**SHORT COMMUNICATION**

**ELEVATION OF ALCOHOL DEHYDROGENASE ACTIVITY IN THE SUBCLINICALLY SCORBUTIC GUINEA-PIG**

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**SUMMARY**

1. Because it has been shown that a majority of alcoholics are subclinically scorbutic, the metabolism of ethanol was studied in subclinically-scorbutic guinea-pigs.

2. Hepatic alcohol dehydrogenase activity was raised maximally by ethanol within 2 days.

3. In twenty-three subclinically-scorbutic guinea-pigs fed ethanol for 2 weeks, the alcohol dehydrogenase activity (±SD) was 11.5 ± 1.2 units/g of liver protein compared with 8.6 ± 0.6 units/g of liver protein in twenty-three healthy animals fed ethanol.

4. The NAD⁺/NADH ratio in subclinically-scorbutic guinea-pigs and healthy guinea-pigs fed ethanol, shows that there is more NAD⁺ available for oxidation of alcohol in subclinically-scorbutic guinea-pigs. These results may explain the increased tolerance of alcoholics to alcohol.

Key words: alcohol dehydrogenase, ascorbic acid, ethanol, redox ratio, guinea-pigs.

It is an old observation that alcoholics have an upper limit of tolerance which is usually 3–4 times the amount of alcohol that can be consumed by the occasional drinker. The reason for this elevated biochemical tolerance has often been sought in an adaptive increase of the enzymic processes involved in the oxidation of ethanol. Nutrition also affects the rate of ethanol metabolism. Because alcoholics as a group are subclinically scorbutic (O'Keane, Russell & Goldberg, 1972), attempts were made to determine whether the scorbutic state could affect the metabolism of ethanol. The pathway of ethanol oxidation is the same in guinea-pigs and man, so an experiment was set up to measure the activities of liver alcohol dehydrogenase (EC 1.1.1.1) in normal and subclinically-scorbutic guinea-pigs. Alcohol dehydrogenase is the initial and rate-limiting enzyme in the pathway of ethanol oxidation.

**MATERIALS AND METHODS**

Male guinea-pigs, weight range 240–500 g, were used in the experiments. They were divided into four groups, which were maintained as follows. Group A were fed diet 18 (Universities
Federation of Animal Welfare Handbook, 1957) *ad libitum* and given tap water as their sole drinking fluid; group B were fed diet 18 and given 10% (v/v) ethanol (Burroughs 95% ethanol) in tap water as their sole drinking fluid; group C were fed a scorbutic diet (Universities Federation of Animal Welfare Handbook, 1957) while drinking only tap water, and group D were fed the scorbutic diet while drinking the 10% ethanol solution. The guinea-pigs were caged individually and given fresh food and liquid every day. The guinea-pigs in groups B and D were also injected intraperitoneally with 2 ml of 30% (v/v) ethanol in 0.09 M-saline daily. The guinea-pigs were maintained under these conditions for different time intervals—2 days, 1 week, 2 weeks and 3 weeks. Healthy guinea-pigs must be fed the scorbutic diet for 2 weeks to become subclinically scorbutic. Therefore in the first two experiments (2 days and 1 week) the guinea-pigs were fed the scorbutic diet for 12 days and 1 week before the alcohol injections to make a total time of 2 weeks in which the scorbutic diet was given in all experiments.

At the end of each experiment the guinea-pigs were killed by cervical dislocation and the alcohol dehydrogenase activity in the liver was measured by the method of Bonnichsen & Brink (1955). In some cases the leucocyte and liver ascorbic acid amounts were also measured by the 2,4-dinitrophenol method (Gibson, Moore & Goldberg, 1966).

In a further experiment, at the end of 2 weeks the guinea-pigs were killed by cervical dislocation and the livers were rapidly excised for measurement of alcohol dehydrogenase and of nicotinamide adenine dinucleotide (NAD⁴⁺) and reduced nicotinamide adenine dinucleotide (NADH) by the method of Jedeiken & Weinhouse (1955). All results are expressed as the mean±SD.

**RESULTS**

The mean leucocyte ascorbic acid amounts for all guinea-pigs fed diet 18 (groups A and B) was 44.80±26.9 µg/10⁸ leucocytes compared with 11.1±6.6 µg/10⁸ leucocytes in the guinea-pigs fed the scorbutic diet (groups C and D). The mean liver ascorbic acid amounts in guinea-pigs fed diet 18 was 171.0±20.0 µg/g liver wet wt. compared with 46.0±4.1 µg/g wet wt. in guinea-pigs fed the scorbutic diet. Therefore the guinea-pigs given the scorbutic diet were subclinically scorbutic since they had significantly lower (*P*<0.005) leucocyte and liver ascorbic acid amounts without clinical evidence of scurvy.

