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Elevated Blood Acetaldehyde in Alcoholics with Accelerated Ethanol Elimination

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LINDROS, K. O., A. STOWELL, P. PIKKARAINEN AND M. SALASPURO. *Elevated blood acetaldehyde in alcoholics with accelerated ethanol elimination.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1,119–124, 1980.—Alcoholics and controls given ethanol (1.2 g/kg body weight) were analyzed for blood and breath acetaldehyde using the more sensitive and reliable semicarbazide method. The acetaldehyde levels in controls were almost undetectable (<2 μM), but were found to be elevated (10–110 μM) in 6 of 8 alcoholics. Breath acetaldehyde and blood acetaldehyde co-fluctuated during the experiments. Fructose infusion transiently increased blood acetaldehyde, but only in 4 of the alcoholics. The apparent discrepancy between our finding and the simultaneously reported low acetaldehyde level in alcoholics (Eriksson and Peachy, this volume) may be explained by the different status of the alcoholics tested. Our alcoholics were tested on the day after hospital admission and eliminated ethanol 55% faster than controls. It is suggested that elevated blood acetaldehyde occurs regularly after uninterrupted drinking in heavy alcohol abusers with fast ethanol elimination, possibly combined with reduced liver aldehyde dehydrogenase activity, but that the phenomenon may rapidly disappear upon abstinence and hospital treatment, which reduces disturbances in hepatic functions and the ethanol elimination rate.

Blood acetaldehyde Alcoholics Ethanol elimination

A MAJOR difficulty in evaluating the possible contribution of acetaldehyde to various actions of alcohol [7,16] has been the lack of accurate and reliable methods to determine acetaldehyde from human blood [2]. Korsten *et al.* observed significantly higher blood acetaldehyde levels in alcoholics than in controls [5], but the reported levels probably have to be corrected for artefactual acetaldehyde formation from ethanol during perchloric acid blood deproteinization [1, 13, 17]. In view of the potential importance of proving the existence of abnormal acetaldehyde metabolism in alcoholics in the research on alcoholism and alcohol-related disorders, we decided to re-investigate the blood acetaldehyde levels in alcoholics by the use of newly developed improved analytical techniques. Blood was analyzed for acetaldehyde using the semicarbazide method [14] by which artefactual ethanol-derived acetaldehyde production can be minimized. Advantage was also taken of our recent observation that blood acetaldehyde can be estimated from breath analysis [15], which completely excludes technical artefacts. By these techniques we were indeed able to demonstrate transient and variable elevation of blood acetaldehyde in the majority of the alcoholics tested.

METHOD

Subjects

The alcoholics were 40–56 year old males of skid-row type. They were admitted to the hospital the day before the experiment. All had a history of heavy drinking for at least 10 years and reported uninterrupted drinking for at least two weeks before admission. On the day of hospital admission they received 20 g ethanol *per os* every third hour until 10 p.m. in order to minimize the time of abstinence before the experiment. Fatty changes were seen in 6, and elevated transaminases in 5 of 8 alcoholics that were investigated. Control subjects were healthy males, 20–48 years old. Informed consent was obtained from all the subjects tested.

Ethanol Infusion Experiments

The subjects had a light standard morning meal. Thereafter a flexible venous indwelling catheter (Venflow; Viggo Ab, Sweden) was inserted into an antecubital vein, and control blood and breath samples were taken. Ethanol (1.2 g/kg body weight, as 7% solution in saline) was infused into an antecubital vein of the other arm during a 40–70 min period.

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Subsequently, a test dose of galactose (0.5 g/kg) was infused within 1–2 min to measure the galactose elimination time. A second dose of ethanol (0.4 g/kg in the first two experiments, 0.5 g/kg thereafter) was infused 3 (in the first two experiments with alcoholics) or 4 hours later, during a 25–45 min period in order to re-elevate blood ethanol levels. Then fructose infusion was started (0.7 g/kg per hour) and continued until the termination of the experiment.

