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Concentration-time profiles of ethanol and acetaldehyde in human volunteers treated with the alcohol-sensitizing drug, calcium carbimide

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1 The disposition kinetics of ethanol and its toxic metabolite acetaldehyde were investigated in 10 healthy male volunteers who ingested 0.25 g kg^{-1} ethanol after an overnight fast. This dose of ethanol was given 2 h after they swallowed a tablet of either calcium carbimide CC (50 mg), a potent inhibitor of low K_m aldehyde dehydrogenase (ALDH), or placebo according to a single-blind crossover design.

2 The pulmonary blood concentrations of ethanol and acetaldehyde were estimated indirectly by means of a gas chromatographic method modified for analysis of end-expired breath. This non-invasive sampling technique allowed replicate determinations at 15 min intervals.

3 The distribution volume of ethanol (V) was 0.64 ± 0.023 l kg^{-1} after CC and 0.68 ± 0.026 l kg^{-1} after placebo treatment ($P > 0.05$). The zero order slope of the blood-ethanol decay profile (k_o) decreased by about 5% when low K_m ALDH was inhibited. The elimination of ethanol from the body ($V \times k_o$) was 1.9 ± 0.051 mmol kg^{-1} h^{-1} after CC compared with 2.11 ± 0.056 mmol kg^{-1} h^{-1} in placebo control experiments ($P < 0.001$). The area under the ethanol concentration time curve (0 → 180 min) increased after CC treatment implying a change in clearance.

4 The disposition of acetaldehyde was markedly different in subjects pretreated with CC. The peak blood-concentrations, estimated by analysis of breath, ranged from 40–242 μmol l^{-1} compared with 1.7–6.5 μmol l^{-1} after placebo. The apparent elimination half-life of acetaldehyde after inhibition of ALDH was 23 min on average with a range of 18–31 min. The area under the acetaldehyde-time curve (0 → 180 min) was increased significantly after CC pretreatment.

5 The calcium carbimide-ethanol interaction caused intense facial flushing in all subjects tested beginning 20–30 min after drinking. Despite abnormally high concentrations of acetaldehyde in blood and breath after pretreatment with CC, the elimination kinetics of ethanol were not markedly changed from the placebo control trial. Our results do not support a significant role of acetaldehyde in regulating *in vivo* oxidation of ethanol in humans.

Keywords ethanol acetaldehyde ALDH metabolism calcium carbimide kinetics breath analysis flush reaction

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Introduction

The low K_m isoenzyme of liver aldehyde dehydrogenase (ALDH), mainly located in mitochondria, plays a predominant role in maintaining very low concentrations of acetaldehyde in blood and liver tissue during the metabolism of ethanol (Lundquist, 1983). If this enzyme is inactive or lacking as in 40–50% of Japanese, the blood-concentrations of acetaldehyde rise to abnormally high levels after alcohol consumption (Ikama *et al.*, 1983; Yoshida & Dave, 1985). This elicits a range of unpleasant effects such as facial flushing, tachycardia, fainting, sensations of warmth and shortness of breath (Mizoi *et al.*, 1979). A similar response occurs if subjects drink alcohol after treatment with alcohol-sensitizing drugs, such as disulfiram or calcium carbimide; these work by partially blocking the low K_m isoenzyme of liver ALDH (Sellers *et al.*, 1981).

In both these situations, the disposition kinetics of acetaldehyde are markedly different from those operating during normal biotransformation of ethanol. Moreover, the inhibition of ALDH by ethanol-sensitizing drugs might influence the elimination kinetics of ethanol (Dawson, 1983; Cronholm, 1985). The oxidation of ethanol to acetaldehyde by alcohol dehydrogenase (ADH) is a reversible process and abnormally high hepatic concentrations of acetaldehyde should therefore favour the backward reaction i.e. reduction once again to ethanol (Cronholm, 1985). The role of acetaldehyde in the regulation of ethanol oxidation *in vivo* has not been thoroughly investigated in healthy volunteers after treatment with alcohol-sensitizing drugs such as calcium carbimide.

