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Acute alcoholic fatty liver—Metabolism or stress

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Mallov and Bloch [1] first reported that a large single dose of ethanol, given to the rat either by stomach tube or by intraperitoneal injection, provoked a significant accumulation of triglycerides (TG) within 12–16 hr. Numerous investigators have confirmed this observation (for references, see Hawkins and Kalant [2]), but the mechanism has not yet been clarified. Since the effect is abolished by hypophysectomy or adrenalectomy [1], the accumulation of hepatic TG was first attributed to stimulation of mobilization of fatty acids from peripheral adipose tissue to the liver, under the influence of adrenalin released as a consequence of intoxication. Others have pointed out, however, that in the fasting animals the only significant source of fatty acids would be the peripheral adipose tissue. The accumulation of TG in the liver was attributed to impairment of oxidation of the mobilized fatty acids as a consequence of the increased NADH/NAD ratio secondary to the oxidation of ethanol [3].

One method of distinguishing between these two possibilities appeared to be the use of pyrazole, an inhibitor of alcohol dehydrogenase activity [4–6]. If the accumulation of hepatic TG required the active metabolism of ethanol, it should be prevented by a dose of pyrazole which drastically reduced alcohol oxidation during the experimental period. In contrast, if the lipid accumulation were due to a direct effect of ethanol *per se*, rather than to its metabolism, the administration of pyrazole should have no effect upon it.

Unfortunately, the results of such experiments have been contradictory. Morgan and DiLuzio [7] found that pyrazole completely prevented the alcohol-induced acute fatty liver. Bustos *et al.* [8] found that it had no effect whatever on the TG accumulation measured at 16 hr after the alcohol. Numerous other groups [9–12] have obtained intermediate results with reduction of TG accumulation under some conditions and not under others. The effects of the pyrazoles appear to be complex, and a more detailed analysis of their effects upon the acute alcoholic fatty liver has been presented elsewhere [13].

In order to avoid the complications introduced by the inhibitors of alcohol oxidation, a simple expedient has been adopted. Since alcohol oxidation reaches maximal velocity at quite low blood alcohol concentrations, any effects on fat metabolism due to the metabolism of ethanol should be fully demonstrated at sustained low concentrations of alcohol. In contrast, any effects secondary to intoxication should be demonstrable only at higher alcohol levels, and should be proportional to the level. In the present study, hepatic TG accumulation has been examined in rats given the same total dose of ethanol, but in one case as a single dose producing marked intoxication, and in the other as divided doses which did not produce gross intoxication.

The subjects used were adult male Wistar rats purchased from High Oak Farms, Guelph, Ontario. The mean body weights of the animals in different experiments varied from 135 to 265 g, but within any given experiment the weight range was not more than ± 15 g difference from the mean. The animals were housed in group cages, and fed standard Purina Rat Chow and water *ad lib.* up to the time of their selection for the experiment.

In the first experiment, animals were selected at random and distributed among four matched groups. Chow was removed at 4:00 p.m. on the day preceding the experiment, but water remained available *ad lib.* throughout the experiment. Treatments were begun at 9:00 a.m. the next day according to the following schedule. Group 1 received a dose of ethanol (4·8 g/kg) given by stomach tube as a 20% (v/v) solution in water. Group 2 received an equal volume of an equicaloric solution of glucose in water, also as a single dose by intubation. Group 3 received the same total dose of alcohol as group 1, but divided into four doses of

Table 1. Liver TG levels in rats receiving ethanol or equicaloric glucose in single and divided doses (experiment 1)*

Group	Treatment	TG levels (mg/g liver)
1	Ethanol, single dose, 4·8 g/kg	13·68 ± 0·98
2	Glucose, single dose	6·71 ± 0·69
3	Ethanol, four divided doses	10·24 ± 0·83
4	Glucose, divided doses	7·37 ± 0·54

* Results are expressed as mean ± standard error, with ten animals per group. P values were determined by Student's *t*-test (see text for P values).

1·2 g/kg spaced at intervals of 3 hr. Group 4 received the same dose of glucose solution, also divided into the same series of doses at corresponding times. In groups 1 and 2, the stomach tube was also passed at the same times as the fractional doses in groups 3 and 4, but no solution was administered ("dummy gavage").

In each of the remaining experiments only two treatment groups were used, viz. those receiving the same single and divided doses of ethanol as in experiment 1.

At 16 hr after the first administration of alcohol or glucose, weighed portions of liver were extracted [14] and analyzed for TG content [15]. In the first experiment, blood alcohol measurements were made at intervals during the 16-hr period. For this purpose, samples (0·05 ml) of tail tip capillary blood were deproteinized and used for gas chromatographic analysis by the method of LeBlanc [16].

Experiment 1. At 16 hr after the beginning of alcohol or glucose treatment, the liver TG levels in the two glucose control groups did not differ significantly from each other. The animals receiving ethanol in divided doses had a significantly higher level ($P < 0·01$) than the corresponding glucose group (Table 1), but those receiving the same total amount given as a single dose had a still higher TG level, which differed significantly from those of the three other groups ($P < 0·001$, 0·02 and 0·001 vs groups 2, 3 and 4 respectively).

