Application of the Galactose Tolerance Test for the Early Diagnosis of Fatty Liver in Human Alcoholics

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Abstract: SALASPURO, M. P. Galactose Tolerance Test in the Diagnosis of Fatty Liver. *Scand. J. clin. Lab. Invest.* **20,** 274-280, 1967. Intravenous galactose tolerance tests with and without an alcohol load have been used to study the liver function of fifteen healthy persons and twelve chronic alcoholics whose diet has for long periods been inadequate. The results are related to other liver function tests and to the microscopic appearance of liver biopsy specimens. In patients with normal liver function the T $^1/_2$ value for galactose without ethanol averages 12.7 minutes, with a standard deviation of $\pm$ 2.5 minutes. The mean prolongation of the T ½ value induced by ethanol was 28.5$\pm$ 5.0 (S.D.) minutes.

All but one of the alcoholics had typical fatty infiltration of the liver. The breakdown of galactose without ethanol was equal to that of normal persons. In sharp contrast to the controls, however, the previous ethanol load did not influence the galactose elimination rate in these patients with fatty livers. It is concluded that fatty liver should always be suspected when the ethanol-induced prolongation of the T ½ value for galactose remains below 17 minutes.

All other liver function tests normalized during the first week after admission to hospital.

Index-words: Alcohol ethyl; alcoholism; fatty liver; galactose; liver; liver diseases; liver function tests

Abnormal accumulation of fat in the parenchymal cells is one of the commonest pathological changes in the liver. Prolonged alcohol intake is generally regarded as the chief aetiological factor in the genesis of hepatic steatosis. Whether this kind of fatty liver is caused by inadequate food consumption (8, 13, 24) or is due to the alcohol itself (16) is still a matter of debate. None of the common liver function tests is sensitive enough for the early diagnosis of fatty liver (33) and the best method available is still the blind liver biopsy (15).

In normal human and rat livers ethanol causes a shift in the redox state of the cytoplasm in a more negative direction (3, 5, 26, 31). In fatty liver induced by a choline-deficient diet, the capacity of the liver to use extramitochondrial reducing equivalents has changed in such a way that no shift in the redox state of the liver can be observed during ethanol oxidation (28). Using the galactose tolerance test as a measure of the redox potential of the liver, we were later able to prove that the main reason why ethanol no longer causes a shift in the redox state of the liver in a more negative direction is apparently an unknown effect produced by the lack of choline (27, 29). In the present study an attempt was made to relate our previous observations on rats to human alcoholics. Intravenous galactose tolerance tests with and without ethanol loading have been performed on normal human subjects and on alcoholics who have been living for long periods on an inadequate diet. The results are related to other liver function tests and to the microscopic appearance of liver biopsy specimens.

METHODS

The galactose test was made during the first week after admission to hospital. The experiment was

Table I. *Galactose T ½ values in fifteen healthy subjects with and without ethanol intake*

Patient No.	T ½ values for Galactose I (min)	T ½ values for Galactose II (min)	Galactose II minus Galactose I (min)	The ratio of Galactose II to Galactose I
1	14	33	19	2.4
2	16	53	37	3.3
3	13	41	28	3.2
4	15	40	25	2.7
5	12	44	32	3.7
6	8	44	36	5.5
7	13	39	26	3.0
8	13	39	26	3.0
9	12	41	29	3.4
10	14	37	23	2.6
11	14	44	30	3.1
12	10	33	33	3.3
13	9	37	28	4.1
14	17	44	27	2.6
15	10	31	21	3.1

Galactose I = Galactose test without ethanol load Galactose II = Galactose test with ethanol load

begun in the morning with the patient in the recumbent position and fasting for the previous night. 30 % sterile galactose solution was administered as a single intravenous injection. The total amount of galactose was 350 mg/kg body weight. Blood samples were taken from a finger-tip at 10-minute intervals during 60 minutes. The galactose concentration of the blood samples was determined enzymically according to de Verdier & Hjelm (38) and Hjelm (9), by means of the galactose-oxidase reagent produced by AB Kabi (Stockholm, Sweden). The values were plotted against time on semilogarithmic paper and the T ½ value for galactose was determined according to Tengström (34). In this method, the normal T ½ value is 12.0±2.6 (S.D.) minutes and the highest normal value 17 minutes. In this paper this version of the galactose tolerance test is called Galactose I. In Galactose II test 300 mg of ethanol per kg body weight was given as a 15 % solution per os 15 minutes before the galactose injection; otherwise the test was the same as Galactose I.