This paper describes a non-invasive technique for determination of ethanol and acetaldehyde in end-expired alveolar air by gas chromatography. This method of analysis was used to study the disposition kinetics of ethanol and its more toxic metabolite acetaldehyde. In a cross-over design experiment, healthy volunteers were pretreated with either a placebo tablet or calcium carbimide (50 mg) before they drank a small dose of ethanol.

Methods

Subjects and conditions

Ten healthy men all nonsmokers with moderate drinking habits took part in this study as paid volunteers. Their mean age was 25.6 years (range 21–35) and their mean body weight was 74.5 kg (range 64–86). Each of the subjects served in two experimental sessions separated by 5–7 days. In a single-blind crossover design study they swallowed either a tablet of calcium carbimide (50 mg) (Dipsan®, Lederle, USA) or placebo about 2 h before they drank 0.25 g kg^{-1} ethanol. The men arrived at the laboratory at about 06.30 h after fasting overnight. They were given a physical examination and control specimens of venous blood were taken. A coded tablet was swallowed at about 07.00 h, consisting of either placebo or calcium carbimide. At 09.00 h they drank 0.25 g kg^{-1} body weight ethanol (96% v/v) diluted with lemonade to 250 ml. This small dose of ethanol was finished within 5 min. The study was approved by the Karolinska Hospital ethics committee.

Determination of ethanol and acetaldehyde

Ethanol and its metabolite acetaldehyde were determined in end-expired alveolar air by gas chromatography as described in detail elsewhere (Jones *et al.*, 1984; Jones, 1985). Porapak Q was used as the stationary phase and the retention times of ethanol and acetaldehyde were 1.8 and 3.1 min respectively.

This gas chromatographic breath-analyzer was calibrated by means of a breath simulator device (MK 2A Smith & Wesson Ltd, USA). The simulator was charged with 500 ml of a known strength aqueous solution of either ethanol or acetaldehyde and these were equilibrated at 34 ± 0.05° C which is approximately the same temperature as end-expired breath in healthy men (Jones, 1982). The actual concentration in the standard air-vapour mixture was calculated from the water/air partition coefficients of ethanol (2585) and acetaldehyde (159) determined from experiments *in vitro* (Jones, 1983; Jones *et al.*, 1985).

The outlet from the simulator was connected to the inlet of the gas sampling valve and after circulating the air-vapour mixture for a few seconds to flush-out the system a sample (2 ml) was trapped for analysis by gas chromatography. The chromatograms were displayed on a Hewlett Packard model 3392A recording integrator. Care was taken not to deplete the strength of the simulator charge when acetaldehyde standards were generated. This was done by renewing the solution more often. The theory and application of this technique of generating known strength air-vapour mixtures by means of a breath-simulator device was published by Dubowski (1979).

The relationships between detector response and the concentration of ethanol and acetaldehyde in air-vapour-samples emerging from

the simulator were linear and the regression lines passed through the origin. The standard deviation calculated from replicate determinations of ethanol and acetaldehyde increased with their concentrations in the vapour analysed. The coefficients of variation ranged from 3.3–1.7% for the assay of ethanol-vapour which correspond to blood-ethanol concentrations of 2–10 mmol l^{-1}. And for blood-acetaldehyde equivalent to concentrations within the range 3.6–179 μmol l^{-1} the coefficients of variation calculated from analysis of air-vapour-samples ranged from 7.4–3.3%.

Sampling of end-expired alveolar air

At 15 min intervals timed from start of drinking, samples of breath were captured with the aid of a highly reproducible gas-sampling device. Each subject took a moderately deep inhalation of room air and then expired as much breath as possible through one arm of a heated (50° C) T-piece made of copper. A Wright Respirometer was connected to another arm of the T and the remaining end led to the inlet of the gas sampling valve (Valco Instruments Ltd, USA). The volume of breath blown into this arrangement during a prolonged exhalation was noted from the dials on the respirometer. In this way we could guarantee obtaining alveolar equilibrated breath for analysis when the men reached their vital capacities. The gas sampling loop was positioned within the oven of the gas chromatograph which was maintained at 130° C. The copper T-piece was wrapped with heating tapes to minimize the risk of condensation of water-vapour from the breath specimen and subsequent loss of alcohol and acetaldehyde.

