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# Aldehyde Oxidizing Capacity of Erythrocytes in Normal and Alcoholic Individuals

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MARING, J.-A., K. WEIGAND, H. D. BRENNER AND J.-P. VON WARTBURG. *Aldehyde oxidizing capacity of erythrocytes in normal and alcoholic individuals.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 135–138, 1983.—The acetaldehyde metabolizing capacity in blood of alcoholics and nonalcoholics was investigated by an improved head-space gas chromatographic method. Great individual and interindividual variability was observed. The mean acetaldehyde oxidizing capacity of 3.51 nmoles/min/ml erythrocyte suspension in alcoholics was significantly lower than the mean of 5.20 nmoles/min/ml in nonalcoholics. Furthermore, treatment of alcoholics with aldehyde dehydrogenase inhibitors reduced the acetaldehyde oxidizing capacity significantly (mean of 1.67 nmoles/min/ml). No acetaldehyde could be detected in blood of nonalcoholics who ingested 0.25 g ethanol/kg body weight whereas levels of 2–14 μM were detected in blood of alcoholics. After disulfiram, an elevation to 7–103 μM in blood of alcoholics was observed.

Blood acetaldehyde  Human erythrocytes  Acetaldehyde oxidizing capacity  Alcoholics

MOST of the findings in recent years suggest that acetaldehyde is likely to be a mediator of alcohol toxicity. However, the difficulties encountered in the determination of this compound in human tissues render essential a reevaluation of the earlier reports on acetaldehyde levels in blood [1]. It was reported that acetaldehyde levels are higher in blood of relatives of alcoholics than in controls [9] and of alcoholics compared to nonalcoholics [6,7]. Besides an accelerated ethanol metabolism a reduced aldehyde dehydrogenase activity in alcoholics as compared to normal subjects could lead to such differences. The presence of aldehyde dehydrogenase in human erythrocytes has been demonstrated [2, 3, 8, 11] as well as their capacity to metabolize acetaldehyde to acetate [10]. A change in the aldehyde oxidizing capacity might serve as a marker for alcoholism and possibly also contribute to the observed differences in blood acetaldehyde levels. We therefore started to investigate the acetaldehyde metabolizing capacity of erythrocytes of alcoholics and nonalcoholics.

## METHOD

### *Patients*

Fourteen alcoholics, age 24 to 54, were investigated. All were hospitalized and abstinent for at least two weeks prior the collection of blood. Blood was taken in the morning after an overnight fast. Macrocytosis was not observed in any of the blood samples tested. All the alcoholics investigated were involved in a treatment program with aldehyde dehydrogenase inhibitors. Therefore blood was also collected after 4 days of treatment and tested for the acetaldehyde metabolizing capacity. Nitrefazol was a gift from Dr. P. Zimmerman (Merck, Darmstadt, Germany).

### *Drinking Experiments*

The subjects ingested in the fasted state 0.25 g/kg body weight ethanol in 200 ml of water with some drops of orange essence to improve the taste. Blood samples were collected at different intervals over a period of 3 hours and analysed on the same day according to a method described previously [12]. Acetaldehyde concentrations were also determined in end expiratory samples of breath collected in special plastic bags (L. Etzlinger, Geneva, Switzerland) which seal automatically at the end of the sampling. Five ml of breath were taken out at room temperature by puncturing the bag with a gas tight syringe and injected in the gas chromatograph with the same conditions used for the head-space technique for blood.

### *Acetaldehyde Oxidizing Capacity*

Blood samples were centrifuged for 10 min at 5000 rpm. After thoroughly discarding plasma and buffy coats, the erythrocytes were washed 3 times with an equal volume of physiological saline. The erythocytes were then resuspended in physiological saline to give a hematocrit of 50%. Aliquots of this suspension were incubated in head-space vials at 37°C with 100 μM acetaldehyde. After 10 min the reaction was stopped by precipitation with 0.1 ml perchloric acid. The residual acetaldehyde content was then determined by head-space gas chromatography. Standards were prepared by adding the same amount of acetaldehyde to erythrocyte suspensions and immediate precipitation with perchloric acid. The recovery of acetaldehyde in these standards was identical to that of standards in saline. Acetaldehyde stock solutions in physiological saline (1 mg/ml) were prepared with freshly distilled acetaldehyde and stored in head-space vials at 4°C.

