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Lack of Difference in Blood Acetaldehyde of Alcoholics and Controls after Ethanol Ingestion[1]

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ERIKSSON, C. J. P. AND J. E. PEACHEY. *Lack of difference in blood acetaldehyde of alcoholics and controls after ethanol ingestion.* PHARMAC. BIOCHEM. BEHAV. **13**: Suppl. 1, 101–105, 1980.—Ethanol and acetaldehyde (AcH) metabolism were studied in male Caucasian alcoholic subjects and matched controls following 1 g/kg ethanol, which was administered after a 10 day, ethanol-free period. The rate of ethanol elimination was higher ($p>0.05$) in the alcoholics (0.120 g/kg/hr) than in controls (0.108 g/kg/hr). Blood AcH concentrations were measured in either the supernatants of whole blood deproteinized with perchloric acid (PCA) or from the supernatants of PCA-treated plasma obtained from blood added to isotonic semicarbazide. There were no differences between the alcoholic and control subjects for AcH in blood dripped directly into the PCA. The blood AcH concentrations decreased from 22 μM (controls) and 23 μM (alcoholics) to 7 μM (controls) and 3 μM (alcoholics) at 1 hour and 7 hours after the start of drinking, respectively. No significant AcH was found in blood first taken into heparinized tubes before deproteinization with PCA, after correction for artifactual AcH formation was made. As well, no significant AcH was measured by the semicarbazide method after correction for artifactual AcH. These results suggest that elevated blood AcH levels after ethanol ingestion cannot be taken as a general marker of alcoholism.

Ethanol Blood acetaldehyde Alcoholism marker

BLOOD acetaldehyde (AcH) levels, measured after ethanol ingestion, are reported to be increased to a greater extent in alcoholics compared with controls [9,19]. Impaired mitochondrial AcH oxidation [9] and increased rate of ethanol metabolism [19] have been used to explain the higher blood AcH concentrations in the alcoholics. In addition, blood AcH levels are reported to be increased in nonalcoholic individuals with alcoholic relatives [14], which suggests that the previously reported raised blood AcH levels in alcoholism were not necessarily the result of alcoholism, but could be involved in the etiology of alcoholism.

These reports of increased blood AcH in alcoholics and relatives of alcoholics need to be reevaluated [1–3] in view of new information concerning the determination of human blood AcH [2–4,17]. The accurate measurement of blood AcH levels in man depends upon rapid deproteinization of the blood sample (to avoid rapid disappearance initiated by the blood sampling) and correction for artifactual AcH formation (which occurs during treatment of the blood).

The goal of the present study was to test for the possibility that elevated AcH is a general marker of the alcoholic phenotype, with special emphasis on the control of analytical problems involved in the determination of blood AcH in man.

METHOD

Subjects

Our subjects were paid male Caucasian volunteers consisting of 10 alcoholic and 10 control, nonalcoholic drinkers, matched for age (alcoholics: 40±9 years, controls: 39±9 years), weight (alcoholics: 70.8±9.8 kg, controls: 77.8±9.6 kg), height (alcoholics: 172±6 cm, controls: 175±14 cm) and per cent body fat (alcoholics 20.8±3.3; controls: 19.0±5.9).

The alcoholics were steady drinkers (daily or almost daily ethanol ingestion, n=3), binge drinkers (days or weeks of continued drinking alternating with periods of abstinence, n=6) and episodic drinkers (2 to 3 days drinking per week on a regular basis, n=1). The alcoholics reported daily ethanol intake in excess of 130 g (239.7±99.5) for a minimum of 10 years (20.6±5.9). For controls, the reported daily ethanol intake was less than 70 g (44.2±15.8) over 14.7±6.7 years.

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All subjects were abstinent with respect to ethanol and other drugs for at least 10 days prior to the experiments, did not manifest withdrawal symptoms or brain abnormalities, and had normal cardiovascular, hepatic and renal function.

Procedures

On the morning of the experiments, the subjects received 1 g/kg ethanol orally as a 15% (v/v) solution in unsweetened orange juice. The ethanol drink was taken over 30 min, 1 hour after a 400 calorie breakfast consisting of 12 g protein, 23 g fat and 35 g carbohydrate.

