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Title: The Effects of L-Ascorbic Acid, Thiamine HCl, or L-Cysteine on Ethanol and Acetaldehyde Blood Levels and Disposition.

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The effects of L-ascorbic acid, thiamine HCl, or L-cysteine on acetaldehyde blood levels and disposition were investigated in acetaldehyde-treated rats. Rats were treated with ascorbic acid (2 mmoles/kg), thiamine (0.24 mmole/kg), or cysteine (2 mmoles/kg) one hour before the administration of acetaldehyde (6 mmoles/kg). The results show that each of these nutrient factors lowered acetaldehyde blood levels but by different mechanisms. Ascorbic acid and thiamine lowered acetaldehyde blood levels by increasing the apparent volume of distribution. Cysteine lowered the blood levels by direct interaction with acetaldehyde. Ascorbic acid and thiamine increased the half life of acetaldehyde by 300% and 250%, respectively. Cysteine had no significant effect on either the half life or the volume of distribution of acetaldehyde.

Disulfiram (1 mmole/kg/3 days) was used to inhibit the metabolism of acetaldehyde. The effects of ascorbic acid, thiamine, or cysteine on acetaldehyde blood levels and disposition were then investigated in the disulfiram-acetaldehyde-treated rats. Ascorbic acid and thiamine reduced the half life of acetaldehyde by 55% and 40%, respectively.

Both agents increased the total body clearance, and reduced the volume of distribution of acetaldehyde. Acetaldehyde blood levels were lowered by thiamine, but not by ascorbic acid. Cysteine had no significant effect on either the blood levels or the kinetic parameters of acetaldehyde in the disulfiram-acetaldehyde-treated rats.

When ethanol (2 gm/kg) was used as an endogenous source of acetaldehyde, ascorbic acid and thiamine showed no significant effects on either the ethanol or acetaldehyde blood levels. Both agents, however, reduced the total amount of acetaldehyde relative to ethanol. Cysteine increased the blood levels and the half life of acetaldehyde. It reduced the total body clearance, and increased the total amount of acetaldehyde relative to ethanol, an effect opposite to those of ascorbic acid and thiamine.

The effects of ascorbic acid, thiamine or cysteine on the blood levels and disposition of ethanol and acetaldehyde were investigated in disulfiram-ethanol-treated rats. Only ascorbic acid was effective in lowering ethanol and acetaldehyde blood levels. It reduced the half life of ethanol by 20%, and that of acetaldehyde by 24%. Ascorbic acid also increased the total body clearance of ethanol by 27%.

It seems that the protective effects of ascorbic acid, thiamine or cysteine against acetaldehyde-induced mortality, as formerly reported (Moldowan and Acholonu, 1982), may be due to the reduction in acetaldehyde blood levels. Because ascorbic acid and thiamine increased the half life of acetaldehyde (in acetaldehyde-treated rats), their use in chronic acetaldehyde exposure (such as in chronic alcoholism) may lead to acetaldehyde accumulation. Cysteine lowered

acetaldehyde blood levels without affecting the half life or the volume of distribution. It is less likely to lead to acetaldehyde accumulation. However, the beneficial effect of cysteine seems to be limited to exogenously administered acetaldehyde. Because cysteine reduced the clearance and metabolism of acetaldehyde generated from ethanol, its use in chronic alcoholism may not be recommended.

The protective effect of ascorbic acid against disulfiram-ethanol-induced lethality may be due to its ability to lower the ethanol and acetaldehyde blood levels. The reduction in blood levels is coupled with increase in ethanol and acetaldehyde disposition.

The Effects of L-Ascorbic Acid, Thiamine HCl, or L-Cysteine on Ethanol and Acetaldehyde Blood Levels and Disposition.

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INTRODUCTION

Alcoholism and alcohol related illness is among the major medical and public health problems in the world. It has been estimated to affect in excess of ten million persons in the United States alone. The etiology of ethanol-induced illness is not fully understood, but evidence exists (James et al., 1970; Truit and Walsh, 1971; Lieber, 1981) that implicates acetaldehyde (AcH) as a primary mediator in many of the ethanol-induced diseases.

Acetaldehyde is the key intermediary metabolite of ethanol, potentially more toxic than ethanol, and also a toxic component of cigarette smoke (Sprice et al., 1975). The toxic effects of acetaldehyde on the body tissues assume greater significance with the increasing realization that despite its rapid metabolism in vivo, blood levels of AcH may be elevated by both heavy ethanol drinking (Majchrowicz and Mendelson, 1970) and heavy cigarette smoking (Horton and Guerin, 1974; Rylander, 1973; Egle, 1970). The relationship of acetaldehyde to the adverse effects of alcohol and cigarette smoking was stated in the 1970 review of James et al., and is strengthened by the recent reports establishing that heavy drinkers are generally also heavy smokers (Walton, 1972).

In the case of alcoholism, acetaldehyde has been implicated in alcoholic cardiomyopathy (Schreiber et al., 1974; James and Bear, 1968; James and Bear, 1967), in alcohol addiction (Davis and Walsh,

1970), in ethnic sensitivity to alcohol (Ewing and Rouse, 1974), in alcohol cerebellar degeneration (Dreyfus, 1974), in the aging process (Bjorksten, 1971), in disulfiram-ethanol reaction, and in alcoholic fatty liver (Lieber, 1977).

In the case of heavy cigarette smoking, acetaldehyde has been implicated not only in cardiac disease (Schreiber et al., 1974; James and Bear, 1967), but also in pulmonary disease by way of its highly toxic action on cilia of the respiratory tract (Kensler and Battista, 1974), an effect believed to be related to the premalignant state of the tracheo-bronchial tree (Kensler and Battista, 1974; Auerbach et al., 1961; Ballenger, 1960).

Because of the high prevalence of alcoholic fatty liver cirrhosis, and alcoholic cardiomyopathy, this review will be centered on the influence of acetaldehyde on the hepatocyte, cardiovascular system, and on the disulfiram-ethanol reaction.

Influence of Acetaldehyde in the Alcoholic Fatty Liver, Hyperlipemia, and Hyperuricemia Produced by Chronic Ethanol Consumption

About 90 to 98% of the ingested ethanol is metabolized in the liver to yield acetaldehyde (Lieber, 1981); the rest is eliminated by the kidney and lung. All the known pathways of ethanol oxidation in the liver, namely, alcohol dehydrogenase (ADH) pathway, microsomal ethanol oxidizing system (MEOS), and the catalase system result in production of acetaldehyde. It is generally accepted that at least 90% of the acetaldehyde formed from ethanol is in turn oxidized by the liver to acetate or acetyl-CoA. Levels of acetaldehyde after a single

dose of ethanol are usually low, except in the presence of an aldehyde dehydrogenase inhibitor, such as disulfiram or cyanamide (Merchner and Tottmar, 1978).

In alcoholics the blood acetaldehyde levels are higher than in nondrinkers given the same alcohol load (Karsten et al., 1975). The reason for this may be due to a combination of increased acetaldehyde production secondary to the accelerated ethanol metabolism (Van Wae and Lieber, 1977), and reduced acetaldehyde metabolism (Hasumura et al., 1976). The increased acetaldehyde levels in turn enhance several functional disturbances of the mitochondria. These include the following: inhibition of the citric acid cycle (Rubin and Lieber, 1967), beta oxidation of fatty acids and chylomicrons (Lieber et al., 1967; Lieber and Schmid, 1961), and the inhibition of oxidative phosphorylation (Cederbaum et al., 1974). This increase in acetaldehyde blood levels may also impair the functions of various shuttle mechanisms involved in the disposition of reducing equivalents, generated in the cytosol of the hepatocyte (Cederbaum et al., 1974). The inability of the hepatocyte to dispose the reducing equivalents may lead to an increase in NADH/NAD ratio. This is reflected by an increased lactate/pyruvate ratio that could result in hyperlactacidemia (Lieber et al., 1962a; 1962b), due to increased production and decreased utilization of lactate by the liver. Hyperlactacidemia may contribute to acidosis and induces an impairment of the kidney to excrete uric acid, leading to secondary hyperuricemia (Lieber et al., 1962a). The increase in NADH/NAD ratio may also induce an increase in the synthesis of alpha-glycerophosphate (Nikkile & Ojala, 1963), which favors the hepatic triglyceride accumulation in the liver. In

addition to the enhanced synthesis of triglycerides, the inhibition of fatty acid oxidation by acetaldehyde and by ethanol (Lieber et al., 1961; Lieber and Schmid, 1961; Blomstrand et al., 1973) may lead to the accumulation of dietary fat, or fatty acids derived from endogenous synthesis in the absence of dietary fats (Lieber and Spritz, 1966; Matsuzake and Lieber, 1977).

Because of its toxic effects on mitochondrial function, acetaldehyde plays a significant role in the development of alcoholic fatty liver, and hyperuremia produced by chronic ethanol consumption.

Influence of Acetaldehyde in the Development of Alcohol Cardiomyopathy

In addition to damage of liver mitochondria, acetaldehyde has been shown to impair myocardial protein synthesis (Sorrel et al, 1977; Schreiber et al., 1972; Perin and Sessa, 1975). The impairment of protein synthesis is thought to be mediated, in part, by changes in the redox state induced by high acetaldehyde levels (Perin and Sessa, 1975). This effect of acetaldehyde on myocardial protein synthesis may be involved in the genesis of the alcohol cardiomyopathy (Bing and Tillmans, 1977) which is characterized by arrhythmias, increased heart rate, cardiomegaly and changes in ECG.

Of particular interest is the continuing evidence pointing to the release of catacholamines from tissue storage sites by acetaldehyde, at concentrations which arise in man after alcohol intake (Schneider, 1974; Truitt and Walsh, 1971). Acetaldehyde has been shown to produce a dose-related increase in blood pressure and the contraction of the nictitating membrane (an organ containing only alpha-adrenergic

receptors, Walsh, 1969). The positive ionotropic and chronotropic effect of acetaldehyde was first demonstrated by Kumar and Sheth, 1962 and was later supported by Walsh et al., (1969), who also noted that the positive ionotropic and chronotropic effects of AcH were inhibited in the presence of the alpha adrenergic blocker, phentolamine, or when the store of norepinephrine was depleted by reserpine pretreatment. It therefore follows that the cardiovascular effects of acetaldehyde are mediated by the displacement of catecholamines from their storage sites. Acetaldehyde may, thus, be an important contributor to the adverse effects of excessive catecholamine release (Truitt and Walsh, 1971; James et al, 1970) known to occur with heavy drinking of alcohol (Iriji, 1974; James and Bear, 1970) and heavy smoking of cigarettes (Cryer et al., 1976). These adverse effects are related to some of the symptoms observed in alcoholic cardiomyopathy (arrhythmias, increased heart rate, cardiomegaly and changes in ECG). Acetaldehyde may therefore contribute to the development of alcohol cardiomyopathy through induction of catacholamine release and impairment of myocardial protein synthesis.

Acetaldehyde as a Mediator in Disulfiram-Ethanol-Reaction

Disulfiram is a drug used as an adjunct in the treatment of chronic alcoholism. Its therapeutic benefit is based on the alcoholic's fear of the unpleasant reaction that results, known as disulfiram-ethanol reaction (DER), after ethanol ingestion (for review see Haley, 1979; Kwentus and Major, 1979).

Although the mechanism underlying the DER is not fully understood, it is believed that the main factor leading to the reaction is the increased acetaldehyde levels in the blood, caused by inhibition of acetaldehyde dehydrogenase. In addition, a decreased neuronal content of norepinephrine, caused by inhibition of dopamine beta hydroxylase by disulfiram, may contribute to the symptoms of the DER (Kitson, 1977; Truitt and Walsh, 1971). The increased acetaldehyde levels lead to the disulfiram-ethanol reaction, characterized by blurred vision, hot, flushed and scarlet face, palpitation, and tachycardia. Hypotension observed in the DER is thought to be caused by the decreased norepinephrine store (Faiman, 1979). The symptoms of DER range from minor to severe reaction. Several deaths as a result of the disulfiram-ethanol reaction have been reported (Cahill, 1972; Fernandez, 1972).

Other drugs reported to induce a disulfiram-ethanol-like reaction are; calcium cyanamide (Collins and Brown, 1960; Ferguson, 1954), chlorpropamide, tolbutamide, phenylbutazone, metronidazol, pyrogallol, Caprinus atramenterias (edible mushroom) and animal charcoal. These drugs interact with ethanol to induce an increase in acetaldehyde blood levels. The interaction between these drugs and ethanol has been reviewed by Truitt and Walsh, (1973).

Acetaldehyde Condensation with Biogenic Amine

Acetaldehyde can also condense with some biogenic amines to give rise to compounds that are implicated in alcohol addiction (Cohen and Collins, 1970; Myers and Melchier, 1977). Among these compounds are

the tetrahydroisoquinolines and specifically, tetrahydropapaveroline. Tetrahydropapareroline (THP) is a benzyltetrahydroisoquinoline alkaloid, and a requisite intermediate in the biosynthesis of morphine (Davis and Walsh, 1970). This alkaloid (THP) has been isolated from brain and liver homogenates, the perfused adrenal gland, and from the urine of Parkinson's patients receiving L-dopa therapy (Kricka and Clark, 1979).

Dopamine has been shown to condense with its aldehyde to form tetrahydropapavenoline and to condense with acetaldehyde to form salsoline (Kricka and Clark, 1979). Both alkaloids have been implicated in alcohol addiction (Myers and Melchiar, 1977).

One can see that the toxic effects of acetaldehyde are varied. In the liver, it induces a metabolic derangement of mitochondrial function, and contributes to the pathogenesis of the alcoholic fatty liver. Acetaldehyde impairs the function of the cilia of the respiratory tract, leading to a premalignant state of the tracheobronchial tree. In the cardiovascular system, acetaldehyde inhibits protein synthesis and displaces norepinephrine from storage sites, leading to the development of alcoholic cardiomyopathy. Acetaldehyde condenses with biogenic amines to produce alkaloids that are implicated in the development of addiction.

Acetaldehyde is therefore a toxicant in its own right. Because it is produced by ethanol and cigarette smoke, it may contribute to the complication of chronic alcoholism and chronic cigarette smoking. Therefore, methods to control acetaldehyde blood or tissue levels may be useful against ethanol induced toxicity. These methods may also be

useful in the study of the mechanism of toxic actions of acetaldehyde and ethanol.

In previous work (Moldowan and Acholonu, 1982) it was found that ascorbic acid or thiamine lowered acetaldehyde or disulfiram-ethanol-induced lethality in mice. Sprince et al. (1975), had previously reported that ascorbic acid or thiamine decreased acetaldehyde-induced mortality in rats. The mechanism of protection of ascorbic acid, thiamine or cysteine against acetaldehyde-induced toxicity and lethality is not known.

It seems very likely that the protection by L-ascorbic acid, thiamine, or cysteine against acetaldehyde toxicity and lethality may be due to a reduction of acetaldehyde concentration at the reactive site. The main objective of this research was to investigate whether ascorbic acid, thiamine, or cysteine can lower acetaldehyde blood levels or acetaldehyde disposition (metabolism, distribution, or elimination) in rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 7-8 weeks old, weighing 230 to 330 grams were used. The rats were feed a commercial diet (Oregon State University Rodent feed), and given free access to tap water. They were housed in cages in groups of five at a room temperature of 22°C. The room was on a 12 hour light/dark cycle.

Drug and Reagent Sources

L-ascorbic acid was purchased from Baker Chemicals Corporation (Phillipsburg, N.J.). Thiamine-HCl was obtained from Sigma Chemical Company. L-cysteine free base was obtained from Nutritional Biochemical Corporation. Disulfiram was from Aldrich Chemical Company Inc. Gas chromatographic packing (15% Carbowax 20 M, on Celite 80/100) was purchased from Applied Science Lab. Acetaldehyde was obtained from Mallinckrodt. All other chemicals used were analytical grade.

Experimental Design

Four sets of experiments were performed to determine the acute effects of the vitamins (ascorbic acid and thiamine) and the amino acid (cysteine) on the disposition of ethanol and its metabolite, acetaldehyde. In each case, ascorbic acid was administered as a 14.10% solution of normal saline at a dose of 2 mmoles/kg (0.356 g/kg body weight). The dose of thiamine was 0.24 mmole/kg body weight (82.80 mg/kg), given as a 7% solution of normal saline, while that of

cysteine was 2 mmoles/kg body weight (0.242 g/kg), given as a 10% solution of normal saline. All chemicals and drugs were administered intraperitoneally in a volume range of 0.12 to 3.5 mls.

The first experiment, investigated the effects of ascorbic acid, thiamine or cysteine on the exogenously administered acetaldehyde. Groups of rats were first treated with either ascorbic acid, thiamine, cysteine, or normal saline. One hour later, acetaldehyde (6 mmoles/kg body weight) was given. Serial blood samples were collected from each rat at 5 , 10, 15 30, 60, 90, 120, 150, 180, 240 and 300 minutes post acetaldehyde treatment. These samples were immediately transferred to ice-cold polycarbonate tubes containing propanol and thiourea (see below). The blood acetaldehyde was extracted, then quantitated by the head-space technique describe above.

The second series of experiments were carried out to investigate whether disulfiram interfered with the effects of ascorbic acid, thiamine or cysteine on acetaldehyde blood levels. In this study, disulfiram was administered in a dose of 1 mmoles/kg body weight (308 mg/kg), IP, for three consecutive days. Two hours after the last dose, ascorbic acid, thiamine or cysteine was administered. This was followed by the administration of acetaldehyde, 6 mmoles/kg body weight, one hour later. Blood samples were collected, and acetaldehyde was extracted and analyzed by the GLC head-space technique. The control rats were treated with normal saline in place of the nutrient factors.

The third series of experiments were performed to elucidate the influence of ascorbic acid, thiamine or cysteine on the blood levels and disposition of endogenously generated (from ethanol) acetaldehyde.

Rats were treated with either ascorbic acid, thiamine or cysteine (IP), one hour before the administration of ethanol. The dose of ethanol was 2 g/kg body wt, given as a 20% (w/v) solution of normal saline. The control rats were treated with normal saline one hour before ethanol administration. Blood samples were collected from the tail tips of the rats at various time intervals (30, 60, 90, 120, 150, 180, 210, 240, and 300 minutes) post ethanol treatment. These samples were immediately transferred to ice-cold polycarbonate tubes containing propanol and thiourea. Blood ethanol and acetaldehyde were extracted, then quantitated by the head-space technique described previously.