Sampling

Approximately 1.5 ml blood was dripped directly into chilled plastic tubes containing 1.5 ml heparinized isotonic phosphate buffer (pH 7.0) with 6 mM semicarbazide (from Hopkin and Williams Ltd., Chadwell, Essex, England) to trap acetaldehyde. The tubes were stored on ice and semicarbazide-plasma samples processed and analyzed for acetaldehyde and ethanol by head space gas chromatography as previously described [14,15]. Control samples (taken from the patient before ethanol was given) were added to semicarbazide solution containing ethanol in order to correct for any spontaneous formation of acetaldehyde. Storage of the semicarbazide buffer was found to cause an increase in the spontaneous formation of acetaldehyde from ethanol on addition of blood. However, provided freshly prepared solution (0–20 hours old, stored at 4°C) was used, the artefactual acetaldehyde production was found to be quite low (<2 μM) at the ethanol concentrations used (10–30 mM). The values given have been accordingly corrected.

For determination of acetaldehyde from plasma samples, whole blood was immediately centrifuged for 30 sec in heparinized tubes, plasma separated and analyzed for acetaldehyde by head space gas chromatography after incubation at 37°C in closed vials as previously described [11].

Breath samples were collected by having the subjects blow 4.0 liters through cold semicarbazide solution, which was analyzed for ethanol and acetaldehyde as before [15]. The control subjects were able to blow 4.0 l by a single expiration, but for most of the alcoholics 2 expirations were necessary. For conversion of breath acetaldehyde to blood acetaldehyde, a blood:breath partition ratio of 190 was used [15].

RESULTS

Ethanol Elimination Rates

Ethanol was administered intravenously in order to avoid the effects of individual variations in gastrointestinal absorption on ethanol distribution in the body. Blood ethanol levels varied between 32–14 mM during the experiment (Fig. 1). The rate of ethanol elimination was calculated by extrapolation of the near-linear part of the clearance curve, and was found to be 107 mg/kg body weight per hour in controls (Table 1). In the alcoholics, however, marked acceleration of ethanol elimination was found. Their elimination rates ranged from 121–195 mg/kg per hour, their average elimination being 55% faster than in the controls.

Fructose infusion moderately increased the rate of ethanol elimination both in controls (average+14%) and in alcoholics (average: +17%).

Blood and Breath Acetaldehyde

In the control subjects, barely detectable (0.5–1 μM) acetaldehyde levels were observed in the early phase of the experiment, coinciding with peak blood ethanol, but in general, the blood acetaldehyde levels in the controls were below the detection limit (0.5 μM) (Fig. 2, bottom right). In sharp contrast to this, remarkable elevation of blood acetaldehyde was found in the majority of the alcoholics tested (Fig. 2). However, large variations in the individual response, as well as marked fluctuations during one experiment were registered. Since blood and breath acetaldehyde levels co-fluctuated, the fluctuations cannot have been caused by a technical artefact. Because of the large individual variations, individual acetaldehyde curves are shown. It can be seen that, in general, peak ethanol and acetaldehyde coincide, but acetaldehyde levels decline more rapidly. An increase in acetaldehyde levels was seen after the start of fructose infusion, but this effect was mostly transient in spite of the continued fructose infusion. Furthermore, in 3 alcoholics (V.L., O.S. and O.H.) a rather unexpected increase in acetaldehyde was seen before fructose infusion in spite of declining ethanol levels.

TABLE 1

ETHANOL ELIMINATION RATES IN CONTROLS AND ALCOHOLICS*

		Ethanol Elimination (mg/kg Body Weight Per Hour)	
		−Fructose	+Fructose
Controls	(n=4)	107 ± 19	122 ± 14
Alcoholics	(n=9)	166 ± 23†	195 ± 38‡

*Ethanol (1.2 g/kg) and fructose was given intravenously as outlined in Fig. 1. Means ± S.D. are given.

†=$p<0.001$, ‡=$p<0.005$ for difference between alcoholics and controls.