The first specimens of breath were analysed 15 min after the men had finished their drinks and consequently this first result should be considered with caution. There is a risk of contamination of the breath sample from ethanol dissolved in the mucous membranes of the mouth from the alcoholic beverage consumed. This suggests that time to reach the peak concentrations of ethanol, which is a useful parameter reflecting the rate of absorption is difficult to interpret. This mouth-ethanol effect is one draw-back with the use of breath analysis in pharmacokinetic studies. Its significance can, to some extent, be eliminated by washing the mouth with warm water before sampling or waiting at least 20 min after the end of the drinking (Caddy *et al.*, 1978).

Kinetics of ethanol and acetaldehyde

Concentration-time profiles were plotted for ethanol and acetaldehyde after placebo and calcium carbimide pretreatments. Ethanol kinetics were evaluated by the method proposed by Widmark (1932) based on the assumption of zero order elimination kinetics after reaching the peak concentration of ethanol in blood. This model is suitable after the intake of a moderate dose of ethanol under fasting conditions (Jones, 1984). The best fitting straight line was drawn through the points on the rectilinear descending portion of each blood-concentration (BAC) time profile. The y-intercept corresponds to the theoretical blood-ethanol concentration at zero time (C_o) assuming instantaneous absorption and distribution of the alcohol dose into the total body-water compartment. The x-intercept gives an estimate of the time to reach zero blood-ethanol (min_o) but neglects the exponential nature of the BAC profile when ADH is no longer saturated with substrate. The rate of ethanol disappearance from blood (k_o) was calculated from the relationship C_o/min_o (mmol l^{-1} h^{-1}) and is therefore the zero order slope of the decay profile. The apparent volume of distribution (V) was calculated as dose/C_o and is therefore expressed as l kg^{-1}. The elimination of ethanol from the body (turnover) was calculated as the product of slope (mmol l^{-1} h^{-1}) and V (l kg^{-1}) and has units of mmol kg^{-1} h^{-1}. Areas under the curves (0 → 180 min) were calculated by the trapezoidal rule (Rowland & Tozer, 1984).

The concentration-time profiles of acetaldehyde after pretreatment with placebo and CC were plotted on semi-logarithm paper. The areas under the acetaldehyde time-profiles were calculated by trapezoidal rule from 0→180 min (Rowland & Tozer, 1984). After CC treatment, an apparent half-life of elimination of acetaldehyde was also computed to give an estimate of the rate of removal of this metabolite from the body. Because the ethanol-acetaldehyde reaction is reversible, the values obtained include loss of acetaldehyde by reduction to ethanol.

Results

Gas chromatography of end-expired alveolar air

Figure 1 gives an example of the gas chromatograms obtained from analysis of breath in one test subject typical for the group. The pre-drink traces show endogenous breath volatiles. Before the intake of ethanol the concentration of acetaldehyde in breath was below the limits of detection. The other endogenous volatiles were identified as methanol, ethanol, acetone and isoprene (2- methyl-1,3-butadiene) confirming previous work with this sampling device (Jones, 1985).