Blood samples were collected from an arm vein through a heparinized, indwelling catheter (Butterfly® No. 19, Abbott), before ethanol ingestion and up to 7 hours following the start of drinking. Approximately 1 ml of blood was dripped through this catheter directly into 4 ml 0.6 N ice-cold perchloric acid (PCA); a second blood sample was withdrawn by syringe into heparinized tubes from which 1 ml of blood was pipetted into the PCA. In addition, most subjects consented to give three further 4-ml blood samples, taken before drinking, and at 1 and 2 hours after commencing drinking, which were taken from the opposite arm by repeated venipunctures using a Butterfly® catheter with the seal missing in order to allow for direct dripping of the blood into the PCA. This latter procedure for the collection of blood samples has been recommended for human blood AcH determinations [3], and in the present study, this method of blood collection gave results which were comparable to those initial samples obtained by direct dripping from an indwelling catheter. Consequently, only the AcH values on the blood samples obtained by the indwelling catheter with direct dripping into PCA are reported in the text.

In addition to the PCA analysis, blood AcH was analyzed by the semicarbazide [16] and chloralhydrate [21] methods. Blood samples were either collected by dripping the blood directly into isotonic semicarbazide or chloralhydrate reagents through the indwelling catheter as well as the catheter used for separate venipunctures, or they were withdrawn by a syringe, placed in collection tubes and then pipetted into the reagents. All samples were immediately centrifuged and the plasma was treated with PCA, as described before [16,21].

Artifactual AcH formation, which occurs during protein precipitation with PCA [17], is a function of the ethanol concentration at different blood dilutions [3]. The spontaneous formation of AcH at blood dilutions and ethanol concentrations corresponding to the conditions of the present study was calculated for the before-drinking samples, in the manner described elsewhere for the correction of artifactual AcH formation [3]. The average AcH formation curves were used to calculate a correction value which was subtracted from the AcH levels measured in the samples collected after the start of drinking. The formation of artifactual AcH was similarly controlled for during the semicarbazide and chloralhydrate methods by adding ethanol, in concentrations corresponding to the levels measured during the present study, to the semicarbazide and chloralhydrate reagents used for the control blood samples.

AcH and ethanol concentrations were measured by head-space gas chromatography from deproteinized supernatants [4]. Head-space and GC conditions were as follows: 1 ml supernatant was incubated at 60°C for 18 minutes in 24 ml sealed "head-space" bottles. A 1.0 ml head-space sample was injected into a Hewlett-Packard Model 5711A gas chromatograph equipped with a flame ionization detector. A glass column (1.8 m×4 mm i.d.) containing Chromosorb 102 on 60–80 mesh was used. The operating conditions were: injection temperature, 150°C; column temperature, 120°C; detector temperature 150°C; nitrogen flow rate 60 ml/min. AcH and ethanol were measured by peak areas obtained with a Hewlett-Packard Model 3380A integrator.

RESULTS

For the semicarbazide method, the uncorrected blood AcH concentrations were approximately 10 μM at 1 to 2 hours after the start of drinking. There were no differences in the AcH values between the alcoholics and control subjects; the combined AcH values are illustrated in Fig. 1. As well, there were no differences between the AcH values from blood dripped directly into semicarbazide reagent compared with blood pipetted into the reagent from collection tubes. In contrast to what has been reported earlier [16], artifactual AcH formation was observed with the semicarbazide method. As demonstrated in Fig. 1, this artifactual AcH production increased with the age of the semicarbazide buffer solution. Thus, it seems that after appropriate correction, no *in vivo* AcH is found during these experimental conditions. With the chloralhydrate method, the corresponding non-corrected values (1.0±5 μM, n=5) were much lower. The artifactual AcH formation observed with this method was in the same order (almost negligible), so that no significant levels of *in vivo* blood AcH were detected.

The average blood ethanol elimination curves are shown in Fig. 2. It is interesting to note that, in spite of a higher ethanol elimination rate in the alcoholics (0.120±0.015 g/hr/kg) compared with the controls (0.108±0.012 g/hr/kg), calculated from the total time necessary to eliminate this dose of ethanol, there were no significant differences between the average slopes of the elimination phase of the blood ethanol curves. This observation demonstrates the pitfall of using only the slope as the measure of the average ethanol elimination rate.