In the comparison of these galactose tests with other liver function tests the results of other tests were obtained with the following methods:

Serum bilirubin was determined according to MacDonald (18), serum alkaline phosphatase according to Bessey, Lowry & Brock (2) and serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum lactic dehydrogenase (SLDH) according to Bergmeyer & Bernt (1), Wróblewski & La Due (41) and Wróblewski & La Due (40), respectively.

Serum proteins, albumin, and globulins were determined electrophoretically according to the Beckman microzone system. The bromsulphthalein (BSP) test was made with the administration of 5 mg of BSP per kg body weight, and the determination of the bromsulphthalein concentration was done according to Seligson & Marino (30). The T ½ value for BSP was calculated from a semilogarithmic graph. Values above 6.3 minutes are regarded as abnormal.

Percutaneous liver puncture was performed according to Menghini (21). The degree of fatty infiltration in the liver biopsies was classified from zero to four +. The amount of fatty cells and fat globules were calculated in ten separate fields of microscope and related to the amount of normal cells.

MATERIALS

Normal values for Galactose I and Galactose II were determined on 15 male patients with no clinical suspicion of hepatic disease. Some of them were admitted to hospital for examination and some of the patients were recovering from an uncomplicated heart infarction. Liver biopsies were not performed on them, but the possibilities of diabetes, alcoholism, malabsorption, and malnutrition were ruled out. The mean age was 45 years with a range of 21 to 61 years.

The alcoholics had all consumed great quantities of alcohol for years. Alcohol consumption had mostly been periodic with intervals of one week to several months. Adequate knowledge of their food consumption could not be obtained, but in all of them the diet had been inadequate with regard to protein and vitamins. Twelve male alcoholics were examined. Their mean age was 48 years, with a range of 33 to 54 years.

RESULTS

Table I summarizes the Galactose T ½ values with and without ethanol in fifteen control subjects. The T ½ value without ethanol averages 12.7 minutes, with a standard deviation of ± 2.5 minutes, that is of the same order as the one reported by Tengström (34). The mean T ½ value for Galactose II was 40.0 ± 5.6 (S.D.) minutes. The mean prolongation of T ½ value induced by ethanol was 28.0 ± 5.0 (S.D.) minutes, and the ratio of Galactose II to Galactose I 3.1 ± 0.4 (S.D.).

The results in Table II indicate that the breakdown of galactose without ethanol is somewhat increased in some of the alcoholics. This observation supports our previous observations in experiments with rats on a choline-deficient diet (29), but the reason for it remains to be elucidated. Only one of these patients has an abnormally high T ½ value. In sharp contrast to the controls, the influence of ethanol on the galactose breakdown in these alcoholic subjects was very slight. The prolongation of T ½ value was below thirteen minutes in ten of the twelve patients. Only two had values approaching the mean normal value of 28.0 ± 5.0 (S.D.) minutes, and both of them had only a slight fatty infiltration in the liver biopsy. In the light of these results fatty liver can always be suspected when the difference between Galactose I and Galactose II remains below 17 minutes.