Disulfiram was employed in the fourth set of experiments to increase the blood acetaldehyde levels after ethanol administration. The influence of ascorbic acid, thiamine or cysteine was then investigated on the elevated acetaldehyde and ethanol blood levels. The disulfiram suspension was administered to the rats at a dose of 0.5 mm/kg (154 mg/kg body weight), IP, for two consecutive days. Twenty four hours after the last dose of disulfiram, ethanol (2g/kg) was administered. One hour before the administration of ethanol, ascorbic acid, thiamine, cysteine or normal saline was given. Serial blood samples were collected at 1, 3, 5, 7, 8, 9 and 10 hours after ethanol administration. Blood ethanol and acetaldehyde were extracted and quantitated as described above.

The last experiment investigated the ability of ascorbic acid, thiamine and cysteine to reduce acetaldehyde concentration at a physiological pH of 7.4 in vitro. Four polycarbonate tubes were assembled in an ice bath. To each tube was added 1-propanol solution

(0.25 ml of 0.76 g/L). A 72 mM concentration of either ascorbic acid, thiamine, or cysteine was added to each of the tubes. Normal saline was added to the control tube. Acetaldehyde (1 ml of 30 mg/L) was added to each of the four tubes. Enough phosphate buffer solution, pH 7.4, was then added to each of the tubes to make a total volume of 11.03 ml. (1.5 ml of the solution was pipetted and transferred to a serum bottle, sealed with a rubber septum, and equilibrated for 20 minutes in a shaking water bath at 37°C. The bottles were then equilibrated in a separate water bath at 60°C for 10-15 minutes. A 1 ml sample of vapor above the mixture in the serum bottle was drawn with a gas-tight syringe. This sample, containing free acetaldehyde and propanol (the internal standard), was injected into the GLC for quantitation as described previously. The peak height ratios of acetaldehyde/propanol in the ascorbic acid-, thiamine-, or cysteine-containing solutions were compared to that of the saline control. The result obtained is as shown in Figure 11.

Extraction Process for Ethanol and Acetaldehyde from Blood Samples

Acetaldehyde and/or ethanol were extracted from blood, then analyzed by using the GLC-head-space techniques described by Brien and Loomis (1978). 100 to 200 microliters of blood was collected from each rat at various time intervals (from 5 to 60 minute intervals). Immediately after collection, the blood sample was added to ice-cold polycarbonate tubes (50 ml tubes), containing 1-propanol (50 microliters of a 0.076% w/v solution) and thiourea (20 microliters of 7.6% w/v solution). 1-propanol was used as the internal standard, while

thiourea was used to inhibit non-enzymatic acetaldehyde formation (Erickson, 1980). The blood was immediately hemolyzed with 1.87 mls of water, and blood protein precipited with 64 microliters of concentrated perchloric acid. The mixture was then centrifuged at 4500 RPM for 10 minutes at 4°C. One and one-half (1.5) ml of the supernatant was pipetted to a serum bottle, sealed, and incubated in a water bath for 10 to 25 minutes at 70°C. After incubation, one ml of the vapor above the mixture in the serum bottle (head-space volume) was drawn with an gas-tight syringe. This sample, containing acetaldehyde, ethanol and propanol, was injected into the GLC for quantitation.

Instrumentation

Sample analysis for ethanol and acetaldehyde was conducted on a Varian, Series 3700 GLC, equipped with a flame ionization detector and a 10 mv potentiometric recorder. A glass column, packed with 15% carbowax 20 M, on celite 80/100, was used. The operating temperature conditions were: injection port, 160°C, detector, 170°C, and column, 75°C. The gas flow rates were: hydrogen, 33.5 ml/minute (40 psi inlet pressure); air, 300 ml/minute (60 psi inlet pressure) and nitrogen, 33.5 ml/minute (60 psi inlet pressure). The detector sensitivity was as follows: range, 10^{-12}, attenuation, from x2 to x32 depending on the sample. The mean retention times for acetaldehyde, ethanol, and propanol were 0.51 ± 0.09, 1.04 ± 0.12, and 2.09 ± 0.15 minutes, respectively.

Ethanol and Acetaldehyde Quantitation

Aqueous Standard Curves

Standard curves for ethanol and acetaldehyde were prepared for the quantitation of blood ethanol and acetaldehyde concentrations. Six aqueous standard solutions were prepared containing 60, 100, 200, 400, 800 and 1600 mg/l of ethanol, and 2.5, 5, 10, 20, 30 and 40 mg/l of acetaldehyde.

Seven polycarbonate tubes labeled from 0 to 6 were assembled in an ice bath. The following were added to each of these tubes:

1. 1-propanol (internal standard): 50 ul of 0.075% w/v
2. thiourea: 20 ul of 7.6% w/v
3. percholonic acid (PCA) = 64 ul of conc. acid.

200 ul of each standard solution of ethanol or acetaldehyde was then added to separate tubes. For example, 200 ul of 60 mg/l standard solution was added to the tube numbered one, and 200 ul of 100 mg/l standard solution added to tube number two, etc. Tube zero served as a control, and to it was added 200 ul of distilled water. Enough distilled water was then added to each of the tubes to make a total volume of 2.21 ml.

One and one half ml of the mixed solutions was pipetted to a serum bottle. This was sealed with a rubber septum and incubated in a water bath from 15 to 25 minutes at a temperature of 70°C. After incubation, a one milliter sample of air-vapor above the mixture in the serum bottle (head-space volume) was drawn with a gas-tight syringe. This sample, which contained acetaldehyde and propanol, or ethanol, AcH and propanol, was injected into the GLC for quantitation.

From the resulting chromatogram, the peak-height ratios of ethanol/1-propanol or acetaldehyde/1-propanol of each of the standard solutions was calculated. These were then plotted against their respective concentrations.

The curve generated (aqueous standard curve) from the plot was subjected to a linear-regression analysis to test for linearity. The results showed that the ethanol and acetaldehyde aqueous standard curves were linear within the concentration range used. The correlation coefficient was 0.995 ± 0.003 (SE), and 0.993 ± 0.005 (SE) for ethanol and acetaldehyde, respectively.

Blood Standard Curves

In order to normalize the variations that could arise from the blood, standard curves were prepared by using freshly collected rat blood. The procedure used is exactly the same as described above, except that 200 ul of blood collected from the control (untreated) rat was added to the polycarbonate tubes containing 1-propanol (50 ul of 0.075% w/v) and thiourea (20 ul of 7.6% w/v). Following the addition of blood, 200 ul of the ethanol or acetaldehyde standard solution was added to each separate tube. Immediately after this, the blood was hemolyzed by the addition of enough cold distilled water to make a total volume of 2.15 ml. The blood protein was then precipitated out by the addition of 64 ul of concentrated perchloric acid (PCA). This mixture was centrifuged at 4500 RPM for 10 minutes at 4°C. One and a half (1.5) mls of the supernatant was pipetted into a serum bottle, sealed and incubated in a water bath for 10 to 25 minutes at 70°C. After incubation, a one milliliter head-space-volume was drawn with a gas-tight syringe for the GLC analysis.

From the resulting chromatogram, the peak-height ratios of ethanol/1-propanol or acetaldehyde/1-propanol of each standard solution were calculated. These were plotted against their respective concentrations. The curves generated (blood standard curves) from the plot were subjected to a linear-regression analysis. The results showed that the blood ethanol and acetaldehyde standard curves were linear within the concentration range used. The correlation coefficient was 0.987 ± 0.015 (SE) and 0.990 ± 0.005 for ethanol and acetaldehyde, respectively.

There was a significant difference between the blood standard curves and aqueous standard curves when performed simultaneously. The differences were not consistent, but fluctuated between higher and lower peak height ratios for each of the standard solutions. In order to normalize these biologic variations, blood standard curves, rather than the aqueous standard curves, were used in these experiments for the quantitation of blood ethanol and acetaldehyde concentrations.

There was less day to day variation in the standard curves of ethanol than there was for acetaldehyde. The coefficient of variation [CV = (SD/mean)(100)] for ethanol was 8%, while that for acetaldehyde was 20%. Because of these large day to day variation, standard curves of AcH and ETOH were performed for each experiment.

Analysis of Unknown Sample

Blood acetaldehyde and ethanol in the treated and control (untreated) rat were extracted, then analyzed using the GLC (head-space techniques described above). The peak height ratios of ethanol/

1-propanol or AcH/1-propanol of the unknown samples were calculated. The blood ethanol or blood acetaldehyde concentration was obtained by extrapolating the resultant peak-height ratios to their respective concentration on the standard curve.

Preparation of Solutions

Ethanol Standard Solutions

A stock solution of ethanol (3 g/L) was prepared from 95% ethanol. This was done by diluting 3.88 ml of 95% ethanol to 1000 ml with distilled water. (Density of ethanol equals 0.816). The other ethanol standard solutions (100 mg/L, 200 mg/L, 400 mg/L, 800 mg/L and 1600 mg/L) were prepared directly from the stock solution. For example, 100 mg/L standard solution was prepared by measuring 1.67 ml of 3 g/L (0.3% w/v) ethanol and then adding enough distilled water make 50 ml. The 200 mg/L standard solution was prepared by measuring (1.67 ml x 2) mls of the stock solution (0.3% w/v ethanol) and adding enough distilled water to make 50 ml solution, and so on.

Acetaldehyde Standard Solutions

Acetaldehyde solutions were prepared in the cold room at 1°C, and kept cold in the ice-bath until they were used.

A stock solution of acetaldehyde (400 mg/L or 0.04 % w/v) was first prepared by measuring 507 ul of pure acetaldehyde and adding enough cold distilled water to make 1000 ml. (Density of acetaldehyde is 0.788 g/ml). All the glassware and pipettes were kept cool in the cold room before they were used. The acetaldehyde standard solutions

(40 mg/L, 20 mg/L, 10 mg/L, 5 mg/L and 2.5 mg/L) were prepared from the stock solutions. For example, 40 mg/L was prepared by measuring 20 ml of the 0.04% w/v acetaldehyde and adding enough cold distilled water to make 200 ml. The rest of the standards were then prepared either from the 40 mg/L or the 400 mg/L stocks.

Preparation of Disulfiram

Disulfiram (10% w/v) was prepared by suspending it in a mixture of propylene glycol and water (1:5). This suspension was achieved by trituration of the disulfiram granules in a glass mortar followed by the addition of propylene glycol, a little at a time. Water was then added in fractions, while trituration in the mortar continued. After the addition of water, the mixture was further triturated for another 5 to 10 minutes before use.

Pharmacokinetic Methods

Ethanol

The blood concentrations and other pharmacokinetic parameters for ethanol or acetaldehyde were evaluated for each rat. The parameters for ethanol and its metabolite, acetaldehyde, were determined by using the one compartment open pharmacokinetic model with zero-order elimination process (Ritchie, 1980). The elimination rate constant (K) was determined by taking the slope of the terminal phase of the concentration time profiles. The half life ($t_{\frac{1}{2}}$) = C/2K, where C is the concentration of ethanol or acetaldehyde at time zero, obtained by the backward extrapolation of the terminal limb of the concentration-time plot to the ordinate. The area under the concentration time curve

($AUC^{0\rightarrow\infty}$) of ethanol or acetaldehyde from zero to the last sample time was determined by the trapezoid rule. The $AUC^{T-\infty}$ from the last sample time to infinity was determined from the equation $AUC^{T-\infty} = C^2/2K$, where C^2 is the square of the concentration at the last sample time. The total body clearance was determined from this equation.

$$CL_{Total} = Dose/AUC^{0-\infty}$$

Acetaldehyde

The kinetic parameters for acetaldehyde, when administered directly to the rats (P), were determined using the two compartment open pharmacokinetic model with a first order elimination process. Thus, $t_{\frac{1}{2}} = 0.693/Beta$ where Beta is the slope of the terminal limb of the concentration-time curve (Ritschel, 1980). The area under the curve (AUC) was determined by the trapezoid rule to the point of last sample time, then from the last sample time to infinity, the equation $AUC^{T-\infty} = Cx/\beta$ was used, where Cx is the concentration of acetaldehyde at the last sample time. The Beta phase volume of distribution (Vd) equals $Dose/AUC^{0-\infty} \times \beta$. Total body clearance ($Cl_{total}$) of acetaldehyde was calculated by dividing the dose of acetaldehyde by the area under the curve. All the pharmacokinetic parameters were calculated by the procedure given by Ritschel (1980).

Statistical Analysis

All the data generated were subjected to statistical evaluation using the student's t-test, where feasible. The non-parametric Mann-Whitney test was used when it was not possible to assume that the samples were derived from a normal population (Snedecor & Cochran, 1980).

RESULTS

Blood Levels and Disposition of Exogenous Acetaldehyde:

The effects of ascorbic acid, thiamine, or cysteine on exogenous acetaldehyde blood levels and disposition (as measured by the half life, ($t_{\frac{1}{2}}$), Beta phase volume of distribution (Vd_b), or the total body clearance (Cl_{total}) were investigated. These agents have been reported to protect animals against the lethal effects of acetaldehyde (Moldowan and Acholonu, 1982; Sprince et al., 1975), but their mechanism of protection remained unknown. This study was done to investigate the mechanism of protection by ascorbic acid, thiamine, or cysteine against the lethal effects of acetaldehyde. It was designed to elucidate the influence of these nutrient factors on acetaldehyde blood levels and other kinetic parameters (as shown in Table 1).

After the administration of acetaldehyde (6 mmoles/kg) to rats, the resultant concentration-time plot of acetaldehyde showed a bi-phasic elimination process (Fig. 1). The first phase was a rapid distributive phase followed by a much slower elimination phase. This is typical of a two compartment open pharmacokinetic model with first order elimination process (Klassen, 1980). The result obtained from the kinetic evaluation of the acetaldehyde concentration-time profile is shown in Table 1.

L-Ascorbic Acid

Figure 1a shows the effects of ascorbic acid on acetaldehyde blood concentration. Essentially, the administration of ascorbic acid

Figure 1a Effect of L-ascorbic acid on acetaldehyde blood levels in acetaldehyde-treated rats.

Acetaldehyde (6 mmoles/kg) was given to the rats one hour after the administration of ascorbic acid (2 mmoles/kg). All the agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of 10 rats for the control and 9 for ascorbic acid treatments.

* Significantly different from control ($p < 0.05$).
** Significantly different from control ($p < 0.01$).
(Student's T-test)

Figure 1b Effect of thiamine HCl on acetaldehyde blood levels in acetaldehyde-treated rats.

Acetaldehyde (6 mmoles/kg) was given to the rats one hour after the administration of thiamine (0.24 mmole/kg). All the agents were administered by intraperitoneal injection. Each point represents the mean (± standard error) of 10 rats in the control and 9 in thiamine treatments.

* Significantly different from control ($p < 0.05$).
** Significantly different from control ($p < 0.01$).
(Student's T-test)

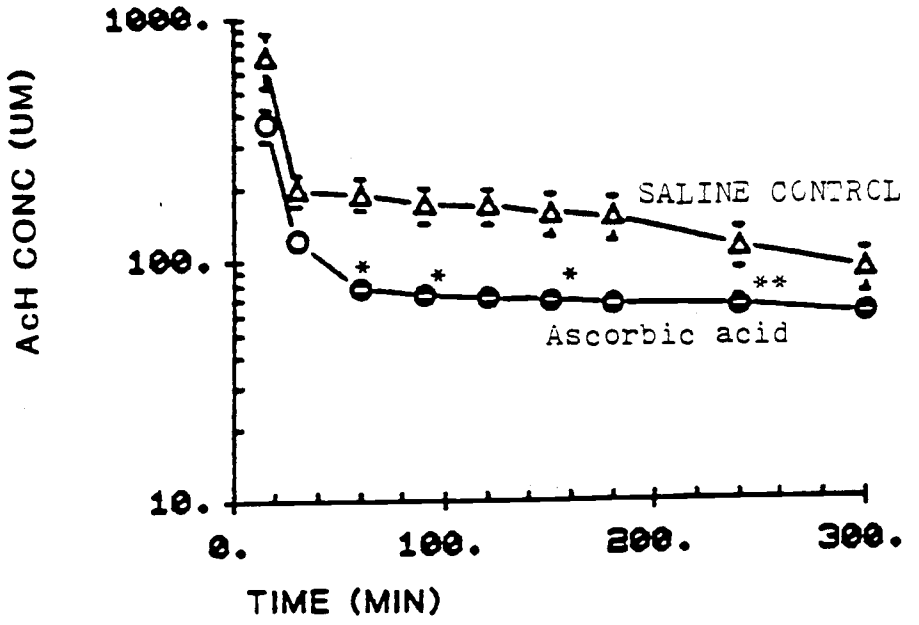
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Fig. 1a

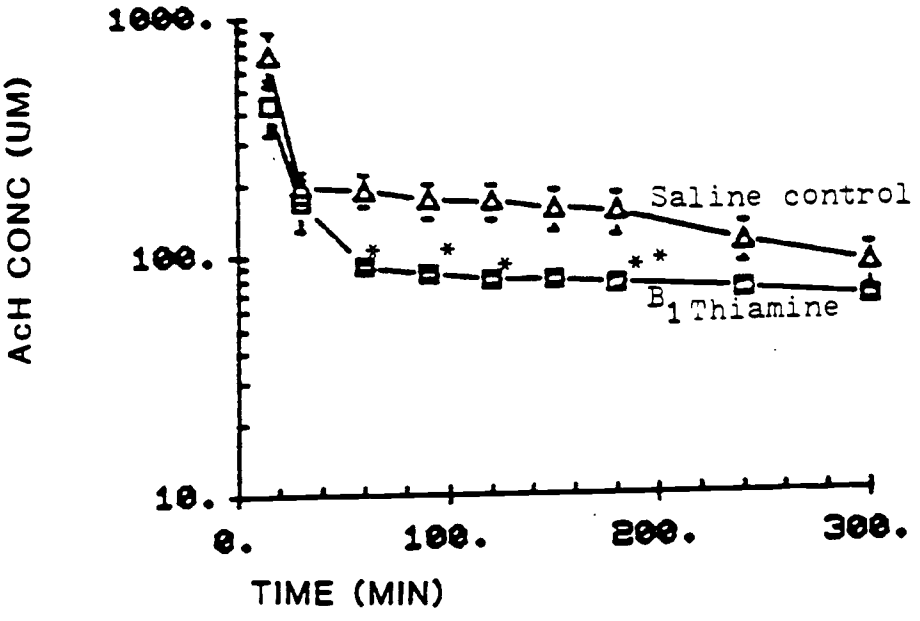
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Fig. 1b

Table 1 The effects of L-ascorbic acid, thiamine HCl, or cysteine on the pharmacokinetic profile of acetaldehyde in rats.