Blood AcH concentrations determined by direct sampling into PCA are demonstrated in Fig. 3. No differences in either corrected or uncorrected AcH values were observed between the alcoholics and controls. The magnitude of the average correction was similar for both the alcoholic and control subjects and varied between 1 to 8 μM, depending upon the ethanol concentration and blood dilution. The slopes of the AcH elimination curves for the alcoholic and control subjects (Fig. 3b) were similar to the slopes of the corresponding ethanol elimination curves (Fig. 2).

The AcH concentrations measured in blood pipetted into PCA from collection tubes, are shown in Fig. 4; there were no differences in AcH values between the alcoholic and control subjects. A comparison of the uncorrected (Fig. 4a) and corrected (Fig. 4b) AcH values demonstrates that most of the AcH measured by these methods was artifactually formed. Thus, most if not all of the *in vivo* blood AcH had disappeared while taking the blood into collection tubes prior to pipetting into PCA.

DISCUSSION

State of Human Blood Acetaldehyde Determination

The results of the present study confirm the need [1, 3, 17] for a reevaluation of earlier reported human blood AcH determinations; the AcH values reported in earlier studies have

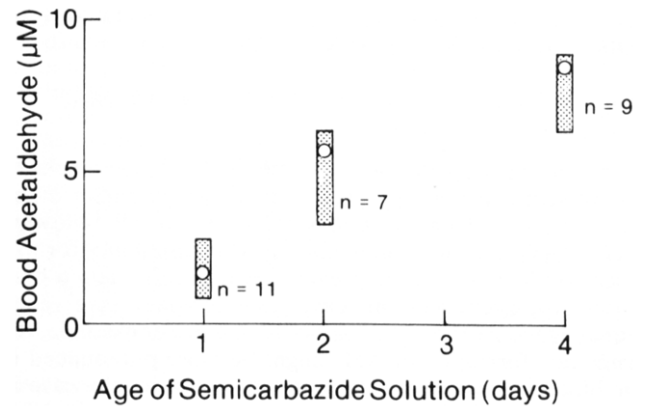

FIG. 1. Blood acetaldehyde determined with the semicarbazide method (see Method section). Dashed range: Non-corrected blood acetaldehyde±SD (obtained 1–2 hr after start of ethanol drinking). (○): Corresponding artifactual acetaldehyde (from control blood, i.e., before ethanol ingestion, treated with semicarbazide containing ethanol).

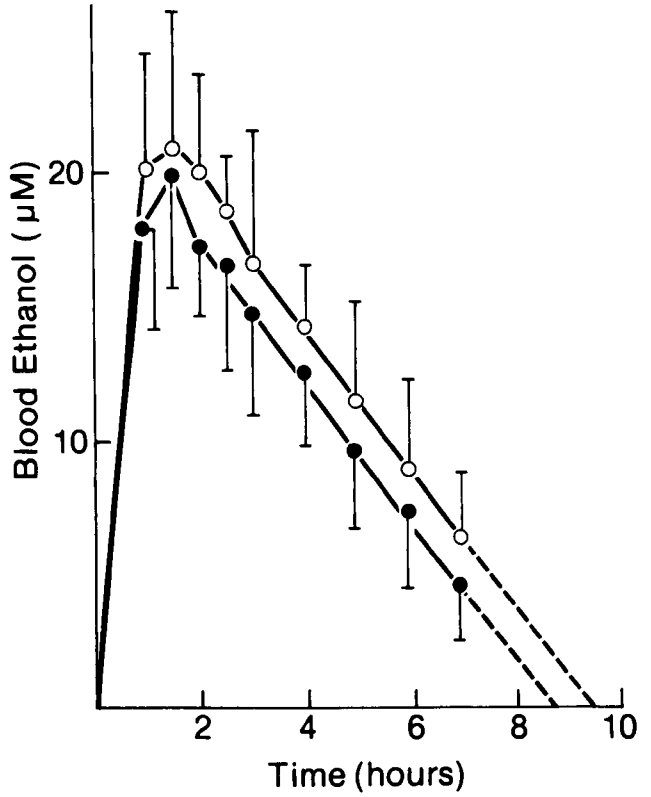

FIG. 2. Blood ethanol (1 g/kg) elimination in control subjects (○) and alcoholics (●). Values are means±SD (n=10+10).