In Table III are given the results of the other

Table II. *Galactose* T ½ *values and the degree of fatty infiltration in liver biopsies of twelve alcoholics*

Patient No.	T ½ values for Galactose I (min)	T ½ values for Galactose II (min)	Galactose II minus Galactose I (min)	The ratio of Galactose II to Galactose I	Degree of fatty infiltration
1	19	19	0	1.0	++++
2	14	17	3	1.2	++++
3	6	9	3	1.5	+++
4	7	11	4	1.6	++++
5	13	21	8	1.6	+++
6	15	24	9	1.6	+++
7	11	21	10	1.9	+++
8	8	19	11	2.4	+
9	13	24	11	1.8	++
10	8	21	13	2.6	++++
11	7	24	17	3.4	+
12	8	29	21	3.6	0

Percentage of the histological section occupied by fat globules
+ = less than 10 % ++ = 10 to 30 % +++ = 30 to 50 % ++++ = 50 to 70 %

Table III. *The results of other liver function tests in twelve alcoholics. The tests were performed during the first week after admission to hospital*

Patient No.	Serum bilirubin (mg/100 ml)	Serum alk. phosphatase (m IU/ml)	SGOT (m IU/ml)	SGPT (m IU/ml)	SLDH (m IU/ml)	Serum albumin (g/100 ml)	Serum gamma-globulin (g/100 ml)	Serum $\alpha_1+\alpha_2$ globulins (g/100 ml)	T ½ values for BSP (min)
1	0.4	41	40	20	130	3.41	1.60	1.28	6.0
2	0.8	36	17	10	119	4.55	1.08	0.87	5.4
3	0.6	28	12	11	142	4.32	0.98	0.86	4.3
4	0.9	31	63	50	220	4.16	1.77	1.14	5.0
5	0.4	37	15	11	120	3.88	0.97	1.08	4.5
6	0.9	40	17	10	114	3.41	1.21	0.73	5.5
7	0.4	16	17	14	120	4.07	2.11	1.43	3.5
8	0.8	22	20	28	130	4.16	0.79	0.77	4.3
9	1.0	30	20	22	130	3.71	0.68	0.94	4.0
10	1.5	36	44	58	330	5.67	1.01	1.29	6.0
11	0.7	18	16	13	140	4.82	1.09	0.88	3.5
12	0.8	28	11	14	119	3.90	0.90	1.15	4.5

Normal values in our hospital: Serum bilirubin: 0.30-1.50 mg/100ml; SGOT: 2.5-19 m IU/ml; SGPT: 2.5-17 m IU/ml; SLDH: 96-240 m IU/ml; Serum alk. phosphatase: 13-48 m IU/ml; Serum albumin: 3.87-4.84 g/100 ml; Serum γ-globulin: 0.80-1.45 g/100 ml; Serum α_1-+α_2-globulins: 0.54-1.02 g/100 ml; T ½ value for BSP: 3.2-6.3 min.

liver function tests in the alcoholics. It should be emphasized that despite moderate fatty infiltration of the liver in some of the patients, all of them still had a normal liver excretory function as assessed by the BSP test. SGOT and SGPT were elevated in five of the patients and SLDH in one of them, but the values normalized during the first week in hospital care. Serum albumin was within the normal range in every patient and serum globulins were slightly elevated in three patients.

DISCUSSION

Alcohol is oxidized to acetaldehyde and acetate by two enzymes, alcohol dehydrogenase and acetaldehyde dehydrogenase, and both these reactions lead to the transfer of hydrogen to NAD, which is reduced to $NADH_2$ (17, 39). This induces a change in the $NADH_2$/NAD ratio to a more reduced direction, indicating that the redox state of the liver becomes more negative (6). According to Hohorst, Kreutz & Bücher (10), the redox potentials of biological redox systems can be measured by determination of the concentration ratios of metabolites forming redox pairs, for instance the lactate/pyruvate ratio. Consequently, ethanol oxidation greatly increases the lactate/pyruvate concentration ratio in perfusion experiments with normal livers (5). This change has also been observed in human subjects (3, 31, 37).

In fatty livers induced by a choline-deficient low-protein diet, ethanol no longer increases the lactate/pyruvate ratio in the medium during rat-liver perfusion (28). This study indicates that the fatty liver has a greater capacity to oxidize the cytoplasmic $NADH_2$ or that the control of the cytoplasmic redox state is different. This change in the metabolism of the choline-deficient fatty liver is probably not associated with the well-known lipotropic activity of choline. Because the role of phospholipids is very important in mitochondrial structure (23) and great mitochondrial changes have been demonstrated in choline deficiency (20, 25), it may be that some kind of alteration in mitochondrial function has taken place during the development of choline-

deficient fatty liver. This question, however, needs much more detailed investigation.