	Animal Treatments			
Parameters	Saline	Ascorbic Acid	Thiamine	Cysteine
N	10	9	9	5
Beta (hr^{-1})	0.227 ± 0.03	0.087±0.023**	0.077±0.013**	0.174 ± 0.036
$T_{\frac{1}{2}}$(hrs)	3.67 ± 0.51	14.64±4.29***	12.94±2.44***	4.375 ± 0.73
AUC mcg.hr/ml	60.00 ± 9.00	76.00 ± 14.00	81.00 ± 13.00	41.00 ± 4.00
Vd_B(L/Kg)	31.14 ± 7.13	65.24 ± 6.45*	59.19 ± 4.07*	40.64 ± 3.87
CL_{total} (ml/min/Kg)	105.53 ± 28.6	81.51 ± 18.47	68.86 ± 12.24	110.77 ± 9.00

Rats were treated with acetaldehyde (6 mmoles/kg) one hour before the administration of ascorbic acid, thiamine, or cysteine. All the agents were administered by intraperetoneal injection. The data represent the mean ± standard error.

* Significantly different from the control as determined by student t-test ($p < 0.025$).
** Significiantly different from the control as determined by Mann-Whitney test ($p < 0.01$).
*** Significantly different from control as determined by students t-test ($p < 0.005$).

Beta = Slope of the terminal phase of the concentration-time profile.
$T_{\frac{1}{2}}$ = Half life of acetaldehyde
AUC = Area under the concentration-time curve from zero to infinity.
Vd_B = Beta phase volume of distribution of acetaldehyde
Cl_{total} = Total body clearance of acetaldehyde
ACH = Acetaldehyde
NS = Normal saline (control)

(2 mmoles/kg) prior to acetaldehyde, caused a decrease in the acetaldehyde blood levels. The reduction of acetaldehyde blood levels by ascorbic acid was significant ($p < 0.025$) at 60 minutes post treatment through to 240 minutes. At this time interval, the reduction in acetaldehyde blood levels was 50%. After 240 minutes post treatment, the reduction in blood level decreased from 50% to 37%.

The influence of ascorbic acid on the kinetic parameters of acetaldehyde is shown in Table 1. Ascorbic acid significantly ($P < 0.005$) increased the half life ($t_{\frac{1}{2}}$) of acetaldehyde by about 300%, and the volume of distribution by about 100% ($p < 0.025$). The Area Under the Curve (AUC) and the total body clearance (CL_{total}) of acetaldehyde were not significantly altered by ascorbic acid.

Thiamine-HCL:

The influence of thiamine on the mean acetaldehyde blood concentration is shown in Figure 1B. Thiamine (0.24 mmole/kg) administered one hour before acetaldehyde resulted in a significant ($p < 0.025$) decrease in acetaldehyde blood levels: about 50% of the control at 60 minutes through to 180 minutes post treatment. At 300 minutes post treatment, the reduction in acetaldehyde blood levels decreased from 50% to 30% as compared to the saline control.

The kinetic parameters for acetaldehyde following thiamine treatment are shown in Table 1. Thiamine significantly ($P < 0.005$) increased the half life ($t_{\frac{1}{2}}$) of acetaldehyde by 250%, and the apparent volume of distribution (Vd_B) by 90% ($P < 0.025$). The total body clearance (CL_{total}) of acetaldehyde was lowered by thiamine from

105.53 ± 28.60 (SE to 68.86 ± 12.24 ml/min/kg. The Area Under the Curve (AUC) was not significantly altered.

L-Cysteine

The effect of cysteine on the mean blood concentration of acetaldehyde is shown in Figure 2. Cysteine (2mmoles/kg), as with ascorbic acid and thiamine, lowered the mean blood levels of acetaldehyde. The reduction in acetaldehyde blood levels by cysteine was significant ($P < 0.025$) from 60 minutes post treatment to 200 minutes post treatment.

The kinetic parameters of acetaldehyde after cysteine treatment are shown in Table 1. Cysteine lowered the blood levels of acetaldehyde, but showed no significant effect on any of the kinetic parameters as compared to the control.

Exogenous Acetaldehyde and Disulfiram

The effects of ascorbic acid, thiamine, or cysteine on acetaldehyde blood levels after disulfiram-acetaldehyde-treatment were investigated. Disulfiram, a known metabolic inhibitor of acetaldehyde dehydrogenase, was used in these experiments to reduce acetaldehyde metabolism. This drug was also employed to see if it had any effect on the activity of ascorbic acid, thiamine, or cysteine toward acetaldehyde blood levels or disposition. These experiments were necessary because earlier work (Moldowan and Acholonu, 1982) indicated that disulfiram could influence the protective effect of these nutrient factors in acetaldehyde-treated animals.

Figure 2 Effect of L-cysteine on acetaldehyde blood levels in acetaldehyde-treated rats.

Acetaldehyde (6 mmoles/kg) was administered to the rats one hour after the administration of cysteine (2 mmoles/kg). All the agents were administered by intraperitoneal injection. Each point represents the mean (± standard error) of 10 rats for the control and 5 rats for cysteine treatments.

* Significantly different from the control ($p < 0.05$). (Mann Whitney Test)

Figure 3 Effect of L-ascorbic acid on acetaldehyde blood levels in disulfiram-acetaldehyde-treated rats.

Disulfiram (1 mmole/kg) was administered once a day for three days. Ascorbic acid (2 mmoles/kg) was administered two hours after the last disulfiram dose and one hour before the administration of acetaldehyde (6 mmoles/kg). All the agents were given by intraperitoneal injection. Each point represents the mean of five rats for the control and four for ascorbic acid treatments.

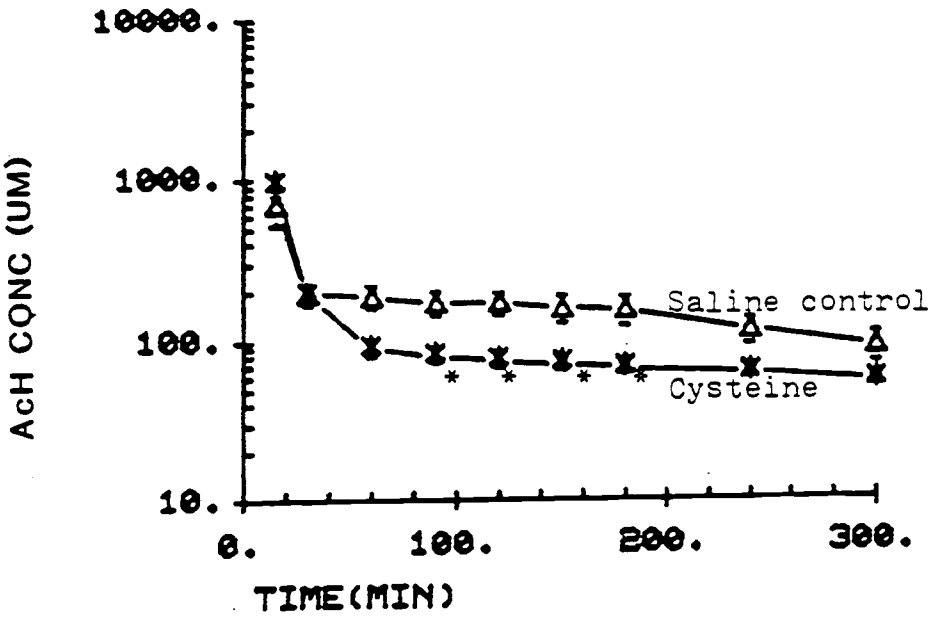
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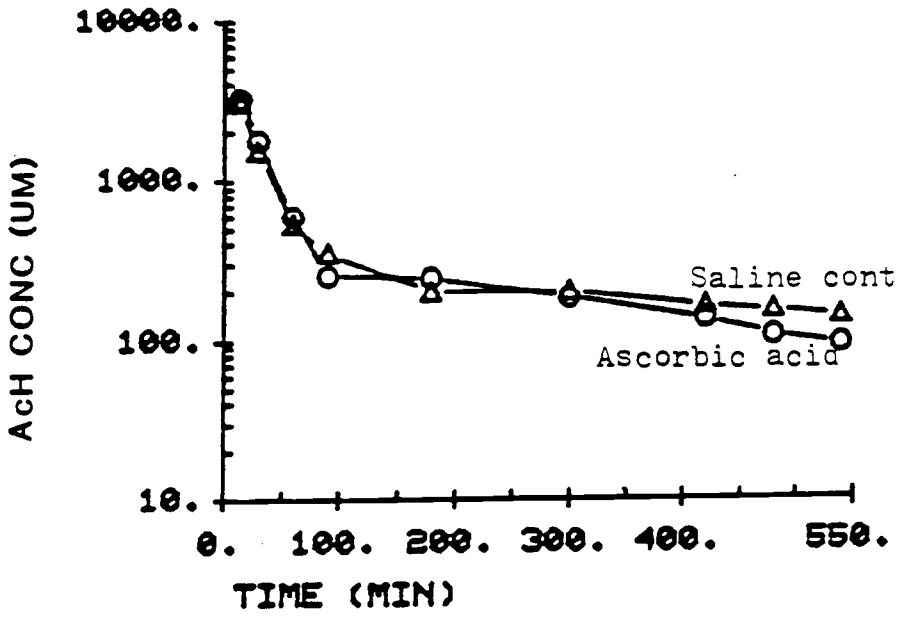
Fig. 2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TIME (MIN)

Fig. 3

Effects of Disulfiram on Exogenous Acetaldehyd Blood Levels and Disposition

Pre-treatment of rats with disulfiram (1 mmole/kg) for three days before the administration of acetaldehyde (6 mmoles/kg) resulted in a significant ($p < 0.015$) increase in acetaldehyde blood levels. Fifteen minutes post acetaldehyde treatment (Table 2), the blood levels of acetaldehyde in disulfiram treated rats were over four times those of control (non-disulfiram treated) levels. At 90 minutes post acetaldehyde treatment, the acetaldehyde blood level was increased ($P< 0.01$) by 101.3%. After nine hours, the blood acetaldehyde levels in disulfiram-treated rats still remained high. No acetaldehyde could be detected in the blood of the controls after five hours of treatment.

The effects of disulfiram on the kinetic parameters of acetaldehyde are shown in Table 3. Disulfiram (1 mmole/kg) significantly ($P< 0.01$) increased the half life ($t_{\frac{1}{2}}$) of acetaldehyde by 142% and the Area Under the Curve (AUC) by 258% ($p < 0.01$). Both the volume of distribution (Vd_B) and the total body clearance (Cl_{total}) of acetaldehyde were significantly ($p < 0.01$) reduced by the high disulfiram dose (1 mmole/kg x 3 doses). The reduction in the volume of distribution was by 47% of the control, while that of the body clearance was by 79%. The lower disulfiram dose (0.5 mmole/kg x 2 doses) increased ($P< 0.01$) the half life ($t_{\frac{1}{2}}$) of acetaldehyde by 102%. This lower dose also reduced ($p < 0.05$) the total body clearance (Cl_{total}) of acetaldehyde by 38% but, had no significant effect on the Area Under the Curve (AUC) and the volume of distribution (Vd_B).

Table 2 Effects of disulfiram on acetaldehyde blood levels in acetaldehyde-treated rats.

Time (mins)	Control	Disulfiram
15	702.30 ± 182.05	3028.30 ± 578.50*
30	198.10 ± 29.13	1536.00 ± 216.60*
60	190.25 ± 29.25	536.02 ± 99.14*
90	172.60 ± 28.10	347.40 ± 91.03*
120	170.38 ± 28.10	-- --
150	157.72 ± 30.13	-- --
180	151.80 ± 31.48	203.20 ± 46.90*
240	112.83 ± 21.45	-- --
300	94.60 ± 18.20	199.80 ± 46.23*
420	-- --	165.20 ± 36.18
480	-- --	151.60 ± 32.79
540	-- --	141.18 ± 31.13

Disulfiram (1 mmole/kg) was administered once a day for three days. Acetaldehyde (6 mmoles/kg) was administered two hours after the last dose of disulfiram. The data represent the mean ± standard error of 10 rats in the control and 5 in the disulfiram treatments.

* Significantly different from the control ($p<0.01$) Mann-Whitney test

-- Levels not determined

Table 3 The effects of disulfiram on the pharmacokinetic properties of acetaldehyde in disulfiram-acetaldehyde-treated rats.

Parameter	Control	Disulfiram[a]	Disulfiram[b]
N	10	5	5
$B(hr^{-1})$	0.227 ± 0.03	0.086 ± 0.018*	0.122 ± 0.036*
$t_{\frac{1}{2}}$(hrs)	3.67 ± 0.51	8.88 ± 1.43*	7.41 ± 1.67*
AUC (mcg.hr/ml)	60.00 ± 9.00	215.60 ± 28.00*	65.86 ± 6.31
Vd_{Beta} (L/Kg)	31.14 ± 7.13	16.54 ± 2.92*	42.15 ± 10.42
CL_{total} (mL/min/Kg)	105.53 ± 28.60	21.69 ± 2.53*	65.44 ± 8.04*

a. Rats were treated with disulfiram, 1 mmoles/kg for three days. Acetaldehyde (6 mmoles/kg) was administered two hours after the last disulfiram dose.
b. Rats were treated with disulfiram (0.5 mmole/kg) for two days. Acetaldehyde (6 mmoles/kg) was given 24 hours after the last disulfiram dose.

Disulfiram, acetaldehyde and saline were administered by intraperitoneal injection. The data represent the mean ± standard error of 10 rats in the control and 5 in disulfiram treatments.

*Significantly different from the control ($p < 0.01$). (student's t-test).
The kinetic symbols are defined in Table 1.

L-Ascorbic Acid

The effect of ascorbic acid on the mean blood concentration of acetaldehyde in disulfiram-treated rats is shown in Figure 3. Ascorbic acid showed no significant effect on acetaldehyde blood levels in the disulfiram-acetaldehyde-treated rats. However, it increased the metabolism of acetaldehyde as evidenced by the significant ($p < 0.01$) reduction of the half life ($t_{\frac{1}{2}}$) (Table 4). Ascorbic acid (2 mmoles/kg) significantly reduced the $t_{\frac{1}{2}}$ of acetaldehyde to a value similar to that of non-disulfiram-treatment (Tables 1 and 4). The $t_{\frac{1}{2}}$ of acetaldehyde was 3.67 ± 0.51 (SE) hours before disulfiram treatment, and was 8.88 ± 1.43 hours after disulfiram treatment. Ascorbic acid reduced the $t_{\frac{1}{2}}$ from 8.88 ± 1.43 (SE) hours to 3.97 ± 0.24 (SE) hours. Ascorbic acid also reduced ($p < 0.05$) the volume of distribution (Vd_B) by 52%, and increased ($p < 0.05$) the total body clearance (Cl_{total}) of acetaldehyde by 48% as compared to the control (Table 4). The mean Area Under the Curve (AUC) was lowered, but not statistically significant, probably due to large individual variations.

Thiamine HCl

The concentration-time plot of acetaldehyde in the disulfiram-acetaldehyde-treatments with and without thiamine is shown in Figure 4. Thiamine at a dose of 0.24 mmole/kg significantly lowered ($P< 0.01$) the mean acetaldehyde blood levels. The reduction in acetaldehyde blood levels was by 12% at five hours post acetaldehyde treatment and by 55% at nine hours post treatment.

Table 4 The effects of L-ascorbic acid, thiamine HCl or L-cysteine on the pharmacokinetic properties of acetaldehyde in disulfiram-acetaldehyde-treated rats.

Parameter	Control	Ascorbic	Thiamine
$B(hr^{-1})$	0.086 ± 0.013	0.176 ± 0.011	0.149 ± 0.021
$t_{\frac{1}{2}}$(hrs)	8.88 ± 1.43	3.97 ± 0.24**	5.35 ± 0.76*
AUC (mcg.hr/ml)	215.60 ± 28.00	164.20 ± 31.70	161.00±18.50***
Vd_{Beta}(L/Kg)	16.54 ± 2.92	7.95 ± 1.15**	13.34 ± 2.19
CL_{Total} (ml/min/Kg)	21.69 ± 2.53	32.15 ± 6.15**	30.23 ± 3.66

Rats were treated with disulfiram, 1 mmoles/kg (308 mg/kg) for three days. Ascorbic acid (2 mmoles/kg), or thiamine (0.24 mmole/kg) was administered two hours after the last dose of disulfiram and one hour before the administration of acetaldehyde (6 mmoles/kg). All the agents were administered by intraperitoneal injection. The data represent the mean ± standard error of 5 rats in the control disulfiram-saline), 4 in ascorbic acid and 8 in thiamine treatments.

*Significantly different from the control ($p < 0.05$)

**Significantly different from the control ($p < 0.025$)

***Significanlty different from the control ($p < 0.01$) (Student's t-test)

B = slope of the terminal phase of the concentration time profile of acetaldehyde

Figure 4 Effects of thiamine HCl on acetaldehyde blood levels in disulfiram-acetaldehyde-treated rats.

Rats were treated with disulfiram (1 mmole/kg) for three days. Thiamine (0.24 mmole/kg) was administered one hour before the administration of acetaldehyde (6 mmoles/kg). All agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of five rats for the control and eight for thiamine treatments.

* Significantly different from the control ($p < 0.01$). (Mann-Whitney Test)

Figure 5 Effect of L-cysteine on acetaldehyde blood levels in disulfiram-acetaldehyde-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Cysteine (2 mmoles/kg) was given one hour before the administration of acetaldehyde (6 mmoles/kg) and 24 hours after the last disulfiram dose. each point represent the mean (± standard error) of five rats in control and three in cysteine treatments.