Hepatic alcohol dehydrogenase activity was increased maximally within 2 days of feeding and injecting ethanol. Initially the activity (expressed as units/g of protein) was 7.0±0.8; after 2 days of ethanol injection it rose to 8.6±0.5 in group B and 11.6±1.3 in group D (*P*<0.002). After 8 days of injection these activities were 8.8±0.8 in group B and 12.2±1.1 in group D (*P*<0.001). After 14 days the values were 8.4±0.5 in group B and 11.2±1.0 in group D (*P*<0.02), finally after 21 days the values were 8.5±0.4 in group B and 11.0±1.1 in group D (*P*<0.001). At each time interval the activity of hepatic alcohol dehydrogenase in the guinea-pigs fed the scorbutic diet with alcohol was significantly higher than in guinea-pigs fed the normal diet with alcohol.

The NAD⁺/NADH ratios and alcohol dehydrogenase activities of experimental and control animals killed after 2 weeks are shown in Table 1.

**DISCUSSION**

Under the conditions of ethanol feeding used in the present work, hepatic alcohol dehydrogenase activity is increased maximally in guinea-pigs within 2 days. It increases to even higher
Alcohol dehydrogenase and scurvy

values in subclinically-scrobutic guinea-pigs. As alcoholics have been shown to be subclinically scrobutic (O'Keane et al., 1972) the increased activity of this important enzyme could partly explain the increased tolerance of alcoholics for alcohol.

Because alcohol dehydrogenase is the rate-limiting enzyme in the pathway of ethanol oxidation, it has been presumed that factors which affect the rate of ethanol metabolism in vivo do so through alterations in the rate of this initial oxidation reaction (Videla & Israel, 1970). The main factors which determine the rate of the alcohol dehydrogenase-catalysed dehydrogenation of ethanol are the concentration of the substrate, the activity of the enzyme and the availability of NAD⁺. The concentration of the substrate influences the rate of this reaction only at concentrations below which the enzyme is 'saturated'. It has been shown in dogs that the rate of ethanol metabolism is proportional to the concentration of ethanol in blood at concentrations below about 10 mg/100 ml; above this concentration 'saturation' of the enzyme occurs and the rate of ethanol metabolism is independent of the ethanol concentration (Marshall & Fritz, 1953). Because the enzyme is 'saturated' at such low substrate concentrations its availability becomes the most important determinant of the rate of ethanol oxidation at the ethanol concentrations usually found in animal and human studies. Thus in subclinically scrobutic states where the activity of alcohol dehydrogenase is increased the rate of alcohol metabolism may also be significantly increased.

The preceding statement is likely to be true only if one assumes an optimum supply of NAD⁺ for the dehydrogenation reaction. During the oxidation of ethanol to acetaldehyde and the formation of acetyl-CoA from acetaldehyde, NAD⁺ is reduced to NADH. If the supply of NAD⁺ is not to be exhausted, NADH must constantly be reoxidized to NAD⁺ via some other oxidation–reduction reaction. It is known that there is some disparity between the rates of production of NADH and its oxidation to NAD⁺ as evidenced by the decrease of the NAD⁺/NADH ratio which occurs in the liver during ethanol metabolism (Smith & Newman, 1959).

Thus metabolic systems involved with the oxidation of hepatic NADH may secondarily affect the rate of ethanol metabolism by influencing the availability of the oxidized co-factor. Dehydroascorbic acid is reduced to ascorbic acid in several reactions that occur in the cell. During this reaction NADH is reoxidized to NAD⁺. Thus in the subclinically scrobutic state the amount of NAD⁺ present in the cell is increased and more is available for alcohol meta-

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Liquid</th>
<th>Alcohol dehydrogenase activity (units/g of protein)</th>
<th>NAD⁺/NADH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18 Tap water</td>
<td>Tap water</td>
<td>6.8±1.0 (12)</td>
<td>1.16±0.03 (7)</td>
</tr>
<tr>
<td>B</td>
<td>18 10% ethanol in tap water</td>
<td>8.7±0.7 (23)*</td>
<td>0.76±0.03 (7)*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Scorbutic diet Tap water</td>
<td>7.2±0.6 (12)</td>
<td>1.64±0.04 (7)*</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Scorbutic diet 10% ethanol in tap water</td>
<td>11.5±1.1 (23)*</td>
<td>0.99±0.04 (7)*</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses show the number of animals in each group.
* Significance with respect to group A, P<0.001.
bolism. The results (Table 1) show that when ethanol is fed to the subclinically scorbutic animal there is a significant increase in the NAD$^+$/NADH ratio compared with the healthy animal. Thus this whole cycle is stimulated by a lack of ascorbic acid and it has already been shown that alcoholics do lack ascorbic acid.

ACKNOWLEDGMENT

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REFERENCES