One of the key reactions in the metabolism of galactose is the one involving the conversion of UDP-galactose to UDP-glucose. This reaction is catalyzed by the enzyme UDP-galactose-4-epimerase. Maxwell (19) has shown that the coenzyme in this epimerization is NAD and that $NADH_2$ is a potent inhibitor of the enzyme. As mentioned above, during ethanol oxidation great amounts of $NADH_2$ are formed. This results in decreased capacity of the liver to oxidize galactose in the incubation of rat-liver homogenate (11). A pronounced impairment of galactose tolerance caused by ethanol has also been observed in normal individuals (32, 35, 36). Because ethanol did not affect the redox state of choline-deficient fatty livers of rats, the rate of galactose breakdown was used as a measure of the redox potential of these livers with and without ethanol (27, 29). In these experiments ethanol caused almost total inhibition of galactose elimination in normal animals. However, as expected, ethanol did not influence the galactose breakdown of choline-deficient rats at all.

Hepatic steatosis of alcoholics is believed by some authors to depend on inadequate food consumption, particularly of lipotropic agents (13, 24). Moreover, ethanol is known to increase choline-requirements (14). Dietary cirrhosis induced by choline deficiency in rats is regarded by Hartroft (8) as the experimental equivalent of alcoholic cirrhosis in man. On the other hand, alcohol itself can provoke fatty liver despite adequate diet in both human subjects and rats (12, 16, 22). In the present study all the alcoholics had consumed inadequate food for long periods. Using the galactose tolerance test as a measure of the redox state of the liver, it was possible to demonstrate that ethanol did not increase the $NADH_2/NAD$ ratio in these patients. According to our animal experiments, this may indicate some kind of metabolic alteration in liver function induced by choline deficiency. For the present, only three of the alcoholics have had lipotropic therapy during hospital stay and in all of them the galactose tolerance test with ethanol was normalized in a month. It seems, however, that the disappearance of fat from the liver does not progress so fast. These observations remain to be elucidated. Investigation of the rate of galactose breakdown with ethanol in human alcoholics without adequate food consumption will also be most interesting.

Stenstam (32) has reported that ethanol did not decrease the rate of galactose oxidation in three cases of pernicious anaemia. Various malnutritional stages and absorption disturbances as well as diabetes are common aetiological factors in the pathogenesis of human fatty liver (4, 15, 33). In two patients with grave malabsorption I have also observed that ethanol did not decrease the rate of galactose oxidation. Later on, fatty infiltration of the liver was demonstrated in the biopsy specimens. These alterations in liver function may perhaps be caused by protein and choline deficiency due to disturbances of absorption. Tygstrup & Lundquist (35) have reported that ethanol did not decrease galactose elimination in cirrhotic patients. They suggest that the concentration of $NADH_2$ in the cirrhotic livers is elevated owing to deficiency of $NADH_2$-oxidizing enzymes (7), and that no appreciable further inhibition results from combustion of ethanol. This hypothesis can also be used to explain the observations of Stenstam (32) in subacute and acute hepatitis, but the possibility that changes in liver metabolism induced by primary or secondary choline deficiency may play a part cannot be excluded until further investigations have been made.

There are two main disadvantages inherent in the existing liver function tests. They are not capable of demonstrating early alterations in liver function during the development of protein-deficient fatty liver and they do not provide any information about the aetiology of hepatic injury. The most valuable diagnostic aids in hepatic steatosis have up to the present been palpation of the liver, and liver biopsy (15). The rate of elimination of galactose in the absence of ethanol was likewise within normal limits in every alcoholic patient except one. Only the performance of Galactose II test gave definite information about the changed capacity of the liver to regulate the

redox state in the cytoplasmic compartment of the liver cell. This test was pathological in every patient with fatty degeneration of the liver. Definite information about the test in various hepatic and other diseases is still lacking, but it can already be used in the diagnosis of early fatty liver in human alcoholics whose food consumption has been inadequate for long periods.

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