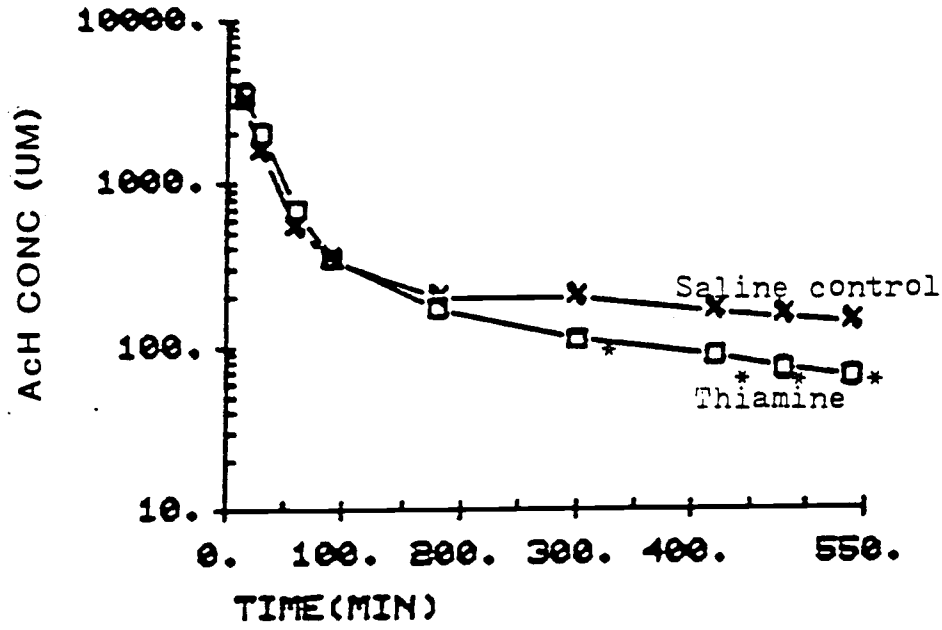
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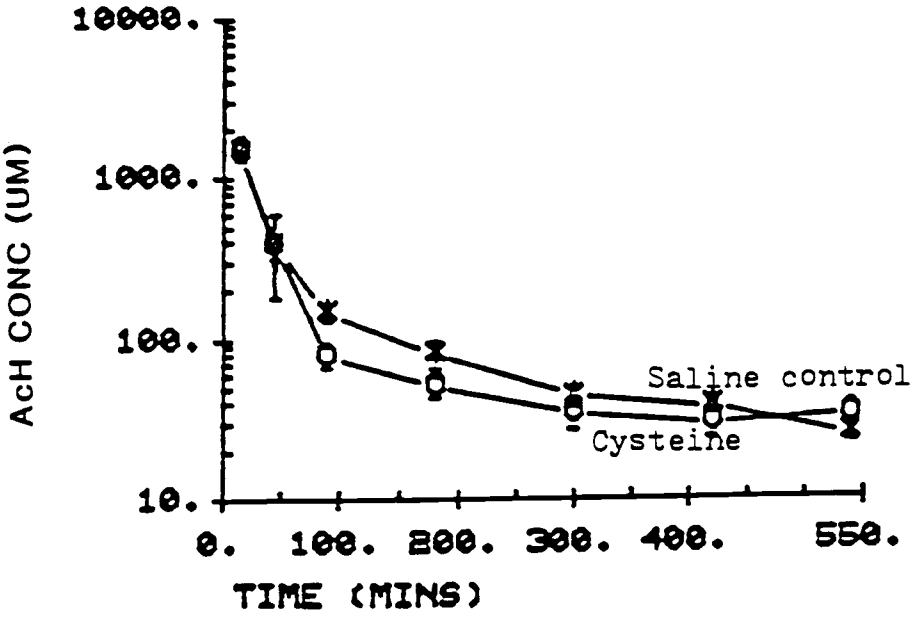
Fig. 4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TIME (MINS)

Fig. 5

Thiamine, like ascorbic acid, lowered ($p < 0.05$) the half life of acetaldehyde by 40% (Table 4). It also reduced ($p < 0.01$) the Area Under the Curve (AUC) by 25%. The total body clearance (CL_{total}) and the apparent volume of distribution (Vd_B) for acetaldehyde were significantly reduced ($p < 0.05$) by thiamine. The reductions were by 39% and 9% for the Cl_{total} and Vd_B, respectively.

L-Cysteine

The effects of cysteine on acetaldehyde blood levels in disulfiram-treated rats is shown in Figure 5. Essentially, cysteine had no significant effect on acetaldehyde blood levels. The metabolism and disposition of acetaldehyde in the disulfiram-acetaldehyde-treated rats were unaffected by cysteine (Table 5).

Blood Levels and Disposition of Endogenous Acetaldehyde

Experiments were performed to elucidate the influence of ascorbic acid, thiamine, or cysteine on the blood levels and disposition of endogenously generated (from ethanol) acetaldehyde. The rationale for this study arose from the observation that these nutrient factors, protected mice against disulfiram- ethanol-induced mortality (Moldowan & Acholonu, 1982; Sprice et al., 1975). The cause of death or toxicity in disulfiram-ethanol reaction is generally attributed to abnormal increase in the blood acetaldehyde levels (Faiman, 1979). Because these nutrient factors protected mice against the lethal effects of disulfiram-ethanol reaction, the mechanism of protection may be due to a reduction in acetaldehyde blood levels (Moldowan & Acholonu, 1982). This study was designed to investigate the influence of ascorbic acid,

Table 5 The effect of L-cysteine on the pharmacokinetic properties of acetaldehyde in disulfiram-acetaldehyde-treated rats.

Parameter	Control	Cysteine
$B(hr^{-1})$	0.122 ± 0.036	0.135 ± 0.056
$t_{\frac{1}{2}}$(hrs)	7.405 ± 1.67	7.01 ± 2.39
AUC (mcg.hr/ml)	65.86 ± 6.31	69.56 ± 9.04
Vd_{Beta}(L/Kg)	22.15 ± 10.42	37.56 ± 10.05
CL_{Total} (ml/min/Kg)	65.44 ± 8.04	69.69 ± 7.13

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Cysteine (2 mmoles/kg) was administered one hour before the administration of acetaldehyde (6 mmoles/kg) and 24 hours after the last dose of disulfiram. The data represent the mean ± standard error of five rats in the control and three in cysteine treatment.

thiamine, or cysteine on endogenously generated acetaldehyde blood levels and disposition.

Kinetics of Ethanol and Its Metabolite, Acetaldehyde

Blood ethanol and acetaldehyde analysis was done after the administration of ethanol (2 g/kg), IP, to rats. The concentration-time plots for ethanol and acetaldehyde are shown in Figure 6 A and 6B respectively. The pharmacokinetic parameters of ethanol were determined by using the one compartment open pharmacokinetic model with a zero order elimination process (Ritchie, 1980). The pharmacokinetic parameters of acetaldehyde (generated from ethanol) were also determined by using the zero order elimination process (Creasy, 1979). This is because: (a) the concentration-time profile of the endogenous acetaldehyde follows closely to that of the parent compound, ethanol (Figure 6A and 6B): (b) the rapid distributive phase of the exogenous acetaldehyde is not seen in the endogenous acetaldehyde (Figures 1 through 5 and Figures 6A and 6B).

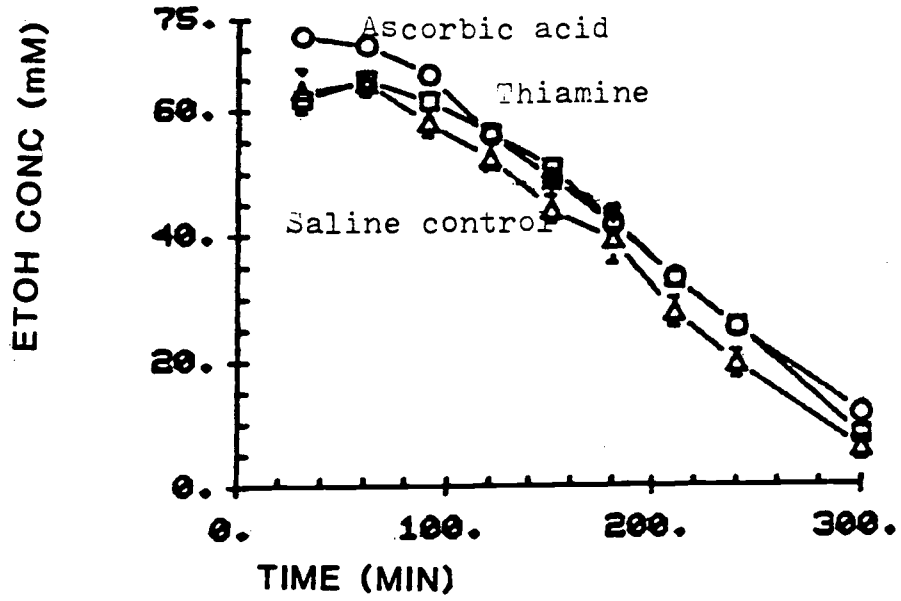
The maximum acetaldehyde blood levels obtained from the administration of 2g/kg ethanol was 115.28 ± 7.83 (mM). The normal reported range of blood acetaldehyde from ethanol oxidation is from 1 to 100 uM (Weiner, 1979) when the GLC head-space acetaldehyde analysis is used. Results obtained in this study is slightly above this range, because of higher incubation temperature of 70°C used instead of the recommended temperature of 56°C (Weiner, 1979). It was observed from this analysis that increasing the temperature yielded higher acetaldehyde/propanol peak height ratios. The higher values were needed so that the effects of ascorbic acid, thiamine or cysteine on them could be

Figure 6a The effect of L-ascorbic acid or thiamine HCl on ethanol blood levels in ethanol-treated rats.

Rats were treated with ethanol (2 g/kg in 20% w/v solution) one hour after the administration of ascorbic acid (2 mmoles/kg). Ascorbic acid, normal saline, and ethanol were given by intraperitoneal injection. Each point represents the mean of 14 rats in the control and seven in each of the ascorbic acid and thiamine treatments.

Figure 6b The effect of L-ascorbic acid or thaimine HCl on acetaldehyde blood levels in ethanol-treated rats.

Rats were treated with ethanol (2 g/kg in 20% w/v solution) one hour after the administration of thiamine (0.24 mmole/kg). All the agents were administered by intraperitoneal injection. Each point represents the mean of 14 rats for the control, seven each for ascorbic acid and thiamine treatments.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Ascorbic acid
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Fig. 6a

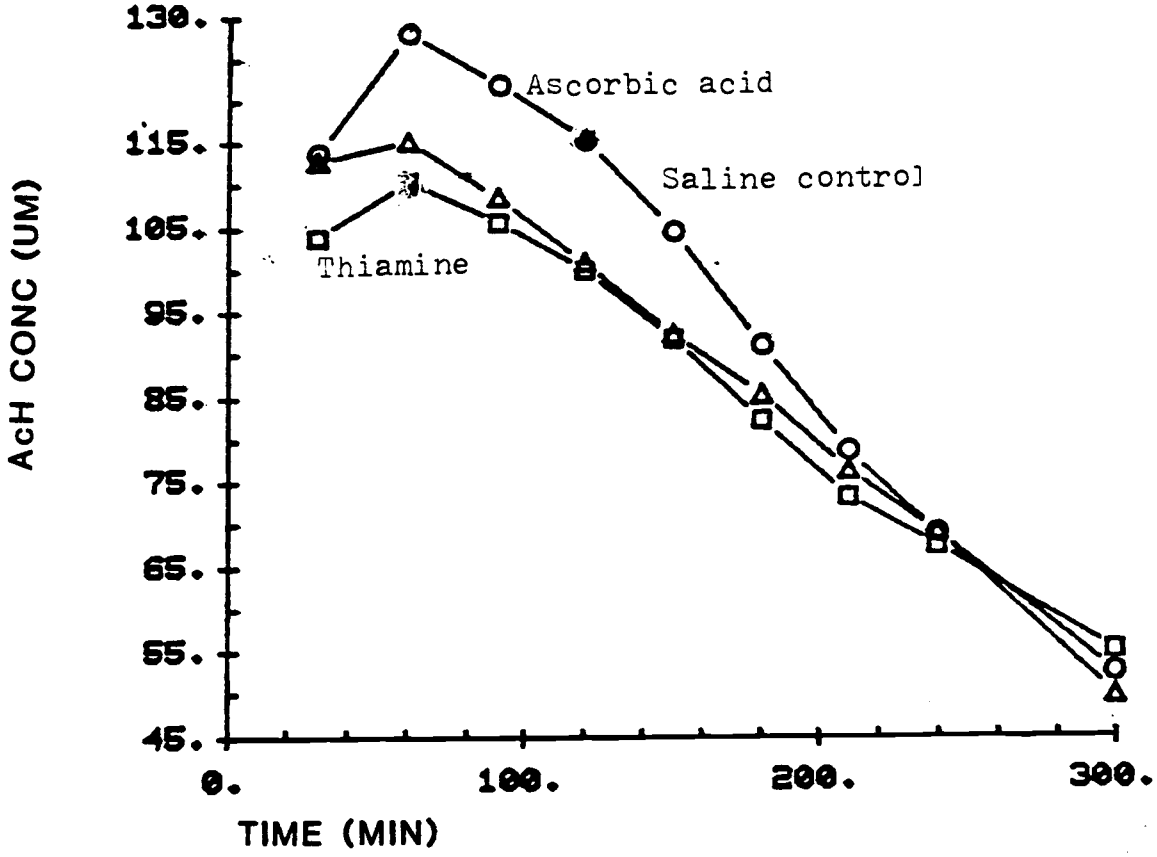
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Fig. 6b

determined. The pharmacokinetic parameters of ethanol generated from the concentration-time profiles are shown in Table 6. That of its metabolite, acetaldehyde, are shown in Table 7.

L-Ascorbic Acid and Thiamine HCl

The effects of ascorbic acid (AA) or thiamine (B1) on ethanol and acetaldehyde blood concentration are shown in Figure 6. Neither ascorbic acid nor thiamine had any effect on the metabolism of ethanol. The blood levels of ethanol in the ascorbic acid- or thiamine- treated rats remained essentially the same as that of control throughout the time course (Figure 6A).

The effects on the kinetic parameters are shown in Table 6. The maximum concentration (C_{max}) of ethanol in ascorbic acid treated rats is higher than the control by about 10%, but this is not statistically significant ($P < 0.05$). The half life ($t_{\frac{1}{2}}$), total area under the curve, and the total clearance of ethanol remained unchanged by ascorbic acid and thiamine pre-treatments.

Ascorbic acid significantly increased ($p < 0.05$) the acetaldehyde elimination rate constant (K) by 58%. The $t_{\frac{1}{2}}$ of acetaldehyde was reduced from 4.72 ± 0.46 hours to 3.56 ± 0.19 hours, while the AUC was reduced from 25.00 ± 2.00 mcg.hr/ml to 22.0 ± 2.00 mcg.hr/ml (Table 7). These reductions, however, are not statistically significant due to large individual variations. The ratio of AUC-ETHANOL/AUC-ACETALDEHYDE was significantly increased ($P < 0.05$) by thiamine as was with ascorbic acid. This means that the total amount of acetaldehyde relative to ethanol was reduced by ascorbic acid and thiamine.

Table 6 The effects of L-ascorbic acid, thiamine HCl, or L-cysteine on the pharmacokinetic profile of ethanol in rats.

Parameters	Animal Treatments			
	Saline Control	Ascorbic Acid	Thiamine	Cysteine
Body Wt (g)	293 ± 8	298 ± 12	288 ± 11	276 ± 10
C_{Max}(mM)	64.6 ± 2.1	70.75 ± 2.0	65.3 ± 4.0	50.28 ± 8.0
t_{Max}(Mins)	60	60	60	150
K(hr^{-1})	15.72 ± 0.8	15.20 ± 0.84	17.22 ± 0.60	13.70 ± 1.80
$t_{\frac{1}{2}}$(hr)	2.65 ± 0.075	2.92 ± 0.124	2.75 ± 0.07	3.17 ± 0.175
AUC (mg.hr/ml)	9.05 ± 0.48	10.47 ± 0.35	9.8 ± 0.33	9.30 ± 1.12
CL_{total} (ml/min/Kg)	3.85 ± 0.25	3.21 ± 0.110	3.42 ± 0.114	3.79 ± 0.46

Rats were treated with ethanol (2g/kg in 20% w/v solution) one hour after the administration of ascorbic acid, thiamine, cysteine or normal saline. All the agents were administered by intrperitoneal injection. The data represent the mean ± standard error of 14 rats in the control, 8 in ascorbic acid, 7 in thiamine and 5 in cysteine treatments.

C_{Max} = Peak Concentration
t_{Max} = Time of Peak Concentration
K = Elimination Rate Constant
AUC = Area Under the Concentration Time Curve
CL_{total} = Total Body Clearance
$t_{\frac{1}{2}}$ = Half Life

Table 7 The effects of L-ascorbic acid, thiamine HCl or L-cysteine on some pharmacokinetic profiles of acetaldehyde generated from ethanol in rats

	Animal Treatments			
Parameters	ETOH & NS	ETOH & AA	ETOH & B1	ETOH & CYS
Body Wt (g)	293 ± 8	298 ± 12	288 ± 11	276 ± 10
C_{Max}(UM)	115.3 ± 7.3	128 ± 15	111 ± 6	119 ± 4
T_{max}(mins)	60	60	60	150
K(hr^{-1})	16.14 ± 1.8	25.60 ± 3.60*	16.00 ± 2.30	21.60 ± 1.80*
$t_{\frac{1}{2}}$(hr)	4.72 ± 0.46	3.56 ± 0.190	4.33 ± 0.30	4.355 ± 0.36
AUC (mcg.hr/ml)	25 ± 2.00	22 ± 2.00	23 ± 1.10	28 ± 2.00
$\frac{\text{AUC-ETOH}}{\text{AUC AcH}}$	404.80±43.70	497±35.37*	442.52±21.81*	339.25±43.09*

Rats were treated with ethanol (2g/kg in 20% w/v solution) one hour after the administration of ascorbic acid, thiamine, cysteine or normal saline. All the agents were administered by intrperitoneal injection. The data represent the mean ± standard error.
* Significant difference from the control ($p < 0.05$). (student's T-test)

L-Cysteine

Figures 7A and 7B show the effects of cysteine on the concentration-time plots of ethanol (A) and its metabolite, acetaldehyde (B), respectively. The administration of cysteine (2mmoles/kg) one hour before ethanol (2g/kg) resulted in an apparent reduction in the absorption of ethanol from the peritoneal cavity. Thirty minute post ethanol treatment the blood ethanol level was significantly ($p < 0.01$) lowered by 22% of the control. The peak concentration was not significantly altered, but the time of peak (t_{max}) shifted from 60 minutes to 150 minutes post treatment (Table 7). The total body clearance and area under the curve of ethanol were not altered by cysteine.