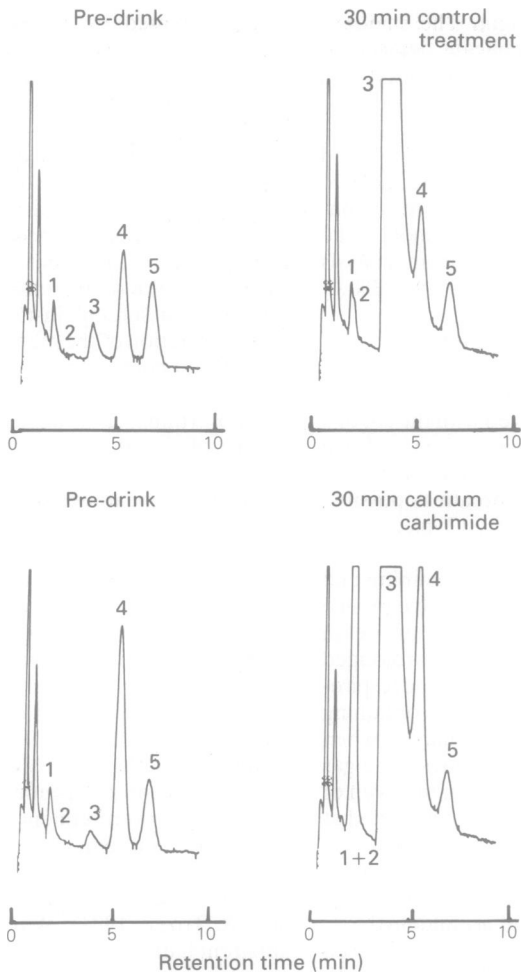

Figure 1 Gas chromatograms obtained from analysis of end-expired alveolar air in one test subject. The pre-drink tracings show the presence of various low-molecular endogenous volatiles; 1 = methanol, 2 = acetaldehyde (below detection limit), 3 = ethanol, 4 = acetone, 5 = isoprene. The 30 min traces show the changes after placebo pretreatment (control) or calcium carbimide tablet (50 mg) swallowed 2 h before the subjects drank 0.25 g kg^{-1} ethanol within 5 min.

After drinking, the ethanol peak on the chromatogram increased dramatically as expected although the acetaldehyde peak still remained insignificant in placebo control trials. Pretreatment with the tablet of CC before ethanol brought about a pronounced rise in breath acetaldehyde. The small endogenous methanol peak merged with the acetaldehyde response on the chromatogram under these conditions (Figure 1).

Time course of drug-ethanol flush reaction

About 20–30 min after intake of this small dose of ethanol, the subjects given calcium carbimide began to flush in the face and neck and this often spread over the shoulders to reach the back and extremities. The reaction was most intense at about 30–60 min after drinking and was almost gone after 120 min. The most intense flushing coincided with the peak concentrations of ethanol and acetaldehyde in blood and breath. Some of the volunteers even complained of difficulties in taking deep inhalations and felt faint on standing up. There were marked increases in heart rate and decreases in diastolic blood pressure at or near the time of most intense flush. Three hours after the start of drinking some of the subjects were pale in the face and complained of headache.

Ethanol disposition after placebo and calcium carbimide treatment

Figure 2 shows blood-ethanol profiles for the 10 individual subjects in both test situations. The time course of blood-ethanol maintained a somewhat higher elevation after CC pretreatment compared with the placebo control situation. The BAC curve after CC reached zero concentration later (extrapolated value assuming linear decay) and the areas under the curves were significantly increased ($P < 0.01$) implying a slower clearance.

The blood-ethanol parameters presented in Table 1 were calculated from individual curves and confirm the general impressions gleaned from Figure 2. A higher peak concentration of ethanol was noted after this small orally administered dose when ALDH was inhibited by treatment with the alcohol-sensitizing drug. The rate of disappearance of ethanol from blood as reflected in the zero-order slope (k_o) was about 5% slower after treatment with CC. This was also supported by the slower elimination of ethanol from the body as a whole defined as the product of k_o and the volume of distribution (V) mmol