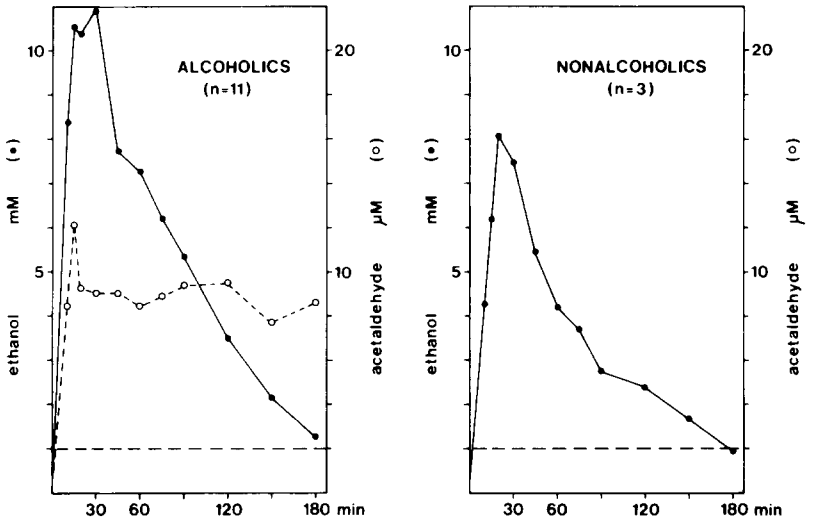


FIG. 1. Mean levels of ethanol (●−●) and acetaldehyde (○−○) in blood after oral administration of 0.25 g ethanol/kg body weight. Both alcoholics and nonalcoholics were in the fasted state. The dashed line represents the limit of the method for accurate acetaldehyde determination (2 μM).

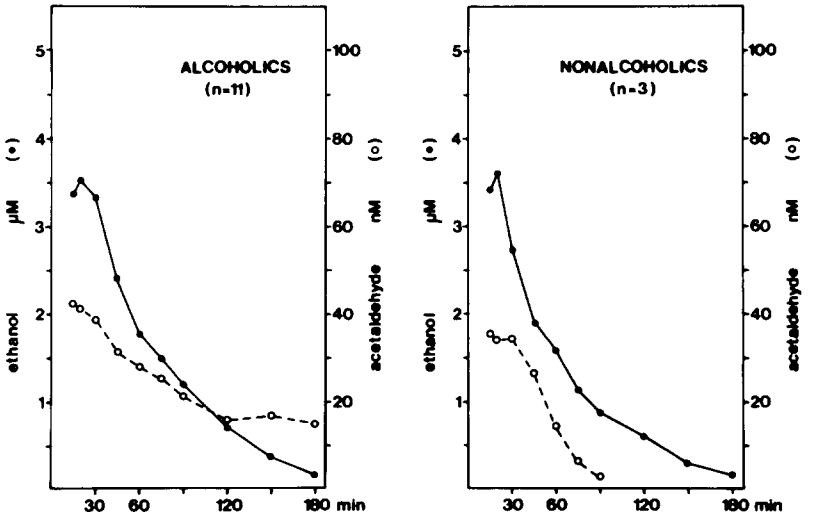


FIG. 2. Mean levels of ethanol (●−●) and acetaldehyde (○−○) in breath after oral administration of 0.25 g ethanol/kg body weight. Both alcoholics and nonalcoholics were in the fasted state.

## RESULTS

In drinking experiments 11 of the alcoholics tested showed elevated acetaldehyde values in blood as compared to the nonalcoholics (Fig. 1). Values in blood ranged from 2–14 μM whereas the acetaldehyde levels in the nonalcoholics were below the limit for accurate determination with the method used (2 μM). The blood acetaldehyde reached its maximum in the absorption phase of the alcohol after about 15 min. After 3 hours, blood ethanol had dropped to about 1 mM while blood acetaldehyde still was about 8 μM (Fig. 1). Analogous results were obtained for acetaldehyde concentrations of end expiratory breath (Fig. 2).

The acetaldehyde oxidizing capacity showed great individual and interindividual variability (Fig. 3). The lowest oxidizing capacity found in nonalcoholics was 4 nmoles/min/ml erythrocyte suspension. Some of the alcoholics investigated also showed an oxidizing capacity between 4 and 5 nmoles/min/ml. However the mean value for alcoholics was significantly lower than that for nonalcoholic individuals. Three of the alcoholics were treated with disulfiram and 11 with nitrefazol. This treatment significantly reduced the aldehyde oxidizing capacity in these individuals. However, we still observed great individual variations as shown in Table 1 for the disulfiram treated patients. The

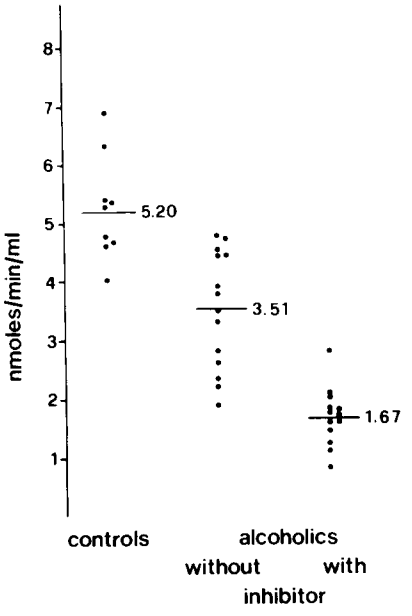


FIG. 3. Aldehyde oxidizing capacity of erythrocytes in suspension. $p<0.01$ for difference between controls and alcoholics without inhibitor. $p<0.01$ for difference between alcoholics with and without inhibitor.