Cysteine increased the acetaldehyde blood levels, particularly after the peak concentration was reached (Figure 7B). The acetaldehyde blood levels were increased ($p < 0.01$) by 37% of the control from 150 minutes to 300 minutes post treatment. The half life of acetaldehyde was not significantly lowered, but the ratio of $\frac{\text{AUC-ETOH}}{\text{AUC-AcH}}$ was decreased ($p < 0.05$) by 16% of the control. This result is opposite to that found after ascorbic acid and thiamine treatments.

Endogenous Acetaldehyde and Disulfiram

Influence of Disulfiram on Ethanol and Acetaldehyde Blood Levels and Disposition

The mean concentration-time profile of ethanol and its metabolite, acetaldehyde in disulfiram- and non-disulfiram-treated rats is shown in Table 8. The peak concentration (C_{max}) of ethanol in the

Figure 7a Effect of L-cysteine on ethanol blood levels in ethanol-treated rats.

Rats were treated with ethanol 92 g/kg in 25% w/v solution) one hour after the administration of cysteine. Each point represents the mean (± standard error) of 14 rats for the control and five for the cysteine treatments.

*Significantly different from the control ($p < 0.05$). (Mann Whitney Test)

Figure 7b Effect of L-cysteine on acetaldehyde blood levels in ethanol-treated rats.

Rats were treated with ethanol (2 g/kg in 20 w/v solution) one hour after the administration of cysteine. Each point represents the mean (± standard error) of 14 rats for the control and five for the cysteine treatments.

*Significantly different from the control ($p < 0.01$). (Mann Whitney Test)

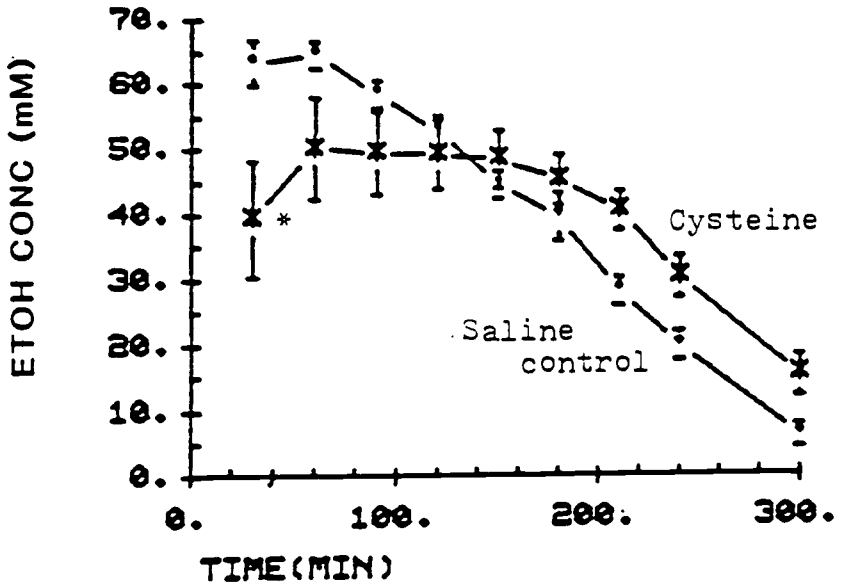

ETOH CONC (mM)
70.
60.
50.
40.
30.
20.
10.
0.
0.
100.
200.
300.
TIME(MIN)
Cysteine
Saline
control

Fig. 7a

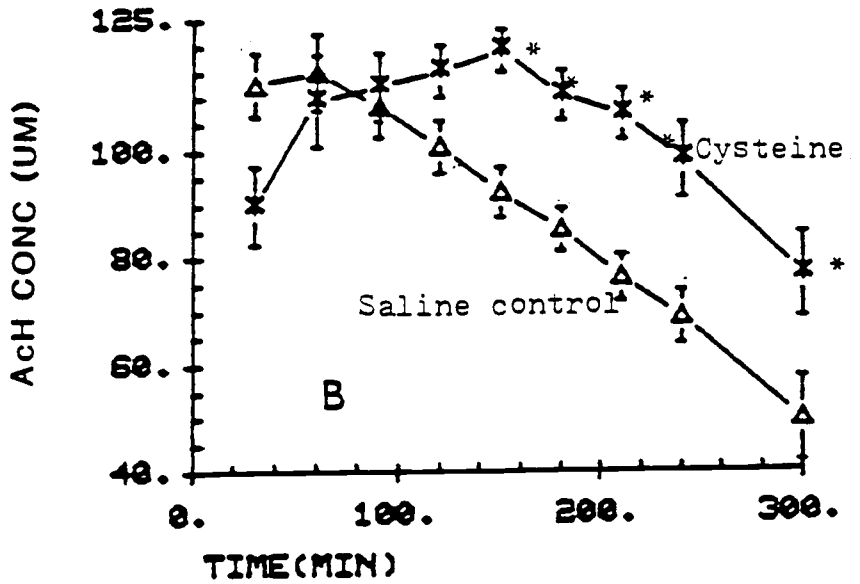

AcH CONC (UM)
125.
100.
80.
60.
40.
0.
100.
200.
300.
TIME(MIN)
Cysteine
Saline control
B

Fig. 7b

disulfiram-treated and non-disulfiram-treated rats was essentially the same, 65.62 ± 1.61 mM and 64.60 ± 2.09 mM, respectively, and occurred at the same time (60 minutes post ethanol treatment). From 180 minutes post ethanol treatment and beyond, the ethanol blood levels became significantly higher ($p < 0.01$) in the disulfiram pre-treated rats than the non-disulfiram treated rats. This is made more apparent by the increase in the area under the curve of ethanol from 9.05 ± 0.48 (SE) mg.hr/ml, in the non-disulfiram-treated rats, to 20.07 ± 1.91 mg.hr/ml in the disulfiram-treated rats (about 120% increase).

The kinetic parameters of ethanol in the disulfiram-ethanol-treated rats are shown in Table 9. The half life of ethanol was significantly increased ($p < 0.01$) by 57% as a result of disulfiram pre-treatment. The area under the curve of ethanol was increased ($P< 0.01$) by 120%, while the total body clearance was significantly reduced from 3.85 ± 0.25 ml/min/kg to 1.75 ± 0.15 (SE ml/min/kg (a decrease of about 55%).

Unlike ethanol, the peak concentration of acetaldehyde in the disulfiram-ethanol-treated rats was significantly increased ($P< 0.01$), measuring about seven times that of control (Table 8). The acetaldehyde blood levels in the disulfiram pre-treatments remained high after 10 hours of analysis. The increase in acetaldehyde blood levels by disulfiram is made more vivid by comparing the area under the curve of acetaldehyde in the disulfiram-treated and non-disulfiram-treated conditions (Table 9). The area under the curve of acetaldehyde in the disulfiram treatments is 10 times that of the non-disulfiram treatments. Acetaldehyde half life was also increased ($p < 0.05$) by disulfiram by about 47%.

Table 8 Effects of disulfiram on ethanol and acetaldehyde blood levels in disulfiram-ethanol-treated rats.

Time (mins)	Control		Disulfiram	
	ETOH conc (mM)	AcH conc (um)	ETOH conc (mM)	AcH conc (um)
30	63.35 ± 3.50	112.79 ± 6.00	-- --	-- --
60	64.60 ± 2.90	115.29 ± 7.30	65.62 ± 1.62	786.16 ± 83.71*
90	58.40 ± 2.16	108.57 1 5.80	-- --	-- --
120	52.70 ± 2.43	100.93 ± 4.90	-- --	-- --
150	44.45 ± 2.13	92.43 ± 4.60	-- --	-- --
180	39.57 ± 3.68	85.36 ± 4.10	59.12 ± 2.04*	662.17 ± 76.30*
210	28.14 ± 2.15	76.36 ± 4.40	-- --	-- --
240	19.76 ± 2.23	68.96 ± 5.00	-- --	-- --
300	6.06 ± 1.79	49.75 ± 8.00	44.80 ± 2.79*	574.17 ± 63.63*
420	-- --	-- --	32.97 ± 2.95	481.50 ± 61.12
480	-- --	-- --	25.08 ± 3.63	382.33 ± 50.19
540	-- --	-- --	19.24 ± 4.52	293.17 ± 64.40
600	-- --	-- --	10.65 ± 4.13	217.00 ± 57.48

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Ethanol (2 g/kg in 20% w/v solution was administered 25 hours after the last disulfiram dose. Numbers in parenthesis represent the number of rats used. The data represent the mean ± standard error. ETOH = Ethanol, AcH = Acetaldehyde.

* Significanlty different from the control ($p < 0.01$). Mann-Whitney test.
-- Levels not determined

Table 9 The effects of disulfiram on the pharmacokinetic properties of ethanol and its metabolite, acetaldehyde, in disulfiram-ethanol-treated rats.

	Control (14)		Disulfiram (7)	
Parameters	Ethanol	Acetaldehyde	Ethanol	Acetaldehyde
$K(hr^{-1})$	15.72 ± 0.84	16.14 ± 1.80	6.54 ± 0.72***	81.24 ± 16.80**
$t_{\frac{1}{2}}$(hrs)	2.70 ± 0.08	4.72 ± 0.46	6.34 ± 0.616**	6.94 ± 0.76*
AUC	9.05 ± 0.48 (mg.hr/ml)	25.00 ± 2.00 (mcg.hr/ml)	20.07 ± 1.91** (mg.hr/ml)	253.00 ± 35.00[a] (mcg.hr/ml)
$\frac{\text{AUC ETOH}}{\text{AUC AcH}}$	404.81 ± 43.78		86.30*** ±	
CL_{total} (ml/min/Kg)	3.85 ± 0.250		1.75 ± 0.15***	

Rats were treated with disulfiram. 0.5 mM/kg, for two days, IP. Ethanol, 2g/kg in solution, was administered to the rats 24 hours after the last dose of disulfiram. Data represent the mean (± standard error).

*Significantly different from the control ($p < 0.05$). (Student's T-test)

**Significantly different from the control ($p < 0.01$). (Student's T-test)

***Significantly different from the control ($p < 0.001$). (Student's T-test)

AcH = Acetaldehyde

L-Ascorbic Acid

The effects of ascorbic acid on the ethanol and acetaldehyde blood levels are shown in Figures 8a and 8b, respectively. Ascorbic acid lowered the ethanol blood levels in the disulfiram-treated rats. This effect is significant ($P < 0.025$) at 8, 9, and 10 hours post ethanol treatment. Three hours after administration of ethanol, ascorbic acid lowered the ethanol blood level by 16% when compared to the control. At nine hours post treatment, ascorbic acid decreased ethanol blood level by 72% ($p < 0.025$). The total body clearance of ethanol was increased ($p < 0.05$) by 27% as a result of the ascorbic acid treatment (Table 10).

Like its effects on ethanol blood levels, ascorbic acid significantly reduced ($p < 0.05$) acetaldehyde blood levels at 8, 9, and 10 hours post ethanol treatment (Figure 8B). The reduction of acetaldehyde blood levels by ascorbic acid was by 67% at 9 hours and 76% at 10 hours post-ethanol treatment.

The effects of ascorbic acid on the kinetic parameters of acetaldehyde in the disulfiram-ethanol-treated rats are shown in Table 11. The half life of acetaldehyde generated from ethanol in the disulfiram-treated rats was 6.94 ± 0.76 (SE) hours in the saline control. This value was reduced to 5.25 ± 0.07 (SE) hours by ascorbic acid treatment (a reduction of about 24% of the control). The area under the curve of acetaldehyde was also reduced by about 42% of the control as a result of the ascorbic acid treatment. The reduction in AUC, and $t_{\frac{1}{2}}$ of acetaldehyde by ascorbic acid is not statistically significant ($p < 0.05$), probably due to high individual variations.

Figure 8a Effect of L-ascorbic acid on ethanol blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Ascorbic acid (2 mmoles/kg) was given 24 hours after the last disulfiram dose. One hour after ascorbic acid, ethanol (2 g/kg in 20% w/v solution was administered. Each point represents the mean (± standard error) of seven rats for the control and four for the ascorbic acid treatments.

*Significantly different from the control ($p < 0.05$). (Student's T-test)
**Significantly different from the control ($p < 0.025$).

Figure 8b Effect of L-ascorbic acid on acetaldehyde blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Ascorbic acid 2 mmoles/kg) was given 23 hours after the last disulfiram dose. One hour after ascorbic acid, ethanol (2 g/kg in 20% w/v solutuion was administered. All the agents were administered by intraperitoneal injection. Each point represents the mean (1 standard error) of seven rats for the control and four for the ascorbic acid treatments.

*Significantly different from the control ($p < 0.05$). (Student's T-test)

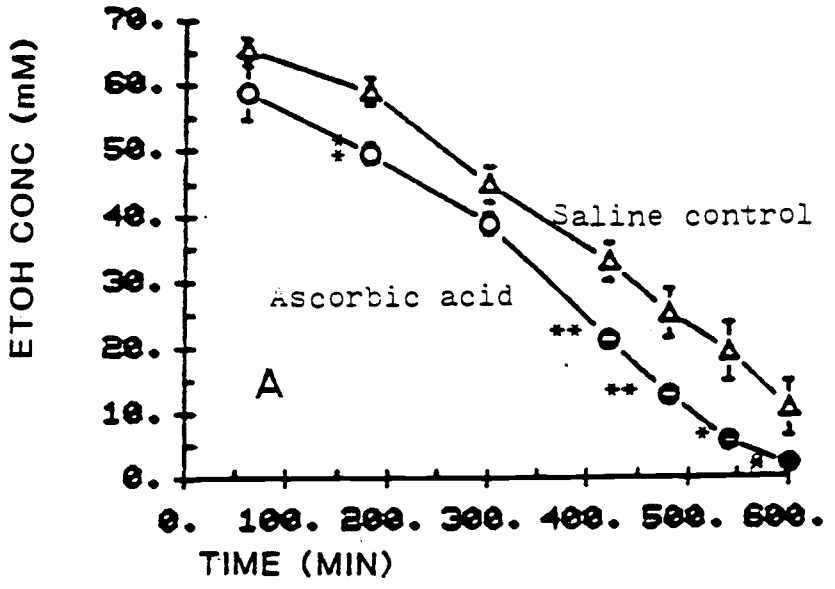

ETOH CONC (mM)
70.
60.
50.
40.
30.
20.
10.
0.
Saline control
Ascorbic acid
A
0. 100. 200. 300. 400. 500. 600.
TIME (MIN)

Fig. 8a

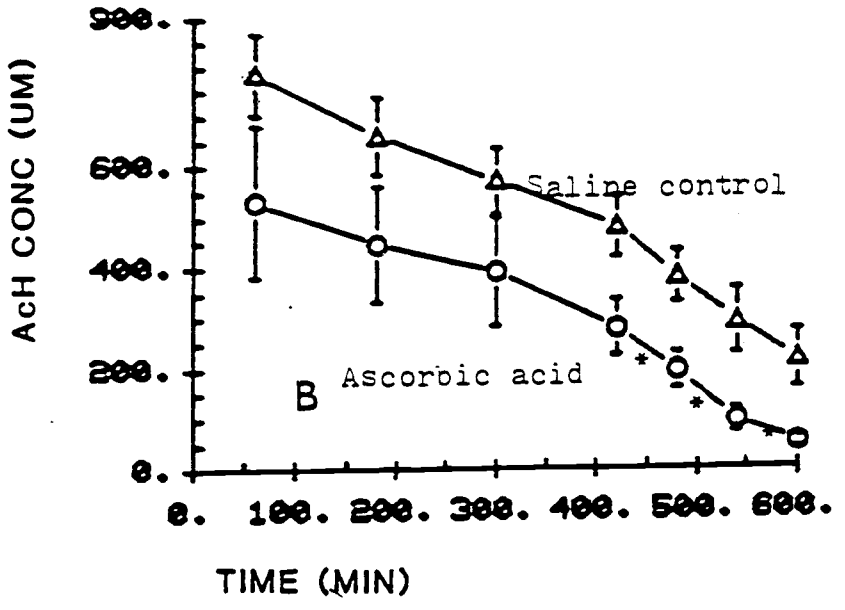

AcH CONC (UM)
800.
600.
400.
200.
0.
Saline control
B
Ascorbic acid
0. 100. 200. 300. 400. 500. 600.
TIME (MIN)

Fig. 8b

Table 10 The effects of L-ascorbic acid, thiamine HCl, or L-cysteine on some pharmacokinetic properties of ethanol in disulfiram-ethanol-treated rats.

Parameter	NS	Ascorbic Acid	Thiamine	Cysteine
$K(hr^{-1})$	6.54 ± 0.72	6.78 ± 0.36	7.74 ± 1.44	6.42 ± 0.48
$t_{\frac{1}{2}}$(hrs)	6.34 ± 0.62	5.01 ± 0.09	6.74 ± 0.45	6.73 ± 0.56
AUC (mg.hr/ml)	20.07 ± 1.91	14.54 ± 0.6*	26.3 ± 2.1	22.95 ± 2.9
CL_{total} (ml/min/Kg)	1.75 ± 0.15	2.230 ± 0.089*	1.27 ± 0.09	1.77 ± 0.017

Rats were treated with disulfinom (0.5 mmoles/kg) for two days. Ascorbic acid, thiamine, cysteine or saline control was given 24 hours after the last disulfiram dose. One hour later, ethanol (2 g/kg in 20% w/v solution) was administered. all the agents were given by intraperitoneal injection. The data represents the mean ± standard error) of 6 rats in the control, 4 in ascorbic acid, 3 in thiamine and 4 in cysteine treatments.