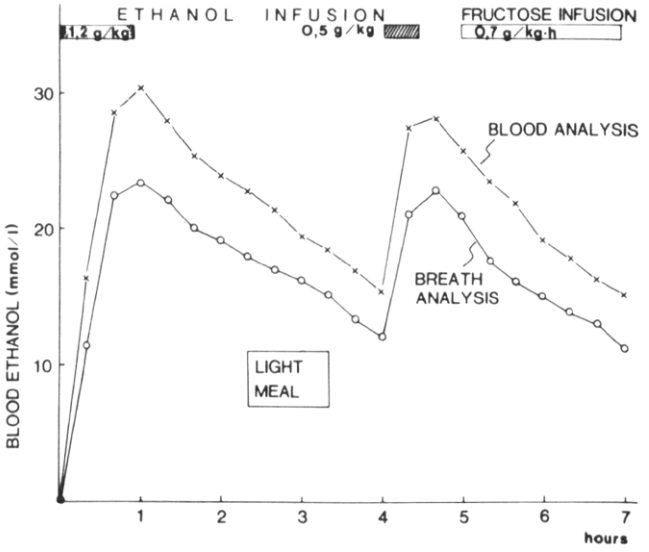

FIG. 1. Ethanol clearance curves of alcoholics. The figures represent mean blood values from seven alcoholics as analyzed directly or indirectly from breath samples. Ethanol and fructose were infused intravenously as indicated in the figure. The light meal consisted of 2–3 sandwiches and a glass of milk.