The blood ethanol curves for groups 1 and 3 are shown in Fig. 1. There was a striking, even though completely predictable, difference between them. After the single dose of 4·8 g/kg, a peak blood alcohol level of about 400 mg/180 ml was attained between 2 and 4 hr after gavage, and was followed by a linear descent to about 50 mg/100 ml at 12 hr. In contrast, the divided dosage schedule showed the expected rise after each dose, with linear decline during the interval preceding the next dose. The highest level recorded was 122 mg/100 ml at 10 hr, and the final portion of the curve was almost exactly superimposable over the corresponding part of the curve for group 1. During the first 12 hr, the mean blood alcohol level in group 3 did not fall below 16 mg/100 ml.

Closely similar results of the liver TG analyses were obtained in a preliminary experiment in which group 1 received a single ethanol dose of 4 g/kg and group 3 received three doses of 1·2 g/kg and one dose of 0·4 g/kg separated by 4-hr intervals. The results are not shown here because the blood ethanol level in group 3 fell to 10 mg/100 ml or less just before the second, third and fourth doses.

Experiment 2. Five separate replications of this exper-

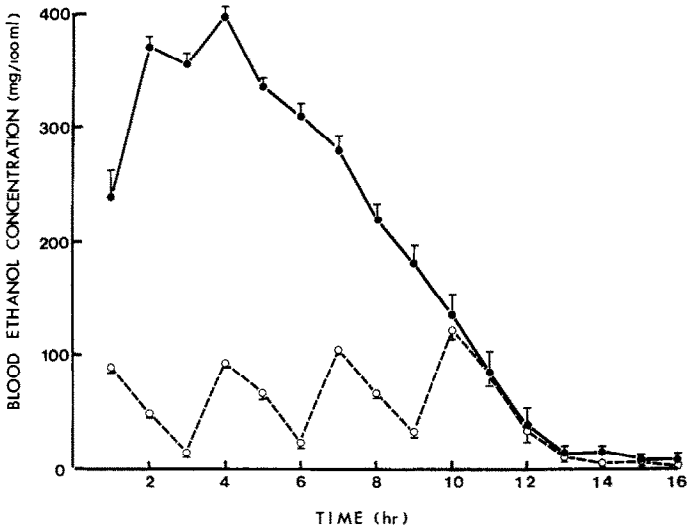

Fig. 1. Concentration of ethanol in blood of rats at various times after administration of ethanol by stomach tube: ○, doses of 1·2 g/kg at 0, 3, 6 and 9 hr; ●, single dose of 4·8 g/kg at 0 hr and passage of stomach tube without fluid at 3, 6 and 9 hr. Vertical bars indicate S.E.M.; N = ten values at each point.

Table 2. Effect of ethanol on liver TG levels in rats receiving ethanol in single and divided doses

Experiment	Holding time* (days)	Hepatic TG levels (mg/g) Single dose	Divided doses	P†
2	1	20·1 ± 1·4‡	17·0 ± 1·1	NS
	1	14·3 ± 1·08§	15·4 ± 1·2	NS
	3	36·4 ± 2·8	25·4 ± 1·8	<0·005
	5	13·8 ± 1·7	8·3 ± 0·85	<0·001
	7	27·8 ± 1·9	21·9 ± 1·8	<0·05
3	1	16·7 ± 1·7	17·2 ± 1·2	NS
	7	17·4 ± 0·74	13·6 ± 1·1	<0·02

* Holding time refers to the duration of stay after arrival in the animal quarters before intubation with ethanol.

† P values are for the difference between single and divided doses. NS = not significant, $P > 0{\cdot}05$.

‡ Results for all groups are expressed as mean ± S.E.M.

§ For this group, $N = 9$; for all other groups, $N = 10$.

iment were carried out. In each one, group 1 received a total amount of 4·8 g/kg of ethanol, as a single dose at 0 time, while group 2 received four doses of 1·2 g/kg each, separated by intervals of 3 hr. Group 1 underwent dummy gavage at the same time as the later doses of group 2. In two replications, the animals had been in the laboratory for only 1 day before the experiment. In the other three replications, the animals had been in the laboratory for 3, 5 and 7 days before the day of the experiment.

As seen in Table 2, there was no significant difference in the hepatic TG content between the animals of groups 1 and 2 in those replications conducted on the day after arrival in the laboratory. In contrast, there was a highly significant difference in the three replications carried out at 3, 5 and 7 days after arrival. In all of these cases, the hepatic TG level was higher in animals receiving the alcohol as a single dose.

Experiment 3. A final experiment was done deliberately to test the effect of the length of holding time after arrival in the animal quarters. Forty animals, obtained at the same time, were divided randomly into two equal groups. One group was subdivided into groups of 10, started on an overnight fast and used the next day on the same single and divided alcohol dosages as in experiment 3. The other group was held for 7 days, then used in another replication. As seen in Table 2, there was no significant difference between the single-dose and divided-dose groups tested the day after arrival, but a clearly significant difference was observed when the test was conducted 7 days later.

