The Effect of Ethanol on Galactose Elimination in Rats with Normal and Choline-deficient Fatty Livers

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Abstract: SALASPURO, M. P. & SALASPURO, A. E. The Effect of Ethanol on Galactose Elimination in Rats with Normal and Choline-deficient Fatty Livers. *Scand. J. clin. Lab. Invest.* **22,** 49-53, 1968.
A study has been made of the ethanol inhibition of galactose oxidation in nephrectomized rats in vivo, and the influence of choline deficiency and the fat and carbohydrate content of the diet on the rate of galactose breakdown, with and without ethanol, has been elucidated.
Ethanol induced almost total inhibition of galactose oxidation in normal rats; however, no inhibition was observable in choline-deficient rats with fatty livers. This observation was independent of the fat and carbohydrate content of the low protein, choline-deficient diet. Choline supplementation restored the normal effect.
The results are discussed in the light of earlier studies on the metabolism of choline-deficient fatty livers of rats.

Key-words: Alcohol ethyl; fatty liver; galactose; liver

During ethanol oxidation in normal livers, NAD is reduced to $NADH_2$ and a diminution occurs in the ratio of NAD to $NADH_2$ (4). $NADH_2$ is a potent inhibitor of UDP-galactose-4-epimerase (12), which is one of the key enzymes in the metabolism of galactose. As a result, inhibition of galactose oxidation by ethanol can be demonstrated by means of both in vivo and in vitro studies (23, 24, 9).

In fatty rat livers induced by a choline-deficient high fat diet, low in protein, ethanol no longer reduces the NAD/$NADH_2$ ratio (21). Ethanol does not diminish the rate of galactose oxidation in these rats (19).

The elimination of galactose is mainly a function of the liver (18); in human subjects, only small amounts are excreted by the kidneys. In rats, however, the urinary excretion of galactose rises to from 60 to 80 per cent (unpublished observation). In a preliminary study (19), the blood elimination curve of galactose, corrected by the urinary excretion, was used in calculation of the rate of galactose oxidation in the rat. As the complete collection of rat-urine is very difficult, the results were not quite exact. In the present study, the rate of galactose oxidation, without and with ethanol, was determined in vivo, in nephrectomized rats, and elucidation was also made of the role of choline, fat and carbohydrate in regulation of the redox state of the liver.

MATERIALS AND METHODS

In all the experiments, male albino rats of the Wistar strain from our own laboratory stock were used. Initially, the rats weighed 110-140 g., and were 6-8 weeks old. They were kept in wire-bottomed cages, ten in each.

Four different diets were administered, and specified amounts of each were given to the respective groups of animals, to ensure that in all the groups the daily caloric, amino-acid, vitamin and salt intakes were identical. The variables were the fat, carbohydrate and choline intakes. The detailed composition of the diets is given in Table I.

Table I. *Composition of diets*

	Lard	Starch sugar	Choline
Fat 4 per cent	0	8560	0
Fat 30 per cent	1840	4520	0
Fat 50 per cent	2760	2490	0
Fat 50 per cent +choline	2760	2490	20

The diets were isocaloric (40 calories per day). Each rat received the following diet daily: Casein, 490 mg; cystine, 31 mg; salt mixture, 184 mg; Arachis oil, 400 mg as well as vitamine mixture*

*For details see the text.

The content of fat in the diet varied from 4 to 50 per cent. On increase in the fat, the amount of dietary carbohydrate was reduced in isocaloric amounts. The mixture of starch and sugar contained equal amounts of pure maize starch and sucrose. The salt mixture corresponded to that used by György & Goldblatt (6). One group taking the diet containing 50 per cent of fat was given 20 mg/rat/day of choline. The following vitamin supplements were added to each daily portion: thiamine 25 μg, riboflavin 20 μg, pyridoxine 25 μg, calcium panthothenate 100 μg, nicotinic acid 100 μg, and cod liver oil one drop. Every week, each animal was given 5 mg of α-tocopherylacetate. On the fifth and eleventh days after commencement of the experiment, each animal was given 20 mg of choline to prevent the renal lesions that may prove fatal in rats during the first few weeks on a diet deficient in lipotropic factors (8). Some rats received a standard laboratory diet, and were used as controls.