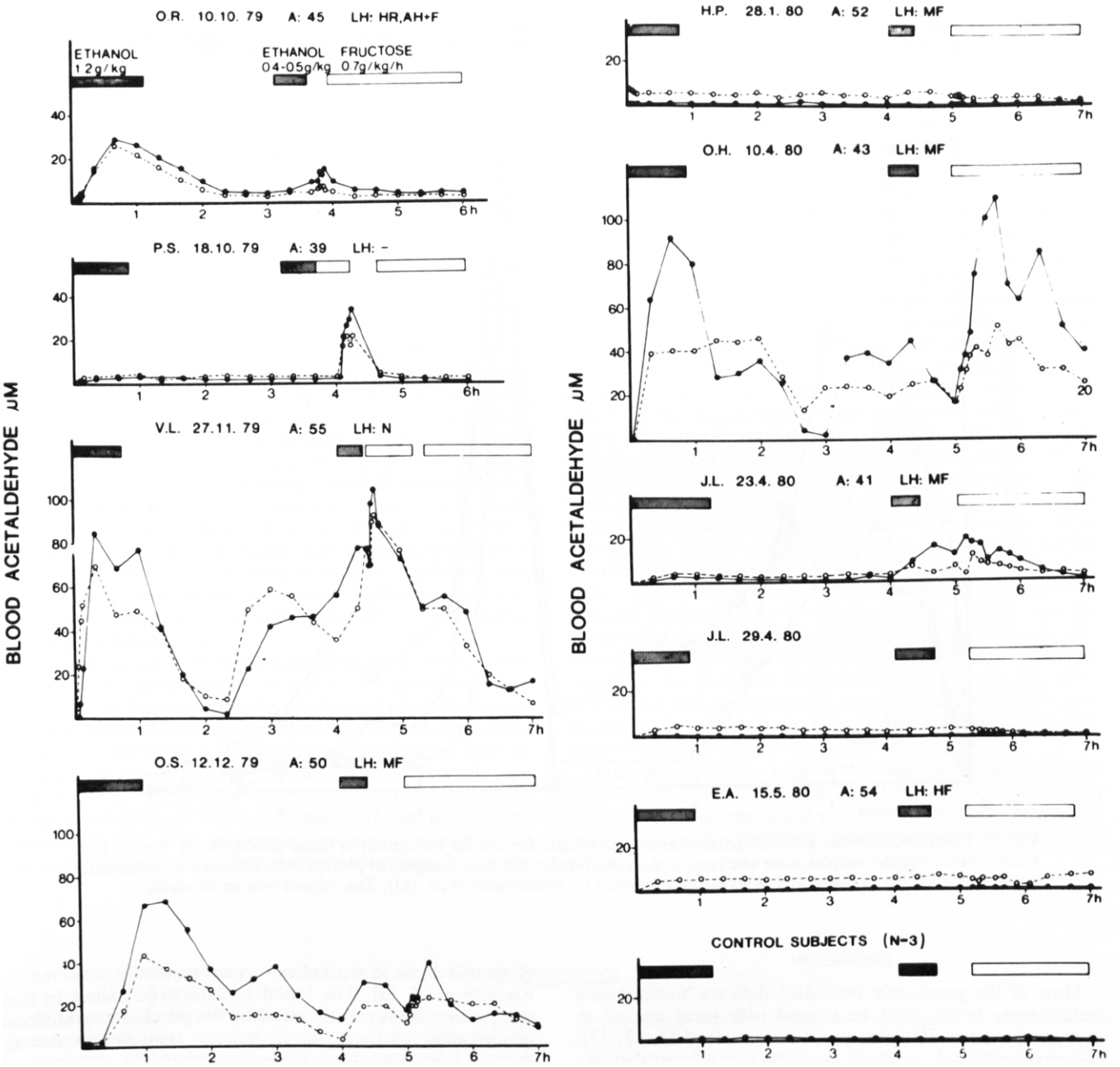

FIG. 2. Blood and breath acetaldehyde in alcoholics and controls. Curves from individual alcoholics (n=8) and the average from three control subjects (bottom, right) are shown. Exact duration of ethanol and fructose infusions (see Fig. 1) for each separate experiment is indicated by bars. One of the alcoholics (J.L.) was tested twice with a six-day interval. Concentrations of acetaldehyde in antecubital venous blood (●——●) and pulmonary blood (○---○) (calculated from breath samples) were determined as described in Method. Abreviations: A: age in years; LH: liver histology; N: normal; MF: moderate fatty liver (40–60% of hepatocytes occupied by fat); HF: heavy fatty liver (more than 90% of hepatocytes occupied by fat); AH+F: alcoholic hepatitis and fibrosis; BAH: beginning alcoholic hepatitis.

Method Comparison

Recently, several alternative methods which circumvent or reduce artefactual acetaldehyde production have been described [11, 14, 19]. We compared the semicarbazide method with the direct analysis from rapidly separated plasma [11] (Fig. 3). The values obtained by the two methods gave rather similar results, especially when taking into account the rather low acetaldehyde levels found in the experiment with this alcoholic. Also, breath acetaldehyde levels correlated with the blood values, although the calculated pulmonary blood values in general were slightly higher, in concordance with our previous report [15]. Generation of acetaldehyde from ethanol in the airways [10] may contribute to this effect. On the other hand, when the acetaldehyde levels are high as in several of our alcoholics, breath acetaldehyde seems to give underestimates of blood acetaldehyde. This may be explained by the incapability of the alcoholics to produce deep breath samples resulting in incomplete equilibration.