* Significantly different from the control ($p < 0.05$). (Mann-Whitney test).

Thiamine HCl

Figure 9 shows the effects of thiamine on ethanol (9a) and acetaldehyde (9b) blood levels in disulfiram-ethanol-treated rats. Thiamine induced an increase in the ethanol blood levels, but had no effects on acetaldehyde blood levels. Eight hours post ethanol treatment, the ethanol blood levels were increased ($p < 0.05$) by 56% of the control, and after ten hours, the ethanol blood level was raised ($p < 0.05$) by 160%. Compared to the saline treated rats, thiamine increased the area under the curve for ethanol from 20.07 ± 1.91 (SE) mg.hr/ml to 26.30 ± 2.10 (SE) mg.hr/ml (Table 10). The half life ($t_{\frac{1}{2}}$), and total body clearance of ethanol were not significantly affected by thiamine.

Thiamine showed no effect on acetaldehyde blood levels in the disulfiram-ethanol-treated rats (Figure 9b). All the kinetic parameter of acetaldehyde generated from ethanol (Table 11) were also not affected by thiamine treatment. Though the blood levels of ethanol were increased by thiamine the ratio of the AUC-ETOH/AUC-AcH was not significantly increased.

L-Cysteine

Unlike ascorbic acid and thiamine, cysteine had no significant effect on ethanol blood levels (Figure 10), in the disulfiram-ethanol-treated rats. However, cysteine lowered the mean acetaldehyde blood levels at one hour post ethanol treatment ($p < 0.05$). From eight hours post ethanol treatment until the last sample time (10 hours), acetaldehyde levels in the cysteine treated rats remained the same as the control rats (Figure 10b).

Figure 9a Effect of thiamine HCl on ethanol blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Thiamine (0.24 mmole/kg) was given 24 hours after the last disulfiram dose. One hour after thiamine, ethanol (2 g/kg in 20% w/v solutuion) was administered. All agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of seven rats in the control and three in the thiamine treatments.

*Significantly different from the control ($p < 0.05$). (Mann Whitney test)

Figure 9b Effect of Thiamine HCl on acetaldehyde blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Thiamine (0.24 mmole/kg) was given 24 hours after the last disulfiram dose. One hour after thiamine, ethanol (2 g/kg in 20% w/v solution was administered. All agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of seven rats in the control and three in the thiamine treatments.

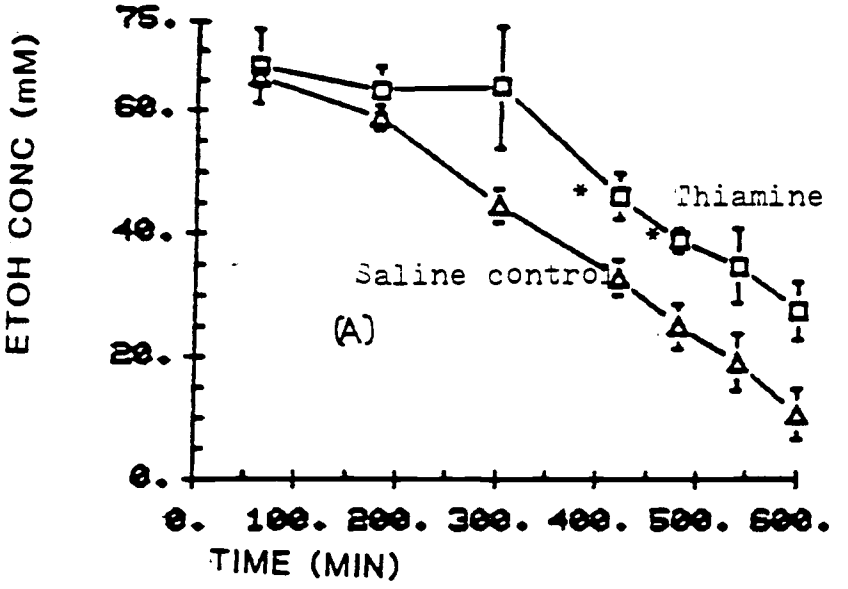

ETOH CONC (mM)
75.
60.
40.
20.
0.
0. 100. 200. 300. 400. 500. 600.
TIME (MIN)
Thiamine
Saline control
(A)

Fig. 9a

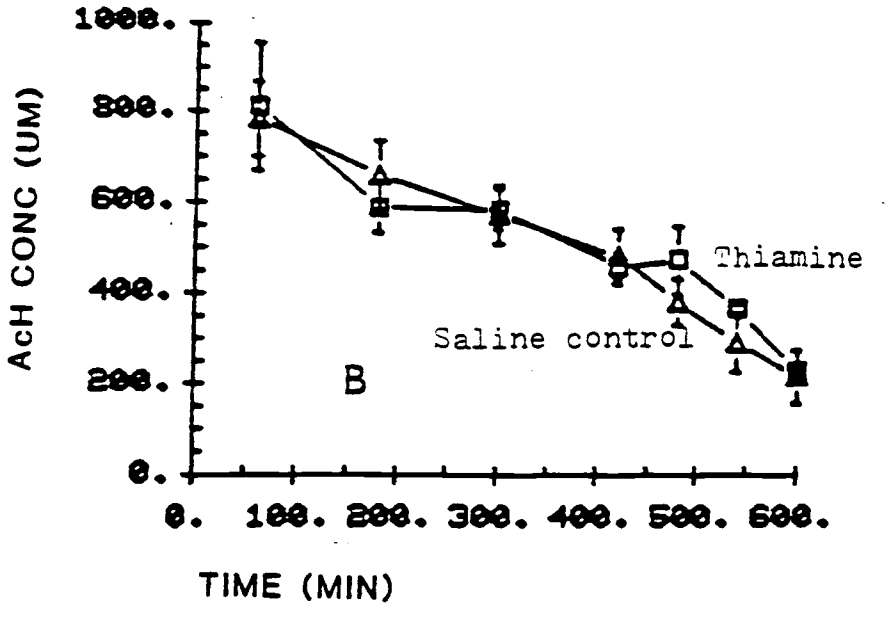

AcH CONC (UM)
1000.
800.
600.
400.
200.
0.
0. 100. 200. 300. 400. 500. 600.
TIME (MIN)
Thiamine
Saline control
B

Fig. 9b

Figure 10a Effect of L-cysteine on ethanol blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.05 mmole/kg) for two days. Cysteine (2 mmoles/kg) was given 24 hours after the last disulfiram dose. One hour after cysteine, ethanol (2 g/kg in 20% w/v solution) was administered. All the agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of six rats for the control and six for cysteine treatments.

Figure 10b Effect of L-cysteine on acetaldehyde blood levels in disulfiram-ethanol-treated rats.

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Cysteine (2 mmoles/kg) was given 24 hours after the last disulfiram dose. One hour after cysteine, ethanol (2 g/kg in 20% w/v solution) was administered. All the agents were given by intraperitoneal injection. Each point represents the mean (± standard error) of six rats for the control and six for cysteine treatments.

*Significantly different from the control ($p < 0.05$). (Mann-Whitney Test)

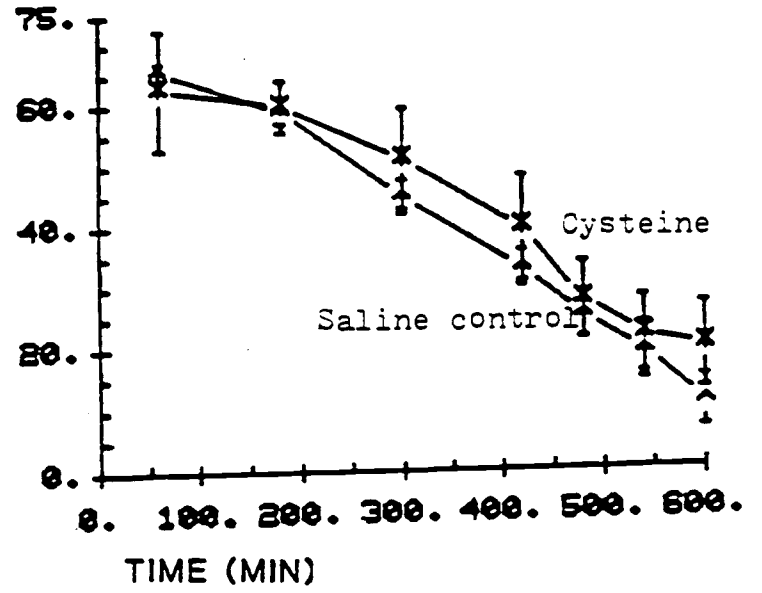


Fig. 10a

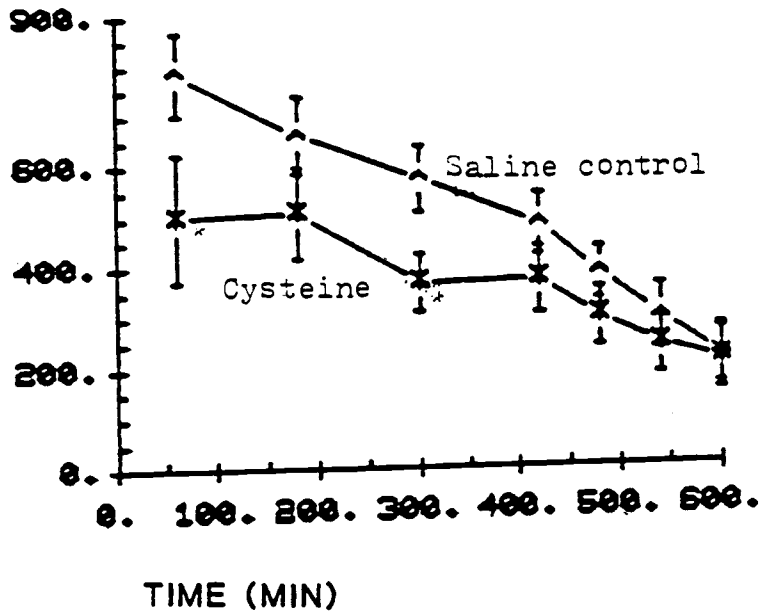


Fig. 10b

Table 11 The effects of L-ascorbic acid, thiamine HCl, or L-cysteine on some pharmacokinetic properties of acetaldehyde in disulfiram-ethanol-treated rats.

Parameter	Control	Ascorbic Acid	Thiamine	Cysteine
$K(hr^{-1})$	81.00±16.30	78.66±15.00	72.60±46.00	54.66±5.58
$t_{\frac{1}{2}}$(hrs)	6.94±0.76	5.25±0.07	6.79±0.17	6.98±0.071
AUC (mcg.hr/ml)	253±35.00	143±40.00	246±24.00	182±30.00
$\frac{AUC\ ETOH}{AUC\ ACH}$	86.30±6.84	125.92±35.10	109.54±15.66	135.91±16.57*

Rats were treated with disulfiram (0.5 mmole/kg) for two days. Ascorbic acid, thiamine, cysteine, or normal saline (control) was given 24 hours after the last disulfiram dose. One hour later, ethanol (2 g/kg in 20% w/v solution) was administered. All the agents were given by intraperitoneal injection. The data represent the mean (± standard erro).

*Significantly different from the control ($p < 0.05$). (Mann-Whitney N = number u of rate used test).

The effects of cysteine on the kinetic parameters of ethanol and acetaldehyde in the disulfiram-ethanol-treated rats are shown in Tables 10 and 11, respectively. Cysteine showed no effects on any of the kinetic parameters of ethanol and acetaldehyde. However, it increased ($p < 0.05$) the ratio of AUC-ETOH/AUC-AcH by 56%. This shows that the total amount of acetaldehyde relative to ethanol was reduced by cysteine.

The In Vitro Effect of L-Ascorbic Acid, Thiamine HCl, or L-Cysteine on Acetaldehyde Solution

The ability of ascorbic acid, thiamine, or cysteine to reduce acetaldehyde levels at a physiological pH of 7.4 was investigated *in vitro*. The result obtained is shown in Figure 11. Each bar on the graph represents a relative amount of acetaldehyde remaining free in the solution after the equilibration with ascorbic acid, thiamine or cysteine, as compared with the control (NS). Ascorbic acid and thiamine had no significant effect on the acetaldehyde solution. They could not reduce the relative amount of acetaldehyde from the solution. Cysteine significantly lowered the relative amount of acetaldehyde from the solution by about 90%. A similar result on the acetaldehyde complexing potential of cysteine has been reported by Nagasawa *et al.*, 1980.

Figure 11 The *in vitro* effect of L-ascorbic acid, thiamine HCl, or L-cysteine on acetaldehyde solutuion.

A mixture of acetaldehyde and ascorbic acid, thiamine, or cysteine in a phosphate buffer pH 7.4 was equilibrated at a temperature of 25°C for 15 minutes. Thereafter, it was equilibrated for 15 to 30 more minutes at a temperature of 70°C. A GLC head-space analysis was performed to determine the relative amount free acetaldehyde in the solution.

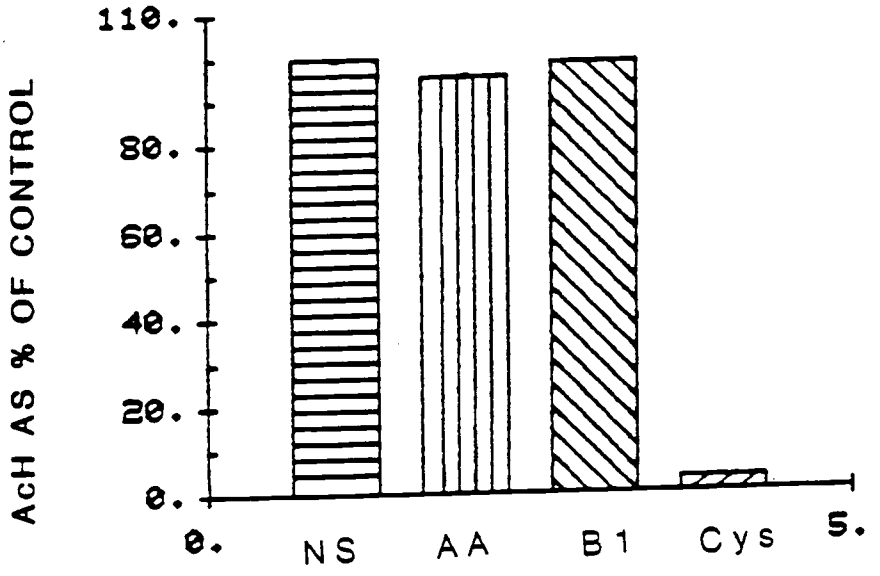


Fig. 11

DISCUSSION

Ascorbic acid, thiamine, or cysteine have been shown to reduce acetaldehyde or disulfiram-ethanol-induced lethality (Moldowan and Acholonu, 1982; Sprince et al., 1975). Whether or not the protective effect of these agents was due to alteration in the blood acetaldehyde and/or ethanol levels is not apparent from the literature. The mechanism for their protection against acetaldehyde and disulfiram-ethanol induced mortality was not known. Therefore, the effects of these compounds on acetaldehyde blood levels and disposition were investigated.

Our results show that the effects of these nutrient factors on the blood levels and disposition of AcH varied, depending on the source of acetaldehyde (exogenous or endogenous) and on whether disulfiram was added in the treatment regimen. When acetaldehyde was exogenously administered, each of these nutrient factors, ascorbic acid, thiamine or cysteine, induced a reduction in acetaldehyde blood levels. The reduction in blood levels was coupled with increase in acetaldehyde half life and volume of distribution in ascorbic acid or thiamine treatments, but not in cysteine. When disulfiram was added to the treatment regimen, the effect of ascorbic acid or thiamine on the half life and volume of distribution of exogenously administered acetaldehyde was reversed. The blood acetaldehyde levels were lowered by thiamine, but not by ascorbic acid. Cysteine had no effect on either the blood levels or the kinetic parameters of acetaldehyde in the disulfiron-acetaldehyde-treatments.

When the source of acetaldehyde was endogenous (from ethanol), ascorbic or thiamine showed no significant effects on acetaldehyde or ethanol blood levels. However, they increased the ratio of AUC-ETOH/AUC-AcH, indicating that the total amount of acetaldehyde relative to ethanol was reduced. Cysteine increased the blood levels and half life of acetaldehyde. It reduced the clearance of acetaldehyde and increased the total amount of acetaldehyde relative to ethanol, an effect opposite to those of ascorbic acid and thiamine. In the presence of disulfiram, only ascorbic acid was effective in lowering the ethanol and acetaldehyde blood levels.

Effects of L-Ascorbic Acid on Acetaldehyde Blood Levels and Disposition

Exogenous Acetaldehyde

The effects of ascorbic acid on the blood levels and disposition (as measured by the metabolism ($t_{\frac{1}{2}}$), distribution (Vd_B), and elimination (Cl_{total}) of exogenously administered acetaldehyde were investigated. The results obtained indicate that ascorbic acid induced a reduction in acetaldehyde blood levels at 60 minutes through 240 minutes post acetaldehyde treatment. Beyond 240 minutes, the decrease in acetaldehyde blood levels was insignificant. The reason for this was because the metabolism of acetaldehyde in the ascorbic acid-treated rats was slower than the control (Figure 1). Therefore, the rate of fall in blood acetaldehyde levels is less in the ascorbic acid treatments. The mechanism by which ascorbic acid may have reduced the metabolism of acetaldehyde is discussed below.

From this result, ascorbic acid seems to exhibit two opposite effects on the disposition of acetaldehyde. First, ascorbic acid increased the apparent volume of distribution of acetaldehyde. The increase in volume of distribution is probably responsible for the observed decrease in acetaldehyde blood levels at 60 minutes to 240 minutes post treatments. Second, ascorbic acid reduced acetaldehyde metabolism by increasing the half life. Increased volume of distribution (Vd_B) reflects an increase in the disposition of the toxicant from the blood. Reduction in metabolism (increased $t_{\frac{1}{2}}$) reflects a reduction in the disposition of the toxicant.