Table II. *Amounts of total liver-lipids in various rat groups*

Diet	g fat/100 g liver
Normal laboratory diet	7.3± 0.9
Fat 4 per cent	14.0± 8.13
Fat 30 per cent	25.2± 8.8
Fat 50 per cent	27.9± 6.8
Fat 50 per cent +choline	9.0± 3.3

The results are given as means± S.D. of five experiments.

After the animals had been on the diet for 40 days, the main renal vessels were ligated under light pentobarbital anaesthesia. Immediately after the ligation, 10 per cent galactose solution was injected into the femoral vein to the amount of 70 mg/100 g body weight. During the following three hours, blood samples for galactose estimation were taken from the tail every 30 minutes. The galactose concentration of the blood samples was determined enzymically according to Hjelm (7) with the galactose-oxidase reagent produced by Kabi (Stockholm, Sweden). The rate of galactose oxidation was calculated as the amount injected/$t_{c=o}$. In this quotient $t_{c=o}$ is the intercept with the time axis of the rectilinear part (90 to 180 minutes after galactose injection) of the plasma concentration-time curve. $t_{c=o}$ was related to the time when galactose oxidation was expected to start after pentobarbital anaesthesia. Because the recovery from the anaesthesia is gradual, this way of calculation may have caused an error of about ten per cent in the results. When ethanol was used,

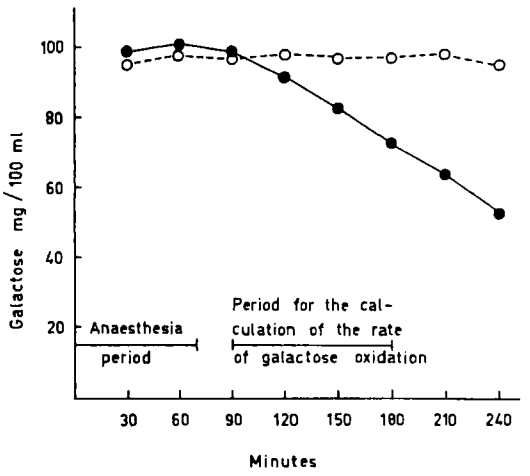

Fig. 1. Disappearance of galactose from the blood of normal rats and the influence of pentobarbital anaesthesia and ethanol

●—●—●—● Without ethanol

○---○---○---○ With ethanol

Table III. *Influence of ethanol on the rate of in vivo galactose oxidation in nephrectomized normal rats and in rats with fatty liver*

Diet	Rate of galactose oxidation without ethanol mg/100 g/h	Rate of galactose oxidation with ethanol mg/100 g/h	Inhibition per cent
Normal laboratory diet	13.2 ± 2.0	1.4 ± 1.8	89
Fat 4 per cent	31.8 ± 3.9	30.7 ± 3.5	4
Fat 30 per cent	33.6 ± 3.9	29.8 ± 2.8	11
Fat 50 per cent	35.8 ± 4.4	34.2 ± 3.2	5
Fat 50 per cent + choline	15.4 ± 2.5	2.4 ± 1.5	84

Experimental details are given in the text. The results are given as means ± S.D. of five experiments.

it was injected intraperitoneally 30 minutes before the galactose. The amount was 250 mg/100 g rat.

After completion of the experiment, the rats were killed by decapitation. The livers were rapidly removed, and the total lipids determined by the method of Entenman (3).

RESULTS

Table II presents a summary of the amounts of total liver-lipids in the animals given the different diets. The results were of about the same order as those reported by Glynn, Himsworth & Lindan (5). The degree of fatty infiltration correlated with the fat content of the diet. The fat content of the liver was restrained almost within normal limits by the choline supplement.

In Fig. 1, the galactose concentration of the blood samples of the control rats has been plotted against time. During the period of pentobarbital anaesthesia, no oxidation of galactose was observable. After the animals had been awakened, the plasma concentration-time curve was rectilinear, and the rate of galactose oxidation could be calculated during the subsequent period of 90 minutes. The inhibition of galactose oxidation during pentobarbital anaesthesia occurred in all the rat groups, in experiments both without and with ethanol.