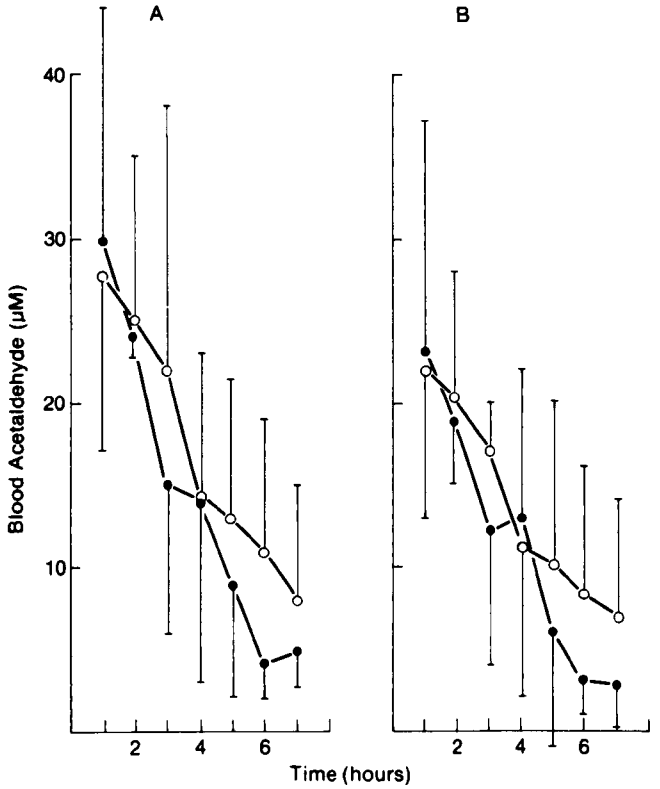

FIG. 3. Blood acetaldehyde in control subjects (○) and alcoholics (●) after ethanol (1 g/kg) ingestion. Acetaldehyde was measured from blood immediately (<5 sec) deproteinized in perchloric acid (see Method section). Values are means±SD (n=7–10). (A) Uncorrected values for AcH. (B) AcH values corrected for artifactual formation.

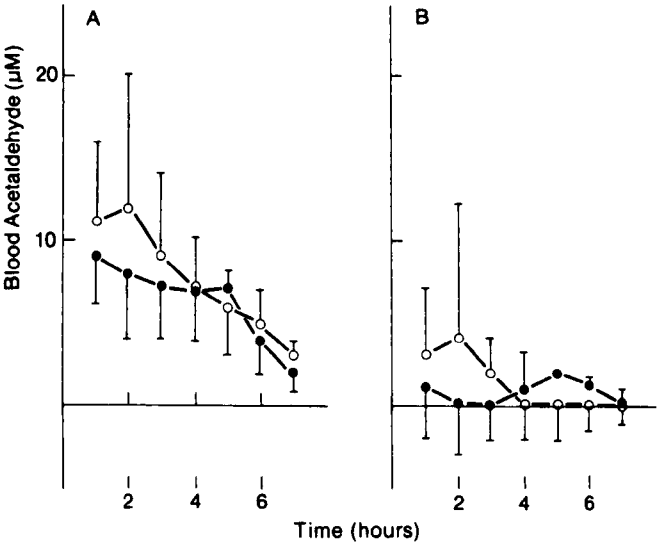

FIG. 4. Blood acetaldehyde in control subjects and alcoholics. Conditions as in Fig. 3 except that the blood was taken into heparinized tubes and thereafter (about 30 sec) deproteinized in perchloric acid. Values are means±SD (n=9–10). (A) Uncorrected AcH values. (B) corrected AcH values.

most likely reflected the combination of artifactually formed AcH and residual *in vivo* AcH. Several new plasma methods for the measurement of AcH have been described [12, 16, 21] to overcome these analytical difficulties, and, in particular, the spontaneous formation of AcH. The common feature with these methods is that the proteins are not precipitated until the cells, which contain most of the capacity for AcH production, have been separated by rapid centrifugation [12] and treatment with isotonic semicarbazide [16] or chloralhydrate [21]. The present results (Fig. 1) demonstrate, in contrast to previous results [16], that correction for artifactual AcH formation is necessary with the semicarbazide method. However, the magnitude of artifactually formed AcH is minimized by using fresh semicarbazide solutions.