The present results indicate clearly that intoxication *per se*, as distinct from the metabolism of ethanol, contributes significantly to the TG accumulation in the liver after a single dose of alcohol. This is consistent with the earlier observations of Horning *et al.* [17], which indicated that the level of TG accumulation was related to the dose of alcohol, even over a dosage range far above that required to saturate alcohol-oxidizing capability.

It seems clear that the metabolism of ethanol does contribute to the accumulation of TG, since group 3 in experiment 1 showed a higher level than did the corresponding glucose controls. However, the results of all three experiments also leave no doubt that an additional factor is introduced by the administration of alcohol as a single large dose. In the divided-dose group in experiment 1, the lowest blood alcohol level found during the first 12 hr was well in excess of the estimated K_m for disappearance of ethanol *in vivo* in the rat, given as 2·7 mM (12·4 mg/100 ml) [18]. Ethanol metabolism via the alcohol dehydrogenase (ADH) pathway should therefore have been proceeding at virtually maximal velocity in both groups [2]. The additional TG accumulation in group 1 as compared to group 3 must therefore reflect some influence of intoxication *per se*. As noted previously [8], there was considerable variation in mean hepatic TG levels from one experiment to another, for as yet unknown reasons. However, the results of single and divided dosage were always compared within individual experiments. The fact that the single-dose effect can be prevented by the administration of beta-adrenergic blocking agents [19] is consistent with the assumption that intoxication causes release of adrenalin, which increases mobilization of fatty acids from adipose tissue.

There is also a considerable body of evidence [20] that large doses of ethanol stimulate the secretion of adrenal corticosteroids, in experimental animals as well as in man. However, human studies [21, 22] have indicated that adrenal stimulation occurs only at blood ethanol levels above 100 mg/100 ml. In an earlier study [23], we found increased steroid output *in vitro* by adrenal glands from rats which had received a single 4 g/kg dose of ethanol, but not by glands from animals which had received the same total amount of ethanol divided into two equal doses separated by an interval of 1·5 hr. It is therefore reasonable to assume that the single dose of 4·8 g/kg in the present work was much more stressful than the divided dosage, and that this difference is causally related to the difference in TG levels.

An unexpected observation was that of the relationship between length of acclimation to the laboratory and the presence of an alcohol single-dose effect. Conceivably, animals which had been in the laboratory for only 1 day failed to develop TG accumulation when given the ethanol as a single dose. It seems more probable, however, that animals which were not acclimated would have a higher level of adrenalin secretion, and even those which received the alcohol in divided doses would have a greater stress response than animals treated similarly after a period of acclimation to the laboratory.

An incidental inference from the results of experiment 1 is that the microsomal ethanol-oxidizing system (MEOS) which operates *in vitro* [24] must have been playing little or no role *in vivo*. According to the kinetics of MEOS, it should have been most likely to function at the high blood levels found in group 1, and not at the lower levels prevailing in group 3. In contrast, the ADH pathway should have been

functioning maximally in both groups. Therefore the total amount of ethanol metabolized during the first 10–12 hr should have been greater in group 1 than in group 3 if MEOS were functioning. The fact that the two blood ethanol curves were virtually superimposable from 10 hr onward suggests that, in fact, MEOS was not contributing to the ethanol disappearance.

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Stabilization of total and free ribosomes associated with 3-methylcholanthrene-induced adult rat liver growth

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Several investigators [1–4] have suggested that the accumulation and distribution of ribosomes within the cytoplasm play an important role in the modulation of protein synthesis which accompanies most situations of accelerated growth and development. While it is well established that increases in ribosomal RNA synthesis are responsible for much of the ribosome formation observed during rapid growth, there are few reports on the role of ribosomal RNA degradation in this process. Similarly, there is little information on the degradation rates of ribosomes in their two topographic states in the cell, i.e. free in the cytoplasm or bound to the endoplasmic reticulum, during induced growth. A complete understanding of the mechanism(s) of ribosome accumulation and distribution during growth and development requires that such studies be undertaken.

In rats, liver growth and an accumulation of cellular RNA follow the administration of certain drugs such as the barbiturate, phenobarbital and the polycyclic hydrocarbon, 3-methylcholanthrene (3-MC) [5–7]. Although RNA synthesis is stimulated shortly after adult rats are injected with 3-MC [7, 8], these increases in RNA synthesis are insufficient, in themselves, to account for the accumulation of RNA which accompanies the subsequent liver growth [7]. We [7] and others [9] have proposed that RNA stabilization may also be associated with 3-MC-induced adult rat liver growth. In the present experiments, we have investigated this contention more directly by studying the effect of 3-MC administration on the turnover of total and free rat liver ribosomes. Our results indicate that the half-life of ribosomes from 3-MC-treated rats is increased when the