Table III presents the hepatic galactose elimination capacity of the various rat groups without and with ethanol. In accordance with preliminary data (19), the breakdown of galactose without ethanol was increased more than twofold in rats on a choline-deficient, low protein diet, either with or without the addition of fat. When the diet was supplemented by choline, the rate of galactose oxidation was equal to that of normal rats. In normal rats, ethanol caused the total inhibition of galactose oxidation. In sharp contrast to the control rats, ethanol did not exercise any influence on the breakdown of galactose in rats on a choline-deficient, low protein diet. After the addition of choline to the diet, ethanol again inhibited galactose oxidation. It can be concluded that the main reason why ethanol ceases to influence the rate of galactose oxidation in rats with fatty livers is because of the effect induced by choline-deficiency.

DISCUSSION

It is believed that the conversion of galactose to glucose proceeds through the following four stages (10):

1. galactose+ATP $\rightleftarrows$ galactose-1-phosphate+ADP
2. galactose-1-phosphate+UDP-glucose $\rightleftarrows$ glucose-1-phosphate+UDP-galactose
3. UDP-galactose $\rightleftarrows$ UDP-glucose
4. UDP-glucose+pyrophosphate $\rightleftarrows$ glucose-1-phosphate+UTP

These reactions are catalysed by the enzymes galactokinase, galactose-1-phosphate-uridyltransferase, UDP-galactose-4-epimerase and UDP-glucosepyrophosphorylase, respectively. In this reaction chain, stage 3 requires nicotinamide adenine dinucleotide as a coenzyme, and $NADH_2$ is a potent inhibitor of the reaction (12). The ratio of NAD to $NADH_2$ is responsible for the inhibition, rather than the absolute amount of reduced pyridine nucleotides (17).

Recently evidence has been presented for the existence of a new pathway for galactose oxidation in mammalian liver (1). In this pathway, galactose is converted to galactonolactone by an enzyme, galactose dehydrogenase in the presence of NAD. Calactonolactone is further converted to d-xylulose by a series of reactions. The reaction product, $NADH_2$, is a strong competitive inhibitor of galactose dehydrogenase (2).

During ethanol oxidation large amounts of $NADH_2$ are formed, and for this reason one might think that the pathways for galactose oxidation could be inhibited. In fact, a diminished capacity of the liver to oxidize galactose during ethanol metabolism in rat-liver homogenate has been reported (9). Pronounced impairment of galactose oxidation caused by ethanol has also been observed in normal individuals (23, 25, 24).

In an earlier study, it was observed that ethanol did not affect the $NAD/NADH_2$ ratio of choline-deficient fatty livers of rats (21). In the present study, ethanol induced almost total inhibition of galactose elimination in normal rats. On the contrary, as was expected, ethanol did not in any way influence the galactose breakdown of choline-deficient rats. This study indicates that the fatty liver possesses a greater capacity to oxidize the cytoplasmic $NADH_2$, or that the control of the cytoplasmic redox state is different. It is probable that this change in the metabolism of the choline-deficient fatty liver is not associated with the well-known lipotropic activity of choline. As the role of phospholipids is very important in mitochondrial structure (14), and large mitochondrial changes have been reported in choline deficiency (16, 13), it may be that some kind of alteration in mitochondrial function has taken place during the development of choline-deficient fatty liver.

The proportionate variation of the amounts of fat and carbohydrate in the diet did not exert any influence on the galactose elimination, either with or without ethanol. After the addition of choline to the low protein, high fat diet, the rate of galactose oxidation, both with and without ethanol, was again the same as that observed with the control rats. It can be concluded that ethanol no longer induced a shift in the redox state of the choline-deficient fatty liver apparently by reason of the lack of choline.

The rate of galactose oxidation without ethanol in choline-deficient rats was more than twice that in the normal or choline-supplemented groups. The reason for this phenomenon remains to be elucidated, although it may indicate the strengthening of some pathway for galactose oxidation during the development of choline-deficient fatty liver.

No galactose oxidation was discernible in any group of rats during the pentobarbital anaesthesia period of the experiment. This may find its explanation in various barbiturates being known inhibitors of liver respiration (22); consequently, the redox state or the $NAD/NADH_2$ ratio of the liver is not suitable for galactose oxidation.

It is believed that the hepatic steatosis of alcoholics may depend on inadequate food consumption, particularly of lipotropic agents (11, 15). Indeed, it has recently been proved that alterations similar to those presented in this study occur in the functioning of the human liver in alcoholics with hepatic steatosis, and in various malnutritional stages (20). On application of the galactose tolerance test, with and without ethanol, as a measurement of the redox potential of the liver, valuable

information about possible fatty infiltration of the liver is obtainable by virtue of a simple metabolic test.

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