The AcH levels after correction, which were measured in the present study with the semicarbazide (Fig. 1) and chloralhydrate methods, are extremely low, which is in agreement with a recent report of almost negligible (<2 μM) human blood AcH levels as determined using the rapid centrifugation method [12]. Observations [10,18] of slightly higher (2 to 6 μM) AcH concentrations with the semicarbazide method may be explained by the lack of correction for artifactual AcH. Significant AcH levels in excess of 80 μM have been determined with the plasma methods in flushing individuals [18], in experiments with aldehyde dehydrogenase inhibitors [12], in certain alcoholics [10] and in a single control subject [20].

One may ask why no *in vivo* AcH was detected with the plasma methods, or with the PCA method involving the collection tube (Fig. 4), when significant AcH levels were obtained with the PCA method involving direct sampling of blood into the PCA (Fig. 3). It is unlikely that the AcH values, obtained with direct PCA treatment, is due to an "initial burst" of AcH formation which is not accounted for in the correction procedure [3]. Rather, rapid AcH disappearance during blood sampling would appear to be a more obvious explanation. That this is not the whole answer, however, is demonstrated by the present results, since there are no differences in the blood AcH levels with blood dripped directly into the semicarbazide solution compared to blood pipetted into the reagent from collection tubes. One explanation is that, during normal conditions, there is little, if any, free plasma AcH which can be trapped by the semicarbazide reagent, or can be determined by the other plasma methods. It is proposed that the *in vivo* AcH is "loosely" bound and is unavailable to the semicarbazide but, at least partly, is releasable by direct treatment with PCA. Such a possibility is supported by the observation (Eriksson, unpublished observation) that it is not possible to recover, with the semicarbazide method, the AcH which is added to rat blood and which has been shown to bind incompletely to hemoglobin [5]. Even if this type of hemoglobin binding has not been demonstrated in human blood [5], there are indications of AcH binding to blood proteins [6, 7, 15]. As proposed elsewhere [3], loosely bound AcH may become rapidly stabilized during blood sampling and the tightly bound AcH, which is not released by PCA treatment, could be centrifuged away with the protein precipitation.

Blood Acetaldehyde Levels in Alcoholic and Matched Nonalcoholic Individuals

In contrast to previous observations [9,19], the results of the present study do not show a tendency to higher blood AcH levels in alcoholics compared with controls following oral 1.0 g/kg ethanol administration. One possibility for this discrepancy might be the previous lack of information concerning the circumvention of analytical problems involved in human blood AcH determinations [2–4,17]. For example, the artifactual formation of AcH might be more pronounced in the blood of alcoholics. However, this was not the case in the present study and no conclusions regarding the possible differences in the rapid AcH disappearance reaction can be drawn from the present data.

In addition to explaining the contradictory AcH results on the basis of analytical difficulties, it might be fruitful to look for explanatory differences in the subjects used in these studies. The assumption is that with certain types of alcoholics, AcH levels are indeed elevated either with direct or coincidental (indirect) relation to the alcoholism. The relation would be indirect if the alcoholics chosen for the experiments included individuals belonging to ethnic groups which are "sensitive" to ethanol and among which alcoholism could be more common and based on factors other than AcH induced sensitivity [13]. Unfortunately, previous studies do not state the ethnic origin of their subjects [9,19]. However, in the study of relatives of alcoholics compared with controls only Caucasians were used [14].

In addition to the possibility of elevated AcH being involved in the etiology of alcoholism [14], increased AcH could be the direct result of alcoholism. Increased AcH could be due to ethanol-induced impairment of mitochondrial AcH oxidation [9] or nutritional deficiency causing a decrease in aldehyde dehydrogenase activity. Indeed, decreased cytosolic aldehyde dehydrogenase activity in alcoholics has recently been observed [8]. A factor which would enhance the effect of impaired AcH oxidation is the ethanol-induced adaptive increase of ethanol metabolism. The magnitude of this effect is, in turn, highly dependent on the elapsed time from the last drinking period [11]. Thus, the elevated blood AcH concentrations measured in skid-row alcoholics on the day following admission [10] probably reflect the combined effect of a greatly increased (>50%) rate of ethanol metabolism and aldehyde dehydrogenase deficiency. However, even if this condition could be associated with elevated AcH concentrations, it may be concluded that an elevated AcH level cannot be taken as a general marker of alcoholism.

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