The exact mechanism by which ascorbic acid influenced the disposition of acetaldehyde is not known. It may be related to the physiological and biochemical effects of ascorbic acid and acetaldehyde on norepinephrine activity. Acetaldehyde, at a low concentration of 0.1 to 3 mM, induces the release of norepinephrine from the storage sites (for review of NE-releasing effects of acetaldehyde, see Truitt and Walsh, 1971). The releasing of norepinephrine (NE) by acetaldehyde, like tyramine, may accelerate the synthesis of NE (Bhagat et al., 1965). Increased synthesis of NE would be necessary so that the depleted store, induced by acetaldehyde, would be replenished.

Ascorbic acid facilitates the synthesis of NE by increasing the activity of tyrosine hydroxylase (Nakashima et al., 1972) in the conversion of tyrosine to dopa. Ascorbic acid also is involved in the activity of dopamine beta hydroxylase, an enzyme that catalyzes the conversion of dopamine to NE (Deana et al., 1975). It is considered as an essential cofactor of dopamine beta hydroxylase. Because of its involvement in NE synthesis, the increased availability of ascorbic

acid may act like a catalyst in the production of NE. Thus, the combined effects of acetaldehyde and ascorbic acid may increase the activity of norepinephrine. In this case, acetaldehyde induces release of NE from the storage site, and ascorbic acid replenishes the depleted store by increasing the synthesis of norepinephrine.

The increased activity of norepinephrine may then constrict the mesenteric blood vessels and reduce splanchnic and hepatic blood flow (Weiner, 1980). It therefore follows that the decreased perfusion of the liver, induced by NE, may be responsible for the observed decrease in the metabolism of acetaldehyde by ascorbic acid (Weiner, 1979).

Ascorbic acid significantly increased the apparent volume of distribution of acetaldehyde. This increase in acetaldehyde volume of distribution by ascorbic acid can also be explained by the increased activity of norepinephrine. It has been reported that NE reduces the blood volume by causing a loss of protein-free fluid to the extracellular space (Weiner, 1980). The vasoconstrictor effects of NE at the post-capillary vessels is thought to be responsible for the loss of the protein free fluid from the blood. Because of the increased activity of NE, the blood acetaldehyde could be lost to the extracellular fluid compartment as a part of the protein-free fluid of the blood. It follows therefore, that ascorbic acid, by increasing the activity of NE, increased the $t_{\frac{1}{2}}$ of acetaldehyde. Through the same mechanism, it reduced acetaldehyde blood levels by increasing the volume of distribution. Further study is needed to verify this as well as effects on metabolism and hepatic blood flow.

Perfusion Rate Hypothesis

From the above discussion it seems very apparent that the rate of flow to the liver may be an essential limiting step in the metabolism of acetaldehyde. Because of this, hypothesis can be made that the metabolism of acetaldehyde depends on the perfusion rate of the liver, if other things (including liver status, the status of acetaldehyde dehydrogenase, NADH/NAD ratio, and the status of mitochondria) remain constant.

There are several ways by which the perfusion rate hypothesis could be tested.

1. By the comparison of blood and liver acetaldehyde levels before and after the administration of ascorbic acid. This could be done as described by Brien and Hoover, 1980. If the hypothesis is true, less amount of acetaldehyde would be in the liver of the ascorbic acid pre-treatmented rats. The reason for this would be due to a decreased perfusion of the liver induced by both ascorbic acid and acetaldehyde (and mediated by NE).
2. By inhibiting the synthesis of norepinephrine with a known inhibitor, and then, comparing the effects of ascorbic acid on the disposition of acetaldehyde before and after inhibition. If the hypothesis is true, the disposition of acetaldehyde would be increased if norepinephrine synthesis is inhibited.

Disulfiram is an example of a norepinephrine synthesis inhibitor (Goldstein et al., 1964; Masacchio et al., 1966a). Experiments show that disulfiram, at a concentration of 10^{-5}M, inhibits by 100% the synthesis of norepinephrine from dopamine (Goldstein et al., 1964). The result obtained when disulfiram was used to inhibit the metabolism

of acetaldehyde, as described below, supports the hypothesis of the perfusion rate dependence of acetaldehyde metabolism. Disulfiram was used to inhibit the metabolism of acetaldehyde, but its secondary effect may have inhibited NE synthesis (not measured) to give the results obtained in the disulfiram-acetaldehyde-treatments.

Exogenous Acetaldehyde and Disulfiram

The effects of ascorbic acid on acetaldehyde blood levels and disposition were investigated, when disulfiram was used to inhibit the metabolism of acetaldehyde. Under the influence of disulfiram, ascorbic acid significantly increased the metabolism of acetaldehyde. This was done by reducing the half life ($t_{\frac{1}{2}}$) of acetaldehyde, and by increasing the total body clearance (Cl_{total}). The combined reduction in half life, and the increase in total body clearance of acetaldehyde, indicate that ascorbic acid was effective in enhancing the disposition of acetaldehyde in disulfiram-acetaldehyde-treated rats.

Of greater significance is the observation that disulfiram reduced acetaldehyde volume of distribution. This is very important because it shows that the increase in acetaldehyde blood levels by disulfiram and by other drugs known to have disulfiram-ethanol-like reaction, may not only be due to inhibition of acetaldehyde metabolizing enzyme. A decrease in the volume of distribution concentrates the toxicant more in the plasma water than in the larger pool of the total body water (Klassen, 1980). Thus, the smaller the volume of distribution, the higher the blood levels of the toxicant and vice-versa, if other things (metabolism and clearance) remain constant.

Like its effects in non-disulfiram treatments, ascorbic acid showed two opposite effects on acetaldehyde disposition. The effects of ascorbic acid observed in disulfiram-treatments were the reverse of the non-disulfiram-treatments. First, ascorbic acid increased the metabolism of acetaldehyde by reducing the half life. It also increased the elimination of acetaldehyde by increasing the total body clearance. Second, ascorbic acid reduced the apparent volume of distribution of acetaldehyde, but had no effects on the blood levels attained in the presence of disulfiram (Table 4).

A decreased volume of distribution would normally increase the blood levels of a toxicant (Klassen, 1980). The lack of effect of ascorbic acid on acetaldehyde blood levels, in the presence of reduced volume of distribution, can be explained by the increased metabolism and clearance of acetaldehyde.

With the perfusion rate hypothesis, as stated above, the increased metabolism (reduced $t_{\frac{1}{2}}$) and elimination (increased Cl_{total}) of acetaldehyde by ascorbic acid can be explained. In the absence of disulfiram norepinephrine activity is increased (Truitt and Walsh, 1971). This is because of the possible increase in the synthesis and release of NE by ascorbic acid and acetaldehyde, respectively. Increased NE activity constricts the mesenteric blood vessels and reduces perfusion of the liver (Weiner, 1980). The reduced perfusion of the liver is thought to be responsible for the observed reduction in acetaldehyde metabolism in experiment one. In the presence of disulfiram, however, the synthesis of NE is inhibited (Goldstein et al., 1964; Muscchio et al., 1966a). The perfusion of the liver is therefore normalized or enhanced. With the enhanced perfusion of the

liver, ascorbic acid may increase the metabolism of acetaldehyde by any of the following mechanisms described below.

Significance of the Perfusion Rate Hypothesis

The significance of the perfusion rate hypothesis lies in the fact that most of the acetaldehyde that escapes metabolism by the liver at the "first pass" must be recirculated through the liver before metabolism could take place. This means that substances that increase impedance to liver blood flow may limit the metabolism and clearance of blood acetaldehyde.

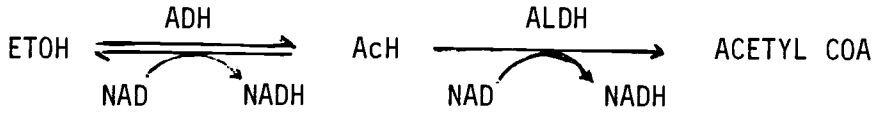
Possible Mechanisms for the Increased Disposition of Acetaldehyde by L-Ascorbic Acid in the Disulfiram-Acetaldehyde-Treatments

The mechanism by which ascorbic acid increased the disposition of acetaldehyde in the disulfiram-acetaldehyde-treated rats is not known. There are several possible mechanisms that may be involved. These may include:

1. Reduction of acetaldehyde by alcohol dehydrogenase.
2. Direct interaction of ascorbic acid with acetaldehyde.
3. Facilitation of the high-K_m acetaldehyde dehydrogenase activity.
4. Increased disposition of disulfiram by ascorbic acid.

1. Reduction of Acetaldehyde by Alcohol Dehydrogenase

The increased disposition of acetaldehyde by ascorbic acid in the disulfiram-acetaldehyde-treated rats may be explained by the influence of ascorbic acid on alcohol dehydrogenase (ADH) (an enzyme that catalyze the oxidation of ethanol to acetaldehyde).



ETOH = Ethanol; ADH = alcohol dehydrogenase;
ALDH = acetaldehyde dehydrogenase; AcH = acetaldehyde.

Krasner et al. (1974), reported that the rate of clearance of ethanol from the blood was associated with the level of leukocyte ascorbic acid. He postulated that the activity of alcohol dehydrogenase may depend on the level of ascorbic acid in the blood. Alcohol dehydrogenase has an equilibrium characteristic that favors the reduction of acetaldehyde to ethanol at a high acetaldehyde levels (Tottmar and Marchner, 1976). Ascorbic acid may facilitate the activity of alcohol dehydrogenase in the reduction of acetaldehyde to ethanol.

Greshman (1975), showed that acetaldehyde could be reduced to ethanol during the oxidation of ethanol. He, however, calculated that the rate of reduction of acetaldehyde was 2% the rate of oxidation under physiological conditions. This value seems too small to account for the 55% reduction in acetaldehyde half life by ascorbic acid in the disulfiram- acetaldehyde-treated rats. Other mechanisms may also be involved.

2. Direct Interaction Between Ascorbic Acid and Acetaldehyde

The second possible mechanism by which ascorbic acid increased the disposition of acetaldehyde is through direct interaction with acetaldehyde. Ascorbic acid has been shown to condense or react with formaldehyde (Lugg, 1942). Acetaldehyde, in common with other

aldehydes, could react with ascorbic acid by way of hemiacetal formation with the hydroxyl group of ascorbic acid in the 2, 3, positions, as formally proposed by Sprince et al. (1979).

The direct interaction between ascorbic acid and acetaldehyde was investigated in vitro at a physiological pH of 7.4 (Fig. 9). Ascorbic acid did not reduce the concentration of acetaldehyde in the solution. The same experiment was repeated with thiamine and cysteine. Cysteine was very effective in sequestering acetaldehyde from the solution. The effects of cysteine on acetaldehyde blood levels and disposition will be discussed later. This result shows a lack of direct interaction between ascorbic acid and acetaldehyde in vitro. Therefore, direct interaction of ascorbic acid and acetaldehyde may not be the principal mechanism by which ascorbic acid increased the disposition of acetaldehyde in the disulfiram-acetaldehyde treated rats.

3. Facilitation of the high-Km Acetaldehyde Dehydrogenase Activity

An interaction between ascorbic acid and the High-Km acetaldehyde dehydrogenase could be another mechanism by which ascorbic acid increased the disposition of acetaldehyde (Veiner, 1979). The high-Km acetaldehyde dehydrogenase is one of the two isoenzymes of acetaldehyde dehydrogenase. The low-Km enzyme is found primarily in mitochondria, and is responsible for the oxidation of acetaldehyde below 200 microliters (Weiner, 1979). The High-Km acetaldehyde dehydrogenase can be found in the mitochondria, cytosol, or in the microsomal fraction of the cell (Weiner, 1979). The activity of the High-Km acetaldehyde dehydrogenase is more apparent in high acetaldehyde concentrations (Marchner and Tottmar, 1978), and it has

been shown to be resistant to the inhibitory effects of disulfiram (Marchner and Tottmar, 1978).

Because the high-Km acetaldehyde dehydrogenase is resistant to the inhibitory effect of disulfiram, ascorbic acid may interact with it to increase the metabolism of acetaldehyde. The stimulatory effect of ascorbic acid on High-Km acetaldehyde dehydrogenase activity has not been investigated. However, evidence exists which suggest that ascorbic acid could stimulate the microsomal enzyme metabolizing system (Basu, 1982). Several studies have been done to correlate ascorbic acid levels and the rate of drug metabolism in animals (Conney et al., 1961; Street and Chadwick, 1975) and in humans (Rosenthal, 1971; Beattie and Sherlock, 1976). In most of these studies, the slowest rate of drug metabolism were found in those with the lowest ascorbic acid levels. This suggests that ascorbic acid can facilitate drug metabolism, probably by stimulating the microsomal enzyme-metabolizing system. Since the high-Km acetaldehyde is also a part of this system, its activity may be stimulated by ascorbic acid. Therefore, ascorbic acid may facilitate the activity of the disulfiram-resistant high-Km acetaldehyde dehydrogenase to increase the metabolism of acetaldehyde.

Effects of Thiamine HCl on Acetaldehyde Blood Levels and Disposition

Exogenous Acetaldehyde

Experiments were performed to investigate the effects of thiamine on acetaldehyde blood levels and disposition. The result obtained indicates that thiamine (0.24 mmole/kg) significantly reduced

acetaldehyde blood levels at 60 minutes through 180 minutes post acetaldehyde treatment (Figure 1B). At 240 minutes and beyond, the reduction in acetaldehyde blood levels by thiamine was insignificant. The reason for this was because the metabolism of acetaldehyde (as reflected by $t_{\frac{1}{2}}$) in thiamine treated rats was slower than the control. As a result of this, the rate of fall in the blood acetaldehyde levels is less in thiamine treatment than in the control. The decrease in acetaldehyde metabolism by thiamine is made more apparent by the increase in acetaldehyde half live ($t_{\frac{1}{2}}$) (Table 1).

Thiamine, like ascorbic acid, exhibited two opposite effects on the disposition of acetaldehyde. First, thiamine increased the distribution of acetaldehyde (as measured by the apparent volume of distribution). Second, it reduced the metabolism of acetaldehyde by increasing the half life. Increased distribution indicates increases in disposition while decrease metabolism indicates reduction in disposition.

The increase in acetaldehyde volume of distribution is probably responsible for the observed reduction in acetaldehyde blood levels at 60 minutes through 180 minutes post acetaldehyde treatments. The reason for the decrease in acetaldehyde metabolism by thiamine is not clear. But its mechanism may be similar to that of ascorbic acid, that is, through a reduction in the perfusion rate of the liver. Danford and Munro (1980), reported that the administration of thiamine reduces blood flow rates. Whether or not the reduction in flow rate is mediated by catecholamines is not known. But a decreased flow rate induced by thiamine may be the mechanism involved in the observed reduction in acetaldehyde metabolism by thiamine.

Exogenous Acetaldehyde and Disulfiram

The effects of thiamine on acetaldehyde blood levels and disposition were investigated in disulfiram-acetaldehyde-treated rats. The results show that thiamine, unlike ascorbic acid, lowered the blood levels of acetaldehyde. Not only were the blood acetaldehyde levels reduced, the metabolism of acetaldehyde was increased. This was evidenced by the reduction in acetaldehyde half life (Table 4) and by the increase in the total body clearance of the toxicant. This result indicates that in the presence of disulfiram, the effectiveness of thiamine in disposing blood acetaldehyde was enhanced. Thiamine alone partly reduced the blood acetaldehyde levels, but increased the half life (reduced metabolism). In disulfiram-pretreated conditions, thiamine lowered the blood acetaldehyde levels, and also reduced the half life (increased metabolism).

The mechanism of interaction between disulfiram and thiamine that led to the observed increase in acetaldehyde metabolism and clearance is not known. The mechanism involved may be similar to that of ascorbic acid, and could be explained, at least in part, by the perfusion rate hypothesis. In the disulfiram pre-treated conditions, the perfusion rate to the liver may have increased. The reason for the increase in flow rate to the liver is because of the inhibition of norepinephrine synthesis by disulfiram (Kitson, 1977; Truitt and Walsh, 1971), and also because of the depletion of norepinephrine store by acetaldehyde (Schneider, 1974; Truitt and Walsh, 1971). When the NE store is depleted and synthesis inhibited, acetaldehyde may then act directly on the cardiovascular system to induce vasodilation (Truitt and Walsh, 1971), with a subsequent increase in flow rate.

With the enhanced perfusion to the liver, thiamine may increase the metabolism of acetaldehyde through its metabolite, thiamine pyrophosphate. Thiamine phyrophosphate, the physiologically active form of thiamine, is known to complex and transfer acetyl groups to lipoic acid for the subsequent formation of acetyl coA (White et al., 1978). Thiamine could increase the metabolism of acetaldehyde by forming a pyrophosphate which may complex with acetaldehyde with a subsequent transfer to the lipoic acid cycle (Sprince et al., 1975).

In an in vitro experiment, thiamine HCl was unable to reduce acetaldehyde concentration in the buffered solution (Figure 11). This may suggest that there is no direct interaction between acetaldehyde and thiamine HCl. The metabolite, thiamine pyrophosphate, may be responsible for the increased disposition of acetaldehyde. Further experiments may be needed to determine the effect of thiamine pyrophosphate on acetaldehyde concentration in a buffered solution. More studies are also needed to investigate whether thiamine pyrophosphate could enhance the metabolism of acetaldehyde through the lipoic acid pathway.

Effects of L-Cysteine on Acetaldehyde Blood Levels and Disposition

Exogenous Acetaldehyde

The effects of cysteine on the blood levels and disposition of acetaldehyde were investigated. Our results show that cysteine at a dose of 2 mmoles/kg was effective in lowering the blood acetaldehyde levels in rats treated with acetaldehyde (6 mmoles/kg). Unlike ascorbic acid and thiamine, cysteine lowered the blood levels of

acetaldehyde without affecting the half life ($t_{\frac{1}{2}}$) and the apparent volume of distribution (Vd_B). This means that the mechanism for reduction in acetaldehyde blood levels by cysteine could be different from those of ascorbic acid and thiamine. Ascorbic acid and thiamine reduced the blood acetaldehyde levels by increasing the apparent volume of distribution. Because cysteine had no effect on the volume of distribution of acetaldehyde, and on the $t_{\frac{1}{2}}$, the reduction in blood acetaldehyde levels could not be due to an increase in acetaldehyde metabolism.