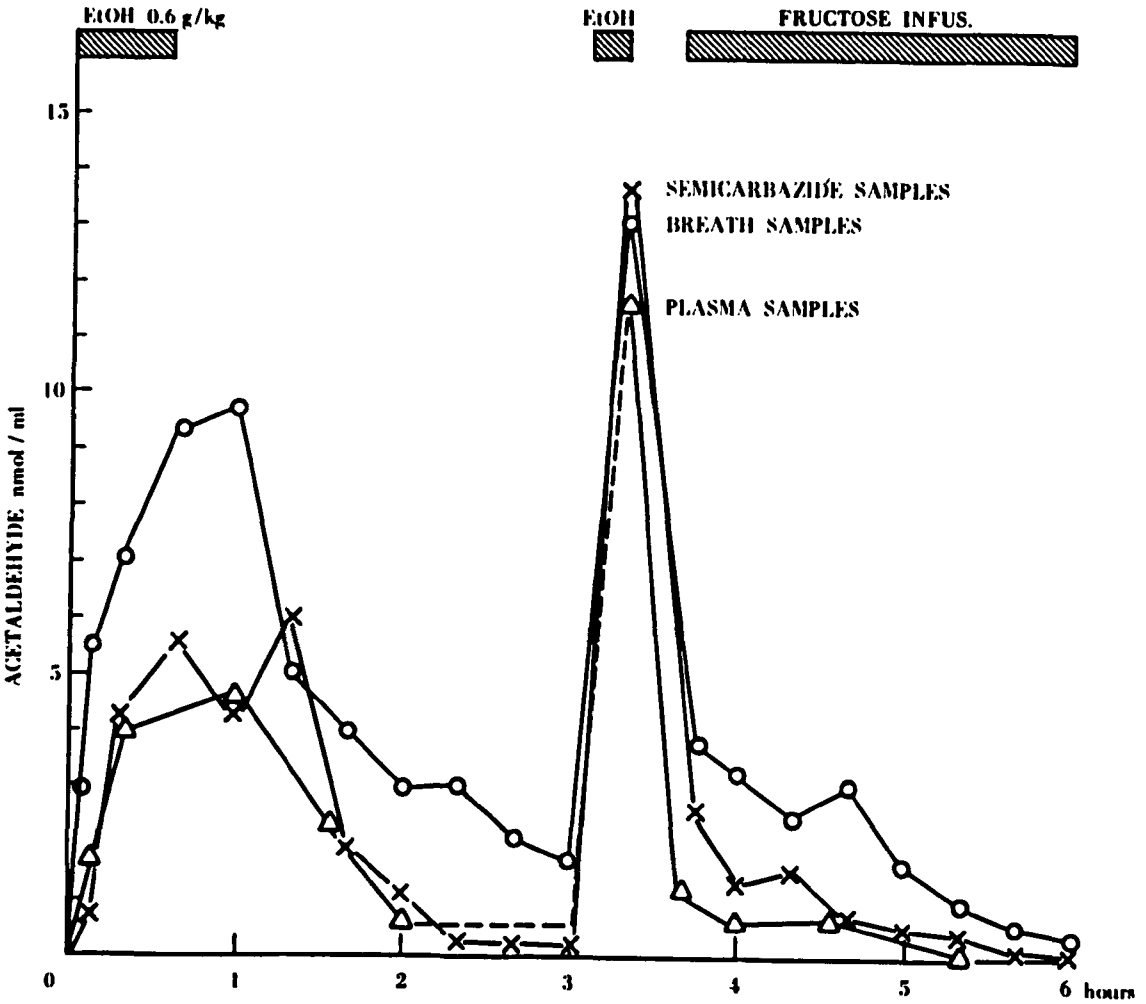

FIG. 3. Comparison of the semicarbazide, plasma and breath method for estimation of blood acetaldehyde. Breath and blood semicarbazide samples were analyzed as described under Method. Samples of plasma were obtained by immediate rapid centrifugation of blood and analyzed as described by Pikkarainen *et al.* [11]. The subject was an alcoholic.

DISCUSSION

Most of the previously published data on human blood acetaldehyde levels must be treated with great caution in view of the methodological problems involved [2, 13, 17]. With respect to non-alcoholic controls, our present data are in good agreement with other recently published studies in which improved methods have been used [11, 14, 15]. These studies demonstrate that during normal ethanol metabolism in healthy caucasian individuals blood acetaldehyde levels are extremely low (<5 μM). In contrast, elevated blood acetaldehyde seems to occur quite frequently in non-alcoholic orientals [9].

The major question to which we will address ourselves in this discussion therefore will be: To what extent does elevation of blood acetaldehyde occur in individuals after sustained alcohol abuse, and is this phenomenon a consequence of alcohol abuse, or not. We will also demonstrate how the seeming discrepancy between our data and the recent observation of absence of elevated acetaldehyde in alcoholics [3] may be logically explained.

The first evidence for higher acetaldehyde concentrations in alcoholics was described by Truitt [18]. A clear elevation of acetaldehyde in alcoholics was subsequently observed by Korsten *et al.* [5]. The blood acetaldehyde values in that study were, however, obtained by the perchloric acid blood precipitation method, which causes spontaneous acetaldehyde formation from ethanol even in the presence of thiourea [1,13]. However, even if the values are corrected for this artefact, the absolute difference between controls and alcoholics would persist, provided the blood ethanol levels were equal and the samples identically treated. Thus, generally speaking, our data would support the observation of elevated blood acetaldehyde in alcoholics given ethanol intravenously. However, a closer comparison between the two studies reveals a number of fundamental differences. Korsten *et al.* found a remarkably consistent acetaldehyde level in all alcoholics tested, (39–46 μM). We found a remarkable variation among individuals as well as during the experiment, and in three of the alcoholics, no increase was seen. Also, the alcoholics investigated by Korsten *et al.* had been hospitalized for up to two weeks and, possibly as a consequence of this, did not eliminate ethanol faster than controls. The remarkably high blood acetaldehyde levels in the controls (22–30 μM) as compared to our study also

suggest the involvement of a large technical error. It remains to be demonstrated whether the higher ethanol doses (peak blood ethanol 50 mM) used by Korsten *et al.* somehow could "stabilize" the blood acetaldehyde levels, thus explaining the differences between the two studies.