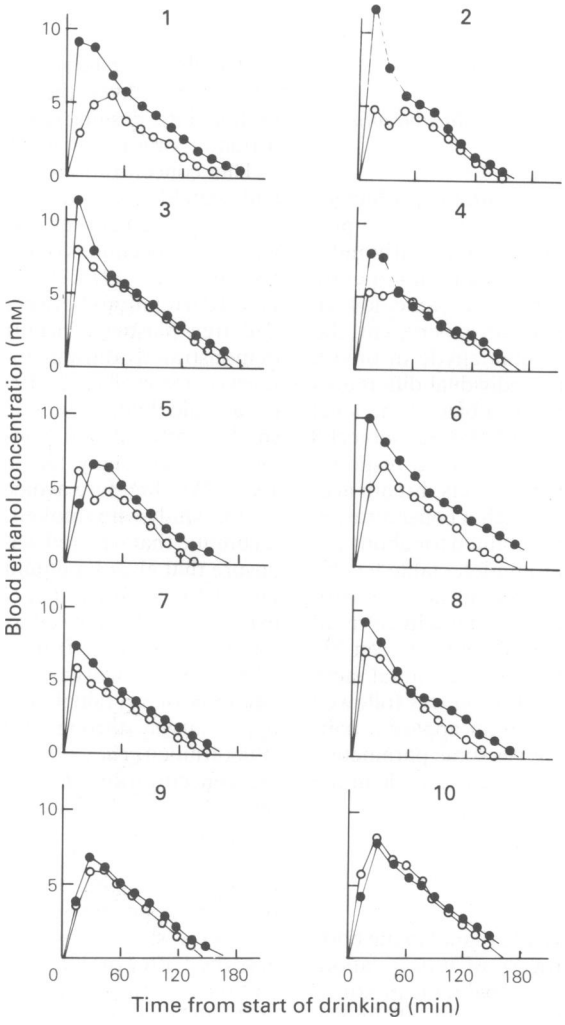

Figure 2 Blood ethanol profiles determined indirectly by analysis of end-expired alveolar air in 10 healthy volunteers in two test situations. After an overnight fast, the subjects drank 0.25 g kg^{-1} ethanol after intake of placebo tablet (control treatment, ○) or calcium carbimide (50 mg, ●) 2 h earlier.

Table 1 Blood ethanol parameters (mean ± s. e. mean) in 10 healthy fasting volunteers who received 0.25 g kg^{-1} ethanol (5.43 mmol kg^{-1}) 2 h after pretreatment with placebo or calcium carbimide (50 mg)

Blood ethanol parameter	*Pretreatment*		*Student's t-test*
	Calcium carbimide	*Placebo*	*d.f. = 9*
Peak blood ethanol concentration (mmol l^{-1})	9.5 ± 0.58	6.7 ± 0.41	$P < 0.01$
C_o (mmol l^{-1})	8.5 ± 0.30	8.0 ± 0.32	$P > 0.05$
k_o (mmol l^{-1} h^{-1})	3.0 ± 0.15	3.1 ± 0.14	$P > 0.05$
Min_o (min)	172 ± 4.5	155 ± 8.4	$P < 0.001$
V (l kg^{-1})	0.64 ± 0.023	0.68 ± 0.026	$P > 0.05$
Turnover (mmol kg^{-1} h^{-1})	1.90 ± 0.051	2.11 ± 0.056	$P < 0.001$
Area under curve (mmol l^{-1} h)	12.06 ± 0.587	9.36 ± 0.518	$P < 0.01$

d.f. = degrees of freedom in paired t-test. C_o = zero time intercept; k_o = slope of pseudolinear elimination phase; Min_o = zero concentration intercept; V = apparent volume of distribution; Turnover = ethanol elimination rate.

kg^{-1} body weight h^{-1} ($P < 0.001$).

Acetaldehyde-time profiles after placebo and calcium carbimide

Figure 3 shows concentration-time profiles of acetaldehyde after volunteer subjects were pretreated with placebo or calcium carbimide. Strikingly different disposition kinetics operate in these two test situations for the same dose of ethanol. In the ethanol-placebo experiment, the peak concentrations of acetaldehyde in breath were low but definite inter-individual differences in response were evident. In subject 4 the level barely rose above background whereas subject 8 showed a much higher acetaldehyde response after drinking. In the other subjects, breath acetaldehyde quickly rose to reach a concentration plateau which was then maintained for about 1–2 h before dropping again to undetectable levels.