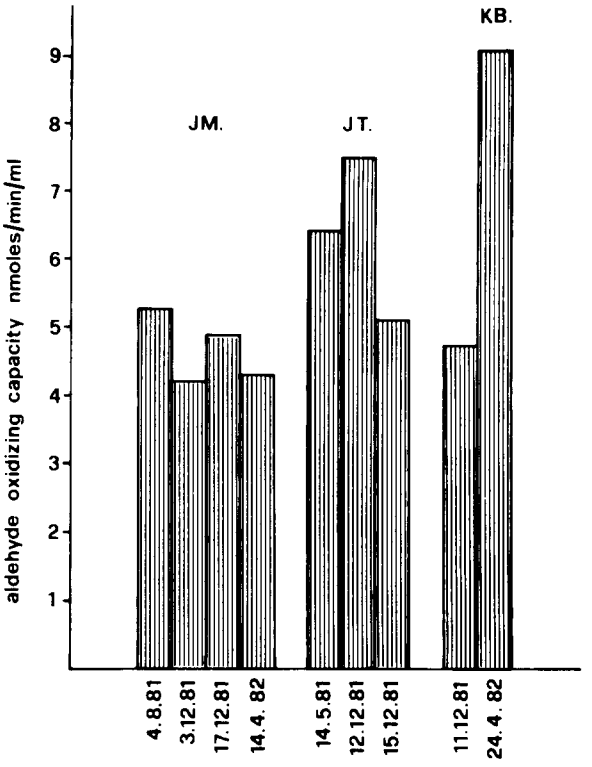


FIG. 4. Individual variability of the aldehyde oxidizing capacity. Three nonalcoholic individuals were tested at different dates. The reported variability is greater than the methodological error.

intraindividual variability observed in the nonalcoholic subjects (Fig. 4) was greater than the methodological error itself. The addition of glucose or pyruvate to the assay mixture did not influence the oxidizing capacity.

## DISCUSSION

The results of the present study clearly show that blood of alcoholics has a lower capacity to oxidize acetaldehyde than the blood of nonalcoholics. These individuals also showed a tendency to have higher blood acetaldehyde levels after an oral ethanol load, thus confirming previous findings [6,7]. These effects could be due to a difference in the activity of the aldehyde dehydrogenase, individuals with higher blood acetaldehyde levels having a lower activity of the enzyme. A recent publication [5] reports that the liver aldehyde dehydrogenase activity measured in biopsies of non-cirrhotic alcoholics was lower than that of control samples. The impaired aldehyde oxidizing capacity of erythrocytes in alcoholics observed by us could possibly be indicative of a reduced activity of the hepatic cytosolic enzyme. The oxidizing capacity was indeed lowered further when the alcoholics were treated with aldehyde dehydrogenase inhibitors, thus leading to even higher blood acetaldehyde levels in drinking experiments. Some of the alcoholics exhibited an oxidizing capacity in the lower range of the control subjects and we could only measure breath acetaldehyde in the control drinking experiment before treatment of these individuals, suggesting that the oxidizing capacity of the liver may not be significantly lower. In Japanese a correlation between the activity of the aldehyde dehydrogenase from erythrocytes and the sensitivity to alcohol has been demonstrated [4]. Nevertheless, to what extent the aldehyde oxidizing capacity of erythrocytes reflects the activity of the hepatic cytosolic enzyme remains to be defined in more detail.

TABLE 1

ALDEHYDE OXIDIZING CAPACITY* OF ERYTHROCYTES IN ALCOHOLICS WITH AND WITHOUT TREATMENT WITH DISULFIRAM

| | Patient | | |
|---|---|---|---|
| | No 2 G.S. | No 4 I.B. | No 5 H.H. |
| before treatment | 3.84 | 3.50 | 4.80 |
| after treatment† | 0.84 | 1.10 | 2.80 |
| inhibition (%) | 78.9 | 68.6 | 41.7 |

*The aldehyde oxidizing capacity is expressed as nmoles acetaldehyde oxidized/min/ml erythrocyte suspension.

†On the 4th day of treatment.

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