The large fluctuations of acetaldehyde levels observed during the course of our experiments may, at a first glance, seem difficult to explain. It must, however, be pointed out that blood acetaldehyde levels result from the small difference between the rate of its (mainly hepatic) rate of production (=ethanol oxidation) and its subsequent oxidation [6]. Since the fraction of unmetabolized acetaldehyde appearing in the blood may be less than 1% of all acetaldehyde produced from ethanol, subtle changes in the rate of ethanol oxidation or acetaldehyde oxidation will greatly influence this fraction. Thus, it seems obvious that the fructose-induced increase in blood acetaldehyde is mediated by the stimulation of ethanol oxidation by fructose. Accordingly, the transient or initially stronger elevation of acetaldehyde by fructose may reflect a similar initially more marked stimulation of ethanol oxidation, as was recently observed [20].

In those alcoholics exhibiting elevated acetaldehyde, peak acetaldehyde generally coincides roughly with peak blood ethanol, i.e. at about the termination of ethanol infusion. Again, this effect probably can be explained from a small, albeit sufficient increase in ethanol oxidation rate occurring as a consequence of a more complete saturation of the ADH pathway and of possible other alternative alcohol oxidizing pathways [12].

The near-absence of blood acetaldehyde in the controls and in two of the alcoholics as well as the absence of any stimulation by fructose, despite accelerated ethanol elimination, suggests that in these individuals the hepatic enzymatic capacity to metabolize acetaldehyde exceeds the rate of acetaldehyde production even in the presence of fructose.

In the three alcoholics with the highest blood acetaldehyde levels (Fig. 2: V.L., O.S. and O.H.) an unexpected pronounced (V.L.) or moderate increase in acetaldehyde was seen during the first ethanol clearance phase. Since this phenomen occurred after the patients received the light meal (Fig. 1) it might have been caused by a meal-stimulated ethanol oxidation rate, for instance by increasing the supply of oxidized 3-carbon metabolites from carbohydrate breakdown in the liver.

Eriksson and Peachey [3], using reliable techniques, were unable to find any elevation of acetaldehyde in alcoholics. The apparent discrepancy between our and their results may, however, be explained by a fundamental difference in the status of the subjects tested. The alcoholics tested by Eriksson and Peachey were hospitalized and alcohol-free for at least a week before the experiments and eliminated alcohol only slightly (10%) faster than the controls. In our study, the alcoholics were drinking until the day of the experiment and metabolized alcohol 55% faster than controls. Deceleration of ethanol elimination occurs in hospitalized alcoholics [8] and also normalization of certain liver abnormalities such as steatosis and certain enzyme deficiencies is likely to occur. Reduced liver aldehyde dehydrogenase activity in alcoholics was recently reported [4], suggesting deranged acetaldehyde oxidizing capacity. Preliminary examination of liver enzyme activities from alcoholics in our study support this view. Furthermore, when one of our alcoholics was reinvestigated under identical conditions six days after the initial experiment, the previously observed elevation of acetaldehyde was no longer observable (Fig. 2, J.L.). Taken together, these observations suggest that the combination of reduced hepatic aldehyde dehydrogenase activity and the accelerated acetaldehyde production rate explains the elevation of acetaldehyde seen in our subjects. During hospitalization the alcoholic normalization of the ethanol elimination rate and probably also of hepatic aldehyde dehydrogenase activity occurs, so that elevated acetaldehyde concentrations after ethanol no longer are seen.

Our results further suggest that high blood acetaldehyde concentrations are simply the result of excessive drinking in some individuals and is not involved in the etiology of alcoholism, but may contribute to organ damage after alcohol abuse.

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