Pretreatment with CC brought about a pronounced increase in breath acetaldehyde in all subjects and this effect was elicited between 20–30 min after the end of drinking. An initial rapid rise to reach a peak concentration was followed by an elimination curve with an apparent half-life of 18–31 min. Table 2 gives some parameters related to the disposition of acetaldehyde in the two test situations.

Discussion

The metabolic breakdown of ethanol in the body can be considered a process of bioactivation. This follows because acetaldehyde, the proximal metabolite of ethanol oxidation by all known pathways, is potentially more toxic than the parent drug (Von Wartburg & Bühler, 1984). Two main factors regulate the concentrations of acetaldehyde generated during metabolism of ethanol in the liver; the rate of ethanol oxidation itself and the ensuing destruction of acetaldehyde formed (Nuutinen *et al.*, 1983).

The concentration-time profiles of ethanol and acetaldehyde measured in breath resemble those reported earlier involving direct determinations in specimens of plasma and whole blood (Brien *et al.*, 1978; Peachey & Sellers 1981). Good correlations between breath acetaldehyde and free plasma concentrations were recently demonstrated during the drug-alcohol flush reaction (Stowell *et al.*, 1984). The concentration of acetaldehyde generated in the breath of smokers after alcohol consumption might, however, be artificially too high (Jauhonen *et al.*, 1982). We therefore avoided selecting volunteers for this study who smoked. The breath-sampling technique that we used was carefully designed to ensure that alveolar equilibrated breath was obtained for analysis. The 2 ml aliquots of end-expired breath analysed were definitely not contaminated with air from the upper airway and mouth. Some workers have shown that small amounts of acetaldehyde are produced in the upper airway through the action of microbes (Pikkarainen *et al.*, 1981). We feel confident that the concentration of ethanol and acetaldehyde measured in end-expired breath reflects the concentrations present at the alveolar-capillary membrane of the lungs. This is closer to the conditions affecting brain than a sample of blood from an upper arm vein.

This investigation assumes that CC is effectively absorbed after oral intake in the form of tablets and that pharmacologically active concentrations of the drug exist in the circulation before giving ethanol. We decided to wait 2 h before administration of ethanol as recommended by Peachey *et al.* (1981). Nine of the ten subjects showed

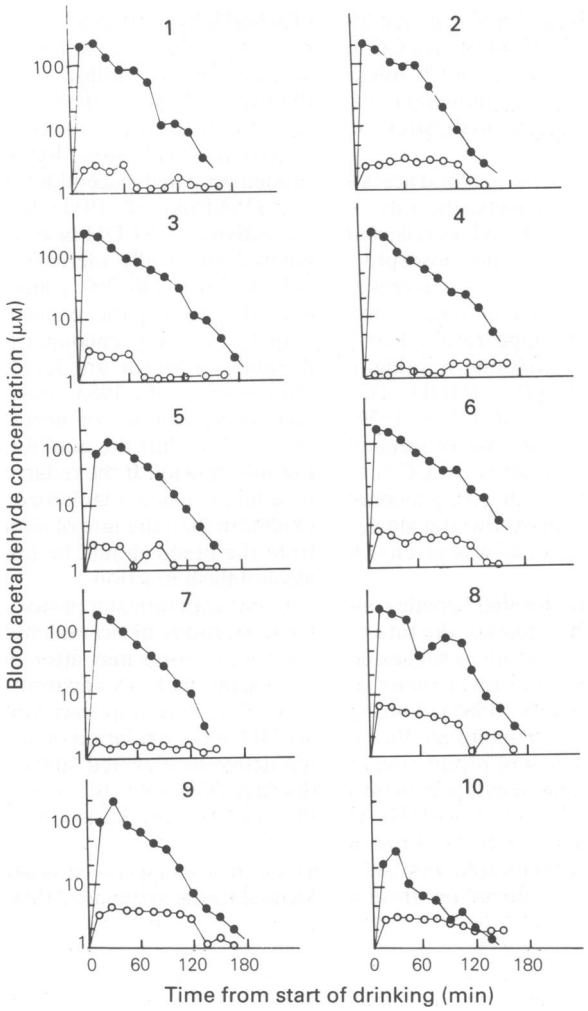

Figure 3 Blood acetaldehyde profiles determined indirectly by analysis of end-expired alveolar air in 10 healthy volunteers. After an overnight fast the subjects drank 0.25 g kg^{-1} ethanol after intake of placebo (control treatment, ○) or calcium carbimide (50 mg, ●) 2 h earlier.

