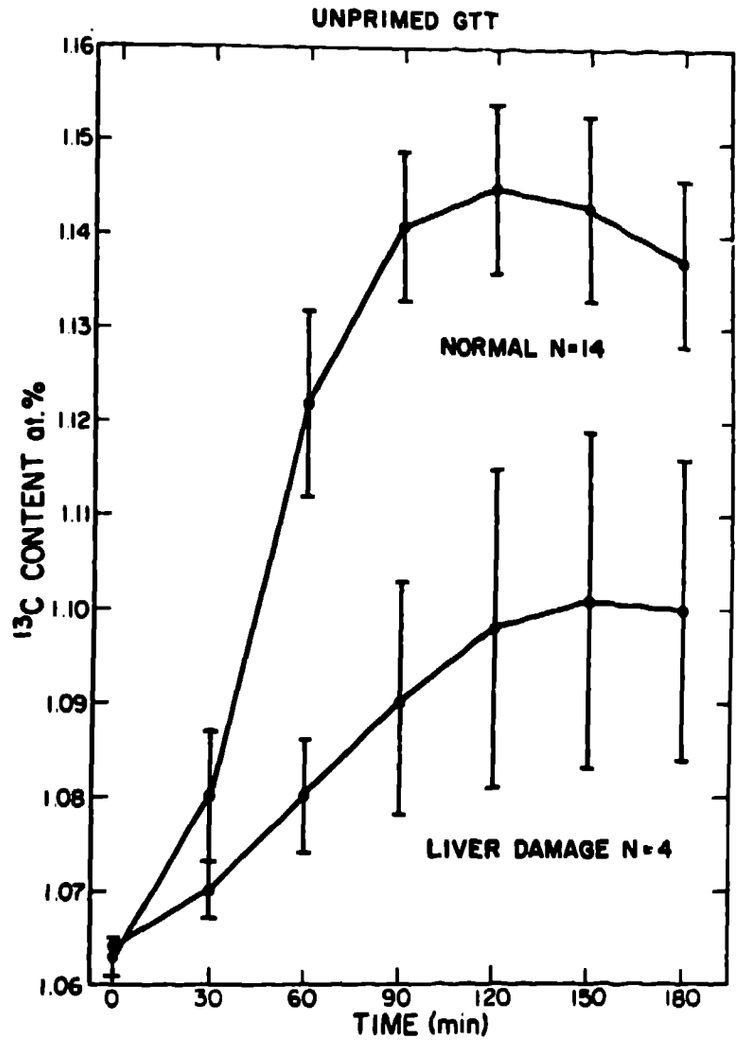
Fig. 5. Oral galactose tolerance test in 14 normal subjects and 5 subjects with liver damage. The conditions were as described in Fig. 1.


NORMAL N=4
1.16
1.15
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
1.06
^{13}C CONTENT at.%
UNPRIMED
PRIMED
0
30
60
90
120
150
180
TIME (min)


PROBABLY NORMAL N=5
UNPRIMED
PRIMED
^{13}C CONTENT at.%
1.16
1.15
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
1.06
0
30
60
90
120
150
180
TIME (min)


PROBABLE LIVER DAMAGE N=5
1.16
1.15
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
1.06
^{13}C CONTENT at.%
UNPRIMED
PRIMED
0
30
60
90
120
150
180
TIME (min)


LIVER DAMAGE N=4
1.16
1.15
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
1.06
^{13}C CONTENT at.%
PRIMED
UNPRIMED
0
30
60
90
120
150
180
TIME (min)


UNPRIMED GTT
1.16
1.15
1.14
1.13
1.12
1.11
1.10
1.09
1.08
1.07
1.06
^{13}C CONTENT at.%
NORMAL N=14
LIVER DAMAGE N=4
0
30
60
90
120
150
180
TIME (min)