The mechanism for reduction in the acetaldehyde blood levels by cysteine could be explained by direct interaction. Cysteine may interact with acetaldehyde by forming a non-toxic complex. Chemically, cysteine by way of its -sH group, complexes with acetaldehyde to form L-2-methyl-thiozolidine-4-carboxylic acid (L-MTCA) (Sprince, 1975). Sprince suggested that this complex may serve as a non-toxic metabolic detoxification product as well as a protectant by generating free intracellular -SH groups to complex acetaldehyde more effectively.

The ability of cysteine to reduce acetaldehyde concentration in a buffered solution was investigated *in vitro*. The result obtained shows that cysteine was very effective in reducing the amount of acetaldehyde in the solution (Figure 11). This *in vitro* result indicates the existence of direct interaction between cysteine and acetaldehyde. Our result is in agreement with that of Nagasawa *et al.*, 1980.

Exogenous Acetaldehyde and Disulfiram

Experiments were performed to investigate whether disulfiram could influence the effects cysteine had on acetaldehyde blood levels and disposition. The results show that cysteine had no effect on the blood level or the disposition of acetaldehyde in the disulfiram-acetaldehyde-treated rats. The reason for the lack of effect may be due to a direct interaction of cysteine with disulfiram. Disulfiram could interact with the sulfhydryl group of cysteine, as it does with all the sulfhydryl-containing enzymes (Eneanya et al., 1981). The loss of the sulfhydryl group may render cysteine unable to react with acetaldehyde. Moreover, one of the known metabolites of disulfiram, carbon disulfide, is known to interfere with primary amino acids such as cysteine (Eneanya et al., 1981). Thus, either disulfiram or its metabolite has the potential to interfere with the ability of cysteine to react with acetaldehyde.

Endogenous Acetaldehyde

L-Ascorbic Acid and Thiamine HCl

The effects of ascorbic acid and thiamine on ethanol and acetaldehyde blood levels were determined in ethanol-treated rats. The result obtained shows that ascorbic acid and thiamine had no effect on either ethanol or acetaldehyde blood levels. The kinetic parameters of ethanol and acetaldehyde were not affected by ascorbic acid or thiamine treatments. However, the ratio of AUC-ETOH/AUC-AcH was increased by both agents. This means that the total amount of acetaldehyde relative to ethanol was reduced by both ascorbic acid and

thiamine. The lack of effects by ascorbic acid or thiamine on the endogenous acetaldehyde levels may be due to low levels of acetaldehyde produced from ethanol. Because of the low acetaldehyde levels, the GLC analysis may not be as accurate as it would be in higher levels. The lower levels may carry greater inbuilt errors.

L-Cysteine

The effects of cysteine on ethanol and acetaldehyde blood levels are shown in Figures 7A and 7B, respectively. Unlike ascorbic acid and thiamine, the absorption of ethanol in cysteine treated rats was delayed. As a result of the delayed ethanol absorption, the ethanol blood level was reduced by cysteine at 30 minutes post ethanol treatments. The peak concentration of ethanol was extended over a period of two hours. The result is in agreement with that of Beauge et al., 1976. Beauge noted that the absorption of ethanol was delayed by cysteine treatments. Despite the delayance in ethanol absorption, the kinetic parameters were not significantly altered by cysteine (Table 7).

Cysteine also induced an increase in acetaldehyde blood levels. It increased the half life and the area under the curve of acetaldehyde, suggesting a reduction in acetaldehyde metabolism and clearance. The reason for the decrease in acetaldehyde metabolism by cysteine is not clear from these experiments. The mechanism involved seems to be linked to the direct effects of cysteine on ethanol absorption and distribution. Acetaldehyde metabolism follows very closely to that of ethanol (Weiner, 1978). Thus, a reduction in ethanol distribution, for example, would increase both ethanol and acetaldehyde blood levels.

Endogenous Acetaldehyde and Disulfiram

L-Ascorbic Acid

Under the influence of disulfiram, ascorbic acid lowered the blood ethanol and acetaldehyde levels. The reduction in blood levels may be due to the increase in ethanol metabolism (reduced $t_{\frac{1}{2}}$) and clearance (Table 10). The mechanism for the reduction of ethanol and acetaldehyde blood levels by ascorbic acid may be similar to those described for the exogenous acetaldehyde. Specifically, the increase in disposition of ethanol and its metabolite, acetaldehyde, may be due to the effects of ascorbic acid on the high-Km acetaldehyde dehydrogenase. The high-km acetaldehyde dehydrogenase is resistant to the inhibitory effects of disulfiram (Marchner and Tottmar, 1978). Ascorbic acid could increase the activity of this enzyme by stimulating the activity of the microsomal enzyme metabolizing system (Conney et al., 1961; Bettie and Chadwick, 1976). As a result of the increase in acetaldehyde metabolism, the clearance of ethanol would also be enhanced. The kinetic equilibrium that exists in ethanol oxidation would be shifted to the right as a result of enhanced clearance of acetaldehyde.

Thiamine HCl

The effects of thiamine on ethanol and acetaldehyde blood levels and disposition were investigated in disulfiram-ethanol-treated rats. The results obtained show that thiamine increased ethanol blood levels (Figure 9A). It showed no effect on the metabolism and clearance of either ethanol or acetaldehyde.

This result was very disappointing, because, in the preliminary studies (Moldowan and Acholonu, 1982), thiamine was very effective in protecting mice against disulfiram-ethanol induced mortality. The reason for the lack of effect on ethanol and acetaldehyde disposition is not clear. It may be related to species differences (rats versus mice) since the protective effect of thiamine was demonstrated in mice rather than rats.

Blood acetaldehyde levels in various species treated with disulfiram and ethanol have been shown to vary (Truitt and Walsh, 1971). For example, in disulfiram-ethanol-treated animals, the maximum blood acetaldehyde level reported in mice was 1.84 ug/ml, while that of rats was 22.4 ug/ml (Truitt and Walsh, 1971). The significance of this species variation is the fact that thiamine was unable to protect mice against the lethal effect of acetaldehyde at high acetaldehyde concentrations (Moldowan and Acholonu, 1982). Ascorbic acid gave protection at all concentrations of acetaldehyde in mice. Thus, at high acetaldehyde levels, the protective effect of thiamine was lost. This seems to suggest that there is an acetaldehyde concentration limit at which thiamine could offer protection. If this is true, then it may be possible that the amount of acetaldehyde produced in rats (in the disulfiram-ethanol-treatments) was above the concentration limit at which thiamine offers protection. This may be the possible explanation for the lack of effects of thiamine on the disposition of acetaldehyde in the disulfiram-ethanol-treated rats. More specifically, the inhibitory effects of disulfiram on acetaldehyde metabolism in rats, resulted in acetaldehyde levels that overwhelmed the ability of

thiamine to clear it from the body. More studies are needed to clarify this.

L-Cysteine

The effects of cysteine on endogenously generated acetaldehyde were investigated in disulfiram-ethanol-treated rats. Cysteine showed no effects on ethanol blood levels (Figure 10A). The mean acetaldehyde blood levels in cysteine treated rats were lower than the control at most time intervals (Figure 10B). These, however, were not statistically significant, probably due to large individual variations.

Cysteine had no effect on the kinetic parameters of either ethanol or acetaldehyde in the disulfiram-ethanol-treated rats (Table 10 and 11). Nagasawa et al. (1980), have reported that cysteine had no effects on acetaldehyde blood levels in disulfiram-ethanol-treated rats. The reason for these results may be due to a direct interaction of cysteine with disulfiram. Disulfiram may interact with the sulfyhydryl group of cysteine, as it does with all the sulfhydryl containing enzymes (Eneanya, et al., 1980). The lost of the sulfhydryl group of cysteine may render it unable to react with acetaldehyde.

Of interest is the observation that the delay in ethanol absorption and distribution by cysteine (Figure 7A) was lost in the disulfiram-treated rats (Figure 10A). The physiologic or biochemical effects of disulfiram that modulate the influence of cysteine, ascorbic acid or thiamine, on ethanol or acetaldehyde disposition are not clearly defined, and may need further investigation.

Possible Mechanism for the Protection of L-Ascorbic Acid, Thiamine HCl, or L-Cysteine against Acetaldehyde or Disulfiram-Ethanol-Induced Mortality

In previous work (Moldowan and Acholonu, 1982), it was found that ascorbic acid or thiamine lowered acetaldehyde or disulfiram-ethanol-induced mortality in mice. Their mechanism of protection was not known. We have, therefore, explored these mechanisms by investigating the effects of these nutrient factors on acetaldehyde blood levels and disposition.

The results obtained show that ascorbic acid, thiamine or cysteine were effective in lowering the blood acetaldehyde levels in acetaldehyde-treated rats. The reduction in acetaldehyde blood levels was the probable mechanism for their protection against acetaldehyde-induced lethality. Of greater significance is the observation that ascorbic acid and thiamine increased the half life of acetaldehyde. This means that the metabolism of acetaldehyde was reduced by ascorbic acid and thiamine treatments. The reduction in acetaldehyde blood levels was due to the increase in the apparent volume of distribution. This observation is important because it shows that these protectants could also lead to acetaldehyde accumulation in the body when used in chronic or sub-chronic acetaldehyde exposure to the body.

The protective effects of cysteine, as of ascorbic acid or thiamine, is due to the reduction in acetaldehyde blood levels. Unlike ascorbic acid or thiamine, cysteine reduced acetaldehyde blood levels without increasing the half life or the apparent volume of distribution. The reduction in acetaldehyde blood levels by cysteine may

be due to a direct interaction between them. In an *in vitro* experiment, cysteine was effective in reducing the relative amount of acetaldehyde in the buffered solution. The reduction in acetaldehyde levels in solution may be due to complexation or sequestration. Thus, the effects of cysteine on acetaldehyde levels are reproducible *in vivo* and *in vitro*, suggesting a direct interaction between them. Because cysteine lowered acetaldehyde blood levels without increasing the half life, it is less likely to induce acetaldehyde accumulation in a chronic or subchronic acetaldehyde exposure to the body.

The protective effects of ascorbic acid against disulfiram-ethanol induced mortality could also be explained by the reduction in ethanol and acetaldehyde blood levels. Ascorbic acid reduced the half life of ethanol and acetaldehyde in the disulfiram-ethanol-treated rats. The reduction in half life indicates that the metabolism of ethanol and acetaldehyde was increased by ascorbic acid treatments. The increase in ethanol and acetaldehyde metabolism by ascorbic acid may be responsible for the observed reduction in blood levels.

It therefore follows that the protective effect of ascorbic acid against disulfiram-ethanol-induced mortality, may be due to the ability of ascorbic acid to facilitate the metabolism of both ethanol and acetaldehyde. The increase in acetaldehyde metabolism may be due to a direct effect of ascorbic acid on the high-Km acetaldehyde dehydrogenase. This, however, is speculative. More work is needed to investigate whether ascorbic acid has any stimulating effect on the microsomal enzyme metabolizing system, and specifically on the high-Km acetaldehyde dehydrogenase.

The mechanism by which thiamine protected animals against disulfiram-ethanol-induced mortality could not be explained by the result of these studies. The effects of thiamine on the blood levels and disposition of ethanol and acetaldehyde were investigated in the disulfiram-ethanol-treated rats. The result obtained shows that thiamine increased the blood ethanol levels, but had no effect on acetaldehyde blood levels. The reason for the no effect is thought to be due to high acetaldehyde levels generated from the disulfiram-ethanol reaction, that overwhelmed the ability of the rat to clear it.

In the previous work (Moldowan and Acholonu, 1982), thiamine had no protective effect against high acetaldehyde levels. It offered protection only at lower acetaldehyde doses. Thus, the high acetaldehyde levels generated from the disulfiram-ethanol-treated rats may have overwhelmed the ability of thiamine to clear it from the body.

The protective effect of cysteine against disulfiram-ethanol-induced mortality could also be explained by the ability of cysteine to reduce acetaldehyde blood levels. The reduction in acetaldehyde blood levels may be due to a direct interaction between cysteine and acetaldehyde. Cysteine may form a complex with acetaldehyde to facilitate its elimination from the body.

SUMMARY AND CONCLUSION

Ascorbic acid, thiamine, or cysteine have been shown to reduce acetaldehyde or disulfiram-ethanol induced mortality in animals. The mechanism by which these agents offer protection was not known. In order to elucidate this, we have determined the effects of ascorbic acid, thiamine or cysteine on acetaldehyde blood levels and disposition. The results obtained are summarized in Table 12.

From the results obtained, it is clear that each of these nutrient factors was capable of reducing acetaldehyde blood levels, but with different mechanisms. Ascorbic acid and thiamine reduced acetaldehyde blood levels by increasing the apparent volume of distribution. The metabolism of acetaldehyde was diminished by ascorbic acid or thiamine treatments. This is evidenced by the increase in acetaldehyde half life. The reduction in acetaldehyde metabolism by these agents may be due, at least in part, to a reduction in the liver perfusion rate, mediated by an increase in norepinephrine activity.

Cysteine reduced acetaldehyde blood levels, but had no effects on the half life or the apparent volume of distribution. The reduction in acetaldehyde blood levels by cysteine may be due to the formation of a non-toxic complex. An *in vitro* experiment showed that cysteine was effective in sequestering acetaldehyde from the pH 7.4 buffered solution.

Disulfiram was used to inhibit the metabolism of acetaldehyde. The effects of ascorbic acid, thiamine or cysteine were then determined. The results show that ascorbic acid and thiamine increased acetaldehyde metabolism, and the total body clearance. The reason for

the increase in acetaldehyde metabolism may be due, in part, to an increase in the perfusion rate of the liver, mediated by the reduction in norepinephrine (NE) activity. Disulfiram inhibits NE synthesis (Goldstein et al., 1967). Acetaldehyde releases NE from the storage sites (Truitt and Walsh, 1971). When norepinephrine store is depleted and synthesis inhibited, acetaldehyde exhibits a secondary vasodilation, with the resultant increase in perfusion rate (Truitt and Walsh, 1971).

With the enhanced liver perfusion, ascorbic acid may increase acetaldehyde metabolism by facilitating the activity of the disulfiram-resistant high-Km acetaldehyde dehydrogenase. Thiamine may improve acetaldehyde metabolism by forming a phyrophosphate which channels the acetaldehyde into the citric acid cycle, through the lipoic acid pathway.

The lack of effect by cysteine on acetaldehyde blood levels and disposition, in the disulfiram-acetaldehyde treatments, may be due to an interaction between cysteine and disulfiram. Disulfiram may block the active complexing center (the sulfhydryl group) of cysteine. The lose of the sulfhydryl group (-SH) may diminish the ability of cysteine to sequester acetaldehyde.

In the disulfiram-ethanol treatments, ascorbic acid reduced the blood ethanol and acetaldehyde levels. The reduction in ethanol and acetaldehyde blood levels may be due to an increase in the metabolism of ethanol and acetaldehyde. The half life of ethanol was significantly reduced; that of acetaldehyde was lowered, but not statistically significant. The mechanism by which ascorbic acid increased the metabolism of ethanol and acetaldehyde may be similar to that of the

exogenously administered acetaldehyde, that is, through the facilitation of the high-km acetaldehyde dehydrogenase.

Thiamine and cysteine had no significant effect on acetaldehyde blood levels and disposition in the disulfiram-ethanol treatments. However, the ratio of area under the curve of ethanol/area under the curve of acetaldehyde (AUC-ETOH/AUC-AcH) was increased by both thiamine and cysteine. This means that the total amount of acetaldehyde relative to ethanol was reduced by thiamine and cysteine treatments.

The effects of ascorbic acid, thiamine, or cysteine on acetaldehyde blood levels and disposition were determined in ethanol-treated rats. The result shows that ascorbic acid and thiamine had no effect on the blood acetaldehyde levels. Cysteine altered the metabolism of ethanol and that of acetaldehyde. It increased acetaldehyde blood levels, increased the half life, and reduced the total body clearance. The mechanism involved in this reaction is not clear from these results but may be related to a direct effect of cysteine on ethanol absorption and distribution. The metabolism of acetaldehyde follows very closely to that of ethanol.

From these results, it seems that the protective effect of ascorbic acid, or thiamine against acetaldehyde or disulfiram-ethanol-induced mortality was due to the ability of these agents to reduce acetaldehyde blood levels. Because these agents also reduce the metabolism of acetaldehyde, their use in chronic acetaldehyde exposure (such as in chronic alcoholism), may lead to acetaldehyde accumulation in the body.

The protective effect of cysteine may also be due to its ability to reduce acetaldehyde blood levels. Because cysteine did not

increase the half life of acetaldehyde, it is less likely to induce acetaldehyde accumulation. However, the effects of cysteine on the endogenously generated acetaldehyde (in the absence of disulfiram), suggest that cysteine would not be an agent of choice for protection against acetaldehyde toxicity in chronic alcoholism.

Table 12 SUMMARY TABLE: Effects of L-ascorbic acid, thiamine HCl, or L-cysteine on acetaldehyde blood levels and disposition.

	Endogenous AcH		Exogenous AcH	
Parameters	ETOH	DS+ETOH	ACH	DS + AcH
Ascorbic Acid				
Blood Levels	±	↓	↓	±
$t_{\frac{1}{2}}$	±	±	↑	↓
Vd_{Beta}	0	0	↑	↓
CL_{total}	±	±[a]	±	
AUC	±	↓	±	±
AUC-ETOH / AUC-AcH	↑	±	0	0
Thiamine				
Blood Level	±	±	↓	↓
$t_{\frac{1}{2}}$	±	±	↑	↓
Vd_{Beta}	0	0	↓	↓
CL_{total}	±	±	±	↑
AUC	±	±	±	↓
AUC-ETOH / AUC-AcH	↑	↑	0	0
Cysteine				
Blood Levels	↑	±	↓	±
$t_{\frac{1}{2}}$	↑	±	±	±
Vd_{Beta}	0	0	±	±
CL_{total}	↓	±	±	±
AUC	↑	±	↓	±
AUC-ETOH / AUC-AcH	↓	↑	0	0

± = No statistical significant affect
↑ = Increase
↓ = Decrease
0 = Not applicable
DS = Disulfiram
AcH = Acetaldehyde
ETOH = Ethanol

[a] Ascorbic acid significantly increase the clearance of ethanol, but not of acetaldehyde

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