Table 2 Disposition of acetaldehyde in 10 healthy fasting volunteers who received 0.25 g kg^{-1} ethanol 2 h after pretreatment with placebo or calcium carbimide (50 mg); mean ± s.e. mean

Blood acetaldehyde parameter	*Pretreatment*		*Student's t-test*
	Calcium carbimide	*Placebo*	*d.f. = 9*
Peak plasma concentration (μmol l^{-1})	190 ± 20	3.4 ± 0.51	$P < 0.001$
Area under curve (μmol l^{-1} h)	166 ± 19	4.9 ± 0.71	$P < 0.001$

d.f. = degrees of freedom in paired *t*-test.

intense facial flushing after this small dose of ethanol and dramatic increases in breath-acetaldehyde confirms pharmacologically active CC in the body. Different bioavailability of CC might explain, at least in part, the inter-individual differences in peak level of acetaldehyde after the same ethanol dose.

Despite a 40–100 fold increase in blood acetaldehyde seen in individual subjects, the rate of disappearance of ethanol from blood, as reflected in the slope of the pseudolinear postabsorptive phase, was diminished by only 5% on average. The combined effect of a smaller distribution volume and a decrease in slope resulted in a statistically significant retardation of ethanol elimination from the body ($P < 0.001$). This supports the work of Brien *et al.* (1978, 1979) who investigated the effect of dose of ethanol and time of administration relative to CC pretreatment on the efficacy of the drug-alcohol flush reaction. These workers showed a slower elimination of ethanol when CC was given 4 h before drinking.

The mechanism whereby elevated hepatic concentrations of acetaldehyde decrease the rate of ethanol disappearance from blood, as suggested by others, may be linked to the reversibility of the ADH reaction (Cronholm, 1985). During normal biotransformation of ethanol a delicate balance exists between the rate of production of acetaldehyde and its subsequent catabolism into acetate through the action of low K_m ALDH. At a blood-ethanol concentration above 1–2 mmol l^{-1}, the ADH enzymes are saturated with substrate and the metabolism of ethanol occurs at a constant velocity resulting in steady production of acetaldehyde (Lundquist, 1983). Boosting the rate of ethanol oxidation by feeding fructose accentuates the outflow of acetaldehyde from the liver and the levels in peripheral blood also rise (Nuutinen *et al.*, 1984). Inhibition of ADH isoenzymes with 4-methyl pyrazole causes a sudden drop in the acetaldehyde output from the liver (Lindros *et al.*, 1981). Similarly, a change in the activity of ALDH isoenzymes by CC treatment dramatically increases the blood-acetaldehyde level (40–200 μmol l^{-1}). This vastly exceeds the hepatic concentration of acetaldehyde normally encountered during ethanol metabolism which are less than 10 μmol l^{-1} (Nuutinen *et al.*, 1983, 1984). An abnormally high concentration of acetaldehyde might be expected to shift the equilibrium reaction back towards ethanol. If the reduction of acetaldehyde to ethanol has a regulatory role in the ADH oxidation then the rate of elimination of ethanol from the blood should be less during the drug-alcohol flush reaction.

In our experimental protocol, abnormally high concentrations of acetaldehyde were sustained for about 30–60 min after drinking thereafter decreasing with an apparent half-life of 18–31 min. This may implicate other isoenzymes of ALDH in the oxidation of acetaldehyde becoming active as elevated substrate concentrations develop. Alternatively, CC may not totally inhibit the available low K_m isoenzymes.

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